



# JOURney

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# Letter From the Editor

Dear Reader,

We are excited to present to you the fourth annual volume of JOURney, the University of North Carolina at Chapel Hill's first interdisciplinary Journal of Undergraduate Research!

JOURney started as an idea of founder Gabi Stein, to create a journal that would showcase the outstanding, diverse range of student research done by UNC-Chapel Hill undergraduates. With the support of the Office of Undergraduate Research, JOURney became an official publication, providing students a place to publish SURF projects, partial and full honors theses, and independent research.

This year, JOURney has continued to expand, despite unprecedented challenges with the unseen global developments of spring 2020. We have gained many successes along the way as we built teamwork and resilience. With all the excellent work submitted, we are able to exhibit 6 original pieces of work here that explore interesting, topical, and complex areas of research. We congratulate the student authors on all of their efforts put into researching and publishing their work!

We are so thankful of everyone who has helped JOURney continue to meet its mission of celebrating and supporting the research conducted early in students' academic careers. We would like to thank the Office for Undergraduate Research, which has provided JOURney unwavering support since its inception. Specifically, we would like to thank Associate Dean and Director Troy Blackburn (Department of Exercise and Sports Science), Associate Director Monica Richard, Associate Director Boots Quimby, and Business Services Coordinator Denise Carter. We would also like to thank our entire editorial board and publicity team, who have spent many dedicated hours making this edition. As the current Co-Editor-In-Chiefs, we have enjoyed helping JOURney expand as an organization. We look forward to continue watching it grow in the future.

We leave you here to explore the work presented in this journal. We hope you will enjoy the knowledge collectively presented to you by students of UNC-Chapel Hill.

Sincerely,

Mili Dave

Harrison Jacobs

2019-2020 JOURney Co-Editor-in-Chiefs

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# Social Sciences

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# Life, Liberty and the Pursuit of Happiness When Evicted:

## *Lessons from San Francisco's Implementation of Tenant Right to Counsel*

by Jona Bacari

High rates of urban poverty and homelessness are among the most pressing issues facing cities in the United States. Deeply connected is also the phenomenon of eviction, which has also been rising along with the market price for a rental unit in these cities. Due to stagnating income and soaring housing costs, the communities most vulnerable to eviction are low-income communities of color, as well as folks with disabilities, mental health issues and domestic violence survivors. Evictions result in significant material hardship and deteriorating health, quality of life, income and lessened prospects for rehousing due to its reporting on credit scores. The ability to fight back an eviction in court is inherently an issue of access to legal aid as numerous statistics have shown that most landlords are represented by attorneys while an overwhelming majority of tenants represent themselves. Recognizing this uneven standing between tenants and landlords in the court of law and the importance of having legal representation, San Francisco voters approved a measure in June 2018 that gave all tenants with an eviction notice the right to a city-funded full-scope representation attorney. Through numerous interviews with legal service providers, housing attorneys, and tenants' rights activists that have been engaged in the efforts to enact and fully implement the Right to Counsel for eviction cases, this paper will argue that this measure, although necessary, is only a first response to a growing crisis in evictions and homelessness, and that more concerted efforts are needed to ensure full implementation.

### **Introduction**

Eviction is one of the direct, yet frequently overlooked, causes of homelessness and poverty. It is an issue that involves big cities like New York and San Francisco and smaller, but increasingly more urban, cities like Greensboro, and Durham in North Carolina. Greensboro, in particular, currently ranks seventh in the country for its high eviction rate, according to Princeton University's Eviction Lab, the first nationwide database of evictions.<sup>1</sup> Many reasons explain the rise in eviction rates, particularly in the last decade. Family income has stagnated or fallen while housing costs have soared. According to Harvard University's Joint Center for Housing Studies, nearly a third of American households were cost-burdened in 2017, meaning that families were spending over 30% of their total income in housing.<sup>3</sup> Sociologist Matthew Desmond in his groundbreaking study, *Evicted: Poverty and Profit in the American City*, has found that between 1991 and 2013, the number of renter households dedicating 70% or more of their income toward housing grew from 901,000 to 1.3 million. If the data includes households with cost burdens higher than 100 percent of family income, the estimates grow to 2.4 million in 1991, and 4.7 million in 2013.<sup>3</sup> These numbers shed light on the magnitude of eviction, although they fail to capture the harsh reality that ensues after a household is evicted. Losing a home sends families to overcrowded shelters, and often, the streets. An eviction, and the ensuing homelessness often triggers depression and other illnesses.



To borrow Matthew Desmond's words, eviction "compels families to move into degrading housing in dangerous neighborhoods, uproots communities, and harms children. Eviction reveals people's vulnerability and desperation, as well as their ingenuity and guts."<sup>4</sup>

Despite the prevalence of evictions in the United States, there has been little effort on the part of the federal and state governments to tackle this issue. Recognizing that eviction stands at the heart of a variety of other social problems, New York City became the first city to launch the Right to Counsel program (RTC) in late 2017.<sup>5</sup> On June 5, 2018, San Francisco became the second city in the United States to guarantee city-funded full-scope representation in housing court to renters at-risk of eviction in a ballot measure titled "No Eviction without Representation Act" that is often referred to as Proposition F.<sup>6</sup> In essence, the passage of Proposition F mandated that the City of San Francisco fund ten nonprofit organizations that provide free legal representation to tenants facing eviction, AIDS Legal Referral Panel (ALRP), Asian Law Caucus, Asian Pacific Islander Legal Outreach, Bay Area Legal Aid, Eviction Defense Collaborative (EDC), Homeless Advocacy Project, Justice & Diversity Center of the Bar Association of San Francisco, Legal Assistance for the Elderly, Open Door Legal, and Tenderloin Housing Clinic. Gloria Chun, an attorney at the Justice and Diversity Center of the Bar Association regarded the passage of Proposition F, as a step toward the "right to civil counsel movement." Even the language utilized by activists for civil legal aid is one that characterizes Proposition F as part of a "civil Gideon," mirroring *Gideon v. Wainwright*, the Supreme Court case whose ruling established the constitutional right to an attorney for indigent defendants in criminal court. This demonstrates, thus, the inherently progressive nature of a measure like Proposition F that solidifies housing as a human right for all households, not as a privilege reserved for the economically advantaged.

While a measure can be written and passed with the best of intentions, the reality of implementing Proposition F, or the Tenant Right to Counsel has been marked by challenges that have limited its reach in curbing San Francisco's high eviction rates. For this reason, I set out to interview legal service providers and housing advocacy nonprofit organizations that interact with evictions and Proposition F on a daily basis. More specifically, in February 2020, I conducted eleven interviews with Carolyn Gold, Deepa Varma, Fred Sherburn-Zimmer, Claire LaVaute, Tim Iglesias, Bao-Tran Dang, Teresa Friend, J. Scott Weaver, Bill Hirsh and two individuals who wished to remain anonymous out of privacy concerns.<sup>7</sup> The first portion of the interview was focused on the issue of eviction, as it has troubled San Francisco and as they have interacted with it through their work with a particular focus on the difficulties associated with tackling it both on a small, as well as major scale. The second portion of the interview revolved around Proposition F, its history and impact on the work of the organization. Lastly, the interviewees were asked to state their views on Proposition F, what it has achieved, its strength and areas for further development. As such, this report will be structured in two main parts. Part I will contextualize eviction in the broader socioeconomic context of San Francisco and the law, while also providing background information on eviction proceedings in court and the civil legal aid landscape in San Francisco. Part II will focus substantively on Proposition F, the history and mechanics of how city-funded full scope representation is implemented in practice, while identifying some key areas for further development and analyzing their implications. In this report, I will largely argue that the provisions of Proposition F are only a start to the full implementation of tenants' right to counsel and that the City of San Francisco, in light of the magnitude of the city's housing crisis, needs to engage in more concerted efforts to tackle its alarming eviction rates.

### **Brief Methodological Considerations**

While twelve interviews were conducted, not all interviews were of the same length primarily because of the limited availability of the interviewees. Some of the interviews were conducted in-person and were recorded, mimicking a proper oral history interview,

while some interviews were conducted over the phone and were subsequently not recorded.<sup>8</sup> As such, direct quotations from the interviews will be limited, whereas other content from the interviews has been paraphrased. In addition, due to client-attorney confidentiality, many of the examples of the cases were provided without any identifying details. Given the highly personal nature of lived experience, the takeaways from these interviews have been informed by and speak directly to the working experiences of those interviewed. They should not be construed as applicable to meanings and contexts outside those in which they were given.

### **Housing Rights, Housing Fights**

An eviction is a process by which a landlord ejects the tenant from the property that is being rented.<sup>9</sup> This involuntary move is motivated by a variety of reasons such as failure to pay rent, breach of leasing contracts, causing a disturbance in the property, as well as other less direct influences such as raising rent for prospective tenants or the owner wanting to move into the property. According to a report prepared by San Francisco Rent Board, a department within the City of San Francisco that promulgates the provisions of the San Francisco Rent Ordinance to approximately 170,000 rental units, there have been 10,111 eviction filings from 2012-2017.<sup>10</sup> Between these years, 2015-2016 saw the largest increase in eviction filings with 2,376 notices received by the Rent Board.<sup>11</sup> While these statistics are significant, they do not convey a city-wide picture because not all rental units fall under the Rent Ordinance. Estimates that consider all eviction filings in housing court from all rental units, from both private and subsidized housing markets are closer to about 3,000.<sup>12</sup> In addition, the figures are only the “tip of the iceberg” as they account only the formal evictions that went through judicial channels. Many more informal evictions, or informal attempts to pressure a tenant into vacating the unit occur frequently. In my interview with Fred Sherburn-Zimmer, Executive Director of the Housing Rights Committee of San Francisco, they explained that tenants will often come in with informal eviction notices, thinking they are about to be formally evicted. “Most tenants are tricked into moving out in San Francisco—so verbal evictions, fake notices, harassment. Or just telling a new immigrant who doesn’t speak English that they are being evicted, or you’re going to call immigration on them. The last one is technically illegal in California now, but they still do it.”<sup>13</sup> While there is certainly a tremendous amount of anecdotal evidence for this phenomenon, its informality renders it difficult to capture in data.

Eviction as a phenomenon in San Francisco has taken a distinct geographical form. A report released by the Eviction Defense Collaborative in 2016 listed their caseload proportionally by supervisor district. The districts that had seen the most evictions filed that year were District 6 (Tenderloin, Treasure Island, and South of Market neighborhoods), District 10 (Bayview/Hunters Point, Potrero Hill, and Visitacion Valley), and District 9 (Mission, Bernal Heights).<sup>14</sup> It goes without saying that these districts are among San Francisco’s poorest, with household incomes averaging between \$54,000 and \$83,839 annually.<sup>15</sup> For additional context, the Department of Housing and Urban Development has estimated that a family of four in the San Francisco metro area making less than \$129,150 annually qualifies as “low income.”<sup>16</sup> The arrival of the “tech boom,” and “techies,” a group connoting software designers, engineers, and programmers in large firms like Google, Facebook, Salesforce and Apple, has significantly contributed to tenants being priced out of their units.<sup>17</sup> Indeed, the Anti-Eviction Mapping project has found that for every year between 2011 and 2018, 69% of no-fault evictions, i.e. evictions triggered through no faulty action of the tenant, occurred within four blocks of known shuttle stops, most of which have been established by private bus lines operated by the big companies in Silicon Valley.<sup>18</sup> Thus, the city’s transformation has come at an increasingly high cost for its local residents as “techies,” with their high salaries have given landlords a clear incentive to seek these kinds of renters at any and all costs.

Another finding from the EDC’s 2016 report sheds light on the interconnectedness between eviction, poverty and race.

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It showed that districts predominantly made up of African American and Latino communities, such as District 10 and District 9, disproportionately reached out to the Eviction Defense Collaborative. For example, tenants from District 10 sought assistance thirteen times more than tenants in District 2, one of the wealthiest districts of the city.<sup>19</sup> As sociologist Matthew Desmond aptly writes, “if incarceration had come to define the lives of men from impoverished black neighborhoods, eviction was shaping the lives of women. Poor black men were locked up. Poor black women were locked out.”<sup>20</sup> This finding is consistent with the majority of existing literature on eviction, including a 2014 report from the United Nations Human Rights Council establishing that forced evictions can both be discriminatory and lead to discrimination as the victims of evictions belong to specific groups, “the poorest communities facing discrimination, the marginalized and those who do not have the clout to change the decisions and designs of the project leading to their displacement.”<sup>21</sup>

Although eviction rates have decreased since 2012, the decrease conveys a false sense of hope, because the fallout of an eviction on the household is difficult to measure, and even more difficult for the family or individual to overcome. Jane was informally evicted in the spring of 2019. She was renting a studio adjacent to the landlord’s residence. Her landlord claimed they needed the unit so that a family member could move in, and proceeded to harass her to move out. She was in her final months of her graduate program and was scheduled to graduate in two weeks. Because no rental unit in the city was both affordable and available at such short notice, she had to move back in with her family in San Mateo, about 30 minutes driving distance away. She recognized that she “was lucky because if my parents didn’t live here, I would have been screwed. I would have had to either go to court or I would have had to stay in my unit, which would have been terrible” as her landlord had been verbally harassing her prior to the eviction.<sup>22</sup> Throughout her recounting the story, Jane highlighted the degree to which this process caused her a tremendous amount of stress. Unfortunately, similar is the reality of mothers who are evicted, who report higher levels of material hardship, parenting stress and who are more likely to suffer from depression. The effects of losing a home can cause tremendous amount of despair and hopelessness, which the AIDS Legal Referral Panel has explicitly recognized in their organization’s services, “taking on landlord/tenant cases, we help prevent hopelessness and homelessness.”<sup>24</sup> When I asked Bill Hirsh, Executive Director of ALRP why the terms were mentioned in one sentence, they responded that the reality for many folks living with HIV is one with fewer and smaller support networks. If they lose their housing, recovery is almost impossible because in addition to housing, they will have also lost access to healthcare and other supportive services that have enabled them to survive thus far.<sup>25</sup>

An eviction notice prompts a series of challenging questions to struggling families such as where to stay, what happens to the household possessions, will the children be able to continue attending the same school, how long will the eviction be on record and so forth. If rent nonpayment caused the eviction, the costs ensuing the removal of the tenant pose additional financial burden to the household who was already struggling to make ends meet. In fact, the fallout in the first year following an eviction may result in additional hardships such as relationship dissolution and moving into poorer, distressed and higher-crime neighborhoods.<sup>26</sup> In addition, a 2016 study by sociologists Matthew Desmond and Carl Gershenson showed that disruptions caused by an eviction and general housing instability contributed more significantly to poorer job performance among low-income workers in Milwaukee.<sup>27</sup> Thus, evictions aggravate and often even cause job insecurity among the working poor. Although less research efforts have been focused on studying evictions compared to other issues in housing justice, existing literature has repeatedly demonstrated the undeniable link between high eviction rates and urban poverty.<sup>28</sup>

While tenants clearly lose in an eviction, landlords often emerge victorious despite some of the short-term costs associated with the removal process.

According to Tim Iglesias, a professor at the University of San Francisco School of Law, one of the hardest concepts for law students in his eviction lawyering class is the extent of profitability in being a landlord. If the property is mortgage-free, the landlord earns revenue with every rent check while incurring marginal expenses on insurance and maintenance. Any expenses as a result of an eviction notice are cloaked by considerable tax breaks that help landlords and owners offset some of these costs.<sup>29</sup> For some landlords, this is a second occupation and their primary occupation salaries cover some of the costs incurred during an eviction, which allows them to pay less in income taxes. If there is a mortgage on the property, the payments are passed down to the tenants, meaning that the landlords are able to obtain equity on the property. Because of rising living costs in most of America's large and mid-sized cities, being a landlord has therefore become a profitable avenue. When studying a trailer park with high tenant turnover rate in Milwaukee, Matthew Desmond noted that the trailer park owner made an annual income of \$447,000 and belonged to the top 1% of income-earners. Most of his tenants belonged to the bottom 10%.<sup>30</sup>

Compared to other cities, San Francisco appears more favorable to tenants because of its strong implementation of rent control, meaning that if the unit is rent-controlled, the rent can only increase with inflation.<sup>31</sup> However, the passage of the Costa-Hawkins Act in 1995, a state law that limits a locality's ability to institute rent control, protects the landlord's right to raise the rent when a tenant moves out.<sup>32</sup> In practice, this has resulted in San Francisco becoming a city with vacancy decontrol.<sup>33</sup> Deepa Varma, Executive Director of the San Francisco Tenants Union, pointed out that in the Bay Area, the number of vacant units is actually greater than the number of homeless individuals, which demonstrates the magnitude of this issue.<sup>34</sup> When combined with rising property values and soaring market rate prices, the landlords have a tremendous incentive to evict long term tenants with stabilized rent. Fred Sherburn-Zimmer noted that the elderly are particularly vulnerable. In addition, it is impossible for individuals whose income stems only from benefits such as Supplemental Security Income (SSI) or disability (SSDI) to afford a rental unit at market rate, when the average rate for single-room occupancy hotels (SROs) is about \$1,500.<sup>35</sup> As such, it is evident that San Francisco's eviction crisis is taking a toll on its most vulnerable communities.

The 2019 Annual Eviction Report published by the San Francisco Rent Board provides a complex matrix of the reasons behind eviction notices. While nonpayment of rent is frequently cited as a primary reason why tenants are evicted, it constituted only 5.3% of total eviction filings with the Rent Board. Instead, the most frequent were notices due to breach of the rental agreement (29.4%), committing a nuisance (19.1%), owner/relative move-in (13.3%), capital improvement work (11.4%), and Ellis Act or withdrawal of unit from the rental market (9.7%).<sup>36</sup> Although these statistics only reflect the properties protected by the SF Rent Ordinance, they convey a landscape of evictions that has been confirmed by the Homeless Advocacy Project and their work with individuals and families that are either homeless or at imminent risk of homelessness. Housing attorney Bao-Tran Dang commented that landlord attorneys are filing less frivolous eviction cases in housing court, but the eviction notices that are filed due to nuisance are much more severe.<sup>37</sup> Nuisance laws limit a tenant's ability to use the property if the use interferes with other property owners and they were initially instituted during the industrial revolution in the mid-eighteenth century to prevent investors from establishing factories in housing neighborhoods.<sup>38</sup> In contemporary practice however, these nuisance laws have resulted in domestic violence survivors and individuals with mental health issues and other disabilities being disproportionately forced out of their homes. During the interview, John shared with me a story of a client who had been evicted because of nuisance, as their landlord had determined that the tenant's son, who had autism, was a nuisance.<sup>39</sup> In addition, long-term housing activist and tenant attorney, J. Scott Weaver, commented on leases and rental agreements becoming more convoluted in restrictions that are difficult to follow for certain tenants.<sup>40</sup> or example, some properties

limit the number of visitors to two at a time, and thus a tenant who has invited their friends may be involuntarily violating their rental agreement.<sup>41</sup> Oftentimes rent-controlled units are more frequently accompanied by rules and restrictions that tenants have to abide by, otherwise they risk being evicted. However, little attention is drawn to how these rules restrict a tenant's ability to enjoy their home and give the landlord significant leverage over their tenants.

Should a landlord decide to file an eviction in court, the tenant is up for a relatively short, but arduous battle. Typically, the tenant will receive a Summons & Complaint for "unlawful detainer" and along with the notice, the court will pass along information to the Eviction Defense Collaborative. The tenant will have to file an official response to the notice within 5 days, otherwise a default judgment is entered. According to Cary Gold, Director of Litigation and Policy at the Eviction Defense Collaborative, about 400 to 600 cases per year are defaults.<sup>42</sup> After a response is filed, the case is scheduled for a Mandatory Settlement Conference (MSC) between 8-13 days from the tenant's answer. The MSC is a chance for landlords and tenants to resolve the issue. If an agreement is not reached in the MSC, then the case is scheduled for a jury trial the following Monday.<sup>43</sup> The timeline of an eviction case thus is particularly labor-intensive for housing attorneys because between the filing of a Summons & Complaint and the jury trial, there are about 21 days to prepare. In addition, clients are sometimes difficult to work with because of the intense stress and trauma they may be going through, which further complicates the work of a housing attorney.<sup>44</sup> For example, Jane pointed out that "if you're evicted, your first thought isn't going to be 'I need an attorney,' it's 'I need shelter.'"<sup>45</sup> It certainly means that tenants are almost always not their best advocates and self-representation predominantly results in the court ruling in favor of the landlord.

