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Letter From the Editors

This fall semester marks a new beginning for the Georgetown community and beyond. The ability of many student researchers to regain their footing in their research studies after returning to an in-person environment demonstrates the adaptability and passion for science of the Georgetown student body. Students wrote literature reviews and analyzed data in their never-ending pursuit to answer noble questions in their fields of interest. They were able to produce exceptional articles due to the amalgamation of the knowledge and skills learned from this past year's virtual environment and the return to an in-person education. Those accomplishments are not only inspiring to the research community, but show the students' passion to further scientific research despite the various challenges they face.

In this issue, we are met with a diversity of research, tackling a variety of important topics ranging from gene editing technology to climate change. This publication presents many fascinating and thought-provoking studies: CRISPR and COVID-19, climate change and fungi in salt marshes, energy security in Poland, and the wellbeing of college students serving in a resident assistant role.

We hope this issue will serve as a testament to the true commitment that will always find a way to move forward despite the pandemic that continues to challenge the safety of conducting traditional research. In fact, because of these challenges, students are using new ideas, topics, and methods to further the scientific field and the growth of our students and the scientific community here at Georgetown. As we transition to our third publication, we hope to continue contributing to the scientific student experience at Georgetown University. Through resources on how to get involved in research and showcasing the work of our community members, we hope students will learn more about the myriad research opportunities at Georgetown. Please join us in commending the students who have advanced the ongoing research at Georgetown University both in this publication and beyond.

Nesreen Shahrour

Nesreen Shahrour
Editor-in-Chief

Layan Shahrour

Layan Shahrour
Executive Editor

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Letter From the Editor-in-Chief Emerita

The Ice Age, Stone Age, and Bronze Age all engender defining periods in history, each described by an overarching narrative. Most recently, we have found ourselves in the Information Age, characterized by rapidly developing technologies such as the internet, giving us access to information within the matter of seconds. At the beginning of 2020, however, we stumbled upon a pandemic that left people around the world baffled. We watched in admiration as scientists collaborated, innovating ways to understand and tackle the novel coronavirus. This turning point marks the beginning of the Biological Age.

The rapid development of the COVID-19 vaccines using mRNA technology demonstrates the contributions biology has made for everyone around the globe. These recent biological discoveries and technological innovations including vaccine technology, gene therapy, 3D brain mapping, and stem cell technology have been spearheaded by scientists across the world. Research has already started to test gene therapy to treat different cancers and sickle cell anemia, brain mapping is being used as a tool to understand Alzheimer's disease and schizophrenia, and stem cell technology is being adapted to generate organoids and perhaps replacement organs. In the future, mRNA vaccine technology might be used to treat other diseases such as diabetes. With rapid innovation, research and medicine has the continued potential to ameliorate the lives of a myriad of people.

Student scientists are the next generation of researchers ushering in the Biological Age. Throughout the pandemic, first, virtually and, more recently, in person, students at Georgetown University have continued to rise to the challenge to conduct research safely, demonstrating resilience and determination. Our issue is joined by articles ranging from direct impacts to students in the Georgetown University community–understanding mental health afflicting Resident Assistants–to impacts on a global scale–understanding CRISPR's applications in defense against pathogens. These articles are a testament to the extensive research conducted by students within the Georgetown University community. Join me in commending Georgetown University student researchers for driving discoveries in the research field as we enter this new biological age.

Danya A. Adams
Editor-in-Chief Emerita

GEORGETOWN SCIENTIFIC RESEARCH JOURNAL

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Energy Security in Poland: Where the Energy Sector Falls Short and Where It Can Go

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Energy Security in Poland: Where the Energy Sector Falls Short and Where It Can Go

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Abstract

In the aftermath of World War II, Poland rebuilt its energy sector through the use of fossil fuels, thus establishing a dependence on coal power. This reliance has slowed its transition to environmentally friendly energy sources, leading to increased greenhouse gas emissions. These characteristics of Poland's energy sector serve as a roadblock to diversifying the nation's energy sources and have subsequently resulted in its average energy security and poor environmental sustainability rankings in the top 25 largest on the 2020 International Index of Energy Security Risk. This ranking highlights the areas of improvement necessary for the nation to achieve greater energy security. This paper outlines Poland's current energy security status and provides policy recommendations that the nation's federal government can employ to improve overall energy security by diversifying their energy sources and improving the environmental impact of the energy sector.

Keywords: Poland, Energy Security, Policy

1. Introduction

Following World War II, Poland's economy and infrastructure had been devastated due to warfare raged across the country. The country had been ravaged physically and economically, leading to a period of modernization and rebuilding. This period created a dependence on coal that is still central to the country's energy sector today.[1] During the 20th century, coal-burning plants and factories contributed to the rebuilding of the nation's economy. However, the economically favorable outcome of coal reliance has stifled Poland's transition to cleaner energy sources in the 21st century.[1] Poland's energy security, determined by reliability, affordability, and environmental impact of energy sources,[2] has been negatively affected by its reservations about transitioning to cleaner energy sources. A lack of progress in energy diversification, along with coal's prominence as a part of Poland's energy supply and economy, have contributed to the country's current energy security status and its energy security rating compared to other nations.[3,4]

2. Energy Security Assessment

According to the 2020 International Index of Energy Security Risk, Poland ranked 11th in the world for coal consumption and 10th for coal production.[3] As of 2020, coal makes up 48% of Poland's current energy mix and 79% of Poland's power generation mix of energy sources.[3] Energy mix is defined as the combination of direct energy use, while power generation mix is defined as the combination of energy sources used to generate electricity. Coal contributed to the largest percentages of Poland's current energy mix and power generation mix.[3] Comparatively, natural gas makes up 16% of the country's energy mix and 7%

of the nation's power generation mix.[3] Nuclear energy did not contribute to Poland's energy mix or power generation mix.[3] Moreover, Poland, as of 2018, was a net importer of petroleum, natural gas, and coal, showing dependence on foreign energy supplies.[3] These factors contributed to Poland's energy security risk score of 967 on the 2020 International Index of Energy Security Risk evaluation.[3] Comparatively, the Organization for Economic Co-operation and Development (OECD) had a group average score of 884.[3] To give more context to Poland's energy security risk score, Poland's score ranked 12th out of 25 scores associated with countries considered "large energy users".[3]

Poland has also been evaluated by the World Energy Council and assigned a trilemma score, which is an evaluation of the energy security, energy equity, and environmental stability of a country scored on an A through D scale. Poland scored a trilemma score of 70.4 out of 100, with a rating of a B in energy security, a B in energy equity, and a C in environmental sustainability.[4]

Polish energy security ranked 37th out of 101 countries due to its import dependence, lack of electricity generation diversity, and energy storage capacity.[4] Between 2010 and 2020, Polish import dependence has been trending towards more importation, which is a threat to energy security because foreign suppliers have control over energy supply and access.[4] Diversification of electricity generation was the nation's second-lowest key metric score, well below 35 out of 100, with 100 being the highest level of possible diversification of energy in a country.[4] These metrics are determined relative to the other nations' ranking and evaluation.[4] Due to Poland's reliance on coal for power generation, there is limited use of renewable energy sources, natural gas, and nuclear power.[3] Energy storage capacity was found to be below 50 out of 100, which indicates a need for improvement because the country relies heavily on new production or importation of energy, as compared to relying on readily available stores. All three of these scores factored into an overall energy security score of 62.7 out of 100.[4]

The energy equity of the country, which is defined by accessibility and affordability, was an obvious strength of their energy sector at 84.7 out of 100. This metric explains Poland's relatively higher trilemma score, as well as its B grade in energy equity.[4] As of 2020, Poland scored a 100% in access to electricity, demonstrating that an overwhelming majority of citizens have access to electricity.[4] Electricity prices also scored in the 90s, which shows electricity is provided at affordable prices to the population.[4] However, this affordability is almost overshadowed by the nation's environmental stability performance; the country ranked 63rd out of 108 countries with a score of 65.9 out of 100 in environmental sustainability.[4] A major indicator of its poor score is its low metric of electricity generation from decarbonized sources; this is by far Poland's lowest key indicator score, falling below 25.[4] This metric contributes to Poland's C rating in environmental sustainability.[4]

3. Domestic Energy Ambitions

Poland is currently trying to implement policies that increase energy security through domestic energy initiatives and transitions to cleaner energy sources.[5] This can be seen in the federal government's plans of Poland's Energy Policy by 2030 (PEP2030), which has since been updated to PEP2040.[6]

PEP2040, officially released in 2020, plans to reduce coal reliance by cutting the fuel from 80% to 60% of the national power generation mix; this will then be followed by an ambitious decline to 22% by 2040.[6] This reduction in coal reliance is fueled by planned investment in new energy sources such as nuclear energy. The Ministry of Energy expects to have six new nuclear plants producing domestic energy, with the first being launched in 2033; each subsequent plant will launch every two years until 2043 to produce a total capacity of 6-9 Gigawatts.[7] The government plans to roll out Generation III and III+ nuclear plants, which they claim will still supply affordable energy

to citizens.[7] Generation III and III+ plants are nuclear plants that have safety envelopes constructed on Western safety standards and require relatively large electric grids.[7] Generation III power plants have reactor technology that give them the potential to function for upwards of 60 years.[8] This transition towards nuclear energy can be seen as a long-term goal of creating energy security because it diversifies Poland's energy mix while also moving the country away from coal reliance. Since nuclear energy is currently not contributing to Poland's energy or power generation mix, this would be a large step for Poland in achieving more energy security through diversification.[3]

Additionally, nuclear power development would contribute to the environmental sustainability aspect of energy security because nuclear power is notably cleaner than coal; a coal plant on average will put out 704 to 709kg of sulfur dioxide per Gigawatt hour (GWh), 717-721kg of nitrous oxides per GWh, and 150kg of dust per GWh.[9] According to PEP2040A, a nuclear plant will emit zero air pollutants through the technology of Generation III and III+ reactors.[7] Nuclear plants, however, create high, intermediate, and low-level radioactive waste that must be properly contained and managed through compaction.[9] Radioactive waste is not a significant safety concern because Generation III and III+ reactors are regulated by the Nuclear Regulatory Commission and equipped with technology that allows them to run safely for approximately 60 years.[8] Radioactive waste is not a negative tradeoff of nuclear energy because Poland's future reactors are expected to represent about 10% of energy generation, which will be more environmentally friendly than coal.[7] The government claims nuclear energy will be Poland's main strategy for reducing greenhouse gas emissions while also allowing the country to diversify their energy mix, thus increasing their energy security.[7]

PEP2040 plans to reduce Poland's emissions by 30% by 2030, in comparison to its 1990 emission levels, with a mixture of investments in renewables and natural gas. Nuclear energy is its largest investment to reduce emissions.[7] This progression towards ambitious environmental sustainability is a relatively new development. Poland's reliance on coal has led it to shy away from accepting legally binding carbon emission reduction plans, as seen in 2015 when Poland vetoed an amendment to the Kyoto Protocol, an international treaty between state parties that promotes actions to address climate change and reduce greenhouse gas emissions through the United Nations Framework Convention on Climate Change.[11,12] The amendment tried to set "a legal framework for carbon emission reductions" that would remain in place until 2020 and the European Union's (EU's) entry into the Paris Climate Agreement.[11] Poland refused until 2018, when they ratified the amendment.[11] In 2019, Poland was also left out of a EU 2050 climate agreement, which looked to push states to climate neutrality by 2050.[13] Poland was steadfast against coming to terms with the agreement due to worries over financing their nuclear energy transition, claiming that Poland would reach climate neutrality at its own pace; the country even mentioned the idea of reaching neutrality by 2070.[13] The outcomes of this negotiation are part of the important context surrounding the slow-moving progression towards a more environmentally sustainable future, as funding and EU assistance becomes key to pushing Poland towards their nuclear diversification goals.

