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Letter From the Editors

We are proud to present to you volume nine of the Florida Atlantic Undergraduate Research Journal. The FAURJ is an interdisciplinary, peer-reviewed journal that is published annually. FAURJ is published online as well as in print and its mission is to showcase high quality undergraduate research in all fields, supply undergraduates with an idea of the standard of research, and promote inquiry-based activities at Florida Atlantic University.

The FAURJ is student-run by the Council for Scholarship and Inquiry (CSI), a registered student organization at FAU in collaboration with The Office of Undergraduate Research and Inquiry. The organization promotes and fosters a culture of research at the university across all disciplines. CSI strives to enhance scholarship, creativity, critical thinking and academic excellence of undergraduate students at FAU. Opportunities sponsored by CSI include publication in the FAURJ, student workshops, promotion of faculty-student interaction and peer mentoring. Undergraduate research provides students the ability to create and pursue innovative ideas under the mentorship of faculty members, which distinguishes FAU as a prestigious institution and shapes the young minds of the future.

We would like to thank all of the students who have submitted their work for the 2019-2020 publication. You all have shown immense dedication to your research and we wish you the best in your future endeavors. We hope you enjoy this edition of FAURJ, and encourage you to stay curious!

Mikaela Jonsson, Isis Majeska
Cover Design Artist Statement

This microscopy image of primary neuronal culture from a mouse cortex was taken on an epifluorescence microscope at 40X magnification. It depicts neurons reaching out to other neurons to form functional connections, known as synapses. Something about this image reminded me of the famous painting of “God Reaching Out to Man,” known widely as the Creation of Man by Michelangelo. If you compare them side-by-side, you can observe the parallels between them. Hence, Neuron Reaching Out to Neuron seemed a fitting name to me. Oscar Wilde once stated, “Life imitates Art far more than Art imitates Life,” and I think this image illustrates this well. Science is the study of the physical and natural world, and biology is the science of life. We see beauty in life as a projection of the art that inspires us.

Florida Atlantic University Harriet L. Wilkes Honors College Biological Chemistry ’20

Christy LaFlamme
Florida Atlantic University Harriet L. Wilkes Honors College
Biological Chemistry’20
POLYSPECIFIC ASSOCIATIONS FROM CAMERA TRAPPING DATA OF THE LESULA MONKEY (CERCOPITHECUS LOMAMIENSIS) IN THE LOMAMI NATIONAL PARK AND BUFFER ZONE IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Aaron Mencia, Charlene Fournier & Kate M. Detwiler

Dorothy F. Schmidt College of Arts and Letters & Charles E. Schmidt College of Science

Abstract

Polyspecific associations are common among African Cercopithecus monkeys in the forest canopy and may provide benefits by maximizing foraging success and reducing predation risk. The ground dwelling lesula monkey (Cercopithecus lomamiensis) is endemic to the Congo Basin in Central Africa and provides a unique opportunity to study interspecies interactions on the forest floor. The objectives of our study were to investigate the type and frequency of associations between lesula and heterospecifics, and if variation in patterns of association occurs among survey sites. We also assessed lesula's behavior when in association. Camera trap videos containing lesula with heterospecifics were organized into events. We scored behaviors in an ethogram using continuous focal sampling. We found that lesula occurred in polyspecific associations with multiple sympatric species and were observed most often with the blue duiker (Philantomba monticola). Results indicated that relaxed behaviors were the most common responses of lesula when in polyspecific associations.

Introduction

Biodiversity monitoring has revealed a diverse animal community in the Lomami National Park (LNP) in the Democratic Republic of the Congo (DRC) (Hart et al., 2012). The lesula monkey (Cercopithecus lomamiensis) is found only in the LNP and buffer zone making it endemic to the area. The species was first documented in 2012 after intense field observations in the LNP area. Due to the relatively recent discovery of the lesula monkey, camera traps were placed in its range to learn more about its behavioral ecology and unique terrestrial niche among the Cercopithecus genus, a group of African monkeys commonly referred to as the guenons.