It goes without saying that even with representation in jury trial, a judgment in favor of the tenant is not guaranteed. Although tenants could, and still can, use the possibility of a jury trial as a bargaining chip with the landlord, the increasing lack of representation among jury pools might significantly disadvantage the tenant, when people of color are disproportionately evicted. The migration of the "techies" and other professionals earning high wages and paying market rate rent has made it more likely for jury pools to be unsympathetic toward tenants in rent-controlled units. Cary Gold noted that there is almost a sense of libertarianism within their progressive views that may decrease their ability to empathize with a tenant struggling to make rent that is much lower than theirs.<sup>46</sup> Deepa Varma also noted that judges tend to be landlords, and thus are more likely to rule for an eviction.<sup>47</sup> Because the burden of proof in most civil cases is preponderance of evidence, landlord attorneys simply have to argue that it is more likely than not that the tenant engaged in activities warranting an eviction. From a legal perspective, this is an easier evidentiary standard for landlord attorneys to meet, but harder for the defense.<sup>48</sup>

Legal services for tenants facing an eviction in the city of San Francisco are provided by a number of organizations, some of which have been mentioned earlier. The Eviction Defense Collaborative is largely the main agency serving low-income tenants in court. In addition to the EDC, other organizations that provide legal assistance include Bay Area Legal Aid, Asian Law Caucus, AIDS Legal Referral Panel, Asian Pacific Islander Legal Outreach, Legal Assistance to the Elderly, the Tenderloin Housing Clinic and so on. However, most of these organizations have restrictions on client eligibility as often implied in their name, and the decision to take on a client is always dictated by their capacity. Referrals between agencies in this network are frequent, but tenants may have to go through multiple intake meetings and screenings, which can be frustrating and time-consuming. In my interview with Cary Gold, she pointed out that prior to the Tenant Right to Counsel about half of the clients that EDC referred to the Homeless Advocacy Project never even made it to their intake on Tuesday afternoon.<sup>49</sup> This lack of a centralized intake system and organizations operating at near capacity has resulted in many tenants not being able to access the legal help they need to remain in their home.

In large part, this became the major argument for the passage of the Tenant Right to Counsel.

### To the Ballot and Beyond

In 2011, David Chiu, then President of the Board of Supervisors, introduced an ordinance co-sponsored by Supervisors Jane Kim and Scott Wiener with the aim of declaring San Francisco a “right to counsel” city.<sup>50</sup> The ordinance created a year-long pilot program through a \$100,000 grant that would provide free legal services to a limited number of civil cases that dealt with “basic human needs” such as child custody, shelter, sustenance, safety and health. A major motivator for this ordinance was the disproportionate number of self-represented low-income clients in civil cases. More specifically, the press release cited the following statistics:

*Over 4.3 million civil court users in California are self-represented, and the statistics are especially severe in family law court where 80 percent of litigants do not have counsel at the time of disposition. Statewide 90 percent of eviction defendants are self-represented, and in domestic violence and restraining order cases 90 percent are self-represented. San Francisco data reinforces the urgent need for counsel in high-stakes civil cases. In 2009, 95 percent of child support cases were filed by those who were self-represented. Legal services organizations in San Francisco are unable to meet the increased demand in eviction cases against the City’s most vulnerable, low-income families living in public housing units. The number of eviction lawsuits filed this year by the San Francisco Housing Authority has increased by 400 percent from those filed in 2010. Legal services organizations provide more than \$30 million in pro*

*bono civil representation per year, but the need is far greater.*<sup>51</sup> These figures reveal the inherent injustice in a legal system built on principles of rights and liberties, but that at the same time is not accessible by the more marginalized communities.

The City awarded the contract for the pilot program to the Justice & Diversity Center, who was able to hire an additional staff attorney through the grant. This additional staff attorney supervised other pro bono attorneys providing limited and full-scope representation to clients earning less than 200% of the federal poverty level, as well as increased pro bono representation by volunteer attorneys and developed training materials.<sup>52</sup> The John & Terry Levin Center for Public Service and Public Interest at Stanford Law School conducted an analysis of the outcomes of the pilot and found significant evidence for its success. Among the tenants who received full-scope representation, 62.92%

had the case dismissed or were able to remain in their home and 34.83% reached a settlement.<sup>53</sup> Thus, “an estimated 114 tenants receiving full-scope representation were able to avoid homelessness.”<sup>54</sup> A partial explanation for why full-scope representation resulted in such successful outcomes is that the

possibility of going to trial can dramatically improve client outcomes.<sup>55</sup> The report reiterated this observation when analyzing the effect of repeat players in the courtroom. In particular, it noted that “landlord attorneys who are repeat players know that there is a good chance that the tenant will not be represented at trial, and some consequently take a hard line when it comes to the settlement negotiations.” Therefore, landlords’ knowledge that there will be an attorney advocating for the tenant all the way up to jury trial incentivizes them to try to reach an agreement earlier. In addition, even clients who received limited scope representation faced better outcomes, albeit at a lesser rate. Thus, the total number of tenants, both full-scope and limited scope, who were able to avoid homelessness as a

result of the pilot program reached about 609.<sup>56</sup> If the cost of sheltering someone for the night is about \$30, this means that an estimated \$18,270 were saved each night as a result of the pilot program.<sup>57</sup> According to David Chiu, the initial grant of \$100,000 has leveraged over \$2.12 million in pro bono legal services, as it reduced the number of individuals seeking homeless shelters and other support services.<sup>58</sup> Thus, there is strong evidence that increasing access to legal aid is one of the most successful and cost-effective interventions to reducing homelessness.

Given the success of the pilot program, Chiu, other supervisors and housing activists pushed for

increased city funding in eviction defense in light of an exacerbating housing crisis. “In 2013, 550 tenants lost their case simply because they didn’t respond to a lawsuit or show up to court to defend themselves. Of the tenants who did file a response and requested a jury trial, over 1100 arrived at court without attorney representation. Many more tenants are likely pressured out because they are unaware of their rights.”<sup>59</sup> As such, fighting evictions rose to the top of the agenda for San Francisco voters, particularly in renter-heavy districts like District 5. Undoubtedly, the success of the pilot program had given voters and legislators a model that could potentially be employed to tackle the city’s high eviction rates. In late 2017, a group of housing advocates and the Democratic Socialists of America of San Francisco began collecting signatures in order to put a city-funded, full-scope tenant right to counsel program on the ballot.<sup>60</sup> Jane<sup>61</sup> recollected how smoothly this ballot signature collection campaign went. “It was easy going up to people like ‘This is just to put it on the ballot, you’ll get to vote on it. But if you’re evicted, we want to give you free lawyers who will help support you in court because too many people are getting evicted’ – I have collected signatures for other measures and none of them have been as popular as Prop F was, because it resonates with most people living in the city. Eviction is the scariest thing.”<sup>61</sup> Undoubtedly, this reflects the fact that more San Francisco residents are renters, and that the housing justice movement has grown tremendously since 1978, when there were about eight activists fighting for rent control.<sup>62</sup> Cary Gold also commented that it was difficult for those opposing the measure to render campaign whose premise is that individuals should not have access to an attorney popular among voters. “The landlords are doing a public relations campaign too, but they’re not bad, they’re not evil – and running a campaign that says ‘Well, we think it’s good for tenants to have to go to court without a lawyer while all the landlords have one is not going to have a great appeal.”<sup>63</sup> The main political figure behind the activism was Dean Preston, now Supervisor for District 5 and founder of Tenants Together, a California-based tenant rights group. All these combined efforts came to fruition on 5 June 2018, when San Francisco voters approved Proposition F with a majority of 55.74%.<sup>64</sup>

While the measure was widely supported among renters, several voices spoke against it, particularly the San Francisco Chronicle and the San Francisco Apartment Association. One of the main criticisms against Proposition F is the measure’s lack of any income criteria, meaning that the measure could apply for renters of all income levels.<sup>65</sup> However, what legal service providers have seen in practice is that most clients who ask for legal assistance at the EDC or the other ten legal service providers selected by the City qualify as low-income.<sup>66</sup> Based on the experiences of the Homeless Advocacy Project working with clients in the Tenderloin area, most indigent clients in unlawful detainer proceedings also face mental health issues.<sup>67</sup> It is often the case that tenants who can afford to hire private counsel will choose to do so rather than seek free legal assistance. According to Deepa Varma, the absence of income limits in the measure helped solidify its success<sup>68</sup> on the ballot, as the measure appealed to the 60-64% of renters in San Francisco and its proponents did not frame it as a poverty program.<sup>69</sup>

Proposition F established a city-funded tenant right to counsel, but the total amount of funding was not specified.<sup>70</sup> San Francisco Mayor London Breed and the Board of Supervisors allotted a total amount of \$5.6 million to the program from the City’s budget to last through fiscal year 2020.<sup>71</sup> Of all the legal service providers, the Eviction Defense Collaborative became the lead agency in implementing Proposition F through a central intake system called the Justice Portal, with about 47 housing attorneys listed across the eleven legal service providers.<sup>72</sup> If a tenant shows up to the EDC office with an eviction notice in their hand, they fill out an online form with demographic information and meet with an intake staff member to check the information. The referral database then determines an appropriate referral based on their information and based on whether there is capacity in the other agencies to take on an additional client.<sup>73</sup> A major difference between the legal aid landscape in San Francisco before and after Proposition F is that there is no more screening for meritorious defenses.

Because of capacity issues, legal service providers would also screen for the possibility that a defense could be built on the facts of the case, and they considered this feasibility as well when deciding to accept a client. With the current system, if a tenant meets the eligibility criteria and the agency has capacity to accept them, regardless of the presence or absence of any meritorious defenses, the agency has to accept.<sup>74</sup> If no referral can be made, then the EDC conducts a more thorough intake which includes the facts of the case and equips the tenant with a response containing all their affirmative defenses.<sup>75</sup> Therefore, the implementation of Proposition F has not necessarily resulted in a significant increase in the eviction caseload of legal service providers, but it has resulted in a centralized intake system for tenants seeking legal assistance that has resolved some of the communication gaps between agencies.

On 24 February 2020, Supervisor Dean Preston held a hearing on the implementation of the Tenant Right to Counsel, urging the City to increase funding for the program. According to data from the Mayor's Office of Housing and Community Development, eviction rates have declined, and more than 700 San Francisco households at risk of eviction in the past six months were able to retain their housing.<sup>76</sup> Specifically, there has been a 10% decrease in eviction filings from 2018 to 2019, which reflects housing attorney Bao-Tran Dang's observation during our interview. Furthermore, an estimated 67% of clients receiving full-scope representation under Proposition F were able to stay in their homes. This figure is not too far off the success rate of the pilot project in 2011, strongly suggesting that access to legal counsel is one of the key determinants in the outcome of an eviction proceeding.<sup>77</sup> The rate was even higher for African Americans who sought legal assistance as four out of five, or 80% of tenants were able to stay in their homes.<sup>78</sup> The hearing also revealed that the program is predominantly being utilized by lower income tenants (85%) compared to moderate (9%) and above moderate income earners (6%).<sup>79</sup> These numbers reflect the powerful advantage that the right to counsel provides to marginalized communities facing more powerful landlords, and attorneys who know how to tip the balance of the scales. As Supervisor Preston stated in the hearing, "providing tenants with a guaranteed attorney stops unfair displacement and gives San Francisco residents a fighting chance when facing eviction."<sup>80</sup>

When asked about what they would change or improve about the implementation of Proposition F, all of my interviewees stated funding levels, first and foremost. With the current level of funding, only two thirds of tenants facing an eviction notice end up receiving full-scope representation, and they only have one chance for a referral.<sup>81</sup> Combined with the impossibility of denying services to a referred client if when there is agency capacity, this has resulted in some attorneys "spending more time on cases that are hopeless and ... not being able to spend the time on cases that might have stronger defenses that didn't get an attorney simply because there wasn't one in the system on the day they came in."<sup>82</sup> Housing attorney Bao-Tran Dang echoed similar views, because with Proposition F, attorneys have to be ready to litigate a case with any given client, effectively eroding the agency's discretion supporting a client through to jury trial or not.<sup>83</sup> In particular instances, such as when clients have unreasonable demands, or if a case is unwinnable and there are no substantial arguments to be made, this discretion allowed agencies with limited resources to make important, albeit difficult decisions regarding how to best allocate their time and labor.

If Proposition F were running at full capacity, a significant portion of these challenges would be mitigated, because the supply of legal assistance would meet the demand. According to J. Scott Weaver and other proponents, the program could use about 20 to 30 more attorneys.<sup>84</sup> The most recent press release stated that the program would need between 17 and 22 additional attorneys.<sup>85</sup> However, one of the difficulties associated with finding new attorneys to hire even if there is funding, is that fewer attorneys seek to work in civil legal aid compared to other areas of law. In fact, the Eviction Defense Collaborative has two open attorney positions that have yet to be filled. According to Fred Sherburn-Zimmer, this reflects the shortage of attorneys in legal aid organizations.<sup>86</sup>



Bill Hirsh referred to this as a crisis in the ability of legal service providers to recruit and retain staff, largely driven by the low salaries in this field.

*While that worked for people who were committed to a cause, and were able to suck it up and not make a lot of money, increasingly with the cost of living in the Bay Area, it is no longer possible for folks to make it on those low salaries... It is a crisis, you could all the best intentions in the world, the best progressive politics, but if you don't have the bodies to actually provide those legal services, folks are not going to get served.<sup>87</sup>*

As such, a clear shortage of tenant attorneys who are willing to work with low-income clients as opposed to working in private practice also stands in the way of Proposition F reaching full implementation. The extent to which these difficulties in hiring and retaining staff are caused by the limited funds of Proposition F is difficult to determine given that the program is still relatively new and subject to political debate. However, as Supervisor Preston and all of my interviewees have reiterated, an adequate level of funding is largely the main concern for all proponents for an accessible Tenant Right to Counsel for all.

A different aspect of working with low-income clients that could significantly benefit from increased funding is the practice of increasing the number of social workers within legal services providers. The premise of incorporating social work in eviction defense is so that the circumstances that led to the eviction notice in the first place can be properly addressed. Teresa Friend, director of the Homeless Advocacy Project, explained the necessity of this joint effort:

*If somebody is being evicted, because they are hoarding, because they are behaving badly, because they're not getting along with their neighbors... It doesn't really do any good to represent them in the eviction case, if you don't address the underlying issue, if they can't get their place cleaned up, if they can get some kind of support and assistance to interact differently with their neighbors or to stop doing whatever it is that is causing the trouble... We try to address the underlying issues so that people aren't just at risk of being evicted right away again.<sup>88</sup>*

Housing attorney Bao-Tran Dang also commented on the value of this approach by sharing information about a case where her client was near the end of life and was about to be evicted due to hoarding. However, as her health deteriorated due to old age, she found herself unable to take things out. The client had also been living in this house for decades, as she had raised her children and grandchildren there. With the help of a social worker, they were able to organize a deep clean of the unit as well as get in touch with Adult Protective Services. Thus, this case was not simply about saving her housing, but the fight was “so that she could be there and not be homeless in her last few years.”<sup>89</sup> Cases like these exhibit the emotional complexity involved with fighting for clients to keep homes to which they may have grown attached. This is one of the things that have surprised Claire LaVaute, volunteer attorney with the Eviction Defense Collaborative, most in her work. Specifically, “the clients have all wanted to be able to pay the arrears they owe and/or otherwise meet their obligations.... I find that astounding, given the usurious rents and the often disrespectful way they have been treated.”<sup>90</sup> In a justice system that can feel dehumanizing, perspectives like these act as reminders that a tenant facing an eviction is a human being first, that they are more than their eviction notice, and that they are worthy of respect regardless. Thus, a holistic implementation of Proposition F is ethically imperative and much more effective in curbing subsequent evictions. One way of instituting this would be through the incorporation of social work practice within eviction defense.

Several of the interviewees have commented on the comparison between implementing the tenant right to counsel for eviction defense and the public defender model. In practice this would mean that rather than having a lead agency conduct referrals to other legal service providers, there would be one central agency and location. Specifically, Cary Gold commented that while the current system is as efficient as can be, there are still individuals who fall through the cracks from

one referral to another.<sup>91</sup> However, the public defender model does not necessarily account for the challenges of the current system if funding is not increased to an adequate level to sustain the full implementation of the right to counsel, the program risks being underfunded and overworked, similar to many of the public defender offices in the country.

## Conclusion

To conclude, this report has shown the implementation of the Tenant Right to Counsel is a necessary response to San Francisco's substantial eviction rates and the imbalance of power between tenant and landlord in court and other matters. Given the continued growth of the rental market in San Francisco, powerful profit-making incentives signal landlords to evict their tenants as vacancy decontrol enables landlords to raise rents for their future tenants. This practice is particularly harmful to low-income neighborhoods like the Tenderloin and Bayview/Hunters Point who have seen evictions become one of the most powerful tools for gentrification.

The passage of a measure like Proposition F in 2018 is an important first step in recognizing that access to legal aid should be a universal human right. Without access to an attorney, it cannot be reasonably expected that the client be able to exercise their rights in court and beyond. When 90% of landlords on a national average are represented by attorneys in housing court, compared to only 10% of tenants, this expectation is unrealistic.<sup>92</sup> As evidenced by the data compiled by the Mayor's Office for Housing and Community Development, the implementation of Proposition F has so far been successful in both reducing eviction rates and securing better outcomes for their clients, the majority of whom qualify as either low income or extremely low income.<sup>93</sup> Furthermore, according to the most recent press release, San Francisco has inspired other cities like Newark, Cleveland, Santa Monica and Philadelphia to implement similar measures as they face higher eviction and homelessness rates.<sup>94</sup>

Despite these early successes, the funding allocated to implement Tenant Right to Counsel needs to increase if it is to be accessible to every tenant facing an eviction in the city of San Francisco. Currently, only about two thirds of tenants in eviction proceedings are able to benefit from city-funded full-scope representation. Higher levels of funding could solve the challenges that nonprofit legal service providers face in recruiting and retaining attorney staff. This funding would also be most helpful in incorporating social work practice in eviction defense as clients almost always have underlying issues that need to be addressed as well. For the one third of tenants who are currently not being served, this increased funding is paramount to give them an equal footing in fighting for their home, or to prevent a more severe eviction fallout from occurring. Given the rising prevalence of homelessness and urban poverty, as well as eviction's direct ties to both, the political will should recognize the importance of adequate funding and support for necessary measures like the Tenant Right to Counsel. Only then might stable housing and a home be recognized as essential to life, liberty and the pursuit of happiness.

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**Jona Baçari** is an international Morehead-Cain scholar in her third year of studies, majoring in History. Shortly after joining Carolina, she became involved with the Community Empowerment Fund, an organization in Chapel Hill and Durham which supports folks in meeting their goals related to housing, savings, employment, benefits, access to healthcare and so forth. Through her relationships with members of the community, she has seen firsthand the consequences and systemic causes of homelessness and poverty. Given her interest in housing and economic justice, news of the right to counsel being implemented in New York City and San Francisco sparked deep curiosity. A trip to San Francisco and several interviews with attorneys and activists working on the ground to fight predatory evictions were inspirations for this independent research project.

# The Impact of Maternal Engagement in Child Development in Bangladesh

by Rehnuma Riana

Maternal and child health and development has been a topic of concern in Bangladesh for decades. Although progress has been made, there is still a need for more research and analysis to allow for a better understanding of disparities in childhood development. Early childhood development can have a huge impact on social, emotional and cognitive functioning in adulthood. Moreover, there are many factors which can enhance or hinder development. Specifically, maternal sensitivity, responsiveness, and engagement have been shown to influence early childhood development. The primary focus of this study is to examine the relationship between maternal engagement and child development among children in Bangladesh. A logistic regression was conducted using the UNICEF Multiple Indicator Cluster Survey (MICS), 2017, which is a nationally representative dataset. The sample consisted of 4,510 children between the ages of three and four years old. Preliminary results indicated a significant association between child development and maternal engagement. Children with engaged mothers are 1.23 times as likely to be developmentally on-track (95% CI: 1.04, 1.45). Further analysis will evaluate the effect of covariates such as maternal education, socioeconomic status, and other relevant variables.

## The Impact of Maternal Engagement in Child Development in Bangladesh

The developmental period during childhood is arguably the most important time of an individual's life. During this critical interval of a few years, children learn essential skills that allow them to be successful within different domains of life: physical, social and cognitive. Previous research suggest that maternal engagement can be an important factor contributing to children being developmentally on track (Tramonte 2015). However, there is much to be explored about disparities amongst different groups of mothers and how these factors can impact their level and quality of engagement with their child. The purpose of this paper is to explore these disparities in greater context as well as to understand the differences in physical, emotional and cognitive development among children growing up in Bangladesh in relation to their interactions with their biological mother.

