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Government Website Inaccessibility

and the Future of Accessibility in Information Technology

E-Cigarette Taxes and Health
The Economics of E-Cigarettes: How Taxation Affects Health Outcomes

Invertebrate Competition Dynamics
Investigating variations in competition across different ecosystems

Investigating PEPCK Paralogs from E. histolytica
Investigation into PPi-dependent PEPCK Paralogs in E. histolytica

CORNELL UNDERGRADUATE RESEARCH JOURNAL

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Letter from the Editor

Dear reader,

We are very excited to present the fourth issue of the Cornell Undergraduate Research Journal (CURJ). CURJ is Cornell's peer-reviewed bi-annual publication of exemplary research work done by undergraduates across many disciplines. Our goal is to give all students the opportunity to showcase their work to their peers and to the general public, foster intellectual discussion and collaboration, and provide a wide range of academic perspectives.

This semester has been particularly exciting for us. With our former editor-in-chief's graduation, many members of our staff who have long been dedicated to CURJ are now leaders on our team, working hard to make the journal the best it can be. Additionally, this semester came with an influx of new members, many of them freshman, passionate about what we do and with the skills and new ideas needed to keep pushing CURJ forward. These changes did not come without challenges, but we are very proud of the team we have built, and their ability to meet any challenge head-on.

CURJ has always focused on ensuring the Cornell community can learn about various subjects. From the efficacy of masks on particle dispersion, inaccessibility online, to metabolic enzyme functions, this issue is amongst our most diverse. All of our manuscripts shine a light on the importance of disseminating information that students with all interests can learn from. To turn the page and be transported from the world of invertebrates to how disabilities are not accommodated in a digital world presents a novel outlook on the world around us. What has always made us proud of CURJ is the dedication of graduate reviewers and faculty advisors who bring the hard work of each Cornell student's unique manuscript to the forefront. Leading the ever growing teams that stitch these subjects together has been a pleasure and hopefully brings CURJ one step closer to the club that our founder, Victoria Alkin, wanted it to become.

This journal is only possible due to the diligent work of all the student researchers that have submitted their projects to CURJ. Many students participate in research at Cornell, but the amount of time and effort that goes into completing an independent project is an amazing accomplishment that only so many can achieve. In addition to giving thanks to our staff members that have been working for weeks to develop the Fall 2023 issue, we would also like to applaud each student that submitted their work for publication this semester. The primary purpose of CURJ is to highlight these projects and publish them for a greater audience to see, and none of that would have been possible without the contribution of our student researchers.

We are delighted to share the fourth issue of the Cornell Undergraduate Research Journal and hope you enjoy reading the edition as much as we enjoyed making it.

Sincerely,

Chris Cizmeciyan

Minji Kim

Connor Rosenthal

CURJ

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CONTENTS

Government Website Inaccessibility and the Future of Accessibility in Information Technology

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Abstract

Although the government has made efforts to mandate the accessibility of federal websites, such guidelines have ultimately failed at addressing the needs of the disability community. The law requiring the viewability of federal websites, Section 508, is enforced by the Access Board, a government agency. However, the guidelines for evaluating website accessibility are vague and do not include provisions for people with cognitive disabilities. Such insubstantial laws deny disabled people essential government benefits, opportunities to interact with the government, and knowledge about the legislation that affects them. Upon examining this literature, the practical impacts of exclusionary language were revealed. After reviewing relevant legislation, I explored research papers detailing the disparities caused by inaccessible legislation. Lastly, I examined current projects to develop my policy recommendations. I contend that stricter legislation and website testing boards led by people with disabilities will promote the proper administration of accessibility laws. I found that the approach employed by the Department of Justice to determine the accessibility of federal websites excludes the varied ways that disabled people interact with technology. The Department of Justice must clearly present and frequently release its accessibility assessments to promote accountability. Further, accessibility measurements must be led by people with disabilities, and the testing boards should be a coalition of communities, internet accessibility tools, and legislators to promote the agency of the most impacted. The institution of these initiatives would remove barriers restricting information from the people that the laws are intended to affect

Keywords: Accessibility, disability rights, legal analysis, policy, internet access.

Government Website Inaccessibility and the Future of Accessibility in Information Technology

When Joel Price, a blind Floridian, sought to discover information about the local legislation and governmental processes of the City of Ocala, he ended up feeling "segregated, rejected, and isolated as he was left excluded from participating in the community services, programs and activities" (Price v. City of Ocala, 2019). Price attempted to interact with Ocala's website to learn more about the quality of life in the city, as he was considering relocating to the region (Price v. City of Ocala, 2019). However, he was denied this knowledge, as the documents' format was unreadable to blind individuals (Price v. City of Ocala, 2019). Price's story exemplifies a familiar and exclusionary narrative; in a world of constant innovation, the legislation defining how governments should make their resources accessible perpetuates ableist narratives about who is fit to participate in society. The government has obstructed access to the information essential for community engagement by implementing policies that employ ableist language.

Price's account reveals the detrimental impacts of

the government's dismissal of accessible website design as a mere legal obligation. The Information Technology and Innovation Foundation, a think tank for science and technology policy, "found that 30 percent did not pass an automated accessibility test for their homepage, and nearly half (48 percent) failed the test on at least one of their three most popular pages" (Johnson & Castro, 2021, Key Takeaways section). Local and state governments have neglected accessibility accommodations despite this research; the Department of Justice (DOJ) released a report in January 2023 detailing the "compliance with Section 508" of "Chief Financial Officers (CFO) Act federal agencies," and the DOJ had not done so "in ten years" (U.S. Department of Justice, 2023, pp. i, as cited in Jenkins & Vu, 2023). The report found that although "90% of 58 agency domains tested have an accessibility statement," agencies' claims of accessibility are inadequate as "32 (55%) of the accessibility statements tested require remediation to meet requirements" (U.S. Department of Justice, 2023, p. 9). The findings also indicated that "73% of the PDF documents" downloaded from federal websites were "untagged," meaning that they "lack any kind of markup information that helps make content accessible to people who rely on assistive technologies to access the written and visual information" (U.S. Department of Justice, 2023, p. 9). The government's failed attempts to remedy inaccessibility exclude disabled individuals from technological progress.

Furthermore, the government has failed to fulfill its obligations in acknowledging systemic failures. The institution of accessibility regulations has significantly increased from the government's first acknowledgment of mandating equitable internet access. However, this legislation has perpetuated ableism, which is evident in the first conceptions of internet accessibility policy. Society is becoming increasingly dependent on web-based content, and accessibility legislation must be as progressive as technological innovation to ensure the inclusion of the disability community.

In this paper, I will explore the multifaceted implications of the barriers to web accessibility by revealing how the government has avoided responsibility. I will first describe the legislation governing the accessibility of government websites, which will provide a framework for analyzing its deficiencies. Next, I will argue that the government's failure to establish a clear ruling for web accessibility requirements under the ADA and the government's reductionist perspectives that undermine systemic obstacles are the primary failures of accessibility legislation. I will highlight the many consequences of inaccessible technology and argue that iterative legislation addressing the diverse ways that people with disabilities interact with website content must be prioritized.

After contextualizing these insufficiencies, I will maintain that these disparities impact the disability community's ability to engage politically. Due to the emphasis on virtual environments and the new technologies populating our world, much of the information vital for individuals to participate in civic engagement is only available via the Internet. Virtual spaces can potentially increase societal involvement, and therefore, the guidelines governing their usage should be reformed to center those affected by inaccessible resources.

Lastly, I will argue for policy recommendations directly addressing these inadequacies. I will describe the need for the mandated release of DOJ website accessibility reports and the use of conspicuous criteria ensuring the enforceability of accessibility guidelines. I will affirm that Section 508 should be revised to include those with cognitive disabilities and that such adaptations should be sourced directly from user-experience interviews and assessments.

Ultimately, I support the institution of governmental supervisory boards composed of people with disabilities. The board, harmonizing technical accessibility tools with personal experiences, will advise legislators in developing DOJ accessibility reports. Policy

itself cannot mitigate the rampant issue of inaccessible government information; advocacy for an attitudinal shift through disability awareness should be prioritized in making such recommendations. I will emphasize that these integrative solutions must prioritize the disability community over fulfilling legal requirements. These guidelines should acknowledge technological change while upholding the need to develop inclusive provisions for all disabled people.

Present Legislation Mandating Accessible Federal Websites

The following laws and policies define accessible media and how federal and state governments should implement guidelines to ensure equal access to government information. In the next section, I will describe Title II and Sections 504 and 508 of the Rehabilitation Act of 1973 and the role of this legislation in establishing the modern framework for assessing accessibility guidelines. I will describe how governments precisely fail to implement accessibility guidelines. The legislative information will form the foundation of my critique.

Title II serves as the primary legislation enforcing accessibility standards. "Title II applies to State and local government entities, and, in subtitle A, protects qualified individuals with disabilities from discrimination on the basis of disability in services, programs, and activities provided by State and local government entities. Title II extends the prohibition on discrimination established by section [*sic*] 504 of the Rehabilitation Act of 1973" (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). Section 504, "the first disability rights law related to technology," requires the government to equitably present information through such media" (Jaeger, 2012, p. 47). While Title II and Section 504 are paramount to note as the first legislation recognizing the necessity for government information accessibility, these requirements did not specifically pertain to websites, as the writing of these laws occurred before the widespread presence of the Internet.

Section 508 expanded upon the standards for accessibility described in Section 504. The 1998 amendment of the Rehabilitation Act of 1973 was designed to mandate government websites to "make their electronic and information technology (EIT) accessible to people with disabilities" (Section508.gov, 2020). Section 508 also applies to "federal employees and members of the public alike" (29 U.S.C. § 794d, 1998, as cited in Johnson & Castro, 2021, p. 2). "The Access Board is an independent federal agency that promotes equality for people with disabilities through leadership in accessible design and the development of accessibility guidelines and standards" (U.S. Access Board, n.d.). In "2017, the Access Board issued a final rule that updated accessibility requirements covered by Section 508" (Section508.gov, 2020). The Access Board, as an enforcement mechanism for Section 508, is a crucial step towards the tangible implementation of accessibility guidelines. However, Section 508 considers the disability community as homogenous and overlooks the needs of cognitively disabled people, as I will contend in the next section (Jaeger, 2012, p. 52).

The Inadequacy of Accessibility Guidelines for Federal Websies Ambiguity in Accessibility Recommendations

Despite this legislation, the government has failed to develop policies considering the impacts of inaccessible government information. This disregard perpetuates ableist stereotypes about who is deemed fit for societal engagement. Legislators may claim that such legislation

intends to mandate website equity, but disabled individuals continue to be denied essential information despite this legislation. This section will address the inadequacies of these policies and establish a framework for analyzing the impacts of exclusionary legislation.

As exemplified through the ambiguous language in accessibility recommendations, the government considers website accessibility a burden rather than a need for the disability community. Section 508 states, "a final rule specifying technical standards under the ADA has not been adopted. If you're subject to the ADA, you have more flexibility in determining how to make your website compliant with the ADA's general requirements of nondiscrimination and effective communication" (Section508.gov, 2017). Accessibility regulations do not include information on what website specificities, such as font size and color contrast, constitute accessible design. Professor of Information Studies Paul Jaeger argues that "the revision of the Section 508 requirements did not result in a consensus on guidelines for accessibility for cognitive disabilities, noting that any such guidelines would not be specific or measurable" (Jaeger, 2012, p. 52). Improvements to accessibility legislation must begin with an attitudinal change; "in an ableist context, interdependence will always get framed as 'burden,' and disability will always get framed as 'inferior'" (Mingus, 2017).

Failure of the Government to Accommodate Cognitive Disabilities

Current policies fail to recognize the multitude of ways the disability community encounters hardships in interacting with websites. Blanck contends that legislation must acknowledge the nuances within the disability community by specifying accessibility requirements for cognitively disabled people (Blanck, 2014, p. 45). "Cognitive disability, as compared to sensory or physical disability, necessitates that substantive web content and complexity is the focal point at which designers make the leap towards web equality" (Blanck, 2014, p. 45).

A website may be viewable with certain assistive technologies for some individuals, but different disabilities require varying guidelines to promote access (Blanck, 2014, p. 45). Requirements for websites that are viewable by cognitively disabled people could include "providing content that is not unnecessarily dense" by giving users the option to view the "original" information on a webpage in different "formats" like "screen reader software" along with "video, text, and image description" (Blanck, 2014, p. 164). However, government agencies have asserted that reshaping standards to accommodate cognitive disabilities would require a "'fundamental alteration;' if a defendant successfully asserts the 'fundamental alteration' or 'undue burden' defense, they may escape liability under the ADA" (Roberts v. KinderCare Learning Centers, 1996, p. 86, as cited in Pavlicko, 2021, p. 973). The absence of comprehensive standards furthers the idea that detailed accessibility requirements are onerous and unnecessary to define.

Thus, legislative ambiguity allows the government to avoid responsibility while disabled individuals are still unable to access essential services. This unfair standard is illustrated through the case of the Florida Department of Economic Opportunity Office (DEO) of Unemployment Compensation, where they were "cited by the Department of Labor for violating Federal statutes, including Title II of the ADA, for requiring unemployment compensation applicants to file claims online and complete an online skills assessment as part of the claims-filing process even though the State's Web site was inaccessible" (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). The government's failure to mandate a specific guideline contributes to similar incidents by failing to adopt specific requirements for federal entities.

While the absence of a concrete rule impacts the relevance of the Title II mandate, a legal requirement is nevertheless present. As evident in the citing of the Florida DEO, "without a uniform interpretation of the ADA's scope, uncertainty surrounding website accessibility litigation will continue as the utilization of online technology continues to increase" (Pavlicko, 2021, p. 956). Flexible measures for holding agencies accountable have allowed the government to escape its responsibility to enforce equity. "Section 508 also requires the Department of Justice (DOJ) to submit biennial reports to the president and Congress evaluating the extent to which the electronic and information technology federal agencies use is accessible" (29 U.S.C. § 794d.(d), 1998, as cited in Johnson & Castro, 2021, p. 2). "However, it does not require DOJ [sic] to make these reports available to the public" (Section508.gov, 2021, as cited in Johnson & Castro, 2021, p. 2). The government diverts the responsibility of fulfilling accessibility requirements to the disability community, so the government itself does not have to carry the financial and emotional burden of holding its agencies accountable. In 2010, the DOJ "conducted interviews with agencies in which they merely asked the agencies how accessible their web-based content is rather than actually evaluating the websites" (Jaeger, 2012, p. 137). While these measures appear to be a positive step towards enforcing website accessibility, these interviews did not involve any protocol ensuring their work would benefit the disability community. Ultimately, the interviews lacked the assurance that agencies were presenting accurate information.

The Undermining of Comprehensive Accessibility Legislation

The government has employed reductionist perspectives on issues of website accessibility by approaching the issue solely financially. Research indicates that "simply allotting more financial investment to accessible design might not lead to the desired outcome of higher accessibility of government websites" (Bai et al., 2020, p. 858). Channeling monetary resources into rural counties is counterintuitive to solving inadequate web accessibility in these localities. It is a disrespectful measure for governments to implement, acting as if money solves the social consequences of inaccessible websites. Such actions consider accessibility a financial issue when it affects the livelihood of disabled individuals.

The Societal and Personal Repercussions of Web Inaccessibility

Through understanding the failures of accessibility legislation, we can now witness how the lack of key resources for civic engagement excludes the disability community from societal participation. Website accessibility is not merely a legal issue; the current accessibility legislation suppresses the political voice of the disability community.

The Denial of Voter Education Resources

As Professor of Information Studies Jonathan Lazar argues, "without access to the Internet and the services it offers, persons with disabilities will not have equal access to information" that is "available to the rest of society. That denial relegates those who are excluded to second-class citizenship and ensures that the social integration envisioned by the ADA cannot occur" (Lazar et al., 2015, Title III of the ADA—Places of Public Accommodation section, para. 19).

This lack of consideration establishes barriers to political involvement and often renders it impossible. Jaeger contends that "low levels of access to e-government leave people with disabilities unable to equally participate, or

participate at all, in these online manifestations of government" (Jaeger, 2012, p. 110). If a website is unreadable by assistive technologies, individuals cannot view deadlines, track mail-in ballots, or analyze candidate platforms to make informed voting decisions.

The Prevention of Education Regarding Government Services and Political Action

The COVID-19 pandemic normalizes virtual engagement with government officials, which initially appears favorable for the disability community. "Many people with disabilities face difficulties in traveling to government offices and many individuals with disabilities have a greater need for social services" (Jaeger, 2012, p. 110). While online spaces have eliminated the physical barriers that Jaeger describes, many individuals are not allowed virtual engagement due to the absence of regulatory adaptations to our technology-centric society. Maintaining reliable and transparent communication between the people and their government is vital to a thriving democracy. The Internet has granted the ability for "citizens to watch local public hearings, read minutes from community meetings, or take part in live chats with government officials on the Web sites of State and local government entities" (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). Inadequate policy has transformed virtual spaces into a mechanism for exclusion. For example, identifying the requirements for voter registration is already an overcomplicated task that disincentivizes voting.

Burdensome voting guidelines communicate an antidemocratic message that if someone cannot properly navigate the proper resources due to their disability, they should not have the right to vote. This message extends to engagement in political leadership. Websites serve as the primary platform for community leadership information; "individuals interested in running for local public offices can often find pertinent information concerning candidate qualifications" (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). The Chief Executive Officer of Miami Lighthouse for the Blind and Visually Impaired, Victoria Jacko, along with a group of researchers, determined if candidates running for political office had websites that were accessible (Miami Lighthouse for the Blind and Visually Impaired, 2022, Research Rationale section). The 2022 study examined "16 top midterm candidates" (Miami Lighthouse for the Blind and Visually Impaired, 2022, Individual Candidate Scores section, as cited in Abrams, 2022). If people encountered problems navigating website content, no contacts were listed on the websites that could help (Miami Lighthouse for the Blind and Visually Impaired, 2022, What We Uncovered section). The inability to access government content communicates to disabled individuals that they are unworthy of inquiring about policies affecting their communities, voting, and advocating for issues transforming their livelihoods.

Government websites "include a variety of information about issues of concern to the community and how citizens can get involved in community efforts to improve the administration of government services," but direct action from the disability community is inhibited by inaccessible educational resources (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). The government encourages a disconnect between itself and the disability community by allowing information on civic engagement to be hidden by inaccessible websites.

According to Jaeger, "In many states, taxes can only be paid online, [sic] and social services can only be applied for online" (Jaeger, 2012, p. 110). Disabled individuals may be unaware of these

requirements due to inaccessible websites and fail to apply for programs, including "unemployment benefits and food stamps," which "are available through State Web sites" (Nondiscrimination on the Basis of Disability; Accessibility of Web Information and Services of State and Local Government Entities, 2016). While the government allegedly designed these services to promote equity, the hardships that disabled individuals encounter while attempting to access such services exemplify the longstanding difficulty of gaining government benefits. Considering the consequences of inaccessible government content, I will next present recommendations designed to address the inadequacies of current policy.

Policy Reccommendations to Improve Government Website Accessibility Mandate the Adoption of DOJ Reports and Clear Standards of Assessment

Increasing the frequency of the release of the DOJ reports is a prominent recommendation among scholars. Johnson and Castro contend that "Congress should also require the [sic] DOJ to collect and share data on the number of accessibility complaints agencies receive each year" (Johnson & Castro, 2021, p. 14). However, merely increasing the accessibility of this data will fail to contribute to substantial change. Further, "federal agencies (and other groups) are at liberty to develop test processes that incorporate all the baseline tests and any additional test criteria specific to their needs" (Section 508 ICT Testing Baseline, 2022). This means that government agencies can utilize any program for accessibility testing they choose if it adheres to the requirements outlined in Section 508 (Section 508 ICT Testing Baseline, 2022). Section 508 empowers the government to make the best choices for Disabled people without any consultation into the failures of accessibility tools and why a human-focused perspective on disability is necessary. A 2022 study from the Umeå Student Conference in Computer Science tested the effectiveness of popular accessibility assessment tools and found that "there is a vast difference in how many errors the evaluators find on a website, and there are discrepancies in how the errors are classified by the tools" (Björkman, 2022, pp. 16, 23). Accessibility tools may be helpful for an initial review. However, none of these examined tools were highly recommended, and one tool was even found "confusing" as the accessibility violations were hard to find (Björkman, 2022, pp. 22-23). This was especially noted in the case of Wave, lauded as one of the premier tools (Björkman, 2022, p. 22; Regents of the University of Minnesota, 2023).

The DOJ has not framed its audits around the experiences of disabled individuals, and the automated approach to enforcing Section 508 must be adapted to focus on the lived experiences of disabled people. I contend that the approach to accessibility testing can first involve an initial examination of the website content using accessibility tools, which can assist the testing boards in finding initial issues. The tools used will vary by agency, as agencies may find different tools more effective based on the precise goal of the accessibility testing (such as reducing "repetitive content" or developing "keyboard accessible" webpages) (Section 508 ICT Testing Baseline, 2022).

As accessibility tools have shown to be problematic, the DOJ must use a comprehensive interview process to report the accurate status of website accessibility (Björkman, 2022, p. 23). I assert that the interviews should be conducted with individuals with various disabilities, including those with cognitive and sensory disabilities. Disabled individuals will lead the process; panel members will gather via an interactive panel discussion with the DOJ in which user experience (UX) simulations will occur. The UX sessions must be adaptable,

as not all disabilities are the same, which current legislation continuously denies. The participants will be given a prompt for a specific page they should navigate to, which will help evaluate the accessibility of each website feature. Similarly, the panel will be directed to click on menus and respond to questions concerning their viewability, font size readability, and color contrast. Participants should be encouraged to divert from the interview questions, as a set of questions cannot cover the broad scope of experiences the panel members may have during the session.

The information collected from the interviews and the observations of the DOJ during the sessions must be published bi-annually. I contend that this frequency is necessary due to the history of the failure of the DOJ to communicate the status of government website accessibility (Johnson & Castro, 2021, Key Takeaways section). The DOJ should work with website developers to include several website prototypes, one featuring the reports as an announcement on government website homepages, another prototype as a separate menu, and a final layout including the reports in the location where the government has their accessibility information available. The information should be released via easily navigable reports; the location will be determined from the interactive interviews and tested separately for accessibility compliance. During the interview process, individuals may respond with feedback that will be used to select the website format. This approach is an essential step for the government to take in improving website accessibility, as legislators will integrate the needs of disabled individuals in enforcing accessibility rather than incorporating the sole judgment of the government.

Involve a User-Focused Approach

Policymakers must include diverse types of disabilities in the legislation developed from universal design principles. Universal design is a comprehensive approach, "also called inclusive design, design for all, or life span design" (Maisel & Ranahan, 2022). The cost of interacting with accessibility experts and private testing boards may pose a financial challenge for governments in rural localities. Governments cannot use financial excuses for universal design, which requires thought and engagement with those marginalized by internet inaccessibility. The instantiation of universal design in government website development will establish a precedent for greater inclusion, as rather than relying on preconceived notions of what constitutes accessibility, developers and government leaders are supporting how the disability community can best be served by legislation.

Conclusion

In this paper, I have asserted that governmental efforts to promote accessibility encourage ableist stereotypes and prevent the disability community from accessing essential information. I described the history of website accessibility legislation and how governments have allegedly promoted inclusion through Title II and Sections 504 and 508 of the Rehabilitation Act of 1973. Using this legislative framework, I analyzed the insufficiencies of governmental regulation. I argued that the language of accessibility legislation uses ambiguity to fulfill legal requirements while failing to provide sufficient protection for disabled individuals' interactions with the Internet. I asserted that the legislative neglect of cognitive disabilities and the government's reductionist view in considering the importance of accessibility legislation have resulted in the denial of the disability community to access essential resources.

After establishing an understanding of the failures of accessibility legislation, I described how these insufficiencies contribute to the isolation of the disability community and the suppression of their participation in society. I contended that regulations impact how disabled individuals are afforded the opportunity for

political leadership and voting and exclude the voices of the disability community. As a result, legislation is developed without considering how disabled people are impacted, and the inaccessibility of web-based governmental education resources contributes to the cycle of exclusion. I emphasized the need for virtual government services to institute accessible design. Online spaces may serve as a mechanism to reach larger audiences than in-person activities, as disabled people still experience the harmful effects of COVID-19.

Lastly, I presented policy recommendations developed from the inadequacies previously discussed. I argued that defining the standards used in compiling the DOJ reports, instituting universal design to ensure flexibility in website design, and using a governmental accessibility advisory board can promote the voices of those involved in accessibility regulation. As demonstrated by the experience of Joel Price, the government will continue to restrain vital information to ostracize the disability community. Therefore, I contend that policy formation must be developed with the needs of the disability community to protect the well-being of individuals such as Joel Price, ensuring that disabled people are leading the way for accessibility measures in technological change.

