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# Undergraduate Research Journal

Guadalupe Abonce
Rechael Acheampong
Alexandra Aringer
Katelynn Bergman
Tiffany Gomez
Daniel Gutierrez
Sarah Ibrahim
Janine Medina
Dagoberto Partida
Jonathan Phillips
Camilla Reyes
Amanda Xayprasеuth
Sharon Zhao
Julia Zumaya

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# Undergraduate Research Journal

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# From the Administration

So far, 2020 has provided an intense study in deviation. As a research university, we made rapid adjustments when COVID-19 swept through the country, disrupting normal operations, upsetting our plans and work in progress. Yet, this year has also given us a lesson in rigor and adaptability. Despite the challenges, our students, faculty, and research teams worked to move forward. And when we couldn't, we learned from that. In other words, no matter where we turned, we strengthened our skills as researchers. Even in a pandemic, we seized opportunities to bring new learning to light.

You will see in this 14th volume of the UC Riverside Undergraduate Research Journal that our students are delivering truly remarkable work. The published papers this year span the disciplines of Psychology, Biomedical Sciences, Bioengineering, Environmental Sciences, English, Dance, and Ecology. One paper examines how feelings of shame and guilt impact procrastination. Another studies our respiratory system's response to toxins in organic dust.

Timeless and timely—these examples are but two highlights from this year's journal reminding us that our scholarly pursuits are critical at any time but especially now as we turn to science for help against a viral enemy. At UCR we are stewards of transformation; we create knowledge through student-centered research endeavors, providing the next generation with skills to address tomorrow's challenges.

To all of the students who contributed to this year's Journal, congratulations on your impressive work. I also offer my heartfelt gratitude to the faculty mentors and staff members whose dedication, guidance, and perseverance made these projects possible. All of your efforts inspire pride in our university and hope for the future.

Sincerely,

Kim A. Wilcox
Chancellor

The Fourteenth Annual UC Riverside Undergraduate Research Journal features some of the very best faculty-mentored undergraduate research and scholarship from America's fastest rising university. The Undergraduate Research Journal adds to the university's impressive 66-year legacy of high-impact research, which fully integrates with the undergraduate student engagement experience.

In many ways, UC Riverside represents the university of tomorrow. Our inclusive culture, world-renowned faculty, and rigorous and future-focused environment produces young researchers who are prepared to tackle the dynamic challenges that face not only California's Inland Empire, but the world at large. The students whose work is presented in the journal are enriching the environmental, economic, social, and cultural future of California, the nation, and the world.

Congratulations to each of the scholars featured in this journal as this is the culmination of a lengthy process of discovery filled with excitement, frustration, and anticipation. Thank you to our students, faculty, and staff who made the publication of the Undergraduate Research Journal possible. In particular, I would like to acknowledge the Student Editorial Board for leading the peer-review process, the Faculty Advisory Board for its consultation and advice, and Gladis Herrera-Berkowitz for her role in bringing this journal to fruition.

I hope you enjoy and are enriched by the discoveries revealed in this year's Undergraduate Research Journal.

Wishing you all the best,

Jennifer Brown, Ph.,D.
Vice Provost and Dean of Undergraduate Education

# Undergraduate Research Journal Editorial Board

**Phuong-Anh Do**
Editor in Chief
*Biology*

It is with great pleasure that we present UC Riverside's 14th Edition of the Undergraduate Research Journal. It has been an honor to work alongside talented individuals across disciplines and backgrounds, all of whom have contributed to the success of the Journal. Congratulations to the authors, Student Editorial Board, and Faculty Advisory Board — your diligence, dedication, and efforts towards the editing process have ensured the quality and success of the Journal. The research presented in this Journal represents the creative culture and academic achievements of the UCR student body and contributes to the legacy of excellence within UCR's undergraduate research. We are proud and extremely humbled to have been part of an outstanding team that has made this year's Journal a success.

Sincerely,
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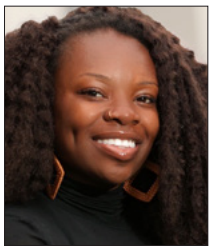

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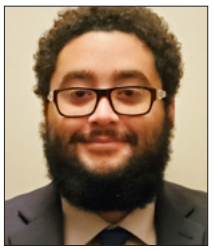

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# From the Faculty Advisory Board

Since UCR's Undergraduate Research Journal started, it has published over 150 scholarly articles across many fields. These papers represent the commitment of our undergraduate students to performing independent research as part of their undergraduate experience. The Undergraduate Research Journal thus fills a critical need. More often than not, undergraduate research forms part of a larger work with many contributors, which can mean a dilution of the student's contributions as well as a longer time between completing the work and publication. With the Undergraduate Research Journal, our students can publish their work and get first-author credit. They can publish before the end of the academic year, and gain the experience of seeing their manuscript go through a peer-review and publication process just like a standard research journal. When the paper becomes a part of a student's professional experience, it contributes to their record of scholarly achievement. The Journal submission and review process is run by undergraduates who form the Student Editorial Board, working with members of the Faculty Advisory Board. The preparation of this issue has been especially challenging because students and faculty have been able to meet only virtually. We owe a debt of gratitude to the students for their professionalism and dedication for the review and preparation of the articles in this issue. We are also grateful for the participation of the members of the Faculty Advisory Board in guiding the reviewers. If you are interested in publishing your undergraduate research at UCR, consider submission to our next issue!

Prof. Morris F. Maduro
Chair of the Undergraduate Research Journal
Faculty Advisory Board
Professor of Biology

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# About the Cover

## Amanda Xaypraseuth

Amanda is a fourth year Bioengineering major who enjoys balancing her time between science and the arts. Throughout her time at UCR, she has participated in ASUCR's Marketing Committee, served as the Bourns College of Engineering Day's Graphic Designer, and created graphics for various organizations and friends. Currently, she's dedicating her time towards developing an online women's magazine and exercising her interest in Product Design to further develop her design skills.

## THE PATH OF LEAST RESISTANCE

Located near Page, Arizona, this crescent-shaped meander is better known as Horseshoe Bend. As I approached this spectacle, the Type A in me gave up the fight for constant control of the situation and succumbed to the grandeur of the scene. I pondered and took a long stare at what was in front of me when I remembered how this greatness was created — like how I would navigate my life moving forward — by following the path of least resistance.

# Evaluating Greenhouse Gas Emissions from Soil Application of Anaerobic Organic Digestate Compared with Conventional Manure

**Guadalupe Abonce, Sharon Zhao, Michael Rodrigues, & Francesca Hopkins**
*Department of Microbiology*

## ABSTRACT

The state of California is investing in anaerobic digesters to reduce methane emissions from agriculture. However, little is known about the impact of anaerobic digesters on nitrous oxide ($N_2O$) and carbon dioxide ($CO_2$) emissions from soils after land application of digestate. The purpose of this study was to compare soil $CO_2$ and $N_2O$ emission fluxes from anaerobic digestate treatment in conjunction with manure, manure treatment, and a control group without treatment on agricultural soils from two dairy farms. In addition to comparing treatments and sites, we tested the effects of temperature at either 23°C or 28°C to compare predicted future average temperatures. Soil samples were placed in mason jars with 18 jars per location: three manure treatments x 2, temperatures x 3 replications per treatment, and incubated for six weeks according to the temperature treatment. Soils were watered once a week to maintain 65% water holding capacity. Cavity ring-down spectrometers were used to collect gas emissions in a closed-loop system, and elemental analyzers were used to evaluate soil and treatment nutrient composition. We hypothesized that three main variables — manure, lower temperatures, and soils with low-nutrient content in conjunction with anaerobic digestate would all lead to lower emissions. Anaerobic digestate has been found to reduce greenhouse gas emissions while also being a nutrient-rich energy source. Microbial soil communities are also more active in warmer temperatures, which may increase the production of gas emissions. Overall, the results were inconclusive for either argument.

**KEYWORDS**: *Greenhouse Gas Emissions; Anaerobic Digestate; Manure; Agriculture; Soil; Incubation*

**FACULTY MENTOR**

**Francesca Hopkins,** *Department of Environmental Sciences*

Dr. Francesca Hopkins is an Assistant Professor in the Department of Environmental Sciences. She received her Ph.D. from University of California, Irvine in 2013. During her post-doc, Dr. Hopkins was a part of NASA's Postdoctoral Fellow Program, in 2014, and was announced as one of UC Irvine's Top 50 Graduate and Postdoctoral Scholar Alumni, in 2016. Currently, her research involves anthropogenic greenhouse gas emissions and their contribution to climate change and finding ways to improve our sustainability. Other research areas include trace gas emissions, terrestrial carbon cycle, and isotope biogeochemistry.

**Guadalupe Abonce**

*Department of Microbiology*

Guadalupe Abonce is a fourth year Microbiology major. She has been researching under Assistant Professor Francesca Hopkins for three months. She works at Harkins as a playcenter team member. She plans to enroll in the Pathologists Assistants Program in Loma Linda University.

**Sharon Zhao**

*Department of Microbiology*

Sharon Zhao is a fifth year Microbiology major. She has been researching under Assistant Professor Francesca Hopkins for over a year. She plans to pursue a career in environmental microbiology with the government and continue research in her field.

# Evaluating Greenhouse Gas Emissions from Soil Application of Anaerobic Organic Digestate Compared with Conventional manure

## INTRODUCTION

In 2017, California's agricultural industry emitted approximately 8% of the state's total greenhouse gas (GHG) emissions. Most were due to methane ($CH_4$) and nitrous oxide ($N_2O$). Compared to 1g of carbon dioxide ($CO_2$), $CH_4$ has a global warming potential of 25 g and $N_2O$ has a global warming potential of 298 g over a 100 year time frame8. California's massive dairy industry is a large contributor to GHG; its emissions rose between 2000-2007 and have remained constant every year since[3].

For this study, we examined the impact of supplementing the traditional land application of manure with anaerobic digestate (AD) on soil GHG emissions of $CO_2$ and $N_2O$. AD is the decomposition process of organic matter without the presence of oxygen[2]. It is low in carbon, nutrient-rich, and may increase net primary productivity of crops and increase carbon sequestration in soils[3]. AD is thought to have low mineralization activity due to the stabilization of organic matter after the anaerobic process. As a result, this reduces the amount of labile carbon, which could also lower $N_2O$ emissions[16]. Anaerobic digestion of manure may reduce $CO_2$ and $N_2O$ emissions during field application in comparison to conventional practices.

**Figure 1** depicts how carbon is assimilated into the soil through decomposition or sequestration; carbon is also emitted from the soil by root respiration or mineralization[13]. Nitrogen is assimilated into the soil through leaching and decomposition. It is emitted from the soil as excess during nitrification and denitrification[1]. These processes are conducted by soil microbial communities, and the rates of production respond to temperature. Increased temperatures lead to increased microbial activity[13]. However, a consensus is lacking on the temperature sensitivity of soil carbon and nitrogen decomposition after land application of organic amendments[6,18].

In this study, we sampled agricultural soil from two dairy farms in California and amended the soils with manure or a combination of manure and AD. To examine the effects of temperature, we incubated these soils at two different temperatures in the laboratory. Our hypotheses are (1) the more nutrient-rich soil will release more emissions of $CO_2$ and $N_2O$, (2) the combination of manure and anaerobic digestate will have less GHG emissions compared to only manure, and (3) the higher temperature setting would release more GHG emissions due to stimulated microbial activity.

## METHODS

### Materials

The purpose of this procedure was to determine soil and amendment carbon and nitrogen content. Soil samples were taken on-site at two California dairies in Tulare County (Central Valley) and Marin County (San Francisco/Bay Area). Soils were sampled up to 20 cm in depth and air-dried. Afterward, soils were homogenized

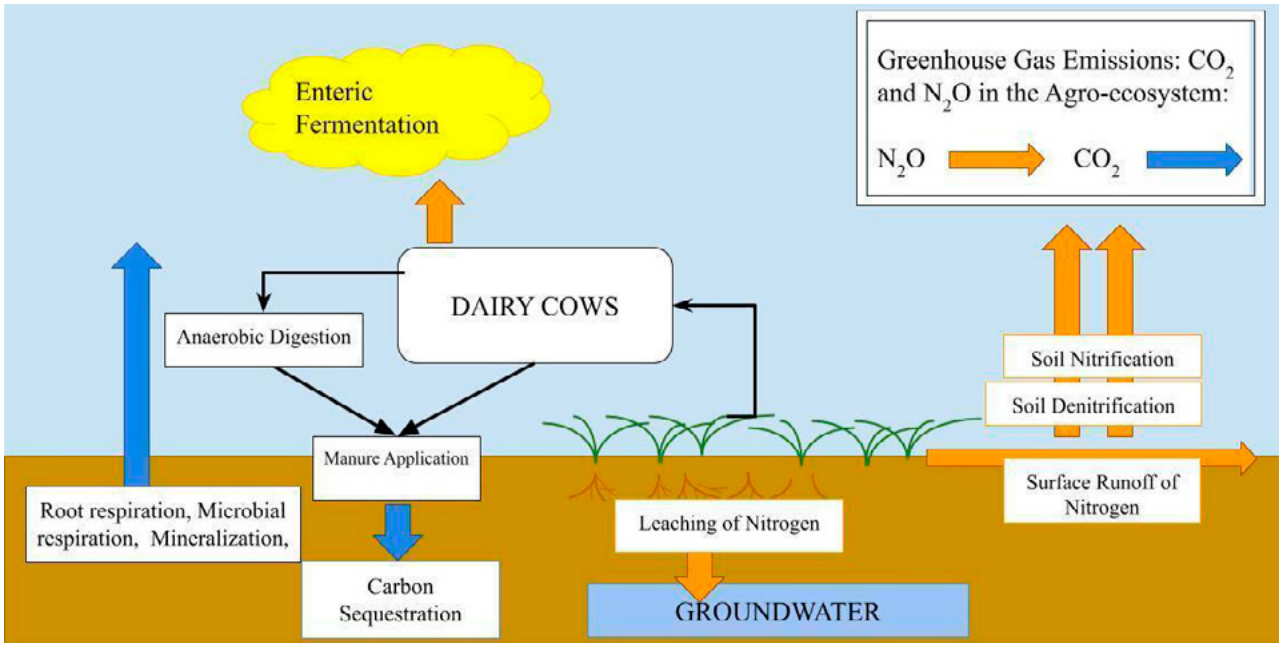


**Figure 1.** A representation of $CO_2$ and $N_2O$ molecules circulating the ecosystem with a focus on agriculture and soil.[10]

and sieved to 2 mm. Solid manure was obtained in June 2019 from a Riverside County dairy farm, then dried and grounded to 2 mm. AD was obtained in September of 2019 from the Marin County site. In this experiment, "the anaerobic digestate" treatment is a combination of ⅓ parts digestate slurry from Marin County and ⅔ parts solid manure from Riverside County. In addition, the liquid portion of the AD was used.

We used a Fisher Scientific Flash Elemental Analyzer and a Shimadzu TOC-V to analyze the nutrient content in our soils and manure amendments. The elemental analyzer is used for solid samples, and the Shimadzu TOC-V is used for liquid samples. Soil and solid manure samples were ground to 100 µm and oven-dried for 24 hours at 105°C to remove moisture; only the AD was analyzed for TOC as a liquid. The AD we used was heavily diluted, mostly containing water, which may have affected the calculated values of total carbon and total nitrogen **(Table 1)**.

## Experimental Design

Soil samples were placed in mason jars with 18 jars per location (Marin and Tulare): 3 treatments (solid manure, liquid anaerobic digestate + manure, control with no amendment) with 3 replicates. Soils were incubated for 6 weeks at designated temperature groups (23° or 28°). The mason jars were filled with 250 g of air-dried soil and were incubated for 38 days at 65% water holding capacity (WHC) for optimal microbial activity. The amendments were applied to a 560 kg N/ha nitrogen application rate. During the experiment, jars were weighed once a week to determine the amount of water loss. Water was added to maintain a 65% WHC before each measurement.

## Soil Emission Measurements

Soil emission fluxes were measured using a Cavity Ringdown Spectroscopy (CRDS) in a closed-loop chamber system. CRDS uses a single-frequency laser and a photodetector to create a continuous traveling light wave. It is able to detect small amounts of light through its three-mirror cavity and emit an amplified signal correlated to the frequency inside the cavity[5,7]. Compared with the traditional method of Gas Chromatography (GC), CRDS overall has performed better than GHG and has a more consistent linear response with $CO_2$[5]. A chamber system amplifies measurements significantly; therefore, smaller emissions can be detected given low instrumental precision[10].

The three CRDS instruments, on an average of five minutes, had a precision rate as follows: G2308, $N_2O$ < 3.5 ppb +/- 0.05%, G2210i and G2401 for $CO_2$ < 200 ppb and < 20 ppb of reading, respectively[12]. Given that a shorter enclosure time reduces systematic errors in chambers, the measurement time we used was 10 minutes[5]. Measurements took place frequently for the first two weeks and then biweekly for the remainder of the experiment.

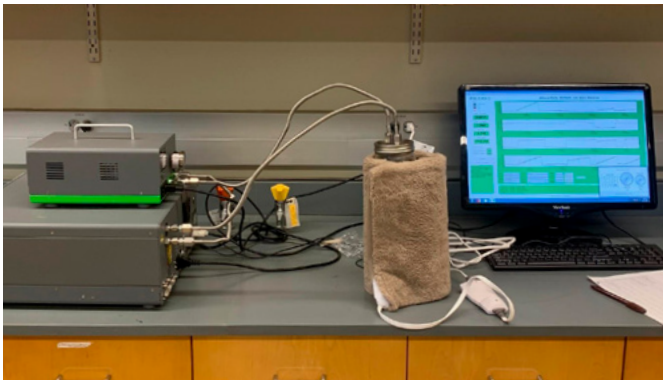

**Figure 2.** Our set up for measuring an elevated jar and a side view of the CRDS

In order to keep the elevated temperatures consistent, a heating pad was kept on a low setting and wrapped around each elevated mason jar for each measurement. A 12" stainless steel tube collected gas from the jar lid into the CRDS and is represented on the computer screen. A 6" stainless steel tube generated recirculating air inside the jar's headspace. This was generated by a recirculating pump on top of the CRDS. The recirculating pump also has a tube attached to the CRDS **(Figure 2)**.

## Flux Measurements & Calculations

The flux measured from the CRDS is in gas units using equation (1), which is then converted into mass concentration (mg/time) using the Ideal Gas Law in equation (2).

Moles of $CO_2$ or $N_2O$ varied by the slope (ppm/sec) =

***End Time - Start Time***

Equation 1

**PV = nRT**

Equation 2

Our calculations yielded the following results: 101325 Pa × 6.697 × 10-4 $m^3$ (headspace) = (mol of $CO_2$ or $N_2O$) * 8.314 (R Constant) * 298.15 K

Then, the actual flux (mass/time/area) of both $CO_2$ and $N_2O$ were calculated using the Closed Chamber System Equation in equation (3), where J is the actual flux (mg $CO_2$/g soil), V (headspace volume), A (soil area), and ΔC (change in gas concentration) over ΔT (change in time)[9]:

$$J = (V/A) * (\Delta C/ \Delta T)$$

Equation 3

We converted J to calculate the cumulative emissions to mass/g soil basis. Using the data from **Table 1**, we normalized emissions by dividing the mass of C/N per g of soil to the final form of mass emitted/ mass C or N. After three replicates of each condition, the cumulative emissions were averaged to calculate the standard deviation and standard error.

For our statistical analysis, we used a paired sample *t*-test for 2-means to make direct comparisons and to find the p-values. For our overarching factors, a statistical analysis of 3-Factor Fixed Analysis of Variation (ANOVA) was done using the XRealStats resource package. Both were done in Excel with an alpha of 0.05.

## RESULTS

### Normalized Emissions for Carbon Dioxide and Nitrous Oxide

Average fluxes for $CO_2$ and $N_2O$ were separated into **Figure 3** and **Figure 4.** In the figures, the two locations were separated into two graphs to visualize any statistical significance when using the *t*-test. Both Marin and Tulare had no statistical significance between the ambient and elevated temperatures for both GHGs. The Marin soil also had no statistical significance when comparing manure treatments and AD treatments for both gases **(Figure 3; Figure 4)**.

For Tulare, $CO_2$ emissions were significantly reduced for ambient temperature AD compared to ambient manure (p-value of 4.7E-02) (**Figure 3**). When analyzing the $N_2O$ emissions for Tulare, statistical significance was seen between elevated temperature manure and elevated temperature AD (p-value of 7.0E-03), with higher $N_2O$ emissions from the manure only treatment (**Figure 4**). However, data referring to $N_2O$ should not be absolute as the standard error was high, indicating large variability in emission rates within a treatment.

### Three-Factor Fixed ANOVA

When looking at the overall factors that may influence the fluxes of $CO_2$, we used ANOVA to identify statistical significance in location ($p$ = 8.4E-11), temperature ($p$ = 2.4E-04), and treatment ($p$ = 7.9E-10). Significance was also seen when location and treatment interacted ($p$ = 4.5E-05) and when all three experimental factors interacted ($p$ = 1.3E-02). However, there was no significant interaction of temperature and location or temperature and treatment alone. For $N_2O$, the only statistical significance came from treatment ($p$ = 1.9E-03). All other experimental factors and interactions were insignificant.

### Carbon and Nitrogen Soil Content Analysis

The results of the elemental analyzer and Shimadzu TOC-V were used to determine the amount of carbon and nitrogen content in soils and amendments **(Table 1)**. Marin County soil had a higher carbon and nitrogen soil content compared with Tulare County soil.

## DISCUSSION

In this experiment, we only saw that significant differences in GHG emissions from the Tulare location soils from Marin had no significant treatment effects. Normalized $CO_2$ emissions were higher

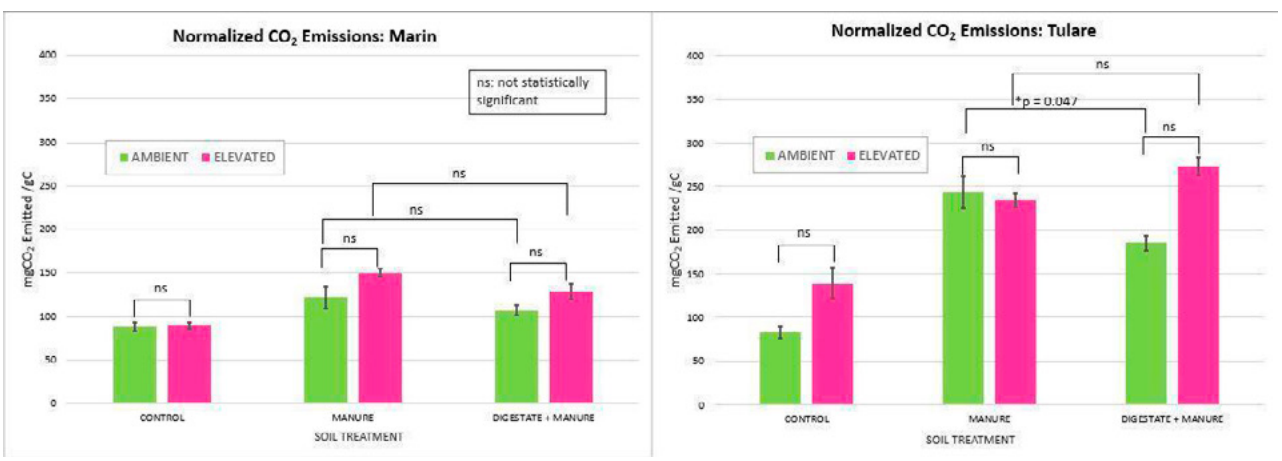


**Figure 3.** Side by side comparison of Marin County (left) and Tulare County (right) showing temperature differences and different soil amendments affecting average emissions of $CO_2$.

from Tulare for AD under both temperatures and for manure at the elevated temperature compared to Marin. Thus, the location plays a major role in the stability of carbon and nitrogen in the soil. In **Figure 3**, we saw an increase in $CO_2$ emissions in Tulare AD, as a result of temperature. This indicates that the interaction between this location and this treatment is sensitive to temperature and will have implications for this site's future soil management when average temperatures are warmer.

$N_2O$ emissions are significantly less from soils amended with manure compared to those amended with AD at elevated temperatures **(Figure 4)**. The lack of statistical significance in $N_2O$ emissions may indicate rapid $N_2O$ loss immediately after application and before measurement, or there might not have been enough AD in our mixture to see a difference. A possible reason for AD having high $N_2O$ emissions is its high concentration of $NH_3$ and $NH_4^+$, correlating to the loss of nitrogen through nitrification rather than denitrification activity. There were only statistical differences found between the controls; however, this is likely due to the Marin soil's higher nitrogen content **(Table 1)**. The technology may also be a factor in large standard error values for $N_2O$. CRDS showed a more consistent linear response to increasing concentrations of $CO_2$ but not $N_2O$[5]. Even microbial soil communities will naturally leak NO and $N_2O$ as byproducts. These are dependent on factors that affect microbial growth such as soil type, WHC, and tillage practice[17]. Due to the variable possibilities and large variability between replicates we observed, it is difficult to quantify $N_2O$ emissions.

Since our temperature analysis lacked findings, its effect on $CO_2$ & $N_2O$ is unclear. A lack of temperature effect is seen in several other studies. For example, researchers in Agriculture and Agri-food Canada saw larger $N_2O$ emissions correlated with an increase of N-fertilizer application and higher daily minimum temperatures; however, annual estimates of $N_2O$ emissions were dependent on the timing of rainfall and snow melts[17]. Another study by Jansen at the University of Iceland discovered that $CO_2$ and $CH_4$ emissions did increase with temperature, while $N_2O$ emissions did not vary between temperatures. Instead, NO2 emissions were driven by water fluctuations[11].

**Table 1.** Initial amounts of carbon and nitrogen found in Tulare and Marin Counties depicted by percentage.

| | Total Carbon* | Total Nitrogen* |
|---|---|---|
| Tulare County Soil | 1.79% | 0.24% |
| Marin County Soil | 4.21% | 0.35% |
| Solid Manure | 18.32% | 1.71% |
| Anaerobic Digestate | 0.1060% | 0.0418% |

*The units are in a per mass scale of soil.

Based on our results, land application of AD does not appear to decrease soil GHG emissions compared to traditional manure applications. However, the production of AD is still beneficial as it captures methane, and the application of AD does not appear to change soil GHG emissions. More research needs to be done

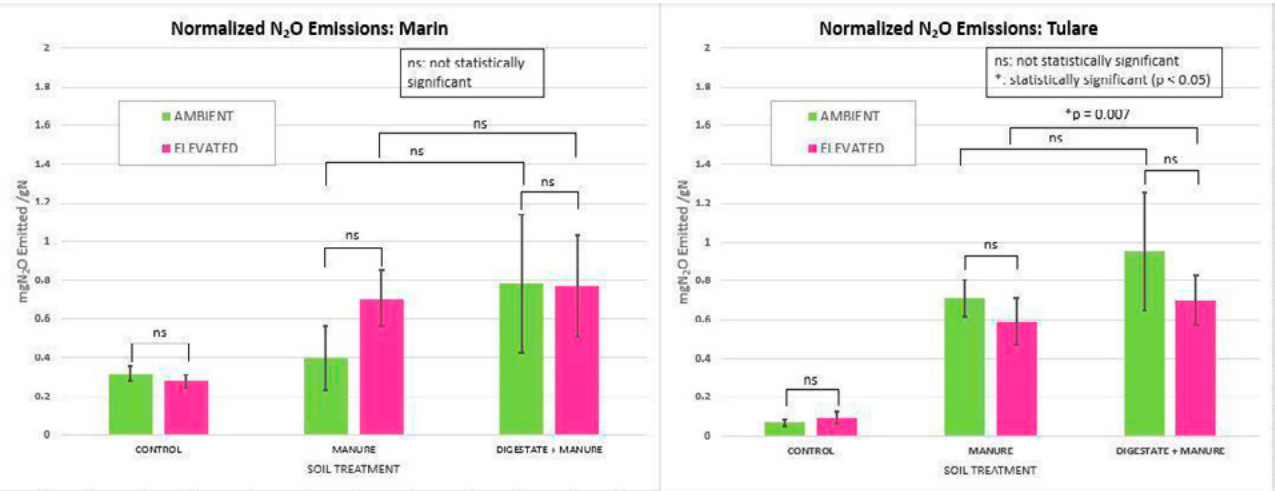


**Figure 4.** Side by side comparison of Marin County (left) and Tulare County (right) showing temperature differences and different soil amendments affecting average emissions of $N_2O$.

since a laboratory experiment cannot fully represent a field-scale experiment. In the field, factors can easily alter soil respiration, soil dynamics, and microbial community structure resulting in an impact on GHG emissions[7].

## CONCLUSION

The purpose of this study was to compare the effects of GHG emissions from soils amended with a manure treatment and manure + anaerobic digestate treatment on 2 different agricultural soils under different temperature conditions. We used CRDS to collect $CO_2$ and $N_2O$ emissions and elemental analyzers to measure the nutrient content of soils and amendments. However, findings were lacking in statistical significance and in evidence to support all our hypotheses. Future studies should include more AD, which may reveal differences not currently seen since the AD treatment used here is only ⅔ manure. Furthermore, since the elevated temperature did not show strong differences, it should be set higher. However, this shows that GHG emissions from amended soils are stable in an increased climate. The focus on the properties and factors of a single location would narrow the variety of data and its implications. These results demonstrate the importance of factors such as site-specific management, location, and even how future changes in temperature will affect carbon and nitrogen cycling in the area when considering the land application. The intention of land application should both benefit the soil and result in the reduction of harmful GHG emissions.