In the past decade, the use of camera traps has exponentially increased in wildlife field studies as a tool for monitoring unhabituated and cryptic species that would have been inaccessible otherwise (Pebsworth & Lefleur, 2014; Boyer-Ontl & Pruetz, 2014). Camera traps have provided new insight into surveying rare species, monitoring remote primate populations, and documenting elusive primate behavior, such as resource use and mixed species interactions (Pebsworth & Lefleur, 2014; Tisovec et al., 2014; Boyer-Ontl & Pruetz, 2014). For example, Prasad et al. (2010) documented through camera traps the ecological interaction between arboreal primate species and terrestrial frugivores by confirming which species consumed the fruit dropped by the primates. This type of association contributes to natural forest regeneration through seed dispersal and the health of an ecosystem (Prasad et al., 2010).

In this study, the use of camera traps provided a unique opportunity to study mixed-species associations between the elusive lesula monkey and sympatric terrestrial mammals. Arboreal guenons typically form interspecies associations among one another (Buzzard, 2010; Cords, 1990; Noe & Bshary, 1997). Lesula is one of the rare guenon species that spends most of its time on the forest floor, which makes this study important by providing insight on which sympatric species lesula may associate with (Arens et al., 2020). Additionally, this study contributes to the limited number of publications regarding the LNP and buffer zone and even fewer that report on lesula's ecology.

Primate studies involving associations between multiple species, or polyspecific associations, typically focus on overlapping (sympatric) tree dwelling (arboreal) primate species; however, this study has a unique opportunity to record associations between the ground dwelling lesula monkey species and heterospecifics. Furthermore, the lesula monkey is relatively understudied and no study has investigated mixed species group behavior. The objectives of our study were to use ground camera trap data to: (1) document polyspecific associations between lesula and sympatric species, (2) report the frequency of these associations, (3) investigate if variation in association patterns exists among survey sites, and (4) analyze the behavioral responses of lesula individuals when found in close proximity associations with heterospecifics.

Methods

Study site

LNP is an 8,874 km² area of undeveloped lowland rainforest that lies within the 21,000 km² TL2 landscape (named after the Tshuapa, Lualaba, and Lomami Rivers bordering the area) (Figure 1). This area of the Congo Basin is relatively understudied, and little is known beyond the knowledge of local hunters. Since 2007, members of the Frankfurt Zoological Society’s TL2 Project have surveyed the area and found a biodiverse ecosystem with a number of endemic animal species. The project documented bonobos (Pan paniscus), confirmed the presence of okapi in the park (Okapia johnstoni), documented the threatened and commonly hunted African forest elephant (Loxodonta cyclotis),
and discovered the lesula monkey (Cercopithecus lomamiensis), which is a unique species that spends most of its time on the forest floor, unlike other guenons. Through collaborations with the Congolese Institute for the Conservation of Nature (ICCN), local hunters and villagers, and regional governments, the LNP was established in 2016. The TL2 Project is ongoing, and patrol teams regularly survey the park and buffer zone for illegal hunting, document species, and contribute to the conservation of its biodiversity.

**Camera trap surveys**

The Florida Atlantic University (FAU) Primatology Lab has an ongoing collaboration with the TL2 Project of the Frankfurt Zoological Society in the DRC (Hart, 2020). Our study is part of the Lesula Project, a larger project on the lesula monkey based on camera trap surveys that were conducted along the forest floor over a three-year period in the LNP and buffer zone in the DRC. We used both Moultrie and Bushnell cameras of similar standards and they were programmed to take videos at a resolution of 1280x720 pixels. Camera models consisted of Bushnell trophy cam, Moultrie M100, and Moultrie M880. The lesula species was recorded at three survey sites, Losekola, Okulu, and E15, located between the Lomami and Tshuapa Rivers (Figure 1). Two survey sites (Losekola and E15) were located inside the protected park area, and one site (Okulu) was located in the buffer zone, where mammals are heavily hunted (Figure 1). Forty-one cameras were placed in Okulu between October and December 2013, 41 cameras in Losekola between January and November 2014, and 20 cameras in E15 between August 2015 and January 2016. We recorded the sampling effort as the total number of days the camera traps were running. The video length for each camera varied from 20 to 90 seconds, the difference in video durations were caused by camera trap models used and its deterioration of quality after extensive exposure outdoors. The Primatology Lab at Florida Atlantic University (FAU) received the camera trap videos from the TL2 Project (Hart, 2020).