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The Effect of E-Cigarette Tax on Health Outcomes

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Abstract

The alarming rise in the use of electronic cigarettes (e-cigarettes) among teens in the US has become a major health concern, driving many states to take action, including Illinois, which implemented a 15% tax on e-cigarettes. This research aims to evaluate the effectiveness of taxation to control e-cigarette use, measured by improvement in health outcomes in Illinois, from before and after the state implemented the 15% tax. Additionally, a comparison was done with neighboring states of Michigan and Missouri. Using difference-in-difference hypothesis testing, a statistically significant decrease in reported rates of asthma, depression, toothache, and bleeding gums, was noted when comparing health outcomes before and after the implementation of the state tax in Illinois as compared to Michigan. A decrease in the reported rate of asthma was noted to be significant in the comparison with Missouri as well.

Introduction

Since electronic cigarettes (e-cigarettes) were introduced in the market in 2007, vaping has been on the rise in the US. It reached "epidemic proportions" in 2018, when the US Surgeon General issued a call to action to address the epidemic of e-cigarette use in adolescents, while asserting the health risks associated with e-cigarette use (*Surgeon General's Advisory on E-Cigarette Use among Youth*, 2018). E-cigarettes or vapes are also called electronic nicotine delivery systems (ENDS) as they insufflate nicotine. The CDC warns that the nicotine present in e-cigarettes is highly addictive. Nicotine can be toxic to developing fetuses, and harmful for teen and adult brain development until the mid-20s (About Electronic Cigarettes (E-Cigarettes), n.d.). Per the CDC, e-cigarettes produce an aerosol by heating a nicotine-containing liquid along with other component chemicals, which users inhale into their lungs as they vape (About Electronic Cigarettes (E-Cigarettes), n.d.).

Initially, e-cigarettes were marketed as a nicotine-cessation device, which led to their popularity, especially with teens and young adults. In mid-2019, the US experienced an e-cigarette product use-associated lung injury (EVALI) outbreak due to lack of long-term data on health risks, coupled with a lack of regulation of e-cigarettes. In 2019, clusters of lung injury were reported with e-cigarette use, especially in Illinois and Wisconsin, with the key symptoms being respiratory, gastrointestinal, and constitutional complaints (*Outbreak of Severe Pulmonary Disease Linked with E-Cigarette Product Use*, 2020).

E-cigarettes have many detrimental effects on public health. The National Academies of Science, Engineering, and Medicine published a report that reviewed over 800 studies in January 2018, which asserted that using e-cigarettes causes health risks (The National Academies of Sciences, Engineering, and Medicine, 2018). The report stated that e-cigarettes contain and emit a number of potentially toxic substances harmful to human health, and youth who use e-cigarettes are at a heightened risk of asthma exacerbations, cough, and wheezing. Also, e-cigarettes produce a number of toxic chemicals such as acetaldehyde, acrolein, and formaldehyde, which can lead to lung and cardiovascular diseases (Ogunwale

et al., 2017). Acrolein, used in e-cigarettes, is a herbicide primarily used to kill weeds, and can cause acute lung injury, chronic obstructive pulmonary disease, asthma, and lung cancer. Two primary ingredients found in e-cigarettes—propylene glycol and vegetable glycerin—have been identified as toxic to cells in a study at the University of North Carolina (Sassano et al., 2018). The U.S. Surgeon General as well as the National Academies of Science, Engineering, and Medicine have warned about the risks of inhaling secondhand e-cigarette emissions, which are released when an e-cigarette user exhales the chemicals created by e-cigarettes as they contain nicotine, volatile organic compounds such as benzene, and chemicals such as diacetyl that are linked to serious lung disease (The National Academies of Sciences, Engineering, and Medicine, 2018).

As e-cigarettes are a major health hazard amongst youth, there is focus both from regulating the supply side as well as controlling the demand. In the US, for decreasing the demand and controlling the use of tobacco, taxation has proved to be an efficient strategy. While regulation concentrates on the supply side of the e-cigarette market, taxation focuses on the demand side, providing a unique advantage of creating public revenue (Mainous et al., 2015).

This study aims to evaluate the effectiveness of taxation of e-cigarette in Illinois by comparing various health risk factors including asthma, headache, depression, and bleeding gums before and after the implementation of the policy. As the e-cigarette tax was implemented in 2019, we compared and analyzed health data from 2017 and 2021 to assess the effectiveness of the policy. Additionally, we compared the Illinois data with neighboring states Michigan and Missouri, as neither Michigan nor Missouri have implemented any tax policies to curb e-cigarette sales. Our hypothesis is that there is a correlation between current e-cigarette usage and taxation, and therefore the health risks would improve after the policy was put into effect. Therefore, we expect rates of asthma, breathing difficulties, headache, depression, toothache, and bleeding gums to decrease in Illinois as compared to Michigan and Missouri between 2017 and 2021.

Literature Review

As vaping has become rampant in the past decade, there has been significant interest in researching the relationship between prevalence of e-cigarettes and the various variables that affect its usage including price and health risks. Several studies have investigated the use of vaping products and its impact on health and safety including Thirión-Romero et al. (2018), Bircan et al. (2021), King et al. (2020), Obisesan et al. (2019), Akinkugbe (2018), and Alhajj et al. (2022). Additionally, papers including Corrigan et al. (2021) and Yao et al. (2020) looked into the price elasticity of demand in an effort to estimate sensitivity to price increase amongst teens, while Jun et al. (2021) evaluated the effect of policy on prevalence of vaping.

Thirión-Romero et al. (2018) conducted an investigation into the respiratory impact of e-cigarettes and found that vape aerosols pose cytotoxic effects on lung tissue, similar to that of a tobacco cigarette, as they contain various respiratory toxins. Known toxins such as formaldehyde, acetaldehyde, metallic nanoparticles, and acrolei have been detected in e-liquid and aerosols, and as a result, e-cigarettes could be linked with an increase in symptoms in individuals with asthma (Thirión-Romero et al., 2018). Bircan et al. (2021) further evaluated the link between e-cigarette use and self-reported diagnosis of asthma, COPD, and ACOS. Using a multinomial logistic regression, the study leveraged a representative sample of over 8000 adults from the Behavioral Risk Factor Surveillance System (BRFSS) from 2016 to 2018 while controlling for marital status and employment along with matching variables. The study found that the e-cigarette smokers had increased likelihood of self-reported ACOS (OR=2.27; 95% CI: 2.23–2.31), asthma (OR=1.26; 95% CI: 1.25–1.27), and COPD (OR=1.44; 95% CI: 1.42–1.46) as compared to

non e-cigarette smokers (Bircan et al., 2021). Both Thirión-Romero et al. (2018) and Bircan et al. (2021) found a positive correlation between e-cigarette use and asthma, revealing asthma is a key risk factor for e-cigarette use.

King et al. (2020) investigated the negative health symptoms, including headaches, reported by youth e-cigarette users by conducting a national cross-sectional telephone survey of 975 adolescents between ages 13–17. The study looked into six health issues caused by e-cigarettes and examined various factors including demographics and tobacco use. They found that most of the users had experienced at least one health symptom during their e-cigarette use, with cough being the most common symptom and headaches being more common among the past 30-day users as compared to non-users (King et al., 2020). This paper's findings around correlation between e-cigarette usage and headaches in adolescents indicates that headaches can be considered as a health risk factor for e-cigarette use.

Obisesan et al. (2019) examined the association between e-cigarette usage and depression through a cross-sectional study of 892,394 participants in the Behavioral Risk Factor Surveillance System between 2016 and 2017. Their investigation revealed a higher likelihood for e-cigarette users to report a history of clinical diagnosis of depression as compared to others who never used e-cigarettes. They also found that higher frequency of e-cigarette use was linked with higher odds of reporting depression (Obisesan et al., 2019). Moustafa et al. (2021) further examined the association between depression symptoms and adolescent e-cigarette progression by conducting a longitudinal survey of 1822 teenagers from four Philadelphia area public schools. They found that the e-cigarette use trend was significantly affected by baseline depressive symptoms while holding other variables constant ($b = 0.01$, $z = 4.29$, $p < 0.0001$). Their paper provided evidence that greater depressive symptoms during teenage years were associated with a faster rate of e-cigarette escalation (Moustafa et al., 2021). Studies by Obisesan et al. (2019) and Moustafa et al. (2021) highlights the prevalence of depression as an important variable to consider while evaluating effectiveness of e-cigarette policies.

Akinkugbe (2018) investigated the association between use of e-cigarettes and oral health status by studying the data from 13,650 adolescents aged 12 to 17 years from the Population Assessment of Tobacco and Health study of self-reported current use of e-cigarettes, using survey-adjusted logistic regression. The covariate-adjusted associations between current e-cigarette use on dental problems showed a Prevalence Odds Ratio (POR) of 1.11 (95% CI, 0.79 to 1.55), suggesting an increased risk of dental disorders for the adolescents using e-cigarettes currently (Akinkugbe, 2018). Alhajj et al. (2022) explored and found association between e-cigarette usage and worsening oral health, including oral candidiasis, oral mucosal lesions, halitosis, dental caries, and periodontal disease (Alhajj et al., 2022). Studies by Akinkugbe (2018) and Alhajj et al. (2022) highlight the impact on oral health such as bleeding gums being an important consideration for evaluating the effectiveness of e-cigarette policies.

There are two recent studies that examine price elasticity of demand for e-cigarettes. Diaz et al. (2023) studied the sensitivity of US youth to changes in e-cigarette prices and tax using standardized measures of e-cigarette taxes and prices. They analyzed the cross-sectional 2015–2019 Youth Risk Behavior Survey along with standardized inflation-adjusted e-cigarette price and tax data to evaluate whether changes in e-cigarette price and tax were associated with changes in e-cigarette use. They built two-part demand regression models controlling for demographics and e-cigarette restriction policies. They found that a $0.50 and $1.00 tax increase led to a 6.3% and 12.2% decrease, respectively, in past 30-day e-cigarette use, showing correlation between taxation on e-cigarettes and reduced usage in youth (Diaz et al., 2023).

Corrigan et al. (2021) investigated the question of how sensitive teens' demand for one of the most used brands of e-cigarettes, JUUL, with

respect to change in price. They recruited 300 teenagers from the University of South Carolina (N = 188) and Susquehanna University (N = 112) between 2018 and 2019 and conducted an experimental auction where adolescents bid on a JUUL kit. Their analysis of Price Elasticity of Demand (PED) showed that 10% increase in price leads to as much as a 24% reduction in e-cigarette demand among current teen users, and a 45% reduction among teens who have not used e-cigarettes. This paper concluded that teens are sensitive to increase in price and e-cigarette taxes can be an effective measure at reducing the e-cigarette use among teenagers (Corrigan et al., 2021).

With e-cigarettes becoming a public health concern, several studies have investigated the price elasticity of demand for e-cigarettes. Yao et al. (2020) examined the impact of e-cigarette prices on e-cigarette sales in California. They built fixed-effects models to predict the impact of e-cigarette and cigarette prices on e-cigarette sales separately for each type of e-cigarettes controlling for year, quarter, scantrack market, and California SFAL coverage. A two-tailed p-value < 0.05 was considered to be statistically significant. Their analysis found that when there was a 1% increase in prices of disposable e-cigarettes, reusable e-cigarettes, and cigarettes, there was a decrease in per capita sales of the products by 0.37%, 0.20%, and 0.21% respectively. The study found that e-cigarette sales are responsive to price changes, which suggests that raising prices, such as increasing the tobacco excise tax, can help reduce sales of e-cigarettes (Yao et al., 2020).

Jun et al. (2021) investigated the impact of state regulations and policy on e-cigarette prevalence by performing logistic regressions on 2017 Behavioral Risk Factor Surveillance System and the US e-cigarette regulations-50 state review by the Public Health Law Center. Their paper found that there were significant differences in e-cigarette use based on the number of state laws regulating e-cigarettes, concluding that policy efforts to regulate e-cigarettes could have significant impact on e-cigarette prevalence (Jun & Kim, 2021).

The past studies conducted to evaluate the impact of price and health risks on e-cigarette use have provided a compelling story. The current literature provides sound evidence about health risks associated with e-cigarette usage including asthma, respiratory illnesses, headaches, depression, and oral health issues. Teenagers' demand for e-cigarettes seems to be sensitive to price increase, and e-cigarette tax, with evidence highlighting a reduction in usage of e-cigarettes when the price of e-cigarettes was increased with taxation. However, there have not been any studies conducted to measure the impact of a state that imposed e-cigarette tax. Therefore, our research aims to evaluate the effectiveness of e-cigarette taxes in Illinois by comparing health risks before and after the policy implementation to confirm correlation between current e-cigarette usage and taxation. Further, our study compares the health outcomes with a state that has not imposed e-cigarette taxes to further estimate the effectiveness of taxation as a mitigation strategy for worsening health outcomes with e-cigarette use.

Policy Context in Illinois

In 2019, when many states across the US observed an alarming increase in e-cigarette usage amongst their youth, Illinois imposed a statewide 15% tax on e-cigarettes. One of the primary motivations behind Illinois implementing the 15% vapor tax was because Illinois saw an unprecedented surge in the use of e-cigarettes from 18.4% to 26.7% among high school seniors between 2016 to 2018. This growth in usage by the high school seniors was compounded with a 65% increase in usage among high school sophomores and a 15% increase in usage among 8th grade students (*E-Cigarettes and Vapes*, n.d.). Further, the state got a wakeup call in 2019, when three young people were hospitalized for severe breathing problems after vaping, according to their state Department of Public Health (Azad, 2019).

Another major motivation for establishing the policy was the 18th annual "State of Tobacco Control" report published in 2019 by the American Lung Association. This report appealed to Illinois lawmakers to better regulate e-cigarette access and usage to improve community health. This call for action came after a 135% increase in e-cigarette use among highschool students in two years, along with an adjacent three million kid increase in vaping (American Lung Association, 2020).

The state of Illinois was inclined to impose vape tax after observing the City of Chicago and Cook County impose successful taxes on e-cigarettes in 2016. Chicago was the first major city to also impose such a tax. As compared to the 36% of high school students who smoked combustible cigarettes in 1997, only 20.8% of students are reportedly using e-cigarettes in 2018 (The Heartland Institute, 2020). Further, Chicago generated over $1 million in its first fiscal year, which was then used to fund school-based health service programs (The Civic Federation, 2018).

Illinois was one of three states that imposed such a tax on e-cigarettes in 2019. Additionally, Illinois also bans the sale of vapor products to individuals under the age of 21 years old. In 2017, the overall usage of e-cigarettes in Illinois was around 4.4% and after implementing the tax, usage of e-cigarettes reduced to 3.4% in 2020 (America's Health Rankings analysis of CDC, n.d.). Further, after imposing its tax, Illinois has generated approximately $15 million in fiscal 2020 solely from this tax, which was in turn used to fund the state's Medicaid program (Povich, 2019). Governor of Illinois in 2019, J.B. Pritzker, acknowledged the taxation as a means to control the negative health implications of e-cigarette use and stated, "It's about deterrence" (Povich, 2019). With the reduction observed in Illinois in usage of e-cigarettes along with the state's focus on controlling the negative health implications of e-cigarette through implementation of a health policy, this paper focuses on Illinois and compares with two neighboring states, Missouri and Michigan, both of which do not have taxation controlling e-cigarette usage.

Data Presentation

To better evaluate the impacts of a state-wide e-cigarette tax on youth health, data was pulled from the National Survey of Children's Health (NCHS) (US Census Bureau, n.d.). The survey data was collected via mail and web-based surveys conducted by the US Census Bureau for the three states, Illinois, Michigan, and Missouri. The NCHS provides state-specific data around children's physical and mental health, along with their social surroundings, including their family and neighborhood.

For this analysis, a random sample of 440 observations were extracted for Illinois in 2017 and in 2021, in order to understand the effects of the 2019 state tax on e-cigarettes. The years 2017 and 2021 provide data for two years before and after the implementation of this tax, while also working to exclude any skewed health data as a result of the COVID-19 pandemic. Additional analysis was also done to compare the effects of such a policy to two neighboring states that had not implemented any such policy, such as Michigan and Missouri. A random sample of 425 observations were extracted for Michigan and Missouri in 2017 and 2021. Therefore, 2017 is treated as the "Before" period, and 2021 is treated as the "After" period, while Illinois is the "Treatment" state and Michigan and Missouri are the "Control" states. Additionally, averages from 2018 and 2022 were also extracted for understanding the trends.

Since this policy addresses the variability in children's health as a result of e-cigarette use to assess the efficacy of the state tax, the NCHS data set was modified and condensed to only include several key determinants of e-cigarette use—asthma, headaches, depression, toothache, and bleeding gums. Additional control variables include children's age and sex. Since the data was collected via survey through the Census Bureau,

all variables are based on the parent's subjective answers, and any respondents with missing data were excluded. Additionally, the Illinois, Missouri, and Michigan data were condensed to ensure the similar data sample size per state. The variable "Age" is the reported age of the child and for the variable "Sex", 1 indicates male and 0 indicates female. For the variables "Asthma", "Headache", "Depression", "Anxiety", "Toothache", and "Bleeding Gums", the value of 1 indicates that the child has frequent difficulty with or has been diagnosed with the variable, while a 0 indicates that they have not.

Asthma is a critical health outcome that is heavily affected by e-cigarette use by youth. When comparing 2017 Illinois health data to 2021 health data (Tables 1 and 2), the average occurrence of asthma decreased from 0.1065 to 0.0226, indicating that more families responded "No" to the prevalence of asthma in 2021 as compared to 2017. Comparing this to the respective Michigan health data in Tables 3 and 4, the average value for asthma increased in Michigan from 2017 to 2021. This shows that more families in Michigan reported the prevalence of asthma in 2021 as compared to 2017. Comparing Illinois asthma data to Missouri health data in Tables 3 and 4, the average value for asthma increased in Missouri from 2017 to 2021. Additionally difficulty breathing is a common side effect with both asthma as well as e-cigarette use It is noteworthy that while Illinois saw a decrease in the prevalence of both asthma and difficulty breathing after the taxation policy was implemented as seen in Figure 1 and Figure 2.

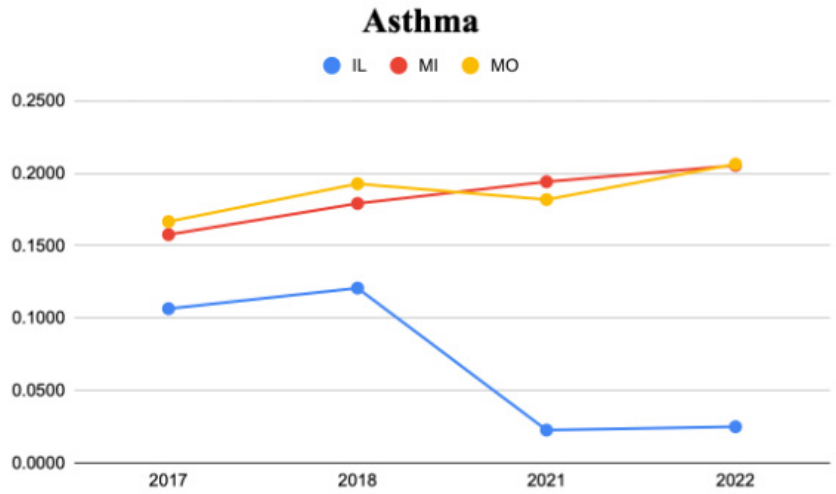

Figure 1: Average prevalence of Asthma for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

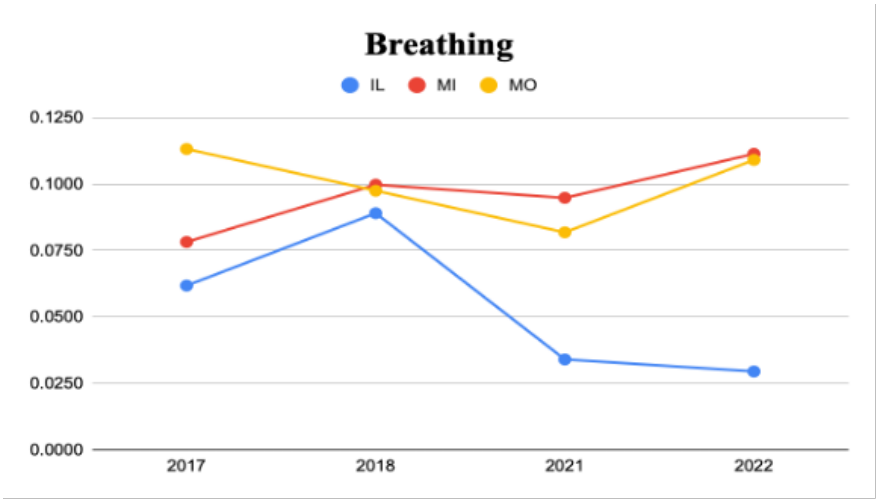

Figure 2: Average prevalence of Breathing problems for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

Table 1: Descriptive Statistics for Illinois in 2017

IL 2017 Data	Average	Std.Dev.	95% CI
Asthma	0.1065	0.3088	0.0100
Breathing	0.0618	0.2851	0.0092
Headache	0.0228	0.1494	0.0048
Gumbleed	0.0185	0.1348	0.0044
Toothache	0.0228	0.1495	0.0048
Depression	0.0477	0.2134	0.0069
Age	9.3832	5.4565	0.1753
Sex	0.5306	0.4996	0.0160

Table 2: Descriptive Statistics for Illinois in 2021

IL 2021 Data	Average	Std.Dev.	95% CI
Asthma	0.0226	0.1489	0.0048
Breathing	0.0340	0.1815	0.0058
Headache	0.0407	0.1979	0.0063
Gumbleed	0.0045	0.0672	0.0022
Toothache	0.0273	0.1631	0.0052
Depression	0.0611	0.2398	0.0077
Age	8.3258	5.4467	0.1747
Sex	0.5271	0.4998	0.0160

Table 3: Descriptive Statistics for Michigan in 2017

MI 2017 Data	Average	Std.Dev.	95% CI
Asthma	0.1576	0.3648	0.0119
Breathing	0.0782	0.2688	0.0088
Headache	0.0468	0.2115	0.0069
Gumbleed	0.0071	0.0842	0.0028
Toothache	0.0260	0.1593	0.0052
Depression	0.0468	0.2115	0.0069
Age	9.4486	5.0764	0.1655
Sex	0.4813	0.5002	0.0163

Table 4: Descriptive Statistics for Michigan in 2021

MI 2021 Data	Average	Std.Dev.	95% CI
Asthma	0.1941	0.3960	0.0127
Breathing	0.0948	0.2933	0.0094
Headache	0.0724	0.2594	0.0083
Gumbleed	0.0183	0.1341	0.0043
Toothache	0.0724	0.2594	0.0083
Depression	0.1400	0.3473	0.0111
Age	9.2045	5.0311	0.1609
Sex	0.5281	0.4998	0.0160

Table 5: Descriptive Statistics for Missouri in 2017

MO 2017 Data	Average	Std.Dev.	95% CI
Asthma	0.1667	0.3731	0.0123
Breathing	0.1132	0.3172	0.0104
Headache	0.0401	0.1964	0.0064
Gumbleed	0.0118	0.1082	0.0035
Toothache	0.0354	0.1849	0.0061
Depression	0.0472	0.2123	0.0070
Age	9.3271	5.3345	0.1745
Sex	0.5176	0.5003	0.0164

Table 6: Descriptive Statistics for Missouri in 2021

MO 2021 Data	Average	Std.Dev.	95% CI
Asthma	0.1818	0.3861	0.0124
Breathing	0.0818	0.2744	0.0088
Headache	0.0250	0.1563	0.0050
Gumbleed	0.0182	0.1339	0.0043
Toothache	0.0455	0.2085	0.0067
Depression	0.0481	0.2141	0.0069
Age	8.2426	5.1210	0.1645
Sex	0.5533	0.4977	0.0160

Headaches are a common side effect of e-cigarette usage in youth. When comparing 2017 Illinois health data to 2021 health data (Tables 1 and 2), the average prevalence of headaches rose from 0.0228 to 0.0407, indicating that more families responded "Yes" to the prevalence of headaches in 2021 as compared to 2017. However, when reviewing the trend in Figure 3, it can be observed that headaches in Illinois rose between 2017 and 2018 and then trended down in 2021 after the policy implementation. Comparing this to the respective Michigan health data in Tables 3 and 4, the average value for headaches increased from 2017 to 2021. Missouri saw an interesting trend of a decrease in 2021 but a spike in 2022. It is noteworthy that Illinois saw a decreasing trend since 2018 and a lower average value for headaches in 2022 as seen in Figure 3.

Depression is a serious health concern among youth and can be further exacerbated by e-cigarette usage. When comparing 2017 Illinois health data to 2021 health data (Tables 1 and 2), the average value for depression increased from 0.0477 to 0.0611, indicating that more families responded "Yes" to the prevalence of depression in 2021 as compared to 2017. Comparing this to the respective Michigan health data (Tables 3 and 4), the average value for depression saw a larger increase, from 0.0468 to 0.14 from 2017 to 2021. Missouri health data in comparison (Tables 5 and 6) only shows a mild increase from 0.0472 to 0.0481. With all three states experiencing an increase, this might be due to the physical, mental, and social effects of the pandemic.