The PEP2040 plan is an ambitious target by Poland, but feelings of uncertainty linger with respect to when it will be achieved. These fears are fueled by the political moves and stances taken by the Polish government, outlined in the previous paragraph, which illustrate the government is not taking on legally binding actions to meet these goals. Nonetheless, Poland promises that the nation will achieve this PEP2040 plan. However, the pace at which it does seems uncertain. Poland's inability to deliver is underscored by the overambitious PEP2030 plan, published in 2009, which needed to be amended in 2019 to extend the

country's deadline to meet the stated goals to 2040.[7] This need to delay dates by a decade throws into question if the nation will meet its own deadlines once again. These fears must be addressed, and ambitions realized to ensure that Poland moves toward joining the rest of the EU in reducing emissions through energy diversification before the devastating impacts of climate change become irreversible.

4. Policy Recommendations

Moving forward, Poland's citizens need to call on their government to move towards environmental sustainability and diversification of energy supply. These are important aspects of energy security that can create assurance of more reliable domestic energy for Poland's citizens. In 2009, Poland's citizens and those of other European nations suffered blackouts and energy shortages due to a dispute between a Russian gas company, Gazprom, and Ukraine, as the two fought over pricing of Russian gas exported through Ukraine.[14] As Poland looks to diversify to cleaner energy sources, their strategy, one shared by many members of the EU, serves to offer a large benefit to the country; diversification will move the nation away from a heavy dependence on one exporter and allow for less devastation in the future if the reliability of one energy source is diminished. In the wake of the 2009 shutdown, these countries should seek to move away from Russian energy export dependence, diversify their energy suppliers, and build up domestic sources in an attempt to improve energy security and avoid future blackouts due to foreign disputes.[14]

One policy recommendation that Poland and its citizens should support is investment in the Energy Bridge Project between Ukraine and Poland. The Energy Bridge will connect Poland to Ukraine across their shared border to create a new nuclear energy supply from the Ukrainian state-owned utility company, Energoatom.[15] The project will connect Energoatom's Khmelnitsky nuclear plant, supplying Poland with nuclear generated electricity that can help the country as they transition away from coal, allowing for greater diversification of energy in Poland.[15] Nuclear is also clean energy that will allow for greater cuts in emissions as the country moves away from coal.[9] Utilizing Ukrainian nuclear energy can help Poland move away from coal faster, moving them closer to the EU goal of carbon neutrality by 2050. Ukraine has a demonstrated history of trade with Poland, which has contributed to the fact that in 2017 and 2018 Poland was Ukraine's second largest economic exportation market, with a trade value of approximately $2,727,594 and $3,257,236 USD respectively .[10] Due to this interconnectedness through trade, the Polish government may see Ukraine as a more reliable partner than Russia, one which Poland can work with in the future.

Poland's government should also invest in carbon capture and sequestration technology. This would increase the energy security of Poland because carbon capture would reduce the number of emissions that the country's coal sector produces, thus making the energy source less environmentally harmful. Specifically, the country should invest in post-combustion carbon capture. The use of post-combustion capture is a technology that allows for fossil fuel-fired power plants to reduce emissions by separating the gas products of the coal energy production and sequestering the carbon dioxide that would usually be released into the atmosphere.[16] Implementation of this technology in existing plants is necessary because Poland is expected to be carbon neutral after 2050 due to its coal dependence, but carbon-capture could speed up that process and improve relations between the member state and the EU.[13] Carbon-capture may be an expensive solution, but the EU might be willing to help fund the initiative if that means Poland will reach its 2050 goal.

5. Conclusion

Poland has much work to do to improve its energy security, as indicated by the Global Energy Institute's and the World Energy Council's evaluations. The Polish government's lack of

movement in 2015 towards legally binding protocols to reduce greenhouse gas emissions through the Kyoto Protocol is a glaring sign that the government is not doing as much as it can, in terms of improving energy security through environmental sustainability. Poland needs to rejoin Europe in its action towards fighting climate change because Poland is currently moving at its own pace, as demonstrated by its refusal to sign on to the 2050 carbon neutrality agreement, unlike its closest EU allies. In addition, by delivering on the PEP2040 ambitions, such as through the development of Poland's nuclear power sector, the country will move towards diversification and more self-sufficiency. Therefore, meeting these goals without further delay or setbacks is imperative to improve Poland's energy security.

In conclusion, diversification and environmental stability are the two aspects of Poland's energy sector that require improvement. Movement away from Russian fuel and towards Ukrainian nuclear energy is a cleaner and more secure option. Additionally, post-combustion capture would make coal plants more environmentally friendly while Poland transitions from coal to nuclear power and other energy sources, thereby improving its overall energy security.

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Impacts of Climate Change on Mycorrhizal Fungi in Salt Marsh Habitats

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Impacts of Climate Change on Mycorrhizal Fungi in Salt Marsh Habitats

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Abstract

Salt marshes are coastal wetlands that cover 2-3% of land surface area.[1] These habitats carry out several essential functions such as providing habitats for many species, acting as a buffer between terrestrial land and ocean waters, and, most importantly, acting as a major carbon (C) storage pool. Arbuscular mycorrhizal fungal (AMF) symbionts are key organisms in salt marsh habitats and are known to influence the following processes and factors: plant zonation, plant resource competition, plant productivity, plant genetic diversity, soil C sequestration, soil C:N:P ratios, saprotrophic bacterial population and diversity, soil stability, and litter decomposition. Under rapidly changing conditions caused by climate change, it is difficult to predict how AMF communities will respond, ultimately altering these factors. This review will explain the role of AMF communities in modulating carbon sequestration by increasing plant and fungal biomass and influencing soil organic matter decomposition. Additionally, this review presents the current knowledge regarding how sea level rise (SLR), elevated CO2 levels, and eutrophication are expected to decrease AMF abundance and diversity by increasing habitat fragmentation, decompositional rates, anoxic conditions and altering soil nutrient stoichiometry. Studying soil fungi is essential for understanding how mycorrhizal communities are predicted to react to a climate change and, consequently, alter salt marsh processes.

Keywords: AMF, sea level rise, eutrophication, salt marsh

1. Introduction

Salt marshes are coastal wetlands that are consistently flooded and drained with salt water due to changing tides. These ecosystems are found worldwide and are often dominated by salt–tolerant smooth cordgrass which is essential for marsh stability. Additionally, salt marsh habitats perform a plethora of essential functions, such as acting as a buffer between terrestrial lands and ocean sea waters, providing habitats for coastal organisms, and most importantly, sequestering carbon (C) at unparalleled rates, which makes it an essential terrestrial C sink.[2]

Salt marsh C sequestration can largely be attributed to its high net primary production and decomposition cycle. It is estimated that the global net primary production from salt marsh vegetation is 0.44×10^{15} g C per year, equivalent to 440×10^{6} metric tons of C per year.[2] Only about 30% of this vegetation is consumed by herbivores, leaving the remaining 70%, around 230 million metric tons of C, to enter the decomposition cycle.[2] However, frequent tidal flooding creates saline and anoxic conditions that slow decomposition, leading to a C storage rate that is 10-100 times greater than terrestrial ecosystems.[3] Regardless of the harsh saline

and anoxic conditions, microbes, such as bacteria and fungi, play an essential role in the degradation of smooth cordgrass detritus, which significantly alters C sequestration rates. Therefore, understanding how these organisms mitigate C–cycling and how they are affected by climatic variation is essential for predicting how salt marsh habitats will respond to climate change.

Salt marsh soils contain all three soil fungal types: symbiotrophs, pathotrophs, and saprotrophs. Symbiotrophs, particularly arbuscular mycorrhizal fungi (AMF), are extremely significant in salt marsh habitats due to their governance of biological processes.[4] AMF communities provide nutrients to their host plant in exchange for photosynthate.[5,6.7] It has been found that AMF communities are able to maintain ecosystem balance and mitigate C sequestration by modulating soil organic matter (SOM) decomposition and nutrient mobilization.[4] However, researchers have now provided evidence to suggest climate change is heavily impacting plant–fungal interactions as well as bacterial–fungal interactions, both of which have a profound influence on C sinks and SOM decomposition. Rising sea levels due to increasing global surface temperatures are significantly impacting salt marsh elevation gradients, leading to the habitat fragmentation of S*partina patens,* a key high–marsh grass. As elevation gradients decline, *Spartina alterniflora*, a foundational low–marsh grass species, is rapidly replacing *S. patens*.[8] These geographical changes, as well as plant species zonation, have leverage over fungal communities consequently affecting decomposition rates.[8]

Soil–fungi interactions are also being remodeled by excessive mineral and nutrient fertilization, a process called eutrophication. This is mostly due to anthropogenic runoff from industrial waste and agricultural fertilizers entering coastal habitats. AMF's ability to acquire nutrients, decompose SOM, and interact with rhizosphere bacteria and its host plant change depending on soil chemistry and nutrient abundance.[4, 9,10] Hence, anthropogenic factors that have increased global surface temperatures, sea level rise (SLR), and eutrophication have had profound effects on microbial interactions in salt marsh ecosystems.[10] In this review, I will outline AMF's role in salt marsh habitats as well as describe their predicted reactions to climate change. Understanding how climate change impacts soil communities will allow us to better predict the outcomes of worsening climate conditions and employ more effective conservation plans in these areas.

2. Mycorrhizal fungi in salt marsh ecosystems

Arbuscular mycorrhizae (AMF) are extremely important mitigators of soil organic matter (SOM). Fungal species that are known to form AMF associations are in the genera *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Clecerocytis*, and *Scutellospora*.[11] These symbionts penetrate within the host plant's root cortical cells that form arbuscular sacs, allowing for nutrient exchange. Furthermore, mycorrhizae extend their hyphae into the soil creating mycelial networks that increase root surface area which improves nutrient acquisition of nitrogen (N), phosphorus (P), improves plant stability in soil, as well as bolsters plant pathogen resistance.[10] In return, host plants provide mycorrhizae with 10-20% of their net photosynthate (fixed C compounds).[5,6,7]

One of the main influential factors in determining AMF association is environmental conditions and micro-climate. Anoxic conditions increase with soil depth, thereby limiting AMF communities to upper soil layers which contain burrowing organisms and fluctuating water tables that provide enough aeration to support mycorrhizal colonization.[12] Hence, elevated grass species, *S. patens,* can host AMF communities and have root colonization rates between 52-68%.[13,14] On the other

hand, *S. alterniflora,* a low, submerged grass with relatively anoxic soils, has root colonization rates of 0-9%.[14,15,16]

Marsh elevation also impacts nutrient availability which alters trade–offs in mutualistic investment. In elevated areas, reduced tidal inundation causes unpredictable nutrient import, resulting in *S. patens* to rely on its AMF association to acquire sufficient amounts of N and P.[17]

Conversely, low marsh grasses are constantly submerged, allowing *S. alterniflora* to have reliable sources of N and P. If nutrients are readily available, the benefit received from AMF association is low, but the association still remains energetically costly, making the symbiont more parasitic than mutualistic.[12]

Additionally, studies have found that plant–fungal associations and community composition differ based on the genetic specificity of both AMF and host plants. Van Der Heijden et al. (1998)[18] was able to show that plants not only respond to the presence of AMF, but response varies amongst fungal taxa. This would suggest that AMF diversity and taxonomic specificity for certain plants have the ability to alter host plant responses.[18] Other studies, like Koch et al (2006)[19], further examined this phenomenon by observing plant responses in relation to intraspecific genetic diversity in isolates from one *Glomus intraradices* population. They found that AMF genetic diversity was able to affect plant species richness, growth, and productivity.[19] Intraspecific variation within a single plant species has also been shown to alter AMF community composition. Eppley et al. (2009)[20] found that different sexes of salt grass *Distichlis spicata* (with both sexes having the same growth rate) had dissimilar AMF colonization rates, suggesting host plant intraspecific genetic variation can alter AMF diversity and abundance. With climate change shifting plant community composition in salt marshes (i.e., replacement of *S. patens* patches by *S. alterniflora*) then we should expect there to be drastic changes to AMF species richness as well as genetic diversity. This will likely have significant impacts on SOM decomposition leading to altered C sequestration rates. Additionally, below ground alterations to fungal communities due to climate change will likely alter above ground responses in host plants.