## ACKNOWLEDGMENTS

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# Fear of Cancer Recurrence Among Black and White Mothers

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## ABSTRACT

Fear of cancer recurrence (FCR), often defined as fear, worry, or concern related to the possibility that cancer may return or progress, is frequently expressed by breast cancer survivors. Previous research on breast cancer survivors suggests that mothers tend to report greater FCR than non-mothers and that FCR differs by ethnicity. This existing body of research often treats motherhood and race as separate entities by which to examine levels of FCR. The purpose of the present study is to investigate the differences in FCR between Black and White mothers. Breast cancer survivors were recruited using Amazon's MTurk service ($n$ =138) and were asked to respond to self-report questionnaires regarding FCR and demographic information. An independent $t$-test revealed that black mothers ($n$ = 37, $M$ = 3.94, $SD$ = 0.58) tended to report greater FCR levels than White mothers ($n$ = 54, $M$ = 3.54, $SD$ = 0.92; $t(88.46)$ = -2.55, $p$ = 0.01). Our findings provide initial support for differences in FCR among mothers of different races. Potential explanations for the observed differences are discussed. Further research is needed to identify the causes of differences in FCR levels among Black and White mothers in order to craft informed interventions for these populations.

**KEYWORDS:** *Fear Of Cancer Recurrence; Breast Cancer Survivorship; Black Mothers; White Mothers*

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# Fear of Cancer Recurrence Among Black and White Mothers

## INTRODUCTION

Among women of all major ethnic groups, breast cancer is the most common form of cancer (Ashing-Giwa et al., 2004). As such, there are over 3.8 million breast cancer survivors living in the United States today (American Cancer Society [ACS], 2019). Though they have entered a period of remission, fears that cancer will return often persist into survivorship. Fear of cancer recurrence (FCR) has been defined by the literature as "fear, worry, or concern relating to the possibility that cancer will come back or progress" (Lebel et al., 2016, p. 3267), and it is one of the most commonly reported causes of distress among breast cancer survivors (Vickberg, 2003; Simard et al., 2013). In the current investigation, we explore differences in FCR by racial identity, particularly among Black and White mothers.

### FCR and Race

Research on the relationship between race and FCR is limited, but the handful of investigations focused on this topic are consistent in their findings. One study regarding worry and FCR in a diverse sample of cancer survivors found that African Americans reported fewer concerns about recurrence and greater psychological well-being than their White and Latinx counterparts (Deimling et al., 2006). Another study mirrored these findings in breast cancer survivors specifically (Janz et al., 2011). Black cancer survivors often report better mental health than White survivors, but they also report poorer physical functioning (Bowen et al., 2007).

### FCR and Motherhood

In addition to race, motherhood is also known to play a role in FCR. Mothers diagnosed with breast cancer face additional hardships that persist into survivorship. Research suggests that breast cancer survivors with children tend to sacrifice their own needs for care in an effort to meet the needs of their children (Billhult & Segesten, 2003), and they display high levels of psychological distress (Schmitt et al., 2008). Additionally, they describe a sense of loss in the quality time spent with their children, guilt for being unavailable, and worries regarding their children's well-being if they should die (Billhult et al., 2003).

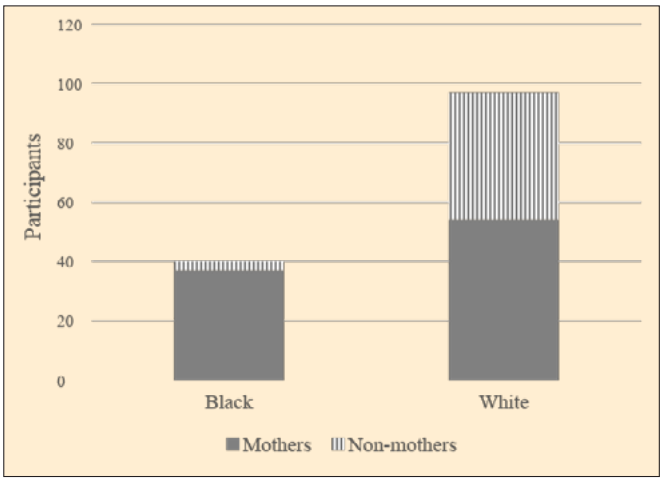


**Figure 1.** Mothers and non-mothers by race.

### Other Correlates of FCR

Young mothers are the most vulnerable to these hardships, likely because they have younger children who are more dependent (Arès, Lebel & Bielajew, 2014). Therefore, FCR in mothers may be exacerbated by younger age and may also be influenced by other demographic variables, such as socioeconomic status.

**Age.** According to a review of the FCR literature, younger age was related to greater FCR in 14 studies, with only two studies reporting a non-significant relationship (Crist & Grunfeld, 2013). Younger people tend to have greater anxiety, higher perceived risk, and more intrusive thoughts regarding their cancer, and these thoughts tend to dissipate over time as people age (Mullens et al., 2004). Young survivors may show higher levels of FCR due to less psychological resilience, as well as the fear that breast cancer may result in more life disruptions (Lebel et al., 2012).

**Socioeconomic status.** There is limited research investigating the relationship between socioeconomic status (SES) and FCR, specifically. However, research has linked lower SES with poorer disease outcomes that may increase FCR. African American women have lower breast cancer survival rates (ACS, 2019) and poorer physical functioning (Bowen et al., 2007) compared to White survivors, often due to late-stage diagnoses and disparities in access and quality of treatment (Brawley & Freeman, 1999). Low income and a lack of private health insurance are also risk factors for a late-stage diagnosis (Lanninn et al., 1998). FCR may be heightened in breast cancer survivors to whom these risk factors apply.

### The Current Study

The existing body of research surrounding FCR in breast cancer survivors often treats motherhood and racial identity as separate entities. To our knowledge, no study has investigated differences in FCR between mothers of different racial backgrounds. The present study aims to fill this gap in the literature. The goal of the current study is to investigate the differences in FCR between Black mothers and White mothers who are breast cancer survivors. We had three hypotheses. First, we hypothesized that mothers as a whole, and particularly young mothers, would report greater FCR than non-mothers. Second, we hypothesized that White breast cancer survivors would report greater FCR in comparison to Black cancer

**Table 1.** FCR, Age, and Subjective SES in Black and White Mothers

| | BLACK MOTHERS | | WHITE MOTHERS | | *t*(90) | *p* |
|---|---|---|---|---|---|---|
| | *M* | *SD* | *M* | *SD* | | |
| **FCR** | 3.94 | 0.58 | 3.54 | 0.92 | -2.55 | .01 |
| **Age** | 33.68 | 4.76 | 37.07 | 8.93 | 2.35 | .02 |
| **Subjective SES** | 7.97 | 1.46 | 7.02 | 1.57 | -2.97 | <.01 |

survivors. Finally, we hypothesized that Black mothers would report lower levels of FCR in comparison to White mothers. An additional goal of the present study was to examine demographic variables, such as age and SES, that might shed light on potential differences between Black and White breast cancer survivors with children.

## METHODS

### Participants

Breast cancer survivors ($N = 138$) were recruited through Amazon's Mechanical Turk (MTurk) service. MTurk is a crowdsourcing platform designed to provide a large, online participant pool for tasks and may be particularly well suited for the recruitment of a diverse sample of young breast cancer survivors (Arch & Carr, 2017). All participants reported their gender as female. Participants ranged in age from 23 to 64 years old ($M = 37.00$, $SD = 10.24$). The sample was primarily White (70%) but also included participants who identified as Black (29%), and Hispanic/Latinx (0.7%). A majority of our sample reported having at least one child (66%). **Figure 1** provides a visual representation of our sample based on both race and motherhood.

### Procedure

Participants were asked to respond to a survey regarding their demographic information and FCR they have experienced as a breast cancer survivor. We asked screening questions to ensure participant eligibility for our study. If participants reported having a breast cancer diagnosis and that they had undergone treatment for breast cancer, but were no longer undergoing treatment, they were eligible for our study. The survey took an estimated 15 minutes to complete and participants were compensated with $2 for their time.

### Measures

**Demographics.** Demographic information obtained included the participants' age, race, and socioeconomic status. Additionally, participants were asked to indicate if they have children. Socioeconomic status was assessed using the MacArthur Scale of Subjective Social Status (Adler & Stewart, 2007). Participants were shown an image of a ladder that represents their standing in society, along with a prompt asking them to report where they feel they belong on the ladder. The lowest rung (coded as a 1) represented those in society with lower status and the highest rung (coded as a 10) represented those with higher status ($M = 6.70$, $SD = 1.89$). This single-item measure of subjective SES outperforms or closely mirrors objective measures of social status, particularly in domains of psychological functioning and health-related outcomes (Adler, Epel, Castellazzo, & Ickovics, 2000; Cundiff, Smith, Uchino, & Berg, 2013).

**Fear of Cancer Recurrence.** Fear of cancer recurrence was measured using the FCR-4, a short form scale adapted from the FCR-7 (Humphris, Watson, Sharpe & Ozakinci, 2018). This four-item scale was designed to measure anxiety and worry about cancer recurrence with low participant burden (e.g., "I am afraid that my breast cancer may recur," "I get waves of strong feelings about the breast cancer coming back"; 1 = *not at all*, 5 = *all the time*). The four items in this scale were averaged to form a composite variable of FCR ($M = 3.47$, $SD = 0.90$, $\alpha = .81$).

## RESULTS

Black and White breast cancer survivors were the primary focus of this investigation, as the majority of our sample identified as either Black or White, with only one participant identifying as Latina. Contrary to our hypothesis, an independent samples *t*-test revealed that Black mothers tended to report greater FCR levels than White mothers (see **Table 1**; all comparisons corrected for unequal variances across groups).

Further analyses revealed demographic differences between Black and White mothers. In our sample, Black mothers were significantly younger than White mothers. Age was negatively correlated with FCR, such that younger survivors reported greater FCR, $r(135) = -.23$, $p < 0.01$. Black mothers were of a higher subjec-

tive SES than White mothers and subjective SES was positively correlated with FCR, such that those with higher subjective SES reported higher FCR, $r(135) = .42$, $p < 0.001$.

Only three Black women who were not mothers participated in our study, leaving our comparison of FCR in mothers and non-mothers by race underpowered. In total, mothers ($n = 91$, $M = 3.70$, $SD = 0.82$) had higher rates of FCR than non-mothers ($n = 46$, $M = 3.02$, $SD = 0.89$), $t(84.43) = 4.35$, $p < 0.001$, and Black women ($n = 40$, $M = 3.88$, $SD = 0.63$) had higher rates of FCR than White women ($n = 91$, $M = 3.70$, $SD = 0.82$), $t(107.89) = -4.20$, $p < 0.001$.

## DISCUSSION

The current study tested three hypotheses regarding the relationship between FCR, race, and motherhood. First, we hypothesized that mothers in our sample of breast cancer survivors would report increased levels of FCR. This hypothesis was supported; mothers reported significantly higher levels of FCR in comparison to those who were not mothers. Second, we expected to see lower levels of FCR among Black breast cancer survivors in comparison to White survivors. Our results did not confirm this hypothesis. Instead, our findings revealed that Black women reported significantly higher levels of FCR than their White counterparts. This finding runs contrary to previous research on this topic (Deimling et al., 2006; Janz et al., 2011). Our third and primary hypothesis was that Black mothers would report less FCR than White mothers. Consistent with the rejection of our second hypothesis, our third hypothesis was also not confirmed. In our sample, Black mothers reported significantly greater levels of FCR than White mothers.

Next, we made an effort to identify potential underlying demographic differences driving the discrepancies in FCR levels between mothers of both races. Our additional analyses revealed that, on average, Black mothers in our sample were significantly younger than White mothers. Past research has revealed that younger survivors experience increased FCR in comparison to older survivors (Lebel et al., 2012), and age was negatively correlated with FCR in our study. This suggests that age may have played a role in our contradictory findings. As such, a mother's age may be an important contributing factor to FCR due to age-related differences in perceived physical or socioeconomic consequences of cancer.

Subjective SES was positively correlated with FCR in our sample, such that those with higher subjective SES also reported greater FCR. Although those with higher SES are more likely to have access to healthcare, their FCR remains high. Black mothers reported significantly higher subjective SES than White mothers and, therefore, reported greater FCR. Greater subjective SES may not be a protective factor for Black mothers against the stress and uncertainty related to cancer recurrence. FCR may persist in Black women despite their SES because of a lack of quality of care or a distrust of healthcare providers, and not because of a lack of access to healthcare (Hausmann et al., 2013).

Past research has suggested that women of color experience more distrust in the health domain in comparison to White women, often arising from uncertainty about the quality of treatment they will receive (Halbert et al., 2006). This uncertainty that many Black women face may be due to past incidents in the medical sector in which physicians provided a lower quality of care to patients of color (Perloff et al., 2006). If that is the case, then it is not entirely surprising that Black mothers exhibited higher levels of FCR in this study. Black women may perceive doctor-patient interactions as stressful. This stress could exacerbate their FCR due to the inevitable increase in time they would spend advocating for themselves with their doctor if their cancer were to return. Further research is needed to identify the specific causes of heightened FCR in Black breast cancer survivors in our sample, particularly those with children, and informed interventions can be crafted for physicians and breast cancer survivors alike.

## LIMITATIONS AND FUTURE DIRECTIONS

The primary focus of this study was to examine FCR, race, and motherhood at a single point in time using a cross-sectional design. Future studies on this topic should consider taking a longitudinal approach in order to examine how FCR changes in these populations over time. Additionally, our participants were gathered from Amazon's MTurk service, which may have masked group differences as all of these individuals voluntarily completed an online survey. The next step in this investigation is to work with a community sample of breast cancer survivors, made possible through partnerships with local medical centers. Furthermore, our conclusions regarding FCR and motherhood are only applicable when comparing Black and White breast cancer survivors, as our sample was only representative of these two races. Additional research is needed with a more diverse sample of breast cancer survivors to understand how FCR compares in the Hispanic and Latinx women, for example.

## CONCLUSION

The findings of the present investigation contradicted current knowledge regarding FCR among African American women and shed new light on differences in FCR levels among Black and White mothers. Further research examining factors beyond age and subjective SES that could explain the higher FCR levels reported by Black survivors are needed. Additionally, our study provides

further support for the notion that mothers and younger women experience greater FCR. Our findings have implications for much-needed interventions that target African American breast cancer survivors with children, as they may be of an increased risk for FCR than previous research would suggest.

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# Ethnic Differences in Perceptions of Mental Illness: Examining Intergroup Relations

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## ABSTRACT

People with mental illness are often stereotyped as dangerous, unstable, or unreliable, and these stereotypes perpetuate prejudice against those who are already vulnerable. However, many of these stereotypes are Eurocentric due to a lack of diversity within psychology. The present, pre-registered research investigates whether depictions of mental illness are idiosyncratic to various racial/ethnic groups, or if these perceptions generalize across groups. Participants reported their endorsement of a series of mental illness descriptions (e.g., "This person spontaneously explodes in outbursts of anger") as they apply to African Americans, Asian Americans, Hispanic/Latinxs, Caucasians, as well as to individuals with unspecified race/ethnicity. Exploratory factor analyses of these descriptions revealed three factors that describe mentally ill people — ashamed, self-destructive, irresponsible — and participants' perceptions of mental illness on these three factors varied by racial/ethnic groups. Participants rated Asian Americans as more ashamed, but less self-destructive and irresponsible than other racial/ethnic groups. Conversely, participants rated Caucasians as less ashamed but more self-destructive and irresponsible than other racial/ethnic groups. Perceptions of mental illness did not differ between Hispanic/Latinxs and African Americans. Additional analyses indicate that, compared to Caucasian participants, non-Caucasian participants rated mentally ill members of their ingroup as more ashamed but less self-destructive and irresponsible. This research indicates that participants from different racial/ethnic groups vary in the extent to which they ascribe different facets of mental illness to their ingroup versus outgroups. Implications for Eurocentric versus more diverse perceptions of mental illness are discussed.

**KEYWORDS:** *Bias, Cross-Cultural, Intergroup Relations, Mental Illness, Perceptions, Stereotypes, Stigma*

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# Ethnic Differences in Perceptions of Mental Illness: Examining Intergroup Relations

## INTRODUCTION

People with mental illness are often stereotyped as dangerous, unstable, or unreliable (Abdullah & Brown, 2011; Corrigan et al., 2014; Link et al., 1987). Such stereotypes are a precursor to discrimination (Eagly & Chaiken, 1993), and perpetuate prejudice against people who are already vulnerable (Corrigan & Bink, 2016). One way in which prejudice can manifest is stigma, where an individual is devalued based upon unfavorable group stereotypes (Hinshaw, 2007). In the context of mental illness, stigma refers to negative perceptions of an individual because they have mental illness symptoms or have been labeled as possessing a mental illness (Corrigan et al., 2014; Link et al., 1987). Once stigmatized as 'crazy,' 'psycho,' or 'sick,' individuals struggling with mental illness may become resistant to seeking assistance (Abdullah & Brown, 2011). More broadly, stigma is defined as a negative label of an outgroup, in the context of stereotypical differences that separate "us" from "them" (Link & Phelan, 2001).

Though existing racial/ethnic dynamics likely exacerbate mental illness stigma, the extent to which mentally ill people are stigmatized may be underestimated because stereotypes about mental illness are primarily Eurocentric, in that mental illness is conceptualized to reflect Caucasian views and perspectives (Katz, 1985). Prior stigma research assumes that perceptions of mental illness are viewed universally, such that individuals of different cultures and racial/ethnic backgrounds are all stigmatized in the same way (Abdullah & Brown, 2011). This restricted focus reflects the lack of psychological research among minorities with mental health conditions, and may not represent stereotypes about mental illness when applied more broadly across different cultures and ethnic/racial groups. Moreover, perceptions of mental illness symptoms, and approaches to treatment, are typically viewed from a Eurocentric perspective, such that the fundamental concept of mental illness reflects a middle-class Caucasian value system (Joseph, 2015; Katz, 1985; Naidoo, 1996).

Further exacerbating the Eurocentric perspective on mental illness, Caucasians have greater representation in media, which includes both stigmatizing (i.e., negative) and de-stigmatizing (i.e., positive) portrayals of mental illness (Frisby, 2017). Taken together, Eurocentrism is pervasive throughout psychological research and mental health and impedes non-Caucasians from seeking help. Mental health professionals are not immune to the effects of stigma (Stubbs, 2014; Hanafiah & Bortel, 2015). Professionals who are not culturally competent may inadvertently adopt a Eurocentric perspective in their treatment of minority patients. These negative, Eurocentric stereotypes of racial/ethnic minority patients' conditions may obstruct minorities from seeking help (Hanafiah & Bortel, 2015; Horsfall et al., 2010).

Contemporary research on mental illness highlights the importance of understanding stigma cross-culturally by acknowledging the values, norms, and social contexts in which diverse individuals operate (Abdullah & Brown, 2011; Corrigan et al., 2014). Additionally, examining mental illness stereotypes through a multiracial/ethnic lens provides the opportunity to ask novel questions. For example, to what extent do mental illness stereotypes depend on the intergroup relationship between perceiver and target? The possibility that intergroup relationships moderate intergroup perceptions (such as stereotypes) has precedent: The ultimate attribution error (Pettigrew, 1979), proposes that people will attribute negative behaviors of outgroup members to internal factors, but attribute negative behaviors of ingroup members to external factors. In other words, when members of the ingroup have negative behaviors, people attribute it to the situation rather than blaming the individual (Pettigrew, 1979). Building on this perspective, given that mental illness stereotypes are negative (and, thus, stigmatizing), this study proposes that people will differentially ascribe descriptions of mental illness to ingroup versus outgroup members.

## THE PRESENT RESEARCH

The goal of this research is to examine mental illness stereotypes from the perspective of race and ethnicity. In doing so, two questions arise: the first question focuses on ethnic and racial differences in mental illness stereotypes, and the second question focuses on whether mental illness stereotypes depend on the intergroup relationship (i.e., ingroup versus outgroup) between the mentally ill person and the perceiver.

## PARTICIPANTS AND METHODS

In the interest of clarity and open research, our hypothesis, methods, and exclusion criteria were pre-registered and are available at https://osf.io/4yn56. A total of 315 undergraduate participants were recruited from the University of California, Riverside. Of these, 26 were excluded for the *a priori* exclusion criteria of missing or incomplete data, leaving a final sample size of 289 (M age = 19.6, *SD* = 2.83; 83 men, 206 women; Participant Ethnicities: 8 African Americans, 103 Asian Americans, 106 Hispanic/Latinx, 32 Caucasian, 40 other). Participants completed five 20-item scales that measured perceptions of mental illness towards different racial/ethnic groups. All five scales shared the same basic structure, such that participants indicated how strongly each item (e.g., "This person is aggressive and spontaneously explodes in loud outbursts of anger.") described a mentally ill person on a 7-point Likert scale (1 = not at all representative, 7 = very representative; see Appendix A).

**Table 1.** Note: Average mental illness endorsement ratings by target group race/ethnicity.

| | Ashamed | | Self-Destructive | | Irresponsible | |
|---|---|---|---|---|---|---|
| | Mean (*SD*) | *SE* | Mean (*SD*) | *SE* | Mean (*SD*) | *SE* |
| General | 5.35 (0.96) | .057 | 4.85 (1.06) | .062 | 4.26 (1.23) | .072 |
| African American | 5.12 (0.97) | .057 | 4.61 (0.95) | .056 | 4.05 (1.10) | .065 |
| Asian American | 5.59 (0.90) | .053 | 4.28 (1.04) | .061 | 3.70 (1.21) | .071 |
| Hispanic/Latinx | 5.37 (0.99) | .058 | 4.68 (0.95) | .056 | 3.95 (1.19) | .070 |
| Caucasian | 4.60 (1.05) | .061 | 4.98 (1.00) | .059 | 4.57 (1.05) | .062 |

All participants began by reporting their perceptions of a general (i.e., not race-specific) person with mental illness. The next four scales, measuring perceptions of African American, Asian American, Hispanic/Latinx, and Caucasian people with mental illness, were presented in random order.

## RESULTS

Exploratory factor analysis utilizing maximum likelihood was conducted on general mental illness perceptions, which revealed three factors: *ashamed* ($\alpha = .81$), *self-destructive* ($\alpha = .89$), and *irresponsible* ($\alpha = .84$). Because the items that comprised each of these three factors demonstrated good reliability, items were then averaged into indices reflecting each factor (see Appendix A for factor loading for each item). Three subsequent mixed-model ANOVAs were conducted, with target race/ethnicity (African American, Asian American, Hispanic/Latinx, Caucasian) as a within-participants factor and participant race/ethnicity (African American, Asian American, Hispanic/Latinx, Caucasian) as a between-participants factor. These ANOVAs were employed to predict perceptions on each of the three factors that emerged in the exploratory factor analysis. The main effects of target race/ethnicity emerged (**Table 1**), such that Caucasian targets were rated as less ashamed, more self-destructive, and more irresponsible than the other target groups, and Asian American targets were rated as more ashamed and less self-destructive and irresponsible than the other target groups. Perceptions of mental illness between Hispanic/Latinxs and African Americans did not differ. No other reliable main effects emerged.

In order to examine the interaction between participant and target race/ethnicity, targets were re-coded as either 'ingroup' or 'outgroup' to each participant for ease of interpretability (**Table 2**). This analysis revealed that African Americans, Asian Americans, and Hispanic/Latinxs rated mentally ill members of their ingroup as more ashamed than did Caucasians. Hispanic/Latinxs and Caucasians rated mentally ill members of their ingroup as more self-destructive than did African Americans and Asian Americans. African Americans and Caucasians rated mentally ill members of their ingroup as more irresponsible than did Hispanic/Latinxs and Asian Americans. No other reliable interactions emerged.

## DISCUSSION

The present research examined mental illness stereotypes across different races and ethnicities. Caucasians were perceived as less ashamed, more self-destructive, and more irresponsible than other groups. In contrast, Asian Americans were perceived as more ashamed, less self-destructive, and less irresponsible than other groups. Additionally, non-Caucasians rated members of their ingroup as more ashamed compared to Caucasians who rated ingroup members as less ashamed, but more self-destructive and irresponsible. Hispanic/Latinxs and Caucasians rated members of their ingroup as more self-destructive than did African and Asian Americans. In contrast, African Americans and Caucasians rated mentally ill members of their ingroup as more irresponsible than did Hispanic/Latinxs and Asian Americans. Notably, the ratings of Caucasians as low in ashamed, but high in self-destructive and irresponsible, provide support for Eurocentric perceptions of mental illness by illustrating the pervasiveness of Caucasian representation within mental health.

## IMPLICATIONS

This work aids literature on mental health stigma by examining depictions of mental illness as they vary by race and ethnicity. These findings add to a growing body of work that highlights the need for diversity and representation within mental health. These results shed light on how individuals stereotype other's behaviors, highlighting a need for greater intergroup communication surrounding mental health. The lack of accurate, de-stigmatizing representation within the media perpetuate negative perceptions of people struggling with mental illness, discourages vulnerable people from seeking help (Abdullah & Brown, 2011). In turn, this underscores

**Table 2.** Note: : Average mental illness endorsement ratings by participant groups for the ingroup and outgroups

| | INGROUP | OUTGROUP | TEST OF DIFFERENCE | | |
|---|---|---|---|---|---|
| | Mean *(SD)* | Mean *(SD)* | *t*(df) | *p* | Cohen's *d* [95% CI] |
| **ASHAMED** | | | | | |
| African American | 5.61 (0.83) | 4.88 (0.88) | 3.04 (7) | .019 | 0.85 [-0.27, 1.97] |
| Asian American | 5.71 (0.96) | 4.90 (0.87) | 9.41 (102) | <.001 | 0.88 [0.59, 1.17] |
| Hispanic/Latinx | 5.81 (0.88) | 5.12 (0.73) | 9.27 (105) | <.001 | 1.97 [1.05, 2.88] |
| Caucasian | 4.88 (0.93) | 5.36 (0.76) | -3.41 (31) | .001 | -0.56 [-1.07, -0.05] |
| **SELF-DESTRUCTIVE** | | | | | |
| African American | 4.33 (1.40) | 4.56 (0.63) | -0.62 (7) | .56 | -0.20 [-1.28, 0.87] |
| Asian American | 4.47 (1.06) | 4.74 (0.95) | -4.04 (102) | <.001 | -0.27 [-0.55, 0.003] |
| Hispanic/Latinx | 4.70 (0.88) | 4.59 (0.74) | 1.64 (105) | .10 | 0.13 [-0.14, 0.41] |
| Caucasian | 4.98 (1.07) | 4.56 (0.96) | 3.90 (31) | <.001 | 0.41 [-0.09, 0.92] |
| **IRRESPONSIBLE** | | | | | |
| African American | 3.47 (1.72) | 3.33 (0.76) | 0.34 (7) | .75 | 0.10 [-0.97, 1.17] |
| Asian American | 3.83 (1.33) | 4.29 (1.01) | -3.94 (102) | <.001 | -0.39 [-0.66, -0.11] |
| Hispanic/Latinx | 3.76 (1.14) | 4.08 (0.78) | -3.84 (105) | <.001 | -0.33 [-0.60, -0.05] |
| Caucasian | 4.30 (1.15) | 3.79 (1.04) | 3.29 (31) | .002 | 0.47 [-0.04, 0.97] |

the importance of raising awareness for a better understanding of non-Caucasian mental illness populations regarding research and approaches to treatment.

Our findings may help mental health professionals in understanding how mental illness symptoms are perceived among different races and ethnicities and, in turn, adjust their approach to treatment. Although it is well understood that treatment approaches are Eurocentric and inappropriate for various minority groups (Katz, 1985; Naidoo, 1996; Sue, 1994), what is not understood is how the Eurocentric perceptions influence this. Understanding stereotypical perceptions versus well-documented symptoms among different minority groups may enhance cultural competency among clinicians and researchers.

## LIMITATIONS

One possible limitation of the present research is that the items used to depict mental illness descriptions may not fully capture the breadth of mental illness symptoms across races and ethnicities. Participants may have felt that none of the twenty items could reasonably be applied to certain racial/ethnic groups, or that one description may apply equally well to all racial/ethnic groups. More broadly, the present research may paradoxically be Eurocentric, in that people from non-Caucasian cultural backgrounds might not understand the concept of mental illness in the manner it is presented here. If a concept is not discussed in one's culture, there would be no logical explanation to ascribe a behavior to mental illness.

Another limitation of our findings is that they reflect only the perceptions of undergraduate participants. The racial/ethnic breakdown of our particular undergraduate sample included few African Americans – corresponding to the racial/ethnic composition of our campus – which is insufficient to draw strong conclusions about their perceptions of mental illness. Future research should seek to include more African Americans, as well as diverse participants on other dimensions, in order to build a more comprehensive and externally valid understanding of mental illness perceptions across races and ethnicities.

Additionally, the three factors – ashamed, self-destructive, irresponsible –were derived based on perceptions of a general (not race-specific) mentally ill person. This method was operational-

ized so that it equally compares all racial/ethnic groups across a common framework. Different factors may have emerged if they were operationalized in terms of specific races/ethnicities. Future research should build upon the foundation laid by the present research to examine the extent to which these three factors persist versus vary across mentally ill people of different races/ethnicities.

## CONCLUSION

The present research indicates that perceptions of mental illness vary across racial/ethnic groups and that racial/ethnic groups vary in the extent to which they ascribe different facets of mental illness to their ingroup versus outgroups. Future research should clearly define stereotypical portrayals of mental illness within minority racial/ethnic groups as members of those groups understand them. Not only does this work extend the existing literature on cross-cultural stigma within intergroup relations, but it also highlights the lack of diverse representation, and emphasizes the importance of recognizing non-Caucasian mental illness symptoms regarding mental health.

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## APPENDIX A – EFA SCALE ITEM LOADINGS

| ITEM | Ashamed | Self-Destructive | Irresponsible |
|---|---|---|---|
| Tries their best to hide mental illness from others | **0.71** | 0.16 | - |
| Afraid of disappointing their family due to others' knowledge of their MI | **0.59** | - | 0.16 |
| Feels as though they have no right to talk about it; others have it worse | **0.56** | 0.17 | 0.16 |
| Hasn't told their family about mental illness | **0.54** | 0.22 | - |
| Experiences sleep disruption, either too much or too little | **0.52** | 0.11 | 0.36 |
| Feels pressure to act 'normal' | **0.49** | -0.14 | 0.13 |
| Experiences frequent chest pains, stomach pains, and/or body aches | **0.46** | - | 0.36 |
| Has thoughts of attempting suicide | 0.17 | **0.68** | 0.18 |
| Aggressive, and spontaneously explodes in loud outbursts of anger | - | **0.63** | 0.35 |
| Dependent on drugs and alcohol | - | **0.60** | 0.31 |
| Experiences "blackouts" or "shuts down" when they're very upset or angry | 0.30 | **0.48** | 0.26 |
| Can still do well in school, work, and/ or extracurriculars (reversed) | 0.24 | **-0.44** | - |
| Has many crying spells | 0.31 | **0.38** | 0.28 |
| Is Unproductive | 0.18 | 0.21 | **0.56** |
| Spends money recklessly | - | 0.24 | **0.53** |
| Calls off of work a lot | 0.28 | 0.27 | **0.49** |
| Is unpredictable and unreliable | - | 0.33 | **0.44** |
| Thinks that their MI is a punishment* | 0.41 | 0.38 | - |
| Copes with difficulties by overeating or undereating* | 0.37 | 0.41 | 0.38 |
| Prays and attends church more frequently to deal with difficulties ‡ | 0.27 | - | 0.24 |

Note: Items indicated (*) were removed from factor loadings due to double loading.
Items indicated (‡) were removed from factor loadings due to not meeting 0.35 criterion.