Declining oral health, including bleeding gums and toothache are known to result from repeated e-cigarette usage. When comparing 2017 Illinois health data to 2021 health data (Tables 1 and 2), the average value for bleeding gums decreased from 0.0185 to 0.0045, indicating that more families responded "No" to the prevalence of bleeding gums in 2021 as compared to 2017. Conversely, the average value for bleeding gums increased per the Michigan health data (Tables 3 and 4), from 0.0071 to 0.0183 from 2017 to 2021. Also, per the Missouri health data (Tables 5 and 6), Missouri also saw an increase

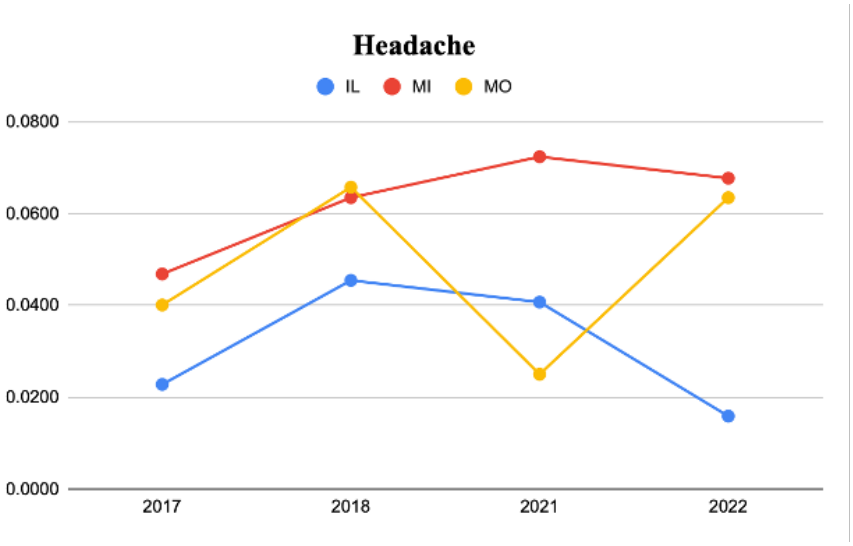

Figure 3: Average prevalence of Headache for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

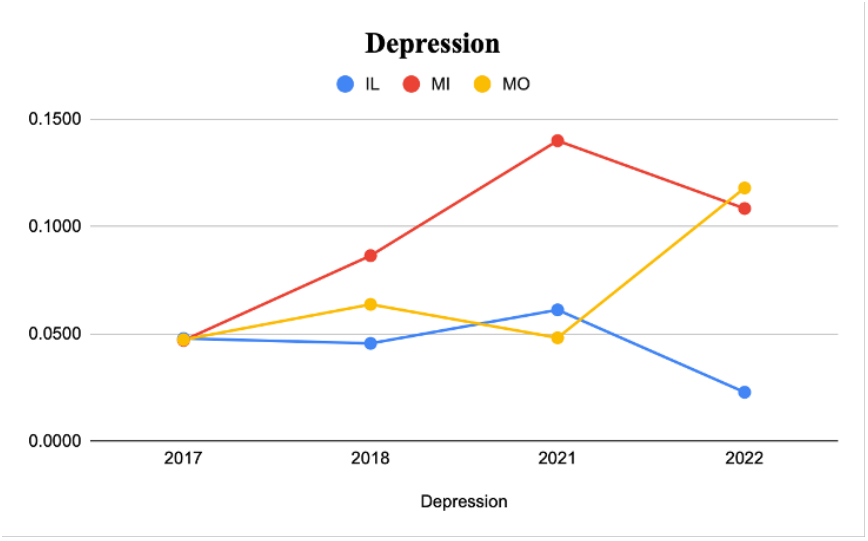

Figure 4: Average prevalence of Depression for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

in the gum bleeds rising from 0.0118 in 2017 to 0.0182 in 2021. This shows that families in Michigan and Missouri reported an increase in prevalence of bleeding gums while Illinois saw a decline in average bleeding gum prevalence after the implementation of the e-cigarette tax, which can also be noted in Figure 5. Reviewing toothache data, Illinois saw a minor increase in toothaches, from 0.0228 to 0.0273, however, Missouri and Michigan saw larger increases in toothache prevalence between 2017 and 2021. Reviewing Figure 6, it is remarkable that Illinois had the lowest occurrence of toothaches in 2022 amongst the three states.

After preliminary analysis of the Illinois, Michigan, and Missouri health data in 2017 and 2021, and reviewing the trend in average occurrence across 2017, 2018, 2021, and 2022, it can be observed that Illinois has experienced an increase in positive health outcomes, specifically asthma, breathing, and bleeding gums. On the other hand, the Michigan health data indicates a decrease in positive health outcomes across all health categories. Missouri health data indicates that they experienced positive health outcomes in 2021 in breathing, headache, and depression, however, those health outcomes became worse in 2022. Thus, the above tables and figures indicate that the e-cigarette taxation did positively affect youth health outcomes in Illinois as compared to its neighboring states. To confirm this hypothesis, further empirical analysis is required.

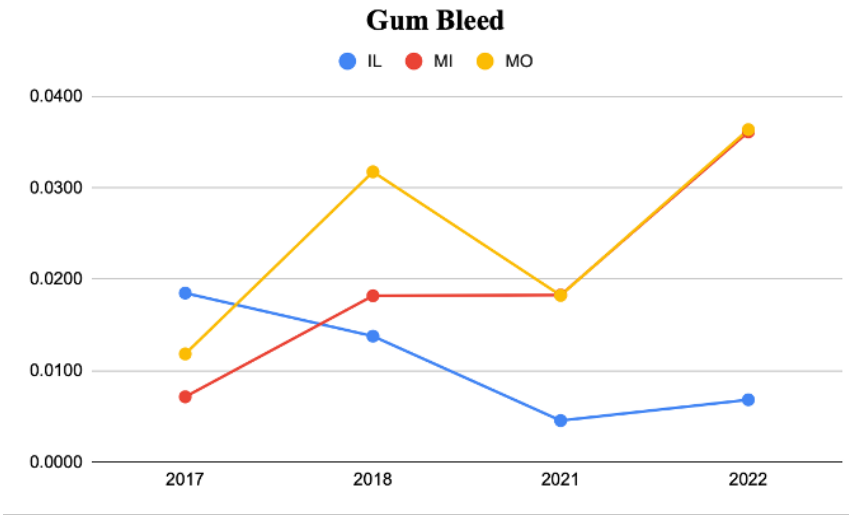

Figure 5: Average prevalence of Gum Bleed for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

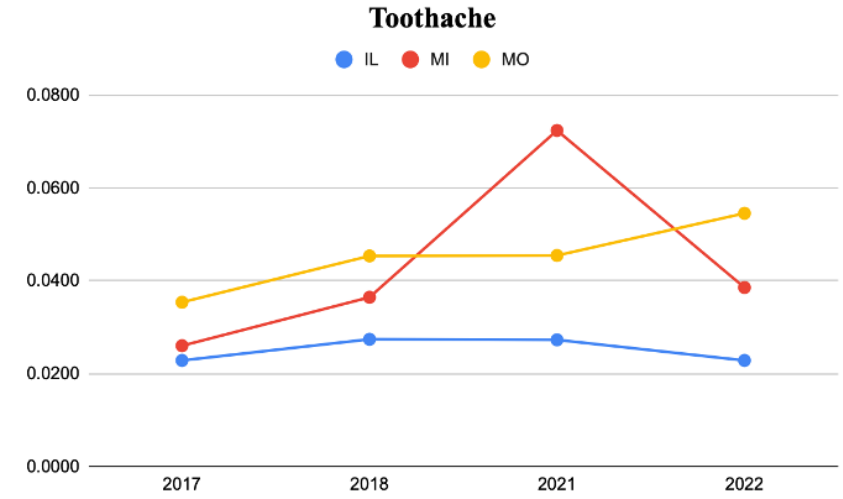

Figure 6: Average prevalence of Toothache for Illinois, Michigan, and Missouri (2017, 2018, 2021, 2022)

Empirical Estimates

To begin the data analysis in order to understand the effects of an e-cigarette tax on health, several hypothesis tests were run. Firstly, two Difference-in-Means hypothesis tests were run in order to understand the changes in the six variables—asthma, breathing, headache, depression, toothache, and gumbleed—in 2017 and again in 2021. The Difference-in-Means tests allows for the analysis of whether or not there was a statistically significant change in the average rates of asthma, headache, depression, and gumbleed between 2017 and 2021. The t-scores were analyzed at the 5% and 10% significance level, and the following conclusions were made, respectively. The null hypothesis indicates no change in the means, while the alternative hypothesis suggests there was a change between 2017 and 2021. The results of the first two hypothesis tests can be seen in the tables below.

Table 7: Hypothesis Testing for Difference in Means in Illinois in 2017 and 2021

Variable	H_0	H_A	t_{actual}	5% Significance Level $t_{critical}$ 1.96	10% Significance Level $t_{critical}$ 1.65
Asthma	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	5.131	Reject H_0	Reject H_0
Breathing	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.724	Fail to reject H_0	Reject H_0
Headache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.518	Fail to reject H_0	Fail to reject H_0
Depression	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.873	Fail to reject H_0	Fail to reject H_0
Toothache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.421	Fail to reject H_0	Fail to reject H_0
Gumbleed	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.943	Fail to reject H_0	Reject H_0

From the above table, it can be noted that in Illinois, there was a statistical difference in rates of asthma at the 5% significance level, and a statistically significant difference in rates of difficulty breathing and gum bleeds at the 10% significance level, between 2017 and 2021. This difference may be attributed to the beneficial effects of the e-cigarette tax. However, there was no statistical difference in rates of headache, depression, and toothache in Illinois between 2017 and 2021, which may be due to the COVID-19 pandemic.

Table 8: Hypothesis Testing for Difference of Means in Michigan in 2017 and 2021

Variable	H_0	H_A	t_{actual}	5% Significance Level $t_{critical}$ 1.96	10% Significance Level $t_{critical}$ 1.65
Asthma	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.397	Reject H_0	Reject H_0
Breathing	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.861	Reject H_0	Reject H_0
Headache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.574	Reject H_0	Reject H_0
Depression	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	4.720	Fail to reject H_0	Fail to reject H_0
Toothache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	3.141	Fail to reject H_0	Fail to reject H_0
Gumbleed	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.450	Reject H_0	Reject H_0

In reference to the above table, it can be noted that in Michigan, there was a statistical difference only in rates of depression and toothache at both a 5% and 10% significance level between 2017 and 2021. However, between 2017 and 2021, there was no statistical difference in rates of asthma, difficulty breathing, headaches, or gumbleeds in Michigan, a state where no taxation policy exists for e-cigarettes.

Table 9: Hypothesis Testing for Difference of Means in Missouri in 2017 and 2021

Variable	H_0	H_A	t_{actual}	5% Significance Level $t_{critical}$ 1.96	10% Significance Level $t_{critical}$ 1.65
Asthma	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.582	Fail to reject H_0	Fail to reject H_0
Breathing	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.543	Fail to reject H_0	Fail to reject H_0
Headache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	1.240	Fail to reject H_0	Fail to reject H_0
Depression	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.061	Fail to reject H_0	Fail to reject H_0
Toothache	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.745	Fail to reject H_0	Fail to reject H_0
Gumbleed	$(\mu_{21}-\mu_{17})=0$	$(\mu_{21}-\mu_{17})\neq 0$	0.767	Fail to reject H_0	Fail to reject H_0

As noted in the above table, in Missouri, there was no statistical significance with the changes in the rates of asthma, difficulty breathing, headache, depression, toothache, or bleeding gums, between 2017 and 2021.

After the analysis of the Difference-of-Means hypothesis tests, a Difference-in-Difference hypothesis test was performed to understand if the change in average values of asthma, difficulty breathing, headache, depression, toothache, and gumbleeds in Illinois was significant as compared to the change in average values of the same in Michigan and Missouri between 2017 and 2021.

Table 10: Hypothesis Testing for Difference in Difference Between Illinois and Michigan in 2017 and 2021

Variable	H_0	H_A	t_{actual}	5% Significance Level $t_{critical}$ 1.96	10% Significance Level $t_{critical}$ 1.65
Asthma	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	3.906	Reject H_0	Reject H_0
Breathing	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	1.765	Fail to reject H_0	Reject H_0
Headache	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	0.379	Fail to reject H_0	Fail to reject H_0
Depression	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	3.195	Reject H_0	Reject H_0
Toothache	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	2.312	Reject H_0	Reject H_0
Gumbleed	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	2.386	Reject H_0	Reject H_0

Table 11: Hypothesis Testing for Difference in Difference Between Illinois and Missouri in 2017 and 2021

Variable	H_0	H_A	t_{actual}	5% Significance Level $t_{critical}$ 1.96	10% Significance Level $t_{critical}$ 1.65
Asthma	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	3.220	Reject H_0	Reject H_0
Breathing	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	0.139	Fail to reject H_0	Fail to reject H_0
Headache	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	1.947	Fail to reject H_0	Reject H_0
Depression	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	0.589	Fail to reject H_0	Fail to reject H_0
Toothache	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	0.329	Fail to reject H_0	Fail to reject H_0
Gumbleed	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CBl})=0$	$(\mu_{TA}-\mu_{TB})-(\mu_{CA}-\mu_{CB})\neq 0$	1.848	Fail to reject H_0	Reject H_0

As observed in Table 10, the difference-in-difference hypothesis test indicates that for asthma, depression, toothache, and bleeding gums, there is a statistical significance between Illinois and Michigan at the 5% significance level, and in addition, the difference between rates of difficulty breathing is significant at the 10% significance level. However, there was no statistical significance noted in the change in rates of headache.

The difference-in-difference hypothesis test shown in the above table highlights that for asthma, there is a statistical significance between Illinois and Missouri at the 5% significance level, and in addition, the difference between rates of headache and bleeding gums is significant at the 10% significance level. However, there was no statistical significance noted in the change in rates of difficulty breathing, depression, or toothache.

Discussion

Per our findings, there has been a statistically significant decrease in the reported rates of asthma, depression, toothache, and bleeding gums from before and after the implementation of the state tax in Illinois as compared to Michigan at 5% significance level. Additionally, there has been a statistically significant decrease in the reported rates of asthma from before and after the implementation of the state tax in Illinois as compared to Missouri at a 5% significance level. It can be further noted that the vaping tax has had a positive effect on the Illinois population, with a decrease in reported rates of difficulty breathing and gum bleeds at the 10% significance level from 2017 to 2021 alongside a decreasing trend in headaches and depression from 2017 to 2022. This supports our initial hypothesis that the vaping tax will help curb the increasing e-cigarette use, thus improving health outcomes for the community. This study also allows for a deeper understanding of the correlation between these variables and other external variables, while acknowledging limitations and the potential for future research.

This study's findings agree with past literature regarding the negative effects e-cigarettes have on health outcomes, including physical health, mental health, and oral health—all of which can affect the community and pose new challenges. Asthma and trouble breathing are the most common ill-effects of e-cigarette use (Hickman, Jaspers, 2020). This study found asthma to be a significant outcome in Illinois as compared to Missouri and Michigan, thus supporting the hypothesis. Additionally, trouble with breathing was also significant at 10% in comparison with Michigan. As noted in Bircan et al. (2021), studies have found a positive correlation between e-cigarette use and self-reported diagnosis of asthma among other respiratory diseases. Also, Thirión-Romero et al. (2018) highlighted the negative effects of formaldehyde on the increased symptoms of asthma. asthma is one of the most common symptoms of e-cigarette use.

Both Obisesan et al. (2019) and Moustafa et al. (2021) highlight the negative effects of e-cigarette use on depression, as Obisesan et al. (2019) noted a higher frequency of reporting depression among e-cigarette users, and Moustafa et al. (2021) indicated that greater depressive symptoms result in increased use of e-cigarettes. The increasing reported rates of depression correlated with e-cigarette use are a result of short-lived bursts of dopamine that leave the user feeling more depressed than before use. Given both of these studies, it can be understood that a rise in e-cigarette use will be followed by a rise in rates of reported depression, resulting in a worsening state of mental health for the population which has larger socioeconomic and health risks.

Akinkugbe (2018), Yang et al. (2020), and Alhajj et al. (2022) highlight the negative consequences of e-cigarette smoking on oral health. E-cigarette use exposes the mouth to more bacteria and can result in dryness, thus resulting in the saliva not being able to protect the gums and teeth, and the aforementioned studies indicate that bleeding gums and toothache are key precursors to other oral diseases and worse health implications (Alhajj et al., 2022) (Akinkugbe, 2018) (Yang et al., 2020). The improvement in health outcomes in Illinois as compared to

Michigan, seen in our findings, also aligns with the price elasticity of demand for e-cigarettes among teens as highlighted by researchers including Yao et al. (2020) and Corrigan et al. (2021).

One notable outcome from the study was that the policy had no significant effect on headaches at 5% significance level in the sample analyzed. However, headaches were significant in the Illinois to Missouri comparison at 10% significance level. As previously stated, King et al. (2020) found headaches to be the most common symptom of e-cigarette use among past 30-day users who were adolescents, ages 13-17. The negative symptoms of headaches can create larger issues for users, as an increase in headaches can directly result in an increase in stress and anxiety.

Though the results of the study mostly support the proposed hypothesis, the underlying effects of bias and the limitations of this study must be scrutinized. One major limitation to this study is the presence of confounding bias, as seen by the COVID-19 pandemic. As the policy in Illinois was only implemented in 2019, following the recognition of the exponential rise in e-cigarette use in 2018, the study analyzed data two years before and after the policy, hoping to minimize the negative effects of the pandemic on the observed health outcomes. However, as seen by the presented data surrounding asthma, the effects of the COVID-19 pandemic are long-lasting and as such have influenced the reporting of health outcomes in Illinois, Michigan, and Missouri. COVID-19 serves as a confounding variable in this study and to address this issue in the future, it would be beneficial to obtain and analyze data in future years, thus mitigating the effects of the pandemic.

Another limitation of this study is the presence of nonresponse bias in our data. As the National Survey of Children's Health is a mail-in and online survey, not every individual is compelled to answer or will leave questions unanswered, adding a layer of bias to the data. To circumvent this, the hypothesis testing was ensured to only use variables with a valid response value, thus excluding any blanks or unanswered questions. This issue could also be addressed by sampling a larger population, across more states, in order to gather more data such that the effects of the nonresponse bias are nearly negligible.

Looking to the future, hypothesis testing can again be used to analyze the effects of e-cigarette usage on other health outcomes, such as physical activity and cardiopulmonary health. Understanding the effects of e-cigarette use on various health outcomes can showcase the true harmful nature of e-cigarettes and the need for policies such as taxes. Additionally, the same conclusions drawn here regarding health outcomes can be extrapolated into economic gains and losses, as a result of various health outcomes and illnesses. Further research can also be conducted to narrow down the most notable negative health outcomes of e-cigarette use by gender and race to better understand health inequities. Such research can inform educational groups to champion for better health at the community and local level.

Conclusion

To summarize, the results of the study validate the initial hypothesis that the implementation of Illinois' e-cigarette tax had a positive correlation on health outcomes, as the reported rates of asthma, depression, toothache, and bleeding gums decreased. However, the change in rates of headaches between Illinois and Michigan was only significant at 10% and insignificant between Illinois and Missouri, possibly due to confounding effects of the COVID-19 pandemic. Using the National Children's Health Survey, a series of difference-in-difference hypothesis tests were run to compare the difference between the change in health outcomes in Illinois, which imposed a state-wide tax, and Michigan, which presents no such tax or bill. The prevalence of both nonresponse bias and confounding variables pave the way for future research to confirm these findings. This research can also be extrapolated to analyze other health outcomes in the context of e-cigarette usage and the economic values of the health outcomes. This study

can serve as a framework that the other states can leverage to implement similar policies. With more states regulating the use of e-cigarettes with such policies, further testing with wider state-to-state comparisons can be performed to examine the outcomes and trends.

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Competition Dynamics in Invertebrates Across Geographical Gradients

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Abstract

Studying competition dynamics of invertebrate species is essential to understanding species distribution, how species become invasive, and dealing with pests and pollinators. However, there is a gap in the literature when it comes to what geographical factors influence invertebrate competition. This study investigates competition dynamics of terrestrial invertebrates across various ecosystems and geographical areas, as well potential predictors for the patterns observed. I used food lures across sites in multiple countries, and recorded the number of visitors and level of consumption to attribute a competition score to each lure. Three different food types were used to account for dietary preferences (Fat, Protein, and Sugar). The results show that there is a significant variation of competition scores across sites. In addition, I identified a significant statistical interaction between sites and food types in relation to competition scores, which suggests that competition is different between the sites, but the pattern of variation differs per food type. A regression was conducted to see if Net Primary Productivity or the geological age of an ecosystem were predictors of the variation in competition scores. Geological age came out statistically significant for the scores in the Fat food type, suggesting that it may be an important driver for invertebrate competition. Idiosyncrasies of the sites are discussed with the aim to identify other factors that may affect competition levels and their patterns. This research contributes to the study of competition dynamics in terrestrial invertebrates and could inspire an approach to predicting interactions and effects of new or introduced species.

Introduction

Studying interactions between species is at the core of ecology, and research on competition dynamics is important as dominant species can influence the distribution of other organisms, such as pests, pathogens, and invasive species (Ribas, 2002). There have been several studies that investigate how invertebrate diversity is affected by geographical characteristics (Kaspari et al., 2004, Guilherme et al., 2019), yet there is little in the literature on the magnitude of competition in terrestrial invertebrates across these gradients. Thus, in this study I aim to investigate the competition dynamics of invertebrate species from varying locations and characteristics. To do so, I analyze competition levels and mechanisms at each site and then evaluate if Net Primary Productivity (NPP) and geological age are predictors for these competition levels.

Net Primary Productivity (NPP) is defined as the result of the gross amount of energy fixed by plants through photosynthesis minus the energy they use for respiration (Woodwell & Whittaker, 1968). This net energy is then available to the plant to grow in biomass, so NPP relates to a measure of energy available for creating an amount of biomass in an area or ecosystem. Since the NPP of a location is affected by its environmental factors (Guilherme et al., 2021), NPP could serve as a proxy for the effect of climate and resources on invertebrate communities. Previous studies show that some environmental variables can be essential in shaping insect distribution at large scales (Kaspari et al., 2000,

2004). For example, precipitation is correlated with ant species turnover (Vasconcelos et al., 2010), and vegetation density is strongly associated with both functional and taxonomic composition of ants (Guilherme et al., 2019). In addition, the energy limitation hypothesis directly links the species richness (number of species in a community) of consumer taxa to NPP as it conveys that NPP limits a taxon's density in an area, and it has been demonstrated on ant abundances (Kaspari et al., 2000). This tells us that as climatic conditions limit NPP, NPP in turn limits the abundance of resources, which then affects the abundance of consumers the ecosystem can sustain, and so forth with higher trophic levels (Guilherme et al., 2021). Thus, the factors that drive NPP of an ecosystem can affect the abundance and richness of its invertebrates. When investigating competition, such information is valuable since larger invertebrate communities could facilitate the development of numerous interactions and of diverse nature. These make NPP an important variable to test as a predictor of competition, so I will use it as one of the two proposed factors of competition in this study.

The second variable is geological age. The geological age of the ecosystem is an important factor to study because it can be a driver at the regional level of the interaction networks between and within species (Trøjelsgaard, 2013). According to Trøjelsgaard (2013), 1) at young islands most organisms are generalists and interactions between them are weak, 2) at mid aged sites there is a peak of speciation, so niches are filled, and 3) at old areas, there is a decline in speciation and species are becoming more of generalists because the fauna are at a point where they cannot survive solely from individual niches so they have to adapt to a wider set of conditions. Furthermore, old islands have unique interactions that have evolved through time and everything is highly connected (Trøjelsgaard, 2013). In relation to and often due to speciation, species richness is also affected by the age of the land. The geographical age hypothesis says that the species richness of organisms increases with the geological age of the ecosystem, and it has been proven applicable in several locations, including Azores islands (Borges, 1999). Furthermore, this hypothesis is also supported by the 'species-pool hypothesis' (Taylor et al., 1990) which predicts that "all else being equal, the larger the local and/or global area of a habitat type and the older its geological age, the greater the past opportunity for speciation and hence, the greater the number of available species that are adapted to that particular type of habitat". So geological age will be a proposed driver of competition in this study.

The purpose of this research is to learn about competition dynamics of terrestrial invertebrates and by doing so, shed light into how species interact in several ecosystems and what forces affect competition. Knowledge of these mechanisms can enhance conservation planning and species distribution modelling by helping scientists see patterns and do predictions more accurately. The big picture goal is to provide information to better protect important species, control pests, and manage invasive species. Thus, this study holds value in the advancement of knowledge of competition dynamics that is needed for solving real world issues.

The objectives of my research are to 1) understand site-based differences in invertebrate competition dynamics and the factors in their differences, and 2) analyze if and how invertebrate competition correlates with NPP and geological age of a site. My hypothesis is that NPP and geological age are significant factors of and positively correlated to invertebrate competition in terrestrial ecosystems, based on the theories and literature above. More specifically, I hypothesize that a site with high NPP and great geological age will experience high competition as it supports many species that have adapted and coevolved to compete for resources in filled niches. In contrast, I think that a low NPP in a young land will lead to low competition as there are still open niches and there are few interactions

due to the low invertebrate abundance in the site. On the other hand, a low NPP and large age may lead to moderately high competition because interactions have developed through time but there is low species abundance so less interactions seen. Finally, a high NPP with a young age may generate moderately low competition as open niches may still be available, but the population is growing fast so many interactions are developed.