**Species analysis**

After receiving camera trap data, we watched all videos using VLC format v. 3.0.8 along with QuickTime player 7 v. 10.5 software (n = 1,885 in Okulu, n = 9,179 in Losekola, n = 3,570 in E15) and logged all species captured into a database (Fournier Korchia, 2020). Throughout the project, the lab recruited FAU undergraduate and high school students to input camera trap data. We created a biological inventory with the known species and listed a description of each species’ physical characteristics accompanied with a video. Lab volunteers used this inventory as a reference list to provide a visual aid for identifying species. We organized the videos in an excel database by survey site and camera trap station and in chronological order by date and time of day. After input, we cross checked the data with an excel database completed by TL2 Project field biologists in the DRC. We then created a cumulative excel database by correcting inaccuracies and inconsistencies in species identification.

**Polyspecific association events**

For the Lesula Project, researchers organized lesula videos into 30-minute events from the same camera, defined as a series of chronological videos where a cluster of activity was observed (Fournier Korchia, 2020). In this study, we used the same event duration for comparison purposes. We differentiated two types of polyspecific associations (close and distant proximity) and recorded an association event when at least one video displayed lesula monkey(s) in close proximity with individual(s) from another species (Table 1). Event data sheets were organized by species, survey site, and in chronological order. We calculated the percentage of polyspecific association events compared to the total lesula events calculated in the Lesula Project. We also compared the number of polyspecific associations between species and survey sites.

**Behavioral Analysis**

Looking at each event, we counted the total number of lesula monkeys recorded in associations along with the total number of lesula monkeys in close proximity to heterospecifics. We determined if the same individual occurred in consecutive videos if it was in the same spot or if multiple videos tracked their activity pattern (i.e., an individual started walking in one video and in the next video a monkey, presumably the same individual, continued to walk in the same direction). We recorded the time (in seconds) each lesula monkey was in a close proximity association with heterospecifics. We then compared the percentage of time lesula monkeys spent in close proximity versus distant proximity within all polyspecific associations.

To understand behaviors of lesula during associations with each species, we sampled all lesula individuals during close proximity using continuous focal sampling. Focal animal sampling is typically done with one animal at a time; however, with camera trap videos, multiple animals can be sampled by replaying the video and selecting a new focal animal until all individuals are sampled. We recorded behaviors following the ethogram in Table 2. During observations, behaviors were scored using a one-zero sampling method. To determine the type of association, we organized each behavior into one of the two categories: relaxed or aroused (Table 2).

**Results**

**Camera trap surveys**

The camera traps ran for a total of 5,960 camera trap days (n = 1,551 in Okulu, n = 2,430 in Losekola, n = 1,979 in E15) (Fournier Korchia, 2020). Researchers recorded a total of 598 lesula events out of the three surveys, which represents a capture rate of 10 events/100 camera trap days (Fournier Korchia, 2020). Here, we recorded 34 events of lesula associating with one species and one event
of lesula associating with two species (Cercopithecus mitis and Philantomba monticola), which represents 5.9% of all lesula events and an association capture rate of 0.6 events/100 camera trap days.

**Polyspecific Association Events**

We recorded lesula in polyspecific associations with multiple bird species (crested guinea fowl, Guttera pucheranii, and two unidentified species), one primate species (blue monkey, Cercopithecus mitis), two duiker species (Weyns’ duiker, Cephalophus weynsi, and blue duiker, Philantomba monticola), and one species of mongoose (long snouted mongoose, Xenogale naso) (Table 3).

Lesula was found to be in association more often with the blue duiker (68%) than any other species across all three surveys (Figure 2a & b). We recorded higher rates of polyspecific associations inside the LNP (E15 followed by Losekola) than in the buffer zone (Okulu) (Figure 3).