# The Power of Words: How Are Depression Symptoms and Labile Self-Esteem Related to Word Use?

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## ABSTRACT

The way people talk about their emotional experiences can reveal information about how well they are functioning. Depression symptoms can include feelings of hopelessness and a saddened mood. Labile self-esteem is the fluctuations a person may experience in their self-esteem. Previous studies have found a relationship between self-esteem, depression symptoms, and word use; however, no research has yet examined the interaction between depression symptoms and labile self-esteem in predicting word use. The present study examines the main and interactive effects of depression symptoms and labile self-esteem in predicting the number of clout (language associated with confidence), achievement (goal-oriented language), and power (words related to superiority) words utilized to describe sad and happy emotional experiences. We predicted that the interaction between more depression symptoms and more labile self-esteem would relate to less use of clout, achievement, and power words when describing sad and happy emotional experiences. Participants answered surveys measuring depression symptoms and labile self-esteem. The Linguistic Inquiry and Word Count (LIWC) software was used to determine how much clout, achievement, and power words participants utilized when describing past sad and happy emotional experiences. The present study found that labile self-esteem was only significantly positively correlated with clout words used to describe a happy emotional experience. In contrast to our hypothesis, participants' self-reported depression symptoms were not linked to clout, power, and achievement words used to describe happy and sad emotional experiences. Finally, there were no significant interactions between labile self-esteem and depression symptoms in predicting words used to express a past emotional experience. The findings in this study provide a greater understanding of how factors that may affect a person's overall wellbeing, such as depression symptoms and labile self-esteem, are linked to how people recall and express past emotional experiences.

**KEYWORDS:** *Depression Symptoms, Labile Self-Esteem, Autobiographical Memories, Word Use, Young Adults, Emotional Experiences, CES-D, LIWC*

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Dr. Davis is an Associate Professor in the Psychology Department. She received her PhD from UC Irvine. Her work examines the development of emotion regulation in childhood, to answer questions about what children can do at different ages to manage feelings, what contexts they do this within, and mechanisms that explain how and why emotion regulation is linked to healthy and unhealthy development. She is a past junior faculty recipient of the Distinguished Teaching Award from UCR's Academy of Distinguished Teachers.

# The Power of Words: How Are Depression Symptoms and Labile Self-Esteem Related to Word Use

## INTRODUCTION

Everyone remembers and talks about their emotional experiences differently. People may recall the joyous cheers and feelings of triumph as they walk across the graduation stage. Similarly, people may remember the profound sorrow and specific events associated with the loss of a loved one. The intricate details of these personal events can be classified as autobiographical memories (Cristofori & Levin, 2015). Tracking the specific linguistic phrases people utilize is vital because it can provide a plethora of information regarding people's personality, emotions, and can reflect how people process a past emotional experience (Tausczik & Pennebaker, 2010). Previous studies have found that people use more positive emotion words, such as "love" and "sweet", to describe a positive event, and more negative emotion words, such as "ugly" and "nasty", to describe a negative event (Kahn, Tobin, Massey, & Anderson, 2007). Past research has also found that people with a higher job position will speak more often and freely make statements about other people, while people with lower job positions are more self-focused and ask more questions (Tausczik & Pennebaker, 2010). Through these past studies, it is evident that people are very particular in their word choice, and how they speak is important. The goal of the current study is to illustrate the relationship between factors that relate to a person's overall wellbeing, such as depression symptoms and labile self-esteem, and how these factors may also relate to words used to describe an autobiographical memory.

A person's sense of self plays a critical role in various facets of their life. Labile self-esteem refers to a person's tendencies to shift or experience fluctuations in self-esteem (Dykman, 1998). Life stressors have a stronger impact on people with a high lability in self-esteem (Roberts & Kassel, 1997). People with high labile self-esteem, or a more unstable sense of self, may consistently change in how they perceive themselves. For example, a person may generally have a high self-esteem, but performing poorly on an exam can act as a catalyst for them to develop a negative perception of themselves. On the contrary, an individual with less labile self-esteem, or a more stable sense of self, may have the same self-esteem from day to day regardless of context or experience. There has been a lack of research examining the relationship between labile self-esteem and word use, thus the current study aims to take a closer look.

Along with labile self-esteem, depression can have detrimental effects on a person's overall well-being. Depression symptomatology can include increased feelings of hopelessness as well as anhedonia-a reduced interest in tasks that were once found pleasurable. Previous studies have illustrated a link between depression symptoms and the way in which people recall previous emotional events. Studies have found that people who report more depression symptoms use more first-person singular pronouns, more negative words, and fewer positive words to describe an emotional experience, in comparison to people who have never experienced a depressive episode (Rude, Gortner, & Pennebaker, 2004).

Past research has demonstrated the detrimental effects of having a highly labile self-esteem and symptoms of low-grade depression on a person's ability to function. Therefore, it is not surprising that previous studies have found a relationship between labile self-esteem and depressive symptomatology. Highly labile self-esteem has been linked to an increased risk for depressive symptoms among asymptomatic individuals following a life stressor (Roberts & Monroe, 1992). Previous studies have illustrated the link between depression symptomatology and word use, along with the link between depression symptoms and labile self-esteem; however, past research has overlooked the interactive relationship between labile self-esteem and depression symptoms, which may be a stronger joint predictor of word use. The research conducted in the present study will provide more knowledge on how specific wellbeing factors can interact and permeate into the way people communicate their past experiences.

### The Current Study

The current study examines the direct and interactive relationships among labile self-esteem, depression symptoms, and words used to describe past happy and sad emotional experiences. My study will investigate four main hypotheses. I hypothesized that participants with more labile self-esteem would utilize fewer clout, power, and achievement words in contrast to participants with more stable self-esteem. I hypothesized that participants would use fewer clout, power, and achievement words as reported depressive symptoms increased. Based on previous studies, I hypothesized that labile self-esteem and depressive symptoms would be positively correlated, such that people reporting high labile self-esteem would report more depressive symptoms. Finally, when examining the interactive relationship between labile self-esteem and depression symptoms in predicting word use, I hypothesized that people with the combination of low labile self-esteem and less depressive symptoms would use more clout, power, and achievement words than people with high labile self-esteem and greater depressive symptoms.

## METHODS

### Participants

Participants were 90 young adults (*Mage* = 19.41 years, *SD* = 1.56, range: 17-26 years old; 62 women), who participated in the study in exchange for research credit for an introductory Psychology course. Racial and ethnic distribution varied; about 41% of partic-

ipants identified as Asian, 19% identified as Hispanic/Latinx, 13% of participants identified being White/Caucasian, 2% reported being Black/African American, 7% identified with more than one ethnicity, and 3% reported being part of an ethnic group not listed above. Approximately 15% of participants did not report their race/ethnicity.

## Procedure

Undergraduate participants came to the Emotion Regulation Lab at the University of California, Riverside for a two-hour single session study. Informed consent was obtained from all participants before the study started. Participants were asked to complete questionnaires that asked about their self-esteem and depression symptoms, as well as other family and personal characteristics. Participants completed various computer tasks and in-person tasks including an interview about their emotional experiences (the Autobiographical Emotion Interview: AEI), described below. At the end of the study, participants were debriefed, thanked, and received research credit for their participation. All procedures were completed in English.

## Measures

**Labile Self-Esteem Scale (LSE).** Participants responded to a five-question survey. The first four items in the survey were directly derived from Dykman's original LSE scale and directly measure the fluctuations in self-esteem a person may experience on a daily basis (Dykman, 1998). These four items included questions such as "How I feel about myself stays pretty much the same day to day" and "Compared to most people, my self-esteem changes rapidly." In addition to these four items, the present study included participants' responses to a single additional item, "I have a high self-esteem," that was not on Dykman's original LSE scale. Participants utilized a 7-point scale (1= strongly disagree; 7 = strongly agree) for all questions. Reliability for the five items for this measure was good ($\alpha = .631$); however, the reliability for the first four items from Dykman's original scale was stronger ($\alpha = .866$).

**CES-D.** Participants completed the Center for Epidemiologic Studies Depression Scale (CES-D) originally developed by former members of the Center for Epidemiologic Studies (CES), Ben Locke and Peter Putnam (Radloff, 1977). This 20-item questionnaire asks participants questions related to depression symptoms including feelings of worthlessness and depressed mood. Participants wrote their responses utilizing a scale from 0 to 3, in which 0 is "rarely or none of the time" and a 3 is "most or all of the time." The written survey included questions such as "I thought my life had been a failure" and "I had trouble keeping my mind on what I was doing." Internal consistency for this questionnaire was strong (Cronbach's $\alpha = .88$).

**Autobiographical Emotion Interview.** The Autobiographical Emotion Interview (AEI) directly asks participants to recall an event in which they felt sad, scared, angry, and happy (Sillars & Davis, 2017). A research assistant would ask about each emotion; in turn, the procedure for each emotion phase of the interview was the same. The research assistant asked the participant to think about a time in which they felt a particular emotion, and to think of all the little details that accompany that event. After leaving the participant alone in the room for 2-3 minutes to think, the research assistant would reenter the room and ask the participant to verbally describe a past emotional experience based on the prompted emotion. Participants were then asked whether the event was something "they could handle" or if the event "was just too much;" however, participants' responses to this question were not included in the data analyses of the current report. Participants' descriptions of past sad and happy emotional experiences were examined for the number of clout, power, and achievement words used. The interview took approximately 15-20 minutes to complete and was recorded.

## Data Processing

LIWC. The Linguistic Inquiry Word Count (LIWC) processing system categorizes various linguistic phrases in a multitude of research settings (Tausczik & Pennebaker, 2010). The LIWC program contains dictionaries that categorize and identify the different types of words people use. Participants' verbal descriptions of their past sad and happy emotional experiences during the AEI, were transcribed into an excel sheet. Participants' responses were distinctly linked to a unique ID number to maintain anonymity. This excel sheet was then analyzed by the LIWC software to determine the number of clout, power, and achievement words participants used to describe past happy and sad emotional experiences. "Clout" language is associated with confidence and status and is characterized by use of pronouns such as "we" and "you," and less use of first-person pronouns such as "I" (Kacewicz, Pennebaker, Davis, Jeon, & Graesser, 2013). Power language terms relate to dominance and can include words such as "superior" and "bully" (Pennebaker, Boyd, Jordan, & Blackburn, 2015). Achievement words reference triumphs and failures; this can include words such as "success", "win", and "better" (Pennebaker, Boyd, Jordan, & Blackburn, 2015).

# RESULTS

Results are organized into sections based on the research questions and hypotheses described above. First, the individual links between labile self-esteem, depression symptoms, and the number of clout, power, and achievement words used to describe a happy and sad autobiographical memory will be described. Next, results will illustrate the link between depression symptoms and labile

self-esteem. Finally, analyses to examine the interaction between depression symptoms and labile self-esteem as a predictor for word use are presented

## Correlations

**Labile self-esteem and word use.** Labile self-esteem was significantly positively associated with the number of clout words people used to describe a happy emotional experience ($r$ = .258, $p$ = .016). This indicates that greater fluctuations in self-esteem were associated with using more confidence and expertise words when describing a happy emotional experience. There was no significant association between labile self-esteem and the number of achievement ($r$ = .030, $p$ = .786) and power ($r$ = -.005, $p$ = .960) words used to describe a happy experience. Labile self-esteem was not significantly associated with clout ($r$ = -.086, $p$ = .424), achievement ($r$ = -.072, $p$ = .505), or power ($r$ = -.048, $p$ = .656) words utilized to describe a sad experience.

**Depression symptoms and word use.** Self-reported depression symptoms were not significantly associated with the number of clout ($r$ = -.034, $p$ = .771), achievement ($r$ = .060, $p$ = .611), or power ($r$ = -.045, $p$ = .705) words participants used when describing a happy emotional experience. Self-reported depression symptoms were also not significantly associated with the number of clout ($r$ = -.069, $p$ = .552), achievement ($r$ = -.007, $p$ = .952), or power ($r$ = .006, $p$ = .956) words used to describe a sad emotional experience.

**Is labile self-esteem associated with depression symptomatology?** Labile self-esteem was significantly positively associated with depression symptoms ($r$ = .676, $p$ < .001), such that greater fluctuations in self-esteem were associated with more depression symptoms, as self-reported on the CES-D.

## Linear Regressions Models Predicting Word Use

For each of the following linear regression models predicting word use, we utilized the same organization of variables. In the first step, we entered both labile self-esteem and depression symptoms to determine if either variable significantly predicted clout, power, and achievement words. In the second step, we entered the interaction between depression symptoms and self-esteem in predicting word use.

**Clout words used to describe a happy experience.** The first step was significant $F$ (2, 71) = 4.047, $p$ = .022, $R^2$=.102. Labile self-esteem was a significant predictor for the number of clout words used to express a happy experience. ($b$ = .9.312, $t$ = 2.828, $p$ = .006); however, depression symptoms did not significantly predict the number of clout words used to explain a happy experience ($b$ = -1.112, $t$ = -2.079, $p$ = .041). When entering the interaction of depression at the second step, this step was not significant F$\Delta$ (1, 70) = .021, $p$ = .886, $R^2\Delta$ = .103, and the interaction effect was also not significant ($b$ = .038, $t$ = .144, $p$ = .886). This finding suggests that labile self-esteem and depression symptomatology did not interact to predict word use. Moreover, incorporating the interaction in step two did not improve the model fit.

**Clout words used to describe a sad experience.** The first step of the model $F$ (2, 73) = .221, $p$ = .802, $R^2$= .006, was not significant. Labile self-esteem ($b$ = -1.018, $t$ = -.300, $p$ = .765) and depression symptoms ($b$ = -.140, $t$ = -.254 $p$ = .800) did not significantly predict clout words used to describe a sad autobiographical memory. The second step was not significant $F\Delta$ (1, 72) = .764, $p$ = .385, $R^2\Delta$ = .016, indicating that labile self-esteem and depression symptoms did not interact to predict clout words used to describe a sad experience ($b$ = .237, $t$ = .874, $p$ = .385).

**Achievement words used to describe happy experience.** The first step of the model $F$ (2, 71) = .129, $p$ = .880, $R^2$= .004 was not significant. Labile self-esteem ($b$ = .004, $t$ = .022, $p$ = .982) and depression symptoms ($b$ = .010, $t$ = .369, $p$ = .713) did not significantly predict achievement words used. The second step of the model was not significant $F\Delta$ (1, 70) = 2.154, $p$ = .147, $R^2\Delta$ = .033. Labile self-esteem and depression symptoms did not interact to predict achievement words used to describe a happy experience ($b$ = -.019, $t$ = -1.468, $p$ = .147).

**Achievement words used to describe a sad experience.** The first step of the model $F$ (2, 73) = .104, $p$ = .902, $R^2$= .003 was not significant. Labile self-esteem ($b$ = -.070, $t$ = -.451, $p$ = .653) and depression symptoms ($b$ = .006, $t$ = .249, $p$ = .804) did not significantly predict the number of achievement words used to describe a sad experience. The second step of the model was also not significant $F\Delta$ (1, 72) = .394, $p$ = .532, $R^2\Delta$ = .005. Labile self-esteem and depression symptoms did not interact to predict achievement words used to describe a sad experience ($b$ = -.008, $t$ = -.628, $p$ = .532).

**Power words utilized to describe a happy emotional experience.** The first step of the model $F$ (2, 71) = .209, $p$ = .812, $R^2$= .006 was not significant. Neither self-esteem ($b$ = .076, $t$ = .523, $p$ = .602) nor depression symptoms ($b$ = -.015, $t$ = -.629, $p$ = .532) significantly predicted power words used. The interaction entered in the second step was not significant for the model step $F\Delta$ (1, 70) = .120, $p$ = .730, $R^2\Delta$ = .008 and for the specific interaction effect ($b$ = -.004, $t$ = -.346, $p$ = .730).

**Power words utilized to describe a sad emotional experience.** The first step of the model was not significant $F$ (2, 73) = .023, $p$ = .977, $R^2$= .001. Labile self-esteem ($b$ = -.027, $t$ = -.209, $p$ = .835)

and depression symptoms ($b$ = .004, $t$ = .178, $p$ = .859) did not significantly predict power words used to express a sad experience The second step of the model was not significant $F\Delta$ (1, 72) = .701, $p$ = .405, $R^2\Delta$ = .010. Labile self-esteem and depression symptoms did not interact to predict power words used to describe a sad emotional experience ($b$ = -.009, $t$ = -.837, $p$ = .405).

## DISCUSSION

The current study examined the direct and interactive relationships among labile self-esteem, depression symptoms, and the number of clout, power, and achievement words utilized to describe past happy and sad emotional experiences. Four hypotheses were investigated. First, we hypothesized that participants with more labile self-esteem would use fewer clout, power, and achievement words to describe sad and happy emotional experiences. Second, that there would be less use of clout, power, and achievement words as depression symptoms increased. Third, I predicted that there would be a positive association between high labile self-esteem and more self-reported depression symptoms. Fourth, I hypothesized that the combination of high labile self-esteem and more self-reported depression symptoms would be linked to fewer clout, power, and achievement words used to describe sad and happy emotional experiences. Overall, hypotheses were partially supported. Each finding is discussed in turn below.

In contrast to our original hypotheses, the present study found that labile self-esteem was positively associated with the number of clout words used to describe a happy experience. In other words, the more people's self-esteem fluctuated, the more clout words they used when talking about a happy autobiographical memory. Previous studies have found that people high in clout language, speak with confidence and greater assertiveness, while people low in clout language are more uncertain and hesitant (Jordan, Sterling, Pennebaker, & Boyd, 2019). It is possible that this relationship emerged because participants with a highly variable sense of self may have referenced autobiographical memories that reaffirm their positive views of themselves. As a result, participants with more labile self-esteem may have had a greater boost of confidence and joy as they described their happy autobiographical memory than participants with low labile self-esteem. These findings not only provide more knowledge on the links between labile self-esteem and word use, but further emphasize the idea that aspects of people's wellbeing, such as self-esteem, may be reflected in the way people communicate and process their past experiences. Moreover, these findings imply the idea that people with more labile self-esteem may speak differently about their past happy emotional experiences and may have a potential bias in the types of memories they recall, in contrast to people with low labile self-esteem; however, more research is needed on the relation between labile self-esteem and other word categories coded by LIWC.

In line with our hypothesis, labile self-esteem and depression symptoms were positively associated, such that greater fluctuations in sense of self were linked to more self-reported depression symptoms. This is consistent with literature indicating that people with more fluctuations in self-esteem have an increased risk for depressive symptoms (Roberts & Monroe, 1992). A key symptom of depression is feeling worthless, and these feelings are often also experienced by people with low self-esteem; therefore, it is not surprising that someone with highly variable self-esteem may concurrently experience more depressive symptoms. The findings in the present study are important because clinicians and researchers can gain a better understanding of the interconnected nature of these variables. Moreover, clinicians can consider both facets of well-being when diagnosing and treating psychopathology symptoms.

The insights from the present study findings are tempered by some limitations. For example, the LSE questionnaire used here included an extra item, "I have high self-esteem." Including this extra question, that was not on the original LSE scale developed by Dykman, may have altered the way participants thought about and reported their labile self-esteem. The first four questions for the scale directly (and reliably) measure labile self-esteem; however, the added fifth item, "I have high self-esteem," appears to only measure overall levels of participants' self-esteem rather than lability in self-esteem. The effects of this extra item are further demonstrated in the scale reliability calculations. The scale containing all five questions had acceptable reliability ($\alpha$ = .631); however, the reliability computed using only the first four questions from the original Dykman LSE scale was improved ($\alpha$ = .866). Thus, the present study may not have received an accurate measure of participants' labile self-esteem because participants answered all five questions. We utilized the original four questions on Dykman's LSE scale in the measure included in the correlations and regression models, ultimately accounting for the possible effects the fifth question may have had on the results.

Since participants had quite a bit of liberty in terms of the memories they recalled and talked about during the AEI, it is possible that participants had a particular bias in recalling memories. In other words, some participants may have disclosed more personal memories while other participants may have been more private. Moreover, some participants may have been more detailed in their responses. As a result, the types of memories participants recalled, and the level of detail provided may have affected the word categorization by the LIWC program. Despite this limitation, it was important to have provided participants with the latitude to speak freely in order to best capture how people would naturally communicate about a past emotional experience. Moreover, responses

were still structured, given that the AEI prompted participants to think about a specific memory linked to a particular emotion.

The present study adds to a growing body of research on the direct and interactive links among depression symptoms, labile self-esteem, and word use. Future research can expand on the link between depression symptoms and words used to describe the experience of other emotions, such as anger and fear. While the present study did not find an interaction between depression symptomatology and labile self-esteem in predicting word use, future research can further examine if these variables interact to predict other linguistic phrases coded by LIWC. Communication is powerful. Tracking word use enables researchers to gain a better understanding of the way people express a past emotional experience and what that means for personal wellbeing.

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# The Effect of Shame and Guilt on Students Writing Habits

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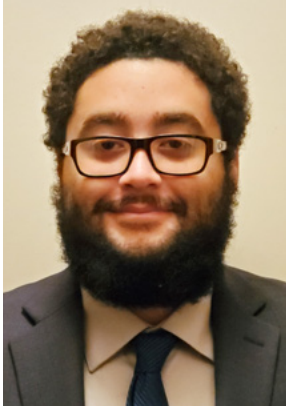
## ABSTRACT

Individuals strive to assuage negative emotions through a myriad of mechanisms, some of which are adaptive while others are not. In the current study, we focus on shame and guilt. Previous research suggests that shame is more associated with defensiveness and the tendency to project negative feelings outward. However, guilt can be an adaptive emotion and is associated with the tendency to take responsibility. The current study explores how such negative emotionality can affect students' perceived and actual work habits by utilizing Google Docs, which keeps a time-stamped record of workers' activity that is accurate to the millisecond. Participants ($n$ = 178) were asked to write an essay into Google Docs. Participants also completed self-reported procrastination scales and the Test of Self-Conscious Affect (TOSCA). Therefore, we can compare participants' self-reported levels of shame and guilt with both their self-reported procrastination and their actual work activity (measured by utilizing the time-stamped data). While both shame-proneness and guilt-proneness are significant predictors of self-reported procrastination, neither predict observed procrastination. Despite this, self-reported procrastination is associated with observed procrastination. Ultimately, this data can be used to better understand students' perceived and actual work habits and motivations from a psychological perspective and can assist in informing others regarding how to best engage with students concerning their writing activity and habits.

**KEYWORDS:** *Motivation; Writing; Shame; Guilt; Habits; Procrastination*



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# The Effect of Shame and Guilt on Students Writing Habits

## INTRODUCTION

Previous research suggests that shame is associated with defensiveness and the projection of negative feelings; in students, this may lead to maladaptive work patterns (Martincekova & Enright, 2018). In other cases, shame is associated with lying and violence (Stuewig, Tangney, Heigel, Harty, & McCloskey, 2010). In contrast, guilt can be an adaptive emotion associated with responsibility and atonement (Stuewig et al., 2010; Tangney, 2002). In students, guilt may then promote responsible behavior towards schoolwork. The differences between shame and guilt have proven to be important in a variety of cases, with these differences becoming important in a variety of contexts that encompass severe mental disorders, our perception of everyday activities, and the variety of topics in-between (Bannister, Colvonen, Angkaw, & Norman, 2019; Parker & Thomas, 2009; Tangney, Stuewig, & Martinez, 2014). While the overarching differences between shame and guilt are plain, there is a significant gap in the psychological literature concerning how these differences relate to academic motivation. Further, there is little psychological literature regarding negative emotionality and writing specifically, and the existing research on emotionality and procrastination is primarily centered around anxiety and/or depression. This is a significant gap to fill as writing is one of the few academic endeavors that continue beyond schooling, making writing an activity that often continues for the rest of the individual's life, albeit in varying capacities. The current study seeks to remedy this and to better understand how these differences relate to students' writing patterns and self-perception in order to provide insight into a task that will likely follow individuals throughout their lives. Of course, this does not mean that emotionality and academic motivation are completely unexplored topics; non-psychological literature explains the importance of such emotionality in writing utilizing qualitative methodology (Ballenger & Myers, 2019). The purpose of the current study is to provide a quantitative foundation for those who seek to research students' shame and guilt using a novel methodology, as multiple fields of study understand the large role that emotionality plays in the lives of students (Hastings, Northman, & Tangney, 2000; Ballenger & Myers, 2019).

Many factors can contribute to an individual's decision to procrastinate. For example, conscientiousness and the simple desire to avoid tasks that the individual finds unpleasant (Fee & Tangney, 2012). However, there are also affective factors that impact motivation, such as shame and self-esteem. Additionally, low self-esteem is associated with an individual's likeliness to procrastinate (Fee & Tangney, 2012). However, this could also be a result of anxiety. Self-esteem can act as a buffer to anxiety in response to a threat (Greenberg, et al., 1992). For example, if an academic assignment is considered a potential threat to an individual's self-image, then low self-esteem may prevent them from protecting against anxiety. Potentially, this could create a positive feedback cycle due to the link between self-esteem and shame, if a decrease in one will lead to an increase in the other. When their negatively impacted work is evaluated, this evaluation may negatively impact their affect, which in turn reduces their ability to buffer against future affective threats. Therefore, with this research, we would expect to find significant differences between the unique relationships that shame-proneness and guilt-proneness have with procrastination. Shame-proneness would be expected to be positively associated with self-reported procrastination since shame causes a negative impact on global self-evaluation (Fee & Tangney, 2012; Martincekova & Enright, 2018; Strelan, 2007). As a result of the comprehensiveness of this judgment, and the relative strength of shame as an emotion, shame-proneness may cause individuals to self-report differently than their less shame-prone peers, even in the absence of any other discrepancy in behavior.

The current study explores how shame-proneness and guilt-proneness can affect students' perceived and actual work habits by utilizing Google Docs, which keeps a time-stamped record of students' writing activity. Participants wrote two essays using Google Docs and completed multiple questionnaire measures of self-reported procrastination, in addition to the Test of Self-Conscious Affect (TOSCA; Tangney, Wagner, & Gramzow, 1989), which measures proneness for both guilt and shame. Therefore, we can compare participants' self-reported levels of shame and guilt with both their self-reported procrastination and how they distribute their time when writing their actual course essays. We expect guilt-proneness and shame-proneness to predict procrastination, with the assumption that procrastination is a result of negative emotionality that leads to self-sabotaging behavior. This viewpoint suggests that shame-prone students prioritize the avoidance of failure over adopting strategies to increase the chances of success overall (Ferradas, Freire, Valle, & Nunez, 2016). Such strategies can appear in layers, with additional strategies being used to rationalize failure in a way that does not impact the workers' sense of self-worth as harshly as it would have otherwise. Therefore, we expect shame to be significantly associated with both self-reported and observed procrastination. Guilt, however, is a more easily externalized emotion and is not as significantly associated with self-worth (Averill et al., 2002; Martincekova & Enright, 2018; Strelan, 2007). Therefore, we would not expect it to be strongly related to self-sabotaging (via procrastination) or our measures of writing habits.

In short, we hypothesize that shame-proneness will be positively associated with both self-reported and observed procrastination, based on two assumptions. First, we expect shame-prone students to also believe themselves to be procrastinators. Second, we expect students who believe themselves to be procrastinators to have different behavioral patterns than those who do not. Inversely, we

hypothesize that guilt-proneness will not be associated with self-reported procrastination. However, guilt is generally a less powerful emotion as it is focused on a specific behavior rather than a global self-evaluation. Therefore, we hypothesize that guilt-proneness will negatively predict observed procrastination with the expectation that guilt-proneness is a stronger predictor of behavior than shame, as guilt and measurement of behavior share a fundamental purpose – to assess specific behavior, while shame forces a more global self-assessment.

## MATERIALS AND METHODS

### Overview

Observed procrastination on writing tasks has been historically neglected by researchers because it is difficult to measure outside of the laboratory. We have attempted to address this gap by introducing a possible new solution - utilizing Google Docs. Google Docs saves a precise record of when participants were working; each time a participant changes the document it is stored as an "edit" along with a precise timestamp recording when it happened. This allowed us to collect an accurate but non-invasive measure of observed procrastination. Further, this allowed us to compare the results from our observed measures with the results from commonly used questionnaires, an important step in bettering our understanding of both types of measures.

### Participants

Participants were recruited from a Social Psychology course; the students were compensated with extra credit. One hundred ninety students submitted at least partial data for the study, but only 177 participants were included in the current data. The main inclusion criteria were that participants had to submit data for at least one essay, but some were excluded based on different factors, such as participants who indicated that they had not followed instructions or whose data contained extreme outliers. Of the two essays, 154 participants participated in the first essay, 153 participated in the second, and 140 participated in both essays. The demographic breakdown can be seen here: 2.2% African American, 27% Asian, 9.6% Caucasian, 47.8% Hispanic/Latino, 1.1% Pacific Islander, 6.7% Middle Eastern, 5.6% mixed race/other. Additionally, the gender breakdown is 23% male, 76% female, 1% either declined to answer or listed "other."