Methods

Study Sites

This experiment was conducted in person in these five geographical locations: San Cristobal, Galapagos; Tiputini, Ecuadorian Amazon Forest; Appledore, ME USA; Craigieburn, South Island, New Zealand; and Whakatīwai, North Island, New Zealand (coordinates in Appendix B). I tested two different areas (high (H) and low (L) elevation within the ecosystem) in each place, so in total this study had ten sample areas (from here on referred to as "Sites" (Table 1)). The choice of these study Sites was constrained by lack of travel funding for this study, and therefore Sites had to be accessible during my time at Cornell and my studies abroad. Within that constraint, I chose Sites that encompassed a wide range of latitudes, geographic characteristics, and varying ecosystem types, to have as ample representation as could be available. This is important for this study because it allows for better analysis in if the proposed drivers of competition are indeed factors that affect interactions of terrestrial invertebrates in general, instead of being only significant regionally. This is supported by how some studies suggest differences in invertebrate abundance and competition with changing latitudes (Procter, 1984; Schemske et al., 2009).

The Galapagos Islands are located 972km west off the Ecuadorian Coast (Wauters, 2014), and the experiment was conducted on San Cristobal island, one of the oldest in the archipelago with estimated emergence of 2.4 Ma (Percy, 2020). Due to the highly variable weather, there is a climosequence strongly dependent on elevation, ranging from very arid at the coastline to humid at the summit (Percy, 2020). This work was conducted on the arid lowlands in Puerto Baquerizo Moreno vs humid highlands around Hacienda Tranquila. The humid highlands have luscious green vegetation, and its main land use is for agriculture (such as banana and sugarcane plantations) (Hamann, 1979). The lowlands are in the Dry Zone (Schofield, 1989) and are arid with fewer plants. The data from the lowlands was collected within the town of San Cristobal.

The Amazon Sites are located in the Tiputini Biodiversity Station within the Parque Nacional Yasuní in the Ecuadorian Amazon. The area of the station is one of the closest to being pristine in the tropical forest of this country and is situated next to the Tiputini River. The Amazon H Site is referred by locals as "Terra Firme" as it is an area farther from and more elevated than the river so the water from precipitation that is not absorbed by the soil and plants is usually flushed out down to the river (Myster, 2014; Nigel et. al., 1999). The Amazon L Site on the other hand, is in the "Flooded Forest", which is an area next to the river that frequently floods from precipitation and water level rise (Myster, 2014). The weather during collection was hot and humid, was generally sunny, but had brief strong precipitation.

Appledore Island is located 10 km off the coast of Portsmouth, Maine in the Gulf of Maine. The data collection Sites were on the Shoals Marine Laboratory housing grounds (Appledore H), and down near the docks (Appledore L). The vegetation in Appledore is mostly herbs, shrubs, and grassy areas (Nichols, 2008). The weather during collection was usually sunny and temperate.

Whakatīwai is a coastal region on the shore of the Firth of Thames, in the Hauraki Gulf, North Island, New Zealand. It is an inhabited area with a low population and its land use mostly going to cattle and sheep pastures. The data collection Sites were within a small grassy program campus

with some residential and food service buildings next to the beach.

Finally, Craigieburn is a range of mountains south of Arthur's Pass in the Canterbury Region, South Island, New Zealand. The vegetation in the data collection area of this Site was predominantly a pure forest of mountain beech (*Nothofagus solandri* var. *cliffortioides*), and some grasses. This is an alpine zone, so the weather was cool and dry.

Study Species

The species studied were land invertebrates of any species. The most common organisms that were observed were ants and flies, but at times the lures would attract other insects like very small arthropods, wasps, and crickets. A list of the orders of species seen per Site can be found in Appendix C.

Experimental Design and Field Methods

As per Wauters et al. 2014's methodology, baits contained 1/2 teaspoon of peanut butter (fat), canned tuna (protein), and granulated sugar or fruit (sugar). These foods were chosen due to their accessibility in all Sites which allows for consistency. The three foods (referred as "Food Types", see Table 1) were placed on a fourth of a 12 cm x 12 cm napkin or toilet paper square. Each bait is treated as one replica as the different food items are used to account for different diet preferences between species (Figure 1). As an observational study, after 15, 30, 45, 60, and 120 minutes of bait placement, the species present and their identity were recorded, and a picture was taken. A group of four baits were placed at four specific Locations (Table 1): 2-10 meters from a building (House), at dense vegetation, on an open area, and on the side of a road. Each bait was placed about 3-10 meters away from the other three baits in the group. There are 2 or 4 groups of four baits per Site, which translates in a total of 4 or 16 baits per Site. The groups were placed as far as possible from each other within the ecosystem borders of the Site. This was done for the ten Sites.

Figure 1. Layout of experiment: pieces of a protein (canned tuna, right), a fat (peanut butter, left), and a sugar (orange, bottom) are placed in different small pieces of paper on the ground. These are the three Food Types (Table 1).

Analytical Methods

A score for competition was given to each food type per experiment:

Competition score = total # of visits during the first 60 minutes x consumption score

For the number of visits, each individual counts as one visit, but if one ant brought its colony, then the pioneer ant and the colony is one unit and thus they count as just one visit. The consumption score was taken by observing how much of the food was consumed or touched during the first 60 minutes (regardless of how much each species ate, and selecting only the highest level of involvement): 1– untouched, 2– touched (an invertebrate was seen eating some of the food, not barely walking over it), 3– food moved around napkin, 4– parts of the food moved off napkin, 5– everything taken.

ANOVA was used in R (R Core Team, 2022) from the package **car** (Fox & Weisberg, 2019) to compare overall competition scores between each Site, Location, and Food Type, as well as a combination of them. The log (score +1) or sqrt(score) were used to account for the zeros (a 0 score means no competition because replica had no visits) and most importantly to make the data be closer to Gaussian distribution. The results are reported based on this form of analysis. The jarque.test function from the

package **moments** (Komsta & Novomestky, 2022) was used to determine which of the above transformations were preferred statistically. The preferred transformation is the one that allows a closer approximation of Gaussian residuals, indicated by a lower test statistic in jarque.test, to better satisfy the assumption of having Gaussian residuals for ANOVA. The function emmeans from the package **emmeans** (Lenth, 2023) indicated which pairs of data had significantly different competition scores (i.e. between Sites, Food Types, or Locations). A multiple linear regression also was run for evaluating the joint effects of the two predictors (NPP and geological age) for corresponding competition levels. For the regression analysis, I used the lm function in the **stats** package (R Core Team, 2022) to perform a multiple linear regression of the relationship between NPP, geological age, and Competition Scores (see glossary in Table 1).

Table 1. Glossary of terms specific to this paper

Term	Definition
Site	With capital "S". The geographical locations where experiments (lures) were laid out. If written as "site" with lower case "s", then it refers to any location on the world, not specific to this study, when making generalizations
Food Type	The kind of food that a lure had to attract invertebrates. There were three food lures placed at each experiment replica: one with a Protein (canned tuna), a Fat (peanut butter), and a Sugar (orange) (Figure 1)
Location	With capital "L". The characteristic of the place where a lure was laid out. There were four variations, so four location types for the lures: dense vegetation, open land, on a road, and near a house/building.
Geological Age	The age of the ecosystem of each Site, based on when ecosystem as it is known today began to form.
NPP	The resulting value of the gross amount of energy fixed by plants through photosynthesis minus the energy they use for respiration (Woodwell & Whittaker, 1968). This net energy is then available to the plant to grow in biomass.
Experiment	The placing of three napkins, each with either the protein, fat, or sugar. These three foods are observed and photographed during the period of 60 minutes. The number of invertebrate visitors are counted and the level of consumption assessed. One experiment means one replica. A zone is an area where the foods are placed at four specific Locations. There are 2 or 4 zones per Site, which translates in a total of 4 or 16 experiments per Site. This is done for the ten Sites.
Competition Score	Competition score = total # of visits during the first 60 minutes x consumption score. See Analytical Methods for details.

Table 2. NPP values from NASA Data as well as different sources of data across several sites

Site	Month	Lateral Coordinates Used	Longitudinal Coordinates Used	2012	2013	2014	2015	2016	NPP of similar locations from different datasets	Author	Year	Location	Average NPP
Galapagos H	February	0.902 S	89.611 W	3.3	3.6	6.5	5.38	6.5	3.5 (in 2016)	Garcia et. al., 2014	2014	Guayas Providence, Ecuador	4.80 (in 2016)
Galapagos L	February	0.902 S	89.611 W	1.6	1.2	2.25	1.42	2.34	1.9	Garcia et. al., 2014	2014	Manabi providence, Ecuador	1.80
Amazon H	April	0.638 S	76.15 W	1.5	3.9	1.3	2.25	3.55	2.9	*Metcalfe et al., 2010	2010	The Caxiuana National Forest, Brazil	2.57
Amazon L	April	0.638 S	76.15 W	1.5	3.9	1.3	2.25	3.55	2.9	*Metcalfe et al., 2010	2010	The Caxiuana National Forest, Brazil	2.57
Appledore H	July	42.99 N	70.615 W	4.1	5.5	6.5	6.5	6.5	5.67	*Magill et al., 1996, Hoeppner& Dukes, 2012	2010	**North East, USA	5.79
Appledore L	July	42.99 N	70.615 W	4.1	5.5	6.5	6.5	6.5	5.67	*Magill et al., 1996, Hoeppner & Dukes, 2012	2010	**North East, USA	5.79
Whakatiwai H	October	37.088 S	175.302 E	6.5	6.5	6.47	5.38	6.5	5.56	*Volder et al., 2007	2003	Grassfield Canberra, ACT, Australia	6.15
Whakatiwai L	October	37.088 S	175.302 E	6.5	6.5	6.47	5.38	6.5	5.56	*Volder et al., 2007	2003	Grassfield Canberra, ACT, Australia	6.15
Craigieburn H	October	43.127 S	171.728 E	1.2	1.6	1.89	1.81	1.51	1.29	Graeme, 2001	2001	Cristchurch hardwood forest, New Zealand	1.55
Craigieburn L	October	43.127 S	171.728 E	1.2	1.6	1.89	1.81	1.51	1.29	Graeme, 2001	2001	Cristchurch hardwood forest, New Zealand	1.55

* From the dataset compiled by Song et. al., 2020 g C / m^2 per day

** Average of northern Maine (Forest) and 2x Waltham, MA, USA (Suburban)

The NPP data are taken from the NASA Earth Observations data set (NASA, 2012-2016), as well as in situ NPP data by individual projects in the areas or similar ecosystems found in the literature (Table 2). The NASA data are averaged over the last 5 years of available data (2012-2016) on the month this study's data was located per Site (Table 3). The geological age of each Site for this study is based on when the present ecosystem began to form (Table 3).

Table 3. The Average NPP (NASA data) and geological age of each site

Site	Average NPP	Ecosystem Age (Myears)
Galapagos H	5.06	3
Galapagos L	1.78	3
Amazon H	2.51	10
Amazon L	2.51	10
Appledore H	5.82	0.018
Appledore L	5.82	0.018
Whakatiwai H	6.27	80
Whakatiwai L	6.27	80
Craigieburn H	1.6	80
Craigieburn L	1.6	80

Results

Overall, the levels of competition in invertebrates vary by Site, which means there are characteristics of each place that shape its competition dynamics. However, it appears that NPP and geological age are not strong factors for competition. To analyse the data, I compared different variables of the study versus competition scores through two-way ANOVAs and a summary of results can be found in Table 4.

Competition Scores Versus Sites

There is a significant difference between the Sites when they are analysed against competition scores without a Site-Food Type interaction in a two-way ANOVA (Two-way ANOVA, $F(9,218) = 5.4547$, $p < 0.0001$; Figure 2). The list of which Sites are statistically different can be found in Table 4. In general, it seems that the Amazon (particularly Amazon H) and Appledore Island have higher competition scores than the other Sites (Figure 2). Meanwhile, the Galapagos, Whakatiwai, and Craigieburn L appear to have very similar ranges of competition in invertebrates. Amazon H has three data points that are much larger than most of its scores (Appendix A1), but statistically only one is considered an outlier, so this Site has a higher mean than the others (Figure 2).

It is important to note that this analysis included all scores from all three food types. Hence, I conducted another two-way ANOVA to analyse if there exists an interaction between Site and Food Type, which would indicate that the impact one variable has on competition depends on a second variable. This analysis appeared non-significant ($F(18,198) = 1,5642$, $p_{\text{interaction}} = 0.072323$, $p_{\text{Site}} = 1.455e-07$, $p_{\text{Food Type}} = 0.003206$). However, when the three high scores in Amazon H were removed, the result of the interaction of Site:Food Type was significant ($F(18,195) = 1.7966$, $p_{\text{interaction}} = 0.027863$, $p_{\text{Site}} = 3.836e-06$, $p_{\text{Food Type}} = 0.005191$). This tells us that when there are no particular anomalous high scores (Appendix A3), competition between invertebrates is different between Sites but the pattern of variation is not the same for all food types. The discussion section explores the nature of these three high scores.

Since Site and Food Type are interacting, I analysed competition scores versus Site for each separate Food Type. As predicted, we get slightly varying patterns in each Food Type across Sites. For Protein (Figure 3a), the Sites had statistically different scores (Two-way ANOVA, $F(9,66) = 3.1439$, $p < 0.001$). However, this time Appledore (H and L) and Craigieburn L seem to have the highest competition scores while the Amazon, Galapagos, and Whakatiwai had low mean scores. For Fat trials (Figure 3b), statistically significant differences also exist but are fewer (Twoway ANOVA, $F(9,66) = 2.2337$, $p < 0.05$). Here, most noticeably is how Amazon H has a very wide spread compared to the other Sites due to the three trials anomalous high scores, which

bring the mean and median up. Apart from the Amazon, Appledore seems to have the highest competition scores, and Craigieburn has the lowest. Finally, sugar lures (Figure 3c) also had a significant difference between the Sites (Two-way ANOVA, $F(9,66) = 4.1778$, $p < 0.0001$). The sugar competition scores are less uniform since Amazon H and Appledore have high medians, while Amazon L, Galapagos H, and Craigieburn have very low medians (close to 0).

Competition Scores Versus Food Type, and Versus Location

Additional comparisons were conducted with competition versus Food Type and competition scores versus Location. The Food Type comparison was significant (Two-way ANOVA, $F(2,225) = 4.5747$, $p < 0.0001$), and it appears that each type has similar spreads with a few outliers (Figure 4, Appendix A4). Protein has the highest median. On the other hand, the comparison with Location type did not have significant differences (Two-way ANOVA, $F(3,60) = 2.4329$, $p = 0.07$; Figure 5).

Linear Regression

The results of the previous section show that the scores are different for each Site. Thus, a multiple linear regression was used to test if NPP and geological age (Age) significantly predicted Competition Scores. The fitted regression model was log(`Competition Scores`+1) ~ NPP + log(`Age (years)`) + `Food Type`. The transformation of taking the log of Competition Scores helps brings the data closer to Gaussian, while the log of Age reduces the leverage of extreme values. Because the geological age of Appledore is very small compared to other Sites, the data for Appledore was removed from the analysis to prevent the skew from affecting significance. In the regression equation, Food Type is used to account for the interaction between Site:Food Type previously determined, which suggested predicted competition scores may likely be shaped by Food Type as well. Because the interaction of Site:Location was not significant (Two-way ANOVA, $F(21) = 1.2836$, $p = 0.1933$), Location was excluded from regression analyses.

The overall regression was not statistically significant ($R^2 = 0.02293$, $F(4,175) = 2.05$, $p = 0.08943$), so NPP and Age may not individually predict competition scores. Another identical regression was run but now removing the three anomalous scores in Amazon H by selecting for only the scores below 23. The overall regression for this version was also not statistically significant ($R^2 = 0.01786$, $F(4,172) = 1.8$, $p = 0.131$) This means that there is not enough statistical evidence that NPP and Age are predictors of all of the competition scores.

However, different results were produced when the linear regression was done for competition scores from each Food Type separately (Table 5). Each of these regressions were conducted with and without selecting for values less than 23. All food regressions except Fat had a nonsignificant overall p value, so again there is not enough evidence that the proposed variables are factors of competition. Only in the regression with Fat competition scores (with score values <23 analysed), there was an overall statistically significant regression value, and here Age was statistically significant ($R^2 = 0.07766$, $F(2,55) = 3.4$, $p = 0.04055$, $\beta_{NPP} = 0.13525$, $p_{NPP} = 0.12275$, $\beta_{Age} = -0.23639$, $p_{Age} = 0.02687$). This means that for competition scores coming from the Fat food type in this study, the geological age of the Site was a significant factor in shaping the competition of its terrestrial invertebrates. Because the estimate is negative, the regression suggests that with higher Age there is less competition seen. Comparing with the box plot, this estimate is unexpected as Galapagos is the youngest Site (when excluding Appledore) and Whakatiwai is the oldest, yet they both show similar ranges in their Fat competition scores.

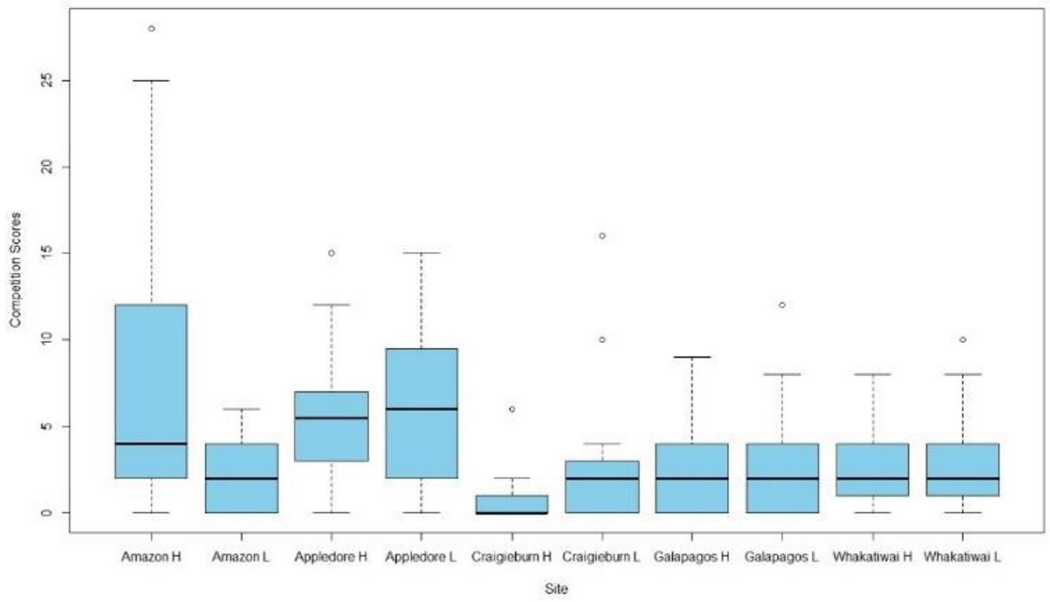
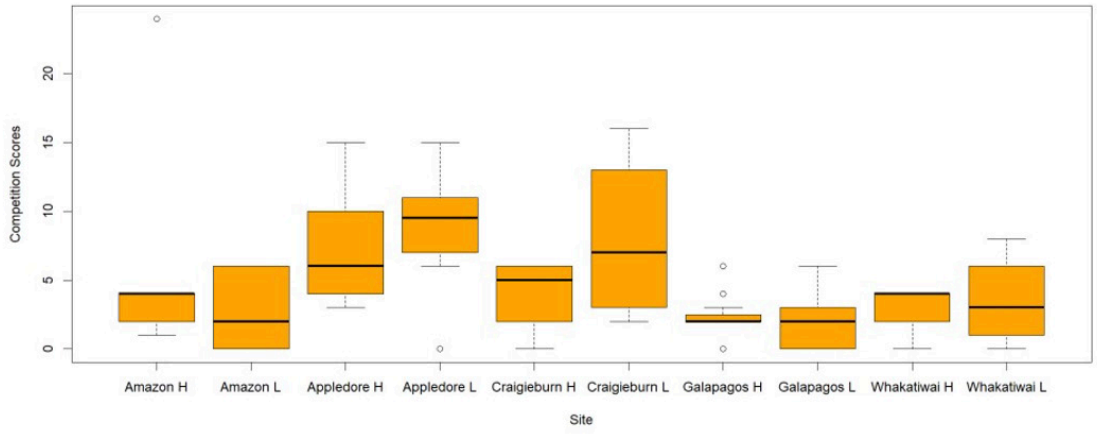
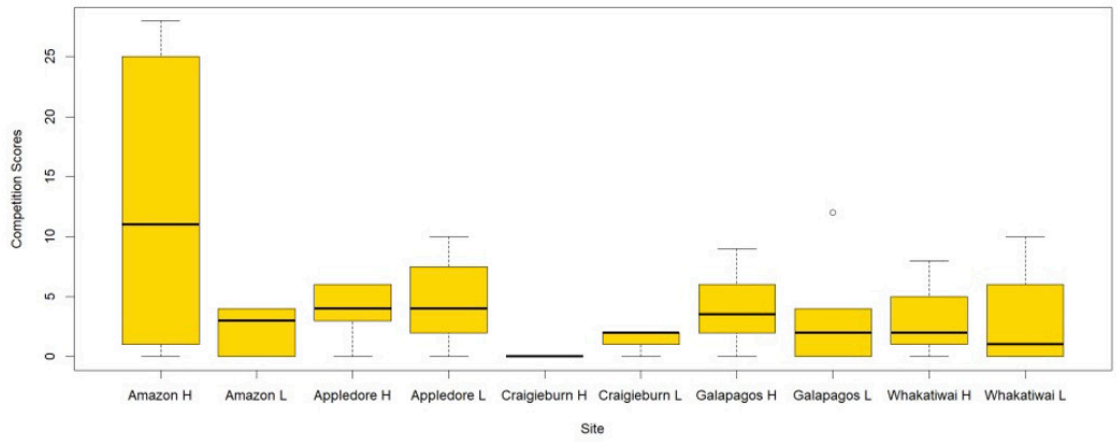


Figure 2. Boxplot of Competition Scores versus Sites. There is a significant difference (Two-way ANOVA, $F(9,218) = 5.4547$, $p < 0.0001$) between the Sites. The following Sites were significantly different to each other: Amazon H / Amazon L, Amazon H / Galapagos H, Amazon H / Galapagos L, Amazon H / Craigieburn H, Amazon L / Appledore H, Amazon L / Appledore L, Appledore H / Galapagos H, Appledore H / Galapagos L, Appledore H / Craigieburn H, Appledore H / Craigieburn L, Appledore L / Galapagos L, Appledore L / Craigieburn H

a)



b)



c)

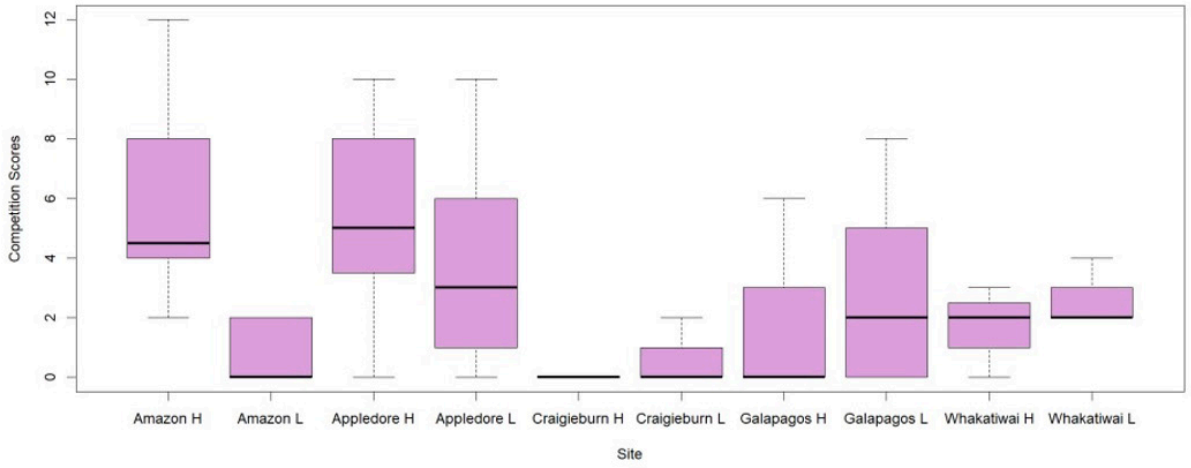


Figure 3. The Boxplots show the competition scores for Sites per Food Type. **a.** For Protein the Sites had statistically different scores (Two-way ANOVA, $F(9,66) = 3.1439$, $p < 0.001$). The following were significantly different to each other: Appledore H / Galapagos L, Appledore L / Galapagos L **b.** For Fat trials there were statistically significant differences (Two-way ANOVA, $F(9,66) = 2.2337$, $p < 0.05$) in only the following: Amazon H - Craigieburn H **c.** Finally, for the sugar lures there was also a significant difference (Two-way ANOVA, $F(9,66) = 4.1778$, $p < 0.0001$) between these Sites: Amazon H - Amazon L, Amazon H - Galapagos H, Amazon H - Craigieburn H, Amazon H - Craigieburn L, Amazon L - Appledore H, Appledore H - Galapagos H, Appledore H - Craigieburn H, Appledore H - Craigieburn L.