**Association types**

We recorded a total of 130 lesula monkeys in association events, of which 43% (n = 56 lesula) were in close proximity with sympatric species. Out of the total time of polyspecific associations (n = 2962 seconds), lesula was in close proximity with heterospecifics 21% (n = 626 seconds) of the time. When in close proximity associations, lesula individuals displayed relaxed behaviors at least once across all species. However, aroused behaviors (n = 6 behavioral scores) were observed with only three of the five species captured in association with lesula: C. mitis, P. monticola, and X. naso (Figure 4). Overall, relaxed behaviors occurred in 93% (n = 69 behavioral scores) of the close proximity associations (Figure 4).

**Discussion**

Similar to arboreal Cercopithecus monkeys, our results confirmed that the lesula species forms polyspecific associations on the forest floor. Of all sympatric species, the blue duiker was found to associate most often with lesula and was the only species captured in association with lesula through all three surveys. Studies on interspecies interactions have discussed driving factors behind polyspecific associations, which include an increase in foraging efficiency and a decrease in predation risk (Heymann & Hsia, 2015; Cords, 1990). One species may exploit the other to locate resources, or both species may collaboratively increase their foraging efficiency. Ungulates have been found to co-occur with primates to benefit from their foraging habits (Heymann & Hsia, 2015; Newton, 1989; Desbiez et al., 2010; Newton, 1989; Tsuji et al., 2007; Koda, 2012). Some primate species are known to leave behind and drop unfinished food items to the forest floor, which are exploited by ground dwelling species, such as antelope and deer (Heymann & Hsia, 2015; Prasad et al. 2010). Similarly, individuals may reduce predation risk by benefiting from antipredator strategies of another species or by having a shared defensive strategy. In a larger mixed-species group, species may gain protection from increased vigilance, dilution, and through primate alarm calls (Rainey et al., 2004; Isbell & Bidner, 2016; Cords, 1990).

The blue duiker species may utilize lesula for protection and foraging purposes. As lesula lives in larger groups than the monogamous blue duiker, it may be an opportunity for the blue duiker to increase vigilance and dilution effect. Additionally, relaxed behaviors were the most common category scored during associations. This suggests that neither species views each other as a threat or as potential competition. Duikers were observed initiating play with lesula or playing while lesula was present. Further analysis should look at possible dietary or home range overlap to find possible explanations of the high association pattern (Waser, 1982; Erinyjery et al., 2016).

Associations were mostly captured between two species; however, we observed one association event composed of three species (blue monkey, blue duiker, and lesula). This event displayed lesula and blue monkey individuals in a close proximity interaction; whereas, one duiker appeared at the end of the event in a distant proximity association. This is the only event recorded with lesula interacting with another guenon species and it contained aroused behaviors. This may suggest a potential competitive interaction for food resources or territory between arboreal guenon species.

When comparing the three survey sites (Okulu, Losekola, and E15), Okulu had the smallest number of association events, which is likely due to the impact of hunting on the mammal population in the buffer zone. Overall, interspecies events were rare, making up less than 6% of all lesula events, which may be due to the use of camera traps as a detection tool. While camera traps are beneficial to study unhabituated species, some limitations may be reported. We expect undocumented associations to have occurred outside of the camera trap detection zone. Additionally, the duration of an association was also limited by the camera detection zone, where animals moving out of sight could no longer be scored.

The understudied lesula monkey is a unique species as it is the only guenon to mostly occupy the forest floor in the Lomami National Park and thus provided us with the opportunity to investigate its interspecies interactions. This study generated new knowledge about the sympatric species that lesula associates with. Future studies that set cameras in the canopy will expand our knowledge on the multiple species in association with lesula. Understanding their mixed species associations provides new insight into lesula’s behavior and ecology and the overall community ecology of the Lomami National Park.
Acknowledgments

Special thanks to our collaborators Drs. John and Terese Hart of the Frankfurt Zoological Society’s TL2 Project for field camera trap data collection and data sharing. Thank you to the undergraduate and high school volunteers of the FAU Primatology Lab’s camera trap team for inputting and organizing data. A special thank you to the graduate student TL2 members: Daniel Alempejievic and Charlene Fournier, for their feedback and guidance. This project was advised by Dr. Kate Detwiler who enabled an already present interest in primate behavior and gave me valuable advice and guidance for the project.