### Procedure

The data was gathered over the course of a single quarter. Participants were asked to take an online survey. Informed consent was obtained from all participants before they participated. Participants were tasked with completing two essays as a requirement for the course but participating in the study required them to write these essays into the Google Docs word processor. Additionally, students had to provide permission to retrieve the relevant data provided by Google Docs to participate. The course required the essays to fit the following parameters: The essays were to be a minimum of five pages long with at least five citations, in APA format. Both essays had identical requirements, but students were given a different choice of topics for the second essay. After the essays were completed, participants allowed researchers to extract the metadata from the document by giving edit-level permission through Google Docs. The metadata consisted of the list of timestamps that were used to create the measures and compute the results. Data on the essay submission times were collected using the timestamps from the online submission portal.

### Measures

**Procrastination.** Procrastination was measured using self-reported and observed measures.

**Observed Procrastination** was measured by utilizing the timestamp measures from Google Docs and the University's online essay submission portal. This data allowed us to compute measures of *Mean Work Time, Submit Time, Essay Start Time,* and *Essay End Time*.

**Mean Work Time** measured the average amount of time participants spent on each essay. This was calculated by utilizing all of the Google Docs timestamps for every edit a participant made in an essay and computing the mean to get their average work time.

S**ubmit Time** was the time that the essay was submitted through the online submission portal for the assignment.

**Essay Start Time** was when the first percentile of the essay was completed. The first percentile of the essay was selected as a timestamp to improve the accuracy of the measure. Using the first percentile instead of the first timestamp prevents a "false-start" as erroneous keystrokes during the creation of the document itself would not be considered a start time in the current study.

**Essay End Time** was when the 99th percentile of the essay was completed. The 99th percentile of the essay was selected as a timestamp to improve the accuracy of the measure. Using the 99th percentile instead of the last edit prevents a "false finish" caused by accidental adjustments made to the essay after it has been completed, such as when students accidentally press a key when sending the metadata to researchers.

**Self-Reported Procrastination** was measured using a selection of self-reported trait-procrastination measures, including the General Procrastination Scale ($\alpha$ = .86), the Irrational Procrastination Scale ($\alpha$ = .85), and the Pure Procrastination Scale ($\alpha$ = .92). The

**Table 1.** Correlation Matrix of All Study Variables. Note * = $p < 0.05$, ** = $p < 0.001$

| # | MEASURE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Composite Procrastination | – | | | | | | | | | | |
| 2 | Shame | .327** | – | | | | | | | | | |
| 3 | Guilt | .005 | .428** | – | | | | | | | | |
| 4 | Work Time – Essay One | .418** | .154 | .147 | – | | | | | | | |
| 5 | Work Time – Essay Two | .335** | -.048 | .005 | .540** | – | | | | | | |
| 6 | Start Time – Essay One | .226** | -.004 | .077 | .662** | .429** | – | | | | | |
| 7 | End Time – Essay One | .400** | .042 | .204* | .703** | .526** | .337** | – | | | | |
| 8 | Start Time – Essay Two | .240** | .068 | .105 | .448** | .642** | .574** | .331** | – | | | |
| 9 | End Time – Essay Two | .237** | -.137 | -.074 | .275** | .793** | .185* | .533** | .354** | – | | |
| 10 | Submit Time - Essay One | .169* | .072 | .144 | .627** | .544** | .280** | .927** | .294** | .547** | – | |
| 11 | Submit Time – Essay Two | .221** | .053 | .066 | .219** | .420** | .007 | .556** | .003 | .695** | .465** | – |

General Procrastination Scale (Lay, 1986) consists of several statements, such as "I generally delay before starting on work I have to do," and each statement must be rated on a 1-5 Likert scale. In this case, a rating of 1 would suggest that the statement is extremely uncharacteristic of the participant while a rating of 5 suggests that the statement is extremely characteristic of the participant. The Irrational Procrastination Scale (Steel, 2010) also consists of several statements, such as "I delay tasks beyond what is reasonable," and is also rated on a 1-5 Likert scale. In this measure, participants are rating how often the statement is true of them. A rating of 1 suggests the statement is rarely or never true of the participant. The Pure Procrastination Scale (Steel, 2010) is also provided on a 1-5 Likert scale and features statements such as "I delay making decisions until it is too late," with a response of 1 suggesting the statement rarely true of the participant and a response of 5 indicating the statement is very often true of the participant. All self-report procrastination measures have strong internal reliability. The scores of these self-reported measures were standardized and then averaged to compute a measure of *Composite Procrastination*.

**Shame and Guilt.** This was measured with the Test of Self-Conscious Affect (TOSCA), a survey that measures proneness to guilt, shame, and blame. It does so by providing the participants with plausible scenarios and responses to those scenarios that indicate shame, guilt, or blame. The participant then rates the likeliness that they would respond similarly to each response on a Likert scale (Tangney et al., 1989). Both shame ($\alpha$ = .83) and guilt ($\alpha$ = .76) subscales have an acceptable internal consistency. Blame was left out of the present study. As an example, one scenario stated "You are taking care of your friend's dog while they are on vacation. The dog runs away." Example responses included: "You would think, 'I am irresponsible and incompetent.'" "You would think your friend must not take very good care of her dog or it wouldn't have run away," and "You would vow to be more careful next time." Each response was rated using a 1 to 5 score from the participant, with a score of 1 suggesting the participant is very *unlikely* to respond in that manner and a score of 5 suggests that the participant is very *likely* to respond in that manner.

## RESULTS

Table one is a correlation matrix showing the relationship between the variables utilized in the present study. The matrix suggests that the observed measures of procrastination were all correlated with each other, except for the submission time of essay two, as that measure did not correlate with the start times of either essay. Additionally, composite procrastination correlated with every measure except for the TOSCA's guilt-proneness subscale. Self-re-

**Table 2.** Summary of Multiple Regression Models

| OUTCOME MEASURE | Shame | Guilt | df | Adjusted $R^2$ | p |
|---|---|---|---|---|---|
| Composite Procrastination | β = .356 | β = -.143 | 175 | .114 | **<.001** |
| | *SE* = .072 | *SE* = .072 | | | |
| | **p < .001** | *p* = **.048** | | | |
| Mean Work Time, Essay One | β = .117 | β = .106 | 151 | .109 | .088 |
| | *SE* = .093 | *SE* = .095 | | | |
| | *p* = .213 | *p* = .264 | | | |
| Mean Work Time, Essay Two | β = -.069 | β = .039 | 150 | -.010 | .772 |
| | *SE* = .096 | *SE* = .094 | | | |
| | *p* = .475 | *p* = .681 | | | |
| Submit Time, Essay One | β = .012 | β = .013 | 175 | .009 | .158 |
| | *SE* = .078 | *SE* = .055 | | | |
| | *p* = .878 | *p* = .096 | | | |
| Submit Time, Essay One | β = .031 | β = .053 | 173 | -.006 | .640 |
| | *SE* = .078 | *SE* = .055 | | | |
| | *p* = .713 | *p* = .528 | | | |
| Essay One Start Time | β = -.047 | β = .102 | 150 | -.005 | .568 |
| | *SE* = .095 | *SE* = .096 | | | |
| | *p* = .618 | *p* = .289 | | | |
| Essay One End Time | β = -.058 | β = .242 | 150 | .031 | **.034** |
| | *SE* = .095 | *SE* = .096 | | | |
| | *p* = .696 | *p* = **.011** | | | |
| Essay Two Start Time | β = .023 | β = .095 | 148 | -.002 | .430 |
| | *SE* = .096 | *SE* = .095 | | | |
| | *p* = .809 | *p* = .320 | | | |
| Essay Two End Time | β = -.137 | β = -.007 | 148 | .006 | .245 |
| | *SE* = .096 | *SE* = .095 | | | |
| | *p* = .154 | *p* = .942 | | | |

ported procrastination correlates strongly with observed measures of procrastination, showing that our observed measures did not fail to measure procrastination. Additionally, shame-proneness correlates strongly with self-reported procrastination but did not correlate significantly with the observed measures of procrastination. Further, guilt-proneness and shame-proneness are highly correlated, but guilt did not correlate with either self-reported or observed measures of procrastination with one exception; guilt did correlate with the end time of the first essay.

Table two describes several linear regression models. These models suggest that both shame-proneness and guilt-proneness are significant predictors of self-reported procrastination. However, neither guilt-proneness nor shame-proneness were statistically significant predictors of any of the observed measures of procrastination, again with one exception, as guilt-proneness was a predictor of when participants completed the first essay.

The main hypotheses of this study were, first, that shame-proneness would be positively associated with both self-reported and observed

procrastination. Second, we hypothesized that guilt-proneness would not be associated with self-reported procrastination but would negatively predict observed procrastination. To assess the first hypothesis, whether shame-proneness is a predictor of procrastination, we ran correlations and multiple regression models. Our multiple regression model revealed that shame-proneness is a predictor of self-reported procrastination, but our correlation matrix did not provide evidence that shame-proneness was associated with observed procrastination. In testing our hypotheses concerning guilt, our data failed to provide evidence for either prediction as the correlation matrix did not identify guilt-proneness as a meaningful correlate of observed procrastination; however, our multiple regression model did identify guilt-proneness as a negative predictor of self-reported procrastination. Since the main question this paper is trying to address is the predictive power of shame and guilt on procrastination and writing habits, these results make it clear that there are separate groupings of variables predicting self-reported procrastination and observed procrastination, despite the associations between self-reported procrastination and our measures of observed procrastination.

## DISCUSSION

As shown from the results, both shame-proneness and guilt-proneness were associated with self-reported procrastination. However, they were not associated with observed procrastination, with one small exception. This means that a person's tendencies towards guilt and shame are not associated strongly with their behavior, but they are associated strongly with how people evaluate that behavior. Importantly, this is not because our Google Docs measure fails to measure procrastination: it does, and this is evident from the significant relationship between self-reported procrastination and all of the measures we computed using the Google Docs metadata. Ultimately, it would seem as if there was a missing variable between shame and observed procrastination that could be uncovered to better understand the connections between self-image and actual working behaviors in students. In other words, shame-proneness predicts whether students believe themselves to procrastinate but does not predict observed behaviors of procrastination. However, self-reported procrastination predicts observed procrastination. Therefore, there may be an unknown variable that would predict both self-reported procrastination and observed procrastination that is also associated with shame-proneness - perhaps some other aspect of negative emotionality, or a working behavior that is only tangentially related to emotionality. One potential direction for further study is self-esteem, as existing literature has linked low self-esteem and high levels of shame, although linking this variable group to procrastination and writing habits represents another gap in the psychological literature (Velotti, Garofalo, Bottazzi, & Caretti, 2017).

The current study, like all others, has a few limitations. One limitation is the unknown impact on the surveys, as they could have been completed at any time during the length of the course. Therefore, some of the survey responses may have been influenced by the students' observations of their work or how they felt while doing the work, while responses completed earlier in the course might better show how they feel generally. Another limitation is the inability to determine the causality of the results as we did not manipulate participants' emotionality, although such manipulation may prove to be fruitful in future studies.

There are several clear implications that we can draw from this data. Google Docs can serve as an appropriate tool for researchers observing students' writing habits in an easy and non-intrusive way, an immense benefit for researchers as collecting similar observational data is historically difficult and disruptive. The current study also provides evidence that shame-proneness and guilt-proneness influence how students evaluate themselves but not how they behave. This means that counselors who are working with students who feel ashamed or guilty of procrastinating perhaps should focus on helping the student come to terms with their behavior and determine whether their feelings are accurate, instead of prematurely prescribing tricks to "fix" their behavior. Ultimately, this is another avenue to be explored in future research, as the current study provides the foundation but cannot specifically draw that conclusion.

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# Analysis of Pulmonary Complement Protein Expression Following Organic Dust Exposure

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## ABSTRACT

Organic dust, as found in agricultural farm work, is ranked among the highest occupational exposure hazards by the CDC. Agricultural dust containing endotoxins, pesticides, mold, and other chemicals, contributes to increased rates of respiratory diseases among these workers. Human bronchial epithelial cells (HBEC), which line upper airways, are frequently exposed to pathogens. Understanding the role of HBEC in inflammation following dust exposure (DE) is necessary to understand the mechanisms underlying inflammatory diseases. The complement system, a nonspecific and non-adaptable defense mechanism, is composed of circulating proteins that promote inflammation by attacking the cell membranes of pathogens and recruiting immune cells that secrete mediators of inflammation. We characterized complement protein expression in DE-treated HBEC using previously generated SWATH proteomics data and Western blotting. Western blotting identified that DE treatment in HBEC mediates the release and activation of C3, while data identified via SWATH-MS proteomics indicated significant upregulation of CD59—a regulator of complement activation. These data suggest that DE-HBEC may regulate complement activation and aim to elucidate the mechanisms by which HBEC promotes the complement system, and thus induce pulmonary inflammation in the presence of organic dust.

**KEYWORDS:** *Human Bronchial Epithelial Cells; Dust Exposure; Complement System; Inflammation; C3; CD59*

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Sarah Ibrahim is a fourth year Biology Major. Her research focuses on studying the effects of dust exposure on bronchial epithelial cells. She has gained experience in biomedical laboratories since her first year and wants to continue pursuing research in the future. Sarah is also interested in addressing health care disparities in underserved communities and intends to pursue a career in medicine.

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**Tara Nordgren**, *Assistant Professor in the Department of Biomedical Sciences*

Dr. Tara Nordgren is an Assistant Professor in the School of Medicine, Division of Biomedical Sciences. She received her PhD and performed postdoctoral training at the University of Nebraska Medical Center, and is broadly trained in lung biology, immunology, lipid signaling, and toxicology. Dr. Nordgren's research interests involve identifying how environmental factors impact inflammation, injury, and repair in the lungs. In particular, she is interested in identifying how agricultural dust exposures elicit lung inflammation, and is exploring the role of bioactive lipids derived from omega-3 fatty acids in promoting inflammation resolution and tissue repair.

# Analysis of Pulmonary Complement Protein Expression Following Organic Dust Exposure

## INTRODUCTION

### The Importance of Identifying Complement Activation

Through participation in everyday activities, lungs are constantly exposed to dangers from dust particles which can result in damaged cells. Lungs have a defense mechanism to help remove dust particles from the respiratory tract; however, repeated exposure to dust can eventually result in lung disease ("What Are the Effects of Dust on Lungs?"). This project investigates the inflammatory effects of dust on human bronchial epithelial cells (HBEC) to better understand the mechanisms of how lung disease occurs. We studied two specific complement proteins, C3 and CD59, to verify whether the complement system, a function of the immunity defense mechanism, is active in the presence of dust. We hypothesize that exposing HBEC to dust will activate the complement system and cause an upregulation of CD59 and proteolytic cleavage of C3 as a defense mechanism to dust toxins. Understanding the immune system's response through complement activation can help other scientific studies further their knowledge in signaling pathways activated in a cell to provide a framework for critical pathways affected by pulmonary diseases.

### Background on the Pulmonary System

The pulmonary system, which consists of lungs and airways, mediates respiration. The lungs and airways directly participate to excrete waste products of cellular metabolism by gas exchange. The process begins as oxygen is brought into the body from the atmosphere. Through a series of branching tubes, such as the bronchus and bronchiole (**Figure 1**), the lungs release carbon dioxide through gas exchange. During the initial process of respiration, not all the particles entering the body's system will reach the lungs primarily because of the airway's function to act as a filter. These air tubes are lined by bronchial epithelial cells that produce mucus to trap passing foreign particles, including dust. Lungs have efficient mechanisms to monitor foreign particles in the respiratory tract. One of the body's mechanisms utilizes tiny hairs, called cilia, that cover the cells of the air tubes, to move the mucus up the throat. The collected particles exit the body's system through coughing or sneezing. Even though large particles that reside in the nose may be removed via bodily protective mechanisms, smaller particles can successfully pass through the filters, reach the windpipe, and enter the air tubes that lead to the lungs

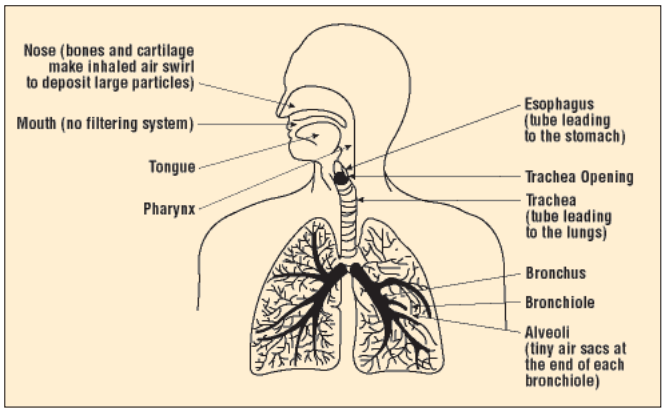


**Figure 1.** Image taken from the Canadian Centre for Occupational Health and Safety showing the pathway dust enters through the air and to the lungs

### The Complement System

Although dust particles that extend to the alveoli and lower part of the airways are captured by specialized white blood cells of the immune system called macrophages, the lungs also have another system that functions to remove dust. The complement system utilizes proteins produced from the lungs to bind and neutralize dust particles ("What Are the Effects of Dust on the Lungs?"). This system is composed of complement proteins that activate inflammation and is one of the most vital mechanisms for protecting the body against pathogenic agents. Complement proteins are found in the lining fluid of the respiratory tract and bind to the surfaces of microorganisms. These proteins mediate opsonization by identifying specific pathogens that are already marked by antibodies used in recognizing antigens. During this process, molecules, microbes, or apoptotic cells are chemically modified to have a stronger attraction to the cell surface receptors on phagocytes to facilitate phagocytosis, and by direct lysis of microorganisms, can activate complement. Additionally, complement fragments, such as CD59, have important immunomodulatory and inflammatory effects including cell activation, chemotaxis, bronchoconstriction, increase of endothelial permeability, and production of pro-inflammatory mediators. Complement proteins are heavily localized in the blood and only a small percentage are activated simultaneously. Accordingly, dysfunctional activation leads to host cell damage. Complement activation is strictly regulated by surface-bound regulators that can accelerate the decay of the convertase CD55, act as a cofactor for the degradation of C3b and C4, or prevent the formation of membrane attack complex (MAC) structures, which permeabilize the membrane through CD59. CD59 is commonly known as a MAC-inhibitory protein, or protectin. The protein's expression by cells is upregulated in response to stimulants, this includes cytokines which are secreted in the presence of inflammation (Morgan). In addition, C3, a complement component of the complement system, has been shown to not only clear pathogens, but also be used for tissue regeneration and synapse pruning to clear debris and control tumor cell progression (Ricklin). The activation of the complement system through the upregulation of CD59 and the proteolytic cleavage of C3 into its alpha and beta fragments leads to a cascade of functions to promote inflammation.

## METHODS

### Human Bronchial Epithelium Cell Culture

To study cells in serum-free culture conditions, we used primary human bronchial epithelium cells isolated from human patients in the sequential windowed acquisition of all theoretical mass spectra (SWATH-MS) experiments, and the human immortalized BEAS-2B bronchial epithelial cell line for C3 activation studies. Cells were purchased commercially from American Type Culture Collection. They were grown on 10 mL culture flasks pre-coated with a coating medium containing 30 µg/mL purified collagen (Vitrogen-100; Collagen Corporation, Palo Alto, California, USA) and incubated with coating medium (5 mL per flask) for 30 minutes in a humidified CO2 incubator at 37°C. The coating medium was then aspirated. Cells were grown to 80% confluency for each exposure study (experiment repeated 4 separate times) in BEGM complete medium (primary cells) or LHC-9 (BEAS-2B). Treatments for cells included a control (medium alone) and a 6 hour treatment of 5% hog barn dust extract (HDE) alone was used to induce airway inflammation. Previous research in our lab indicated that this 5% of HDE dose response is enough to create an inflammatory response without killing the cells.

### SWATH-MS Collection

Cells treated as outlined above were rinsed with 1X PBS and collected for SWATH-MS by scraping in a triton-X lysis buffer that contains protease inhibitors. Protein in lysates (cells from two wells pooled = 600 uL) were quantified by NanoDrop and delivered to the proteomics core laboratory at the University of Nebraska Medical Center.

### SWATH-MS Statistics

Sequential Windowed acquisition of all theoretical mass spectra (SWATH-MS) is an optimal proteomic strategy to identify vital proteins involved in HBEC signaling pathway and progression in an unbiased approach. The experimental scheme of this study is shown in **Figure 2**. HBEC dust-treated and HBEC untreated samples were compared by SWATH-MS to identify differentially expressed proteins that are upregulated and downregulated in the presence of dust exposure. To avoid individual differences and detect true HBEC-related proteins, samples were analyzed by choosing proteins from both groups to determine a quantitative expression ratio between HBEC dust-treated and HBEC untreated tissue groups based on total ion intensity normalization. The targeted identification of peptides in SWATH-MS datasets requires the calculation of z-scores to assert up- and down-regulated proteins. The raw intensity of each protein was transformed by taking the natural log of the intensity followed by a calculation of z-score (Equation 1) in which x is the experimental value, µ is the mean of all experimental values and σ is the standard deviation of all experimental values. Next, the Δz was calculated for each protein in a pairwise manner for each sample (z_HDE-z_Control) and the average Δz was calculated across all samples. The z-test was then conducted for each protein using the paired sample z-test (Equation 2), where Δz_(avg )is the average Δz across all samples, D is the hypothesized mean (null hypothesis) which states that there is no significance between dust and the proteins expressed. Additionally, σ_d is the standard deviation of the pairwise differences per protein, and √n is the square root of the sample size (number of biological samples).

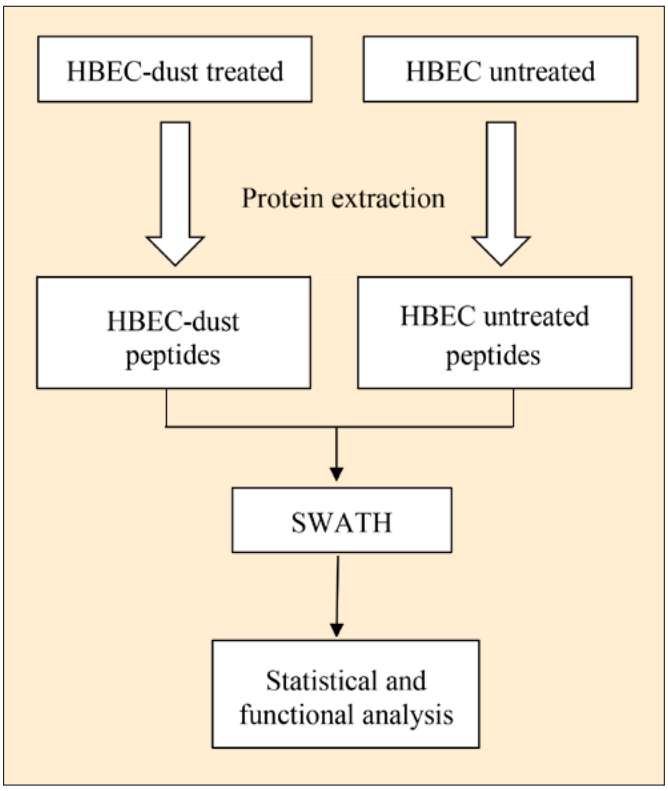


**Figure 2.** Quantitative proteomic workflow of HBEC both dust treated and untreated analyzed using a SWATH-MS approach.

Z-score:

$$Z=(x-\mu)/\sigma$$

Equation 1

Paired sample z- test:

$$z=(\Delta z_{avg}-D)/(\sigma_d/\sqrt{n})$$

Equation 2

### Western Blot to detect C3 proteins

Western Blot technique was used to identify C3 proteins extracted

from BEAS-2B. Supernates and lysates were collected from cells both untreated and treated with HDE. Cells were washed in tissue culture flasks with phosphate buffered saline (PBS), incubated in RIPA lysis buffer for 10 minutes, then scraped and placed into microcentrifuge tubes. Tubes were centrifuged at 10,000 RPM for 7 minutes, and the supernatant fraction was collected. The protein concentration was then determined via NanoDrop. For western blotting, 50 µg of protein was loaded per well into the sample buffer, and ran on a 4-20% SDS PAGE gel at 120 V for one hour. Proteins were transferred to PVDF membranes via semi-dry transfer for 1 hour. After the transfer process, immunoblotting was performed using 5% non-fat dry milk in PBST for 1 hour. Then the primary antibody was added in 5% non-fat dry milk in PBST and incubated overnight on a shaker at 4°C. The membrane was then washed with PBST for 5 minutes and repeated three times. The secondary antibody was then added in 5% non-fat milk in PBST, and incubated for 1 hour at room temperature. Next, the membrane was washed with PBST for 5 minutes and this step was repeated three times. Then, the enhanced chemiluminescence mix was prepared utilizing the proportion of solution A and B provided by the manufacturer, and the membrane was incubated for 1–2 minutes. Finally, the results were visualized by film development in a dark room.

## RESULTS

### SWATH-MS Analysis

There is an estimated equal distribution of up- and down regulated proteins identified by z-scores as seen in **Figure 3**. Positive numbers indicate up-regulated proteins while negative values indicate down-regulated proteins. The p-values for the computed z-test statistic were assigned using the standard normal distribution. 346 proteins were identified to be significantly different using a p-value cutoff of <0.05. A $p<0.05$ represents a rejection of the null hypothesis and further proves the significance between dust and protein expression. Among the 346 proteins, 214 show a $p<0.01$ and the first two proteins (**Table 1**) illustrate the highest levels of up- and down-regulation. The statistical analysis was conducted as outlined in this paper: "Quantitative Proteomics by SWATH-MS reveals Altered Expression of Nucleic Acid Binding and Regulatory Proteins in HIV-1-Infected

### Western Blot Analysis using C3 Antibody

After analyzing western blot on BEAS-2B using C3 antibodies to detect C3 proteins, we saw that when cells were exposed to dust, the complement system was activated whereas in the untreated sample, we saw the full length of C3 (**Figure 4**). Our studies show that when the complement system is activated, enzymatic cleavage of C3 occurs, the alpha and beta chains appear, and the C3 fragment is no longer in its full length. This activation leads to an additional cascade of complement activation. When there is no dust exposure, there is no activation of proteolytic cleavage.

## CONCLUSIONS AND FUTURE DIRECTIONS

### SWATH-MS Analysis

The SWATH-MS proteomics data strongly elucidates our hypothesis that dust exposure activates several cell signaling pathways, specifically the complement proteins such as C3 and CD59, in an effort to reduce invading toxins in HBEC. Through SWATH-MS, we were able to identify proteins expressed during dust exposure and analyze which proteins are up- and down-regulated. For reference, we examine ICAL_HUMAN and DNM1L_HUMAN to show a correlation between dust exposure and their effects on activating specific proteins in HBEC as a response to inflammation. ICAL_HUMAN shows the highest up-regulation and encodes for the protein calpastatin. The protein is a specific endogenous inhibitor of calpain activity through four equivalent inhibitory domains. Calpains are calcium-activated neutral cysteine proteases and play an important role in inflammatory processes. Additionally, calpains are critical for inflammatory cell adhesion, chemotaxis, and inflammatory mediator processing (Zafrani). Previous research has demonstrated that calpastatin expression increases when macrophages were stimulated with TNF-α, a pro-inflammatory cytokine (Hoffmann). Our data shows that in the presence of dust, levels of calpastatin increases in response to high levels of calpains. In comparison to the upregulation of calpastatin, DNM1L_HUMAN (Dynamin-1-like protein) shows the highest down-regulation expression in the presence of dust. Commonly referred to as dynamin-related protein 1 (DRP1), recent studies on injured kidneys have shown deletion of DRP1 promotes epithelial recovery, preserves mitochondrial structure, and reduces oxidative stress (Perry). Mitochondrial dysfunction contributes to programmed cell death called apoptosis, and the study showed that decreased levels of DRP1 attenuates mitochondrial dysfunction. The preservation of mitochondrial structure is important because nearly every cell in the lungs depends on mitochondrial metabolic activities and requires constant supply of energy from oxidative phosphorylation (Cloonan). Hence, a decrease expression of CRP1 in HBEC-dust treated explains cell repair. In addition to these two proteins, the complement system is crucial for protecting the body

**Table 1.** Proteins Identified Using Swath-MS Proteomics

| Accession | Uniprot ID | Δz |
|---|---|---|
| P20810 | ICAL_HUMAN | 14.2038493 |
| O00429 | DNM1L_HUMAN | -16.54900216 |
| P13987 | CD59_HUMAN | 5.815474894 |

against pathogenic agents. We also identified an upregulation of CD59_HUMAN, encoding for CD59 glycoprotein. CD59 is a complement regulatory protein that inhibits MAC formation. The upregulation of this protein in the presence of dust activates the complement system by preventing complement mediated cell lysis (Budding). Previous studies have shown that cytokines secreted in inflammatory lesions can induce CD59 expression, and our data shows a high up-regulated expression of CD59 in the presence of dust exposure. The activation of the complement cascade in the presence of toxins illustrates the system's ability to distinguish between pathological and physiological challenges. In addition to triggering the system, the cascade includes several regulatory mechanisms which use membrane-bound regulators such as CD59 to prevent over-activation of the complement system (Geller). The upregulation of CD59 shows the activation of the complement cascade in dust exposed HBEC and its function to regulate the system. Our results further represent how these pathways activate a defense mechanism to remove toxins from the lungs through specific expression of proteins.

## Western Analysis

Identifying complement activation through proteolytic cleavage of C3 into its alpha and beta chains is important because the production of C3b can facilitate the formation of C5 convertases, and along with the binding of C3b, fuel an amplification that leads to rapid opsonization of the target surface. Therefore, an increase in the formation of C3b initiates the lytic MAC and potent anaphylatoxin C5a, in which C3a binds to the anaphylatoxin receptor C3aR. This function mediates immune adhesion, phagocytosis, and adaptive immune stimulation (Ricklin). This experiment represents our hypothesis that HDE treatment in BEC mediates the release and activation of C3, and thus, proves our hypothesis that the complement system is activated in response to inflammation from dust.