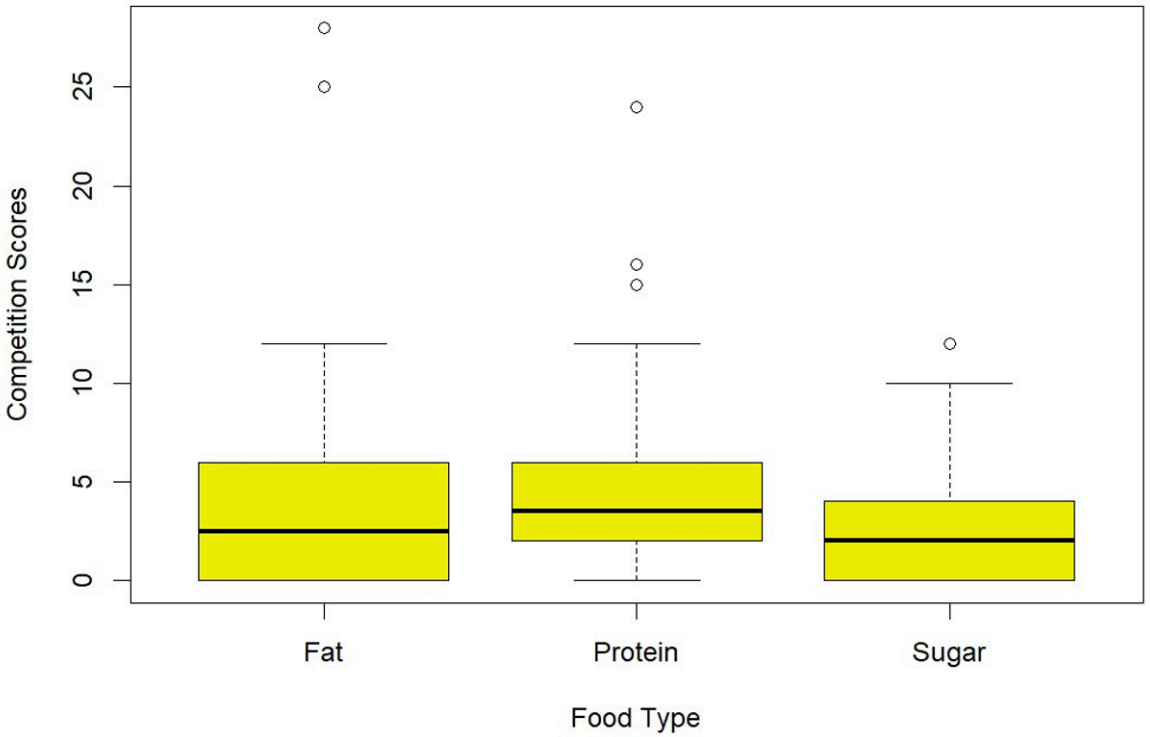


Figure 4. Boxplot of Competition Scores versus Food Type. There was a significant difference (Two-way ANOVA, $F(2,225) = 4.57›47$, $p < 0.0001$) between Protein and Sugar.

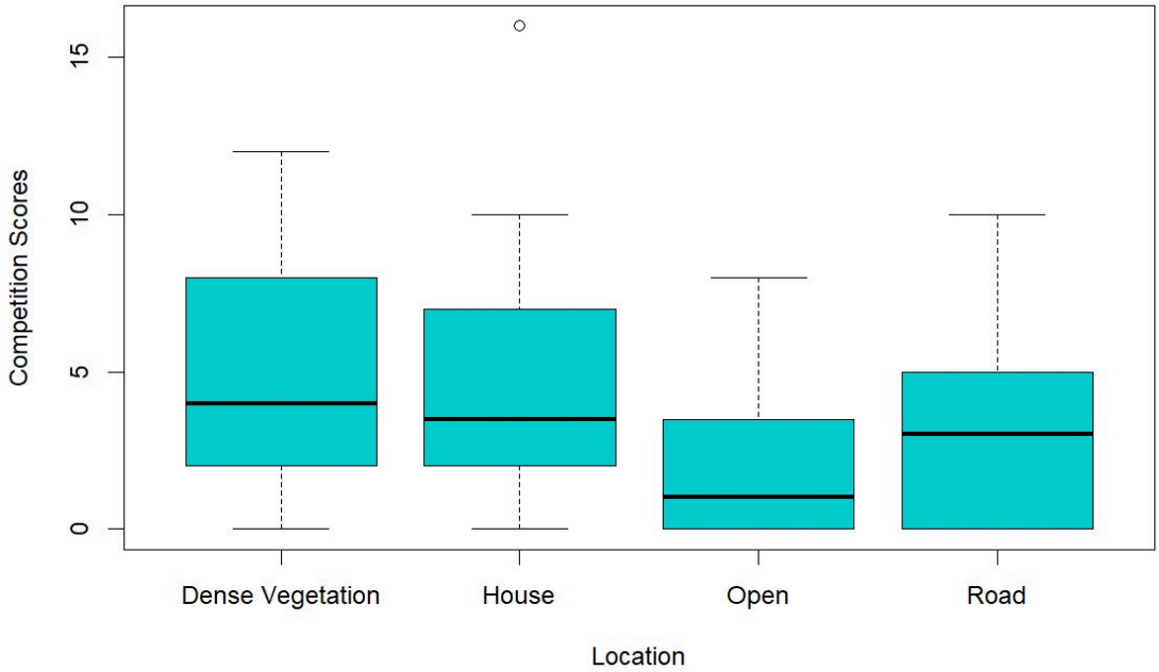


Figure 5. Boxplot of Competition Scores versus Location. There was no significant differences between the Locations (Two-way ANOVA, F(3,60) = 2.4329, p = 0.07)

Table 4. Summary of tests with a Two-way ANOVA. The conversion of competition scores (x) to Sqrt x or Log (x+1) was determined based on which one allowed the data to be closer to a Gaussian distribution. The alpha level to determine significance is defined as 0.05.

Testing	Anova Competition Scores	p value	Significant?	Differences
Competition Scores vs Site * Food Type (All scores)	Sqrt x	• Site: 1.455e-07 • Food Type: 0.003206 • Site:`Food Type`: 0.072323	• Site: Y • Food Type: Y • Site:`Food Type`: N	
Competition Scores vs Site * Food Type (Competition Scores <23)	Sqrt x	• Site: 3.836e-06 • Food Type: 0.005191 • Site:`Food Type`: 0.027863	• Site: Y • Food Type: Y • Site:`Food Type`: Y	
Competition Scores vs Site	Log (x+1)	5.00E-07	Y	Amazon H / Amazon L, Amazon H / Galapagos H, Amazon H / Galapagos L, Amazon H / Craigieburn H, Amazon L / Appledore H, Amazon L / Appledore L, Appledore H / Galapagos H, Appledore H / Galapagos L, Appledore H / Craigieburn H, Appledore H / Craigieburn L, Appledore L / Galapagos L, Appledore L / Craigieburn H
Competition Scores vs Food Type	Log (x+1)	0.01129	Y	Protein / Sugar
Protein Competition Scores vs Site	Log (x+1)	0.00327	Y	Appledore H / Galapagos L, Appledore L / Galapagos L
Fat Competition Scores vs Site	Sqrt x	0.03033	Y	Amazon H - Craigieburn H
Sugar Competition Scores vs Site	Sqrt x	0.0002675	Y	Amazon H - Amazon L, Amazon H - Galapagos H, Amazon H - Craigieburn H, Amazon H - Craigieburn L, Amazon L - Appledore H, Appledore H - Galapagos H, Appledore H - Craigieburn H, Appledore H - Craigieburn L
Protein Competition Scores vs Location	Log (x+1)	0.98	N	NA
Fat Competition Scores vs Location	Log (x+1)	0.73	N	NA

Table 4. Continued

Testing	Anova Competition Scores	p value	Significant?	Differences
Competition Scores vs Site * Location	Log (x+1)	• Site: 6.982e-05 • Food Type: 0.4383 • Site: Location: 0.1933	• Site: Y • Location: N • Site:Location: N	
Competition Scores vs Location	Sqrt x	0.06984	N	NA
Dense Vegetation Competition Scores vs Site	Log (x+1)	0.07684	N	NA
House Competition Scores vs Site	Sqrt x	0.3341	N	NA
Open Competition Scores vs Site	Sqrt x	0.00341	Y	Appledore H - Galapagos L, Appledore H - Whakatiwai H, Appledore L - Whakatiwai H
Road Competition Scores vs Site	Log (x+1)	0.09393	N	NA
Dense Vegetation Competition Scores vs Food Type	Log (x+1)	0.9146	N	NA
House Competition Scores vs Food Type	Log (x+1)	0.1173	N	NA
Open Competition Scores vs Food Type	Log (x+1)	0.1793	N	NA
Road Competition Scores vs Food Type	Log (x+1)	0.3874	N	NA

Table 5. Summary of linear regression analyses and their outputs.

Regression Equation	p value	Overall p value	R^2
All sqrt(Competition Scores) ~ NPP + log (Age + 1) + Food Type	• (Intercept): 8.75e-07 *** • NPP: 0.2782 • log(`Ecological Environment Age (years)`): 0.0015 ** • `Food Type`Protein: 0.4623 • `Food Type`Sugar: 0.0231 *	1.939e-05 ***	0.09787
W/o Appledore log (Competition Scores + 1) ~ NPP + log (Age + 1) + Food Type	• (Intercept): 0.0419 * • NPP: 0.4761 • log(`Ecological Environment Age (years)`): 0.5404 • `Food Type`Protein: 0.9466 • `Food Type`Sugar: 0.0179 *	0.08943	0.02293
(<23) w/o Appledore sqrt(Competition Scores) ~ NPP + log (Age + 1) + Food Type	• (Intercept): 0.0460 * • NPP: 0.3585 • log(`Ecological Environment Age (years)`): 0.5239 • `Food Type`Protein: 0.8951 • `Food Type`Sugar 0.0417 *	0.131	0.01786

Table 5. Continued

Regression Equation	p value	Overall p value	R^2
Protein log (Competition Scores + 1) ~ NPP + log (Age + 1)	• (Intercept): 0.3329 • NPP: 0.6451 • log(`Ecological Environment Age (years)`): 0.0452 *	0.13	0.03642
(<23) Protein sqrt(Competition Scores) ~ NPP + log (Age + 1)	• (Intercept): 0.2748 • NPP: 0.6761 • log(`Ecological Environment Age (years)`): 0.0329 *	0.09977	0.04613
Fat log (Competition Scores + 1) ~ NPP + log (Age + 1)	• (Intercept): 0.00936 ** • NPP: 0.22704 • log(`Ecological Environment Age (years)`): 0.04402 *	0.08464	0.05082
(<23) Fat sqrt(Competition Scores) ~ NPP + log (Age + 1)	• (Intercept): 0.00603 ** • NPP: 0.12275 • log(`Ecological Environment Age (years)`): 0.02687 *	0.04055 *	0.07766
Sugar sqrt(Competition Scores) ~ NPP + log (Age + 1)	• (Intercept): 0.218 • NPP: 0.752 • log(`Ecological Environment Age (years)`): 0.517	0.7874	-0.02644

Discussion

This study demonstrates that levels of competition in invertebrate species vary by Site and Food Type. This variation is not strongly linked to NPP, though perhaps to the geological age of the Site.

Because a significant variation of competition scores between Sites exists (Figure 2), we can assume that there are regional factors that shape the competition of terrestrial invertebrates. This assumption is possible because I used competition scores in this study, which serve as a standard measurement that can be compared throughout Sites and experiments. By having statistical proof that these differences exist, we can move onto the first objective of this study, which is identifying what factors could be significant drivers of competition. First, we will do a qualitative analysis of the Site characteristics.

In general, the Amazon and Appledore Sites had higher medians than the other Sites, which in comparison appeared to have lower scores and similar spreads. When talking about the Amazon in the discussion I refer to Amazon H mainly, since Amazon L is a small percentage of land in the region and works differently as it gets frequent floods (high insect turnover from disturbance). When characterizing Amazon H and Appledore, we see a relatively large amount of biomass in both which relates to abundant food resources for invertebrates. Even though Appledore does not have large trees, its vegetation consists of dense shrubs, herbs, and green grasses (Nichols, 2008). With plentiful resources, these sites may support larger populations of invertebrates which allow for frequent and complex interactions.

In addition, these two Sites have access to high dispersal from outside the system. The Amazon is thousands of years old and it is connected to neighboring ecosystems, so it experiences high dispersal from visitor animals and people that inadvertently carry insects. A study found that dispersal rates affect the diversity patterns of

ants in the Amazon (Guilherme et. al., 2021), and this might also play a role in competition. For Appledore island, the Shoals Marine Laboratory located runs several classes and research all the time outside wintertime, so the island receives many people that come and go in boats from mainland Maine (10km away). Further, Appledore and neighbor islands are nesting grounds for several populations of marine birds (Shoals Marine Lab, 2023) who visit the mainland frequently. Strong dispersal rates allow for the creation of diverse communities and in consequence, create diverse interactions.

High plant biomass plus high dispersal may create a rising invertebrate population until it reaches the carrying capacity (Wisniewski, 1980). Carrying capacity is a term referring to the maximum population level that an environment can support based on its finite resources (Verhulst, 1838). At this level, the resources available will be low and competition could be high as communities engage in intra and inter species competition for food (Wisniewski, 1980). The other Sites in the study did not have this combination of high biomass and dispersal, so this qualitative assessment suggests that levels of dispersal and biomass may be important variables affecting competition in an ecosystem.

On the other hand, the Sites with the lowest competition scores tended to be in Craigieburn. This could be because this is a low alpine zone area and most species from surrounding areas likely do not have the capacity to live at the low temperatures and harsh conditions. Moreover, this area is a pure mountain beech forest, so resource selection is limited. These characteristics could translate in low invertebrate abundance, hence less competitive interactions.

When analyzing competition scores versus Sites with the interaction of Site and Food Type, we saw a statistically significant interaction, which suggests that competition in invertebrates varies by Site, but the pattern of variation is different for each Food Type. This not only supports the methodology for accounting for dietary preferences, but also conveys that invertebrates that prefer one Food Type may behave and interact differently than those with other dietary preferences. The different visitor counts for each Food Type could be because of the composition of species at each Site as some may have more species with preference for one food type over the others.

In terms of Location, it appears that the type of vegetation cover does not affect competition much, possibly since some species can smell out food from large distances and will travel to them (Baker, 2017).

For some analyses, I used competition scores with values less than 23 to remove the three very high scores from the pool. Taking these out illustrates that when ignoring them, competition scores across Sites remain at a range with upper bounds lower than a score of 16. The three anomalous scores were part of the Fat and Protein trials in Amazon H (Figure A4 in Appendix), and were high because in those trials there were large visitor counts (i.e. crickets, wasp, four different ant species, fruit flies, arthropod, etc). Even though these trials included multiple ant species, the number of individuals per species was low (1-10) and no ant colony flooded the food source. This meant that other insects were able to get to the resource, in contrast to many other trials in the study, which suggests that ants (particularly social species) have a competitive advantage to other invertebrates, since normally once the colony settles, less insects visit the lure. Studies support this and suggest a combination of physical and chemical deterrents against competitors (Miner & Rankin, 2023).

As explored so far, the variation in competition scores could be mainly from idiosyncrasies of Sites. Through quantitative analyses, I conducted regressions to test if NPP values and geological age (Age) of each Site are possible predictors of competition scores. At first, NPP and Age did not appear to be significantly predictors. However, when the anomalous high competition scores were removed, the regression revealed that Age

was a significant predictor for the Fat food type scores. This indicates that the geological age of an area could be an important factor in shaping the competition dynamics in invertebrates with a dietary preference of fat. My hypothesis that age could be a predictor of competition is supported. The negative correlation found, on the other hand, is opposite of the hypothesis of positive relationship between age and competition. This is unexpected as the Fat boxplot (Figure 3b) illustrates considerably different range of scores between the two Site with same age (Whakatiwai vs Craigieburn). Perhaps there are other unknown sitespecific variables interacting with geological age that affect competition. Nevertheless, if age is indeed a universal predictor for competition, we could categorise regions into experiencing high or low competition, and then model how movement of species from high competition regions to low, or vice versa, could have an effect on local flora and fauna. This serves to identify potential invasive species that have a competitive advantage by for example being freed from its natural competitors (ecological release) (Kelley et al., 2019).

NPP was found to be non-significant in all the regressions, which contradicts my predictor hypothesis. Perhaps NPP in this study did not represent environmental conditions and biomass well, or these are not impactful to competition. The non-significant results of Age and NPP for Sugar and Protein may indicate that other factors are larger drivers of competition, factors are interacting with each other, or there are no universal drivers of competition in these.

The results in this study may have been limited from its small sample size as NPP and geological age were only one data point per Site (n=10 Sites), making the degrees of freedom small. This may affect the accuracy of the predictor analysis and so for a better regression it is recommended that in future work more Sites are sampled (in different geographical regions with varying geological ages). By having a larger sample size, the predictor relationship of NPP and Age on competition may be determined with higher confidence. I recommend using NPP values that are averages of more current months and years. Additionally, the values of geological ages may differ from the true ages of the ecosystems surveyed, since the geological history of each Site is varied and experienced great changes over millennia, making it complex to pinpoint the start of the ecosystems as we know them today. Therefore, it would be beneficial to use data from novel tools that accurately date each ecosystem. Other suggestions of future work include testing alternative variables such as biomass and a quantification of dispersal, as possible predictors of competition as suggested by the qualitative analyses.

The outcomes and information gathered from this research are valuable to the process of understanding competition dynamics in invertebrates. This study provides an analysis of the factors that may or may not affect competition in various ecological and geographical regions.

Learning about competition allows us to understand the forces structuring invertebrate communities (Parr & Gibb, 2010), predict new community assemblies from introduction or disturbance, and model how organisms will interact in these new arrangements. In real world applications, this information is useful in the movement of species to cooler regions due to global warming, in analysing the impacts of introduced species through the heightened interconnectedness of our modern world (Baker, 2017), or in the dispersal of disease carrying insects. Thus, research on the factors affecting competition dynamics is highly necessary, and this study is a step towards that.

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Appendix A—Comparison Graphs

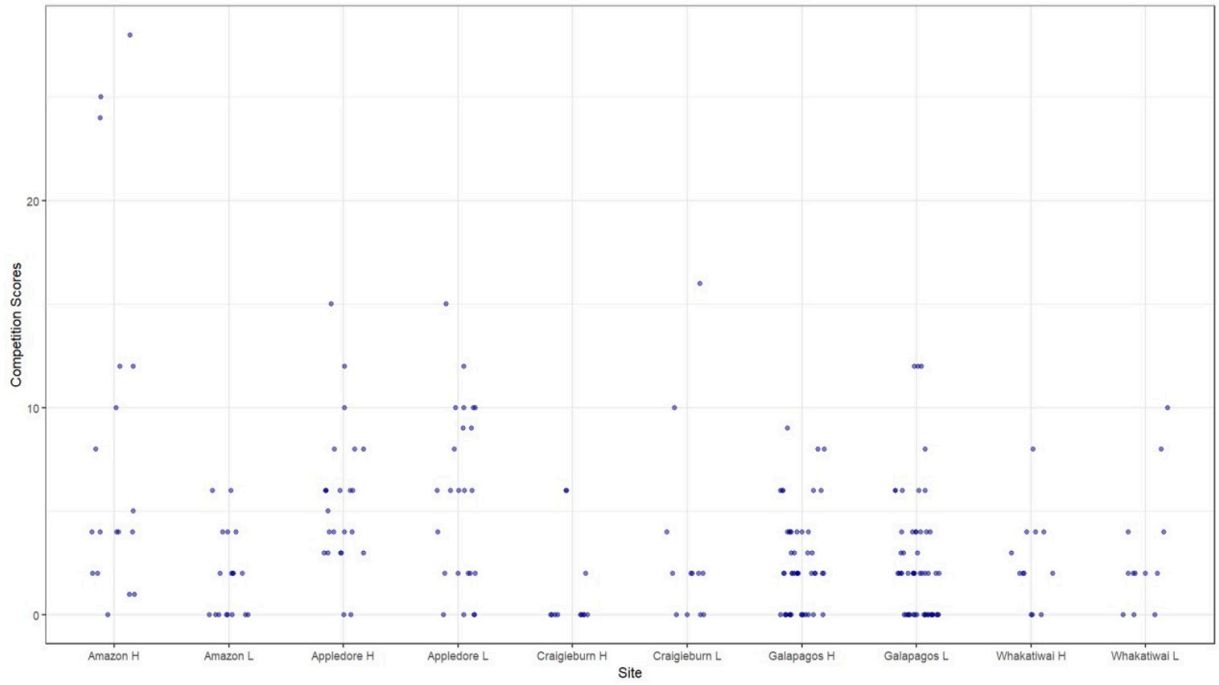

Figure A1. Scatterplot of Competition Scores versus Sites

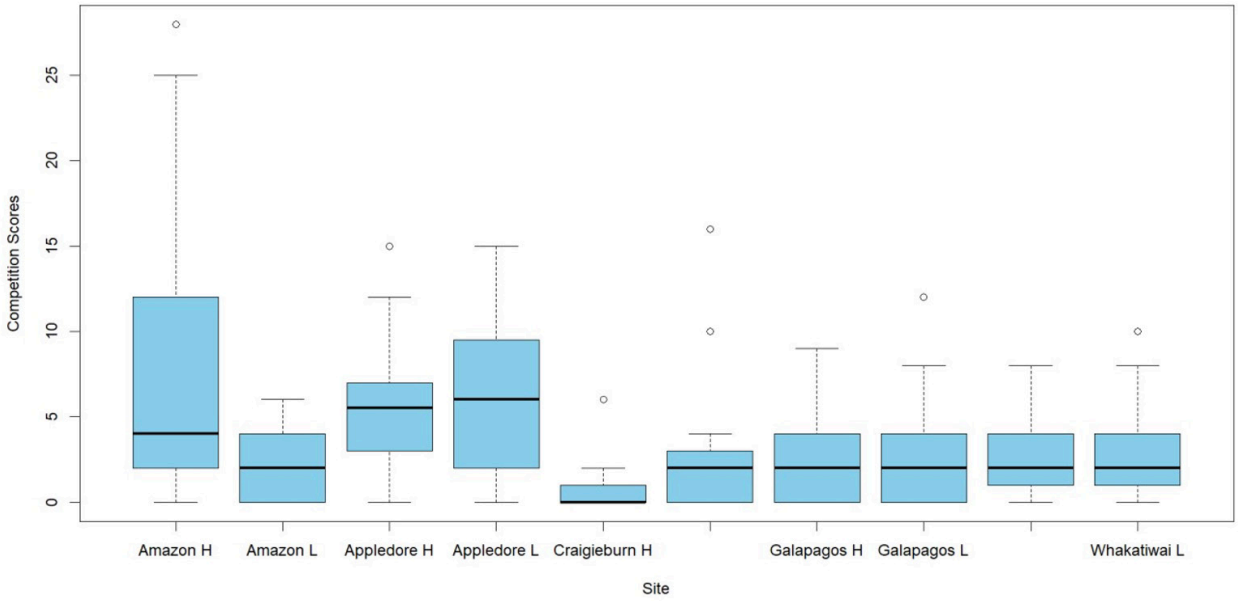

Figure A2. Boxplot of Competition versus Sites

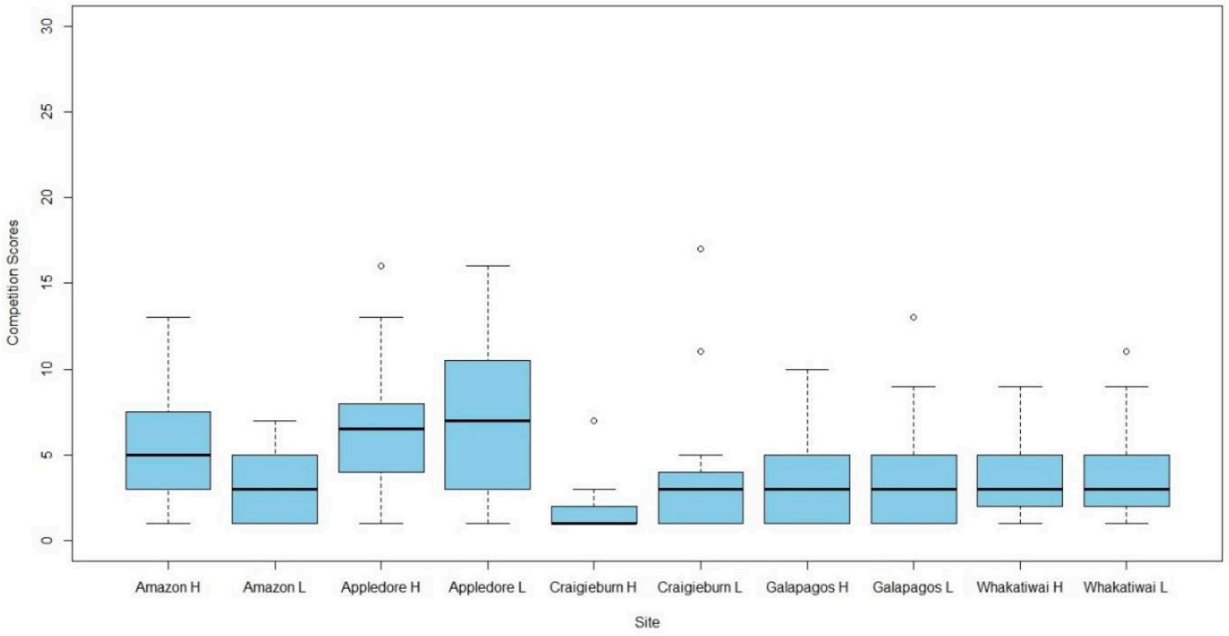

Figure A3. Boxplot of Competition Scores (with values < 23) versus Site. The y axis range was kept the same as Figure A2 for visual comparison.