### Background

Infants are highly sensitive to their relational surroundings, especially maternal sensitivity, responsiveness and engagement. Previous research has shown that poor infant regulatory behaviors and reactivity is associated with poor maternal sensitivity. This suggests that maternal behavior can possibly contribute to changes in the trajectory associated with infant temperament (Crockenberg 2006). Moreover, maternal engagement crosses various domains of development and can have lasting impact that continues beyond childhood years.

However, practices of maternal engagement are culturally constructed and can vary greatly across different regions. In a study conducted by Bronstein et al., results showed that mothers in a number of high-HDI (Human Development Index) countries engaged in more cognitive caregiving activities than mothers in low-HDI countries (Bornstein 2012). That is not to say that maternal engagement was less, rather engagement was varied in what type of activity was being carried out. A greater focus on cognitive caregiving rather than social-emotional caregiving may arise from outward expectations of society which value cognitive skills over others. There is also a pattern that exists in the type of caregiving that is present in all cultures. For example, more than half of the mothers across all countries played with their children and took them outside, however only a third or fewer mothers read books and told stories to their children. Thus, on a global average, more mothers engaged in socioemotional caregiving than cognitive caregiving (Bornstein 2012). It is also important to note that domain focused maternal engagement on early childhood development is greatly associated with factors of time and resource availability. It was concluded by Bornstein and colleagues that caregivers in all countries engage in activities such as not leaving a child alone and taking a child outside, and the next forms of most prevalent engagement include singing to the child, and naming objects with the child.

### **Covariates Impacting Maternal Engagement and Child Development**

While it can be concluded that maternal engagement is an important factor and can span across various domains, research also supports that there are specific factors that alter the quality of maternal engagement in both time and domain specificity. Moreover, it can also change the impact it has on early childhood development. For example, a study conducted by Urke et al., concluded that in rural areas with the poorest wealth quintile samples, maternal engagement was positively and significantly associated with early childhood development—however, this was not reflected in urban and higher wealth quintile samples (Urke 2018). Some factors that may have contributed to this phenomenon is resource availability. Urban regions with greater wealth index have greater access to diverse diets, early learning programs and access to health care which may offset the impact of direct maternal engagement. Another study conducted by Amin and colleagues showed that poverty status was a major determinant in health-seeking behavior. Mothers who come from higher wealth quintiles, were more likely to be trained for antenatal care and post-natal care resulting in better management of maternal engagement in relation to physical health (Amin 2010).

Although socio-economic status can be an important factor that results in changes in maternal engagement, there are also other relevant factors that should be explored. Demographic factors such as age and gender are also significant predictors of maternal engagement. More specifically, they are related to cognitive and socio-emotional activities. (Sun 2016). Another factor worth mentioning is father involvement and engagement from other adults in the household. Research does support that maternal engagement specifically is much more highly correlated to early child development than any other form of engagement. However, this may be due to family structures in the home and the roles of each parent that arise from it. For example, fathers with more highly educated wives are less likely to be involved in cognitive caregiving, and it is possible that more educated mothers lean towards becoming the primary caregiver in the home (Sun 2016). It is important to acknowledge that activities associated with maternal engagement can often be socially and culturally situated, which results in different outcomes of early childhood development. The focus of this paper is on a population of mothers and children in Bangladesh in order to better define these factors of poverty status, low wealth quintiles, and household structure and size as it relates to the relationship between maternal engagement and childhood development.

### **Methods**

This secondary analysis was carried out using the Multiple Indicator Cluster Survey (MICS-UNICEF) for Bangladesh 2012-2013. Three different surveys within the cluster survey were used to pair a child with their biological mother. The surveys used were the household survey, woman survey and child survey. The household survey included information such as the number of people within the household, the number of

children in the household aged 5 or younger, and wealth index quintile. The woman survey included information such as breastfeeding status. Lastly, the child survey included information regarding stunting, age of child, sex of child, access to toys, and engagement behavior from mother and other adults. The sample consisted of 4,510 children between the ages of three and four years old.

#### Independent Variable

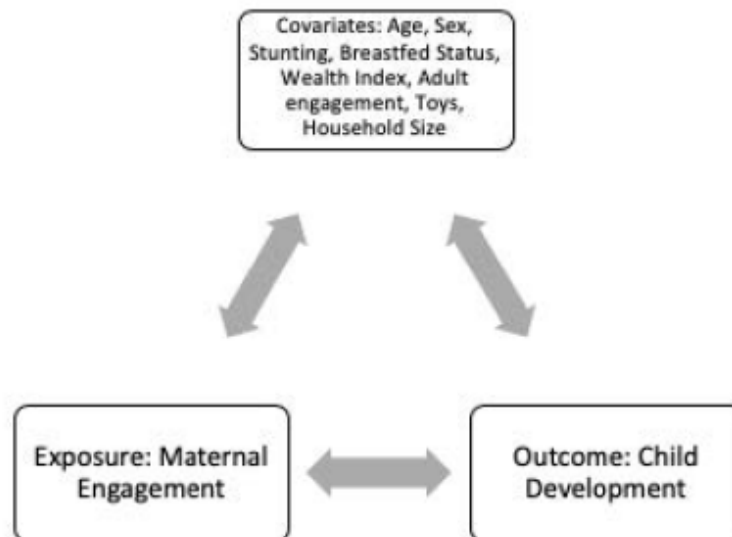
The independent variable in this study was maternal engagement which was categorized through variables in the survey such as number of times mother read to the child, number of times mother counted with the child, number of times mother took the child outside to play etc. A total of five activities were used to create a composite score of maternal engagement. If the mother engaged in three or more activities with the child, a score of 100 adequate engagement was given. If the mother did not engage in three or more activities with the child, a score of 000 inadequate engagement was given.

#### Dependent Variable

The dependent variable in this study was child development which was categorized through variables in the survey such as: child can name and recognize numbers, child can pick up small objects, child can follow simple directions etc. A total of nine variables were used to create a composite score of child development.

#### Statistical Analysis

STATA was used to conduct unadjusted and adjusted logistic regression modeling with covariates of interest including age of child, sex of the child, stunting, breastfed status, wealth index, adult engagement (not mother), toys and household size.



## Results

Overall, children with engaged mothers were 1.23 times as likely to be developmentally on track



(95% CI: 1.04, 1.45) when adjusting for the variables like age, sex, stunting, wealth index, adult engagement, toys and household size. The other covariates that were significant in this analysis was age of child, whether they were three or four years old and household size. Four-year-old children were 2.13 times as likely to be scored as being developmentally on track (95% CI: 1.82, 2.49). Household size was also a significant factor in that families with fewer people in the home were .96 times as likely to be developmentally on track (95% CI: .92, .99).

## Discussion

In developing nations, maternal engagement continues to be an important factor that aids in children being developmentally on track. Increased engagement in all domains of engagement leads to better outcomes overall. More specifically, when assessing early childhood development, it is important to note the age of the child. There is a notable amount of development that occurs between the ages of three and four which may skew the score of being developmentally on track. Moreover, the age of the child can also negate domain-specific engagement with the mother. For example, the mother may be more interested in playing with the child and taking the child outside to foster physical and social development. It can be assumed that activities such as reading to the child and counting with the child may be reserved for older children with greater capacity and interest to engage in such behaviors.

Household size is also a statistically significant factor that impacts the relationship between maternal engagement and child development. Increased number of members in the household can be a factor that takes away time from mom-child interactions. This can take on different forms – firstly it can mean that children are being taken care of by other members of the family for longer durations of time. It can also mean that the mother is spending more time doing household work and taking care of multiple children, thus allocating less time on engagement activities with each individual child. Household size can also reflect distribution of household resources in the form of books, toys, clothing etc.

Similar to the function of resources, other covariates are also important to keep in mind when analyzing this relationship. Although breast-fed status, wealth index, and toys were not significant in the adjusted model, they were independently associated with maternal engagement. This may indicate that these factors moderate the effects of maternal engagement. One example of this can be related to wealth index in that a greater wealth index can account for better outcomes for child development even if maternal engagement is inadequate.

Further analysis would need to be done to account for the different domains of child development individual to conclude whether there are differences between physical, cognitive, and social/emotional development. It can also be beneficial to explore the correlations between domain specific engagement and its effect on different domains of development. In this way, it can be identified which aspects of engagement are most impactful to different types of development. Furthermore, it can be easier to identify which aspects of development occur without adequate maternal engagement as support. Continuous research regarding maternal engagement and child development, especially in developing countries is an essential focus of public health. Due to the fact that there is a lack of healthcare infrastructure, proper nutrition, and access to health care, often times high maternal engagement can mitigate poor health outcomes in early childhood. Education regarding proper maternal engagement can be an important step in ensuring that children are developmentally on track in all domains of life.

## Acknowledgements

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# Humanities

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Physical Disability Is  
At The Core Of  
Richard III's Identity  
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# Bureaucratic Politics and Fractured Messages: *Uzbekistan Religious Freedom Case Study* by Madison Urban

This paper examines intragovernmental politics in the United States (U.S.) to better understand the development of religious freedom policies towards Uzbekistan post-9/11. The unique missions of both the Department of Defense and the Department of State led to the proposal of vastly different, sometimes conflicting, policies. Despite deep political differences, the U.S. and Uzbekistan quickly developed a partnership after the 9/11 attacks shifted America's attention to the Middle East. Military necessity and human rights prerogatives conflicted as U.S. officials struggled to credibly convey their human rights commitment to their Uzbek, autocratic military ally, President Islam Karimov. To better understand the policy process, this paper analyzes government publications, cables from the U.S. embassy in Tashkent, articles from think tanks and the media, and memoirs of the people who oversaw U.S.-Uzbek relations. Synthesizing these accounts, this paper considers the lessons of the past and gives a framework for understanding how to cooperate with Uzbekistan in the years ahead.

**Keywords:** Uzbekistan, Religious Freedom, Human Rights, Bureaucracy, Politics

## Introduction

In 1989 as the Soviet Union fell, Francis Fukuyama famously wrote an article arguing that the end of history had arrived, communism had been defeated and the liberal order had proven itself the only viable governmental structure.<sup>1</sup> However, terrorism, genocide, war in post-Soviet states and the continuation of neo-communist governments dominated international news as the hope of freedom and peace vanished. Former Soviet Bloc countries, like Uzbekistan, retained much of their Soviet-era leadership and while they declared themselves republics with new constitutions, little changed from the days of the Cold War.

War and conflict dominated the 20th century and the Department of Defense grew to dominate the United States (U.S.) foreign policy debate. The primary tools of the Department of State, diplomacy and engagement, take time—they are often painfully slow and require a delicate balance of priorities. In contrast, the precision and speed of military forces which can accomplish tasks and prove dominance is appealing because it plays to an increasingly fast paced society. However, the politics between the two departments has led to conflict within the U.S. government and at times produced fractured policies. In the years after the Soviet Union lost their dominance, the U.S. government attempted to develop relations with newly-formed states and formulate an understanding of the path they might pursue in Uzbekistan. The U.S. government attempted to engage, primarily militarily, with some early success. Their relationship grew after 9/11 thrust the Middle East and surrounding countries into prominence. As the U.S. invaded Afghanistan, due to the differing

priorities of the Department of State and the Department of Defense, there were inconsistencies in the message sent to Uzbekistan about the importance of religious freedom and human right reform. Events in Andijon in 2005 ended the tenuous relationship between neo-Communist government of Uzbekistan and the U.S. Later, the U.S. would reengage with a slightly more cohesive message and attempt to create change through small, incremental engagement. Until 2016, major progress was nearly impossible, but the new president is signaling a desire for reform. Engagement with Uzbekistan across previously impossible economic, political, social, military and diplomatic levels, could lead to changes in religious freedom policy as the U.S. starts to speak as one entity to a more receptive audience.

### **Background: Pre-9/11**

In 1992, when Uzbekistan declared its independence from the Soviet Union, Islam Karimov was elected as President. Karimov began working for the Uzbek Soviet Socialist Republic in 1966 in the State Planning Committee and was elected the First Secretary of the Central Committee of the Communist Party in 1989. A year later, he created and was elected President of the communist state. Unlike Soviet leader Mikel Gorbachev, Karimov actively opposed and “resented perestroika’s tolerance of criticism of the governments.”<sup>2</sup> Therefore, it was no surprise when the Organization for Security and Cooperation in Europe disputed the so-called democratic elections in 1992 that established him as the President of the Republic of Uzbekistan.<sup>3</sup> The authoritarian government he established was “virtually unchanged from the Soviet era”<sup>4</sup> despite the Constitution protecting freedom of religion.<sup>5</sup> Karimov’s regime harshly repressed people involved in any movements remotely considered political, including many religious groups.

In the United States, the International Religious Freedom Act (IRFA) was passed in 1998 which required the U.S. to consider religious freedom and persecution in pursuit of foreign policy goals. IRFA created a new Ambassador-at-Large position, an Office of International Religious Freedom housed in the Department of State and an independent Commission on International Religious Freedom (the Commission).<sup>6</sup> Both the Office and the Commission produce distinct annual reports on the status of religious freedom. The Office details the conditions of religious freedom in every country. The Commission reports more narrowly on specific violators and makes policy recommendations.<sup>7</sup> Yearly, the President must designate countries who have “engaged in or tolerated systematic, ongoing and egregious violations of religious freedom” as Countries of Particular Concern (CPC).<sup>8</sup> As a CPC, a country is subject to a variety of measures which the president can waive for national security reasons.<sup>9</sup> This waiver directly pitted the religious freedom priorities against defense and national security from the beginning.

After the creation of IRFA, Robert Seiple was named the first Ambassador-at-Large for International Religious Freedom. In his first annual report to Congress on the conditions of religious freedom on October 6, 1999, he mentions efforts undertaken by the executive and legislative branches to further religious freedom in Uzbekistan, mainly through dialogue with Uzbek senior leadership. He personally travelled to Uzbekistan within his first year as Ambassador. During the U.S.-Uzbek Joint Commission in 1998, Assistant Secretary of State for Democracy, Human Rights and Labor, Harold Koh, discussed religious freedom with the Uzbek Foreign Minister and the Ambassador-at-Large for Newly Independent States had multiple conversations with senior members of the Karimov government.<sup>10</sup> From the beginning, religious freedom was a right the U.S. discussed with the Uzbeks.

While the Department of State developed policies and procedures to accommodate its Congressionally mandated prioritization of religious freedom, the Department of Defense started to build rapport with the military.<sup>11</sup> In 1995, the U.S. Secretary of Defense William Perry and Uzbek Minister of Defense agreed on a memorandum of understanding that among other things, created an enhanced International Military Education and Training Program (IMET).<sup>12</sup> IMET is an education

program that “focuses on rule of law and non-lethal courses in military management.”<sup>13</sup> U.S. troops first arrived on Uzbek soil in August 1996 and the first teams of U.S. Army Special Forces (Green Berets) arrived in 1997. The Green Berets built the foundational relationships with lower levels of the military hierarchy that paid dividends in the immediate months after the 9/11 attacks on the U.S. homeland. In May of 2000, Central Command (CENTCOM) Commander General Anthony Zinni visited Tashkent requesting more funding, but was denied because of various “human rights concerns and lack of focus on Central Asia.”<sup>14</sup> Zinni circumvented some of the aid restrictions and got some aid to Uzbekistan regardless. Senior diplomats in Central Asia appreciated the time Zinni spent visiting and interacting with people on the ground, emphasizing the importance of engagement and frustration with their own State Department officials who did not prioritize Central Asia.<sup>15</sup>

From the beginning, U.S. policy was characterized by contradictory messages because of divergent policies between the Department of State and Department of Defense. The Department of State preached change and conditioned aid on democratic reform without major engagement. Meanwhile, the CIA and Department of Defense worked on the ground with the military. State was frustrated by the fact that aid had been given by Defense without conditions and because Defense could offer more, their justified protests about human rights were undermined. Defense officials like Zinni felt like conditioning aid on improvement was akin to “delivering health care only to people who are completely healthy.”<sup>16</sup> These frustrations would only grow as Central Asia and Uzbekistan especially became increasingly important after the 9/11 shift to the Middle East.

### 9/11 and the War in Afghanistan

The attacks on the World Trade Centers and the Pentagon by al-Qaeda on September 11, 2001 marked a massive shift in U.S. policy towards Uzbekistan as they prepared to attack al-Qaeda in Afghanistan. Central Asia became a critical region, it was no longer simply the land between Russia and China or a former Soviet state, but a region that demanded attention. Afghanistan is bordered by six states: Tajikistan, China, Pakistan, Iran, Turkmenistan and Uzbekistan. Of those six, Uzbekistan had the greatest combination of willingness to work with the U.S. (unlike Pakistan, Iran and China) and infrastructure development to facilitate the movements (unlike Tajikistan and Turkmenistan).<sup>17</sup> On September 19th, merely eight days after the 9/11 attacks, President George Bush was on the phone with President Karimov.<sup>18</sup> On October 6, 2001, Uzbekistan agreed to give the U.S. access to its airspace and to station troops at Karshi-Khanabad (K2), an airbase just north of the Uzbek-Afghan border after a visit from Secretary of Defense Rumsfeld.<sup>19</sup> <sup>20</sup> The next day, Operation Enduring Freedom commenced.<sup>21</sup> The contacts and rapport the Green Berets built with the Uzbeks on the ground was vital to striking a deal quickly.

The rapid shift in relations with the government of Uzbekistan was cause for concern for many within the human rights and religious freedom advocacy groups. In its first annual report, published post-9/11, the Commission addresses this concern saying that “the promotion of religious freedom also must be evaluated in the context of what is currently the single overriding objective of the United States: protecting its citizens, national interests, and robust democratic government around the globe through, in part, the combating of terrorism and those who support it.”<sup>22</sup> Human Rights Watch and Freedom House, among others, warned that President Karimov’s allowance of the U.S. was built upon a tacit agreement for the U.S. to look the other way on its human rights record and continue its practice of imprisoning activists and religious leaders on terrorism charges.<sup>23</sup> <sup>24</sup>

In the year following the attacks, Uzbekistan seemed to make some progress as a front but behind the scenes the pattern of repressive tactics continued. Chris Seiple, who was in Tashkent, testified that the Uzbeks took the lead in adding human rights components to the Declaration on the Strategic Partnership and Cooperation Framework that was signed in March of 2002, a surprising

move for a country with little appetite for reform.<sup>25</sup> Among other things, the Declaration paved the way for a visit from the UN Rapporteur on Torture in 2003. However, despite Karimov's apparent openness, the rapporteur was denied access to some of the worst prisons, including Jaslyk, a prison specifically for religious prisoners where some of the worst human rights abuses have occurred. The report concluded that torture "is not just incidental, but systematic in this country." Perhaps the most famous of these abuses was the case of Muzaffar Avazov, the 35-year-old head of the Independent Human Rights Organization of Uzbekistan. He was imprisoned in Jaslyk on trumped up charges of membership in an Islamic In the year following the attacks, Uzbekistan seemed to make some progress as a front but behind the scenes the pattern of repressive tactics continued. Chris Seiple, who was in Tashkent, testified that the Uzbeks took the lead in adding human rights components to the Declaration on the Strategic Partnership and Cooperation Framework that was signed in March of 2002, a surprising move for a country with little appetite for reform. Among other things, the Declaration paved the way for a visit from the UN Rapporteur on Torture in 2003. However, despite Karimov's apparent openness, the rapporteur was denied access to some of the worst prisons, including Jaslyk, a prison specifically for religious prisoners where some of the worst human rights abuses have occurred.<sup>26</sup> The report concluded that torture "is not just incidental, but systematic in this country."<sup>27</sup> Perhaps the most famous of these abuses was the case of Muzaffar Avazov, the 35-year-old head of the Independent Human Rights Organization of Uzbekistan. He was imprisoned in Jaslyk on trumped up charges of membership in an Islamic terrorist group, Hizb-ut-Tahrir.<sup>28</sup> He died in custody and when his body was brought to the family for burial, doctors testified that the burn marks that covered his body "could only have been caused by immersing Avazov in boiling water" which was the likely cause of death.<sup>29</sup> Human rights groups around the world condemned the lack of progress in Uzbekistan despite U.S. engagement.