3. Mycorrhizal fungi mitigating C–cycling and decomposition

Because salt marshes have incredibly high net primary productivity and litter accumulation rates, AMF mitigation of saprotrophic bacteria and SOM decomposition is essential for C–cycling.[2, 21] Research has shown that AMF communities can either stimulate or stunt C sequestration, depending on environmental conditions. However, there is debate as to how new climate conditions will alter these processes and whether they will lead to soil C storage or C release.

AMF stimulation of C sequestration can occur through soil aggregation, which is the binding together of micro–and macro–soil particles bound by cohesive forces or organic matter. Mycorrhizae promote soil aggregation by releasing binding agents, such as glomalin–related soil proteins that entangle soil macro–aggregates in dense hyphal networks.[22] Once macro–aggregates are stabilized, micro–aggregates can form, allowing mycelial networks to physically protect C pools and increase C retention.[22] This enmeshment also harbors a large portion of soil microbial biomass and accounts for a large portion of the SOM C pool.[23] Improved nutrient acquisition due to AMF association can support higher amounts of above– and below–ground plant biomass. Consequently, this increase in living and dead organic matter adds to the overall C sink. [24, 25]

The exact mechanism of AMF decomposition is not completely understood, but there are a few

proposed mechanisms. It is thought that mycorrhizal communities excrete hydrolytic enzymes, such as cellulase, pectinase, and xyloglucanase.[22] However, there is no direct evidence to support that these extracellular enzymes have saprotrophic abilities. Another more supported hypothesis is priming, which is AMF's ability to manipulate rhizosphere microbial communities indirectly thereby affecting decompositional rates. Mycorrhizae emit labile C exudates into the soil which stimulate saprotrophic bacterial accumulation, SOM decomposition, and CO_2 release.[24] This causes a release of ammonium (NH_4^+) that AMF rapidly absorbs and transfers to its host plant.[10, 21, 26] Mycorrhizal access to both host plant C and soil C may make AMF communities better rhizosphere competitors, allowing them to alter soil microbe composition.[12] Changing microbial communities would likely lead to shifts in important functional groups such as nitrogen fixation. As a result, altering the diversity and abundance of organisms responsible for important functional processes would have ramifications on soil nutrient levels, plant productivity rates, and plant community structures.[12]

4. Effects of ambient CO_2 levels and sea level rise

Recent anthropogenic activities have caused ambient CO_2 levels to rise significantly, which has coincided with higher-than-average global surface temperatures.[27] This has led to an net decrease in total sea ice, which has ultimately caused global sea levels to rise 0.19 m from 1901 to 2010.[28]

SLR will have profound consequences for salt marsh ecosystems. As described before, *S. patens*, a high elevation grass, and *S. alterniflora,* a low elevation grass, exist at limited elevation ranges relative to mean sea levels.[29] Our current rate of SLR is estimated to be approximately 2.5mm per year. However, through peat accumulation and sediment aggregation, salt marshes gain elevation and can vertically increase 2.08 mm to 4.20 mm each year, allowing them to remain above rising sea levels.[29] Furthermore, elevated ambient CO_2 levels are shown to bolster soil accretion and elevation gain.[30] Elevated CO_2 levels amplify plant growth and photosynthate production, resulting in an increase in fixed C allocations to mycorrhizae, increasing root and fungal biomass.[10] Under higher CO_2 levels, heightened plant growth demands stimulate additional C allocation to AMF communities in order to support nutrient mining. In terrestrial systems, elevated CO_2 levels (550-700 p.p.m.) caused AMF density to increase by 84%.[31] These results support Langley et al. (2009)[30] which found that root thickness increased to 4.9 mm per year under supplemental CO_2 levels compared to 0.7 mm per year under normal ambient CO_2 conditions. If SLR rates and marsh elevation gain remain unchanged, it is predicted that salt marsh ecosystems will be able to persist into the next century.[29]

Although greater biomass and soil accretion rates may seem to negate the issue of SLR, these modified conditions may change the biological mechanisms that govern C sequestration and decomposition. For example, photosynthesis rates diminish with each marginal increase in CO_2 concentration, leading to changes in carbohydrate allocations to AMF communities.[30] Research has shown that under elevated CO_2 levels, mycorrhizal communities exhibit amplified rates of SOM decomposition.[10] Studies have also observed escalating N mobilization rates, suggesting that priming and decomposition rates also increased, leading to soil C losses.[26, 10] Moreover, the assumption that marsh soil accretion rates neutralize flooding from SLR rests on the assumption that SLR rates will remain stagnant. However, with increasing ambient CO_2 concentrations, SLR rates are expected to increase, thereby surpassing the speed of soil accretion.[28] Increasing litter accumulation will also alter soil chemistry which is

an important predictor of AMF's relationships with plants and saprotrophic bacterial communities as well AMF's regulation of decomposition rates.

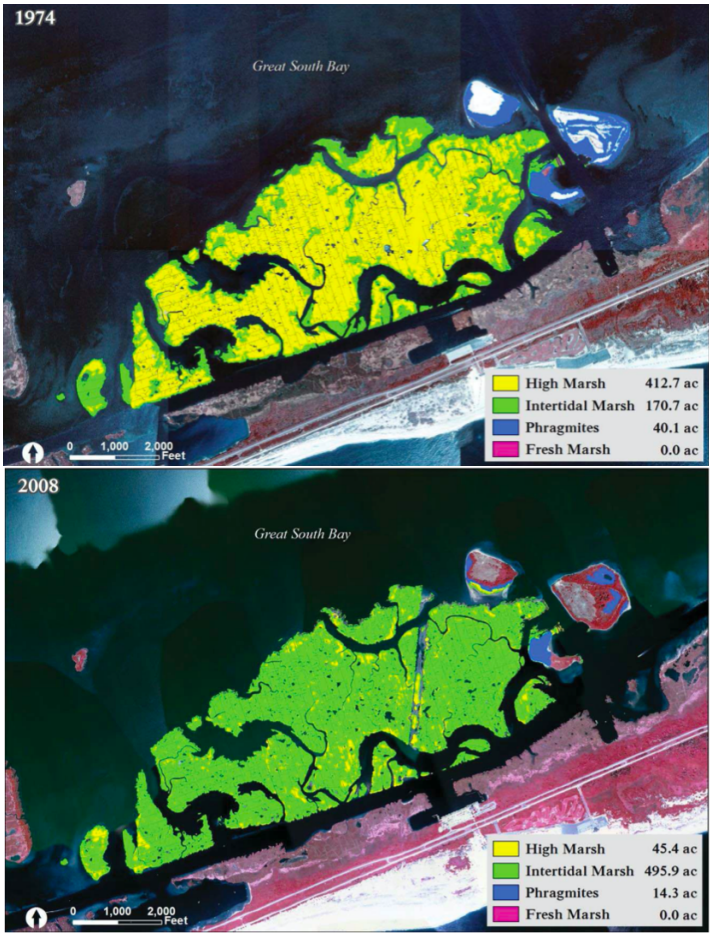

Figure 1. Differences in marsh elevation in the Cedar & Nezera Islands near Long Island NY from 1974 (top) to 2008 (bottom). 1974 displays a high percentage of high marsh habitat (412.7 ac) compared to 2008 (45.4 ac). Adapted from Cameron Engineering & Associates, 2015.[32]

Habitat loss due to human development of shorelines also has added additional pressures on marsh habitats. In the past, salt marsh grasses have been able to escape the threats of SLR by retreating to and colonizing higher elevation areas; but the development of shorelines has obstructed salt marsh grass migration.[29] Figure 1 shows plant composition in the Cedar and Nezera Islands, near the Long Island tidal wetlands, in 1974 (top) and 2008 (bottom).[32] The figure reveals that in 2008, there were more intense regions of fresh marsh development indicated by the darker pink. Moreover, Figure 1 displays the extreme fragmentation of *S. patens* (yellow) and the domination of *S. alterniflora* (green). This phenomenon has been experienced across many North American salt marshes and is not specific to this location. Since 1974, the presence of *S. patens* has decreased by 68%; in 1974 the Cedar and Nezera Islands consisted primarily of high marsh habitat (412.7 acres) but in 2008, only 11% of high marsh elevation remained (45.4 acres). It is important to restate that AMF associations are host–specific and non–resistant to the anoxic conditions of submerged marsh soils. Therefore, these changes would lead to reduced species richness and shifts in community composition of AMF,[33] perhaps causing the extirpation of a particular taxon or a bottleneck effect on AMF genetic diversity.

5. Effects of Eutrophication

Eutrophication is characterized by excessive plant and algal growth due to fertilization of limiting growth factors such as N and P.[34] Eutrophication occurs naturally, but anthropogenic activities, such as sewage runoff and agricultural activities, have amplified this effect.[34] Fertilization of salt marshes greatly transfigures soil C:N:P stoichiometry affecting AMF–plant relationships. Numerous studies have found opposing results on how eutrophication affects AMF's responses to grass zonation, microbial populations, decomposition, and C storage, making it difficult to predict how nutrient adjustments will alter AMF governed factors.

Studies have predicted that anthropogenic addition of N and P will shift plant competitive

dominance as nutrient addition alters nutrient foraging rates, fungal–plant relationships, and fungal–bacterial interactions.[35] Experimental additions of N to *S. patens* and *S. alterniflora* patches displayed a reduction in below–ground root biomass, but an increase in above–ground biomass, suggesting that nutrient foraging was eased. [9, 36, 37] In addition to shifts in biomass production, other studies found that N addition caused spore biovolume and density of extraradical hyphae to decrease.[10, 33, 38] These observations are consistent with the resource–ratio hypothesis, which states that more competitive and successful species will grow in habitats with lower resource levels. It also suggests that when limitations are eased, competition for resources will shift from below–ground to above–ground.[39] For example, when N limitations below–ground are reduced, plants are likely to increase above–ground production to compete for light.[35] Under typical conditions, AMF allows *S. patens* to be competitively dominant in a resource limited environment. However, as N limitations are eased, and above–ground growth is increased, *S. alterniflora* is more successful and energy–efficient.[35] For *S. patens*, as soil nutrient levels increase, nutrient foraging services greatly diminish causing it to be more parasitic than mutualistic.[35, 40] However, more recent studies found conflicting results in that N addition to salt marsh plots did not display signs of increased above–ground biomass.[41] More research regarding eutrophication's influence on above– and below–ground biomass allocation should be investigated.