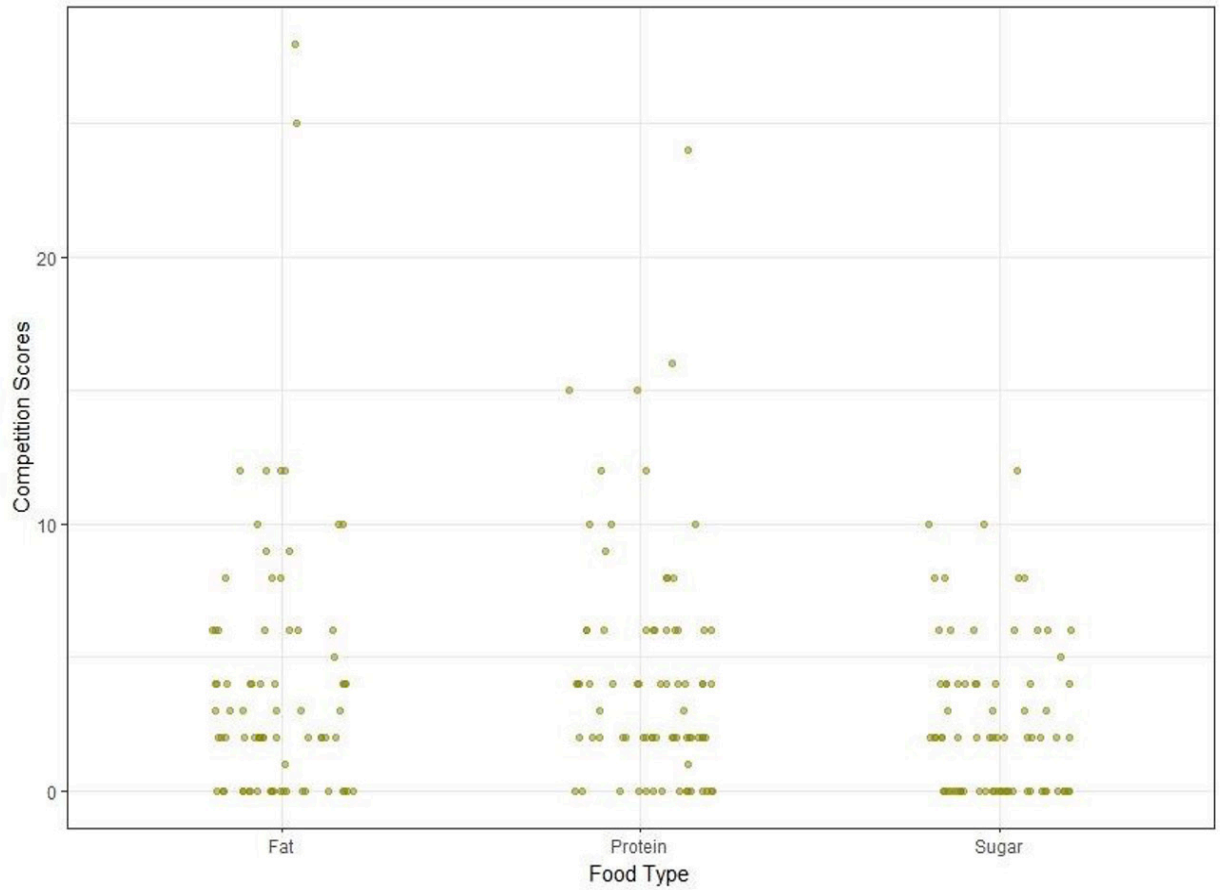

Figure A4. Scatterplot of Competition Scores versus Food Types

Appendix B—Coordinates of Sites

Site	Longitudinal Coordinates	Lateral Coordinates
Galapagos H	0.9014 S	89.6075 W
Galapagos L	0.8868 S	89.5416 W
Amazon H	0.6367 S	76.1503 W
Amazon L	0.6380 S	76.1501 W
Appledore H	42.9871 N	70.6155 W
Appledore L	42.9891 N	70.6152 W
Whakatiwai H	37.0877 S	175.3022 E
Whakatiwai L	37.0873 S	175.3032 E
Craigieburn H	43.1519 S	171.7137 E
Craigieburn L	43.1515 S	171.7121 E

Appendix C—List of the Orders of Species Present on Baits at Each Site

Site	Hymenoptera	Orthoptera	Dermaptera	Diptera	Araneae	Hemiptera	Isopoda
Galapagos H	✓	✓	✓	✓	✓		
Galapagos L	✓			✓			
Amazon H	✓	✓		✓			
Amazon L	✓	✓		✓	✓		
Appledore H	✓			✓			✓
Appledore L	✓			✓			
Whakatiwai H	✓			✓			
Whakatiwai L	✓			✓		✓	
Craigieburn H	✓			✓		✓	
Craigieburn L				✓			

Investigating the Structural, Functional, and Biochemical Properties of PP_i-dependent PEPCK Paralogs from *Entamoeba histolytica*

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Abstract

Phosphoenolpyruvate carboxykinase (PEPCK) is an important metabolic enzyme which functions to interconvert oxaloacetic acid (OAA) and phosphoenolpyruvate (PEP) in the Krebs cycle, a key process of generating cellular energy. There exist three known classes of PEPCK - two of which are nucleotide-dependent, using ATP and GTP. Very little is known about the third, PP_i-dependent PEPCK. Comparing classes, nucleotide-dependent PEPCKs are both functionally and structurally similar (~60-70 kDa) whereas PP_i-dependent PEPCK bears significant functional and structural differences (~130 kDa). This presented work investigates PP_i-dependent PEPCK from a human parasite *Entamoeba histolytica* (*Eh*PEPCK). It is unique from previous work done on another homolog from *Propionibacterium freudenreichii* (*Pf*PEPCK) in that there are three paralogs instead of one. This suggests increased complexity in function and regulation. This work has determined that the interaction between *Eh*PEPCK paralogs gives rise to dimers and heterotrimers, and certain interactions show substrate induced inhibition. Kinetic measurements were completed to determine the metal cofactor of *Eh*PEPCKs, and to determine the kinetic consequences of the aforementioned oligomeric states. The experiments support the conclusion that aggregation causes substrate inhibition, and that dimers are more active than trimers.

Introduction

Phosphoenolpyruvate carboxykinase (PEPCK) is an important metabolic enzyme suggested to be the master regulator of TCA cycle flux (Yang et al., 2009). It operates by removing citric acid cycle anions to be used in other metabolic processes, namely gluconeogenesis, glyceroneogenesis, 1-carbon serine synthesis, or works to replenish the TCA cycle (Yang et al., 2009). PEPCK has implications in glucose-stimulated insulin secretion (a process in diabetes), senescence, tuberculosis, and in cancer and has been thought of as a potential therapeutic target (Jeon et al., 2015; Mendez-Lucas et al., 2014; Montal et al., 2015; Park et al., 2014; Marrero et al., 2010; Yuan et al., 2016; Santra et al., 2016; Yang et al., 2009b). The PEPCK family has classically been divided into two nucleotide-dependent classes: those which utilized ATP, and those that utilized GTP as a phosphoryl donor. *In vivo*, PEPCK is thought to primarily catalyzes the reaction in which oxaloacetic acid (OAA) is converted into phosphoenolpyruvate (PEP) and carbon dioxide using its nucleotide substrate (McLeod and Holyoak, 2021). However *in vitro*, PEPCK can complete the reverse reaction as well, making them bidirectional. More recently, a third PEPCK which utilizes pyrophosphate (PP_i) was discovered, further expanding the family. The two nucleotide-dependent PEPCKs are well characterized in both their structure and function. PEPCK requires two cation metal cofactors for activity, named M1 and M2 (Willard et al., 1969). M1 is an active site cofactor which binds to the enzyme and bridges the substrate and nucleotide binding pockets. M2 binds as a nucleotide-metal complex (McLeod and Holyoak, 2021). In the nucleotide-dependent PEPCKs, the chemical reaction is most activated when the M1 metal is Mn^{2+} and

the M2 metal is Mg^{2+} (Das et al., 2012; Machova et al., 2015; Hebda and Nowak, 1982; Hidalgo et al., 2016; Sokaribo et al., 2020; Escos et al., 2016; Wilkes et al., 1982). The active site of the enzyme contains three important loops (Fig.1). The P-loop binds the nucleotide and positions it correctly for phosphoryl transfer (Matte et al., 1996). The R-loop binds the substrates (OAA/PEP) and once bound, moves to allow closure of the Ω-loop (Holyoak et al., 2006). Finally, the Ω-loop acts as a gate/lid and protectively encloses the active site while the reaction takes place (Johnson and Holyoak, 2010).

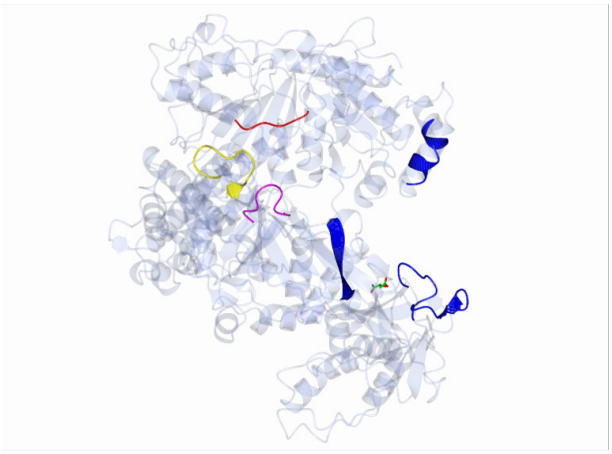

Figure 1. Structure of PPi-dependent PfPEPCK. The P-loop (magenta), R-loop (red), and Ω-loop are shown along with the dimer interface (blue).

Initial studies on the PP_i-dependent class of PEPCK were completed in the 1960s and 1970s (Siu et al., 1961; Lochmuller et al., 1966; Willard and Rose, 1973; O'Brien et al., 1973; Wood et al., 1969; Haberland et al., 1972) In these studies, PP_i-dependent PEPCK from *Propionibacterium freudenreichii* (*Pf*PEPCK) was evaluated. These studies were then re-evaluated to determine the differences between the nucleotide and pyrophosphate using classes (McLeod and Holyoak, 2021). First, PP_i-dependent PEPCKs are approximately twice the size (~130kDa) of nucleotide-dependent PEPCKs (~70kDa), with a mostly conserved (in relation to nucleotide-dependent PEPCKs) core structure including the active site residues/loops (Fig.1). However, this extra mass has manifested as additional "lobes" around this conserved core. Second, *Pf*PEPCKs preferentially use Fe^{2+} as the M1 cofactor instead of Mn^{2+}. Third, although ATP- and GTP-dependent PEPCKs preferentially catalyzed the OAA→PEP reaction, PP_i-dependent PEPCK favors the PEP→OAA reaction (Lochmuller et al., 1966). Fourth, PPi-dependent PEPCK exhibits substrate inhibition where high concentrations of PEP lead to inactivation, whereas ATP- and GTP-dependent PEPCKs do not. Finally, in this substrate-induced inactivated state, the enzyme oligomerizes from monomers to homodimers. In addition to substrate (PEP or OAA), malate was shown to bind to an allosteric site which presumably causes this dimerization. In contrast, nucleotide-dependent PEPCKs are typically monomeric and have not been observed to have any regulation by ligand-induced quaternary structure changes (Fukuda et al., 2004).

The PP_i-dependent class has been relatively understudied despite *Pf*PEPCK having unique characteristics when compared to nucleotide-dependent PEPCKs. A new isozyme of PP_i-dependent PEPCK from *Entamoeba histolytica* (*Eh*PEPCK) was previously studied (McLeod and Holyoak, 2021). *Entamoeba histolytica* is a human parasite and studying it may provide a therapeutic avenue to target this organism (Chou and Austin, 2022). Deeper insight into the structure and function may help to discover how to selectively target this enzyme. In the Chiba et al. study, a unique trimer was observed (McLeod and Holyoak, 2021). However, there was no understanding as to what oligomeric states are possible, how these oligomeric states arise (ligand-induce or paralog interactions), or the paralog/oligomer activities. Here, I structurally and functionally characterize these three isoforms in isolation and in combination to understand these structure-function relationships.

Structural characterization was completed using small angle X-ray scattering (SAXS). SAXS uses X-ray scattering to determine low-resolution structural details on macromolecules. Specifically, structural information that can be obtained are: the radius of gyration (R_g) (mean distance from the center of mass or central axis to the outer edges), estimated molecular

weight, the D_{max} (is the maximum dimension of the molecule), and low-resolution shape information (ie. long rod vs sphere). Further analysis of structural information yields scattering curves and Kratky plots. Kratky plots qualitatively determine the flexibility and degree of unfolding in the sample. Dimensionless Kratky plots normalize scattering profiles by mass and concentration (Hopkins et al., 2017). An unfolded protein displays a plateau with a high q, while globular proteins display a bell shaped curve, and a combination of the two may show characteristics of both. As the oligomerization of *Eh*PEPCK will lead to large changes in size, SAXS accurately assessed the oligomerization states of the paralogs. SAXS data was collected both with and without substrates and known effectors of *Pf*PEPCK to determine how the addition of substrates affects oligomerization state. In addition to structural characterization, kinetic experiments were done to map functional consequences to given structures. Prior to full kinetic measurements, the optimal metal cofactor (M1) was determined as this was shown to be variable between classes. The activity of each paralog in isolation, and in combination was measured (work ongoing). These experiments have revealed a general mechanism of substrate regulation of *Eh*PEPCKs which can then be compared to *Pf*PEPCK to determine similarities and differences.

Materials and Methods

PP_i, PEP, and NADH were purchased from ChemImpex. BME was purchased from Sigma Aldrich. MDH was purchased from Calzyme Laboratories. HEPES was obtained from Gold Biotechnology. Sodium Bicarbonate was purchased from Mallinckrodt Pharmaceuticals. The enzyme was purified previously at Cornell University after recombinant expression. All other chemicals were purchased from the highest grade available.

Small Angle X-ray Scattering of *Eh*PEPCK paralogs (SAXS)

SAXS was used to determine the oligomeric states of *Eh*PEPCKs in isolation, in combination and with different ligands. Screening for optimal conditions was performed using a BioXolver (Xenocs) home-source instrument at Cornell's Laboratory of Atomic and Solid-State Physics. The data was recollected at Sector 7A1 (BioSAXS) at the Cornell High Energy Synchrotron Source (CHESS) to extend signal/noise and resolution limits (Table 1). Final samples were in 50 mM TRIS pH 8.0 and 2 mM TCEP at a final volume of 20 uL and the total concentration of *Eh*PEPCK was 1.25 mg/mL.

Table 1. CHESS SAXS collection parameter details for EhPEPCK structural studies.

Wavelength (Å)	1.103
Detector distance (cm)	1735
Exposure number/sample	10
Exposure length (s)	1
Q range ($Å^{-1}$)	0.000123 - 0.577

Paralog activity measurements using Michaelis-Menten Kinetics

$$PEP + P_i + CO_2 \rightarrow OAA + PP_i$$

The kinetic constants were determined for the paralog *Eh*1, and the combinations *Eh*1+2 and *Eh*1+3 of *Entamoeba histolytica*, with experimentation in progress for every paralog and combination possible for the three paralogs. The assays were done in duplicate at room temperature at a final volume of 1 mL. A Varian 50 Bio UV-Visible Spectrophotometer was used to monitor enzyme activity. The conversion of PEP to OAA in each assay was monitored at 340 nm via a coupled reaction using malate dehydrogenases by observing the change of NADH to NAD^+. An R script from McLeod and Holyoak (2021) was used to determine Michaelis-Menten parameters by fitting data to the substrate inhibition model (Eq. 1)

$$\frac{Vmax * \text{substrate concentration}}{(Km + \text{substrate concentration}) * (1 + (\text{substrate concentration} / Ki))}$$

Equation 1

The standard assay mix for PEP carboxylation was composed of 100 mM HEPES pH 7.5, 20 mM BME, 300 μM NADH, 10 mM KH_2PO_4,

5 mM $MgCl_2$, 250 µM $MnCl_2$ (or other M1 metal substitute), 40 mM $KHCO_3$ (bubbled with dry ice), PEP varying from 2.5 uM to 10,000 uM, 5U of malate dehydrogenase (MDH) and 5 µg of PP_i-dependent PEPCK (*Eh*1, *Eh*2, or *Eh*3). Reactions were initiated with the addition of PP_i-dependent PEPCK. To determine what metal is used most effectively as a cofactor by *Eh*PEPCK, kinetic assays with *Eh*1 were performed with each one using 250 µM of the following metals: iron ($FeCl_3$), manganese ($MnCl_2$), magnesium ($MgCl_2$), calcium ($CaCl_2$), zinc ($ZnCl_2$), copper ($CuCl_2$), and cobalt ($CoCl_2$). As zinc and copper did significantly react in the initial round of testing, they both were not included in final testing, and therefore are not included in the discussion.

Results and Discussion

Paralog oligomerization determined by SAXS

Small-angle x-ray scattering (SAXS) uses X-rays to determine low resolution structural details on macromolecules. SAXS was used here to determine the quaternary state of the paralogs in isolation (*Eh*1, *Eh*2, and *Eh*3) and in combination (*Eh*1+2, *Eh*1+3, *Eh*2+3, *Eh*1+2+3), both with and without substrate (both OAA and PEP) as well as a known allosteric effector of *Pf*PEPCK (malate) (Table 2). It was observed that in isolation, all three paralogs have approximately the same D_{max} values of 140, 139, and 142 Å (respectively) and R_g values of 47.1, 44.2, and 48.4 Å (Table 2). They also have the near identical scattering curves, Kratky plots, and P(r) functions (Fig.2). *Eh*2+3 is approximately the same as the paralogs in isolation, with an R_g of 45.6 Å and D_{max} of 139 Å. The dimer structure from crystallized *Pf*PEPCK was found to have an R_g of 46.4 Å and D_{max} of 139 Å (Manalastas-Cantos et al., 2021). Hence, *Eh*1, 2, 3, and *Eh*2+3's R_g and D_{max} values are, without the addition of substrate or ligand, suggestive of a dimer complex. It is unknown if *Eh*2 and *Eh*3 combine to form a heterodimer, or if they are only present as homodimers.

Table 2. D_{max} and R_g values of Eh1, 2, and 3 in isolation and in combination.

***Eh*PEPCK**	**1**	**2**	**3**	**1+2**	**1+3**	**2+3**	**1+2+3**
D_{max} (Å)	140	139	142	192	192	139	195
R_g (Å)	47.1 ± 0.277	44.2 ± 0.379	48.4 ± 0.358	66.1 ± 0.618	62.4 ± 0.568	45.6 ± 0.381	61.4 ± 0.187
10 mM PEP	**1**	**2**	**3**	**1+2**	**1+3**	**2+3**	**1+2+3**
D_{max} (Å)	Agg.*	160	165	Agg.*	Agg.*	150	480
R_g (Å)	Agg. *	53.5 ± 0.511	53 ± 0.673	Agg.*	Agg.*	56.2 ± 0.607	120.6 ± 1.18
10mM OAA	**1**	**2**	**3**	**1+2**	**1+3**	**2+3**	**1+2+3**
D_{max} (Å)	Agg.*	175	155	Agg.*	Agg.*	150	235
R_g (Å)	Agg.*	57.3 ± 0.694	51.7 ± 0.378	Agg.*	Agg.*	49.1 ± 0.374	72.2 ± 0.257
40 mM Malate	**1**	**2**	**3**	**1+2**	**1+3**	**2+3**	**1+2+3**
D_{max} (Å)	139	144	145	255	250	145	250
R_g (Å)	53.3 ± 0.503	46.5 ± 0.357	48.2 ± 0.373	65.6 ± 0.882	68.6 ± 1.65	45.7 ± 0.304	72.1 ± 0.274

*Aggregation

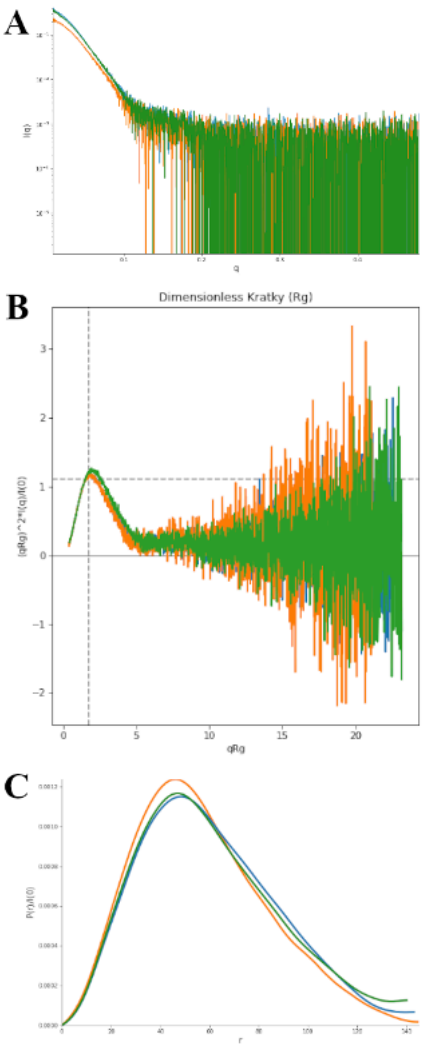

Figure 2. ***Eh*1, *Eh*2, and *Eh*3 in isolation form dimeric structure.** SAXS scattering profiles indicate that each paralog in isolation is the same dimeric structure (when compared to theoretical R_g/D_{max} of known dimeric structure). A) Scattering Curve of *Eh*1 (blue), *Eh2* (orange) and *Eh3* (green). B) Normalized Kratky Plot. C) Normalized P(r).

To determine the molecular origins of the previously reported trimer (McLeod and Holyoak, 2021), each combination was tested. In combination *Eh*1+2, 1+3, and 1+2+3 have approximately the same D_{max} (192, 192, and 195 Å) and R_g (66.1, 62.4, and 61.4 Å) values, which are higher than the aforementioned paralogs in isolation (or *Eh*2+3) (Table 2). This suggests that this complex is a heterotrimer as opposed to *Eh*1 forming a homotrimer with itself as *Eh*2 or 3 is also required to form this structure. Comparing *Eh*1 (dimer) with *Eh*1+2 (trimer), there is a clear change in the structure as observed by the scattering curve (Fig. 3) which is the same structure as *Eh*1+2+3 (Fig. 4). At a sequence level, both *Eh*2 and *Eh*3 share 98% sequence similarity with one another, whereas they only share 90% sequence similarity with *Eh*1. It is therefore not surprising that *Eh*1, being most unique of the three, is the required component for the heterotrimer species.