From 2002-2004, the story of the disjointed U.S. policy seems to be summed up in a cable sent from Brussels to Washington D.C. The cable relayed the conclusions of a conversation between officials from the Department of State and the European Union about Central Asia in February of 2004. Concerns about newly implemented restrictions on non-governmental organizations (NGOs) and broader human rights concerns in Uzbekistan were a primary topic of the conversation. They struggled to articulate how to "maintain a firm line with Karimov while also not pushing him away from the table."<sup>30</sup> Many cautioned against attempting to publicly humiliate the Uzbeks into compliance fearing that they would retreat and ally themselves with Russia or China.<sup>31</sup> Some officials felt that lack of public pressure was a tacit allowance of the violations. There were also national security concerns to be considered and the desire to maintain U.S. access to K2. The question seemed to come down to this: would it be better for the U.S. government to continue to build a space for conversation by partnering with the Uzbeks and developing relationships and educational programs, or would it be better to withdraw funding and attempt change through sanctions? The Department of Defense was the primary ally that had built trust with many in Uzbekistan and the primary ones pushing for engagement. It contributed more money and were "philosophically more consistent" as they brought a common enemy every day.<sup>32</sup> Without the insistence on human rights progress, there was little chance at cooperation. NATO needed access to K2 so Defense was not rushing to criticize Karimov's regime. Therefore, many people in State felt the only way to get their message across was through public criticism. At least until 2004, the U.S. decided to keep their seat at the table, pursue their military objectives and push human rights reform through dialogue through the weaker voice of the Department of State.

In light of concerns of torture and human rights violations, Congress began to condition aid in 2002 on "the Secretary of State determining and reporting to the Committees on Appropriations that the Government of Uzbekistan is making substantial and continuing progress



in meeting its commitments,” based on the agreements in the Declaration on the Strategic Partnerships and Cooperation Framework.<sup>33</sup> However, it was not until July 2004 that the Secretary of State, Colin Powell, did not certify compliance and up to \$18 million in Foreign Military Financing (FMF) and IMET aid was denied.<sup>34</sup> FMF and IMET are two aid programs that the Department of State and Department of Defense collaborate on. State determines who gets the aid and Defense implements the program.<sup>35</sup> State’s refusal to certify compliance led to In light of concerns of torture and human rights violations, Congress began to condition aid in 2002 on “the Secretary of State determining and reporting to the Committees on Appropriations that the Government of Uzbekistan is making substantial and continuing progress in meeting its commitments,” based on the agreements in the Declaration on the Strategic Partnerships and Cooperation Framework. However, it was not until July 2004 that the Secretary of State, Colin Powell, did not certify compliance and up to \$18 million in Foreign Military Financing (FMF) and IMET aid was denied. FMF and IMET are two aid programs that the Department of State and Department of Defense collaborate on. State determines who gets the aid and Defense implements the program. State’s refusal to certify compliance led to turmoil between bureaucracies. Immediately after the cutoff, the Chairman of the Joint Chiefs of Staff General, Richard Myers, criticized the judgment as “shortsighted” because it decreased U.S. military influence. In response, he “boosted nonproliferation aid of \$21 million.”<sup>36</sup> As the Department of State took money away from programs, Defense gave more. Political factors and lack of agreement between the bureaucracies led to the U.S. pushing two policies in Uzbekistan. Despite the apparent disagreement between State and Defense, the head of Human Rights Watch in Tashkent said in 2003 that “the steps taken are basically window dressing used to get the military funding through the US Congress’s ethical laws. Nothing has changed on the ground.”<sup>37</sup>

### **Andijon and Aftermath**

Once aid was conditioned in 2004, the U.S. and Uzbekistan relations cooled. The Andijon massacre on May 13, 2005 would then define Uzbekistan’s relationships with the Western world and drastically change U.S. policy. Protests in Uzbekistan began at the end of 2004 that decried the country’s economic hardships and corruption.<sup>38</sup> According to State Department releases and a now declassified intelligence analysis produced by the Defense Intelligence Agency, the immediate catalyst for the protests in Andijon was the imprisonment and trial of twenty-three businessmen who were falsely charged with membership in an Islamic extremist group. On May 10, people in the city of Andijon began to peacefully demonstrate showing their support for the businessmen.<sup>39</sup> Then, late at night on May 12 and into the early morning of May 13, a number of individuals “attacked a police garrison, seized weapons, and broke into a nearby prison and released several hundred inmates.”<sup>40</sup> In the midst of the prison escape, “up to several dozen prison guards were killed.”<sup>41</sup> Some of the freed inmates went on to storm government buildings.<sup>42</sup> Meanwhile, the protests in the town square revolved around the declining economic conditions. The government quickly sent in “military vehicles and fire indiscriminately into the crowd” of civilians.<sup>43</sup> There was reportedly no attempt to find the individuals who had raided the police and government buildings, but the military opened fire on the populace, the vast majority of whom were unarmed and peaceful. The Uzbek government reported 187 people dead,<sup>44</sup> but unofficial reporting and eyewitness statements say the total amount was significantly higher, perhaps over 700 people.<sup>45</sup> Since the uprisings, there has been no international investigation into the events.

Human rights groups world-wide quickly condemned the actions and called on governments to impose sanctions. Initially after the events, the U.S. response was muted. Their early lack of condemnation was in part due to Defense officials attempting to preserve U.S. access to the

K2 base. Publicly, the offices denied any conflict or debate between Secretary of State Condoleezza Rice and Secretary of Defense Donald Rumsfeld. However, sources familiar with the situation at the time, and later memoirs published by Rumsfeld and Rice, suggest otherwise. A senior diplomat was quoted to say that “there’s clearly inter-agency tension over Uzbekistan” with the Department of State “extremely cool on Karimov” but Defense trying not to retain access to K2.<sup>46</sup> In her book, Rice recalls “crossing swords” over the events with Rumsfeld saying that there was misunderstanding on her position. She insisted on publicly criticizing the events and calling for an international investigation which was taken as her saying that “human rights trump national security.”<sup>47</sup> Indeed, public criticism by Rice and U.S. Congressmen led to Karimov “curtailing certain U.S. military flights”<sup>48</sup> in and out of K2. Ultimately it was the coordinated efforts of the U.S. and the United Nations to help refugee repression that caused Karimov to issue “a formal eviction from the K2 air force base on July 29.”<sup>49</sup> Later, Rumsfeld would write that the U.S. policy post-Andijon caused the U.S. to “effectively tak ourselves out of the region” and drive Uzbekistan “to strengthen ties with Russia and China.”<sup>50</sup> To a degree, this is true. On November 14, within months of the issuance of the order for the U.S. to leave K2, Russia and Uzbekistan “formally signed a new military alliance agreement” which “granted an unprecedented level of military cooperation” between the two countries.<sup>51</sup>

Within months of the massacre and U.S. departure, Karimov changed the legal codes to increase the fines for religious organizations and persons attending religious events in addition to increasing the monitoring of churches, mosques and other places of worship.<sup>52</sup> Furthermore, Karimov began to isolate religious communities from the international human rights and religious freedom communities by censoring mail and kicking out non-governmental organizations.<sup>53</sup> Finally, the UN High Commissioner for Refugees office was shut down after having a presence in Uzbekistan for thirteen years.<sup>54</sup> In light of the escalation in persecution, the Commission openly recommended what should be obvious, that the “U.S. government ensure that it speaks in a unified voice”<sup>55</sup> to try to undo some of the damage. Uzbekistan is the only country of 23 countries in the 2006 report (and in the years to come) with severe religious freedom violations that mentions concern about conflicting messages being sent by the U.S. government. Unique security concerns make Uzbekistan one of the trickiest places to conduct meaningful dialogue about human rights, but despite pushback from the Department of Defense, the massacre in Andijon was too much for the White House to overlook and the U.S. officially designated Uzbekistan as a Country of Particular Concern in 2006.

#### **Northern Distribution Network**

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opportunities for growth.<sup>56</sup> He states that “security and human rights are not and cannot be in opposition to one another in our relationship with Uzbekistan,” and if they are “it is probable that we will achieve neither”<sup>57</sup> as in the first half of the 2000s. He pleads for negotiations to make this principle clear. Berliner’s call was for careful engagement, not allowing human rights on national security to outweigh each other. Instead, he advocates for upholding the two ideas for the advancement of both. Over the next eight years the U.S. would attempt to pursue policy as he proposed with limited success.

In 2009, President Obama granted a waiver on the sanctions that had been in place since the CPC designation in 2006<sup>58</sup> and invited the Uzbek’s to annual bilateral consultations. The U.S. government started to consistently engage in “a face-to-face structured dialogue, based on a jointly developed, comprehensive agenda that facilitate candid discussions on the full spectrum of bilateral priorities.”<sup>59</sup> Cable after cable sent from Tashkent from both military and diplomatic leaders encouraged continued engagement, arguing that “there simply is no alternative to principled engagement and patience.”<sup>60</sup> They acknowledged that abuses would occur during engagement and that the situation would not drastically improve, but pleaded for the U.S. “to take the long view.”<sup>61</sup> There was an explicit understanding among U.S. officials that true change would take new leadership. Nevertheless, they pressed for engagement and developed relations to maintain access. Leading up to 2009 and in the years to come, the U.S. sought to keep its seat at the table and fight for opportunities to release individual prisoners, gain access to non-governmental organizations and hope that one day legislation and judicial progress would follow. The advances the U.S. made over the next decade of engagement was minimal because Karimov had built an oppressive regime full of systematic injustice, torture, and widespread abuse of power at all levels. However, in September 2016, a new opportunity arrived when Islam Karimov died after 27 years of ruling Uzbekistan.<sup>62</sup>

### **A New Era? Shavkat Mirziyoyev**

After Karimov’s death, the long tenured Prime Minister, Shavkat Mirziyoyev, took power as the interim president. Elections, held three months, gave him the presidency with 88.6% of the populous voting for him in what outside observers would call a “tightly controlled election.”<sup>63</sup> In light of his history as a close political associate of Karimov, Western press and human rights organizations were cautious about placing any hope in the potential for reform. Initial releases by Radio Free Europe/Radio Liberty describe him as a “close Karimov ally” who had a “short temper” with reports of him “physically assault[ing] at least one farmer who complained” about his policies and gave little hope for reform.<sup>64</sup> Even more ominously, when a former spokesman for Karimov, Sharaf Ubaidullaev, was asked about his thoughts on Mirziyoyev he believed that he would be “worse” than Karimov because he would likely be more dependent on an oligarchy to keep power.<sup>65</sup> Human Rights Watch was quick to share concerns about the new president publishing a report in June of 2017 that stated that Mirziyoyev’s “previous positions raise concerns about his credibility” because he had previously overseen the implementation of corrupt and abusive labor practices.<sup>66</sup> In spite of these warnings, three years into his rule over Uzbekistan, Mirziyoyev seems to be devoted to reform and increased engagement with the West even though the government is still fraught with problems.

In the Commission’s report on events in 2017, they describe a “general easing of longstanding repressive policies” but in Karimov’s first year, there was no major change in the abusive policies that included continued reports of torture. Leading up to a historic visit at the White House in May 2018, Mirziyoyev dismissed Rustam Inoyatov, the long-serving head of the secret police in Uzbekistan, after publicly criticizing his abuses. He would then go on to remove others from leadership and changed the police’s name in an effort to recreate the service. During

his visit in the U.S., Mirziyoyev agreed to “implement a comprehensive roadmap to advance religious freedom.”<sup>69</sup> The roadmap “includes 52 measures, 40 of which were already implemented in 2018”<sup>70</sup> and signaled his commitment to developing ties with the West and his understandings that to truly do that, he must improve his human rights record, perhaps a lesson learned from Karimov’s failure to do so. From these reforms, in November of 2018, the U.S. removed the CPC designation from Uzbekistan and placed it on a newly created Special Watch List, a step below the CPC designation.<sup>71</sup> Perhaps some of the biggest news from Uzbekistan this summer, Mirziyoyev closed the infamous Jaslyk prison where some of the worst abuses and torture had occurred during the Karimov era.<sup>72</sup> Despite the abolition of torture and some incremental increases in freedom of press, reports of torture and false imprisonment, as well as bloggers being detained for criticism of the government, continue to surface.<sup>73</sup>

Though it has only been three years since Karimov died, much of his governmental structure and leadership, especially on the local level, remain in place. Passing legislation, reforming legal codes, developing ties with the West, and broader economic reform are positive signs, but their full implementation will take years. Developing an institutional structure to oversee and implement reforms will also be necessary. A decreased U.S. military presence in Afghanistan will also allow sustained dialogue without a pressing need to make concessions to gain access. Civil society groups like the American Bar Association and Institute for Global Engagement are engaged with high level officials in Uzbekistan like the Minister of Justice, Chairman of the Supreme Court and the Ambassador to the United States on a variety of issues.<sup>74 75</sup> Mirziyoyev’s aforementioned trip to the U.S. was the first time any Uzbek president has visited the White House.<sup>76</sup> Offers to host upcoming trade forums and Uzbekistan’s hopeful bid to the World Trade Organization are increasing the opportunities for economic engagement.<sup>77</sup> Increasingly, senior government officials from the Department of State, including the current Ambassador-at-Large for International Religious Freedom, Sam Brownback, are travelling to Uzbekistan and inviting Uzbek officials to the U.S.<sup>78</sup> Engagement across different levels of the U.S. government and the private sector will hopefully lead to continued progress on religious freedom and human rights more broadly. Years of engagement and patient progress during the last decade of the Karimov era seem to be paying off as Mirziyoyev is projecting a new, freer image of Uzbekistan to the world and is slowly taking steps to prove his commitment. The same principled engagement across all levels of government that Berliner advocated for in 2008 will continue to be important as the U.S. continues to partner with Uzbekistan, both speaking candidly about ongoing abuses and assisting in the transition away from Soviet-style government.

## Conclusion

Since the beginning of U.S. relations with Uzbekistan, human rights and religious freedom were priorities of the Department of State as they engaged with the Karimov regime. The Department of Defense took a hands-on approach, putting troops on the ground to develop relations with the Uzbek military. In a post-9/11 world where terrorism became the primary focus of U.S. foreign policy, Uzbekistan played a key role in facilitating the U.S. war in Afghanistan. The need for the Uzbek airbase Karshi-Khanabad caused Defense to caution public humiliation or criticism of the government’s abuses. The lack of continuity in the U.S. message about human rights, especially through the lack of conditions of aid given by Defense, frustrated State officials and human rights organizations. The tension came to its peak in 2005 after the Andijon massacre and the official condemnation that terminated the U.S. access to K2 and most diplomatic relations. After seeing the decline in religious freedom post-Andijon with no engagement, State was more willing to re-engage when Defense needed access again to the Afghan border. The idea of consistent, patient engagement, understanding the goal was incremental changes in the face of a regime unwilling to loosen its grip.

Once Karimov died, Mirziyoyev began to show increased willingness to take tangible steps towards reform. Looking forward, the U.S. will need to practice consistent engagement across multiple levels and spheres to truly effect change. The systematic problems within the government and judicial structures will take years to overcome and will take disciplined engagement in economic, social, political, civil society and military spheres. However, Uzbekistan at least appears to be on the right track and proper engagement with the West can increase the likelihood that what was once an oppressive regime could turn into an ally in a strategic area of the world.

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**Madison Urban** is a junior at the University of North Carolina. She is a double major in Public Policy and Peace War and Defense with a minor in Business Administration. Central Asia became a topic of interest for her after she took classes on China and Russia, sparking a passion to explore the politics of the region between the two powers. Desiring to understand human rights policy on a deeper level, she looked at the policies regarding religious freedom across two decades, specifically to develop an even deeper understanding of the politics within administrations as the United States seeks multiple, seemingly conflicting, foreign policy goals.



# Physical Disability Is At The Core of Richard III's Identity in Shakespeare's *The Tragedy of Richard III*

## by Sophie Troyer

In *The Tragedy of Richard III*, feelings toward the disabled and disfigured are apparent through characterization of Richard and his interactions with others. The characters in the play assume that Richard is evil because he is physically marked by disability. Richard has a complex understanding of how he is perceived and is able to use this knowledge to understand his disability and relate to other people. In effect, Richard uses his disability as a tool. While physiognomy, a science at the time, states that deformity reflects inward character, further analysis of Richard's circumstances suggests that this is not true—that instead disability and others' reactions to disability can shape a person's personality. Francis Bacon suggests that disability can bring out components of character, whether positive or negative. Therefore, a person's character and response to disability is a product of a combination of personality and societal or environmental factors on a person, rather than an inherent quality of disability.

Keywords: disability, health, Shakespeare, English, Renaissance

In *The Tragedy of Richard III*, a history by William Shakespeare, Richard III is portrayed as an evil tyrant, bent on being king no matter the cost. Richard's opening soliloquy introduces his physical disability as a disadvantage to him at court. Throughout the rest of the play, his disability is alluded to by others in physical language and insults, and by references to medical language. Richard rarely references his disability directly, while the other characters reference it only in insults. There are also times when Richard seems to draw attention to other bodies as though he is obsessed with the natural human body, but he uses this to draw focus away from his own body. The use of medical and highly physical language in this play suggests that Richard uses his physical disability as motivation to achieve his goals, while the other characters consider his disability to be firmly intertwined with his identity and personality.

On two occasions when Richard explicitly mentions his physical disability, he reveals his own opinions and motivations in relation to his body. In the opening soliloquy, Richard confesses that he is "not shaped for sportive tricks,/ Nor made to court an amorous looking glass," and continues to describe how he looks so ugly that he can't court women, and dogs bark at him as he walks by (1.1.14-15). Here, Richard highlights his lack of ability to participate in the normal activities of a man of his age and stature, thus beginning to paint a picture of his body. To further elucidate his point, he describes himself as "Deformed, unfinished, sent before my time/ Into this breathing world scarce half made up" (1.1.20-21). Medically speaking, Richard is saying he was born prematurely, which is an explanation for the associated "unfinished" deformities. Richard's phrasing is blunt and evocative—he was thrust into the world with physical disabilities causing him to be unprepared for what that life would entail. He recognizes exactly how he appears and how others see him. In addition, Richard reveals his own response to his deformity and how it motivates him: "And therefore, since I cannot prove a lover/To entertain these fair well-spoken

days, / I am determin'd to prove a villain" (1.1.28-30). Since Richard can't participate in normal activities or woo women, as previously mentioned, he recognizes that he could either dwell on his deformity or do something about it. The thing that Richard wants to do is become a power-hungry "villain" who takes it upon himself to advance his own goals. Based on this, Richard's drive to become king and his determination to do so in a villainous manner originates in his perception of his body and understanding of how others perceive his body. In other words, Richard knows that he can't engage with people like a normal person and be seen to have the same value, so he will forcibly gain value by acting as horribly as others expect based on his appearance.

The second time that Richard directly addresses his disability is when he manages to win over Anne and wonders whether he judges his looks too harshly. He is astonished that she would "abase her eyes" on he "that halts and is misshapen thus" (1.2.249, 1.2.253). Richard was so focused on his body that he didn't realize that anyone could see past it. However, he takes this realization to the extreme and gains the confidence from her attentions to buy a mirror and "entertain a score or two of tailors / To study fashions to adorn my body" (1.2.259-260). Although his response can be read as partially ironic, it is revealing when read seriously. Since Richard suddenly has the prospect of marriage, which he didn't seem to think possible before, he becomes eager to present himself well to further influence people's perception of him. It's somewhat surprising how he suddenly becomes willing to look upon his body, which he was so ashamed of in the most recent scene. Perhaps all he is looking for is validation, which seems to come in the form of power which he hopes to gain as a villain. Although he now has the potential for love, it isn't enough at this point and Richard is already using his disability as a tool in his quest for power.

Although the other characters in the play don't explicitly comment on Richard's physical disability, the physicalized nature of their insults and use of animal imagery show that he is perceived as being animalistic and evil, both in nature and in physicality. The characters, especially Queen Margaret and Queen Elizabeth, insult Richard by calling him many foul animal names-- too many to discuss. Both Queen Margaret and Queen Elizabeth call him a "bottled spider" and "bunch-backed toad" (1.3.242-246, 4.4.83). Bottled means swollen, and bunch-backed means hunch-backed, so these insults reference his physical disability. The comparison to a spider and a toad portrays him as an evil pest, since spiders bite and toads are associated with witches. Stanley and Richmond both refer to Richard as a "deadly boar" or "usurping boar" (4.5.2, 5.2.7). Boars are unclean, lowly beasts. This description paints Richard as violent and animalistic. Would such an animal be suited to be king, or just a tyrant to be overthrown? Queen Margaret also refers to him as an "elvish-marked, abortive, rooting hog" (1.3.228). Again, being elvish-marked can be a reference to his disability, being marked physically by elves from birth. But this can also have internal meaning: if he was marked by elves, then this physical mark would indicate something sinister or mischievous inside Richard. The description of him as a rooting hog conveys an image of Richard searching for power like an animal does hungrily for food. Finally, Queen Margaret calls him a dog and a hellhound with venom teeth "that had his teeth before his eyes" (4.4.48-49). This suggests that from a very young age Richard was a horrible, evil beast with a metaphorical venomous bite. Note that York also mentions that Richard "could gnaw a crust at two hours old" (2.4.28). It's interesting that while Richard has other physical disabilities and considers himself half-formed, his teeth came in early. This again paints him as more animalistic and vicious. All of these comparisons to animals demonstrate how the other characters associate Richard's fierce tendencies with his physicality. Elizabeth reminds us of Richard's inability to escape the full reality of who he is when she says, "There is no other way, / Unless thou couldst put on some other shape / And not be Richard, that hath done all this" (4.4.286-287). Richard can try to hide who he is and manipulate people all he wants, but in the end, he is exactly who he is and nothing else. This applies both physically and in terms of personality--his shape, presence, and intangible qualities are fixed, unable to be separated from the whole.