There is also debate about the effects of N addition on elevation gradients and decomposition rates in marsh habitats. Past studies have found that N addition promotes above–ground biomass and decomposition rates.[9, 36] As N is added to soils, there is a subsequent release of CO_2, indicating there is an increase in microbial respiration.[37] This is likely due to a reduction in bacterial–fungal competition. As AMF biomass diminishes with increasing N addition, competition for N between fungi and saprotrophic bacteria is eased, leading to an increase in bacterial populations and decompositional activities.[42] Studies have found that, under these conditions, plants produced lower amounts of polyphenols, which regulate soil microbes that were likely linked to their overall observation of increased CO_2 soil emissions.[1] Furthermore, it was observed that for every atom of nitrogen added to salt marsh soils, 6.1 moles of CO_2 were released.[42] These changes in decomposition rates may negatively affect the soil accretion rates that protect salt marsh habitats from SLR. Yet, more recent studies report that N addition to salt marsh plots did not display signs of increased above–ground biomass.[41] While this study did find that fertilization increased respiration rates, decomposition rates remained stagnant.[41]

N enrichment alone is not the sole determinant in salt marsh grass root biomass.[9] In P–limited soils, N enrichment increases AMF root colonization, spore biovolume, and density of extraradical hyphae.[10] N enrichment to P–limited soils increases plant biomass as it shifts soil N:P ratios leading to P limitations.[10] But, most saline marine systems are N–limited and P–rich meaning N addition would cause AMF associations to be unbeneficial because of a decrease in competition with soil mircobes.[36] Future research is needed to accurately predict how eutrophication will affect these ecosystems long term and elucidate conflicting results.

6. Conclusion

AMF fungal communities are foundational species in salt marsh habitats, moderating C sequestration, plant zonation, nutrient acquisition, soil stability, microbial populations, and decomposition. Recent anthropogenic activities have elevated ambient CO_2 levels, leading to SLR which poses risks to plant community structure as

well as AMF processes.[10, 26, 27] SLR is predicted to diminish AMF abundance, increase decomposition and diminish C pools.[10] Furthermore, anthropogenic N fertilization of marsh soils has led to decreased root and fungal biomass causing competition to shift from below–ground to above–ground.[39] Additionally, it is believed that reductions in AMF biomass and increased N availability will increase microbial respiration and decomposition rates. However, there is still debate about how decomposition rates are impacted by N fertilization.[41] Additional research should be conducted to elucidate the exact mechanisms of AMF driven decomposition, which would allow us to create a clearer picture of how decompositional processes function in salt marsh habitats. Moreover, future studies should observe how climate change conditions such as elevated CO_2 levels, SLR, and eutrophication will impact AMF and plant community structures, decompositional rates, and C storage rates in order to create more consistent results within the current literature as well as provide more concrete directions for conservation efforts.

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CRISPR and COVID-19: Lessons Learned to Prepare for the Next Pathogen

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Abstract

CRISPR-Cas is a gene editing technology that can strengthen a defense countermeasure against an infectious pathogen and can heighten the attack risk of an engineered pathogen. The purpose of this report is twofold: to analyze the advantages of CRISPR for participants within a strategic environment, such as rogue, non-state attackers and defenders coordinated between nation-states and other entities, and to identify the ways in which CRISPR configures a defender's countermeasure against a biological event. In its assessment, this report utilizes the case study of COVID-19 to examine the applications of CRISPR-Cas systems to SARS-CoV-2. This report finds that CRISPR reduces some barriers to entry and exacerbates the possibility for malicious non-state attackers to engineer a pathogen and engender a serious biological event in the very short-term. However, key barriers to entry will continue to pose challenges to attackers comparative to defenders. In this report, "attacker" refers to non-state actors maliciously using CRISPR to engender a biological event while "defender" refers to coordinated entities responding to biological events, whether natural or deliberate. In the short- to mid-term, the use of CRISPR-Cas systems in designing a countermeasure against a biological event is to the advantage of the defender. CRISPR offers more accessible, rapid, and convenient diagnostic testing; a quick and accurate platform to identify viral vectors; and the potential for antiviral therapy. Through enactment of certain policy configurations, the comparative advantage of CRISPR may decisively shift to the defender, including in the very short-term.

Keywords: COVID-19, CRISPR, Biosecurity, Dual-Use Risk Technology

1. Introduction

In December of 2019, a novel coronavirus, now known as SARS-CoV-2, was identified in Wuhan, China.[1] The disease caused by this virus – Coronavirus disease 19 (COVID-19) – spread throughout the world and was eventually characterized as a pandemic by the World Health Organization (WHO).[2] From the first cases in China to the ongoing pandemic, COVID-19 has presented major challenges to economies, governments, health systems, and communities everywhere. As of August 20th, 2021, over 200,000,000 cases have been reported and 4,300,000 deaths have been attributed to COVID-19, according to the WHO.[3]

On December 2, 2020, the first fully tested immunization was approved for use.[4] Various other tested immunizations were approved across the globe shortly after. At this time, around 4,850,000,000 vaccines have been administered,

according to the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University.[5] However, waves of COVID-19 cases continue to rise in several countries, especially due to highly transmissible variants and insufficient population immunity.[6, 7] Furthermore, due to an immense scope of global vaccine inequity, a majority of low- and lower-middle-income countries may continue to experience surges in COVID-19 cases for years to come.[8] These facts drive continued public health measures for countries, as they denote that the COVID-19 pandemic is not over.

The imperative to constrain this virus motivated countries to take innovative defense measures against COVID-19. Among these innovative applications was a gene editing technology that was discovered in the 2010s and is based on natural bacterial immune mechanisms against viruses called CRISPR-Cas systems.[9] The systems are composed of two components: a guide RNA that identifies a target gene and a CRISPR-associated protein that can cut double stranded DNA, allowing for site-specific genome modification that is quick, cheap, and relatively user-friendly.[10] Researchers are now faced with deciding how best to optimize the system for human applications, and the COVID-19 pandemic presented a myriad of pressing public health challenges that CRISPR could help mitigate.

Simultaneously, CRISPR gene editing technology has the potential to be abused for malicious applications to the opposite effect of aiding public health responses. Specifically, CRISPR is often touted for its comparative ease of use and efficiency in editing genes,[11] which could present the opportunity for malicious attackers to genetically engineer pathogens into a biological weapon. As CRISPR reduces barriers of entry due to its low cost and ease of use, more attackers could use CRISPR to cause a deliberate biological event (DBE).

This report will analyze the advantages CRISPR holds for the attacker and defender, as well as how the technology can be leveraged to configure a countermeasure against biological events to enhance global biopreparedness. Utilizing CRISPR during the COVID-19 pandemic represents expanded use of a novel technology for a public health crisis. This report will begin by examining the risk factors that malicious non-state attackers utilizing CRISPR and other developing phenomena pose for the strategic environment. The report will then move to the application of CRISPR from three principal areas: COVID-19 diagnostic tests, pathogenic research, and the potential of antiviral therapy. Finally, the report will draw conclusions pertaining to the global health strategic environment and recommend policy to bolster global biopreparedness.

In this report, "non-state malicious attacker", or "attacker", refers to rogue groups or individuals utilizing CRISPR with malicious intent to engender a biological event. The term "biological event" refers to an outbreak of a disease caused by a pathogen and can be naturally occurring or deliberately released.[12] "Defender" refers to the coordinated entities responding to a natural or deliberate biological event, including, but not limited to, governments, the scientific community, institutions, and sectors. "Biopreparedness" refers to a state of readiness a defender builds for potential biological events.[13] The "strategic environment" is defined as the complex interaction of entities such as "attackers" and "defenders" amid dynamic and contradictory global forces.[14] "Pathogenic surveillance regime" refers to a coordinated program to survey and curtail the infectious progression of a pathogen within a society, usually directed by a government.

2. Anticipating the threat of CRISPR engineered pathogens

CRISPR-Cas systems can be utilized to delete genetic sequences, add genetic sequences, regulate expression of a phenotype, and even combine genetic sequences to produce a novel expression.[15] CRISPR-Cas systems are democratized for use, as gene editing kits are commercially available from laboratory suppliers and genome engineering companies and are relatively inexpensive. Furthermore, CRISPR technologies – especially these gene editing kits – are touted for their ease-of-use compared to other techniques for genomic manipulation.[15] However, the same accessibility, low cost, and ease-of-use of CRISPR-Cas could greatly expand the scope of potential users. In many ways, it is a boon for positive biological applications, and research in the field has flourished due to the democratization of gene editing. In other ways, the technology could pose a biosecurity risk if applied to malicious genomic engineering, rendering it a dual use research of concern. That is, research that advances our understanding of CRISPR-Cas has the potential to be directly misapplied and pose a biological threat.

Regardless of the numerous positive applications, CRISPR could open the door for malicious non-state attackers to engineer optimized pathogenic bioweapons. Prior genomic manipulation techniques posed significant barriers of entry, requiring resources and scientific expertise for performing expensive and complex biological procedures such as constructing zinc-finger arrays or delivering gene editing materials into cells.[16, 17] CRISPR-Cas systems circumvent some of the impediments apparent in other methods like TALENs (Transcription activator-like effector nucleases) or zinc fingers. In fact, the concerns that the broad distribution and low cost of the technology increase the risk for deliberate misuse were stressed in multiple US Intelligence Community threat assessments.[18, 19] These advances in gene editing highlight the modern reality that the tools necessary for genetically engineering a dangerous pathogen are increasingly available, and the biosecurity risk for a DBE could be greater now than ever before. Though potential targets of maliciously released pathogens are diverse,[20] for practicality in this report, discussion regarding the threat of an engineered pathogen refers to that targeting humans.

Beyond the reduced barrier of entry for engineering a pathogen, the attacker may also have certain advantages in releasing that pathogen and causing a DBE. Although there may be several similarities between the defense countermeasure against either a deliberate or natural biological event, a DBE may introduce additional requirements for several factors, from responder safety to attacker attribution.[21] The determination of natural or deliberate origin would presumably be made by a coordinated effort of stakeholders from intelligence communities and clinical professionals with standard criteria for assessing the biological event.[22] However, upon identification of a DBE, it still may prove difficult to discern whether this intentional release is a wild-type or genetically engineered pathogen, which is crucial information for assessing pathogenesis due to environment and natural selection. That is to say, genetically engineered pathogens may experience a comparatively more rapid process of change in selecting for new genes that make the pathogen more competitive while suppressing other genes that are metabolically expensive.[23] A dynamic pathogen like this may confound an efficient countermeasure from the defender. Furthermore, convoluted dimensions and numerous stakeholders in a public health response may inhibit an efficient countermeasure by the defender. A proactive release of a pathogen by an attacker is fundamentally less complicated than a reactive response by a defender.