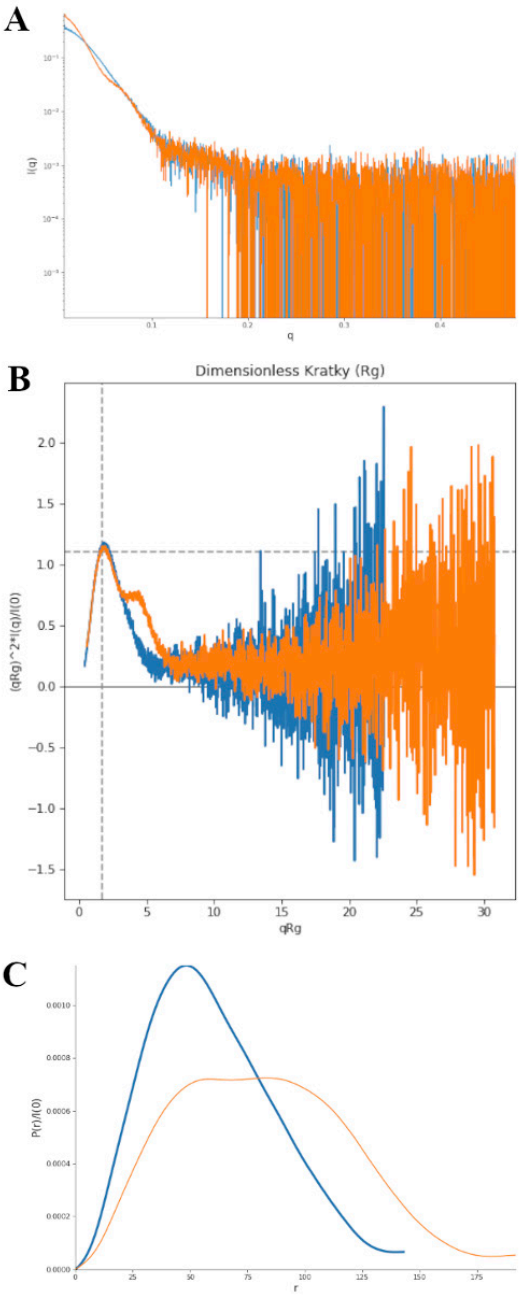

Figure 3. *Eh*1 in isolation forms a dimeric structure, while *Eh*1+2 forms a trimeric structure. *Eh*1 SAXS curve shows the characteristic dimeric scattering curve whereas *Eh*1+2 shows different scatter of the proposed trimeric state. A) SAXS scattering curve of Eh1 in isolation (blue) and with *Eh*1+2 (orange). B) Normalized Kratky plot. C) Normalized P(r).

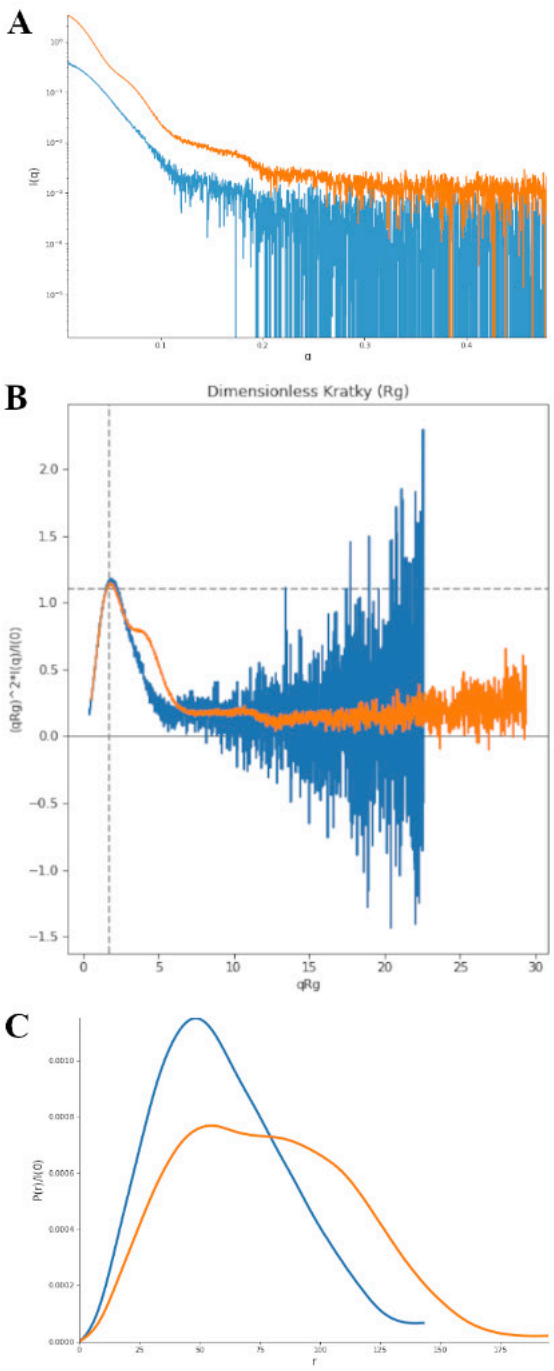

Figure 4. Eh1 in isolation forms a dimeric structure, while Eh1+2+3 forms a trimeric structure. *Eh*1 SAXS curve shows the characteristic dimeric scattering curve whereas *Eh*1+2+3 shows a differing scatter indicative of the trimeric state A) Scattering Curve of *Eh*1 (blue) and *Eh*1+2+3 (orange). B) Normalized Kratky plot. C) Normalized P(r).

Effector molecules induced changes in structure

When substrates or effector molecules bind to their partners, they can often induce structural changes. *Pf*PEPCK for instance, formed a homodimer upon binding of PEP, OAA, and malate (unpublished). Similar experiments were completed here to determine the effect of substrates and malate on *Eh*PEPCK structure. It was found that the effect of OAA and PEP were identical. First, when PEP is present with *Eh*2, *Eh*3 or both *Eh*2+3, an increase in both D_{max} (160, 165, and 150 Å) and R_g (53.5, 53, and 56.2 Å) is observed (Table 2). This suggests that the addition of PEP causes a larger structure to form. However, it is unclear what this structure is. The aforementioned values are greater than the known dimer dimensions, but less than a trimer's, so it could perhaps be an expanded dimer. Second, the addition of 10 mM PEP to *Eh*1, as well as *Eh*1+2 and *Eh*1+3 caused aggregation (the accumulation and/or clumping together of structures) such that the R_g and D_{max} values could not reliably be determined. This suggests that substrates specifically interact with *Eh*1 causes it to destabilize and aggregate, whereas *Eh*2 and *Eh*3 are unaffected. Finally, with *Eh*1+2+3 and 10 mM PEP (only) there is a substantial change compared to the unbound trimer (Fig. 5). This conformational change is evident by all diagnostic plots, and the combination with substrate almost doubles the R_g from 61.4 to 120.6 Å and increases the D_{max} considerably from 195 to 480Å, which points to the probable formation of a much larger structure, whose specifics are unknown but may be a trimer-of-trimers.

The addition of 40 mM malate does not seem to have much effect on the enzyme. In *Eh*1 on, *Eh*2, and *Eh*3, no change was seen in the D_{max} values, which stayed at approximately 140 Å each, which is in contrast to the addition of OAA and PEP, which caused aggregation in *Eh*1. However, an increase in the R_g value of *Eh*1 was seen from 47.1 to 53.3 Å, while *Eh*2 and *Eh*3 saw little to no change. Malate caused a slight increase in size in *Eh*1+2, *Eh*1+3, and *Eh*1+2+3, as the D_{max} values increased from 192, 192, and 195 Å respectively, to 255, 250, and 250 Å. The R_g values for *Eh*1+2, *Eh*1+3, and *Eh*1+2+3 stayed approximately the same, as they changed from 66.1,62.4, and 61.4 Å to 65.6, 68.6. and 72.1 Å respectively.

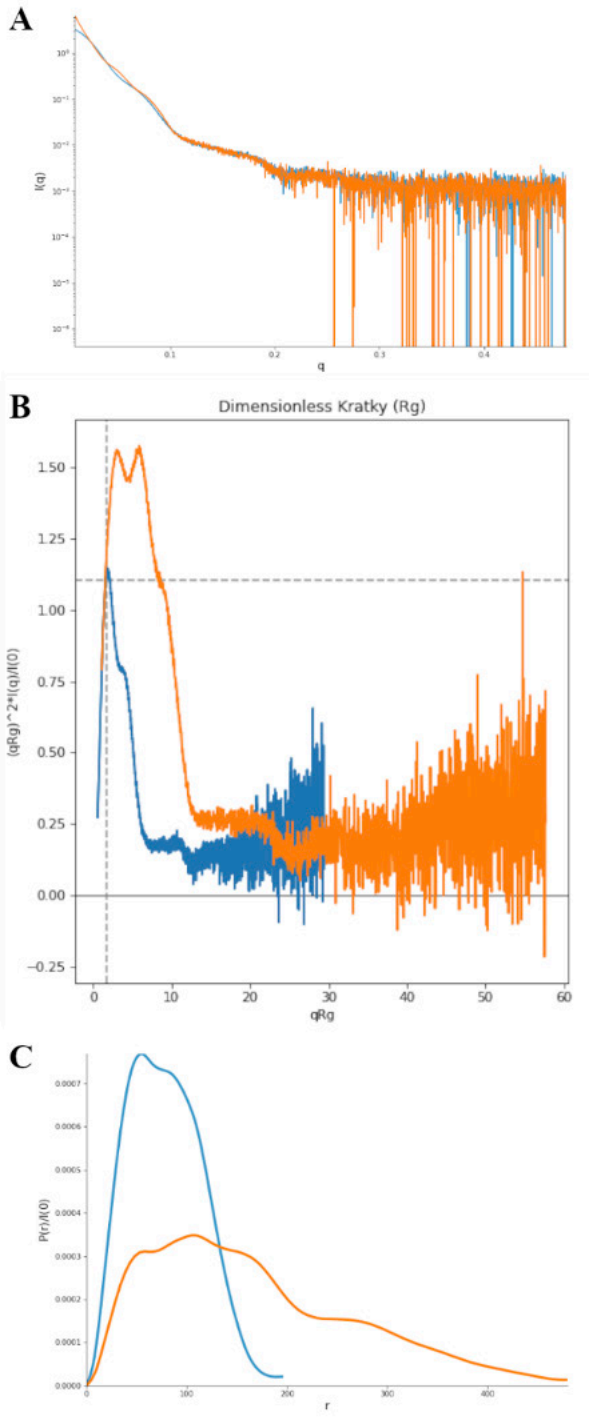

Figure 5. *Eh*1+2+3 forms a proposed trimeric structure, while *Eh*1+2+3 with 10 mM PEP forms an unknown larger structure. A) Scattering Curve *Eh*1+2+3 in isolation (blue) and with 10 mM PEP substrate (orange). B) Normalized Kratky plot. C) Normalized P(r).

Metal Dependency

The SAXS data has revealed the structures of these paralogs both in isolation, and in combination with one another and with the addition of substrate and ligand. However, the activity of these structures is unknown. In order to understand the functionality of each structure, kinetic activity experiments were performed. Before these experiments could be carried out, the optimal conditions for activity had to be determined. Therefore, different metals were tested to see which one functioned as the most activating M1 cofactor. The first experiment done testing metal-dependency to determine if Fe^{2+} is the most activating cation for *Eh*PEPCK as it was in *Pf*PEPCK. Various metals were tested: iron (Fe^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), calcium (Ca^{2+}), and cobalt (Co^{2+}). It was found after testing was complete that manganese was the best M1 Metal cofactor (Table 3).

Activity of *Eh*PEPCK Paralogs

With the optimal metal determined, Michaelis-Menten plots while varying PEP were obtained. *Eh*1 was tested first, and there was an increase in activity at small concentrations of PEP, with the activity gradually decreasing as substrate concentration increases, showing classical substrate inhibition (Fig. 6). The SAXS analysis suggests that *Eh*1 is a dimer in isolation with no evidence of trimer formation but with the addition of PEP, *Eh*1 aggregates suggesting this may be the structural cause of the strong substrate inhibition. Next, *Eh*1+2 and *Eh*1+3 start as a trimer and have stronger substrate inhibition than *Eh*1. Like *Eh*1, aggregation was shown by SAXS and is also likely the origin of the substrate inhibition. Looking at the kinetic constants collected *Eh*1 and *Eh*1+2/1+3 have approximately the same Michaelis-Menten constant (K_M), however, the maximal specific activity of *Eh*1 is over three times greater than that of *Eh*1+2/1+3 (Table 4). While *Eh*1 undergoes substrate inhibition, the determined K_i values suggest that the inhibition is stronger for *Eh*1+2/1+3. Thus, *Eh*1 started as a dimer with higher activity than the trimer, and while both dimer and trimers experienced aggregation with PEP, the trimers appear to be more sensitive.

Table 3. Metal dependency of *Eh*1 PEPCK.

	Fe^{2+}	Mn^{2+}	Mg^{2+}	Co2+	Ca^{2+}
Average rate (µmol/min/mg)	1.59 ±0.10	2.82 ±0.28	2.44 ±0.25	0.050 ±0.03	1.97 ±0.25

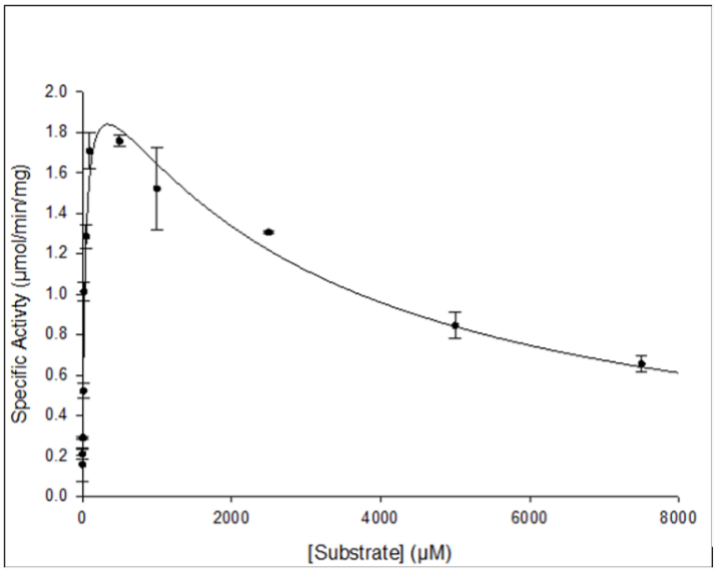

Figure 6. Substrate Inhibition of paralog *Eh*1. *Eh*1's activity was measured with PEP concentration of 2 – 10,000 mM PEP. It undergoes substrate inhibition - Its activity decreases as it undergoes oligomerization.

Table 4. *Eh*PEPCKs and Respective V_{max}, K_m, and K_i values.

*Eh*PEPCK(s)	1	1+2	1+3
V_{max} (µmol/min/mg)	2.3 ± 0.11	0.9 ± 0.14	0.70 ± 0.12
K_m (µM)	39.6 ± 4.8	26.9 ± 8.91	39.5 ± 13.3
K_i (µM)	2960 ± 470	511 ± 166	476 ± 154

Conclusion and Future Directions

Structural analysis showed the oligomerization states of each paralog in isolation and in combination where *Eh*1, *Eh*2 and *Eh*3 are dimers, while *Eh*1+2, *Eh*1+3, and *Eh*1+2+3 exist as heterotrimers. With the addition of substrate, aggregation in *Eh*1, *Eh*1+2, and *Eh*1+3 was observed but not seen in the dimeric complexes and with the trimeric *Eh*1+2+3. Instead, *Eh*1+2+3 formed large structure with over twice the R_g value and 2.5x increase in D_{max} value. Malate did not affect *Eh*2, *Eh*3, or *Eh*2+3. It caused a D_{max} value increase in *Eh*1 and an overall increase in *Eh*1+2, *Eh*1+3, and *Eh*1+2+3, which suggests that it interacts especially with *Eh*1 and its combinations, causing a different, slightly larger trimer to form. These observations are different from what was observed previously in *Pf*PEPCK, which underwent dimerization from the addition of malate, OAA, and PEP, with PEP inducing the least change. SAXS is a useful method, but is low resolution, and prevents the observations of monomers as the high concentrations required force *Eh*PEPCK to dimeric (or trimeric) states. Therefore, the monomer-dimer/trimer transition may also be affected by substrates (or malate). In order to further understand conformational changes, it would be useful to repeat these experiments with crystallography, to observe the exact structures under different conditions.

Kinetically, it was discovered that Mn^{+2} was the most activating M1 cofactor, which is similar to the nucleotide-dependent classes, but

different from *Pf*PEPCK (Fe^{2+}). Of the paralogs tested, *Eh*1 had the highest activity out of *Eh*1, *Eh*1+2, and *Eh*1+3, with over twice the V_{max} of the other two. All three showed activity at low concentrations of PEP but *Eh*1+2/1+3 are most sensitive. From SAXS, this suggests that aggregation is causing substrate inhibition. In order to further understand these paralogs activity, it should be considered to test the kinetic activity of either *Eh*2 or *Eh*3 in isolation. These are both dimers, but do not experience aggregation, and should not suffer substrate inhibition.

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Comparison of Face Masks by Arduino Sensors

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Abstract

The COVID-19 pandemic in early 2020 has created a health crisis around the world. There have been many precautions taken against the virus, such as social distancing and the use of personal protective equipment. Face masks are an essential part of personal protective equipment, especially for essential workers. Although the pandemic has caused a high demand for facemasks, there is limited scientific knowledge available on the various types of masks. Knowledge about the air quality inside the different facemasks could be beneficial for both the COVID-19 pandemic and future disease outbreaks, which will seemingly increase in the future. There are different types of masks, ranging from tight-fitting N95 respirator masks to looser surgical masks to homemade cloth masks. Herein, N95 respirator masks, surgical masks, cloth face masks, and surgical masks on top of N95 respirator masks were compared in terms of CO_2 levels, Volatile Organic Compound levels (VOCs), temperature, humidity, and O_2 concentrations. To record these measurements, Arduino-based sensors were constructed and utilized for the measurements. It was found that the N95 mask and the surgical mask on top of the N95 mask trapped the highest concentration of carbon dioxide, VOCs, temperature, humidity, and the lowest oxygen levels compared to the surgical mask and cloth mask.

Introduction

As a result of the COVID-19 outbreak in early 2020, many individuals have begun to wear many different types of protective face attire. Previously, face masks were mostly used by healthcare workers who cared for patients with respiratory infections or to prevent the spread of infection during surgery. Because of the pandemic, the CDC recommends that "people wear cloth face coverings in public settings and when around people who don't live in your household, especially when other social distancing measures are difficult to maintain." Some examples of protective face wear include N95 respirator masks, surgical masks, and cloth face masks. Healthcare workers and other medical first responders wear surgical masks on top of N95 respirator masks to protect themselves against the coronavirus.

While surgical masks do not seal the area around the mouth and nose, N95 respirator masks protect against viruses and bacteria in addition to sealing the area around the mouth and nose (Smith et al., 2016). Surgical and N95 respirator masks use non-woven fabrics made from plastics such as polypropylene, polycarbonate or polyethylene (Chua et al., 2020). Besides these materials, N95 respirator masks contain several metal parts. The mask uses steel for the staples to secure the straps to the mask and aluminum for the bendable nose clip.

While these aforementioned types of face wear may protect an individual against outside bacteria and disease, essential workers need to wear the face masks for long hours, and this may induce physiological stress on them. It was reported that healthcare workers develop de novo headaches or exacerbation of their pre-existing headache disorders because of prolonged use of N95 respirator masks (Ong et al., 2020; Lim et al., 2006). Wearing an N95 respirator mask triggers different heart rate and discomfort among healthcare providers (Zhu

et al., 2014). Usage of an N95 face mask affects the inhaled gas concentrations as a higher concentration of CO_2 becomes trapped in the masks, thus lowering the O_2 levels available for respiration (Tong et al., 2015). Additionally, higher temperatures and humidity in facemasks can alter heart rate and trigger subjective perception of discomfort (Li et al., 2005).

CO_2 is a colorless, odorless, and tasteless gas that makes up 0.04% of the total gas composition in dry air. While the average outdoor CO_2 concentrations are approximately 400 ppm, the typical average indoor concentration can reach up to several thousand ppm (Satish et al., 2012). As humans breathe, each cell takes in oxygen to complete cellular respiration and generates CO_2, which is removed from the body during exhalation. Exhaled breath is usually made up of around 40,000 ppm CO_2 (Wood et al., 2014). As CO_2 is exhaled inside a mask, the amount of O_2 inside the mask decreases. The Occupational Safety and Health Administration, OSHA, determined the optimal range of oxygen in the air, for humans, to be between 19.5% and 23.5% (Spelce et al., 2016). When O_2 concentrations drop from 19.5% to 16%, the cells fail to receive the oxygen necessary to function properly. The increase in amounts of CO_2 and the subsequent decrease in oxygen levels within a face mask can cause an individual's mental abilities to become impaired in addition to symptoms including dizziness, confusion, fatigue, vertigo, headaches, and tinnitus (Ong et al., 2020). The accumulation of CO_2 in the body initiates respiratory acidosis which also causes headache, confusion, anxiety, and drowsiness (Azuma et al., 2018).

Besides CO_2, Volatile Organic Compounds (VOCs) are also present in human breath. Humans emit different VOCs, such as hydrocarbons, alcohols, ketones, and aldehydes. In most healthy individuals, the most prominent VOCs emitted are isoprene, acetone, ethanol, and other alcohols (Fenske et al., 1999). VOCs accumulate in the face masks and may cause headache, dizziness, and confusion. An increase in temperature and humidity inside the different types of face masks can lead to difficulty in breathing. Shortage of oxygen stimulates the sympathetic nervous system and increases heart rate which results in fatigue, headache, and reduced mental performance in individuals (Li et al., 2005).

There is a great interest in open-source microcontroller development boards such as Arduino for sensing, data acquisition, and educational purposes (Grinias et al., 2016). Arduino is a company that builds low-cost, low-power prototyping boards and supports them through a website. The sensors are easily connected to the platform turned to air quality acquisition systems since it is used as an interface between the sensors and the SD card where data is logged in or between the sensors and a serial monitor. Since the development board uses an open-source operating system, it is easily customized and optimized for the constructed sensor (Arduino, n.d). Arduino-based sensors are small, low-cost and provide highly accurate, reliable results in various settings.

Nowadays, students in science departments are encouraged to use development boards like open-source Arduino platforms to collect data with sensors. These Arduino-based sensors are very portable, expandable, and customizable for various areas of study. This can be done without specialized software or hardware skills. Additionally, code can be developed through the Arduino-friendly Integrated Development Environment. Thus, Arduino sensors can be utilized to measure various parameters of novel face masks or other new devices that may be implemented by doctors or civilians to protect individuals during future pandemics.

The aim of this research article is to compare the different types of face masks, namely the N95 respirator mask, surgical mask, cloth face mask, and surgical mask on top of the N95 respirator mask in terms of CO_2 levels, VOC concentrations, temperature, humidity, and O_2 concentrations. Arduino microcontroller sensors were assembled and utilized for the

measurements inside the different types of masks. N95 masks would have higher levels of carbon dioxide, VOCs, humidity, temperature, but lower oxygen levels than surgical and cloth masks.

Materials and Methods

The experiment was conducted with a subject placing the sensors into their respective masks and then moving around periodically. The data obtained was sent to a computer program that automatically generated graphs.

The experiment was conducted at 26°C (controlled by a thermostat) and was conducted in the same workspace. The subject had correct placement of the mask at all times (never lowered the mask and never had the mask below the nose). The lightweight sensors were secured inside the mask using tape to ensure no infiltration of outside air. The individual conducting the trials was the same throughout the experiment. The subject was a non-smoker who had no cardiovascular issues. All of the experiments were conducted three times and averaged to ensure accurate results and to minimize error.

Due to their ease in programming and connectivity, SGP30 VOC & CO_2, SHT31-D temperature & humidity modules from Adafruit and O_2 sensor from Grove Company were built and utilized. The VOC & CO_2 module was attached to the Arduino and temperature & humidity sensor have been added to the acquisition system. Throughout the experiments, the sensors were powered from a computer via a USB port. The SD-card module was also added as a backup. The program was written in a free and open Arduino software source. The program enabled the sensors' proper use. The data collected was sent to a computer, via a USB port, every minute and was later transferred to Microsoft Excel.

The SGP30 Multi-Pixel Gas sensor could detect a wide range of VOC and CO_2 concentrations. The sensor had a small microcontroller that read the analog voltage, tracked the baseline calibration, and calculated VOC and CO_2 concentrations. The sensor measured CO_2 concentrations within a range of 400 to 60,000 parts per million (ppm), and VOC concentrations within a range of 0 to 60,000 parts per billion (ppb) (Adafruit, n.d).

Along with the SGP30 VOC & CO_2 sensor, the SHT31-D temperature and humidity sensor was utilized because it provided high accuracy and calibration. The sensor collected the data and converted analog temperature (in °C) and humidity (in % relative humidity) signals to digital signals. The sensor had an excellent ±2% relative humidity and ±0.3°C accuracy. Figure 1 shows a photograph of the SGP30 VOC & CO_2 and humidity & temperature sensors sharing an Arduino-based acquisition system.

A Grove-Gas O_2 Sensor was utilized to measure the oxygen concentration in the facemasks. The sensor generated currents that were proportional to the O_2 concentration in the air (Seeed Studio Wiki, n.d). While it has a humidity range of 0-99%, it also had a temperature range of 20°C to 50°C, which made the sensor suitable for this project. To calibrate the sensor, a free code provided by the Grove-Gas company was implemented. Figure 2 shows the O_2 sensor connected to the Arduino board.

The details of codes, schematics for circuits, and examples of the display can be found in supplementary materials (Lankar, 2021). Graphs of the data were created using Microsoft Excel spreadsheets.