References


Tables

Table 1. Definitions of the two type of associations captured between lesula and other species.

<table>
<thead>
<tr>
<th>Type of association</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close proximity</td>
<td>Lesula co-occurs with another species within the detection zone of the camera at the same time (i.e. observer sees animals together on the computer screen).</td>
</tr>
<tr>
<td>Distant proximity</td>
<td>Lesula occurs with another species within the same event (i.e. observer sees another species in the event but not together at the same time).</td>
</tr>
</tbody>
</table>

Table 2. Ethogram of the different behaviors displayed by lesula separated in two categories: relaxed and aroused.

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relaxed behaviors</td>
<td>Focal animal displays a behavior of relative un-alertness.</td>
</tr>
<tr>
<td>Following</td>
<td>Individual walking in the same direction, in close or distant proximity or walking towards the same side of the camera frame.</td>
</tr>
<tr>
<td>Foraging</td>
<td>Individual searching surroundings for food items, such as leaves or fruits.</td>
</tr>
<tr>
<td>Playing</td>
<td>Individual performing non-aggressive chasing, grabbing, and/or wrestling.</td>
</tr>
<tr>
<td>Walking</td>
<td>Individual displaying forward locomotion on four legs at a slow gait.</td>
</tr>
<tr>
<td>Stationary</td>
<td>Individual not moving (sitting, standing, or clinging).</td>
</tr>
<tr>
<td>Aroused behaviors</td>
<td>There is an increased sense of alertness by the focal animal.</td>
</tr>
<tr>
<td>Fleeing</td>
<td>Individual involved in flight behavior, movement in the opposite direction in response to chasing and/or viewing or interacting with another individual.</td>
</tr>
<tr>
<td>Chasing</td>
<td>Individual running rapidly or leaping toward another individual.</td>
</tr>
<tr>
<td>Running</td>
<td>Individual displaying forward locomotion on four legs at a rapid gait.</td>
</tr>
</tbody>
</table>
Table 3. Number of associations between lesula and other species captured during a lesula event at the three camera trap sites.

<table>
<thead>
<tr>
<th>Category</th>
<th>Species</th>
<th>Okulu</th>
<th>Losekola</th>
<th>E15</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bird</td>
<td>N/A</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Primate</td>
<td>C. mitis</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Duiker</td>
<td>P. monticola</td>
<td>1</td>
<td>9*</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>C. weynsi</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Mongoose</td>
<td>X. naso</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2</td>
<td>14</td>
<td>20</td>
<td>36</td>
</tr>
</tbody>
</table>

* The one event where lesula was captured with two heterospecific species (C. mitis and P. monticola) was counted in separate categories in this table (one association with C. mitis and one association with P. monticola).
Figure 1. Map inset: Lomami National Park in the Democratic Republic of the Congo. Enlarged map: The three camera trap survey sites in the Lomami National Park and its buffer zone (Okulu, Losekola, and E15).
Figure 2a. (top) Still frame of lesula (Cercopithecus lomamiensis) standing in front of the blue duiker (Philantomba monticola) in close proximity. Figure 2b. (bottom) Frequency of polyspecific associations between lesula and other species at the three sites combined.

Figure 3. Percentage of polyspecific events between lesula and other species at the three survey sites: Okulu (n = 2), Losekola (n = 13), and E15 (n = 20).
Figure 4: Frequency of the two types of polyspecific associations (relaxed and aroused) based on the analysis of lesula behaviors in close proximity with heterospecifics at the three survey sites (n = 75 behavioral scores).
proposed that agency emerges from initially spontaneous and comparison of goals with outcomes, Kelso (2016) which rely on goal-directedness, mental planning, of adult agency (Chambon, et. al, 2014; Wegner, 2002) disruption in sense of agency. may lead to insights into mental disorders where there is a organization itself, how basic cognitive capacities emerge and agency forms is valuable for understanding how the brain a sense of agency is the feeling of control over one's actions and that those actions affect the environment. While agency has been studied in adults, the process of agency formation is unknown. We conducted a mobile conjugate reinforcement (MCR) experiment to explore agency formation in 16 healthy, 2-3 month old infants. In MCR, an infant is placed supine in a crib and their foot is tethered to a string. The mobile spins when the string is pulled. The larger/faster the infant movement, the faster the mobile spins. We first assess whether our novel motorized mobile replicates previous MCR results (Rovee & Rovee, 1969). Similar to past results, there was a significant increase in kicking rate when the infant was tethered to the mobile. This increase in kicking is a hypothesized signature of agency formation (Kelso & Fuchs, 2016). Our results validate the mobile and allow further analysis of agency formation.