Understanding the complex field of genetics for beneficial human applications is a problem facing researchers in CRISPR for today. From pleiotropic effects where one gene can have multiple expressions to epigenetic effects where expression is regulated by environmental factors, a limitation of CRISPR gene editing is scientific understanding of how genes are associated with specific phenotypic expressions. After all, CRISPR is most often and easily utilized in order to silence genes to create new phenotypes rather than add genes to create new phenotypes because of these limitations in scientific understanding. Furthermore, some have cited the fact that the notable studies utilizing CRISPR are conducted by the most well-resourced and important labs, suggesting that CRISPR still requires a significant level of expertise in order to effectively operate the technology for complicated uses. [24] Thus, the barriers of entry of scientific understanding and a certain level of expertise remains for attackers. As a genetically engineered pathogen is released, it may have unpredictable and unintended effects, again due to environment and natural selection. For instance, an optimized pathogen could be engineered with the intent for higher transmission and lethality and be presumed by the attacker to cause a catastrophic DBE yet perform unpredictably when released because it kills the human host too efficiently to replicate and spread.[24] This theoretical example would unintentionally underperform the attacker's goals. Furthermore, the delivery mechanism to weaponize a biological agent has classically been a limiting factor for malicious use.[21 (p. 90)] This too remains as a limiting barrier of entry for contemporary attackers, particularly if the desired release is within a mass population.

Some barriers of entry persist for the attacker. Even so, a release of a genetically engineered pathogen that exhibits characteristics unintended by the attacker can still be problematic for the defender. If the attacker can surmount these barriers of knowledge and delivery, while harnessing the low cost and ease of use of CRISPR, a resulting DBE may be devastating to the defender. Therefore, it is critically important to bolster global biopreparedness in both prevention and reaction strategies. For example, monitoring supply chains of CRISPR kits and dangerous pathogenic sequences may be worthwhile. However, it seems apparent that not all biological attacks may be preventable due to the lowered barriers of access to gene editing as well as the difficulty of surveillance over genetic and CRISPR materials. Thus, for the remainder of the report, there will be a primary focus on the reaction of the defender, but this is not meant to discount the need for rethought prevention strategies. In the instances of a devastating DBE, an expeditious, prepared response to contain the spread of the pathogen should be a foremost consideration.

3. CRISPR Applications to the COVID-19 pandemic

This report finds that CRISPR-Cas systems can be leveraged to configure an effective countermeasure against a natural or deliberate biological event, particularly in terms of rapidity and feasibility of response. In the case study of CRISPR use in the COVID-19 public health response, three principal applications prevail: diagnostics, research, and potential antiviral therapy. CRISPR diagnostic platforms can provide accessible, rapid, and convenient tests, without sacrificing standards of accuracy. CRISPR-Cas systems as a tool for research can provide an optimized platform to identify and test vectors for countermeasure targets. Finally, CRISPR-Cas antiviral therapy may provide a needed prophylactic treatment option for COVID-19 and other current and future pathogens.

3.1. CRISPR diagnostic platforms

The necessity for testing to curtail the spread of pandemic- and epidemic-prone diseases is of critical importance for adequate public health. When an infectious disease begins to spread, rapid steps must be taken to track those infected in order to protect those who are not. Numerous pandemics have emerged in the past, and in every instance, robust diagnostic testing to support pathogenic surveillance was necessary.[25] In the early stages of confronting pandemic diseases, the rapid development, approval, large-scale manufacturing, and deployment of reliable, fast, and accessible diagnostic tests remains of utmost priority.

Various commentators emphasized the importance of a robust pathogenic surveillance regime during the COVID-19 response: early detection, contact tracing, and isolation of infected individuals for management of contagious individuals to limit their transmission to others. Importantly, this strategy would rely heavily on diagnostic testing, particularly in early stages to prevent a major outbreak.[26] In the United States, the average COVID-19 test sample-to-answer reporting time at one point during the pandemic was 4 days — far beyond what is necessary for contact tracing — in part because the supply chain did not scale up capacity for diagnostic tests and associated materials.[27] Because COVID-19 could spread from individual to individual pre-symptomatically, symptomatically, and asymptomatically, some studies even advocated for prioritizing test accessibility, frequency, and sample-to-answer reporting time over accuracy of positive results.[28]

The gold standard for diagnostic testing of SARS-CoV-2 is the real-time reverse-transcription polymerase chain reaction (RT-PCR) method.[29] However, this method is not without its many challenges. RT-PCR tests require specialized and expensive equipment, a complex molecular laboratory, and highly trained personnel.[30] Comparatively, they are laborious and expensive tests.[31] Moreover, they can produce false negative results,[32] may decrease in sensitivity five days after onset of symptoms,[33] and are susceptible to errors in sample collection.[34] Other common tests such as enzyme-linked immunosorbent assays (ELISA) and rapid antigen and antibody tests are significantly cheaper in comparison to the gold standard RT-PCR test, but they suffer in terms of accuracy and exhibit particularly low sensitivity in the onset and first few days of illness.[35] Rapid antigen and antibody tests are advantageous in accessibility for point of care and low sample-to-answer reporting time (20-60 minutes), but suffer in sensitivity at 50% in comparison to RT-PCR.[36] Furthermore, such an unprecedented demand for diagnostic tests amidst the COVID-19 pandemic led to a shortage in recommended testing supply.[37]

These limiting factors for the gold standard RT-PCR test made it difficult to scale up manufacturing to a level concordant with high demand, often resulting in test shortages and prolonged sample-to-answer reporting time. According to the Infectious Diseases Society of America, test availability and a sample-to-answer responding time within an hour are critical conditions for a positive bearing on care and disease containment.[38] In many cases, diagnostic strategies for adequate surveillance did not meet standards for the containment of COVID-19. Furthermore, gold standard RT-PCR tests don't have the practicality to provide diagnostic testing to endemic regions with limited resources. Without specialized equipment, facilities, and scientists, these complex diagnostic methods become null. However, diagnostics remain critical to limit the disease spread outside of these endemic localities and prevent global or national outbreak.[39] Yet, countries face both global and national disparities in relevant resource distribution, including in diagnostic tests and related

infrastructure or intellectual property.[40] The need for a SARS-CoV-2 test that was rapid, widely distributed, accessible, and accurate drove a dedicated effort to explore innovative diagnostic strategies to address the COVID-19 crisis.

A particularly innovative strategy was CRISPR diagnostics, which relies on the technology's ability to locate specific segments of viral RNA. The approach was established in 2017, and it harnesses CRISPR to quickly pinpoint and tag pathogenic RNA without RNA isolation required by RT-PCR tests that adds hours to the process.[41, 42] Diagnostic testing platforms used this strategy to deliver multiplexed and accurate detection of viral presence, with the reporting mechanism being lateral flow for an easy visual readout.[43] These platforms are called "SHERLOCK" (specific high-sensitivity enzymatic reporter unlocking) and "DETECTR" (DNA endonuclease-targeted CRISPR trans reporter).[43, 44] Upon development, the diagnostic approach was discussed as a revolutionary method to limit disease outbreaks of numerous infectious and non-infectious diseases, but was never approved for use.[45]

The COVID-19 pandemic reoriented defensive efforts within the scientific community towards the common objective to end the public health crisis, as is the case for the efforts of Sherlock and Mammoth Biosciences. These companies emphasized fast, simple, and accessible diagnostic capabilities, and touted the promise of their CRISPR platform to do so, even ceding bitter patent contentions for public health.[46] Thus, Sherlock and Mammoth Biosciences adapted SHERLOCK and DETECTR for SARS-CoV-2.[47, 48,] In March 2020, Sherlock made history with the first FDA authorized use of CRISPR in a diagnostic application, and was soon followed by Mammoth.[49, 50]

When compared with the gold standard RT-PCR tests, CRISPR diagnostic platforms were successful in offering several advantages. These advantages did not sacrifice accuracy. The SHERLOCK adaptation for SARS-CoV-2 is said to take an hour with results that are 100% concordant with the gold standard RT-PCR tests in terms of sensitivity and specificity.[51] The DETECTR adaptation for SARS-CoV-2 is said to be an even faster sample-to-answer alternative to the gold standard RT-PCR tests, yet still comparable in terms of accuracy at 95% positive predictive agreement and 100% negative predictive agreement.[48] Both SHERLOCK and DETECTR exhibit accessibility with ease-of-use qualities such as visual readouts that are converted from CRISPR activity.[52] Furthermore, neither test requires complicated processing through specialized equipment.[53] Because of this profound accessibility, these diagnostic platforms are also promising for important point-of-care testing for SARS-CoV-2, although they currently lack bureaucratic authorization outside of laboratory settings.[48, 54]

These CRISPR diagnostic platforms continue to be promising with each development. Led by CRISPR co-inventor Jennifer Doudna, one research team has recently developed a CRISPR based diagnostic approach that can detect SARS-CoV-2 using only a CRISPR solution and a mobile phone.[55] The tests mix a saliva sample with a chemical solution that allows the CRISPR-Cas system to identify and cut a sequence of SARS-CoV-2, then emit a glow strong enough for a smartphone to detect.[56] Thus, the test is not only capable of reporting a positive or negative result, but also a quantitative estimation of the viral load by measuring the amount of SARS-CoV-2 RNA.[57] Advances like these aim to bring the user interface even closer to patients and practitioners to fill the gaps in diagnostic testing by making diagnosis user-friendly, inexpensive, and portable.

CRISPR diagnostic platforms underscored notable lessons learned in how CRISPR technologies can be harnessed to aid current and

future public health responses. CRISPR diagnostic platforms can provide tests that are more accessible in terms of ease of use and cost, rapid in terms of sample-to-answer reporting time, and convenient in terms of point-of-care, all without sacrificing specificity or sensitivity. Moreover, CRISPR's diagnostic potential to provide unprecedented accessibility is actively being discussed, including over-the-counter CRISPR-based tests for diseases such as COVID-19, HPV, HIV, malaria, Zika, tuberculosis, dengue, and even cancer. [58, 59] CRISPR is naturally multiplexed, meaning that it has high potential for providing a test that detects multiple diseases at once.[60] Clearly, CRISPR can support pathogenic surveillance regimes by providing more efficient avenues for identification of infected individuals, thereby optimizing contact tracing and disease management efforts.

These factors by which CRISPR can augment the standards of diagnostic testing are all policy relevant. CRISPR diagnostic testing is rapid, simple, and cost-effective enough to concur with a timeline relevant to public health. Whereas gold standard RT-PCR sample-to-answer reporting time delays were largely due to supply chain issues for complicated materials and procedural issues for complicated processes, CRISPR tests can help limit pathogenic spread and optimize contact tracing by offering in-clinic results. It is perhaps equally important that detection methods prioritize point of care and accessibility so that testing strategies can feasibly extend to low- and lower-middle-income countries, remote communities that are hours away from the nearest health provider, and any other locality with limited resources or lack of required equipment. This accessibility factor of CRISPR is extremely policy relevant to prevent pathogenic spread out of endemic regions by both limiting the outbreak within these regions and reducing the need to travel outside of these regions. The low $1-2 cost per test would be feasible for consumers and national testing programs, expanding testing capacity in economic terms.[61]

CRISPR-based diagnostics have not been in effect for long — the first authorized use of a CRISPR test was in March 2020. This fact necessitates more studies concerning the trade-off between CRISPR diagnostic testing and alternatives like gold standard RT-PCR tests, particularly in terms of sensitivity and specificity. However, the initial application of CRISPR for diagnostic purposes in the COVID-19 pandemic is promising. The faster, more accurate, more accessible, easier, and cheaper diagnostic potential of CRISPR could improve the pathogenic surveillance of the COVID-19 pandemic and be a boon for global biopreparedness and confrontation of diseases.