The Duchess further points out Richard's lack of princely qualities by comparing her two dead

sons to mirrors of her husband, while Richard is “but one false glass/ that grieves [her] when [she] sees [her] shame in him” (2.2.53-54). To her, Richard’s entire person represents her shame for how he acts. Her use of the mirror as a point of comparison makes it obvious that she sees Richard’s terrible personality and actions embodied in his physical being. Further, multiple characters comment on how they can’t look upon him, likely because they see his body as the embodiment of his evil. In one such instance, Anne describes Richard as the devil: “And mortal eyes cannot endure the devil. / Avaunt, thou dreadful minister of hell” (1.2.46). Here, Anne suggests that she cannot look at him, because there is some physical, visual embodiment of the devil in Richard. Anne also says a bit later that he “doth infect [her] eyes” (1.2.151). Thus, not only is his physical body perceived to embody the devil, but the shift toward medical language suggests a connection between health, the body, and the spirit. She is so repulsed by looking at him that she can feel his evil in that manner. This medical language also appears when Richard is directly called a “defused infection of a man” (1.2.78). Not only do other people feel uncomfortable looking at him because it suggests the presence of evil, but Richard himself is defined as an infection. He is a plague to others and seems to symbolize pure evil. This inward evil and outward physical appearance seem to be inseparable in the minds of others.

Finally, other medical language throughout the play reminds the audience of Richard’s disability even though it isn’t directly referenced. As a result, the human body is always on the audience’s mind. Richard also seems to draw attention to other people’s bodies, perhaps to draw focus away from his own. First, he refers to Elizabeth’s dead sons as “the issue of your womb,” as though they must be referred to in connection to a present body (4.4.296). Then, he says that if he were to have a child with her daughter, those children would be almost hers “save for a night of groans” (4.4.303). This language is very descriptive and draws attention to the natural female body and the process of childbirth. Since this language is so evocative, it could serve to focus the attention from Richard’s body to the bodies of others. This can also indicate that Richard often thinks in terms of the human body, perhaps due to his disability being constantly in his thoughts. There are other instances in the play, however, when characters use medical language to describe England. This analogy functions as a constant reminder of Richard’s physical disabilities throughout the play, as the audience would be able to see him during these references. Buckingham describes England as physically blemished and misshapen: “the noble isle doth want her proper limbs / Her face defaced with scars of infamy” (3.7.125-126). Although Buckingham’s point here is that England is not functioning properly and has been hurt by bad deeds before, the language he uses reminds the audience of physical deformities. It appears this medical language is inescapable—it’s as though in trying to avoid focusing on Richard’s disability, those around him can’t help but think in terms of medical language. Even the citizens use this language, when talking about Richard and the queen’s sons: “And were they to be ruled, and not to rule/ This sickly land might solace as before” (2.3.30-31). Here, the citizens seem to go a step further and draw a connection between Richard and England being “sickly.” Regardless, describing the land that is ruled by a person with a physical disability as sickly appears to be an intentional reference to remind the audience of the connection between physical disability and defects in Richard’s personality.

To understand how the social climate during the 1600s ascribed physical malady with a moral valence that would have impacted the perception of Richard III, it is useful to examine some publications from the time period. A good example of the association of physical illness with sin is seen in the treatment and perception of lepers—this association still lingers today as “lepers” are used to describe social outcasts. And they were social outcasts: as Robert Abbot in *A Defence of the Reformed Catholic* describes leprosy as one of the “maladies of a sinfull soule,” going so far as to single it out specifically (*Defence of the Reformed Catholike*, n.p.). In such a religious climate, leprosy became a way to associate someone as morally unclean, cementing the connection between physical ailment and moral character. Notably, leprosy is perhaps one of the most extreme examples of this, as Abbot goes a

step further to speak about “leprosie of sinne” (*Defence of the Reformed Catholike*, n.p.). This phrasing suggests sin itself is a physical disease and inextricably ties leprosy to all sin. A religious text from the same time period called *The food of the soule* also describes an infection of sin as leprosy: “for if originall sin do continually dwell in vs, and infect all our actions with deadly sinne, as they teach: Gods name can not be sanctified in vs, that are infected with such an vnclean leprosie” (*The food of the soule*, n.p.). The word choice here is significant: the author says that sin dwells in us and infects our actions, much in the same way that a physical illness dwells in us and infects us. *Richard III* also uses the word “infection” in telling ways, so this is an interesting parallel. Additionally, it is important to note that sin is directly referred here as unclean leprosy. Rather than suggesting that sin is a more unclean version of leprosy, this seems to highlight the uncleanness -- both physically and morally -- associated with leprosy. Leprosy is just one example of a way people in the 1600s associated physical illness with immorality.

Not only did religion use physical illness to determine one’s morality, but there was a field of science dedicated to the study of how physical features reflect personality. This science, Physiognomy is described as that “which instructeth a man by the outwarde notes to foretell the naturall motions, and actuall conditions especially in those, which liue after their affection and appetites, rather then gouerning themselues by reason” (*A Pleasant History* 1). Already there appears to be bias in how people are evaluated, as this text says that outward features particularly tell of inward conditions when those people are more driven by emotion and are unreasonable. As such, it is not surprising that this text’s evaluation of signs of good nature and memory include “moyst and soft flesh, being a meane betwene roughnesse and lenity, and meane of stature, and hath a ruddie continuance suffused with White nesse, and a gentle or friendly looke: whose hayre on the head is plaine lying, the head meanly big with an equality” (7). Essentially, good nature is associated with the fair, healthy-looking, and well-proportioned. It is also interesting that this passage ends by saying that this countenance is “framed after a chcerful and mery sort,” effectively claiming that this person has that ineffable quality of looking like they have a good nature and that’s how one can just tell (7). There are other features that seem to be easier to pin down: notably for the analysis of Richard III’s deformities, in the sections titled “condition and judgement of shoulder points” and the “condition and judgement of the backbone” (178). A physiognomer “sildome saw any person beeing crooke backed, which were of a good nature: but that these hauing the like bearing out, or bunche on the shoulders, were rather Trayterous, and verie wicked in their actions” (161). The analysis of those with a crooked back or a hump reaching over their shoulders is essential to the reading of Richard III’s disabilities as the Shakespearean audience would have seen them: his deformities would have been a sign of wickedness. It is also important to note that the language used to describe this evidence gives a strong case for correlation, but correlation does not mean there is a true causal connection. Further, these crooked-backed people were thought to have “a deepe retching with So that it seemeth impossible after Nature, that such deformed persons should possesse in them law | dable actions” (161). This demonstrates that the bias is in fact so strong that people with such deformities are thought to not be capable of doing commendable deeds.

The judgement of the backbone seems to have a strong basis in temperament and feelings that might be associated with femininity or masculinity. First, physiognomy says that the backbone “doth argue strength of the creature,” and then proceeds to describe a muscular backbone ride as associated with masculinity while a soft, fleshy, and narrow one is feminine (178). People with a feminine-type backbone are described as having actions that are “knowne weake, and feeble in conditions, yea these haue a dead minde or faint courage,” while overly masculine backbones are associated with “such a pers to be strong, arrogant, and furious in yre” (178). Richard III never describes what his backbone looks like, but it is possibly that his backbone is more feminine. Perhaps that is why he draws such attention to the female body -- to point out the differences from his own. He may be worried that people think he has he has “weak,” womanly qualities. If so, this is yet another example of Richard directing the audience’s

attention to manipulate how he is perceived to his benefit. Finally, physiognomy addresses a crooked back: “the crookednesse of the backe, doth intimate the wickednesse of conditions: but an equality of the backe, is then a good note” (178). Similar to the analysis of a humped shoulder, a crooked back denotes wickedness while a symmetrical back denotes goodness. This matches the other characters’ assessment of Richard as wicked. Further examination of the crooked backbone, however, provides an interesting perspective on Richard: “if the same be couered with soft flesh, and slender in the gyrtling place: doth denote such a person, to excell in naturall giuftes, and to haue very great pleasure in hunting. Yet some affirme, that the crookednesse of the backe, to declare the wickednesse of conditions” (178). This is particularly notable if it is assumed that Richard’s obsession with female anatomy masks an insecurity -- if he does have a crooked backbone with soft flesh, then this physiognomy analysis rings eerily true. Richard excels in the natural gifts for reading situations and manipulating people, and in fact he essentially hunts people in the play. His potentially positive traits are twisted into wicked ones. Although the analysis of Richard using gendered physiognomy is revealing of his perspectives on the female body, the connection between a crooked back and wickedness and natural gifts again suggests the explanation for his talent at reading and manipulating people is rooted in Richard’s disability.

Francis Bacon agrees with the thesis that disabled people use their deformities as tools. First, the disability itself motivates them: “for as nature hath done ill by them, so do they by nature retaliate and so they have their revenge of nature” (The Essays, 117). Because nature gave them deformities, they will push themselves to become something that perhaps nature may not have intended them to be. For example, perhaps they have the drive to be a king when they were not born to be a king. Bacon is also interested in how the body and the mind interact and whether nature’s impact on one will produce some effect on the other: “Certainly there is a consent, between the body and the mind, and where nature erreth in the one, she ventureth in the other” (117). Bacon is saying that any physical disabilities will be compensated by the mind. Therefore, it makes sense that Richard uses wit and manipulation to gain power and social standing that command respect to compensate for his body. In doing so, he has had great practice developing his understanding of the human condition.

Rather than argue that the deformity tells of an immoral spirit as physiognomy claims, Bacon says that “it is good to consider of deformity, not as a sign, which is more deceivable, but as a cause, which seldom faileth of the effect” (118). This is interesting because it implies that the nature of a person with a deformity is not fixed but forms as a result of living with a disability, perhaps if disabled people were treated better the internal drive caused by the deformity would not be one of malice. Bacon seems to agree: “whosoever hath anything fixed in his person, that doth induce contempt, hath also a perpetual spur in himself, to rescue and deliver himself from scorn” (118). It is because the deformity induces contempt that one develops a “spur” to bitterly pursue a better position through whatever means necessary, like Richard did. Most relevant to the interpretation of Richard as someone who has developed the talent of manipulating others to compensate, Bacon says that the contempt “stirreth in the disabled industry, and especially of this kind, to watch and observe the weakness of others, that they may have somewhat to repay” (118). It seems that Bacon understands the very reasons that Richard pursues a higher calling by manipulating people. Perhaps it is because it is harder for the disabled to gain power without understanding the weaknesses of others, or perhaps because managing a “weakness” their whole lives could give disabled people a better understanding of weaknesses. Bacon also lists a reason that using disability as a tool is so effective: “it layeth their competitors and emulators asleep, as never believing they should be in possibility of advancement, till they see them in possession. So that upon the matter, in a great wit, deformity is an advantage to rising” (118). Perhaps this is one of the reasons why Anne was so willing to listen to Richard, in her already very vulnerable state. Richard is cunning enough to recognize the advantage of people underestimating him and uses it -- coated in flattering words -- as a tool. Finally, it is important to note that Bacon acknowledges that this drive to make something of oneself and better

themselves to get revenge of nature would not inevitably be filled with malice. He says that the disabled “will, if they be of spirit, seek to free themselves from scorn—which must be either by virtue or malice—and therefore let it not be marvelled, if sometimes they prove excellent persons” (118). Although some people who constantly face mistreatment use this motivation in a negative manner, some people use the motivation as a reason to be a better person overall. The difference may be a question of natural disposition that a harsh environment exaggerates. Therefore, it is not inevitable that a physical disability will be a signal of an immoral person or a cause of immorality. Regardless, the experiences of a person with a disability tend to develop them into driven individuals capable of using their disability as a tool.

Richard’s disability is such an integral component that it melds with Richard’s power-hungry and sinister character. He recognizes how people perceive him, and so he decides to use his disability as a tool to commit evil acts. The other characters in the play compare Richard to animals and reference his violent character, communicating to the audience how they perceive Richard. The connection between medical disability and evil or misfortune is also frequently presented using an analogy of England’s poor state as though it is wounded. The analogy insinuates that England’s problems are because of Richard’s defects as a ruler and his infectious qualities. The inseparability of Richard’s character defects and physical defects in the minds of the other characters seems to perpetuate a cultural stereotype of the association between physical disabilities and evil. These cultural stereotypes even had a basis in physiognomy, which was thought to be a science during the 1600s. However, a more psychological interpretation by Francis Bacon posits that those with disabilities become driven or power-hungry as a result of how they are viewed by society. Although the perception of visible physical disabilities has largely changed since Shakespeare’s time, feelings of pity and horror are still evoked, and the impact of perceptions of certain conditions, such as leprosy or STDs, still lasts as a stigma today. While the assumptions made about physical disability in the play are erroneous and problematic, lessons can still be taken from the analysis of this play. Most notably, how you treat someone with a disability can cause them to feel their disability on a deeper level, which can create an emotional wound.

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**Sophie Troyer** is a senior majoring in biology and English who is interested in pursuing an MD-PhD in Immunology. She enjoys learning about topics in the health humanities because the field is so relevant to the reality of the patient experience, the range of interactions that patients have with the health care system in medicine. Sophie also conducts biology research in the Kesimer lab and has completed a senior honors thesis. During her time at UNC, Sophie has been an OUR Ambassador and Co-Editor-in-Chief of Carolina Scientific. This paper was written as a research project during a Shakespeare class, and it became personal when she realized that certain themes in Richard III aligned with her interest in health humanities, as well as her personal health experiences. Sophie is very passionate about the patient experience and hopes to reflect that in her future career. She would like to pursue medical research as a physician-scientist so that she can use her experiences to improve the lives of others who are going through similar health struggles.

# Natural Sciences

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SAVing Submerged  
Aquatic Vegetation:  
*Understanding Preferential  
Utilization of N Forms to  
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Carolina*

by **Elizabeth Farquhar**



# Chronic Alcohol's Effect on Anti PD-1 Immunotherapy

by Ali Khan

Programmed cell death 1 (PD-1) receptor targeted immunotherapy has emerged as an effective treatment for a variety of advanced malignancies. Anti-PD-1 antibodies have been FDA-approved for use in 13 cancer types since 2014, with trials in other types ongoing. The persistent responses and low side effect profile of anti-PD-1 immunotherapy makes this one of the most significant advances in modern cancer treatment. However, only a minority of patients respond to anti-PD-1 immunotherapy. Efficacy of anti-PD-1 immunotherapy requires adequate T cell responses in the patient. Thus, identification of modifiable risk factors that regulate fundamental mechanisms involved in T cell tumor responses could impact anti-PD-1 response rates across a variety of tumors. Understanding environmental or behavioral factors that reduce response rates to anti-PD-1 immunotherapy is, therefore, critical. Chronic alcohol use is common in the general population and alters T cell function. However, little is known regarding the effect of alcohol on responses to anti-PD-1 immunotherapy. In this proposal we directly address this significant need by assessing the impact of chronic ethanol on responses to anti-PD-1 immunotherapy in two different tumor models of cancers that are highly prevalent in humans and approved for anti-PD-1 immunotherapy. We find that chronic alcohol blocks the efficacy of anti-PD-1 treatment in a mouse model of bladder cancer (BBN963), increasing mortality and tumor growth to control/no anti-PD-1 levels. Preliminary findings suggest chronic ethanol reduces T cell infiltration into the tumor microenvironment. These data are compelling and warrant further investigation to clarify the effect of ethanol and investigate its mechanism of action. These studies could have immediate and direct clinical implications regarding the treatment of patients receiving anti-PD-1 immunotherapy across a variety of tumor types.

Key Words: PD-1, immunotherapy, chronic alcohol, cytokine, chemokine

## 1. Introduction

### 1.1 Cytokines, Chemokines, and T-cell responses to Cancer

Cytokines are small secreted proteins released by cells that influence the interactions and communications between cells. They are regulators of host responses to infection, immune response, and inflammation. Some of these proteins enhance immune responses, i.e. proinflammatory, whereas others which reduce inflammation and promote healing anti-inflammatory, both of which are essential for injury and infection response. Cytokines are identified in release of free serum and micro-vesicles (MVs). MVs are a reservoir that are often not measured in plasma studies but can transmit signals that are easy to detect [1]. Attention has focused on blocking cytokines, when they are harmful to the host, particularly during overwhelming infection. Interleukin (IL)-1 and tumor necrosis factor (TNF) are proinflammatory cytokines and when administered to humans, they produce fever, inflammation, tissue destruction, and, in some cases, shock and death [2]. Reducing the biological activities of IL-1, TNF, and other cytokines is accomplished by several different, but highly specific, strategies, which involve neutralizing antibodies, soluble receptors, receptor antagonist, and inhibitors of proteases that convert inactive precursors to active, mature molecules [3].

Chemokines, a subtype of cytokines, are small proteins that control the migration of the immune cells

cells to specific organs. They bind to G protein-coupled receptors that trigger intracellular signaling which facilitates the movement of immune cells such as T-cells into certain parts of the body. The chemokine receptors play a vital role in the regulation of T cell homing to inflammatory areas. In the presence of tumor cells, chemokines migrate T cells, NK cells, B cells, and immature DCs to the tumor, allowing these immune cells to attack and suppress the tumor from spreading to other parts of the body. Chemokines are considered tour guides for the immune cells to find and kill cancer cells affecting the host [4].

Once the chemokines direct the immune cells to the tumor cell, T-cells, a subtype of white blood cells, play a key role in fighting cancer. T-cells work in both direct and indirect ways to fight cancer. Natural killer T-cells are cytotoxic T-cells that need to be activated and differentiated to perform a task, killing tumor cells directly by apoptosis. In addition, helper T-cells indirectly assist in killing tumor cells by recruiting other immune cells and organizing an immune response [5].

### **1.2 Anti PD-1 Immunotherapy**

Immune checkpoint inhibitors have emerged as a treatment for different cancers, promoting immunotherapy as a revolutionary option for cancer treatment. Today, years of research have advanced our understanding of the role of the immune system in targeting and fighting tumors, as well as, reveal the tumor cells' ability escape these detection mechanisms. To protect the host from any threat, the immune system has evolved to identify and induce damage to harmful invaders, while allowing healthy cells to continue to thrive. The programmed cell death protein 1 (PD-1) pathway is one of the most intensively investigated regulators with its strong emphasis on optimizing T-cell function and maintaining the immune system's homeostasis. PD-1 detects any cells in the body that may be ruinous, emitting a signal for the T-cell to act accordingly. However, tumor cells attach and block the PD-1 protein attached to the T-cell to suppress immunity and evade immune surveillance. As a result, anti PD-1 pathway agents have been proposed to readily bind to the PD-1 proteins and allow T-cells to successfully detect and kill tumor cells [6]. Since 2014, anti PD-1 antibodies have been FDA-approved for use in 13 cancer types with continuing clinical trials on other malignant types. Conversely, few patients are responding to the anti PD-1 immunotherapy. For the anti PD-1 immunotherapy to bind accurately, the immune system must properly be functioning without the interference of external mechanisms such as chronic ethanol use that may hinder its effectiveness. With improper binding of the anti PD-1, PD-1 proteins could still attach to tumor cells and deactivate its targeted cell death function. Therefore, it is vital to understand how environmental or behavioral factors affect the response rate to the anti PD-1 immunotherapy [7, 8].

### **1.3 Chronic Alcohol Use and its Immunosuppressive Effects**

A risk factor investigated in this experiment is chronic alcohol and its effect on the anti PD-1 immunotherapy. Alcohol use is extremely common today with about 86.3% of people in the United States ages 18 or older reported that they drank alcohol at some point in their lifetime. 70.1% reported that they drank in the past year and 55.9% reported that they drank in the past month [9]. While common alcohol use seems harmless, chronic alcohol (i.e ethanol), alcohol misuse, is a worldwide health concern that may lead to damage in almost every organ of the body. According to the Centers for Disease Control and Prevention, nearly 88,000 people die from alcohol-related causes annually in the United States, and globally, in 2012, 3.3 million death, 5.9% of total deaths, were traced to chronic alcohol consumption [10].