## Importance of Data and Future Directions

We were able to identify CD59 by SWATH-MS to show how it is upregulated in the presence of dust. In addition, through western blot analysis we further concluded that proteolytic cleavage of C3 in its alpha and beta chains occur in the presence of dust to activate the complement system. This reinforces the concept that in the presence of dust, the body responds through the activation of the complement system to enhance the ability of antibodies and phagocytic cells to clear toxins, promote inflammation, and finally aid in attacking the pathogen's cell membrane. Our data can be used in other lung research studies to identify up- and down-regulated proteins to understand the effects of dust exposure in HBEC and lead to potential treatments to prevent lung disease through anti-inflammatory drugs. In our future experiments, we plan to use immunofluorescence staining for C3 and CD59 on mouse bronchial epithelial cells to locate the expression of these proteins in the presence of dust exposure. This will aid in detecting where the antibodies are located on the tissue and generate a better understanding of the mechanisms underlying tissue inflammation. In addition, we will further study dietary supplements such as omega-3-fatty acids, which are known to have anti-inflammatory properties, in preventing pulmonary diseases.

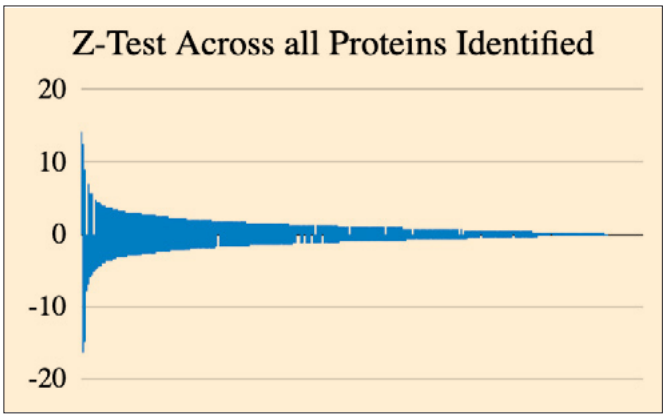


**Figure 3.** Y-axis illustrates an estimate of up-regulated (positive values) and down-regulated (negative values) proteins analyzed by calculating z-test from SWATH-MS.

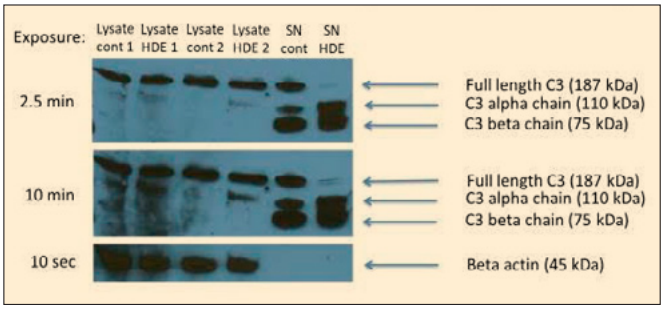


**Figure 4.** Western blot image using lysates and supernatant (SN) identifying C3 proteins in HBEC using 24-hour exposure of 5% HDE.


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# The Benefits of Trait Mindfulness and Flow During a Period of Stressful Preparation

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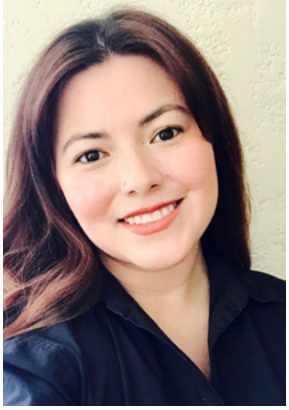
## ABSTRACT

Preparing for an important performance such as a test or job interview can be quite stressful. Considerable evidence reveals that mindfulness meditation (a focus on the present moment) and flow (engaging in activities that fully capture one's attention) are effective strategies for bolstering well-being in stressful situations, including the wait for uncertain news about a performance outcome. However, less research has examined whether mindfulness and flow buffer well-being while preparing for the performance. Ninety-four law graduates preparing to take the 2019 California bar exam completed a survey assessing trait mindfulness, trait flow, well-being, and coping strategies two weeks prior to the exam. Results revealed that trait mindfulness (controlling for flow) consistently predicted well-being as participants studied for the exam, whereas trait flow (controlling for mindfulness) consistently predicted reduced use of several coping strategies (e.g., bracing, proactive coping). These results suggest that cultivating mindfulness may be an effective way to reduce unpleasant emotions while preparing for a performance, whereas flow may facilitate the use of coping strategies that could indirectly affect well-being.

**KEYWORDS:** *Stress; Exam Preparation; Anticipation; Bar Exam; Well-Being; Coping*



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# The Benefits of Trait Mindfulness and Flow During a Period of Stressful Preparation

## INTRODUCTION

Applying for a new job, working on a difficult homework assignment, undergoing a medical procedure, such a biopsy, and taking a difficult exam are all examples of situations in which people have to wait for uncertain feedback or results. Anyone who has ever been in a similar situation can attest to the stress they felt while waiting. Considerable evidence suggests that waiting for news can provoke anxiety, and in turn, this anxiety can result in poorer health and well-being (Howell & Sweeny, 2016; Morin et al., 2003). During waiting periods, people can engage in several strategies in an attempt to minimize distress. Evidence suggests that mindfulness meditation (guided focus on the present with nonjudgmental thoughts) and engaging in flow-inducing activities (activities that capture attention) can be effective in reducing distress in these moments (Rankin, Walsh, & Sweeny, 2019; Sweeny & Howell, 2017). Still, less is known about the period leading up to a stressful life event. The purpose of this study is to examine whether trait mindfulness and flow can buffer well-being while preparing for a stressful performance.

## STRESSFUL UNCERTAINTY

In a situation where a person must wait for news, levels of certainty and control are low. This combination can result in physical and psychological distress (Howell & Sweeny, 2016), and people may not have the appropriate tools to navigate this process successfully. In fact, even when people use various strategies, research suggests they are usually unsuccessful at coping effectively with stressful periods of uncertainty (Sweeny et al., 2016). Nonetheless, two specific strategies can relieve distress during uncertain periods. Mindfulness meditation (a guided focus on the present with nonjudgmental thoughts) and engaging in flow-inducing activities (activities that capture attention) are effective ways of reducing distress and promoting well-being when people face acute periods of uncertainty (Joye & Bolderdijk, 2015; Rankin et al., 2019; Sweeny & Howell, 2017).

The findings just described address the waiting period that comes after a performance (e.g., a job interview or exam) but before receiving feedback. Far less research has focused on the time leading up to an important but stressful event, hereafter referred to as the *preparation period*. Although both periods—preparation and waiting—have similar characteristics, one of the main ways in which they differ is in the individual's sense of control over the outcome (Sweeny & Krizan, 2013). Further, a recent study comparing the role of personality while preparing for an exam and during the wait for exam results found that participants experienced more negative emotion during the preparation period than during the waiting period (Sweeny, Howell, & Kwan, 2020). This finding is in line with past research suggesting that stress levels are higher during the month leading up to a stressful life event than during the month following the event (in this case, a dissertation defense; Laethem et al., 2017). If stress levels are higher during the preparation period, it stands to reason that well-being in response to said stress would be poorer.

## THE CALIFORNIA BAR EXAM

In July 2019, nearly 8000 people took the California bar exam and only half of them passed (State Bar of California, 2019). Although many people take the bar exam every year, it is still a relatively uncommon stressor not experienced by most of the population. However, because this exam is very challenging—judging from the passing percentage—the stress experienced while preparing for it shares features of job-related stress people can experience in any highly demanding job (Demerouti et al., 2001; Shrout, Herman, & Bolger, 2006).

We know from past research that acute stress, which is the kind of stress someone preparing for the bar exam likely experiences, prompts feelings of exhaustion (de Rijk, Schreurs, & Bensing, 1999; Scott, Brandberg & Oehman, 2001), among other well-being deficits. What could make this situation better? The only research we know of to address this question identified social support as a means of ameliorating the negative effects of stress (see Cohen & Wills, 1985; Thoits, 1982), and specifically while preparing for the bar exam (although providing effective support is challenging; Shrout, Herman, & Bolger, 2006). Beyond those findings, we know little about what might increase well-being during this stressful time. Drawing from literature on the positive effects of mindfulness and flow during waiting periods (Joye & Bolderdijk, 2015; Rankin et al., 2019; Sweeny & Howell, 2017), the present study examined the role of trait mindfulness and flow on well-being during the preparation period for a bar exam. We broadly hypothesized that people who are dispositionally more mindful and more likely to find themselves in a state of flow would report better well-being during the weeks leading up to the California bar exam in July 2019. We further hypothesized that flow and mindfulness would reduce the use of most coping strategies, given previous studies showing that more distressed people tend to use coping strategies more (e.g., Sweeny et al., 2016).

## METHOD

### Participants

Recent law school graduates ($N = 94$; 66% female; 53% White, 26% Asian, 13% Latina/o/x, 1% Black, 7% other/multiple; $M_{age} = 28.78$, $SD_{age} = 5.56$) preparing to take the July 2019 California Bar Exam participated in this study. Due to the specific focus of our study, we

recruited participants by emailing the deans of law schools across the U.S. and student bar associations at various institutions. They forwarded our email to their students, and we enrolled those who were interested and preparing to take the California bar exam that summer.

### Procedure

Within two weeks before the exam, participants completed a survey assessing trait mindfulness, trait flow, well-being, and use of various coping strategies. Each participant received an email with a link to the survey. They also completed surveys after the exam while they awaited their result, but those surveys are beyond the scope of this study. Participants received Amazon gift cards as compensation.

## MEASURES

### Mindfulness

Trait mindfulness was assessed with the 15-item Mindfulness Attention Awareness Scale (Brown & Ryan, 2003; Carlson & Brown, 2005; 1 = *almost never*, 5 = *almost always*). Sample items include, "I find myself preoccupied with the future or the past," and "I find myself doing things without paying attention" ($M$ = 3.70, $SD$ = .63, Cronbach's $\alpha$ = .86).

### Flow

Trait flow was assessed with a 34-item revised version of the Dispositional Flow Scale (Jackson & Eklund, 2002; Jackson & Marsh, 1996; 1 = never, 5 = *always*). Sample items include, "I feel just the right amount of challenge, and "I have total concentration" ($M$ = 3.02, $SD$ = .52, $\alpha$ = .90).

### Well-Being

We assessed psychological well-being in a number of ways. We measured perceived stress, which is a person's thoughts about how much stress they're experiencing at any given time, via the 4-item Perceived Stress Scale (PSS-4; Cohen, Kamarck, & Memelstein, 1983; e.g., "In the past few days, how often have you felt that you were unable to control the important things in your life?" "In the past few days, how often have you felt difficulties were piling up so high that you could not overcome them?" 1 = *never*, 5 = *very often*; $M$ = 2.93, $SD$ = .78, $\alpha$ = .70). We assessed worry about the bar exam with three items commonly used during stressful periods of uncertainty (e.g., Rankin & Sweeny, 2019; Rankin et al., 2019; Sweeny & Howell, 2017; "I feel anxious every time I think about my bar exam result," "I am worried about my bar exam result," "I can't seem to stop thinking about my bar exam result"; 1 = *strongly disagree*, 7 = *strongly agree*; $M$ = 5.40, $SD$ = 1.35, $\alpha$ = .82). We assessed positive and negative emotions with an adapted version of the GRID measure (Fontaine, Scherer, Roesch, & Ellsworth, 2007; 1 = *strongly disagree*, 7 = *strongly agree*), which includes 12 negative emotions ($M$ = 4.57, $SD$ = 1.16, $\alpha$ = .87) and 9 positive emotions ($M$ = 4.83, $SD$ = 1.03, $\alpha$ = .83). Finally, we assessed repetitive thought about the bar exam with four items designed to capture various manifestations of targeted ruminative thought (e.g., "How often in the past week have you brought up the bar exam in conversation with family members?" 1 = *not at all*, 7 = *almost constantly*; $M$ = 5.40, $SD$ = 1.30, $\alpha$ = .68).

We also assessed two forms of physical well-being with brief measures of health ("During the past week, would you say your health has been…" 1 = *poor*, 7 = *excellent*; $M$ = 4.27, $SD$ = 1.64) and sleep quality ("During the past week, how would you rate the quality of your sleep?" 1 = *extremely bad*, 7 = *extremely good*; $M$ = 4.06, $SD$ = 1.60).

### Coping

We assessed a set of coping strategies outlined in the uncertainty navigation model (Sweeny & Cavanaugh, 2012), which is a theoretical framework for understanding how people manage the stress of uncertain situations (for all, 1 = *strongly disagree,* 7 = *strongly agree*): preventive action ("I'm putting effort toward trying to minimize problems that would arise if I fail the bar exam"; $M$ = 4.25, $SD$ = 1.85), proactive coping ("I'm thinking about how I'll cope if I fail the bar exam"; $M$ = 3.98, $SD$ = 1.93), distraction (4 items, e.g., "I've been spending time with others to distract myself from thinking about the bar exam"; $M$ = 2.83, $SD$ = 1.44, $\alpha$ = .82), emotion suppression (4 items, e.g., "I've been trying to suppress my feelings about the bar exam"; $M$ = 3.76, $SD$ = 1.43, $\alpha$ = .77), bracing for the worst ("I'm bracing for the worst when it comes to the bar exam," "I'm keeping my expectations low when it comes to the bar exam"; $M$ = 4.49, $SD$ = 1.84, $\alpha$ = .85), positive expectation management ("I'm trying to be optimistic when it comes to the bar exam," "I'm hoping for the best when it comes to the bar exam"; $M$ = 5.67, $SD$ = 1.30, $\alpha$ = .56), preemptive benefit finding (3 items, e.g., "I feel like I would grow as a person if I fail the bar exam"; $M$ = 2.98, $SD$ = 1.46, $\alpha$ = .79), and distancing (4 items, e.g., "The bar exam doesn't really measure anything important"; $M$ = 4.94, $SD$ = 1.44, $\alpha$ = .75).

## RESULTS

We tested our hypotheses with a series of multiple regression analyses predicting well-being and coping from trait flow and mindfulness (both predictors in the same model). **Table 1** presents the results of these analyses. Although results were somewhat inconsistent across measures, our hypotheses were generally supported. As the table reveals, trait mindfulness (controlling for flow) consistently predicted better well-being as participants studied for the exam, whereas trait flow (controlling for mindfulness) more

consistently predicted the (reduced) use of coping strategies. Specifically, more mindful participants also reported less perceived stress, worry, negative emotion, repetitive thought, and bracing, and more positive emotion and better subjective health. Participants who were higher in trait flow reported less perceived stress, negative emotion, proactive coping, and bracing, and better subjective health, better sleep quality, and more positive expectation management.

## DISCUSSION

After finding that the literature on preparation periods was scarce, we asked a simple but novel question: Can the same strategies that promote well-being during waiting periods (mindfulness and flow) help during preparation periods? To answer this question, we used a sample of recent law school graduates preparing to take the California bar exam. We found that trait mindfulness predicted less perceived stress, worry, negative emotion, and repetitive thought,

**Table 1.** Results of Multiple Regressions Predicting Well-Being and Coping from Mindfulness and Flow

| | MINDFULNESS $\beta$ [$CI_{95\%}$] | FLOW $\beta$ [$CI_{95\%}$] |
|---|---|---|
| **PSYCHOLOGICAL WELL-BEING** | | |
| Perceived stress | -.44** [-.60, -.28] | -.45** [-.62, -.28] |
| Worry | -.38** [-.58, -.18] | -.10 [-.30, .11] |
| Negative emotion | -.31** [-.49, -.13] | -.44** [-.62, -.25] |
| Positive emotion | .24* [.03, .45] | .05 [-.17, .27] |
| Repetitive thought | -.38** [-.58, -.18] | -.07 [-.28, .14] |
| **PHYSICAL WELL-BEING** | | |
| Subjective health | .28** [.08, .48] | .27* [.06, .48] |
| Sleep quality | .16 [-.05, .36] | .29** [.08, .50] |
| **COPING STRATEGIES** | | |
| Preventive action | .07 [-.15, .29] | -.01 [-.24, .22] |
| Proactive coping | -.14 [-.35, .05] | -.33** [-.54, -.12] |
| Distraction | .16 [-.06, .37] | -.21$^{+}$ [-.43, .01] |
| Suppression | .06 [-.16, .28] | -.19$^{+}$ [-.42, .03] |
| Bracing | -.24* [-.45, -.04] | -.24* [-.45, -.03] |
| Positive expectation management | .18$^{+}$ [-.03, .38] | .26* [.05, .47] |
| Preemptive benefit finding | .19$^{+}$ [-.02, .40] | .04 [-.18, .26] |
| Distancing | -.14 [-.35, .07] | -.06 [-.28, .16] |

Note: $^{**}p < .01$, $^{*}p < .05$, $^{+}p < .10$. Standardized betas with 95% confidence intervals in brackets.

all of which are detrimental to psychological well-being. Trait mindfulness also predicted higher levels of factors that promote both psychological and physical well-being, namely positive emotion and subjective health. Similarly, we found that a person's natural tendency to experience flow was beneficial, albeit in a slightly different way than mindfulness. Similar to trait mindfulness, trait flow predicted less perceived stress, less negative emotion, better sleep quality, and better subjective health. However, flow was more strongly and consistently associated with the reduced use of coping strategies—less proactive coping and bracing, and somewhat less distraction and suppression.

The bar exam allows researchers to study a large group of people preparing for a stressful performance at the same time. Having said that, it is only one example of a stressful situation that is accompanied by a preparation period. The results of this study are important because they can apply to people who are preparing for other exams, such as the Graduate Record Examination or Medical College Admissions Test, and to those preparing for a master's thesis or dissertation defense. Outside of academics, these findings might also apply to people preparing for job interviews, important speeches and presentations, and other important performances such as a play, dance, acting auditions, and so forth. Overall, the results of this study suggest that cultivating mindfulness may be effective for reducing unpleasant emotions while preparing for a performance. Trait flow, on the other hand, may guide people away from often-ineffective coping strategies (see Sweeny et al., 2016) and thus indirectly affect well-being.

## LIMITATIONS AND FUTURE DIRECTIONS

A clear limitation of this study is the sample size. Although participants were recruited from law schools all over the U.S., we were only able to recruit just under 100 participants due to various logistical constraints. Future research can attempt to replicate these findings in a larger sample. Another limitation is the timeframe we used. It is unclear whether two weeks prior to the exam is the optimal time to assess mindfulness and flow and their associations with well-being. Past studies looking at stress while preparing for a performance assessed it either during the two weeks before the event (Sweeny, Howell, & Kwan, 2020) or during the month prior (Laethem, et al., 2017). Perhaps measuring the effects of trait mindfulness and flow for a longer period of time would more accurately reflect participants' general experiences during the preparation period.

Lastly, it is important to note that our findings addressed a person's natural predisposition to be mindful and experience flow states. Future studies would benefit from implementing interventions that induce both mindfulness and flow while students prepare to take the bar exam or in preparation for other important performances. If cultivating mindfulness and flow are effective ways to manage distress in these challenging moments, interventions to induce these two states could lead to reduced stress, and better well-being during preparation periods.

## ACKNOWLEDGMENTS

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# Role of Perceived Support from Parents and On- and Off-Campus Friends in First- and Non-First-Generation College Students' Life Satisfaction

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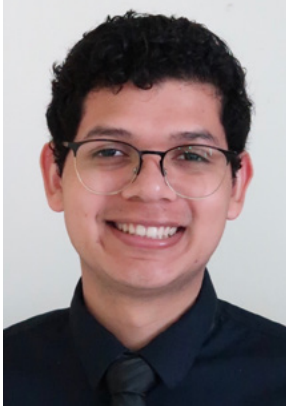
## ABSTRACT

First-year college students, especially first-generation attendees (FGC; neither parent finished college), often have difficulties adjusting to school. The present study examines the social and instrumental support these students receive during their first year of college and its role in their life satisfaction, a dimension of psychological well-being (Jenkins et al., 2013). In this study, 244 first-year college students (107 FGC) completed an online survey that asked about their perceived support and instrumental help from parents and on- and off-campus friends, as well as the students' overall life satisfaction. Results showed that, regardless of college-generation status, students reported feeling more social support than instrumental help from family and off-campus friends. For both FGC students and non-FGC students, there was a positive relationship between perceived social support and help from family and friends and student's satisfaction with life. The findings suggest that university professionals should try to involve families and other supportive persons, including on- and off-campus friends, in students' first-year college experience to help students adjust to this new setting.

**KEYWORDS:** *First Year Experience; Support, Friends and Family; Life Satisfaction*



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Dagoberto Partida is a third year Psychology BS major. He is interested in how communities are adjusting to university demands, specifically in underrepresented students. He has been a research assistant under Dr. Mary Gauvain's Cognitive Development Lab for two years. He is currently assisting on a dissertation project examining cultural differences in parental beliefs between European- and Korean-American families and its role in children's planning skills. Dagoberto plans to apply for PhD programs in developmental psychology this fall.

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# Role of Perceived Support from Parents and On- and Off-Campus Friends in First- and Non-First-Generation College Students' Life Satisfaction

## INTRODUCTION

Life satisfaction, defined as subjective wellbeing about one's life, has been found to predict college student retention (Diener et al., 1985; Krumrei-Mancuso, Newton, Kim, & Wilcox, 2013). The importance of life satisfaction may be especially important for students with lower college retention rates, including first-generation and Latinx students (Becker, Schelbe, Romano, & Spinelli, 2017; Ojeda, Castillo, Rosales, & Pina-Watson, 2014). First-generation college students (FGC) are individuals whose parents or guardians have not attained a bachelor's degree (Davis, 2010). Compared to non-FGC students, FGC students have difficulty adjusting to college as measured by academic performance, sense of belonging, and loneliness (Gibbons, Rhinehart, & Hardin, 2019; Stephens, Hamedani, & Destin, 2014). In addition, FGC students often have fewer financial resources and can experience low levels of social or emotional support about college. Although research has identified the importance of life satisfaction for FGC college student outcomes, the impact of family and friends on students' life satisfaction has received little study (Garriott, Hudyma, Keene, & Santiago, 2015; Navarro, Ojeda, Schwartz, Piña-Watson, & Luna, 2014). In the present study, we investigate FGC and non-FGC students' perceived support and help from family and friends and the relation of these factors to students' sense of life satisfaction in the first year of college.

Some research has found that attending and succeeding in college may not always result in increased life satisfaction for FGC students (Davis, 2010; Garriott et al., 2015). However, other research has shown that individuals who feel supported during college can increase both academic performance and psychological wellbeing (Becker et al., 2017). The present study examines the link between perceived social support and life satisfaction in a college setting. One reason FGC students may not show high levels of life satisfaction in college is that they may feel disconnected from family and friends, perhaps as a result of their educational aspirations. Some students may even feel a sense of guilt in pursuing their educational goals while their family is struggling at home, a feeling that may be especially pronounced for low-income students (Covarrubias & Fryberg, 2015). This so-called family achievement guilt may affect how FGC students perceive and experience college and, in turn, influence students' emotional wellbeing and academic adjustment (Becker et al., 2017).

Many students find the transition to higher education more stressful than anticipated and face many stressors in academic, social, and personal areas (Jenkins, Belanger, Connally, Boals, & Duron, 2013). Support from others may help students adjust to this new setting (Bronkema & Bowman, 2019; Swenson, Nordstrom, & Hiester, 2008). Support, defined broadly, includes reassurance, availability, and loyalty of individuals who are closely connected to the student such as family members and friends (Goldsmith, McDermott, & Alexander, 2000). Friedlander and colleagues (2007) found that college students receive support from multiple sources, including family members and friends both on- and off-campus. Social support can help beginning college students combat feelings of insignificance as well as bolster student autonomy during the transition to a university (Shukla & Joshi, 2017). A student's perception of support from others can also have a buffering effect when confronting distressing situations (Friedlander et al., 2007; Reid, Bowman, Espelage, & Green, 2016).

As this research suggests, social support plays an important role in students' college experiences and may also contribute to life satisfaction or a subjective sense of well-being in the first year of college (Bronkema & Bowman, 2019; Krumrei-Mancuso et al., 2013). Social support is often coupled with helpfulness, which is practical advice that pertains to a specific problem or activity (Goldsmith et al., 2000). However, whether the help someone offers is useful depends on how it is perceived by the individual in need. In this context, perceived helpfulness entails how students view a resource, including another person, as helping them overcome stressors as they begin college. When they occur together, support and helpfulness may boost motivation and enable a student to overcome difficulties in the first year of college. This process, in turn, can help increase a student's life satisfaction during this transition period (Krumeri-Mancuso et al., 2013; Lästch, 2017).

The purpose of the present study is twofold. First, it examines the social support and help that FGC and non-FGC students receive from family and friends in the first year of college. FGC students have different struggles about college compared to non-FGC students, which suggests that the support these students need from family and friends may also differ (Covarrubias & Fryberg, 2015; Garriott et al., 2015). For instance, FGC students who experience familial achievement guilt may be less likely to ask their parents for help, resulting in lower perceived support from parents (Becker et al., 2017). A recent study suggests a more complex picture of types of support FGC students receive, in which they tend to perceive less instrumental help but were just as likely as their non-FGC peers to feel emotionally supported by their parents (Palbusa & Gauvain, 2017). In probing support from friends, we differentiate friends who are on- and off-campus because both may contribute, albeit in different ways, to student adjustment in the first year of college. The second purpose of this study is to investigate how social support and help from family and friends relate to self-reported life satisfaction of FGC and non-FGC students in the first year of college.

## Research Questions

**RQ1:** Do FGC and non-FGC students perceive different levels of support and help from their parents and on- and off-campus friends?
*Hypothesis 1:* Based on previous research suggesting that students receive support from multiple sources and that FGC students have less available resources than non-FGC students do (Dennis, Phinney, & Chuateco, 2005; Friendlander et al., 2007; Nichols & Islas, 2015), we predicted that FGC and non-FGC students will differ in their perceived support of parents and friends.

**RQ2:** Who provides support and help for first-year college students and is there a difference between what is provided by people on- and off-campus?
*Hypothesis 2:* Given previous literature suggesting that first-year college students get much of their support from people with whom they are in established relationships, we expect that both FGC and non-FGC students will report greater perceived support and help from their off-campus family and friends than on-campus friends (Shukla & Joshi, 2017).

**RQ 3 (exploratory)**: Do students report differences in the usefulness of the support and help?
*Hypothesis 3:* With recent studies differentiating the types and utility of support and help students receive (Nichols & Islas, 2015; Palbusa & Gauvain, 2019), we expect FGC and non-FGC students will differ in their perceptions of the usefulness of the support and help they receive in addressing their college concerns.

**RQ 4:** Do perceived support and help from family members and friends relate to first-year college students' reported life satisfaction?
*Hypothesis 4:* In line with research suggesting the positive role of social support in increasing well-being, we hypothesized that perceived support and helpfulness will be positively related to subjective life satisfaction of first-year college students (Becker et al., 2017).

# METHOD

## Participants

An ethnically diverse sample of 244 first-year undergraduate students participated in this study. The ethnic representation was as follows: 53% Asian, 33% Latinx, 5% European American, 5% Mixed, and 3% African American, and 1% unreported. The sample ranged in age from 18 to 21 years (*Mage* = 18.29, $SD_{age}$ = 0.52), and was evenly split by gender (52% female). Almost half the sample (44%) were first-generation college students. They were recruited from the Psychology Department participant pool at a large, public university and received research credit and raffle tickets for movie passes.

## Procedure and Measures

Participants completed an online survey in one sitting using SurveyGizmo. Survey completion time averaged 40 minutes. In the survey, the participants provided demographic information. They also completed questionnaires about *Perceived Support and Helpfulness* and *Satisfaction with Life*, described more later. Finally, they responded to several open-ended questions about their college experience. College-generation status was obtained through university admissions records that included the highest level of education for each parent.

**Perceived Support and Helpfulness.** This measure is a subscale of the *Communication with On-Campus Friends* and the *Communication with Off-Campus Contacts* measures (Cheong, Gauvain, & Palbusa, 2019). This 18-item scale asked participants to rate the extent of support ($\alpha$ = .88; 1 = *not supportive at all*, 4 = *extremely supportive*) and extent of help ($\alpha$ = .89; 1 = *not helpful at all*, 4 = *extremely helpful*) received from friends (on- and off-campus) and parents.

**Satisfaction with Life.** This 5-item scale assessed participants' satisfaction with life and a subjective sense of well-being (Diener et al., 1985). A sample item is "the conditions of my life are excellent." Participants rated items on a 7-point scale ($\alpha$ = .88; 1 = *strongly disagree*, 7 = *strongly agree*). Ratings were summed and then averaged, with higher scores indicating greater life satisfaction.

**Open-ended questions.** Three open-ended questions asked participants to describe concerns they have had in college, the people they have talked to about these concerns, and how talking to them has helped the student in dealing with the concerns.

# RESULTS

To compare perceived support and helpfulness from family members (parents) and on- and off-campus friends in FGC and non-FGC students, independent samples *t*-tests were conducted. Contrary to the prediction, FGC and non-FGC students did not differ in their perceived support and helpfulness from on-campus friends (support: $t(224) = 0.97$, $p = .33$; helpfulness: $t(223) = -0.31$, $p = .75$), off-campus friends (support: $t(218) = -0.04$, $p = .97$; helpfulness: $t(219) = 0.51$, $p = .61$), and parents (support: $t(220) = -0.49$, $p = .62$; helpful: $t(220) = -0.93$, $p = .35$). Thus, data from both student groups are combined for subsequent analyses.

A paired samples *t*-test was used to determine if there was a differ-

ence between the levels of perceived support and helpfulness across different sources of support. All students reported more support from off-campus friends ($M$ = 2.58, $SD$ = 0.68), $t(204)$ = -5.48, $p$ < .001), and parents ($M$ = 2.50, $SD$ = 0.73), $t(205)$ = -2.81, $p$ < .01) than on-campus friends ($M$ = 2.33, $SD$ = 0.64). They also reported greater helpfulness from off-campus friends ($M$ = 2.31, $SD$ = 0.74) than on-campus friends ($M$ = 2.12, $SD$ = 0.66), $t(204)$ = -3.64, $p$ < .001, and parents ($M$ = 2.13, $SD$ = 0.78), t (200) = 2.47, $p$ = .01 (see **Figure 1**).

The open-ended questions were examined to see if students still felt supported even though a clear solution or remedy for their problem or stressor was not received from the contact.