3.2. CRISPR assisted research

The necessity for rapid research on Sars-CoV-2 facilitated by CRISPR-Cas systems enabled quicker development rates for public health countermeasures against infectious pathogens. CRISPR-Cas assisted screening is a process by which researchers can inhibit certain genetic functions in order to find the equivalent of a few needles in a haystack of a complex genome. Researchers often use this CRISPR "knock-out" technique in order to identify the effect of key genes. Researchers can also elicit gain of function by inserting a genetic sequence that the DNA repair mechanisms can utilize as a template when repairing the cuts made by the Cas proteins. These CRISPR "knock-in" techniques can be utilized, for instance, to make experimental procedures more applicable to a desired target.

During the COVID-19 pandemic, these research techniques were heavily utilized to better understand SARS-CoV-2, ultimately in order to develop and test disease countermeasures. Numerous genome-wide CRISPR screening tests

for SARS-CoV-2 were conducted in order to better understand how the virus infected human cells.[62, 63, 64] Some of these tests sought to identify host factors required for SARS-CoV-2 entry with the intent to establish COVID-19 countermeasures that prophylactically or therapeutically targeted the human cell. Other CRISPR screening tests sought to identify viral vectors with the intent to establish potential targets on the virus to inhibit its replication. For instance, researchers discovered both key host and viral factors that modulate SARS-CoV-2 entry into a human cell like the important human ACE2 receptor or the S1/S2 boundary of the SARS-CoV-2 spike protein.[65] For the purposes of this report, understanding the terminology and complex biology behind these factors is less important than understanding the use of CRISPR in discovering them. For SARS-CoV-2 research, CRISPR screening techniques were notably utilized for the background of prophylactic and therapeutic treatment strategies by identifying factors that SARS-CoV-2 relies upon for its replication.

Furthermore, researchers utilized a CRISPR "knock-in" technique to safely apply clinical evaluation to experimental trials. In one study conducted by Sun et al., researchers effectively humanized a mouse by inserting the gene for the host factor that allows SARS-CoV-2 to enter into a human cell, the ACE2 receptor.[66] By eliciting a gain of function outcome that allowed a mouse to become infected by COVID-19, these researchers created a tool that could be used to evaluate the effectiveness of COVID-19 vaccines and therapeutic treatments in clinical trials on non-human subjects. The mouse could then be treated with a vaccine, therapy, or drug that could not yet be ethically tried on human subjects. In the need for rapid development and evaluation of human-targeted prophylactic and therapeutic countermeasures to a pathogen, this is a crucial step to optimize procedure.

This research is the necessary background to continue developing and evaluating COVID-19 therapeutic, vaccine, and drug candidates in clinical trials. The development of these countermeasures remains crucial as nations must continue to cope with COVID-19 cases. Beyond the application of COVID-19, CRISPR can be utilized to identify factors upon which any pathogen relies for replication with these experimental techniques. In fact, researchers are now identifying viral and host factors to target for other critical diseases — such as HIV or malaria – – and discussing the potential of novel countermeasures for these pathogens as well.[67, 68] CRISPR-Cas systems are clearly a promising research tool that can provide an efficient and accurate platform to identify and test viral vectors for needed antiviral therapeutic, vaccine, and drug targets. This tool can be utilized to optimize the configuration of a countermeasure against an emerging pathogen and mitigate its pathogenesis.

3.3. CRISPR therapeutic treatment

CRISPR therapy can potentially provide an important countermeasure opportunity for COVID-19, as well as the ability to reconfigure treatment for other persistent and emergent diseases. Since December 2020, vaccines have been approved and rolled-out across the world in an effort to control the pandemic.[69] However, the mutation rate of SARS-CoV-2 is relatively high, and each variant poses new challenges. Human behavior, motivation, and culture continue to be essential for effective pandemic recovery. Given broad societal challenges such as vaccine hesitancy and vaccine inequity, as well as the fact that vaccinated people can still be infected and spread COVID-19, the pandemic will persist globally.[70, 71] As the world has seen, an outbreak of COVID-19 anywhere threatens to increase the case rate

everywhere. Thus, there is a persistent need for COVID-19 treatment options as the pandemic continues. This need can potentially be filled by CRISPR therapeutic treatment for SARS-CoV-2.

Applying CRISPR-Cas systems as a countermeasure to COVID-19 has demonstrated promising initial evaluations, although the novel therapeutic option has received comparatively less attention than other therapeutic options.[72] One group of Stanford-based CRISPR researchers have developed a CRISPR facilitated therapeutic approach called prophylactic antiviral CRISPR in human cells (PAC-MAN) that can effectively degrade RNA from SARS-CoV-2 in human cells.[73] A significant challenge for the Stanford team is finding a delivery mechanism for the PAC-MAN approach, most effectively targeting epithelial cells in the lung where the virus inflicts the most damage. If this barrier is overcome, the team believes that the PAC-MAN approach can be utilized as a countermeasure to all coronaviruses and emerging variants of SARS-CoV-2. In the same study, the approach was found to demonstrate effectiveness in degrading RNA from influenza in human cells.

Another group of CRISPR researchers utilized a highly reprogrammable CRISPR-Cas system to target SARS-CoV-2 transcripts that code for specific proteins like the spike protein.[74] As mentioned, the spike protein on coronaviruses was identified by CRISPR knock-out techniques to be integral for latching onto and infecting the human cell. Ultimately, the reprogrammable CRISPR-Cas system demonstrated a high degree of effectiveness, with greater than 98% efficiency in silencing these regions of viral transcript. Notably, the group expressed concern for emerging variants of SARS-CoV-2 and the propensity of SARS-CoV-2 to escape from host immunity. The reprogrammable nature of this CRISPR approach is a vital countermeasure for emerging strains of SARS-CoV-2, but also for the system's adaptability to future emerging pathogens. Either the PAC-MAN approach or the reprogrammable CRISPR-Cas system can effectively deactivate SARS-CoV-2 replication cycles.

It should be noted that CRISPR-Cas therapeutic treatment is subject to significant ethical considerations. CRISPR-Cas systems recently brought discussion of gene therapy back into the limelight after tragic setbacks related to other prior gene therapy techniques.[75] However, opportunities associated with CRISPR-Cas systems are not without ethical concerns as well. This is especially true regarding CRISPR germline therapy that causes genetic changes at an early age in all cells, therefore driving through generations via inheritance. Somatic gene therapy through CRISPR, on the other hand, causes genetic changes in only certain cells, but still some commentators remain skeptical and stipulate that ethical distinctions between somatic editing and the more controversial germline editing are not as clear cut as they seem.[76] Furthermore, due to research and development costs, CRISPR gene therapy is likely to be restrictively expensive to lower income individuals.[77] There is also significant concern for limitations to CRISPR like off-target effects — where the system cuts and replaces an unintended sequence and has unpredictable effects. Other limitations include DNA-Damage toxicity, where the editing may trigger effects like early cell death, or immunotoxicity, where the cells may build immunity against the therapeutic treatment.[78] These limitations are especially of concern if the therapeutic target is the human cell.

CRISPR-Cas systems are known to be naturally occurring in bacteria with the purpose of deleting RNA from an infectious virus that hijacks cell functions. These systems can be therapeutically leveraged for somatic gene editing in human cells for the same purpose. Beyond the potential of these systems for this pandemic,

CRISPR-Cas systems can be applied to persistent pathogens like HIV.[79] Before broad application of CRISPR therapies to any disease, careful ethical considerations and clinical trials must be carried out. Thus, CRISPR antiviral therapies have not yet been clinically applied as a countermeasure against COVID-19.[80] Nonetheless, the pandemic persists and there remains great potential: CRISPR-Cas systems can provide a therapeutic treatment for SARS-CoV-2 and emerging variants, setting the stage for an important reprogrammable countermeasure for future pathogens.

4. The role of CRISPR in the strategic environment

CRISPR democratizes gene editing in terms of ease-of-use, cost, and commercial accessibility. It is therefore possible for malicious non-state attackers to engineer a dangerous pathogen and cause a DBE. Some barriers to access remain for the attacker, including scientific knowledge in the field of genetics and mechanism of delivering an engineered pathogen. Nonetheless, the first-mover advantage is prominent as it is far less complicated to release a pathogen than to contain it. Unintended effects caused by a lack of scientific knowledge when engineering a pathogen may be irrelevant and still drive disease through society. Further, the complexity of an efficient and coordinated defense response with varied stakeholders contributes to containment difficulties. This comparative ease of release relative to containment is a key asset for the attacker and gives the CRISPR advantage to the attacker in the very short-term engineering phase and immediately following release.

In the countermeasure response to a pathogen, efficiency and rapidity are key to containment. This report finds that CRISPR can be leveraged to rapidly configure this defense countermeasure. CRISPR diagnostic tests offer certain advantages over gold standard RT-PCR tests, including accessibility, sample-to-answer reporting time, and convenience. These tests are of critical importance for a pathogenic surveillance regime to efficiently and rapidly contain the spread. CRISPR tests are a key asset for the defender – especially in initial outbreak stages – and can be leveraged further to enhance the defense advantage of CRISPR in the short term. CRISPR offers a platform to identify and test viral vectors, aiding the configuration of countermeasures like vaccines and treatments. This efficient platform is to the advantage of the defender in the short- to mid-term. Finally, CRISPR offers the potential development of therapeutic treatment options for the defender's long-term countermeasure strategy since diseases aren't just eradicated. CRISPR can also test the effectiveness of these long-term therapeutic treatments.

Therefore, this report concludes that the decreased barrier to access for malicious actors to engineer a pathogen renders CRISPR more advantageous to a malicious, non-state actor in the very short-term. However, the remaining barriers for the attacker and the CRISPR defense applications against said pathogen suggest a net positive for the defender in the short- to mid-term and long-term response. The short- to mid-term response refers to the direct response to a biological event that can last months to a few years after first cases are identified; the long-term response refers to the several year- to decade-long biological preparation efforts from the defender. With increased research and development (R&D), applied use, and normalization of CRISPR, this net positive can only improve for the defender. Through development of policies that optimize CRISPR for rapid defense applications, the comparative advantage of CRISPR may decisively shift from the attacker to the defender in the very short-term as well.

5. Policy recommendations

Accordingly, this report recommends four policy changes: 1. R&D of CRISPR to break down limitations and build up social norms, 2. The creation of an international platform to promote information sharing, 3. Investment in a manufacturing sector for ready CRISPR diagnostic testing, and 4. Deployment of CRISPR diagnostic tests to endemic regions upon outbreak. These policy recommendations are not intended to be comprehensive. Rather the emphasis of this report is to identify some lessons learned from the applications of CRISPR during the COVID-19 pandemic as a prompt for a rethought biopreparedness strategy for an evolving global health strategic environment.