Alcohol abuse has been linked to an increase susceptibility for opportunistic infections including pneumonia, toxoplasmosis, salmonella infection, and more due to a far weaker immune system. Studies indicate that acute intoxication is associated with attenuation of the inflammatory response while chronic alcohol exposure has a pro-inflammatory effect. Furthermore, ethanol may modify innate immunity with functional alterations of the cells within the immune system. It can affect antigen recognition and intracellular signaling events. As a result, there is significant evidence that ethanol exposure influences the immune system's functions and ability to fight off incoming pathogens [11-13]. Based on these current studies, urgent research is

needed in medical oncology to see associations between excessive alcohol use and efficacy of advanced immunotherapies.

#### 1.4 Experimental Design

In this experiment, we investigate the impact of chronic ethanol on responses to anti PD-1 immunotherapy in two different tumor models that are highly prevalent in humans and approved for this cancer treatment. The BBN tumor and anti PD-1 was injected into mice comparing the experimental group, chronic ethanol, and the control group, water. With a Luminex of the mice blood, we identified and compared cytokine responses to tumor cells. Findings from these studies suggest that chronic ethanol use reduces the infiltration of T-cells into the tumor microenvironment, decreasing the immune system's ability to eliminate these cancer cells. Therefore, blocking the efficacy of anti PD-1 to bind to the PD-1 protein on T-cells, which allows tumor cells to continue to grow in the host.

Based on the data, we will apply this knowledge to conduct a retrospective analysis of human PD-1 immunotherapy groups for alcohol intake and predict immune response based on cytokine levels. This study will provide significant evidence that alcohol use worsens outcomes to anti PD1, emphasizing certain recommendations of physicians for cancer patients to improve their therapy response.

The project I am working on is a continuing collaboration between two labs: Dr. Vincent and Dr. Coleman, who recently found that chronic ethanol blocks the anti PD-1 immunotherapy efficacy in the BBN model. I am investigating the changes in cytokines in plasma in the free and MV compartments between the control and alcohol groups [14].

## 2 Materials and Methods

### 2.1 Chronic Ethanol and Anti PD-1 Treatment

The mouse experiment was performed as previously described by the Vincent laboratory [14]. The injection process for ethanol was administrated by generating two groups: control and ethanol. In the ethanol group, 5 g/kg ethanol was injected per day intragastric for 5 weeks. The mice in the control group received water instead of ethanol for 5 weeks. After 5 weeks, the BBN963 tumor was injected ( $10^6$  cells, intradermal). BBN963 tumor injection occurred in this group after the 5-week ethanol period. The anti PD-1 antibody was given on post-tumor injection days 7, 11, and 14 for both the control and ethanol groups. Another ethanol group with the same anti PD-1 treatment was sacrificed on post-tumor day 10 for flow cytometry and blood collection for Luminex.

### 2.2 Luminex Assay

The Luminex Assays offer a wide selection of analytes for bead-based multianalyte profiling using cell culture supernatant, serum, or plasma samples. The procedure requires to run a full 1 x 96-well assay plate. After planning the plate layout, the Bio-Plex System began to warm up for about 30 minutes. The assay buffer was washed, and samples were diluted to room temperature while keeping the other items on ice until needed. After thawing the samples, they were prepared with the following dilution factors: mouse and rat cytokines were diluted by 1:4 with the Bio-Plex sample diluent, mouse ICAM-1 assay was diluted 1:200 with the Bio-Plex serum-based diluent. Prime wash station for flat bottom plate and prepared 1x wash buffer. After, the 10x stock was mixed by inversion to ensure all salts are in solution then diluted the 1-part 10x wash buffer (60 mL) with 9 parts distilled H<sub>2</sub>O (540 mL). By following Bio-Plex Manager Software prompts, the Bio-Plex System was properly calibrated. A single vial of standards in 500 uL of a diluent similar to the final sample type or matrix was reconstituted, vortexed for 5 seconds, and incubated on ice for 30 minutes. A fourfold standard dilution series and blank were prepared and vortexed for 5 seconds between liquid transfers, changing tips among dilutions. The 10x or 20x coupled beads were vortexed for 30 seconds and diluted to 1x in Bio-Plex Assay Buffer, making sure to protect from external light. While running the assay, the diluted (1x) beads were vortexed for 10-20 seconds, adding 50 uL to

each well of the assay plate. Once samples were made, the plate was washed two times with 100  $\mu$ L Bio-Plex Wash Buffer and all samples, standards, blank, were vortexed, ensuring a proper mix. Then, 50  $\mu$ L was added to each well. Step 4 of running the assay consisted of covering the plate with sealing tape and incubating on the shaker at  $850 \pm 50$  rpm at RT. With 10 minutes left in the incubation, the 10x or 20x detection antibodies were vortexed for 5 seconds and quick spin to collect the liquid, diluting to 1x. The plate was washed three times with 100  $\mu$ L wash buffer and vortexed the diluted (1x) detection antibodies, adding 25  $\mu$ L to each well. Repeating the step 4, the plate was covered with sealing tape and incubated on shaker for its respective time. With 10 minutes left in incubation, the 100x SA-PE was vortexed for 5 seconds and quick-spin to collect liquid. The solution was diluted to 1x and protected from external light. Then, the plate was washed three times with 100  $\mu$ L wash buffer and the diluted (1x) SA-PE was vortexed, adding 50  $\mu$ L to each well. Repeating step 4 with its respective incubation time, the plate was washed three times with 100  $\mu$ L wash buffer and beads resuspended in 125  $\mu$ L assay buffer. Step 4 was repeated, and the plate was shaking at  $850 \pm 50$  rpm for 30 seconds. After, the sealing tape was removed, and the plate read at the Bio-Plex system settings for optimal sensitivity.

### 2.3: Statistical Analysis

Once the Luminex data was gathered, we had obtained a wide array of cytokines with free serum and micro vesicle data extracted from the sample. Each value was an average cytokine level of the respective duplicates. Two sample equal-variance t-tests were used to compare each cytokine's control and ethanol groups. Once conducting the test, it resulted in p-values indicating the probability of the result or one more extreme to occur if our null hypothesis, control and ethanol groups had the same cytokine levels, was true. In addition, we performed a Grubbs' test, also called the ESD method (extreme studentized deviate), to determine whether one of the values in the control or ethanol lists entered was a significant outlier from the rest. Outliers for each free serum and micro vesicle cytokine was calculated and mentioned in the data report. Once we distinguished these values, we re-conducted the two-sample t-tests to observe if any significant differences between the control and ethanol groups among the free serum and micro vesicle cytokines were present. Furthermore, a FDR test was conducted to conceptualize the rate of type I errors in null hypothesis testing when conducting multiple comparisons and control the expected proportion of "discoveries" (rejected null hypotheses) that are false (incorrect rejections) [15].

### 2.4 Literature Search for Future Retrospective Analysis

A literature search for future retrospective analysis was conducted to provide a baseline on patient data extraction and application from the Luminex results. This included searching PubMed for previous studies that included a retrospective analysis of a disease involving the effect of alcohol abuse. Search terms included but were not limited to: "retrospective", "alcohol" "chronic", and "cancer". Once studies were found, the identified studies were narrowed to inquire for papers that had specifically stated variables used from patient records including the distinction between alcohol consumption levels, smoking habits, family history, sociodemographic characteristics, and more. Studies involving cancer-related mortality were preferred, but the search expanded to any chronic disease with detailed alcohol-consumption retro-analysis and possible influential variables.

## 3 Results

The results from the Luminex Assay data indicate cytokines with significant differences in interaction between control and ethanol groups. The cytokines that were significant include: MV-IL-1a, MV-IL-2, MV-IL-12(p70), MV-KC, MV-TNF-a, IL-12(p40), RANTES. These cytokines demonstrated higher or lower levels compared to the control group depending upon if they had presented a pro- or anti-inflammatory response. Table 1 provides an overview of these cytokines, comparing the mean values ( $\pm$  SEM) of the control and ethanol groups and respective p-values. Table 2 is the performed t-test with FDR method to account for multiple comparisons. Cytokines which were found to be significant by the t-test, remained significant to a Q=12% with the FDR method. After providing a summary of the significant cytokines, respective figures of individual cytokine interactions between the control and ethanol group were analyzed. Specifically, Figure 1 displays the distribution of IL-1a cytokine release of micro-vesicles with a decrease in cytokine release among the ethanol group. Figure 2 displays the distribution of IL-2 cytokine release of micro-vesicles with a similar pattern to MV-IL-1a. Figure 3 compares IL-12(p70) cytokine release of micro-vesicles between the control and ethanol groups with an increase in the cytokine release among the ethanol group. Figure 4 compares KC cytokine release of micro-vesicles and provides a similar distribution to MV-IL-12(p70). Figure 5 compares TNF-a cytokine release of micro-vesicles with a response analogous to MV-IL-12(p70) and MV-KC. Figure 6 displays a significant difference ( $p = 0.008$ ) of the IL-12(p40) free-serum cytokine release between the control and ethanol groups, and Figure 7 compares RANTES free-serum cytokine release.

Once conducting two-sample t-tests, confidence intervals and p-values were generated distinguishing if a cytokine type significantly varied between the control and alcohol groups. According to our results, cytokines releasing micro-vesicles were pre-dominantly considered more significant than the free serum, in which there is sufficient evidence to conclude that the levels of control to alcohol samples were different for

those respective cytokines.

**Table 1. Luminex Assay Data of Significant Cytokines' Mean ( $\pm$  SEM) Between Control and Ethanol Groups.**

Type	MV-IL-1a	MV-IL-2	MV-IL-12(p70)	MV-KC	MV-TNF- $\alpha$	IL-12(p40)	RANTES
Control	19.62 (2.69)	52.39 (7.16)	48.90 (3.65)	18.95 (1.95)	48.13 (3.51)	436.90 (53.07)	282.12 (38.49)
Alcohol	12.33 (0.80)	45.21 (4.22)	64.97 (5.00)	27.97 (2.44)	68.87 (7.23)	271.33 (21.69)	206.75 (14.20)
<i>P-value</i>	0.04*	0.04*	0.02*	0.01*	0.03*	0.008**	0.07

**Table 2. FDR method to account for multiple comparisons at Q=12%**

	Discovery?	T-test P value	FDR q value
IL-1a	Yes	0.041	0.07
IL-2	Yes	0.038	0.07
IL-9	No	0.50	0.38
IL-10	No	0.20	0.23
IL-12-p40	No	0.31	0.31
IL-12-p70	Yes	0.02	0.07
IL-17	No	0.16	0.21
Mo <del>gotaxin</del>	No	0.996	0.69
Mo <del>IFNg</del>	No	0.42	0.37
Mo KC	Yes	0.011	0.07
Mo MIP-1b	No	0.16	0.21
RANTES	No	0.51	0.38
<del>TNF<math>\alpha</math></del>	Yes	0.026	0.07

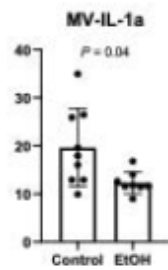


Figure 1. Comparing IL-1a cytokine release of micro-vesicles between the control and ethanol groups

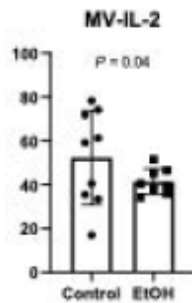


Figure 2. Comparing IL-2 cytokine release of micro-vesicles between the control and ethanol groups

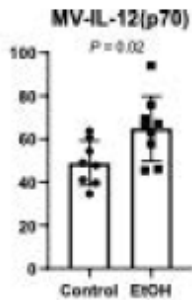


Figure 3. Comparing IL-12(p70) cytokine release of micro-vesicles between the control and ethanol groups

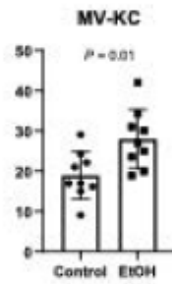


Figure 4. Comparing KC cytokine release of micro-vesicles between the control and ethanol groups

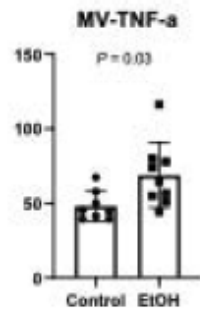


Figure 5. Comparing TNF-a cytokine release of micro-vesicles between the control and ethanol groups

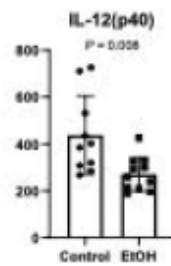
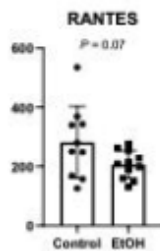
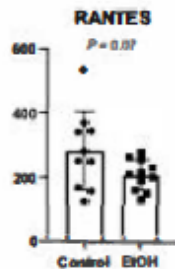


Figure 6. Comparing IL-12(p40) free-serum cytokine release between the control and ethanol groups







**Figure 7. Comparing RANTES free-serum cytokine release between the control and ethanol groups**

#### 4 Discussion

Based on the Luminex results, the cytokines that were initially analyzed include: IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-70, IL-KC, TNF, RANTES, MIP-1a, MIP-1b, Exotaxin, G-CSF, GM-CSF, IFN-g, MCP-1. These cytokines were either pro or anti-inflammatory responses from the anti PD-1 immunotherapy upon the tumor cell invasion. As stated, once confidence intervals and p-values were generated distinguishing if a cytokine type significantly varied between the control and alcohol groups, we had found that cytokines releasing micro-vesicles were pre-dominantly considered more significant than the free serum. Specifically, IL-1a, IL-2, IL-12(p70), KC, and TNF-a were MVs significantly different between the control and alcohol groups. Depending on if the cytokine was proinflammatory or anti-inflammatory determined if the alcohol cytokine levels were greater or less than the control. IL-1a and IL-2 responded with lower ethanol levels than the control. Previously, the Vincent lab had found that increased levels of these respective cytokines had a positive response with the anti PD-1 immunotherapy [14], therefore, providing a possibility that IL-1a and IL-2 are lower due to alcohol, leading to a loss in the anti PD-1 efficacy. On the other hand, IL-12(p70), KC, and TNF-a had higher ethanol levels than the control with unclear implications. Based on these results, MVs could be possible biomarkers or unique mediators of pathology applied to the efficacy of anti PD-1 immunotherapy. In regard to the free serum, there were not as many cytokines that were considered significant as they had far greater variability, resulting in difficulty in obtaining a reliable outcome. In this case, we will need to conduct further experiments to minimize the variability and observe if there are cytokine response differences between the respective control and alcohol groups.

#### 5 Future Directions

##### 5.1 Human Retrospective Study

Planning for next semester, a human retrospective study will be conducted as the data discussed suggests that ethanol is blocking PD-1 interaction with the anti PD-1, allowing tumor cells to invade and replicate within the host. By applying the information of the mice Luminex assay, we may investigate if chronic alcohol had a similar effect on humans. If so, it would indicate that anti PD-1 immunotherapy cancer patients who had consumed an excess of alcohol would have fewer effective results from the treatment compared to cancer patients who had minimally or never drank alcohol. In effect, these results could have immediate and direct clinical implications regarding the treatment of patients receiving anti PD-1 immunotherapy across a variety of tumor types.

## 5.2 Impact of Intestinal Microbiome by Chronic Alcohol Use

Beyond the PD-1 blockade, studies indicate that chronic alcohol use impacts the composition of the intestinal microbiota. The intestinal microbiota is the total collection of microbial organisms within the gastrointestinal tract (GIT), containing tens of trillions of microorganisms of different bacterial species. The microbiota is vital to energy extraction from ingested food and helps form barriers against invading pathogens. The disruption of the intestinal microbiota, dysbiosis, is linked to inflammatory bowel disease, irritable bowel syndrome, food allergies, diabetes, cancer, obesity, and cardiovascular disease. Chronic alcohol use is a factor that causes the dysbiosis of the intestinal microbiota and could affect the immune system's ability to effectively fight against destructive cells, including tumor cells. In a future application, we will examine how alcohol's effect on the dysbiosis of the microbiome impacts the efficacy of the anti PD-1 immunotherapy.

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**Ali Khan** is an undergraduate sophomore at the University of North Carolina at Chapel Hill majoring in Biostatistics in the Gillings School of Global Public Health. He is passionate about research that involves integration of biostatistics, cancer, and public health to advance primary care outcomes. Ali joined as a research student under the Vincent Lab at the UNC School of Medicine this year, conducting biostatistical tests analyzing chronic alcohol's effect on anti-PD1 immunotherapies and fecal microbiome analysis investigating chronic alcohol use causes a reduction in microbiome diversity. He had developed this fond interest in biostatistics after his summer experience in which he collaborated with the Hematology-Oncology and Biostatistics teams in the Levine Cancer Institute to review and edit professional clinical review papers on standardized therapies and genetic biomarkers for newly or relapsed-diagnosed Multiple Myeloma patients. Outside of academics, Ali enjoys playing squash, editing films, and watching horror movies.

# SAVing Submerged Aquatic Vegetation: *Understanding Preferential Utilization of N Forms To Reduce Cyanobacteria Blooms in Lake Mattamuskeet, North Carolina*

## by Elizabeth Farquhar

Lake Mattamuskeet, located on the Albemarle-Pamlico Peninsula in North Carolina, is the state's largest natural freshwater lake and one of the most important waterfowl wintering grounds on the U.S. East Coast. Since the early 1990's, the lake has lost 93% of its submerged aquatic vegetation (SAV), and has shifted to a phytoplankton-dominated lake. The wide-spread loss of SAV has corresponded with an increase in cyanobacterial, harmful algal blooms (HABs) that pose a threat to the recreational users and wildlife of the lake. Therefore, it is important to understand factors that promote cyanobacterial dominance, so environmental managers with Mattamuskeet National Wildlife Refuge and the NC Department of Environmental Quality can most effectively target their SAV-restoration plan at the most problematic bloom organisms. Previous scientific studies suggest that certain nitrogen forms, particularly ammonium, may selectively stimulate the growth of cyanobacteria relative to other, non-HAB forming phytoplankton groups. Our study used experimental nutrient enrichments of ammonium and nitrate to Lake Mattamuskeet waters to assess whether the growth of cyanobacteria is stimulated by ammonium enrichment more than other phytoplankton taxa. Results from two bioassay experiments indicated that ammonium vs nitrate enrichment did not significantly increase the growth in cyanobacteria populations versus other phytoplankton groups. Nutrient reduction strategies to decrease cyanobacteria biomass in Lake Mattamuskeet should consider nutrient loading from both ammonium/nitrate sources and phosphorous, as there was evidence of the system being primarily P-limited in some cases.

**Key words:** harmful algal blooms, bioassays, Lake Mattamuskeet, nitrogen loading, cyanobacteria

### 1. Introduction

Lake eutrophication is becoming an increasingly common condition, as humans contribute excess nutrients to waterways that exacerbate harmful algal blooms (HABs). HABs are often dominated by cyanobacteria that can produce toxins, accumulate quickly due to reduced grazing pressure, and outcompete the rest of the phytoplankton community for nutrients and light (Urrutia-Cordero et al., 2016; Gobler et al., 2012). Not only can HABs lead to hypoxic water conditions and disrupt an ecosystem's food chain, but they occur globally and can have social/economic impacts on the humans interacting with the system. For example, in 2014, HABs in Lake Erie caused an estimated \$65 million of damage to the water drinking system in Toledo, Ohio (Newell et al., 2019). From 2000-2004, there were mass kills of flamingos from the Embakaai crater, Lake Natron, and Lake Manyara in Tanzania due to HABs, which significantly decreased the tourism in the area

(Kimambo et al., 2019). On the East Coast of the United States in the late 1980's, the bay scallop fishery collapsed because the HABs occurring led to widespread scallop deaths and recruitment failure (Bricelj et al., 1989).

In order to reduce these harmful blooms, nutrient reduction is most often the prescribed mitigation method. Nitrogen (N) and phosphorous (P) have long been identified as the primary limiting nutrients in aquatic systems and therefore, the primary agents in causing HABs (Paerl et al., 2016; Glibert et al., 2015; Newell et al., 2019). However, there is a lack of consensus in the scientific community surrounding how different forms of each nutrient may affect community composition of the phytoplankton. Because P is usually only found in the PO<sub>4</sub> form in freshwater systems while N may be primarily found as nitrate (NO<sub>3</sub>) or ammonium (NH<sub>4</sub>), much of the debate is centralized on the effect of different forms of N (Paerl et al., 2016).