For example, a student who had trouble with schoolwork asked their friend for support, who reported: "*He told me that I could do it and that I needed time to rest.*" Another student who expressed feeling lost and unmotivated in academics responded that support: "*… made me realize that I was not alone because they were going through the same exact problems.*"

These responses suggest that while no tangible solutions to resolve an issue were provided, students nonetheless reported feeling supported. An example of a student who received support and found it helpful described that when he was near to failing a class and went to a friend for support, the friend provided support, "*I spoke to my friend since we both had the same class. It helped tons because we helped one another confront difficulties and work together to pass.*" This student mentioned that their friend was supportive and offered a way to help alleviate the problem. Responses to the open-ended questions suggest that students can feel supported even though they report getting varying levels of instrumental support.

Measures of perceived support and helpfulness across the three social groups (parents, on- and off-campus friends) were intercorrelated. A Pearson's Bivariate Correlation showed a positive relation between perceived support and life satisfaction, such that perceptions of support from parents ($r$ = .30, $p$ < .001) and on- ($r$ = .34, $p$ < .001) and off-campus ($r$ = .20, $p$ < .001) friends were associated with higher life satisfaction. Perceived helpfulness from parents ($r$ = .30, $p$ < .001), and on- ($r$ = .31, $p$ < .001) and off-campus ($r$ = .21, $p$ < .01) friends was also associated with higher life satisfaction.

## DISCUSSION

This study examined the role of support and help from families and friends in students' psychological well-being as they transition to college. In contrast to previous studies showing that FGC students are likely to perceive that the support they need is not available (Dennis et al., 2005; Nichols & Islas, 2015), our findings showed that FGC students were just as likely as non-FGC students to perceive support and help from their family and friends. The present findings also revealed a complex nature of support for these students. That is, regardless of college-generation status, first-year students reported that parents and friends are supportive but not necessarily helpful in addressing their college concerns. Students reported receiving more support from off-campus contacts, both parents and friends, than from on-campus friends, with off-campus friends seen as more helpful than parents. These findings are consistent with previous studies that preexisting relationships with people off-campus are a source of support for first-year college students, and that established peer relationships may be especially important (Friedlander et al., 2007; Shukla & Joshi, 2017). The open-ended responses suggest that students may feel greater help from friends relative to parents because they are of similar age and likely have many shared experiences. Indeed, in some cases, the off-campus friends were also students and experiencing similar challenges as the participants.

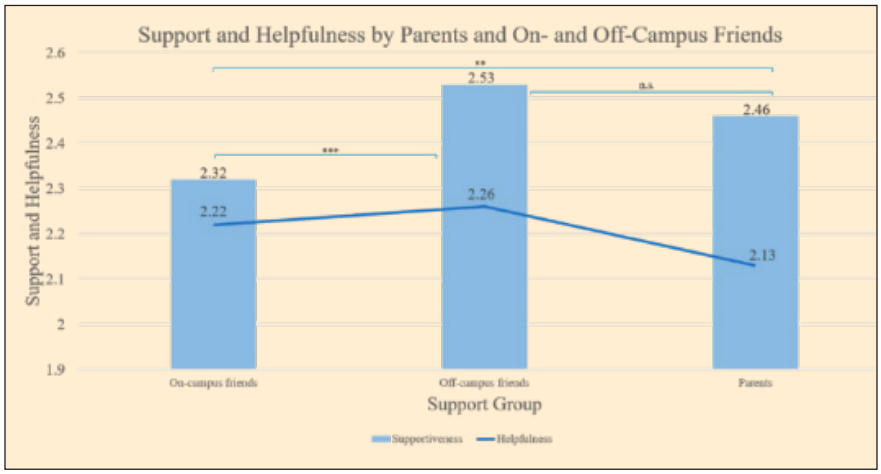


**Figure 1.** Perceived support and helpfulness by support group.

The present findings also showed that both support and the helpfulness of the support were related to greater life satisfaction for first-year students. This is consistent with previous research, suggesting the importance of social support in students' psychological wellbeing (Jenkins et al., 2013). Given the positive role of off-campus contacts (i.e., families and friends) in first-year college students'

psychological well-being, the findings have practical implications for university professionals. Specifically, they suggest involving families and supportive persons from beyond the campus in first-year students' lives during their transition to college. This may be especially important for FGC students who may feel ambivalent or even guilty about attending college because their parents did not have the same opportunity or because they are presently working very hard to enable the student to attend college (Becker et al., 2017).

The study is not without limitations. The data are cross-sectional and, therefore, the direction of the relations is unknown. Moreover, previous studies suggest that other factors, such as staying connected with cultural heritage values, may also contribute to students' life satisfaction (Krumerei-Mancuso et al., 2013; Navarro et al., 2014). Another limitation is the generalizability of our findings due to the institution's demographics. In this institution, the Latinx community is not in the minority; results may differ in institutions with different demographics (Ojeda et al., 2014).

Our findings suggest that students who feel supported by a range of social support personnel have higher life satisfaction. However, whether this pattern remains the same as students advance in their college career is not answered in these data. As to the experience of FGC students, future research might include narrative reports that probe how and why these students consider the support to be helpful. This research may help identify areas in which FGC students need more instrumental support that impact their educational trajectories. Both FGC and non-FGC students benefit from feelings of support from family and friends, and in both groups, these feelings relate to students' life satisfaction. These findings underscore the important role of support systems as students launch their college careers.

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# Blazing Beetles: The Effect of Temperature on the Locomotion of a Namib Dune Beetle

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## ABSTRACT

Deserts represent some of the harshest ecosystems for life to survive in. In order to thrive, species must find novel adaptations either through behavioral or physiological modulation. The Namib desert of Southern Africa is no exception. In the Namib, temperatures can swing from 10 to 30°C throughout the day. Previous studies have indicated that there is temperature dependence in muscle power output in ectotherms. With the Namib being an understudied ecosystem, the present study aimed to investigate if invertebrate muscle output is affected by wild temperature fluctuations. *Onmyacris plana*, a Tenebrionid beetle endemic to the dunes of the Namib, was chosen due to being dorsally flattened, which results in low heat storage capacity. Although the thermoregulatory strategies, running ability, size and metabolic needs of *O. plana* have been studied, there have been no studies on the effect of temperature on the running performance of *O. plana*. We collected 8 beetles from the dunes and kept them in a vivarium in the lab. Beetles were subjected to three temperatures, to represent temperatures commonly experienced throughout the day. They were then placed on a 1-meter trackway in the lab and we made them run while recording them with a high-speed camera. Using the Matlab DLTdv5 digitizing tool we quantified their speed throughout the trials and analyzed differences in speed for the three treatments. We found no significant differences between running ability for beetles running at daytime temperatures and had significant difficulty in getting the beetles to run at temperatures experienced early in the morning. Our findings suggest that the beetles have a threshold muscle temperature which, if met, is sufficient for running at maximal speeds.

KEYWORDS: *Locomotion; Power; Tenebrionidae; Temperature; Behavior*

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Jonathan Philips is a fourth year Biology student at UCR. Over the course of his time here, he has been involved with three research labs and just last year, found his passion for Entomology. Last August, Jonathan studied abroad in Namibia, Africa and was able to study a beetle species native to the Namib desert. In addition to this, he also volunteers in an Entomology lab. Jonathan plans on returning to UCR for graduate school in the field of Entomology.

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# Blazing Beetles: The Effect of Temperature on the Locomotion of a Namib Dune Beetle

## INTRODUCTION

From whales to aphids, temperature has a wide range of effects on the biological systems of animals. For instance, muscle function is greatly affected by temperature. Desert dwelling ectotherms have been analyzed for differences in running performance at different temperatures. In vivo raceway experiments have been done with the southwestern American lizard, *Dipsosaurus dorsalis*. Limb cycling frequency was analyzed at three trial temperatures and stride frequency was found to increase with temperature (Swoap et al. 1993). In ectotherms, it has been found that increased temperatures are associated with increased muscle power output and increased stride frequency.

In the tobacco hawkmoth, *Manduca sexta*, it has been found that maximum muscle power output and the operating frequency for muscle power output were both influenced by temperature with muscle power output being the most temperature-sensitive variable. In addition, it has also been determined that overall temperature dependence of both these variables decreases as temperature increases (Stephenson and Josephson, 1990). This suggests invertebrates have a minimum muscle temperature necessary for maximum muscle power output.

The mechanism by which muscle is affected by temperature is related to the definition of muscular power. Power is defined by the force multiplied by the contractile speed of the muscle. Calcium ion sequestration, which is vital for the crossbridge cycle and therefore contractile speed, is greatly influenced by temperature. Q10, a measurement of change in a system as a consequence of increasing temperature by 10 degrees, was analyzed in frog leg muscle. The Q10 of hind frog leg muscle for the crossbridge cycle and sarcoplasmic reticulum calcium ion sequestration was found to be 3.4 between 0-10 °C (Rall and Woledge, 1990). The rate of the mechanism behind contraction of muscle is restricted by the temperature of the muscle and any increase in temperature at low temperatures.

**Figure 1.** An example of a male *Onymacris plana*. Although there is no difference in the running ability of males and females, females are more rounded and curved around the edges of the elytron.

The Namib desert is home to an impressive array of ultra-psammophilous tenebrionid beetles. The shifting dune system is sparsely vegetated, with Bushman's grass (*Stipagrostis ciliata*) and !nara bush (*Acanthosicyos horridus*, ! is a click in the native language) being the most commonly found (Koch 1961). Tenebrionids can be seen running from shade source to shade source or found taking cover a few inches under the sand of a hummock created by plants. Observational studies on the genus *Onymacris* (Tribe: Adesminii) found that these beetles undergo behavioral thermoregulation. Thermoregulatory strategies include stilting to rapidly increase body temperature, squatting to decrease body temperature, climbing to escape the boundary layer of heat, and burrowing to both raise and lower their body temperatures (Henwood 1975). These beetles have been observed to be very active throughout most of the day, first emerging from the sand mid-morning when the sands heat up and retiring in the late afternoon. Since these beetles are highly active throughout the day, through a range of temperatures, it is possible that muscle power output is only loosely dependent on temperature.

All of the behavioral strategies discussed above have been observed in *O. plana*, a discoid dune beetle with exceptional locomotor abilities (Henwood 1975). Metabolic rates of *O. plana* have been tracked while running on a treadmill. While at speeds ranging from 0-13 cm/s, the volume of oxygen used by the beetle was found to linearly increase. However, from 13-22 cm/s, there was no change in the respiration needs of the beetles (Bartholomew et al. 1985). This suggests that there is a behavioral or physiological trait that enhances the running ability of *O. plana* at high speeds.

The literature value for average speed is 90 cm/s and no difference has been found between running speeds of either sex. Running speed and its effect on internal body temperature has been studied with *O. plana*. It was found that running, no matter the speed, had no effect on the body temperature of the beetle (Nicolson et al. 1984). *O. plana* has the highest muscle mass to weight ratio of the tenebrionids of the Namib desert (Nicolson et al 1984) which results in the incredible speeds of the species. The surface-ar-

ea-to-volume ratio is high for *O. plana*, due to its saucer shape. *O. plana* have a body shape and size that is small enough that their heat storage capacity is minimal (Henwood 1975).

We hypothesized that external temperature would have an effect on the running performance of *O. plana*. We predicted that there would be a positive relationship between temperature and running speed.

## METHODS

### Animals

The investigation that is being reported here was conducted at Gobabeb Training and Research Institute, Namibia. We collected eight specimens by hand (Higham and Russell, 2010) from the dune 2.1 km southwest of Gobabeb. We collected all of our specimens between 9:00 am and 12:00 pm because *O. plana* are most commonly found feeding in this interval. As the sun comes out, *O. plana* will emerge from the sand and begin to stilt to gain heat. Once they have reached a sufficient temperature, they tend to run from bush to bush in search of food.

The specimens were kept in a vivarium with a lid and a hole drilled in the side for ventilation. We also provided them with sand to burrow into and *S. ciliata* to eat *ad libitum*. Of the beetles we collected, seven of them were male and only one of them was female. We continued to look for more females to observe any gait differences, but there were none to be found. It is unknown if females have different behavioral thermoregulatory strategies than males.

### Materials and Experimental design

In order to keep track of individuals, we painted numbers one through eight on the elytra of the abdomen. Our trial temperatures were 10°C, 20°C, and 35°C. We manipulated the temperature of the beetles by exposing them to a freezer and a heat lamp. While we were manipulating their body temperature, they were isolated in a separate plastic container. We used an infrared gun to measure the temperature of the beetles. The temperature was read every minute that they were in the freezer, which was kept at 0 °C. In the freezer, the external temperature of the beetles fell by 3°C/minute. We held the beetles 30 cm away from the heat lamp, which had a surface temperature of 80 °C, to reliably raise their temperature by 10 °C/minute.

The trials took place on a one-meter long raceway set up in laboratory conditions. We randomized all of the trials to prevent skewing of the data from the beetles acclimating to the lab. The running surface was lined with 60 grit sandpaper to provide grip for the pre-tarsus of the beetles. An Edgertronic camera was set up to record high-speed video at 500 frames per second. A computer

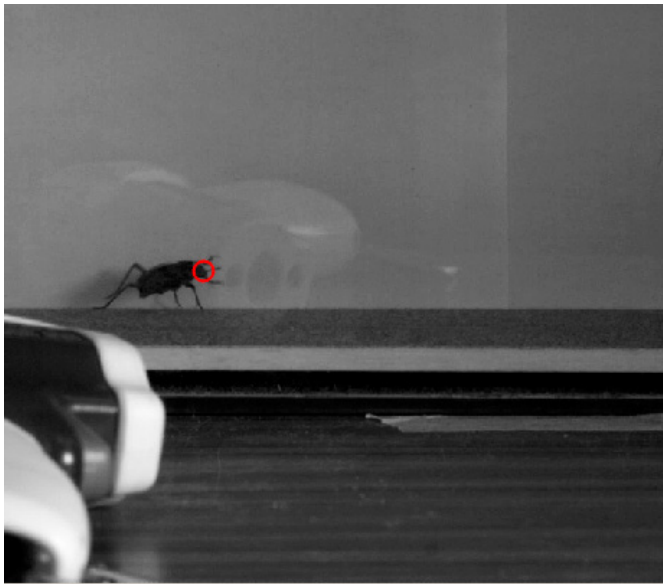

**Figure 2.** An example of the digitization of a trial recorded with high-speed video equipment.

was hooked up to the camera to provide playback. The camera was 1 meter away from the raceway.

In order to properly measure the distance that the beetle ran, we calibrated the camera by placing a ruler in the middle of the raceway and recording a short video of the ruler before beginning trials. Every time we moved the camera, we recalibrated in this manner. We then brought the beetle to the requisite temperature and placed it on the raceway, ensuring that it is always oriented in the correct direction. We used a thin 30 cm metal rod as a motivating stimulus to get the beetles to run. After each attempt at getting the beetle to run, we ensured that the beetles ran at least halfway down the track. (Note: the Edgertronic camera is always recording, we press "trigger" to save a specific interval of seconds before we pressed "trigger".) Our recording interval was six seconds. As the beetles do not tend to run in straight lines, we only recorded the trial if it ran the length of the raceway without hitting the sides. Each trial was replicated three times per individual at a given temperature; every individual was put through the same three temperature treatments.

### Data analysis

To find the maximum instantaneous velocity of the trials, we used the Matlab DLTdv5 digitizing tool (Hedrick 2008). A single point was placed on the tip of the head, the mouthparts.

The software tracked the point through time; and sometimes it was necessary to manually track the point through the frames if the contrast was not high enough for the digitizing tool to track automatically. Each video had a point digitized from the time the

beetle began to run, until it came to rest. We recorded the frame number that we began digitizing on and the last frame that was digitized. We chose to digitize the longest sprint in each trail. Once the trials were digitized, we exported the data to Excel and used the two-dimensional distance formula to find how many pixels the beetle advanced frame-by-frame. We then divided the number of pixels traveled by the frame rate to find pixels/second. Next, we used Excel to find the maximum velocity for each trial. After converting from pixels/second to cm/second, we picked the fastest trial per individual for each treatment for statistical analysis.

### Statistics

We ran a repeated measures ANOVA using R studio (R studio team 2018). A repeated measures ANOVA was utilized because our trials were taking multiple measurements of temperatures effect on the same test subjects in order to see if there is a correlation between temperature and running ability. Our independent variable being tested was external temperature. Our dependent variable was speed of the beetles. Covariables include leg length and total mass of the beetles.

## RESULTS

We had considerable difficulty getting the beetles to run at 10 °C. Of the eight individuals, five of them did not run at all at 10 °C. This suggests that at this body temperature, muscle output is too low for the beetles to run. Due to the surface area of the discoid beetle, heat is easily gained and shed by the beetle. When removed from the freezer, it was imperative that we place them on the raceway immediately. If left on the raceway for longer than a minute, the beetle would warm up beyond 1°C of 10°C. The same is true for 20°C and similarly, the beetles would decrease in temperature at a rate of roughly 4°C/minute when at 35°C. Although, the beetles always ran along the raceway when at all trail temperatures higher than 10°C. Since we were unable to get several of the beetles to run at 10°C, we only ran the repeated measures ANOVA for 20°C and 35°C trials. Our result ($p<0.2973$) for temperature indicates that, at least at higher temperatures, running speed is independent of temperature. We also found that total mass ($p<0.7504$) was an insignificant indicator of running speed. We measured the right hind leg length and used a repeated measures ANOVA to analyze the effect of leg length on running ability. The leg length was found to be an insignificant indicator of running performance ($p<0.6523$).

The average speed of the beetles did increase with each increase in trial temperature, but the standard error was very high for all temperatures, therefore this result was insignificant. The average speed for 10°C is the average for the three individuals that ran at 10°C.

The instantaneous velocity of *O. plana* is erratic and the resulting graph of speed over time can have very sharp peaks and troughs. To smooth the data, we used Excel to find the average speed of every three points. We then graphed the resulting data. Our data does not support our hypothesis. The fact that the beetles did not perform well at 10°C and ran at similar speeds for the other two trial temperatures suggests that at and above a certain temperature, running performance is constant. This is consistent with Stephenson and Josephson's findings that temperature dependence of muscle power output decreases as temperature decreases.

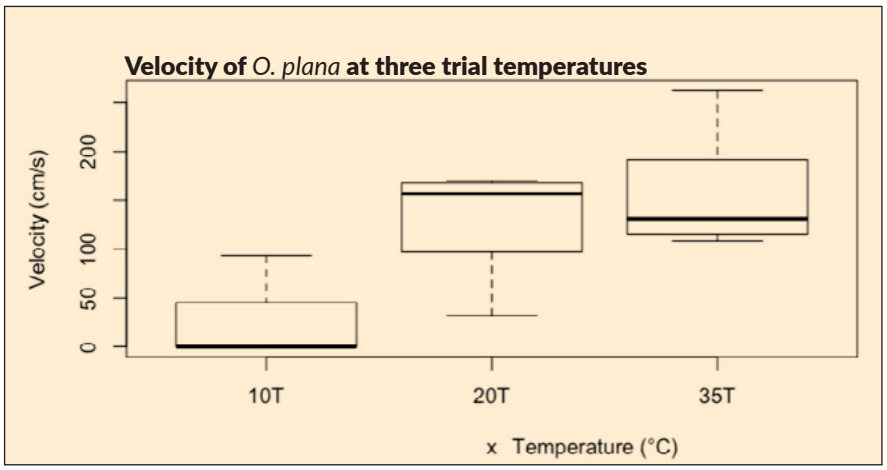


**Figure 3.** The box plot above shows the difficulty in getting *O. plana* to run at 10°C and the similarity in running capability at 20°C and 35°C.

## DISCUSSION

When the beetles ran at lower speeds, they pitched, rolled, and yawed due to the movement of the legs. The femur was lifted higher and swung forward at a smaller angle than it did at higher speeds. At maximum velocity, the beetles lifted their body, so the femur was more parallel with the ground and swung their leg forward at a larger angle, resulting in a very level running stride.

*O. plana* does not need to increase its metabolic rate while traveling at higher speeds. This is representative of a species that has adaptations that allow it to run faster without higher metabolic needs. It has been hypothesized that the saucer shape of the beetle promotes lift when the beetle is at high speeds, thereby reducing the amount

| | N | p-value |
|---|---|---|
| Temperature | 8 | 0.2973 |
| Leg length | 8 | 0.6523 |
| Mass | 8 | 0.7504 |

**Figure 4.** p-values of the three variates.

| Temperature (°C) | Average speed (cm/s) | Standard error |
|---|---|---|
| 10°C | 61.34 | (+/-) 21.319 |
| 20°C | 130.57 | (+/-) 18.286 |
| 35°C | 155.96 | (+/-) 20.983 |

**Figure 5.** Temperature, average speed and standard error of the trials.

of weight that the legs have to push (Bartholomew et al 1984). Our high-speed trials indicate that *O. plana* is level throughout its stride when running at high speed.

Muscle power output was found to be decreasingly associated with temperature as the temperature was raised (Stephenson and Josephson 1990). These findings help elucidate our results. We suggested that there was not much variance in the speeds of *O. plana* at 20°C and 35°C because these beetles have full locomotor function if at or above a threshold temperature. This phenomenon has been observed in other beetles. The effect of temperature on the running ability of other beetles has been studied. Tiger beetles (*Cicindela hybrida*) were found to also have an increased average speed as temperature increased, but the variance was high, and temperature's effect was found to be insignificant much like *O. plana* (Dreisig, 1981). This threshold temperature would be the temperature that would allow the muscle to sufficiently shuttle calcium ions in and out of muscle cells while retracting and contracting. Their ideal locomotor metabolic needs are met at a low enough temperature that the beetles can run at full speeds during most of the day. This would be expected of a beetle that lives in an environment with wildly changing temperatures. In the winter, temperatures in the Namib can reach highs of 30°C and nighttime lows of 10°C.

## CONCLUSION

The locomotor ability of *O. plana* was determined by running the beetles along a raceway at three trial temperatures. We found that there was no significant difference in the velocity of beetles at 20°C or 35°C. We also noted that *O. plana* has a unique locomotor ability; when flipped upside down, the beetle can right itself with use of its legs.

There were notable effects on the willingness to run and right themselves at 10°C, indicating that temperature does have an effect on the locomotor ability of *O. plana* at lower temperatures. It is unknown if this is a behavioral adaptation due to the individual recognizing its decreased running ability, or if it is a physical inability to move. Further investigations are needed. The muscle fiber in

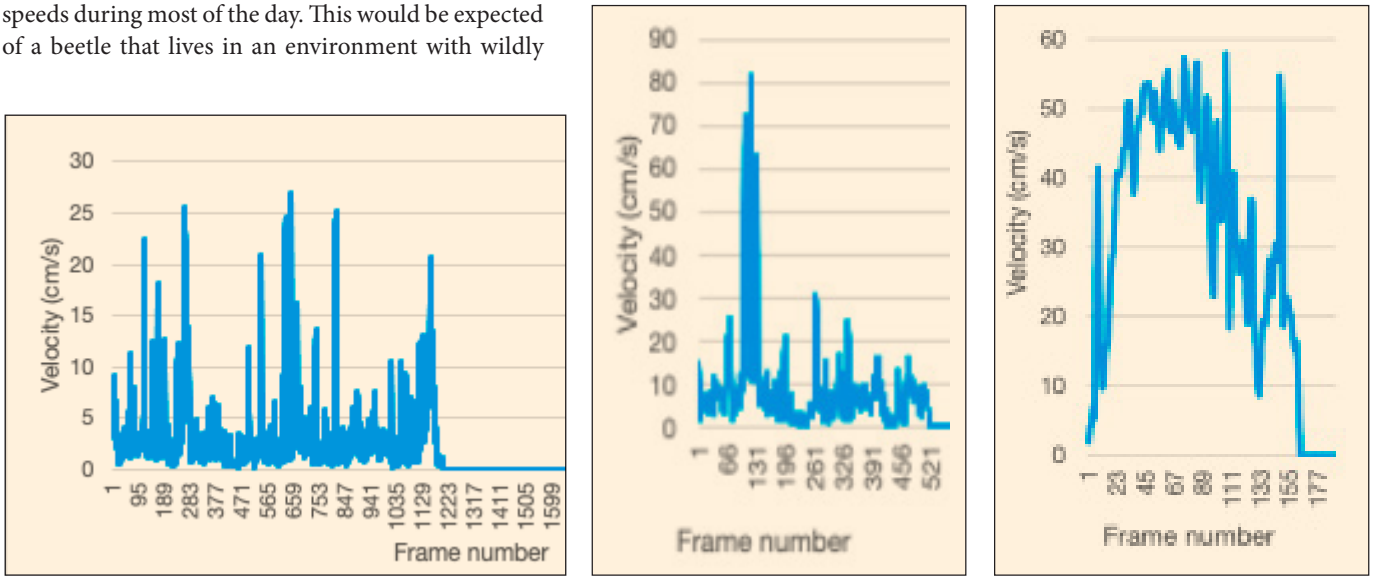


**Figure 6.** A smoothed rendering of velocity at every frame of the video at 10°C (left), 20°C (middle), and 35°C (right). These three representative trials were all by the same individual.

the leg should be directly studied for power output at different temperatures to remove the behavioral component of running speed. We suggest that the beetles are able to run at comparable speeds at the two higher trial temperatures due to the threshold temperature for maximum muscle contractile speed being met. It has been hypothesized that the *O. plana* does not have to increase its use of oxygen at high speeds due to a change in gait and the aerodynamic nature of the beetle's body. Qualitatively, we saw a change in gait and overall body posture when transitioning from a slower to a faster speed. The gait change at higher speeds results in the body traveling levelly and steadily forward.

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# Shakespeare's Violent Women: A Feminist Analysis of Lady Macbeth

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## ABSTRACT

There are numerous examples in which the female characters in William Shakespeare's plays go against the era's gender norms and enact violence. I argue that Lady Macbeth is one of these violent women whose violence defies gender roles, but this violence also simultaneously upholds traditional patriarchal modes of power. Lady Macbeth uses violence that stems from her feminine excess to advance patrilineage and her position within Scotland. In trying to understand her violence, I make use of a feminist analysis of Lady Macbeth by Cristina León Alfar and historical interpretations of the gender norms of the era. Lady Macbeth's violence elucidates the dilemma of the prominence of Shakespeare's female characters. While she has a significant role in the actions of the play, she still maintains hierarchical systems of power that are predicated on women's subjection.

**KEYWORDS:** *Shakespeare; Feminism; Violence*

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Amy Kenny received her PhD in early modern literature and culture from the University of Sussex and has worked as Research Coordinator at Shakespeare's Globe in London, where she was the chief dramaturge for 15 productions, and taught courses on theatrical practice and Shakespearean drama. She also conducted over 80 interviews with actors and directors on architecture, audiences, and performance, as part of an archival resource for future scholarship. She has published articles on dramaturgy, performance of laughter, the senses, and disease in Shakespeare. She is co-editor of The Hare, a peer-reviewed, on-line academic journal of untimely reviews. She recently published her first monograph with Palgrave Studies in Literature, Science, and Medicine, entitled *Humoral Wombs on the Shakespearean Stage*.

# Shakespeare's Violent Women: A Feminist Analysis of Lady Macbeth

## INTRODUCTION

As Derek Cohen notes in *Shakespeare's Culture of Violence*, "though violence is constructed in the established laws and codes as anti-social, though the violent act is punishable by law and called cruel and unnatural, it is the very system that so condemns it that produces it and, occasionally, needs and depends on it" (Cohen, 1). Shakespeare's plays often portray violence that is rewarded or condemned, depending on which system of power it serves and to what ends. This violence is typically rewarded if it is performed for the dominant system in power and harshly repressed if it is performed for the opposition. For example, in *Macbeth*, Macbeth's brutality in service of Duncan's reign is rewarded, and it is his propensity for violence that grants him the crown. However, it is also this inclination towards violence that dubs Macbeth's reign as tyrannical.

This violence that is used to keep patriarchal systems in place is frequently used against women to exclude them from power. Violence in Shakespeare's plays is then typically examined through a lens that further supports the notions of patriarchal dominion against women. As seen in *Othello* and *King Lear*, the violence perpetrated against Desdemona and Cordelia is used as punishment for their tainted female chastity and the refusal of "the subjugation of the female self to the male" (Cohen, 9). Violence, then, is used to perpetuate patriarchal systems of power and is wielded by those in patriarchal society who hold the most power, men. While violence committed by men against other men and women in Shakespeare's works has been thoroughly analyzed, there remains little scholarship on the subject of violence committed by women. These women utilize violence to gain power in a patriarchal society that is dominated by violence. This violence, then, is a means to an end of gaining power and a way for them to further their goals of moving up within their hierarchical society.

The female character that exemplifies these characteristics and that I focus my study on is Lady Macbeth from *Macbeth*. She utilizes rhetorical violence, which I define as verbal harm consisting of insults, mockery, etc. Lady Macbeth uses violence that stems from her feminine excess to advance patrilineage and her position within Scotland. This feminine excess consists of her severe devotion to upholding her role as a wife, and the violence that stems from her feminine excess is gendered through its connection to her reproductive capacities.

## EARLY MODERN GENDER ROLES

Lady Macbeth's unconventional adherence to early modern gender roles is not an anomaly given the fact that these roles were seen as natural for women in the time. Early modern gender roles for women are pervasive in the literature of that era. Women's gender roles in early modern England were informed by both religion and the burgeoning Scientific Revolution. Both Protestant and Catholic thinkers alike believed that while women were spiritually equal to men in the eyes of God, they were still subordinate to them. They also saw marriage as the "natural" vocation for women" (Wiesner, 62) and that the ideal wife was "obedient, silent, pious" (Wiesner, 24). The importance of Protestant and Catholic views on women cannot be understated, as most Europeans were required to attend church, and thus, it was impossible to avoid these views as they were espoused in sermons and manuals by the church (Wiesner, 23). Women who strayed from these expectations were regarded with suspicion.

While the Scientific Revolution challenged old ways of thinking at the time, it did not "challenge inherited ideas about women" from ancient times (Wiesner, 25). This was most predominant in medical theories about women's bodies and reproduction. Aristotelian thinking at the time saw women as incomplete or deformed men. Moreover, they believed women's weaknesses were due to the fact that their reproductive organs were located inside, as opposed to men's who were located outside (Wiesner, 26). One of the predominant theories concerning women's bodies was the role of the uterus in women's dispositions, an idea that emerged from Plato. Often, women's illnesses were attributed to the uterus, especially mental illness. When the uterus was not regularly engaged in sexual intercourse and reproduction, illness was thought to wander the body (Wiesner, 27). The belief of the influence of the uterus and women's reproductive capacities over their behavior was very much prevalent in the early modern period and found its way into many of the works of literature at the time. This claimed significance that the reproductive functions have over women's behavior will be seen in Lady Macbeth's interpretation of her own body.