Keywords: mobile conjugate reinforcement, infant learning, coordination, reinforcement

Introduction

A sense of agency refers to the feeling of control over one's own actions and that those actions affect the environment. Chambon, Sidarus, and Haggard (2014) suggested that humans feel this sense of agency when the predicted consequences of a goal-directed action match the actual consequences of that action. Additionally, a sense of agency may arise when a change in the environment can be traced back to one's own voluntary action and no other plausible causative force. While there is extensive research discussing what sense of agency is and how it relates to adult behavior and neuroimaging, there is a lack of scientific research discussing how a sense of agency emerges in the first place (Kelso, 2016). The understanding that our actions can affect change in the world is a basic and critical capacity for human survival and success. Exploring how sense of agency forms is valuable for understanding how the brain organizes itself, how basic cognitive capacities emerge and may lead to insights into mental disorders where there is a disruption in sense of agency.

In a departure from traditional explanations of adult agency (Chambon, et. al, 2014; Wegner, 2002) which rely on goal-directedness, mental planning, and comparison of goals with outcomes, Kelso (2016) proposed that agency emerges from initially spontaneous infant movement. A young infant may begin moving with no particular goal or plan in mind, but when these spontaneous movements result in some perceptible change in the infant's environment and when infant movement and environmental response are sufficiently coordinated, the infant will suddenly realize that its own movements are making things happen in the world (Kelso, 2016).

Kelso (2016) identified Rovee and Rovee's (1969) mobile conjugate reinforcement (MCR) paradigm as a possible window into the process of agency formation in infancy. MCR involves three phases of experimentation (Rovee & Rovee, 1969). During each phase, the frequency of infant kicking was measured. Phase 1 of MCR was a baseline period in which the infant was placed supine in a crib and observed a stationary mobile overhead. In Phase 2, the infant's ankle was tethered to the mobile by a silk cord that was connected to the mobile. The mobile, composed of colorful wooden figures, moved when the string was pulled. The faster or larger kicks produced by the infant, the more the mobile moved. Although the motion of the mobile has never been measured, conjugate reinforcement refers to the feedback the infant receives because any amount of movement from the mobile is assumed to be directly proportional to the size and frequency of the kicking produced by the infant (Angulo-kinzler, 2001; Kelso & Fuchs, 2016). The string was disconnected in Phase 3, and the infant observed a stationary mobile. Infants kicked at a rate of about 10 kicks per minute during Phase 1, then multiplied their kicking rate by a factor of 3-4 during Phase 2. During Phase 3 the kicking rate gradually returns back to the kicking rate of Phase 1 (Rovee & Rovee, 1969). In order to control for the possibility that an increased kicking rate could be attributed to a generalized state of arousal in response to the movement of the mobile, a control group of infants were also tested. In the control group, Phase 1 and Phase 3 were identical to the experimental group, however during Phase 2 an experimenter moved the wooden figures instead of the infant. Half of the infants in the control group received visual stimulation from the moving mobile and the other half received combined visual stimulation from the moving mobile and somesthetic stimulation from the silk cord that was looped around their ankle; though leg movements did not affect the mobile's movements. The results showed that neither infants in the visual stimulation group nor combined visual and somesthetic stimulation group
increased their kicking rate during Phase 2 from the baseline rate. This confirms that the increased kicking rate during Phase 2 for infants in the experimental group was a result of learned contingency between the infant's leg movements and the mobile, not just a generalized state of arousal in response to the moving figures. Infants only increased their kicking rates when the mobile moved as a result of the infant's movement (not the experimenter's). Therefore, the tripling in infant kicking rate during Phase 2 hints at the emergence of agency in these infants as they realize that they are in control of the mobile's movements (Kelso & Fuchs, 2016).