CRISPR-Cas systems have several technical limitations that include possible off-target effects, immunotoxicity, and DNA-Damage toxicity. These limitations engender social hesitancy due to reasonable ethical concerns, ultimately curtailing the defender's ability to configure a prepared and rapid public health response to a pathogen. In order for CRISPR to be applied to biopreparedness and biosafety on a needed broader scale, limitations and associated hesitancy must be addressed. Thus, the first recommendation in this report is increased R&D funding and efforts to break down limitations regarding CRISPR. Mounting use of the technology after limitations are addressed will build social norms around CRISPR and mitigate public hesitancy for safe applications.

One notable enabling factor for the recent applications of CRISPR was the decision to put patents aside to aid the effort to contain COVID-19.[44] This decision seems to signify that lessened resources devoted to court fights along with the information sharing for a common cause allowed for more efficient development and application of CRISPR. Scientific research builds upon other scientific research, and with an ever-intensifying threat of infectious disease, the scientific community must accelerate R&D of CRISPR for defense applications. Thus, this report also recommends an international platform for information sharing surrounding altruistic CRISPR research efforts for biopreparedness, coordinated by the World Health Organization.

A primary intervention for a defender preventing a widespread outbreak of disease is diagnostic testing to support an efficient pathogenic surveillance regime. This report therefore recommends incentivizing national private sectors to create new manufacturing capacity for ready CRISPR diagnostic tests for priority pathogens. Furthermore, this report recommends that nations deploy these portable CRISPR diagnostic tests to endemic regions upon outbreak. COVID-19 has demonstrated that an outbreak anywhere is a threat everywhere, and the low-cost, portable, and easy-to-use characteristics of CRISPR diagnostic tests could be leveraged to rapidly contain pathogens.

6. Conclusion

The strategic environment for biosecurity is more unstable than ever before. CRISPR further exacerbates an already heightened threat of infectious disease by reducing barriers to entry for genetically engineered pathogens. At the same time, CRISPR is a remarkable tool for defense against either a natural or deliberate biological event. Applied use in the COVID-19 pandemic afforded several lessons learned for how CRISPR can help support pathogenic surveillance regimes, research, and countermeasure development. CRISPR must first overcome technical limitations and social hesitancy, then be broadly applied to biopreparedness strategies like CRISPR diagnostic tests on stand-by. This type of rethought approach is necessary to fully leverage the technology to the advantage of the defender in a transformed biosecurity strategic environment.

Before COVID-19, commentators warned of the emergence of a novel, pandemic-level pathogen. When it arrived, we were not prepared to contain it. Moreover, the interplay between an attacker, a defender, and CRISPR within the contemporary strategic environment does not exist in a vacuum, and there are also several concerning human-driven trends that exacerbate risk: global warming,[81-84] permafrost thaw,[85,86] climate-related migration,[87] deforestation,[88,89] and loss of biodiversity.[90] The critical need for biopreparedness strategies to counteract the increased risk of biological events, whether deliberate or natural, is greater than ever before. When the next pathogen arrives, will we have learned to leverage CRISPR for a prepared and rapid response? Our survival may depend on it.

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The Impacts on Well-being of Undergraduate College Students Serving in a Resident Assistant Role

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The Impacts on Well-being of Undergraduate College Students Serving in a Resident Assistant Role

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Abstract

Resident Assistants (RAs) are an integral part of the residential experience at colleges and universities, but little attention has been paid to how the RA position impacts student workers. This study examines the effect of the RA position on the well-being of undergraduate students working in the RA role. Three surveys collecting anonymous data on student well-being using the Keyes Flourishing Scale were distributed over the course of the fall 2018 academic semester. The SF-12 Health Questionnaire, the Sarason Social Support Questionnaire, the Deakin Coping Scale, and the Perceived Stress Scale were used to collect further data. Analysis was performed on data from 16 student RAs who responded to all three surveys. This diminished sample size prevented statistically significant data, but trends in the data are still evident. Social support remained positively correlated with well-being over the course of the semester. At the end of the semester, mental health was positively correlated with well-being while perceived stress was negatively correlated with well-being. Moreover, RAs in upperclassmen dorms and those with greater prior RA experience had nonsignificant yet overall higher levels of well-being throughout the semester. Though correlations are present between the well-being of student RAs and other factors in their lives, more data are needed to prove significance and further determine the relations between these factors.

Keywords: resident assistant, well-being, college, university

1. Introduction

The resident assistant (RA) position is an integral part of college residential community life and encompasses complex and varied responsibilities. Students hired as RAs provide peer support for fellow students living in campus residence halls and enforce school policies with an overall aim of fostering a community where residents can flourish personally and academically. RAs also act as first responders to student challenges, including substance abuse and physical and mental health crises.[14, 15] This demanding job creates tension in students employed as RAs, who must act as both an authority figure and a friendly peer.[8] This tension means that student RAs may be hesitant to disrupt "social cohesion" by reporting rule breakers.[16] The stress inherent in an RA position might cause a negative impact on the well-being of students in this role.

Studies about RAs have largely focused on the benefits that RAs provide to a college community. These studies have identified factors contributing to burnout in RAs[4], examined the influence of personality[2] and role conflict[3] on RA job performance, identified skills transferable to careers and life post-graduation,[6] and created tools with which to measure RA impacts on community development.[9] In their 2018 paper, McLaughlin recognized the lack of research about self-care in student RAs[10]; however, research into RAs remains focused on job function and institutional benefits. More attention must be paid to students in RA positions, as these students generally face

more stressful situations than their non-RA peers.[11] The RA position is a social one, and evidence suggests that stronger social support systems are tied to better mental health.[5] Coping strategies of student RAs in stressful situations might also influence well-being, as better self-care practices lead to lower stress susceptibility.[13] However, factors correlated with higher levels of well-being in student RAs have not yet been well examined.

This study aimed to examine whether coping skills, perceived stress, and levels of social support improve or detract from the well-being of student RAs over the course of one 15-week semester. The information gained from this study can be used to promote well-being in the RA population. It was hypothesized that higher levels of social support would correlate with lower levels of perceived stress, higher well-being scores, and healthier coping strategies throughout the semester.

2. Methods

2.1 Design

This IRB approved study (2018-0489) was conducted from August 2018 through January 2019. A three-part survey design was used to assess the impacts of serving in an RA role, with surveys sent out pre-RA training, post-RA training, and post-semester. A senior staff member from the Office of Residential Education contributed to the design of the survey and approved its use.

2.2 Participants

Students older than 18 who were entering RA training at the start of the 2018-2019 academic year were invited to participate, and both new and returning RAs were included. This invitation was extended during an in-person information session conducted by the Principal Investigator on Day 2 of RA training, when informed consent was obtained through a written consent form. Surveys were distributed to participants via email: Survey 1 was sent out at the end of Day 2 of RA training (open for 3 days), Survey 2 was distributed at the end of RA training 8 days after the first survey was sent (open for 5 days), and Survey 3 was distributed at the end of final exams for the Fall 2018 semester (open 19 days over the semester break). Sixteen individuals (n=16) completed all three surveys; only their data were analyzed.

2.3 Instruments

Demographic data collected included participant age, gender, class year, previous RA experience, and residence hall placement (Table A1). Participant majors were collected but not presented as this could identify participants within the small population analyzed; given the small sample size, impacts of participant major on any of the measures of interest could not be determined. Additional data were gathered using the following instruments:

The Keyes Flourishing Scale (KFS) measures the general level of well-being in a person.[7] This scale was used to track how the well-being of an RA changed over the course of a semester. Trends in well-being were compared against trends in other aspects of a student RA's life, namely social support, perceived stress, and coping strategies.

The Sarason Social Support Questionnaire (SSSQ) measures the level of social support present in a person's life.[14] This scale was used to determine how social support fluctuated over the course of a semester. Given that greater social support is correlated with better mental health[5], trends in social support were compared to those in well-being.

The Perceived Stress Scale (PSS) measures how an individual subjectively views stress in their life.[1] This scale was used to determine the level of stress that student RAs subjectively experienced, with a focus on whether increased levels of perceived stress are correlated with decreased levels of well-being.

The Deakin Coping Scale (DCS) measures how an individual copes with problems that arise in their life.[12] This instrument was used to determine how student RAs coped with stressors in their lives, and particularly to assess whether

better coping strategies are correlated with higher levels of well-being in student RAs.

2.4 Procedure

Qualtrics served as the platform for survey distribution and data collection. The link to each Qualtrics survey was distributed to participants via email. Each participant initially created a password consisting of an animal and a three-digit number; participants input this password at the beginning of each survey. This was the only item that allowed follow-through of participant survey answers over the course of the study; thus, data were kept anonymous while allowing for trends in an individual's responses to be evaluated over time.

2.5 Data Analysis

At the study's conclusion, data were analyzed via SPSS. All data are presented anonymously and in aggregate (Table A2). Associations between the measures were analyzed using Pearson's Product Moment Correlations.

3. Results

Well-being was positively correlated with social support throughout the semester (Table 1). Well-being was positively correlated with coping skills in the first and second surveys and inversely correlated with perceived stress in the third survey (Table 1). Individual levels of well-being remained stable over the course of the study.

Although not significant, there was an incidental finding of lower levels of well-being in student RAs placed in first year residence halls compared to the well-being of RAs in other residence halls. Results also showed that student RAs with two or more years of prior RA experience had nonsignificant but overall higher levels of well-being compared to student RAs with less prior RA experience.

Table 1. Pearson's Correlations between Student RA Well-Being and Social Support, Perceived Stress, and Coping Skills Across a Semester.

	KFS Time 1	KFS Time 2	KFS Time 3
SSSQ	.682**	.565*	.527*
PSS	-.479	-.492	-.519*
DCS	.571*	.633**	.266

*p < .05. **p < .01.

4. Limitations

This study was limited by its small sample size, as only 16 student RAs responded to all three surveys. Additionally, all participants were enrolled at the same university, leading to potential uniformity among subjects. Response bias may be present as participants who responded to all three surveys might have higher baseline levels of well-being. Follow-through would likely be increased with outside incentive to participate, which was not provided in this study.

5. Discussion

Causality cannot be determined through this study. However, it can be concluded that there is a correlation between higher levels of social support and higher levels of well-being in student RA populations throughout a semester. This correlation may exist because stronger social support systems are linked to better mental health[5], or there could be a third influencing factor not yet discovered. Better coping strategies were correlated with higher well-being during and just after RA training, but not at the end of the semester. Higher levels of perceived stress were inversely correlated with higher levels of well-being only at the end of the semester. A larger study with more diverse participants is needed to validate these findings and ensure significance.

This study suggests a relationship between years of prior RA experience and well-being (Figure A2 and Table A4). It also suggests a relationship between residence hall placement and well-being (Figure A1 and Table A3); this is consistent with Hardy et al.'s finding that student RAs in first year residence halls face higher levels of burnout.[4] Further research should examine the factors influencing well-being for returning versus new RAs, as this information can be used as a recruitment tool to improve RA retention rates.

6. Conclusions

As student RAs are often at the frontlines of student support, universities must take measures to ensure the students in these RA roles are able to flourish. This is necessary not only to preserve the functionality of the RA role, but also to preserve the well-being of students in these roles. The findings of this study indicate that RA programs should foster strong social support networks for their student RA communities to improve overall well-being. This study also suggests that universities should teach healthy coping strategies to their student RAs at the beginning of the semester and help student RAs recognize and reduce perceived stress at the end of the semester. Student RAs work an incredible amount to promote the well-being of students living in university residence halls while serving university interests. It is thus imperative that, in turn, universities care for the well-being of their RAs.