## LADY MACBETH

Lady Macbeth's support to advance her husband's position within Scotland is clear from her entrance into the play. She agrees with the witches' premonition and emphasizes the great things he can achieve if he only had the cruelty to commit the acts to achieve them. While many scholars argue that Lady Macbeth defies the gender roles of the era, Cristina León Alfar argues that Lady Macbeth's character actually parodies the ideal early modern wife (Alfar, 113). She specifically states that Lady Macbeth presents herself as "the image of himself [Macbeth] he seeks" and that Shakespeare does this in order "to put pressure on masculinist and violent structures of relations that depend on women's abject confirmation for their unremitting self-perpetuation" (Alfar, 111). In doing so, Shakespeare calls into question the systems that necessitate women's abjection. While Alfar's argument resonates with my

own, I contend that although Lady Macbeth does portray a heightened version of the gender roles of the era, her actions are not meant to be a parody. Lady Macbeth fulfills her role of the dutiful wife to ensure that Macbeth attains his goals and her dedication to this role manifests itself as rhetorical violence. This violence stems from her feminine excess of fulfilling her role as a wife to extreme ends, and these extreme ends mean supporting her husband no matter the cost. She understands that violence is the only way to ensure movement within the sovereign sphere at the time.

Lady Macbeth's first lines in the play do not consist of her own words, but her husband's, which signals that her character's primary motivations are invested towards her husband. She is first seen reading from a letter that Macbeth has sent her. Once she has finished reading the letter, she immediately establishes that the witches' premonitions will come true but also expresses her worries. She states, "Glamis thou art, and Cawdor; and shalt be / What thou art promised: yet do I fear they nature; / It is too full o' the milk of human kindness / To catch the nearest way:" (1.5.13-16). Through these lines, Lady Macbeth establishes an early connection between violence and attaining power. For Macbeth to achieve his goals, he cannot have too much "o' the milk of human kindness," a phrase that has negative connotations to the female reproductive system. Lady Macbeth continues her questioning of Macbeth's capacity for violence as she states that "thou wouldst be great; / Art not without ambition, but without / The illness should attend it:" (1.5.16-18). Through these lines, Lady Macbeth expands on the idea that Macbeth possesses ambition, but he needs cruelty to make these ambitions a reality; her following "unsex me here" speech demonstrates her willingness to exert cruelty on herself in order to aid him. Just as "human kindness" is considered to be a kind of milk, "cruel ambition" is also associated with the body as Lady Macbeth views it as an "illness." These images of kindness, ambition, and cruelty as having physical manifestations continue as Lady Macbeth declares, "Hie thee hither, / That I may pour my spirits in thine ear; / And chastise with the valour of my tongue / All that impedes thee from the golden round," (1.5.23-26). The line "That I may pour my spirits in thine ear;" suggests that Lady Macbeth possesses a cruelty that Macbeth does not. As Amy Kenny notes in "The Thick Womb," the "spirits" that Lady Macbeth refers to in this speech are typically associated with paranormal spirits, but it is more likely that the spirits she refers to are the more commonly understood spirits of the period that are described as fine, physical substances that govern one's behavior, typically in a malevolent way (Kenny, 63). These spirits are what grant Lady Macbeth the cruelty that Macbeth needs to commit regicide. Lady Macbeth has located cruelty and kindness as traits that manifest themselves physically in the body, thus, providing context for her next famous speech.

Once Lady Macbeth learns of Macbeth's imminent arrival, she prepares by dispelling any weakness from her body, which she associates with the female reproductive system, through verbal demands not unlike the rhetorical violence she uses on Macbeth. Lady Macbeth calls attention to spirits again. She asks them, "Come, you spirits / That tend on mortal thoughts, unsex me here, / And fill me from the crown to the toe top-full / Of direst cruelty!" (1.5.38-41). She wishes to be free from the categories of gender, notably to be freed from the idea that women were the lesser sex because of their leaky bodies. Indeed, as Kenny notes regarding ideas surrounding menstruation in this period, women were seen as "inferior, docile beings without agency over their own bodily functions" (Kenny, 63). Following this transformation, she requests that her body be filled with cruelty, for it to serve as the physical manifestation of cruelty. For this to occur, however, she must be rid of the feminine sensibilities that she believes restricts this cruelty; the spirits must "Make thick my blood; / Stop up the access and passage to remorse, / That compunctious visitings of nature / Shake my fell purpose," (1.5.41-44). Not only is her reproductive system adverse to cruelty, but it is also responsible for the remorse that might impede her goal of urging her husband's purpose. To support her husband's ascent to the crown, she must disown the aspects of her bodily functions that are associated with weakness. Lady Macbeth's desire to disown the "weaker" aspects of her body mimics the rhetorical violence that she utilizes with Macbeth. She uses rhetorical violence upon herself to be able to aid Macbeth with the cruelty he needs to achieve his goals. With these lines, Lady Macbeth is espousing the early modern idea that the female body is inherently lesser than the male body, it is not suitable for cruelty and, thus, needs to be unsexed in order for it to be effective. Lady Macbeth's willingness to alter her body demonstrates the lengths that she will go through to support her husband.

Lady Macbeth continues her association of the female reproductive body with weakness when she asks the spirits to also take the milk from her breasts, which invokes her earlier statement in which Macbeth has too much of the milk of human kindness. She wishes for the spirits to "Come to my woman's breasts, / And take my milk for gall, you murdering ministers" (1.5.45-46). Just as menstrual blood is associated with women being leaky vessels, so is breast milk connected to an understanding of women possessing inherently excessive bodies (Kenny, 64). Lady Macbeth's dedication to her role as a wife is emphasized in these lines as she seeks to deny her duties as a mother to fulfill her duties as a wife, particularly the duty of supporting her husband by possessing enough cruelty in order to encourage him to commit regicide. Her request for her milk to be replaced with gall demonstrates a connection between female reproductive functions and weakness. In this speech, Lady Macbeth tries to disown any aspect of her excessively leaky feminine body as it is not conducive to violence. Once her body is

de-sexed, she can utilize rhetorical violence and play the role of the supportive wife to encourage Macbeth to commit violence.

Lady Macbeth pivots from playing the role of a supportive wife in the conventional way of complimenting him, to using her support as a means of insulting Macbeth and emasculating him. Lady Macbeth begins by insulting Macbeth as she questions his reluctance when she states, "Wouldst thou have that / Which thou esteem'st the ornament of life, / And live a coward in thine own esteem / Letting 'I dare not' wait upon 'I would,'" (1.7.41-44). Lady Macbeth begins her insults by turning his potential lost ambitions on himself and does so by reminding him that he'd be an "a coward in thine own esteem" if he does not complete the deed. She reminds him that he himself deemed the crown as "the ornament of life," and refuses to let him back down from his plans. Lady Macbeth continues her questioning of his initial dedication to his plan after he states "Prithee peace: / I dare do all that may become a man; / Who dares do more is none" (1.7.45-47). Macbeth demonstrates his insecurities with his masculinity as he feels the need to immediately demonstrate that he is the most masculine of all. Lady Macbeth's responds by encouraging him to be this man "When you durst do it, then you were a man; / And, to be more than what you were, you would / Be so much more the man" (1.7.49-51). Lady Macbeth eggs Macbeth on by appealing to his precarious masculinity which she has just questioned, as she has an innate understanding that masculinity and violence dominates the culture of medieval Scotland, a fact that Alfar has noted in her work (Alfar, 121-122). It is Macbeth's unstable sense of masculinity that allows him to be manipulated by Lady Macbeth so readily, and she uses the feminine role of supporting her husband to remind him of his masculine aspirations. Thus, Lady Macbeth's feminine excess of rigidly upholding her role as the supporting wife is demonstrated through her use of rhetorical violence.

In order to encourage Macbeth to commit regicide, Lady Macbeth sets up a hypothetical situation in which her depravity is connected to female reproductive capabilities. Lady Macbeth continues her encouragement of Macbeth's regicide with the infamous lines:

> *I have given suck, and know*
> *How tender 'tis to love the babe that milks me:*
> *I would, while it was smiling in my face,*
> *Have pluck'd my nipple from his boneless gums.*
> *And dash'd the brains out, had I so sworn as you*
> *Have done to this. (1.7.54-59)*

Lady Macbeth presents a fantastical situation in which she is a mother and a dutiful one at that. She nurses the child herself and understands "How tender 'tis to love the babe that milks me." In this hypothetical situation, Lady Macbeth is an ideal mother who cares for her child and demonstrates the maternal warmth that is expected of her; however, things quickly turn for the worse. Lady Macbeth swears that she "would, while it was smiling in my face, / Have pluck'd my nipple from his boneless gums / And dash'd the brains out, had I so sworn as you." Lady Macbeth's hypothetical maternal warmth is replaced by the ultimate maternal cruelty: infanticide. Her attempts to encourage Macbeth to commit the deed result from her playing into the role of the extremely supportive wife, which, as Kenny states "rather than destroying her femininity, this fantasy solidifies her unwavering allegiance to Macbeth, as she yearns to be understood solely as a wife, not a mother…She uses rhetorical infanticide to externalize her inner passions to the audience" (Kenny, 60). This hypothetical situation is a continuation of Lady Macbeth's role as a good wife, but it is a role that requires her to relinquish any maternal instincts, as was demonstrated when she said "take my milk for gall" (1.5.46). The imaginary violence that she conjures up demonstrates that Lady Macbeth understands that violence is what garners someone's power in Scotland, and she utilizes it in this scene in order to demonstrate to Macbeth the extremes that she would go to if necessary. However, Lady Macbeth is incapable of physical violence herself as demonstrated by Act 2, Scene 2, in which she states "I laid their daggers ready; / He could not miss 'em. Had he not resembled / My father as he slept, I had done't" (2.2.11-13). Lady Macbeth can only utilize rhetorical violence to inspire her husband to commit murder, as Alfar notes, "the law of the Father, in fact, precluding her from the power to act, for action is not her role; rather she must only facilitate her husband's acts" (Alfar, 127). Lady Macbeth resists gender roles insofar as insulting her husband, emasculating him, and using hypothetical violence, however, this is also to inspire him to reach the position they feel he deserves; but she does not stray so far as to commit murder herself. This hypothetical violence is used primarily to demonstrate the lengths that she will go to in order to inspire her husband: she would even imagine the violence that is seen as unimaginable for women in order to remind of his purpose.

## CONCLUSION

As Lady Macbeth utilizes rhetorical violence to push Macbeth to regicide, she relies on an inherently masculine form of gaining power to advance her husband's position. She does not advocate for matriarchy or for her own power when is not connected to any man but instead, exerts her efforts to uphold patriarchy. Lady Macbeth advocates for the ascension of her closest male relation to the crown, thus, fulfilling an extreme version of her role as a wife. Lady Macbeth relies on rhetoric to defy gender roles, as her emasculation of her husband goes against the early modern ideal of women as silent and meek. Her violence, however, simultaneously goes against the early modern notion of women as silent and

meek but supports the rule of patrilineage, which fundamentally excludes women from holding power. Lady Macbeth's violence elucidates the dilemma of the prominence of Shakespeare's female characters: while she has a significant role in the actions of the play, she still maintains hierarchical systems of power that are predicated on her subjection.

What makes this topic worthy of further study is that Lady Macbeth is not an anomaly in Shakespeare's corpus. Queen Margaret in *King Henry VI, Part 3,* Goneril and Regan in *King Lear,* and Tamora in *Titus Andronicus* are characters that also exhibit violence and are ideal in continuing the study of Shakespeare's violent women. It is important to note that one thing these characters have in common is they are all in positions of power within the hierarchical society that they live in. A topic that begs further inquiry is a deeper analysis of the links between power, gender, and violence.

While the topic of violence and women in Shakespeare has been discussed as it relates to violence perpetuated by male characters against female characters, more critical discussion needs to focus on violence perpetuated by women in service of patriarchal systems of power. Returning to Derek Cohen, "Elizabethans saw the state as no less patriarchal for being embodied as a female [by Queen Elizabeth I]" (Cohen, 5). Thus, the task remains to further study how Shakespeare's female characters help uphold patriarchal power and the intricacies of power and violence. As women in positions of power become more prevalent, the question remains of who exactly these women are helping, and whether their leadership roles really challenge any of the patriarchal systems of power that remain in place. Examining Shakespeare's violent women can be an avenue for generating these discussions.

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# Development of Quantitative Förster Resonance Energy Transfer (qFRET) based High Throughput (HTS) screening for PD-1/PD-L1 immune-checkpoint Assay

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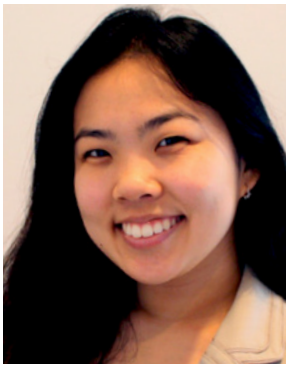
## ABSTRACT

Programmed cell death protein 1 (PD-1) and programmed cell death 1 - ligand 1 (PD-L1) are immune-checkpoint proteins that play an important part in cancer immunity. PD-1 is a protein on the surface of cells that down-regulates the immune system[1] while PD-L1 is a protein on some normal and cancer cells. The interaction of these proteins play a major role in tumor immune escape, inhibiting T lymphocyte proliferation and survival functions. To combat this issue, targeting these immune checkpoint proteins with monoclonal antibodies (mAbs) has become the turning point in cancer treatment. However, limitations were found using mAbs such as the cost of administration, its high molecular weight, and its lack of clinical efficacy. Recently, researchers are investigating small molecule inhibitors to target the PD-1/PD-L1 mechanism instead. With CA-170 as the only small-molecule modulator in clinical trials targeting PD-1, it is essential to research options that can contribute to cancer treatments. This study provides a novel, rapid assessment for PD-1/PD-L1 interaction with the use of FRET-based kinetic analysis. PD-1/PD-L1 binding will be quantified by fluorescence using donor and acceptor pairs, CyPet and Ypet, which were bound to PD-L1 and PD-1, respectively. From this study, we calculated a $K_d$ value of 0.31±0.13 and developed an HTS assay with a Z' value > 0.7, values that validate the robustness and efficacy of this assay. With the development of this type of screening, it will be easy to contribute to small molecule inhibitor discovery and the growing field of cancer immunotherapy.

**KEYWORDS:** *PD-1; PD-L1; Immunotherapy; qFRET; HTS; Oncology*



**Amanda Xaypraseuth**

*Department of Bioengineering*

Amanda Xaypraseuth is a fourth year Bioengineering major. She has been involved in research for three years with experience developed at the UCR School of Medicine, Keck Graduate Institute, and the UCR Department of Bioengineering. Under the guidance of Dr. Jiayu Liao, Amanda's current research focuses on developing a high-throughput screening method for PD-1/PD-L1 interaction to assist in oncological and biopharmaceutical research. After graduating, she aspires to gain more experience in industry before pursuing higher education.

**FACULTY MENTOR**

**Jiayu Liao,** *Department of Bioengineering*

Professor Jiayu Liao obtained his Ph.D. degree from Dept. of Biological Chemistry, School of Medicine at UCLA, where he discovered the first SUMO E3 ligase family of genes, and then conducted his post-doctoral training of human genomics and chemical biology at the Scripps Research Institute. Shortly, he joined the Genomic Institute of Novartis Research Foundation (GNF) as the principle investigator and founding scientist of GPCR platform, where he was involved in the identification of target and side-effect the novel immunosuppressant drug, Gilenya from Novartis, and led a high-throughput screening for S1P1 receptor for the discovery of SEW2871, which led to the second generation of drugs, Siponimod from Novartis and Ozanimod from BMS. Dr. Liao moved to UCR as a founding faculty for the Department of Bioengineering in 2006. His work at UCR mainly focus on development of quantitative FRET technology platform for biochemical/pharmaceutical parameter determinations and high-throughput drug discoveries, focusing on SUMOylation and Ubiquitin-like pathways involved in anti-viruses and anti-cancers.

# Development of Quantitative Förster Resonance Energy Transfer (qFRET) based High Throughput (HTS) screening for PD-1/PD-L1 immune-checkpoint assay

## INTRODUCTION

The World Health Organization predicts that worldwide cancer rates are set to double by 2020[2]. With 10 million new cancers being diagnosed each year worldwide, it is essential to find an effective prevention campaign. A current method being used to address the cancer crisis is cancer immunotherapy, which has successfully brought exponential progress to the development of cancer treatments. Cancer immunotherapy is a method of eliminating cancer cells by enhancing or modulating the host immune system. One of the many types of cancer immunotherapies is using immune checkpoint molecules to regulate the immune balance, and the neutralization of immunosuppressive checkpoints[3]. Among these immune checkpoints, it was found that the blockade of programmed death protein 1 (PD-1) and programmed cell death 1 - ligand 1 (PD-L1) led to one of the most successful immunotherapies by enhancing T cell immune responses against tumor cells[3].

PD-1 is highly expressed by activated T cells, whereas PD-L1 is expressed on several types of tumor cells. The interaction of these two proteins results in the inhibition of T-cell activation and proliferation, providing an immune escape mechanism for tumor cells. Further studies then demonstrated that by blocking the interaction of PD-1 and PD-L1, it subjects tumor cells to attack by cytotoxic T cells **(see Figure 1)**. With the presence of PD-L1 on several types of tumor cells such as lung cancer, breast cancer, melanoma, and ovarian cancer, efforts are being made to develop PD-1/PD-L1 inhibitors for the treatment of these high-risk diseases.

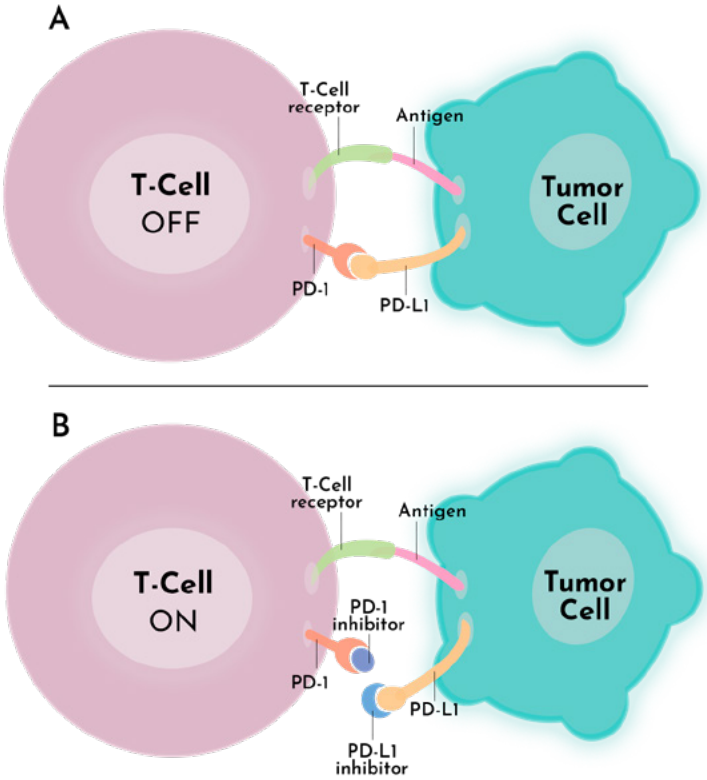



**Figure 1.** PD-1/PD-L1 Pathway Mechanism of Action (a) Tumor Uses PD-L1 as Defense Mechanism. (b) PD-1/PD-L1 Inhibitor Blocks Tumor's Defense Mechanism

Since the discovery of these proteins, monoclonal antibodies (mAbs), which are antibodies that enlist natural immune system functions to fight cancer, have been used for cancer immunotherapy. Ipilimumab was the first mAb monotherapy to be approved for advanced melanoma. By combining a combination of nivolumab (anti-PD-1-mab) and ipilimumab, Bristol-Myers Squibb (an American pharmaceutical company) was able to create the first approved immunotherapy combination with a high response rate. Despite this novel breakthrough, it was found that utilizing mAbs has its disadvantages, such as its production cost, instability, and immunogenicity[4]. Thus, small molecule immune checkpoint inhibitors of PD-1 and its ligand PD-L1 have become an active research field in drug discovery.

Currently, there are very few such inhibitors reported, one reason being the elusivity of the structural information of immune checkpoint proteins. Because of the increasing need to find a small molecule inhibitor to halt the interaction of PD-1 and PD-L1, this study is focused on developing a Förster Resonance Energy Transfer (FRET) based high throughput screening method to provide a rapid assessment for PD-1/PD-L1 interaction, in hopes of making the search for a small-molecule inhibitor more efficient and easier.

FRET has been widely used in biological and biomedical research and is a very powerful tool in elucidating protein interactions in many cellular processes[6]. FRET offers real-time monitoring and spatial information on molecular interactions in living cells[5] while being highly sensitive to nanoscale changes in donor/acceptor separation distance. This distance-dependent physical process measures the energy transfer emitted from the donor as it excites the acceptor. The FRET signal strength is generally determined by two major factors: the intrinsic FRET efficiency of the donor and acceptor and the amounts of the interactive donor and acceptor[5]. This technology can detect particle interactions within a range of 1-10 nm, an ability that allows this study to quantitatively describe the molecular interactive events between PD-1 and PD-L1.

Using this FRET technology by binding donor fluorophore CyPet (cyan fluorescent protein for energy transfer) and acceptor fluorophore Ypet (yellow fluorescent protein for energy transfer) to PD-L1 and PD-1, respectively, this study will provide insight to

how these two proteins interact and are used to develop a high throughput screening method for inhibitor screening. The development of this screening method will be subjected to a $K_d$ measurement and a Z' factor measurement. $K_d$ measurement is an important parameter to quantitatively assess the binding affinity of protein-protein interactions. Although there are traditional methods to calculate for $K_d$, such as surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC), FRET can serve as an additional tool that produces comparable values to these older, aforementioned approaches[5]. The Z' factor is another important value that determines whether the assay results are reproducible, i.e. that the variability of key endpoints of the assay is acceptably low[7]. This statistical method will be used in this study to quantify the potency and efficacy of the assay setup.

With the exploitation of FRET technology, it is obvious to see that there is potential to develop new methods to assist in biological research. This study will report the experimental developments of $K_d$ determination of PD-1 and PD-L1 interaction and HTS assay development using the engineered FRET pair, CyPet and YPet. It is hoped that the use of this developed screening method will be applied in industrial applications, providing a rapid assessment for small molecule inhibitors that have the potential to become a cancer immunotherapy.

## METHODOLOGY

### Cloning and Expression of CyPet-PDL1 and YPet-PD1

The CyPet-PDL1 and YPet-PD1 plasmids used in this study were from the Hungxi Hospital located in China and were cloned into a pET28(b) vector (Novagen). Following cloning, BL21(DE3) *Escherichia coli (E.coli)* cells were transformed via electroporation with the pET28 vectors. The transformed *E.coli* BL21(DE3) were then plated on LB plates supplemented with 50 µg/mL kanamycin to ensure proper transformation.

Single isolated clones of CyPet-PDL1 and YPet-PD1 were then inoculated into loosely capped 10 mL LB tubes and incubated overnight at 37° with 250 rpm (revolutions per minute). Once the LB tubes have been incubated, the 10 mL starting cultures were then inoculated into a 1 L culture of prepared 2 × YT medium (there was 1L of 2 × YT medium made per 10 mL per each type of protein). Then, the 1 L cultures were grown in an incubator at 37° with 280 rpm for about 3 hours, which was the time it took for the culture to reach its exponential phase in its cell cycle. Afterward, the 1 L cultures were induced with 0.1 mM Isopropyl ß-D'1-thiogalactopyranoside (IPTG) and were continued to be incubated at 25° overnight, but no longer than 16 hours. The addition of IPTG enhances the expression of the protein. Aliquots were collected before and after the addition of IPTG for SDS-PAGE analysis.

### Purification of CyPet-PDL1 and YPet-PD1

CyPet-PDL1 and YPet-PD1 cells were harvested by centrifugation for 3 minutes at 4° at 8,000 xg per each 300 g of culture. Post-centrifugation, the 1 L of pelleted cells were washed and resuspended with 40 mL of lysis buffer. Once resuspended, the cells underwent sonication for a 5 minute total process time with 5 seconds on and 5 seconds off intervals in an ice bath. The sonicated cells were then placed in a centrifuge and spun down at 35,000 xg for 20 minutes to separate cellular debris and inclusion bodies, leaving other proteins in solution. The supernatant and pellets were then collected to be examined using 10% SDS-PAGE to verify the successful expression of CyPet-PDL1 and YPet-PD1.

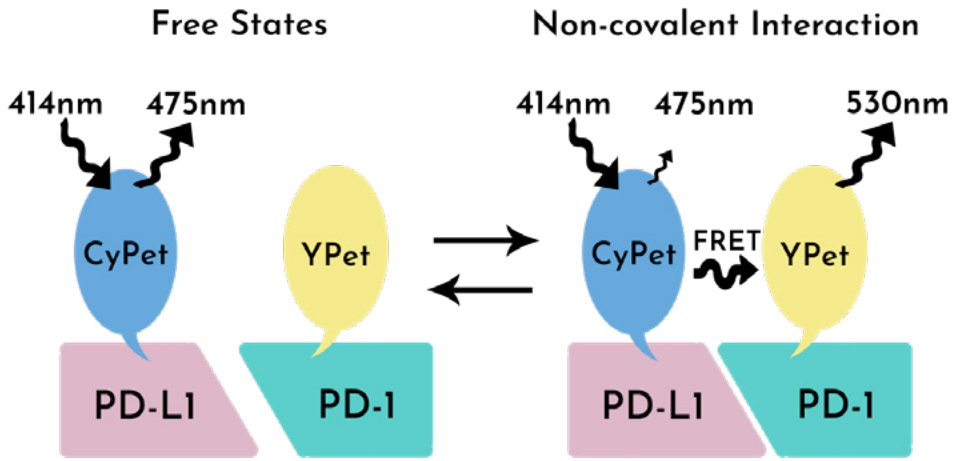



**Figure 2.** Design of FRET-based detection for PD1/PDL1 protein interactions

The supernatant was then subjected to be further purified by Ni-NTA column chromatography to capture histidine-tagged (His-Tag) proteins, PD-1 and PD-L1. To begin purification, 10 mL columns were filled with 300 µL of Ni-NTA agarose beads (QIAGEN). These beads were washed with 1 column volume (CV) of ddH20. The supernatant was then poured into the column and drained to the level of beads. Once finished, 1 CV of Wash Buffer 1 was poured into the column and drained to the level of beads. This step was then repeated with 1 CV of Wash Buffer 2 and 1 CV of Wash Buffer 3. After draining the wash buffers, a 1.5 mL Eppendorf tube was placed under the column to collect protein as it was being eluted with an Elution buffer. Once eluted, the protein solution was injected into a dialysis tube that was clamped at one end. The dialysis tube filled with protein solution was then placed in a large beaker filled with PBS for 16 hours, which is a step needed to remove any remaining imidazole concentration and wash buffer. These protein purification steps were performed according to the QIAGEN standard protocol[8].

## FRET and $K_d$ Measurement Assay

60 µL mixtures of recombinant CyPet-PDL1 and YPet-PD1 and phosphate-buffered saline (PBS) were created to begin FRET measurements. The final concentration of CyPet-PDL1 was fixed to 1 µM and the final concentration of YPet-PD1 was varied from 0 to 4 µM. The rest of the volume was supplemented with PBS, creating a total volume of 60 µL. These mixtures were then transferred to a 384-well plate (Grenier black) to measure the fluorescence emission spectrum of each sample. The two excitation wavelengths used to measure the fluorescence emission (emitted at 530 nm) were 414 nm to excite CyPet and 475 nm to excite YPet **(see Figure 2)**.