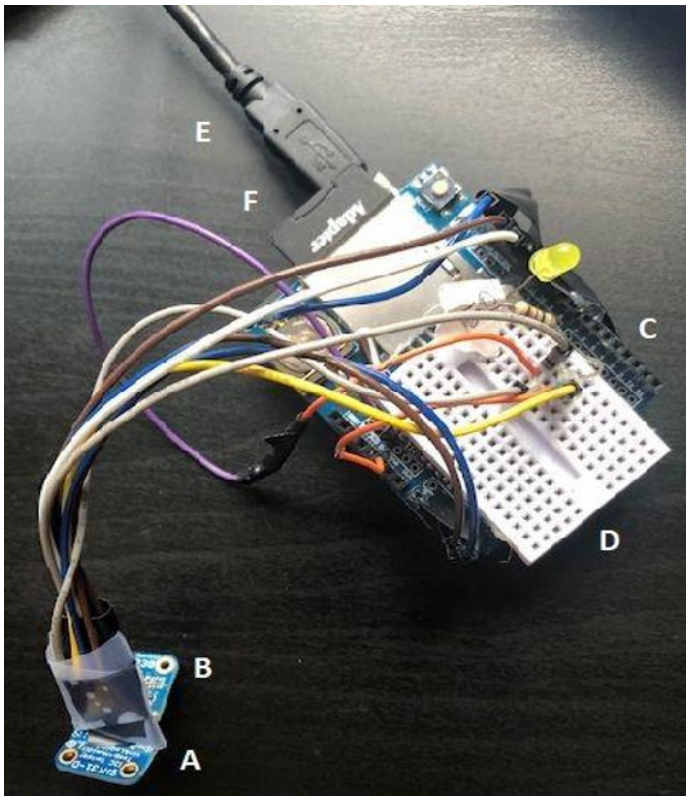

Figure 1: Photo of the VOC & CO_2 and humidity & temperature sensors sharing a development board.
There are six parts in the system: (A) VOC & CO_2 sensor, (B) Humidity & Temperature sensor, (C) Development Board, (D) Data logger shield with SD card and breadboard with signaling LED circuit, (E) USB Port Connection (F) Connection

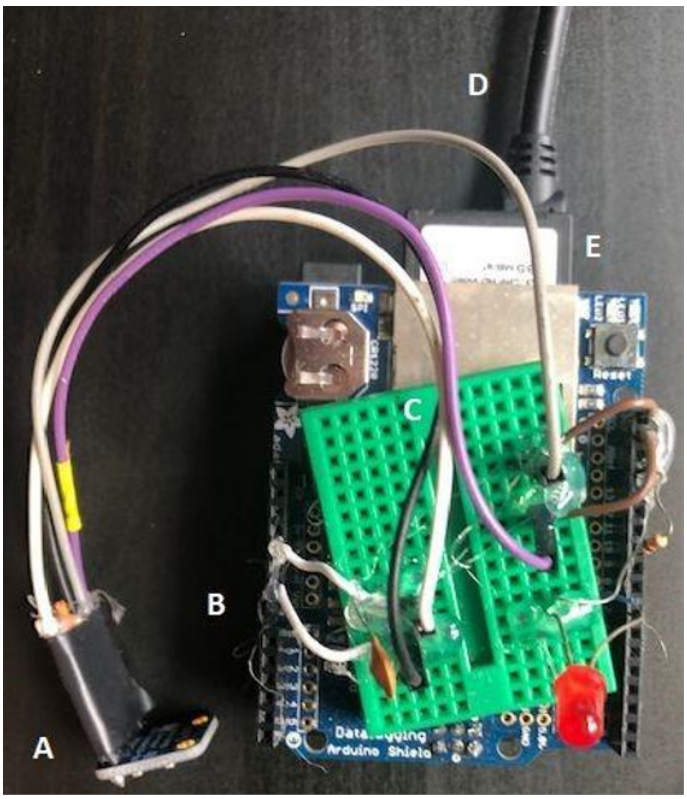

Figure 2: Photo of the Grove-Gas O_2 sensor connected to the Arduino board.
There are five parts in the system: (A) O_2 sensor, (B) Arduino Board, (C) Development Board, (D) Data logger shield with SD card and breadboard with signaling LED circuit, (E) USB Port Connection.

Results and Discussion

Five sensors, measuring VOC, CO_2, O_2, humidity, and temperature levels, were placed inside the four different types of masks to determine the air quality within the masks relative to the air quality outside the mask in the same location. In order to compare the conditions inside the mask with the conditions inside the room, a certain amount of time was allotted for the sensors to take measurements of the room's condition before and after each trial. This can be observed for all the graphs as the flat sections found at the beginning and end display when the masks were not put on and the sensors were measuring the room's CO_2, O_2, humidity, temperature, and VOC concentrations. An example of this can be seen in the Figure 7 for the time intervals ranging from 0-13 minutes and 105-118 minutes. For each parameter in the experiment, since the sensors used in the experiment have a margin of error, the zoxygen concentrations in the N95 mask and the surgical mask on top of the N95 mask's oxygen concentration were not compared to one another. The same was done for the comparison between the cloth and surgical mask.

Figure 3 shows the oxygen concentration (%) over time. The oxygen concentration decreased after all of the different masks were put on. However, on average, the cloth mask had the highest oxygen concentration while the N95 mask with surgical mask had the lowest oxygen concentrations. For the cloth mask, the percent difference between the O_2 concentration inside the mask and outside the mask was around 2.5%. For the N95 with surgical mask, the percent difference between the O_2 concentration inside the mask and outside the mask was around 4%. In all the graphs, there are peaks and troughs that are created by a change in the mask wearer's activity. These peaks and troughs occur at around the same time for each of the four masks for each parameter.

As seen from Figure 4, the highest CO_2 concentrations observed within the masks were observed with the surgical mask on top of the N95 mask, while the lowest accumulated CO_2 concentrations were measured with the cloth mask. For the surgical mask on top of the N95 mask, the percent difference between the CO_2 concentration inside the mask and outside the mask was around 3500%. For the cloth mask, the percent difference between the CO_2 concentration inside the mask and outside the mask was around 1000%.

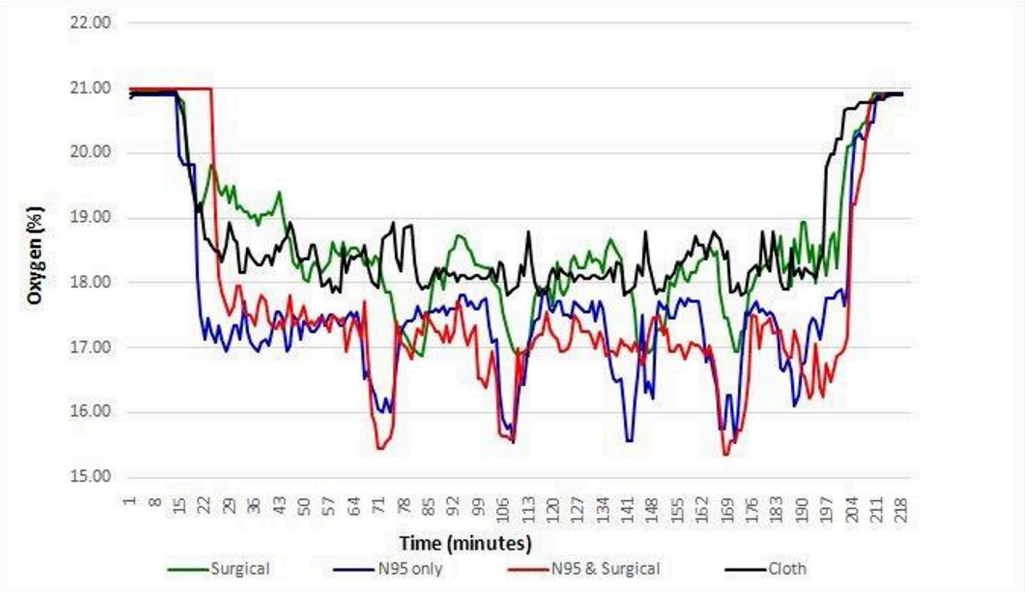

Figure 3: Oxygen (O_2) concentration (%) for different types of masks over time. *The data shown for each mask are the average of the results of 3 different trials. During the experiment, the subject periodically performed physical activity, which led to the O_2 dips at times 71, 106, 141, and 169 minutes.*

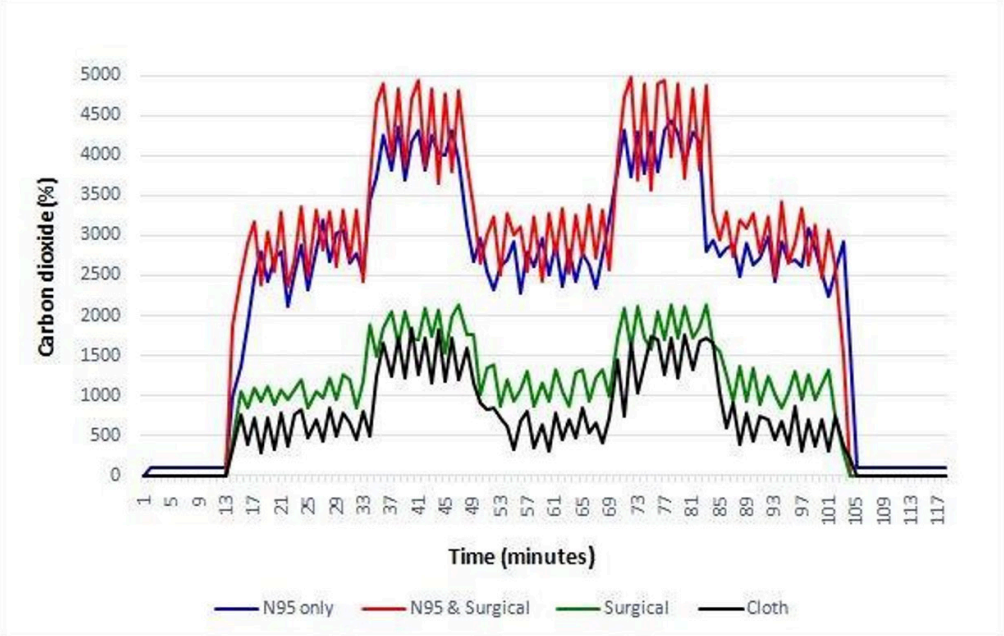

Figure 4: Carbon dioxide (CO_2) concentration (%) for different types of masks over time. *The data shown for each mask are the average of the results of 3 different trials. During the experiment, the subject periodically performed physical activity, which led to the CO_2 increases at times 37-49 and 73-85 minutes.*

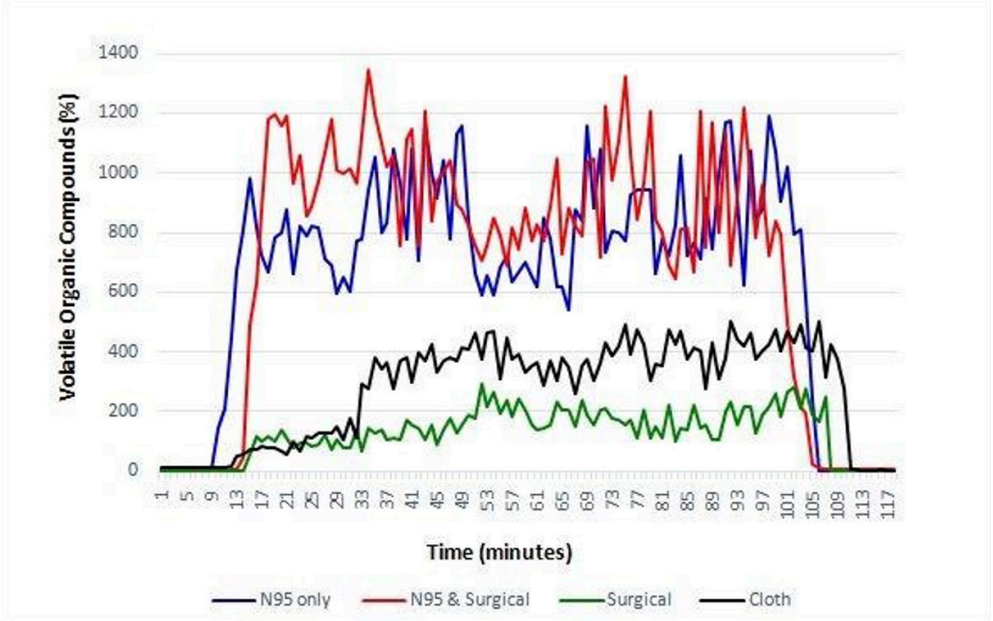

Figure 5: Volatile Organic Compounds (VOC) concentration (%) for different types of masks over time. *The data shown for each mask are the average of the results of 3 different trials.*

Figure 5 shows that the VOC concentration was greatest for the surgical mask on top of the N95 mask, while the VOC concentration was lowest for the surgical mask. For the surgical mask on top of the N95 mask, the percent difference between the VOC concentration inside the mask and outside the mask was around 1000%. For the surgical mask, the percent difference between the VOC concentration inside the mask and outside the mask was around 200%.

As seen from Figure 6, the temperature inside the various masks increased the most for the surgical mask on top of the N95 mask. While the temperature inside the surgical mask and the cloth mask also increased, the temperature increase inside these two masks was less drastic. For the surgical mask on top of the N95 mask, the difference between the temperature inside the mask and outside the mask was around 10.5°C. For the cloth mask, the difference

between the temperature inside the mask and outside the mask was around 7°C.

Figure 7 indicates that the humidity inside the various masks increased the most for the surgical mask on top of the N95 mask and the N95 mask. While the humidity levels inside the surgical mask and the cloth mask also increased, the increase in the humidity levels inside these two masks was less drastic. For the surgical mask on top of the N95 mask, the percent difference between the humidity concentration inside the mask and outside the mask was around 45%. For the cloth mask, the percent difference between the humidity concentration inside the mask and outside the mask was around 35%.

Figure 8 shows the pictures of the different face masks. While surgical and cloth masks are worn by the public, N95 respirator masks and surgical masks on top of the N95 respirator masks are worn by essential workers including healthcare professionals.

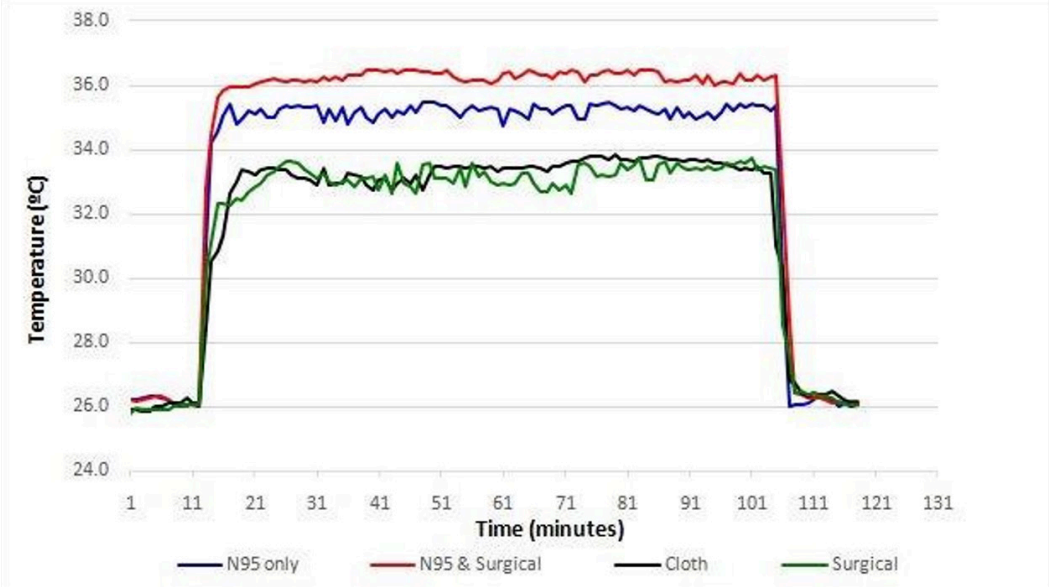

Figure 6: Temperature (°C) for different types of masks over time. *The data shown for each mask are the average of the results of 3 different trials.*

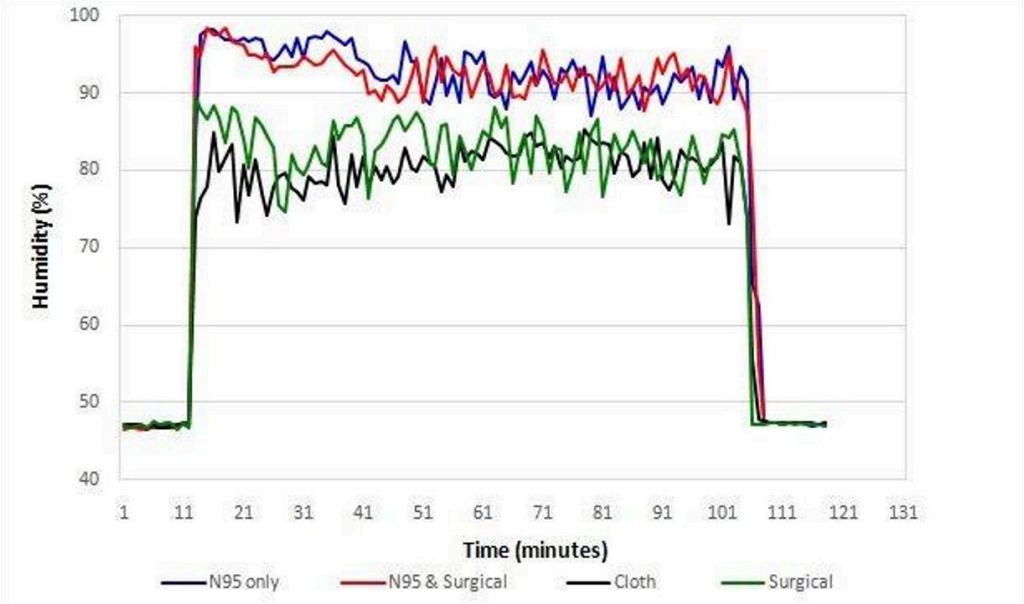

Figure 7: Humidity (%) for different types of masks over time. *The data shown for each mask are the average of the results of 3 different trials.*

Figure 8: Picture of face masks used. *Surgical mask (top left), N95 mask (top right), Cloth mask (bottom left), Surgical mask on top of N95 mask (bottom right).*

Figure 3 shows that the O_2 concentration decreased after all the different masks were put on. However, on average, the surgical mask on top of the N95 mask and the N95 mask alone had the lowest O_2 concentrations because the tighter seal of these masks probably decreased air flow, thus causing O_2 levels to decrease further. In contrast, because the surgical mask and the cloth mask allowed for more air flow, the cloth mask and the surgical mask had the highest O_2 concentration on average. As previously mentioned, OSHA determined the optimal range of oxygen in the air for humans to be between 19.5% and 23.5% (Spelce, 2016). When O_2 concentrations drop from 19.5% to 16%, the cells fail to receive the oxygen necessary to function properly. In Figure 3, the oxygen concentration fell to 15.5% at times for the N95 and the surgical mask on top of the N95 mask. However, for the surgical mask the O_2 concentration at most fell to 16.9%. For the cloth mask, the O_2 concentration at most fell to 17.9%. To mimic real-life situations, measurements were also taken while walking and talking. The dips in all the respective graphs display the moments in which the individual was engaging in these physical activities.

As observed in Figure 4, as the O_2 concentration decreased, the CO_2 concentration increased. This is because CO_2 and O_2 have an inverse relationship as oxygen is breathed in while carbon dioxide is exhaled. Thus, as the CO_2 amount increases, the O_2 amount will decrease. As expected, the highest CO_2 concentrations were observed with the surgical mask on top of the N95 facemask and the N95 facemask alone, while the lowest accumulated CO_2 concentrations were measured with the surgical mask and the cloth mask. The peaks observed in the data display moments in which the individual was engaging in physical activity such as walking and talking.

Figure 5 suggests that the VOC concentration was greatest for the surgical mask on top of the N95 mask and the N95 mask, while the VOC concentration was lowest for the surgical mask and the cloth mask. As aforementioned, since the surgical mask on top of the N95 mask and

the N95 mask had the least air flow, the VOCs generated from the individual's breath were unable to escape to the outside of the mask and consequently accumulated over time. The peaks observed in the data display moments in which the individual was engaging in physical activity.

As seen in Figure 6, the temperature inside the various masks increased the most for the surgical mask on top of the N95 mask and the N95 mask. Since these masks offer less air flow than the surgical mask and the cloth mask, the air inside the surgical mask on top of the N95 mask and the N95 mask was unable to be replenished. While the mean human body temperature is 37°C, the mean human breath is between 31.4°C and 34.8°C. As the breath accumulated inside the mask, so did the temperature of the exhale. Thus, the temperature increased greatly until a certain equilibrium was attained. While the temperature inside the surgical mask and the cloth mask increased, it was less drastic than the N95 respirator mask and the surgical mask on top of the N95 respirator mask due to more airflow.

As displayed in Figure 7, the humidity inside the various masks increased the most for the surgical mask on top of the N95 mask and the N95 mask. Since these masks offer less air flow than the surgical mask and the cloth mask, the air inside the surgical mask on top of the N95 mask and the N95 mask was unable to be replenished. As a result, the humidity increased since water vapor is present in human breath. Humidity levels increased because there is water vapor present in human breath. Similar to the VOCs, if there's inadequate air flow, the humidity levels will increase substantially as the water vapor would be unable to escape to the outside of the mask. The humidity levels inside the surgical mask and the cloth mask did not increase as drastically as the humidity levels for the other types of face masks, as they allow for added airflow. If properly fitted, the temperature and humidity inside the face mask reflect those of a human breath.

It was found that the N95 mask and the surgical mask on top of the N95 mask had the highest CO_2, VOC, temperature, humidity, and the lowest O_2 levels compared to the surgical mask and the cloth mask. This information is not only relevant to the COVID-19 pandemic but can also be applied in the case of future pandemics to prevent disease dissemination. As a result of this experiment, it can be extrapolated that face masks that can interfere with an individual's breathing, such as N95 masks and surgical masks on top of N95 masks, may not be favored in intense physical activity. However, due to their strong protection from viruses, the aforementioned masks may be favored in more sedentary situations.

The entire cost of each sensor was around $40, which is significantly more affordable than commercial sensors. While the sensors used in the experiment were portable and easy-to-use, they provided a good level of accuracy and reliability. The project can be an excellent example of interdisciplinary research for undergraduate students. While computer science and/or physics students could build the sensors and write codes, biology, chemistry, and environmental science students could interpret the data and use the sensors for various applications. Additionally, Arduino-based sensors can be used to measure various health aspects of new face masks or other devices that may be implemented to protect individuals during future pandemics.

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Authors' Biographies

Siddhi Balamurali is a member of Cornell's Class of 2023. I double majored in Biometry/Statistics and Biological Sciences with a concentration in computational biology in CALS. I am originally from Goshen, New York and attended RPI before transferring to Cornell. I currently work at the Center for Computational and Integrative Biology at Massachusetts General Hospital. My research interests include using computational and statistical models in cancer and disease research. In my free time, I love to play the clarinet, paint, draw and hike.

Berk Balkir is a junior pursuing a Bachelor of Arts in Biochemistry and Economics in the College of Arts and Sciences. I am from New York City and am interested in genetics and biochemistry research. I have conducted research in various fields, such as biochemistry, genetics, and chemistry. I hope to attend medical school after graduation.

Iliana Goodhew is from California and received a B.S. in Biological Sciences with Distinction in Research and a minor in Environment & Sustainability from Cornell. She is currently taking a gap year and will be attending grad school next fall. Her main research interests are in biology and ecology, and she has done a lot of research in the past on the ecology of land and marine invertebrate species and also in soil microbiology and the Hawaiian mycobiota. She is currently interested in studying how different organisms can provide us with services that may help us slow down climate change and harness those powers to make the impact greater. During her Cornell experience, Iliana was part of Buckley's Soil Microbiology Lab, competed internships at the Shoals Marine Laboratory and with NSURP, and did an honors thesis. Furthermore, she studied abroad two semesters in Ecuador (including the Galapagos, the Amazon, and the Andes Mountains) and New Zealand taking hands on ecology classes and conducting research. For fun she is involved in a few extracurriculars such as dancing and performing, figure skating, and archery.

Esha Shakthy is a junior from Cary, NC, majoring in Nutritional Sciences in the College of Human Ecology. She works with the Mehta Research Group, examining the intersection of gut microbiome, nutrition, genetics, metabolism, and human health. She also assists at the Lammerding lab, investigating lamin mutations. Esha is a NY-EMT and she serves the Cornell community working with Cornell EMS. She also writes for Cornell Healthcare Review, and is involved with Kappa Omicron Nu Honor Society and Rho Psi Eta. Esha loves dance and music, and is a part of Teszia Belly Dance Troupe, Spicmacay, and BigRedThon.

Kassidy Slaughter is a senior majoring in Industrial and Labor Relations (ILR) and minoring in Information Science: and Law and Society. She is currently conducting research with the Climate Jobs Institute at the ILR School, where she is examining global approaches to the transition to clean energy and how labor rights can be maintained in this transition. She has previously worked with Chris Csíkszentmihályi's research cohort, where she interviewed individuals impacted by the coal industry in Appalachia and explored how clean energy can center workers' livelihoods. She is passionate about discovering how data can be used to support the interests of communities, and she is a teaching assistant for the Business Intelligence Systems course. Kassidy additionally enjoys magazine making, especially focused on Disability rights issues. On campus, she is a member of the Salsa Pa'lante Dance Troupe.

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