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Appendix

Table A1. Demographic information for study participants (n=16). Demographic information is presented both as a percentage (%) of the total participants and as the number (n) of participants in each category.

	%	*n*
Age		
19	43.8	7
20	18.8	3
21	37.5	6
Gender		
Male	43.8	7
Female	56.3	9
Grade		
Sophomore	50.0	8
Junior	6.3	1
Senior	43.8	7
Previous Years as an RA		
0	50.0	8
1	25.0	4
2	25.0	4
Residency Hall Placement		
Freshman	50.0	8
Mixed	31.3	5
Primarily Upperclassmen	18.8	3

Table A2. Well-being in student RAs is correlated with various aspects of the student's life throughout the course of a semester. Correlational data was calculated using Pearson Correlation between well-being (KFS), social support (SSSQ), perceived stress (PSS), physical health (HQ-P), mental health (HQ-M), and coping skills (DCS) from surveys 1, 2, and 3. Well-being was correlated with social support throughout the course of the semester. Well-being was correlated with coping skills in the first survey, but later that correlation became insignificant. In the third survey, well-being was inversely correlated with perceived stress and positively correlated with mental health.
*: Correlation is significant at the 0.05 level (2-tailed).
**: Correlation is significant at the 0.01 level (2-tailed).

Survey 1		KFS	SSSQ	PSS	HQ-P	HQ-M
SSSQ	Pearson Correlation	.682**				
	Sig. (2-tailed)	.004				
PSS	Pearson Correlation	-.479	-.521*			
	Sig. (2-tailed)	.061	.039			
HQ-P	Pearson Correlation	-.129	-.099	.258		
	Sig. (2-tailed)	.633	.715	.335		
HQ-M	Pearson Correlation	.292	.392	-.655**	-.696**	
	Sig. (2-tailed)	.272	.133	.006	.003	
DCS	Pearson Correlation	.571*	.391	-.108	.240	-.138
	Sig. (2-tailed)	.021	.134	.691	.370	.610
Survey 2						
SSSQ	Pearson Correlation	.565*				
	Sig. (2-tailed)	.023				
PSS	Pearson Correlation	-.492	-.371			
	Sig. (2-tailed)	.053	.157			
HQ-P	Pearson Correlation	-.106	.067	.113		
	Sig. (2-tailed)	.696	.806	.677		
HQ-M	Pearson Correlation	.473	.285	-.826**	-.444	
	Sig. (2-tailed)	.065	.284	.000	.085	
DCS	Pearson Correlation	.633**	.349	-.312	.144	.201
	Sig. (2-tailed)	.008	.185	.239	.596	.455
Survey 3						
SSSQ	Pearson Correlation	.527*				
	Sig. (2-tailed)	.036				
PSS	Pearson Correlation	-.519*	-.481			
	Sig. (2-tailed)	.039	.059			
HQ-P	Pearson Correlation	.146	.052	.125		
	Sig. (2-tailed)	.588	.847	.644		
HQ-M	Pearson Correlation	.618*	.382	-.848**	-.221	
	Sig. (2-tailed)	.011	.144	.000	.411	
DCS	Pearson Correlation	.266	.267	.088	.481	.033
	Sig. (2-tailed)	.320	.317	.746	.059	.903

Table A3. Multivariate Tests[a]: Comparison between Well-being and Residency Placement. There were no significant correlations between residency placement and well-being over the course of the semester (Wilks' Lambda: 0.583). There were overall higher levels of well-being in student RAs placed in primarily upperclassman residency halls compared to mixed or freshman residency halls, with student RAs in freshman residency halls consistently having the lowest levels of well-being. See Figure A1 for graphical representation of data.

Effect		Value	F	Hypothesis df	Error df	Sig.
Wellbeing	Pillai's Trace	.103	.692[b]	2.000	12.000	.519
	Wilks' Lambda	.897	.692[b]	2.000	12.000	.519
	Hotelling's Trace	.115	.692[b]	2.000	12.000	.519
	Roy's Largest Root	.115	.692[b]	2.000	12.000	.519
Wellbeing * D9num	Pillai's Trace	.210	.764	4.000	26.000	.558
	Wilks' Lambda	.796	.726[b]	4.000	24.000	.583
	Hotelling's Trace	.249	.685	4.000	22.000	.610
	Roy's Largest Root	.213	1.386[c]	2.000	13.000	.285

a. Design: Intercept + D9num
Within Subjects Design: Wellbeing

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table A4. Multivariate Tests[a]: Comparison between Well-being and Years as an RA. There were no significant correlations between number of previous years of student RA experience and well-being over the course of the semester (Wilks' Lambda: 0.491). There were overall higher levels of well-being in student RAs who had two or more years prior experience as an RA compared to one-year prior experience or no years prior experience. See Figure A2 for graphical representation of data.

Effect		Value	F	Hypothesis df	Error df	Sig.
Wellbeing	Pillai's Trace	.145	1.016[b]	2.000	12.000	.391
	Wilks' Lambda	.855	1.016[b]	2.000	12.000	.391
	Hotelling's Trace	.169	1.016[b]	2.000	12.000	.391
	Roy's Largest Root	.169	1.016[b]	2.000	12.000	.391
Wellbeing * D8	Pillai's Trace	.240	.885	4.000	26.000	.487
	Wilks' Lambda	.761	.879[b]	4.000	24.000	.491
	Hotelling's Trace	.314	.864	4.000	22.000	.501
	Roy's Largest Root	.313	2.031[c]	2.000	13.000	.171

a. Design: Intercept + D8
Within Subjects Design: Wellbeing
b. Exact statistics
c. The statistic is an upper bound on F that yields a lower bound on the significance levels.

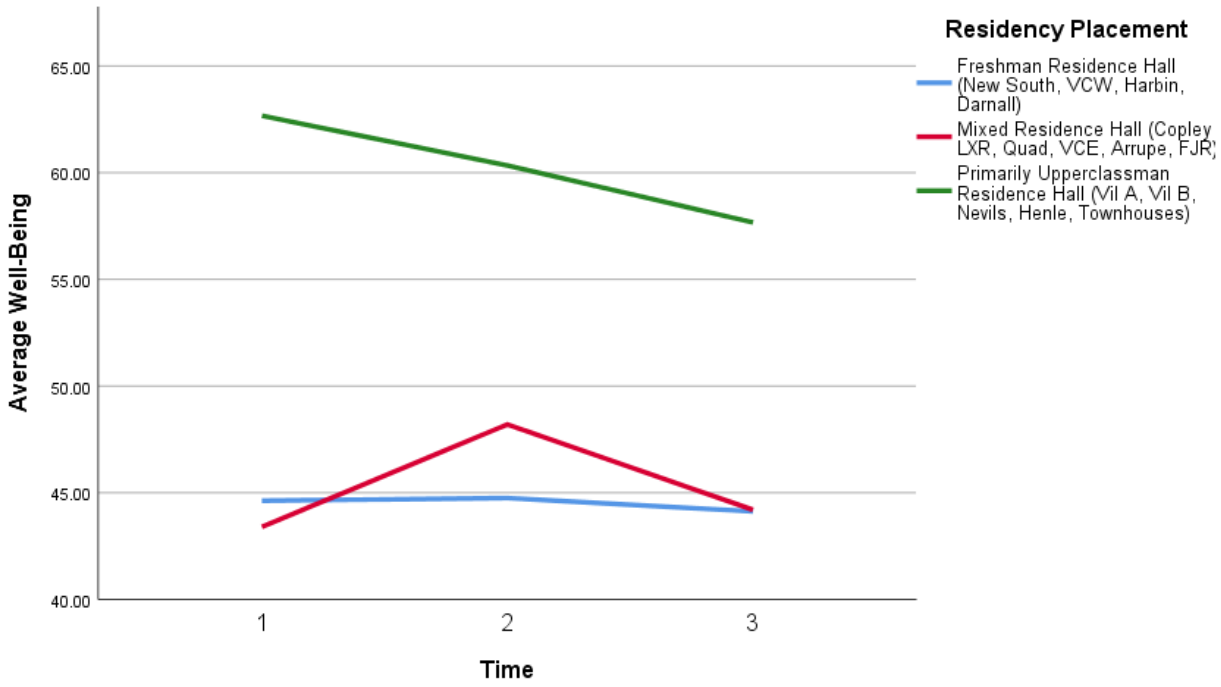

Figure A1. Well-being over time compared to residency placement of the student RA. There were no significant correlations between residency placement and well-being over the course of the semester (Wilks' Lambda: 0.583). There were overall higher levels of well-being in student RAs placed in primarily upperclassman residency halls (green line) compared to mixed (red line) or freshman (blue line) residency halls, with student RAs in freshman residency halls having the consistently lowest levels of well-being.

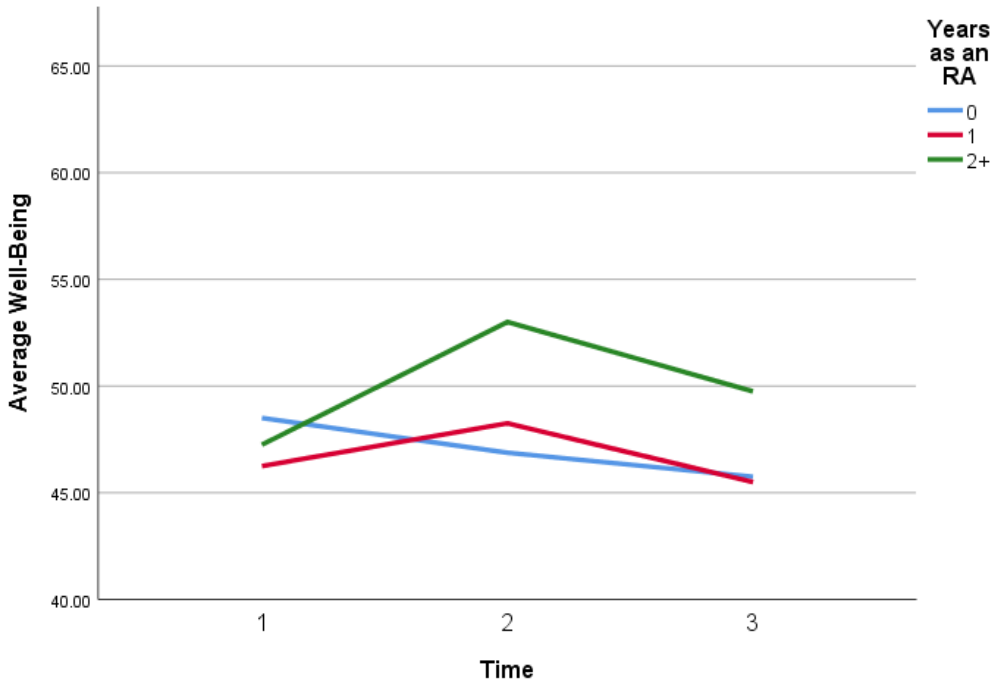

Figure A2. Well-being over time compared to previous years of experience as a student RA. There were no significant correlations between number of previous years of student RA experience and well-being over the course of the semester (Wilks' Lambda: 0.491). There were overall higher levels of well-being in student RAs who had two or more years prior experience as an RA (green line) compared to one-year prior experience (red line) or no years prior experience (blue line).

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