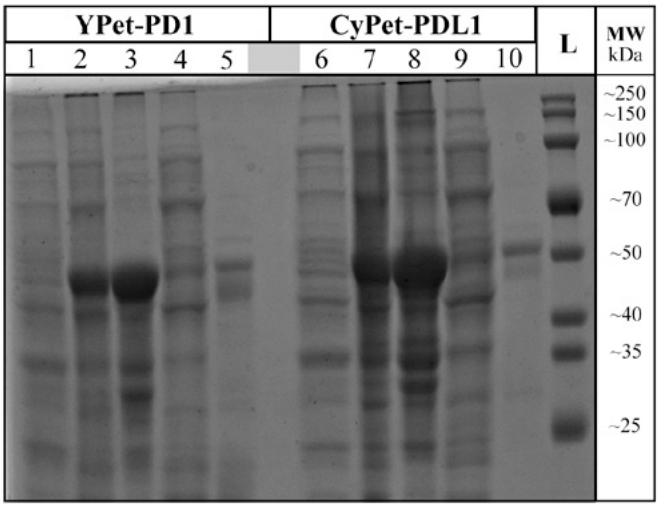



**Figure 3.** SDS-PAGE gel, Coomassie stain for determination of protein expression and validation of protein purification. **1**-YPet-PD1 Uninduced, **2**-YPet-PD1 Induced, **3**-YPet-PD1 Cell Pellet, **4**-YPet-PD1 Cell Supernatant, **5**-YPet-PD1 Purified and Dialyzed Protein, **6**-CyPet-PDL1 Uninduced, **7**-CyPet-PDL1 Induced, **8**-CyPet-PDL1 Cell Pellet, **9**-CyPet-PDL1 Cell Supernatant, **10**-CyPet-PDL1 Purified and Dialyzed Protein, L-Protein Ladder

The fluorescence emission was then calculated by solving for $Em_{FRET}$, which is proportional to the amount of YPet-PD1 bound to CyPet-PDL1. $Em_{FRET}$ was solved by using Equation 1[9], where FLDD is the excited fluorescence signal of the donor, FLAA is the excited fluorescence signal of the acceptor, 'x' is the CyPet ratio factor, and 'y' is the YPet ratio factor. After $Em_{FRET}$ has been calculated for each sample, the $K_d$ value between CyPet-PDL1 and YPet-PD1 was calculated based on the algorithm that Song et. al developed[9]. This data was then processed and analyzed by using the GraphPad Prism software.

$$EM_{FRET}=(EM_{Total}) - (x * FL_{DD}) - (y * FL_{AA})$$

Equation 1

## Z Prime Assay

The Z' factor for this study's assay was done once each day over three consecutive days with an assessment of three reaction conditions that produced a Max, Mid, and Min signal. The reaction was done in a 60 µL volume with 0.5 µM of CyPet-PDL1 and YPet-PD1 for the Max signal, 0.25 µM of CyPet-PDL1 and YPet-PD1 for the Mid signal, and 0.5 µM of CyPet-PDL1 and YPet-PD1 with the addition of Guanidine-HCl for the Min signal. The Max and Mid reactions were supplemented with PBS to achieve a volume of 60 µL whereas the Min reaction was supplemented with Guanidine-HCl. The addition of Guanidine-HCl mimics the presence of a small molecule inhibitor, which will produce no signal of energy transfer emission from CyPet to YPet. After the reactions were created, they were incubated for 30 minutes at 37°C. These reactions were then done in a 384 well plate format and set in a sequence outlined within NIH assay guidelines. After proper plate setup, the fluorescence emissions and the $Em_{FRET}$ of all the reactions were measured and calculated. After calculating $Em_{FRET}$, Equation 2[7] was used to calculate the Z' Factor.

$$Z'=\frac{(AVG_{Max} - 3SD_{Max}/\sqrt{n}) - (AVG_{Min} - 3SD_{Min}/\sqrt{n})}{AVG_{Max} - AVG_{Min}}$$

Equation 2

## RESULTS

### Expression, Purification, and Dialysis of Recombinant Proteins

Induced, un-induced, cell pellet, cell supernatant, and purified samples for both YPet-PD1 and CyPet-PDL1 were examined by using 10% SDS-PAGE to detect the active expression products **(see Figure 3)**. The uninduced bands for YPet-PD1 and CyPet-PDL1 (Lane 1 and 6) show little to no presence of protein. However, once these samples were induced with IPTG (Lane 2 and 7), there was increased protein presence as indicated by the band around 48 kDa and 60 kDa for Lane 2 and 7, respectively, which are the molecular weights for YPet-PD1 and CyPet-PDL1, respectively. The cell pellet and cell supernatant were also collected for examination (Lanes 3,4,8, and 9). As expected, there is protein presence in each sample indicated by bands located around 48 kDa and 60 kDa. Most of the protein is in the cell pellet, therefore the cell pellet samples (Lane 3 and 8) are expected to have a thicker band than the cell supernatant samples (Lane 4 and 9).

The purified and dialyzed protein samples of YPet-PD1 and CyPet-PDL1 were also collected to be examined (Lanes 5 and 10). These lanes have the clearest protein bands compared to the other samples, which have lighter bands scattered down the lane in addition to the protein band. Despite being purified and dialyzed, Lanes 5 and 10 still have a light band beneath the 48 kDa and 60 kDa mark. Because His-Tag purification was used to capture our protein, these light bands can only be presumed to be degraded purified protein. Since the His-Tag is located at the N terminal of the protein, the C terminal can get degraded or cleaved during the process of purification. This phenomenon would yield purified proteins that aren't full-length. Thus, the bands present in Lanes 5 and 10 are YPet-PD1 and CyPet-PDL1 with the lighter bands being the same protein but cleaved at the C terminal.

### $K_d$ Measurements

The $K_d$ Measurement experiment was done by fixing the CyPet-PDL1 concentration to 1 μM and increasing the concentration of YPet-PD1 from 0 to 4 μM in a total volume of 60 μL. After the FRET emission intensity under each condition was calculated, the data were fitted to a nonlinear regression curve fit (see **Figure 4**). The GraphPad Prism software was then used to calculate for our $K_d$ and R2 value, which was 0.31 ± 0.13 μM and 0.9136, respectively. Typically, the smaller the $K_d$ value, the greater the binding affinity of the ligand for its target. The larger the $K_d$ value, the weaker the target molecule and ligand are attracted to.

### Z Prime

The Z' factor for our assay was done once each day over three consecutive days with three reaction conditions that produced a Max, Mid, and Min signal. The Max signal contained 0.5 μM of CyPet-PDL1 and YPet-PD1. The Mid signal contained 0.25 μM of CyPet-PDL1 and YPet-PD1. The Min signal contained 0.5 μM of CyPet-PDL1 and YPet-PD1, including Guanidine-HCl to mimic the presence of a small molecule inhibitor within the reaction. The Z' assay was done 3 times with 3 plates to ensure a lack of variation with our samples **(see Fig**ure 5). The Z' factor for each day and plate was then calculated according to the NIH guidelines, which was well within the criteria of Z' factor ≥ 0.4 and < 1 (see **Table 1**). All conditions of the study's Z' assay, which collectively had an average Z' value of 0.98, met the NIH criteria.

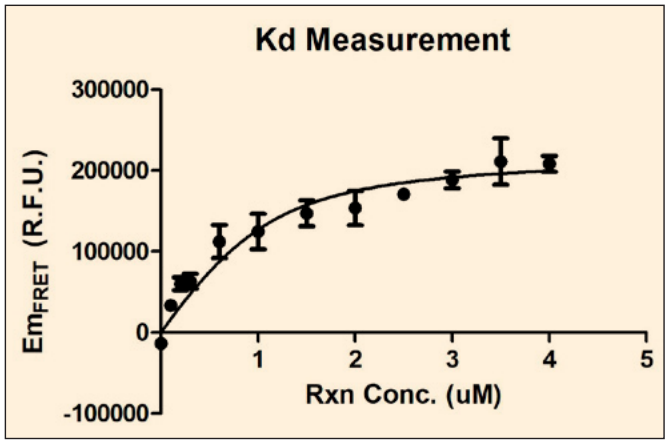



**Figure 4.** YPet-PD1 FRET emission (RFU-Relative Fluorescent Units) vs. YPet-PD1 conc. (μM)

## DISCUSSION

With the growing number of cases of cancer every year, it is important to discover small molecule inhibitors for cancer immunotherapies. This study reports the development of a FRET-based HTS screening method for small molecule inhibitors to assist in PD-1/PD-L1 inhibitor discovery and oncological research. By measuring the $K_d$ and the Z' values of our developed assay, we were able to quantify the robustness and validity of our HTS setup. The $K_d$ value that this assay received was 0.31 ± 0.13 μM, which not only indicates that there is a high binding affinity between the expressed proteins, PD-1 and PD-L1, but also is comparable to previous experimental data[10,11]. This number also validates that the expression of our protein was successful as it is expected that these complementary proteins would bind readily to each other. Further validation was done by running an SDS-PAGE gel, which indicated a thick band around 48 kDa and 60 kDa, the molecular weight of YPet-PD1 and CyPet-PDL1, respectively. The SDS-PAGE gel also

# Development of Quantitative Förster Resonance Energy Transfer (qFRET) based High Throughput (HTS) screening for PD-1/PD-L1 immune-checkpoint assay

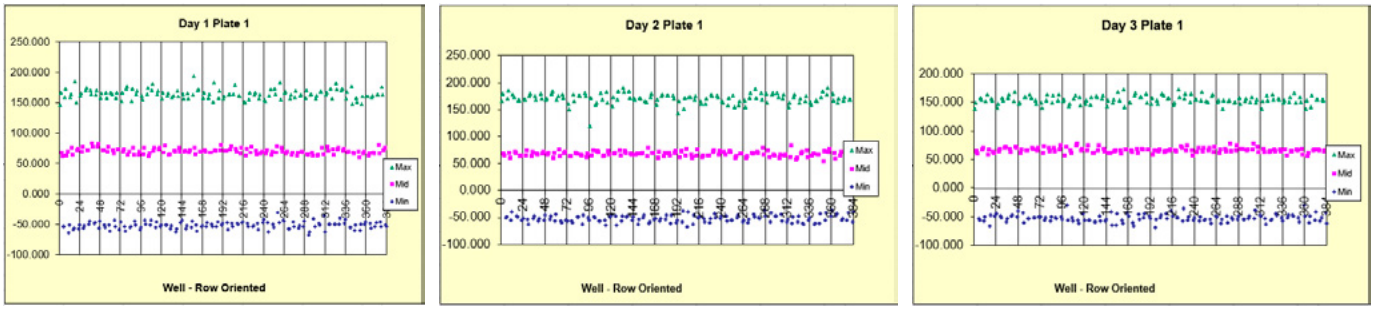



**Figure 5.** Z' for Plate 1 of 3 over days 1, 2, and 3. Each condition has an N of 128 for each plate, for a total of 384 reactions per plate. Plate replicates for each day resembles the figure shown.

showed that there was another light band present within Lanes 5 and 10 in addition to the thick protein band. This light band is usually indicative of protein degradation, but a western blot should be done to confirm what that band indicates. Although the presence of protein degradation may have affected our experimental data, it was confirmed that our purified samples sufficed to perform our experiments due to the comparable $K_d$ value that was measured. The reported Z' assay utilized $Em_{FRET}$ as the metric for hits. The Z' data demonstrated that the signal window between the Max and Min signal fell around 205 R.F.U., as seen by the gap between the Max and Min signal in each graph in **Figure 5**. This size range indicates confidence in each hit, making analysis of the screening data easier and less error-prone. **Figure 5** also demonstrates the lack of variability in our samples, indicated by the horizontal line trend that is present in each plate measurement. It is aspired to see this linearity to validate that the assay can produce accurate and consistent data. Further data analysis determined that the Z' over the 3 days had an average of 0.98 which confirms the assay's fidelity and robustness in the duration of a drug screening.

The robustness of this study's performance does not only hold oncological relevance to PD-1 and PD-L1. It also serves as a platform for other types of assays used in preclinical drug discovery including but not limited to antibody and other protein-protein interaction research. The utilization of FRET technology for HTS development provides a more cost-efficient and robust method for rapid assessment of discovering immunotherapeutic agents. With its wide $Em_{FRET}$ range and Z' factor values that fall within NIH criteria, this FRET-based assay acknowledges and combats the limitations that some HTS methods have, such as the reduction in the quality and specificity of data as throughput increases[12]. For future studies, a western blot can be performed on our purified protein sample to further validate the presence of the correct protein. In addition, protein inhibitor, phenylmethylsulphonyl fluoride (PMSF), can be included in the binding buffer to potentially yield less protein degradation. Once that has been done, an actual screening with drug libraries can be performed to begin the search for a potential small-molecule inhibitor of PD-1 and PD-L1. With its high efficacy and tolerability, FRET techniques are sure to make a mark in biological research and discovery.

**Table 1.** Z' > 0.9 for each plate over three days, all Z' are within the set criteria

| | Z' FACTOR MEASURED | | |
|---|---|---|---|
| | Day 1 | Day 2 | Day 3 |
| Plate 1 | 0.98 | 0.99 | 0.98 |
| Plate 2 | 0.98 | 0.98 | 0.98 |
| Plate 3 | 0.98 | 0.98 | 0.98 |

## ACKNOWLEDGMENTS

I would like to sincerely thank Dr. Jiayu Liao and Vipul Madahar of Bioengineering for the continual guidance throughout this project. This work was supported by the Undergraduate Education Quarterly Minigrant from the University of California, Riverside.

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# Voicing Lyrical Dance: (Re)Considering Lyrical Dance and Dance Hierarchy

**Julia Zumaya & Anthea Kraut**
*Department of Dance*

## ABSTRACT

Lyrical dance intertwines fluid movement aesthetics, emotional narratives, and musicality within competition and commercial dance contexts. However, dance scholars tend to criticize lyrical dance, both directly and indirectly, perceiving it as over-the-top yet underdeveloped. When making such statements, they implicitly contrast lyrical dance with "high art" values that privilege a particular mode of "meaning-making" as rooted in the canon of concert dance forms, such as modern and ballet. However, lyrical dance does not prioritize elements of "high art," meaning that these scholars critique lyrical dance more for what it is *not*. My research, in response, challenges such hierarchical biases by understanding lyrical dance from the perspectives of those who practice it. With IRB-approval, I conducted interviews with ten lyrically trained dancers from both private-sector, competition dance studios and collegiate dance departments in Southern California. My findings assess lyrical dance's values regarding expression, "freedom," connectivity, and affirmation of skill—focusing on the latter for the sake of this article—recognizing that lyrical dancers actively shape each value through their dedication to lyrical dance practices. By voicing the lyrical dancers' perspectives and their reasons for embracing the practice, I aim to show the need to reconsider lyrical dance on its own terms, challenging persisting critiques within scholarship.

**KEYWORDS:** *Dance; Lyrical Dance; Hierarchy; Critique; Culture; Value; Interviews; Identity*

**Julia Zumaya**

*Department of Dance*

Julia Zumaya studies dance at UC Riverside, exploring movement practices, dance making, and critical dance theory. Through the Mellon Mays Undergraduate Fellowship Program, she has researched lyrical dance and dance hierarchy for the past two years with the mentorship of Dr. Anthea Kraut. As a fourth-year senior, she looks forwards to joining PhD programs in Dance Studies, aspiring to publish more works on lyrical dance and relevant dance practices.

**FACULTY MENTOR**

**Anthea Kraut**, *Department of Dance*

Anthea Kraut is a Professor in the Department of Dance, where she teaches courses in critical dance studies. Her publications include *Choreographing the Folk: The Dance Stagings of Zora Neale Hurston* (University of Minnesota Press, 2008) and *Choreographing Copyright: Race, Gender, and Intellectual Property Rights in American Dance* (Oxford University Press, 2015).

# Voicing Lyrical Dance: (Re)Considering Lyrical Dance and Dance Hierarchy

## INTRODUCTION

Percussive yet fluid, emotional yet narrative, lyrical dance moves within a complex division between competition/commercial and collegiate/concert dance frameworks. My research analyzes persisting criticisms of lyrical and its relevant dance contexts to explore a prevailing valuing of "high art" over "othered" dance forms. With the incorporation of self-led interviews, I provide alternate ways of perceiving lyrical dance that are more specific to its own practitioners, institutions, and culture.

## DEFINING LYRICAL DANCE: BACKGROUND RESEARCH

Lyrical dance combines fluid movement aesthetics, personal emotional displays, narrative arcs, and intricate musicality. Lyrical dancers move fluidly, like water, "flowing seamlessly from one move to another" (Bedinghaus 2019) through smooth bodily articulations, particularly of the limbs and torso. Lyrical's artistic intents—or the purposes behind training, choreography, and performance—stem from its etymological "lyric" root. "Lyric" describes both a genre of poetry that "expresses the thoughts and feelings of the poet" (EEB 2017) and the words of a song, which, in ancient Greece, accompanied the lyre (OED 2019). Thus, "lyrical" describes dances that express both musicality and deep, personal emotions. Most commonly, people assume lyrical is dancing to the lyrics of a song, the more frequent use of "lyric." However, lyrical dance also considers the "rhythms and instrumental…cues" (Weisbrod 2010, 104) and the "'tone of the music'" (103). As for emotional expression, lyrical dance concentrates on intense and personal sentiments, "oriented toward the dancer's emotional responses" (Bedinghaus 2019). Lyrical dances contextualize such strong emotions within a story, adding narrative intents to the genre. Furthermore, lyrical's relationship with a song contextualizes all artistic intents between musicality, emotion, *and* narrative.

Critical to defining lyrical dance are its artistic genealogies and contexts. Before becoming "lyrical," it was known as "lyrical jazz," a form of jazz that arose when ballet (and, to a lesser extent, modern) entered Broadway, a venue for vernacular and popular dance, in the mid-twentieth century (Netting 1995; Kraut 2015). With the rise of media and private-sector dance competitions in the 1980s, lyrical jazz entered private-sector studios, where ballet has reigned as foundational. With another large wave of ballet's influence, lyrical became the fusion of "ballet with a particular evolution of jazz dance that removes the Africanist aesthetics" (Weisbrod 2010, 326). To a lesser extent, lyrical incorporates influences from modern, pantomime, acrobatics, and possibly Bharatha Natyam (Weisbrod 2010; Fisher 2014). Particularly with modern's influence in competition and commercial dance spaces, "lyrical" evolved into "contemporary" in the 2000s (Weisbrod 2014).

Lyrical dance persists within competition and commercial dance contexts. Competition dance exists within the private sector, where dancers ages four to eighteen perform in front of a panel of judges to win prizes and awards (Schupp 2019). Commercial dance is "the use of dance in the service of selling a product" (59), whether it be a physical product or an experience, to a ley audience. I consider competition and commercial dance together because lyrical dance also aims to impress judges and entertain audiences, goals that together influence lyrical dance's aesthetics. For example, lyrical dancers train to display precise, "super-human skills" (Weisbrod 2020, 97-98) to impress their audience. Lyrical dancers also convey deep emotions to engage and *move* their audience (Garafoli 2007), using explicit storytelling, gesturing to lyrics, and popular music to help ley audiences understand the performance. Lyrical, developing within competition/commercial dance contexts, reproduces shared values around entertainment and audience.

## HIERARCHICAL ISSUES

Despite recognizing lyrical's artistic aesthetics and intents, some scholars critique lyrical and its competition/commercial spaces for being "over-the-top," "unstructured," and "underdeveloped." I particularly reference Jennifer Fisher (2014), whose article criticizes lyrical dance for not measuring up to "high art" characteristics and values. Fisher's article is the only scholarly publication to date that solely focuses on lyrical dance; so not only is attention to lyrical limited within scholarship (Weisbrod 2010) but lyrical's only considerable presence is through a devaluing of the practice. More common within scholarship, relevant critiques of competition and commercial dance exist that fixate on the absence of "meaning-making" and movement agency, which are artistic intents of "high art," concert dance practices.

Fisher (2014) and other scholars critique lyrical, competition, and commercial dance (lyrical, etc. dance) for valuing virtuosity and spectacle rather than "meaning-making" (Fisher 2014; Netting 1998; Elswit 2012). Additionally, they reduce lyrical, etc. dance to simplistic pedagogy (Fisher 2014) and a mere display of fast movements and poses (Foster 2017). Fisher (2014), Elswit (2012), and Foster (n.d.) critique the emotional expression of lyrical, etc. dance as "over the top," arguing that the movement agency itself does not sufficiently contextualize the emotions. Lastly, scholars present an idea of lyrical as "rootless" (Fisher 2014) or as a "style" rather than an established "technique" (Wesibrod 2010, 105). Instead of valuing lyrical, etc. dance aesthetics and foundations, these scholars focus more on how lyrical, competition, and commercial dancer are not "art dance," concert dance, or "high art." For this reason, I

question to what extent these scholars critique lyrical and its relevant practices for what it is *not* rather than what it *is*.

Reading Fisher (2014) and other scholars, I examine how their criticisms and critiques stem from an implicit comparison of lyrical to "high art," a category of elite dance under which lyrical does not fall. "High art" is subjective because it relies on comparative definitions of what it is "higher" than, prompting the "othering" of arts forms deemed "lowbrow" (Dixon Gottschild 1996; Kealiinohomoku 2001). "High art" institutions of the United States—those with the power to assign cultural value—associate with privileged social categories (Wesibrod 2010), deem themselves "artistic" over other forms, and separate "high art" from capitalist frameworks despite inherently existing in those systems (Dodds 2011). However, lyrical dance is not "high art," nor is it quite "low art" like the vernacular and popular dances of marginalized peoples; instead, lyrical is "middlebrow." The "middlebrow" positionality of lyrical dance in particular, and competition/commercial dance more broadly, is perhaps why scholars can easily critique it while simultaneously supporting a rising movement in Dance Studies to attend to traditionally "low art" dance. In this sense, scholars, as "high art" practitioners, can critique lyrical for not being "high art" without the awareness to also attend to its marginalized cultural values. While lyrical exists between complicated hierarchical stances, I focus on the dichotomy between competition/commercial dance and concert/collegiate dance, spaces of "high art" institutions, practices, and values.

As lyrical waivers between "high art" influences—mainly from ballet, modern, and the middle class—and competition/commercial dance values, lyrical constructs its own cultural values, i.e. aesthetics, intents, training structures, performance contexts, etc. So, to break from a "high art" perception of a non-"high art" dance, I research lyrical's cultural values as embodied, reproduced, and shaped by its practitioners. I question how lyrical dancers' perceptions, experiences, and beliefs further complicate dance scholars' understandings of lyrical dance.

## VOICING THE LYRICAL DANCE PERSPECTIVE: METHODOLOGY AND FINDINGS

With Institutional Review Board approval, I recruited lyrical dancers enrolled in undergraduate dance departments and local dance studios within Southern California. My participants have five or more years of experience with lyrical, contemporary, studio, and/or competition dance training (what I call "relevant dance experience"). Within an approximately 30-minute interview, I asked my ten volunteer participants questions about their understanding, experiences, and perceptions about lyrical in addition to their dancer identity. My interviewees were (at the time of their interview):

**Isabel:** a third-year undergraduate, previously a dance major and now minor, with 13 years of relevant dance experience, 8 years with lyrical, all years at or affiliated with dance studios and competitions

**Zana:** a graduating senior undergrad, dance major, with experience at five other collegiate dance programs, 26 years of relevant dance exp., 12 years with studio dance, and 1 dance experience with lyrical choreography

**Christine:** a second-year undergrad, dance minor, with 16 years of relevant dance exp., 13 years with lyrical, all years at or affiliated with studios and/or competitions

**Silvanna:** a first-year transfer, junior-standing student, dance major, with about 9 years of relevant dance experience, 4 with lyrical, 1 year with competition dance, 1 year with studio dance, a few years affiliated with studio dance

**Samantha:** a first-year transfer, junior-standing student, film major, with 18 years of relevant dance exp., 11 years with lyrical, all years at or affiliated with studio dance

**William:** a graduating senior undergrad, dance major, 15 years of relevant dance experience, 13 years with lyrical, about 10 years with competition dance, all years at or affiliated with studio dance

**Langston:** local dance studio student, 4th grader, with 6 years of relevant dance experience, about 3-4 years with lyrical dance, 4 of which with competition

**Clara:** local dance studio student, also a third-year undergraduate, with 14 years of total relevant experience, about 14 years with lyrical, all at or affiliated with studio and/or competition dance

**Maho:** first-year undergraduate, psychology major, pursuing dance minor, with 7 years of relevant dance experience, 2 years with lyrical, 3 years with competition dance, all at or affiliated with studio and/or competition dance

**Sophia:** local dance studio student, 7th grader, with 6 years of relevant dance experience, 3 years with lyrical, 3 years with competition, all years at or affiliated with studio dance.

**Julia:** third-year undergrad, dance major with creative writing minor, with 6 years of relevant dance experience, 4-5 years with lyrical, 1 year affiliated with studio dance.

As part of my interviews, I also include some autoethnography as a lyrical dancer myself.

Before continuing, I first acknowledge that my research and interviews do not wholly represent lyrical dance. Instead, I incorporate my interviews and reflections to begin to represent the broader community of lyrical dancers and their cultural values. I also acknowledge my bias as a lyrical dancer, which may influence me to focus on beneficial aspects of the practice/culture and minimize lyrical's faults to contrast criticisms and critiques. My purpose is not to prove scholars "wrong" by showing them "rights" but, rather, to show the need to broaden their interpretations and arguments by offering different, more dancer-centric perspectives. I have condensed my findings into four topics: expression, "freedom," connection, and affirmation of skill. For the sake of this article, I will very briefly summarize expression, "freedom," and connectivity before focusing in more depth on affirmation of skill.

To summarize, given lyrical's personal emotive depth, dancers can *explore* their emotional range, providing potentially cathartic and therapeutic experiences to help my participants as adolescents and young adults navigating new realities. Lyrical dance also provides my participants "freedom" within the structures of lyrical choreography and studio and ballet influences. In effect, dancers take agency in interpretation and movement variation within performance. Lastly, lyrical provides dancers with a medium of connection with people (classmates, instructors, teammates, and audience members), community (studio, competition, and commercial dance contexts), and music (song). Dancers achieve such connectivity through lyrical's emotive and interpretive depth within competition and commercial dance environments.

## AFFIRMATION OF SKILL

My participants and I, from our lyrical dance practices, recognize the affirmation of skill through a sense of comfort and confidence. Firstly, we prefer to move our bodies through lyrically informed ideologies, providing us with a sense of comfort. For example, Samantha commented, "for the life of me, I cannot make sharp movements anymore. It's so hard. I just want to make everything seem flowy and flowing to the next move." Christine similarly noted that she is "not a very hard-hitting dancer." For Samantha and Christine, they struggle with sharp movements—the seeming opposite of lyrical fluidity—so they maintain fluid, lyrical undertones to their movements. Additionally, Isabel appreciates lyrical's smoother, calmer energy in comparison to jazz, for example, explaining that "I love jazz music, upbeat music. It's just harder to tap into that 'cause it's more energy." Despite her love for jazz, she prefers lyrical's energy levels, finding it easier for her to embody. William introduces an idea of naturalness with lyrical, stating, "I just choreograph easily, like it just fits my body. It just comes more natural to me." Acknowledging the complex understanding of what "comes naturally," I reinterpret William's statement as an emphasis on his personal inclinations to move in a lyrically informed manner. I similarly appreciate how I feel that I am meant to move in a lyrical-esque form, giving me a sense of belonging and comfort. Christine further recognizes that "my experience with lyrical has always been like it's my comfort zone, and it's my safe place." Christine's comment along with my participants' lyrical predilections suggest that lyrical provides some with a reassuring comfort zone that encourages them to continue practicing and cultivating skill.

Lyrical dancers also value lyrical because it is something they have embodied and mastered, so it is something they can excel at, which informs the dancers' self-confidence. For example, Isabel values the opportunity to succeed through lyrical:

> *And it's something that I think—this will sound kind of cocky—but I'm pretty good at. I am good at teaching and I'm good at explaining it. It's something I don't feel like I'm failing at…. It's nice to be able to do something that other normal people can't do. Like, yeah, I can put my leg here. Yeah, I can jump in the air. Yeah, I can do this.*

I argue that Isabel's feelings should not be perceived as "cocky" but, rather, met with encouragement, for she has found, in lyrical, a source of confidence despite other experiences with failure. Maho and Sophia mentioned how they can see self-improvement and growth overtime when looking back to when they started, so they find confidence knowing that their efforts pay off through constant progress. I appreciate how lyrical gives me the space to engage my skills. As a choir and drama student in high school, I learned additional levels of conveying emotions in performance. As a creative writing student in college, I can incorporate my love for stories into lyrical. Through lyrical, I actively engage with practices I master, boosting my confidence. For Clara, lyrical "it's just one of the things I can do," which reflects how her dedication to training paves the way for mastery. Overall, the confidence and comfort that dancers achieve through lyrical dance practices affirm the value of their skillsets, encouraging dancers to dedicate to lyrical dance practices and mastery.

## SHAPING IDENTITY: IMPLICATIONS

As I have found, lyrical provides expression, "freedom," connec-

tivity, and affirmation of skill for its dancers. However, I must also recognize the role lyrical dancers play in reproducing and shaping lyrical dance values. To demonstrate, I present a quote from Samantha:

> *Out of every dance form that I've ever danced, "ever" being from three to now, I've danced lyrical or contemporary more times than anything else. So, to me, it's more, I guess, natural, and it also has a lot more significance to me because it's what I like to do…. I mean it like I have more training in that, and I've connected with it more because I've had more time with it. Like, for example, people who do more jazz, to them, their values set towards jazz. Mine is more lyrical and contemporary 'cause that's what I've grown up with, that's the path I chose when I did dance.*

The time Samantha has spent with lyrical informs the extent to which she connects with the practice and embodies its values. However, her ability to connect with lyrical values depends on her active choice to practice lyrical dance. Because Samantha and other dancers *dedicate* their time to lyrical dance, *the dancers* reshape that time into connection, value-setting, and embodied knowledge—the sets of skills, ideas, values, and everything else learned through a physical practice. So, yes, lyrical offers dancer the space to express personal emotions, to find freedom from structures, to connect with various people and platforms, and to affirm their skills. However, lyrical dancers only gain these benefits through their decision to commit to the practice. In this sense, the lyrical dancers shape their own embodied knowledge and value sets, giving *themselves* the space to express, be free, connect, and affirm.

Through their reproducing and shaping of lyrical dance, the dancers help cultivate their sense of identity, supported within cultural frameworks and values of lyrical. When I asked my participants if lyrical and/or contemporary are a key part to who they are as dancers, Samantha, Isabel, Christine, Silvanna, Clara, and Sophia agreed enthusiastically; and I concur. Silvanna even gave me a, "Oh, hell yeah!" Through intense, personal expression, lyrical dancers explore their states of being to work through issues and habits, learning about themselves in the process. By valuing freedom from structures, lyrical dancers learn how they generally value agency and, therefore, value their ideas and decisions expressed through such agency. As for connectivity, lyrical dancers learn about themselves through their relationships, valuing the support systems they find through these points of connection. Moreover, by affirming their skills, lyrical dancers learn how they want to move and how they can move. Through a dedication to lyrical dance practices, lyrical not only gives dancers a sense of identity; dancers cultivate their own identities within dance and, potentially, life.

While scholars devalue lyrical dance, almost expecting it to be "high art," lyrical dancers continue to value lyrical's aesthetics, intents, practice, performance, genealogies, and contexts. Instead of dismissing lyrical as "over-the-top," "unstructured," or "underdeveloped," dancers, through their self-initiated dedication to their practice, find significance in lyrical's expressive, freeing, connective, and affirmative qualities. As shown by the lyrical dancers' voices, dance scholars' critiques fail to make space for a more detailed, dancer-centric understanding of lyrical. And, as my participants and I move onto higher education, where scholars critique lyrical and where "high art" continues to reign, we are essentially told to "not do that," which feels like being told "forget everything you know" or "don't be who you are." These negative views of lyrical dance leave dancers feeling stripped of their agency, identities, and worth as they struggle to conform to a new, privileged philosophy in hopes of making it far in the dance world. Moving forward, I hope my research opens space for dance in higher education to move away from hierarchical biases and work towards inclusive approaches to value all dance, including the "middlebrow" lyrical dance.

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# Notes

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