Ammonium is a reduced form of N, so generally it is more energy efficient for phytoplankton to utilize this form instead of NO<sub>3</sub>, the oxidized form. Reduced forms of nitrogen require less energy to convert to organic nitrogen and can move across the cellular membrane easier (Newell et al., 2019). Because NO<sub>3</sub> has to be reduced before phytoplankton can assimilate it, the presence of NH<sub>4</sub> has been shown to inhibit NO<sub>3</sub> assimilation in N-limited conditions, causing phytoplankton to uptake NH<sub>4</sub> before NO<sub>3</sub> (Tada et al., 2009; Glibert et al., 2015). Although it is well understood that NH<sub>4</sub> is the energetically favorable choice of N for phytoplankton, it is not well understood how the presence of NH<sub>4</sub> versus NO<sub>3</sub> would shape the phytoplankton community composition and growth rates, especially regarding cyanobacteria. Because cyanobacteria are the dominant phytoplankton group in HABs, it is important to understand the factors that lead to their persistence and whether certain types of nutrient forms contribute to their dominance more than others.

Because NH<sub>4</sub> generally requires less energy to uptake for phytoplankton and most cyanobacteria have a larger volume to surface area volume than other groups, it has been hypothesized that the presence of NH<sub>4</sub> would contribute to their dominance since they theoretically should be able to uptake nutrients quicker than other species. Donald et al. 2011 conducted an experiment that supported this hypothesis and found that non-heterocystous cyanobacteria did experience faster growth when enriched with NH<sub>4</sub>, while NO<sub>3</sub> favored the growth of cryptophytes and heterocystous cyanobacteria. Newell et al. 2011 similarly found that the growth rates of non-heterocystous *Microcystis* cyanobacteria were higher when enriched with NH<sub>4</sub> over NO<sub>3</sub>, which is likely because of they have a different N-fixing pathway. These experiments demonstrated that different N-forms may evoke different responses from each phytoplankton group as well as within each. While cyanobacteria are especially of interest because of their ties to HABs, the response of other phytoplankton groups should be examined as well, as they may affect nutrient availability.

For instance, one study observed that the growth of the diatom, *Cylindrotheca fusiformis*, completely ceased when its N source was switched from NO<sub>3</sub> to NH<sub>4</sub>/urea due to the lack of a metabolic pathway for processing urea (Hildebrand & Dahlin, 2000; Solomon et al., 2010). A different study found congruent results; diatoms living in cooler waters grew faster when enriched with NO<sub>3</sub> instead of NH<sub>4</sub> (Glibert et al., 2015). Since diatoms often dominate in colder waters and the winter months, it has been thought that spring diatom blooms use up the available NO<sub>3</sub>, leaving NH<sub>4</sub> to be the primary limiting nutrient for summer cyanobacterial blooms (Dugdale et al., 2007). Therefore, the presence of spring diatoms may affect what nutrients are later available for cyanobacteria growth, especially if those cyanobacteria are non-heterocystous. However, a contradictory study found that NH<sub>4</sub> caused two species of *Skeletonema* diatoms to grow faster than NO<sub>3</sub>, suggesting that NH<sub>4</sub> would not affect cyanobacteria more than any other group of the phytoplankton community (Tada et al., 2009). The variety of results observed within the scientific community on NO<sub>3</sub> versus NH<sub>4</sub> enrichment has led to a focus on species-specific responses, as the metabolic pathways for processing each N form may differ greatly (Donald et al., 2013).

Lake Mattamuskeet in eastern North Carolina has recently become eutrophic and subsequently

dominated by cyanobacterial HABs. The shallow, 17,000-ha lake is the center of the Mattamuskeet Wildlife refuge which is comprised of approximately 11 different habitats (e.g. freshwater marsh, open water, and wet pine flatwoods forest), serving as breeding and feeding grounds for its various wildlife (USFW, 2017). Furthermore, the refuge hosts hundreds of thousands of migratory wintering waterfowl every year that depend on its submerged aquatic vegetation (SAV) and wetlands for sustenance and rest (Moorman et al., 2017). However, as of 2017, 37% of the land draining into the lake is intensively farmed, and 1500 ha of the surrounding land are waterfowl impoundments, both contributing excess  $\text{NO}_3$  and  $\text{NH}_4$  into the lake, respectively (Winton et al., 2016). Cyanobacteria blooms outcompete SAV for light and nutrients, which has caused the loss of 93% of the lake's SAV since the early 1990's (Moorman et al., 2017). The eutrophication of the lake has ultimately driven a shift from a macrophyte-dominated, clear water system to a phytoplankton-dominated, turbid system with HABs (Moorman et al., 2017).

The widespread loss of SAV threatens the food supply for migratory bird populations and has changed the way they have been observed using the lake, e.g. sitting instead of actively feeding (Moorman, 2018). Additionally, the increase in phytoplankton has impacted the recreational users and blue crab industry of the lake (Moorman, 2018). The HABs are dominated by the heterocystous cyanobacteria, *Cylindrospermopsis*, which can produce liver toxins that bioaccumulate in the crab and fish population (Moorman, 2018). Because of regular HABs in the lake, community members have been warned to limit their crab intake and swimmers have been cautioned to rinse thoroughly after coming into contact with the water, further exemplifying the effects HABs can have on the community and the need for mitigation (USFW, 2017).

The changing lake has provoked management at the Mattamuskeet Wildlife Refuge to make initial assessments and recommendations on the water quality and changing fauna of the lake (Moorman et al., 2017). They found that the total nitrogen (TN) and total phosphorus (TP) dissolved in the lake were about equally elevated, which compelled researchers to hypothesize and test that the surrounding waterfowl impoundments and agricultural runoff are the main nutrient contributors (Winton et al., 2016; Moorman et al., 2017). While management has completed a watershed restoration plan for overall nutrient loading reduction, it does not consider the potential effects of different forms of nitrogen loading (USFW, 2017). The two main hypothesized main sources of nutrient loading to the lake are the surrounding waterfowl impoundments and agricultural runoff, which contribute mainly  $\text{NH}_4$  and  $\text{NO}_3$ , respectively (Winton et al., 2016). Assessing which form of N is most important to reducing cyanobacterial HABs may assist the Wildlife Refuge in targeting nutrient reductions from these sources and therefore restoring SAV.

Our study aimed to examine the phytoplankton community of Lake Mattamuskeet by using experimental nutrient enrichments to assess the following: 1) whether  $\text{NH}_4$  versus  $\text{NO}_3$  enrichments led to a higher growth rate in cyanobacteria over other phytoplankton groups and 2) whether cyanobacteria would dominate the community composition in groups enriched by  $\text{NH}_4$ . We hypothesize that high availability of  $\text{NH}_4$  relative to  $\text{NO}_3$  will cause cyanobacteria to grow quicker than other groups because of its uptake efficiency, and cyanobacteria will comprise a larger percentage of the community in groups enriched with  $\text{NH}_4$  over  $\text{NO}_3$ .

## 2. Methods



**Figure 1. Sampling sites for this study and sites sampled by the United States Geographic Survey (USGS) for Lugol's preserved phytoplankton community samples.**

Because the lake is separated into two basins as a result of the construction of Highway 94 through the middle, we used water samples from both the east and west side in order to assess whether there are any spatial variations in the phytoplankton community. Using these water samples, we evaluated the growth of different phytoplankton taxa under  $\text{NO}_3$  and  $\text{NH}_4$  enriched conditions.

### *2.1 Establishing a baseline for community composition*

It was necessary to use microscopy to determine the community composition of the phytoplankton of Lake Mattamuskeet to assess whether the community at the time of our experiments was representative of a typical phytoplankton community for Lake Mattamuskeet. Using twelve previously collected water samples from the USGS sites spanning throughout 2017-2019 (Figure 1), six abundant taxa of phytoplankton were identified and quantified for biomass concentration: *Cylindrospermopsis*, *Pseudoanabaena*, *Spirulina*, *Planktolyngbya*, *Scenedesmus*, and pennate diatoms. Of these, *Cylindrospermopsis*, *Pseudoanabaena*, *Spirulina*, and *Planktolyngbya* are potential toxin-producing cyanobacteria, and *Cylindrospermopsis* is a  $\text{N}_2$  fixing species. The 300 mL samples were inverted to homogenize the mixture and then pipetted into a 0.1 mL haemocytometer and covered with a glass slip. Each sample settled in the haemocytometer for 5-10 minutes, and then a  $100 \times 100 \mu\text{m}$  field view was used at 400x magnification to count the number of each abundant species using a Leica DMIRB inverted microscope (Wetzlar, Germany). Counting ceased when 100 individuals were counted or when 50 fields had been counted, whichever occurred first.

Before calculating the biovolume concentration of each species for each sample, the average volume of each species was estimated using the mean measurements the cells from each taxon (e.g. length, radius). We assumed that the volumes of *Cylindrospermopsis*, *Planktolyngbya*, *Pseudoanabaena*, and *Spirulina* could all be estimated using the equation for the volume of a cylinder. Pennate diatoms were envisioned as two facing cones and *Scenedesmus* as four stacked cylinders. The biovolume concentration was then estimated using the following formula:

$$C = VnA/Hfa \quad (1)$$

where  $V$  is the estimated volume of a species,  $n$  is the number of cells counted,  $A$  is the area of the haemocytometer (2.5434 cm<sup>2</sup>),  $H$  the volume of the haemocytometer (0.1 mL),  $f$  the number of fields counted, and  $a$  the area of one field at 400x magnification (0.00060025 cm<sup>2</sup>).

### 2.2 Bioassays

To analyze how NH<sub>4</sub> and NO<sub>3</sub> enriched conditions may affect the community composition of phytoplankton in Lake Mattamuskeet, two bioassays were conducted two weeks apart, on October 11-14th and October 25-28th. For each experiment, water samples were taken from the east and west side of Lake Mattamuskeet, sites 1 and 2, respectively, using two 20 L carboys (Figure 1). General water quality information (temperature, salinity, pH, dissolved oxygen, and turbidity) was measured using a YSI 6600 (Yellow Springs Inc, Yellow Springs, Ohio) multiparameter sonde at the time of collection (Table 3). Each sample was divided into 800 mL and placed into 12 one-liter cubitainers that represented three triplicated treatment groups and a control, as detailed in Table 1.

Table 1. Bioassay Design

	Cubitainer number and treatment received			
West	1 - 3	4 - 6	7 - 9	10 - 12
	Control	50 $\mu\text{mol}$ NO <sub>3</sub> + 5 $\mu\text{mol}$ PO <sub>4</sub>	50 $\mu\text{mol}$ NH <sub>4</sub> + 5 $\mu\text{mol}$ PO <sub>4</sub>	5 $\mu\text{mol}$ PO <sub>4</sub>
East	13 - 15	16 - 18	19 - 21	22 - 24
	Control	50 $\mu\text{mol}$ NO <sub>3</sub> + 5 $\mu\text{mol}$ PO <sub>4</sub>	50 $\mu\text{mol}$ NH <sub>4</sub> + 5 $\mu\text{mol}$ PO <sub>4</sub>	5 $\mu\text{mol}$ PO <sub>4</sub>

Approximately 10 mg of dissolved inorganic carbon (DIC) was added to all cubitainers to ensure carbon limitation did not occur during incubation. Nitrate was added in the form of NaNO<sub>3</sub>, ammonium in the form of NH<sub>4</sub>Cl, and phosphate as K<sub>2</sub>PO<sub>4</sub>. The cubitainers were then incubated in a concrete pool outside to simulate ambient light and temperature conditions; they incubated for about 3 days under a 1 layer neutral density screening to prevent unrealistically high and potentially damaging irradiance levels.

### 2.3 Bioassay Analytical Methods

Nutrient analyses were completed to assess the concentration of dissolved total nitrogen (TN), NH<sub>4</sub>, NO<sub>3</sub>, PO<sub>4</sub>, and silicate (SiO<sub>2</sub>) in 30 mL water subsamples from before ( $T_{\text{initial}}$ ) and after ( $T_{\text{final}}$ ) enrichment/incubation for each experiment. Before samples were run through a



nutrient autoanalyzer (Lachat Quick Chem. IV, Lachat Inc.), they were vacuum filtered (< 25 kiloPascals) through a 25 mm Whatman glass microfiber filter. The material retained on these filters was later used for the analysis of phytoplankton abundance by high performance liquid chromatography (HPLC) analyses of taxa specific accessory pigments. All filtering and subsequent activities were performed under reduced light conditions to prevent photodegradation of phytoplankton pigments.

The glass microfiber filters were collected during filtration, folded in half, blotted dry to remove excess water, and placed into 15 mL centrifuge tubes to be immediately frozen at -20°C until analysis. 1.5 mL of 100% acetone was added to each centrifuge tube and sonicated for 15-30 seconds using a Sonics Ultrasonic Disruptor with microtip to lyse the cells and release the pigments within. They were steeped at -20°C for approximately 24 hours, after which the solutions were extracted and filtered into 2 mL scintillation vials, using a syringe-driven filter.

The 2 mL scintillation vials containing the pigment extracts were then placed into the HPLC machine for analysis (Shimadzu system controller model CBM-20A, solvent delivery module LC-20AB), where 200 µL is extracted from each vial, separated according to pigments, and passed through a photodiode array spectrophotometric detector (PDA) to measure absorbance. Using Shimadzu's LabSolutions Lite software, the individual pigments are identified and quantified using a combination of peak retention time, absorbance spectrum shape/signature, maximum wavelength and the similarity match of the unknown pigment to a standard. This protocol was adopted from Paerl et al. (2013), which can be viewed for further information. The pigments and their concentrations are then translated to phytoplankton biomass using pigments known to be in particular phytoplankton classes, as summarized in Hoek (1977) and Paerl et al. (2013). For our study of Lake Mattamuskeet, a freshwater coastal system, we used the following chart to interpret the raw pigment data provided by the HPLC analysis, which is a simplified version of the previous literature on the topic based on the major groups observed during microscopy:

**Table 2. Phytoplankton photopigments used to interpret the raw pigment data**

<b>Pigment</b>	<b>Major phytoplankton group represented</b>
Chlorophyll a (chl a)	All
Fucoxanthin	Diatoms

<u>Myxoxanthophyll</u>	Cyanobacteria
Alloxanthin	<u>Cryptophytes</u>
Chlorophyll b ( <u>chl b</u> )	Chlorophytes

Although there are several more pigments that may indicate cyanobacterial abundance other than myxoxanthophyll, we chose to focus on that pigment since it is unique to cyanobacteria, while some of the other pigments can be found in other phytoplankton groups (Hoek 1977).

#### 2.4 HPLC Data Analysis

##### 2.4.1 Growth Rates

The HPLC data provided the concentrations ( $\mu\text{g/L}$ ) of each present pigment. We assumed the concentrations were representative of the concentration of its associated phytoplankton class, so we were able to use those concentrations from the HPLC analysis in conjunction with the following exponential growth formula to estimate the growth rates of each phytoplankton group:

$$P_{\text{final}} = P_{\text{initial}}e^{rt} \quad (2)$$

where,  $P_{\text{final}}$  represents the final population,  $P_{\text{initial}}$  represents the initial population,  $r$  is the growth rate, and  $t$  is the incubation time. We were able to solve for  $r$ , using pigment concentration as a proxy for population.

In order to assess statistical significance between the growth rates of different treatment and phytoplankton groups, we performed an analysis of variance (ANOVA) and tukey honest significant difference (HSD) post-hoc test. For all tests alpha was set at 0.05 on MatLab R2019a.

##### 2.4.2 Community Composition

We also used the pigment concentration data in a ChemTax program, which assesses the fraction each major phytoplankton group contributed to the total chl a measured, using the program MatLab R2019a. While the MatLab script for the ChemTax program was adopted from Macky et al. (1996), the input pigment ratio matrix was adapted from Paerl et al. (2014). Our matrix focuses on ten accessory pigments instead of twelve, as they were more representative to the phytoplankton classes observed during microscopy. The algal classes and their ratios of photopigments used for analysis are summarized in Table 3.

Table 3. Dominant algal classes and input accessory pigment ratios used for ChemTax.

Class	Chl a	Fuco	Cisneo	Myxo	Allo	Lut	Zea	Chl b	BCar	Echin
Diatom	1	0.514	0	0	0	0	0	0	0.002833	0
Crypto	1	0	0	0	0.368	0	0	0	0.003667	0
Chloro	1	0	0.038333	0	0	0.14733	0	0.357	0.003	0
Cyano	1	0	0	0.1358	0	0	0.28	0	0.0968	0.076

Crypto = Cryptophytes; chloro = chlorophytes; cyano = cyanobacteria; chl a = chlorophyll a; fuco = fucoxanthin; cisneo = 9' cis-neoxanthin; myxo = myxoxanthophyll; allo = alloxanthin; lut = lutein; zeax = zeaxanthin; chl b = chlorophyll b; BCar = β-carotene; Ech = echinenone

### 3. Results

#### 3.1 Basic water quality information

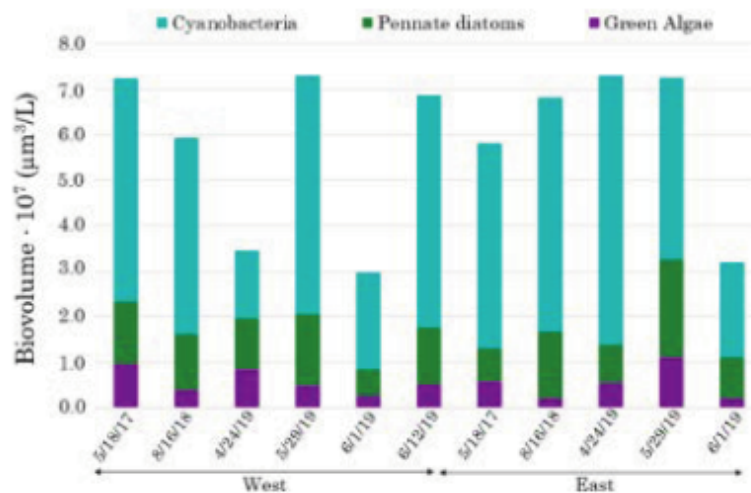
Table 4. Water quality at time of sampling

Table 4. Water quality at time of sampling

	Experimental Day 1 (10/11/19)		Experimental Day 2 (10/25/19)	
	West	East	West	East
Temperature (°C)	16.98	17.65	19.64	19.55
pH	7.66	7.83	8.10	7.96
Turbidity (NTU)	56.4	340	25.1	47.5
Dissolved oxygen %	101.0	98.5	94	103.7
Salinity (ppt)	0.53	0.5	0.56	0.57

Between sampling days, the overall conditions of the lake were comparable (Table 4). However, the turbidity reading for the east side on the first experimental day was more than 6 times higher than any other reading. This is likely due to an abnormal increase in suspended solids caused by elevated wave motion and sampling from the shoreline instead of 2 m from the shoreline. The measurements for temperature, pH, dissolved oxygen, and salinity for both the east and west sides varied from each other by 14.5%, 5.6%, 7.2%, and 5.5%, respectively. This indicates the conditions at the time of sampling for each side of the lake were comparable.

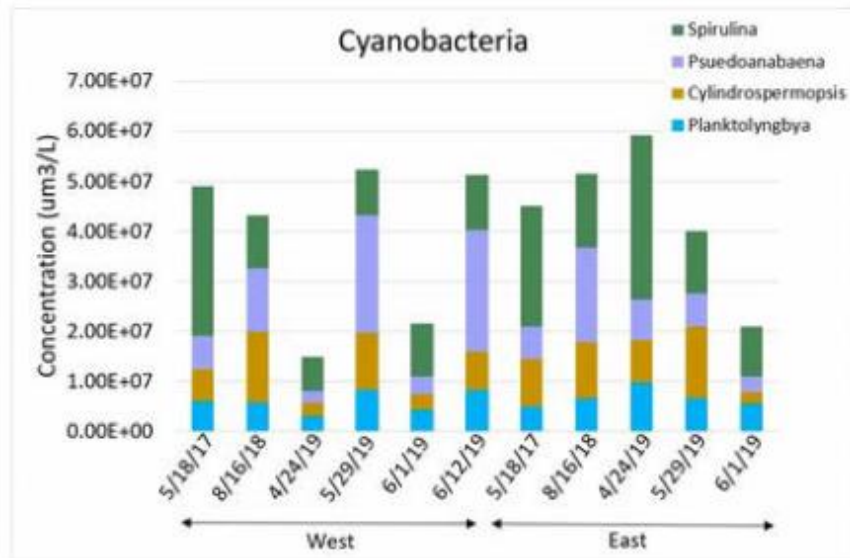
### 3.2 Baseline community composition



**Figure 2.** Overall temporal and spatial phytoplankton community composition of Lake Mattamuskeet. The biomass of *Planktolyngbya*, *Cylindrospermopsis*, *Psuedoanabaena*, and *Spirulina* were summed for the cyanobacteria category.

On average, 69% of the biomass of the lake's phytoplankton community is cyanobacteria year-round (Fig. 2). The east side of the lake showed a more consistent level of cyanobacteria dominance, while the west side showed a small fluctuation between spring and summer months, but they were still on the same order of magnitude.

A closer breakdown of the cyanobacteria groups throughout the time period indicates that *Spirulina* and *Psuedoanabaena* were the dominant two groups during the summer (Fig. 3). The cyanobacterial community on the east side of the lake was comprised of a higher amount of *Spirulina* than the west.



**Figure 3. Temporal and spatial cyanobacterial community composition of Lake Mattamuskeet**

### 3.3 Nutrient concentrations

During the first experimental day, the west side of the lake had  $\text{NO}_3$  levels about 18 times greater than the east side and  $\text{NH}_4$  levels about 4 times greater than the east side (Fig. 4). The  $\text{PO}_4$  levels after incubation did not vary significantly for either side. On the second experimental day, both sides of the lake had similar levels of  $\text{NO}_3$  and  $\text{NH}_4$  to start with (6-8  $\mu\text{g}/\text{L}$   $\text{NO}_3$  and about 17  $\mu\text{g}/\text{L}$   $\text{NH}_4$ ), which generally decreased after incubation (Fig. 5). The average initial total dissolved N level for both sides of the lake was 1500  $\mu\text{g}/\text{L}$ .

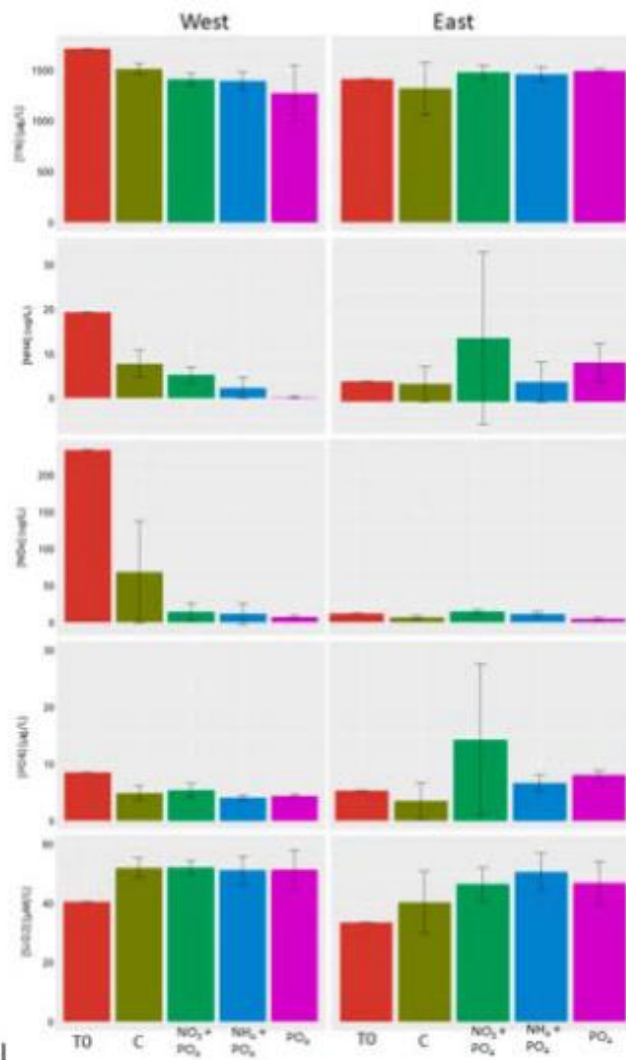
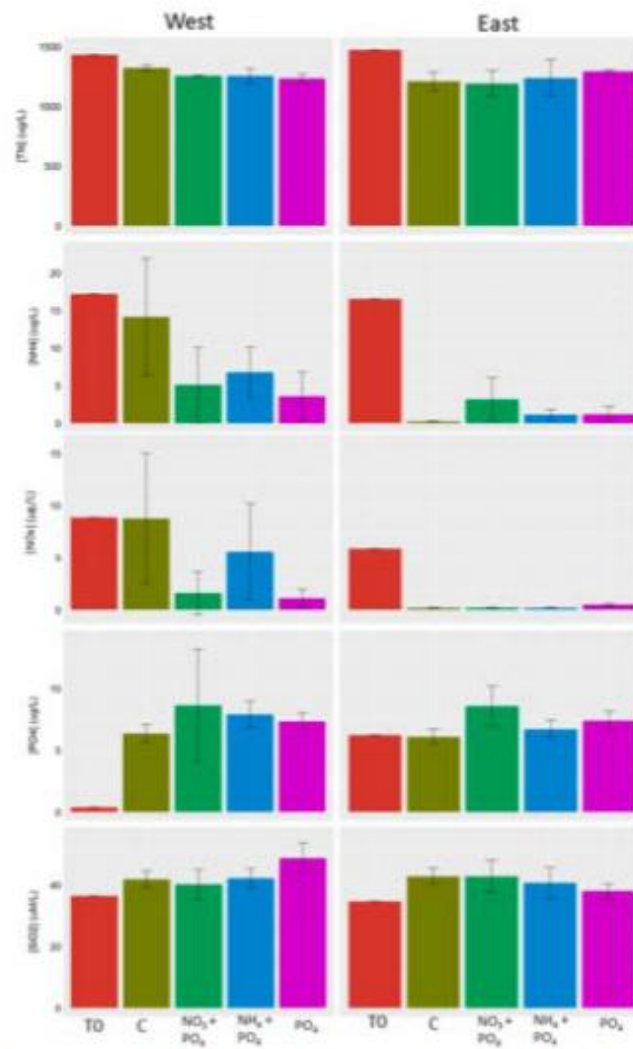


Figure 4. Experiment 1 nutrient concentrations for differing treatments. T0 represents the initial nutrient concentrations measured before incubation and enrichment, and C is the control group. All nutrient levels reported were calculated as the average of the triplicate treatments and error bars represent the standard deviation from the mean.



**Figure 5. Experiment 2 nutrient concentrations for differing treatments. Configuration and axis labels are the same as Figure 4.**

### 3.4 Phytoplankton growth

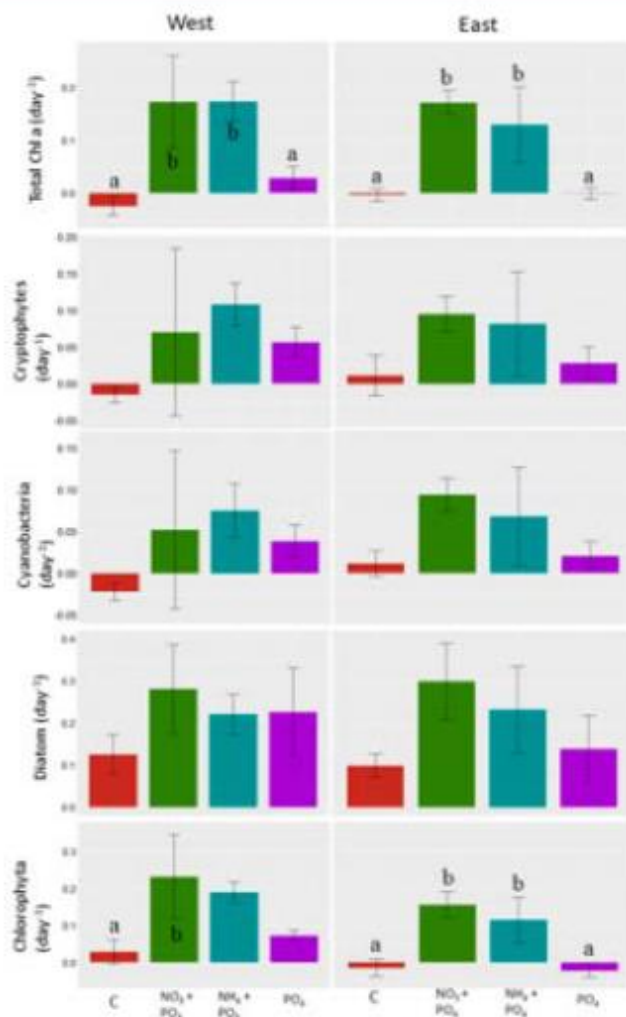
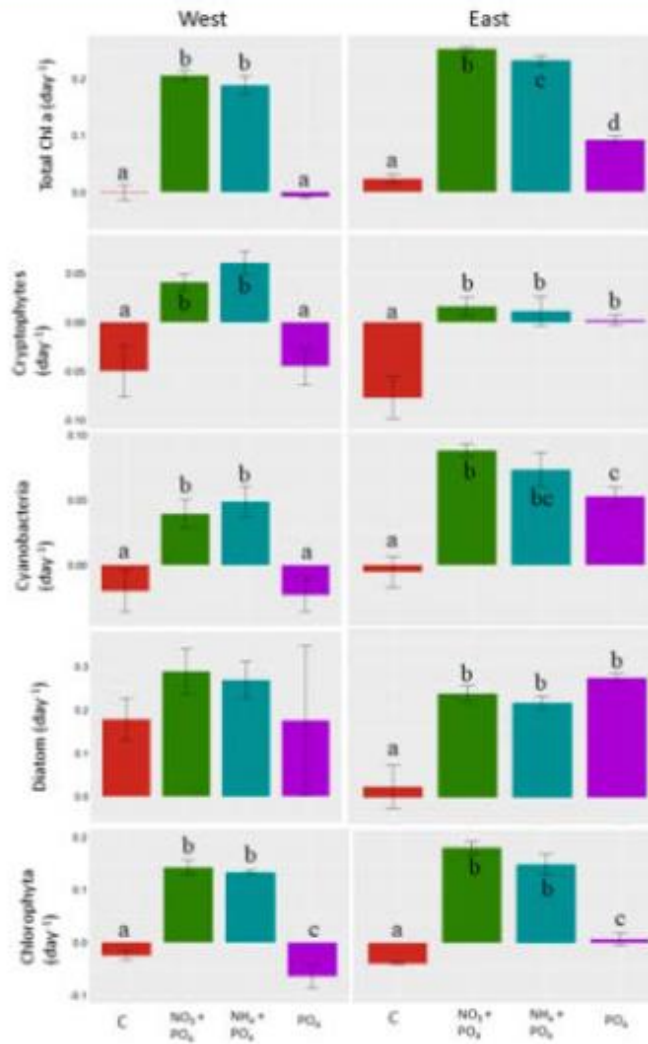


Figure 6. Experiment 1 phytoplankton growth rates. A letter denotes a significant growth rate based on the Tukey HSD post-hoc test (p value < 0.05), completed in conjunction with an analysis of variance (ANOVA) test.





**Figure 7. Experiment 2 phytoplankton growth rates. The configuration and statistical tests completed are the same as Figure 5.**

Overall, whether the phytoplankton received a NO<sub>3</sub> or NH<sub>4</sub> enriched treatment did not result in significantly different growth rates, according to tukey HSD post-hoc tests (Fig. 6 and 7). Similarly, cyanobacteria did not show a significant difference in growth due to enrichment by ammonium versus nitrate. However, cyanobacteria from the east side of the lake seemed to grow slightly faster than those of the west. Diatoms experienced the fastest growth out of the several groups. For experiment 2, chlorophyta and cyanobacteria experienced a significantly higher growth rate for the PO<sub>4</sub>-only treatment in comparison to the control group. This suggests that phosphorous was the primary limiting factor, and N was the secondary limiting factor in these cases. There was no evidence for phosphorous limitation in experiment 1.

### 3.5 Changes in Community Composition

The community composition results from Chemtax showed that the phytoplankton community was dominated by cyanobacteria before and after incubation (Fig. 8). For both experimental days, the percentage of the total phytoplankton community comprised by cyanobacteria decreased for both the NO<sub>3</sub> and NH<sub>4</sub> treatments compared to the control. The only treatment where cyanobacterial dominance increased or stayed about the same was the PO<sub>4</sub>-only treatment. Between the NO<sub>3</sub> and NH<sub>4</sub> treatments, there was consistently a slightly higher percentage of cyanobacteria in the NH<sub>4</sub> treatment, but it was only by 1.6% on average (Fig. 8 and 9).

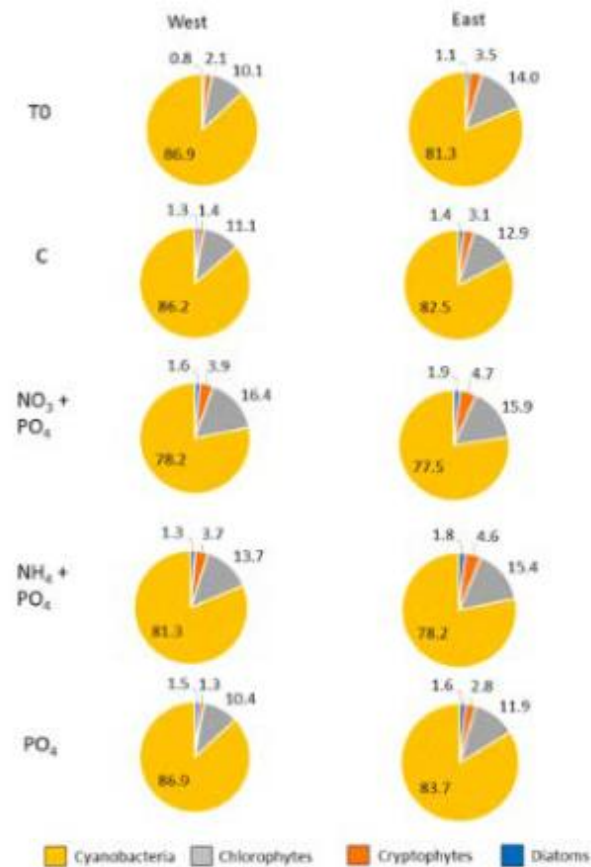
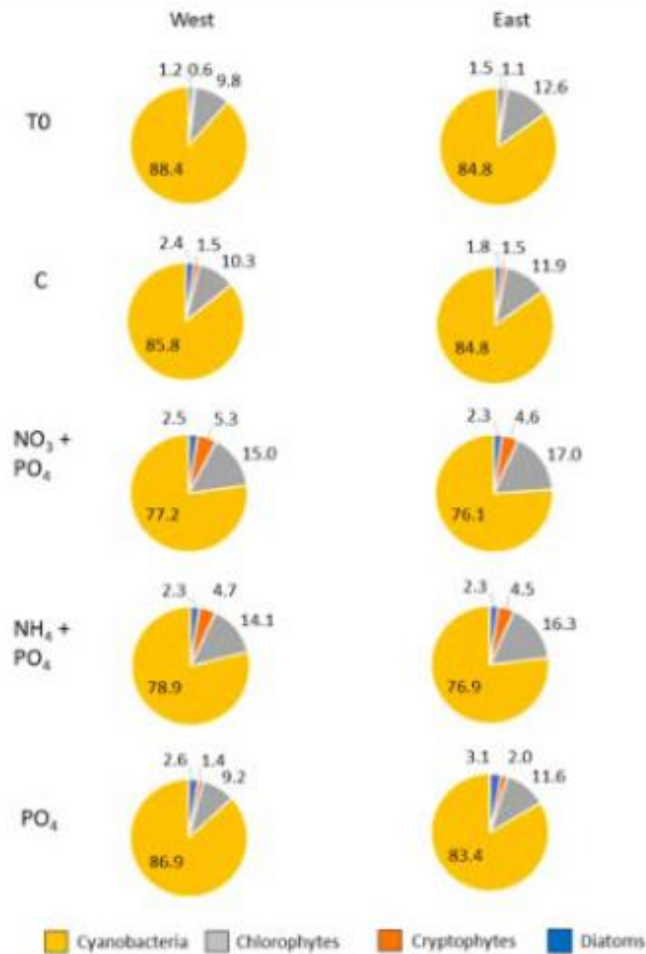


Figure 8. Experiment 1 community composition as a percentage of total chl a. T0 represents the initial community composition measured before enrichment and incubation, while C is the control group.



**Figure 9. Experiment 2 community composition as a percentage of total chl a. The configuration and axis labels used are the same as Figure 8.**

#### 4. Discussion

##### 4.1 Community composition

The dominance of cyanobacteria is a year-round feature of Lake Mattamuskeet, as was demonstrated by the community composition cell counts from water samples taken year-round during 2017-2019 (Fig. 2). Because the four dominant species of cyanobacteria in the lake (*Cylindrospermopsis*, *Planktolyngbya*, *Pseudoanabaena*, and *Spirulina*) can produce harmful toxins, it is especially important to monitor their growth.

Additionally, the ChemTax results from the Tinitial water samples indicate that the lake was dominated by cyanobacteria at the time of sampling as well (Fig. 8 and 9). Therefore, the conditions at which we performed our experiment were consistent with the community composition from the past two years. The HPLC data also reported that Lake Mattamuskeet currently has total chl a levels

that are above the NC Department of Environmental Quality (NCDEQ) standards ( $>40 \mu\text{g/L}$ ).

Although the phytoplankton community was largely dominated by cyanobacteria throughout the entire experiment, cyanobacteria were not significantly and selectively stimulated by additions of  $\text{NH}_4$  (Fig. 6 and 7). In comparison to Newell et al (2019), whose work in Lake Erie suggested that cyanobacterial blooms are driven by  $\text{NH}_4$ , we did not focus on a specific type of cyanobacteria as they did with *Microcystis*. Therefore, different species of cyanobacteria may react uniquely to varying nitrogen forms. Similarly, another study that suggested that cyanobacteria growth is selectively driven by  $\text{NH}_4$  influxes took place in the cooler temperature Wascana Lake, Canada and found that *Microcystis* cyanobacteria was the most likely to exhibit selective growth (Donald et al., 2013). Therefore, it also may be useful to look at temperature as a factor in selective stimulation.

#### 4.2 Nutrient limitation and implication

In Lake Waccamaw, another coastal freshwater lake in NC approximately 200 miles south of Lake Mattamuskeet, a study was done that identified the lake's sediments as its main source of phosphorous influx, causing a near-eutrophic state (Cahoon et al., 1990). Because it is relatively common to observe N-limitation in coastal systems due to the influx of P that is bound to sediments, it is notable that we observed occasional P limitation during our bioassay experiments (Fig. 6 and 7). Furthermore, previous studies suggest that N-P dual reduction is the most effective way to reduce eutrophication (Paerl et al., 2016). Therefore, it is important for management to focus on reducing the inputs of both N-sources to Lake Mattamuskeet, alongside identifying sources of P influxes. When compared to the TN levels in other eutrophic lakes, such as Lake Taihu, China where levels reach around  $3500 \mu\text{g/L}$ , the initial levels of TN in Lake Mattamuskeet are relatively low (Paerl et al., 2014). This implies that eutrophication may be in the early stages at Lake Mattamuskeet, and management should act quickly.

#### 5. Conclusions

- The high total chl *a* values indicate that Lake Mattamuskeet currently has a rich phytoplankton community, with concentrations over the NCDEQ standards ( $>40 \mu\text{g/L}$ ).
- Community composition cell counts done from samples taken throughout the year indicate that Lake Mattamuskeet is a cyanobacteria-dominated system year-round, which was consistent with the community composition observed during our experiments.
- $\text{NH}_4$  vs  $\text{NO}_3$  enrichment did not produce significant growth differences between cyanobacterial groups & other taxa. Cyanobacteria grew equally well with either enrichment treatment.
- Because the system experiences both N and P limitation, management at the wildlife refuge should focus on identifying sources of P loading and decreasing both  $\text{NO}_3$  and  $\text{NH}_4$  inputs to restore SAV-friendly conditions.

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**Elizabeth Farquhar** first became involved in undergraduate research when she began to volunteer in her professor's coral morphology lab sophomore year, which really solidified her interest in marine sciences. Since then, she has participated in the Morehead City, NC environmental science field site, where she began her work on her honors thesis with her mentor, Dr. Nathan Hall. They chose to study the phytoplankton and nutrient dynamics of Lake Mattamuskeet because of the lake's importance to eastern Carolina and its rapidly changing state. In addition to environmental/marine research, Elizabeth also is interested in working with remote sensing and environmental education in graduate school. This upcoming summer, Elizabeth will be participating in an international research experience for undergraduates in Ecuador, where she hopes to gain more field work experience and travel.