One hypothesis about MCR is that the infant learns the relationship between its kicks and the movement of the mobile: every kick moves the mobile, which provides an attractive visual stimulus that reinforces the infant to continue kicking. If agency may be said to emerge when the infant realizes its movements can cause the environment to change (here the mobile), then the MCR model could be used to gain a closer understanding of how the sense of agency emerges by observing the infant in control of the mobile's movements. While emergence of infant agency seems to depend on the coordination of infant and mobile movement, no previous MCR study has measured mobile motion or coordination between infant and mobile.

The aim of this study is to replicate previous results produced by MCR using a novel motorized mobile. Replicating previous MCR results will validate the novel mobile apparatus used here and allow for further analysis of the coordinative basis of the emergence of agency.

Method
Participants

Participants were 16 infants who ranged in age from 78 to 160 days (M=104.93, SD=23.58) and ranged in weight from 10.06 to 17.40 pounds (M=13.49, SD=2.00). All infants were apparently healthy, full term infants (more than 39 weeks gestation) and were tested at a time of day where the mother reported the infant to be awake and active. Infants were recruited through postcards mailed to families informing them of the study. Participants were excluded from the study if they reached an age from 78 to 160 days (M=104.93, SD=23.58) (Table 1).

Procedure

In this experiment, the mobile was controlled by a motor and composed of two blocks decorated in colorful paper that hung above the crib. The mobile spun when a string was pulled, tilting a sensor. Before the start of the experiment the infant was strapped supine in a crib to prevent the infant from rolling over, and a sock with snaps was placed on either the infant's left or right foot. The MCR experiment consisted of four phases: Baseline 1 (2 min.), Baseline 2 (2 min.), Coupled (5 min.), and Decoupled (2 min.). During Baseline 1, the infant observed a stationary mobile overhead. In Baseline 2, the experimenter tugged on the string that caused the mobile to spin and the infant observed the spinning mobile. In the coupled phase, a string that was also connected to the mobile was connected to the sock using the snap. The faster the infant moved and the larger the infant movement, the faster the mobile spun. In the coupled phase the string was disconnected, and the infant again observed a stationary mobile. The infants were videotaped throughout the entire experiment. Using the Behavioral Observation Research Interactive Software (Friard & Gamba, 2016) behavior coding software, one coder viewed the videos and recorded the frequency of kicks in each phase of the experiment for each infant. To test reliability, a second coder independently recorded the frequency of kicks for 13% of the sessions. Inter-rater reliability raised from 20.7% agreement to 61.4% after training. A kick was defined as a vertical or horizontal excursion of the right foot which at least partially retraced its original path in a smooth, continuous motion (Rovee & Fagen, 1976). Further work will use quantitative measures of infant movement.

Results

A repeated measures analysis of variance was conducted on the number of kicks per minute across 2 minutes of each phase: Baseline 1, Baseline 2, Coupled, and Decoupled. The test showed a significant main effect for the phases, F(3, 36)=4.13, p<0.05. The test also showed a significant cubic effect F(1, 12)=17.65 p<0.05. The number of kicks per minute decreased from Baseline 1 (M=6.81, SE=2.12) to Baseline 2 (M=5.50, SE=1.71), and then increased from Baseline 2 to the Coupled phase (M=12.35, SE=2.00), returning to baseline rates in the Decoupled phase (M=8.31, SE=1.76) (Table 1).

In addition, an independent samples t-test between the mean number of kicks per minute, summed across all the infants (and not for each infant), for the Baseline 2 (2 minutes) and Coupled phase (5 minutes) was used to evaluate if the kicks in the Coupled phase was higher than in the Baseline 2 phase. The test was significant in a two-tailed test, t(5)= 3.02, p<0.05. There were more kicks per minute for the Coupled phase (M=8.80, SD=0.85) compared to the Baseline 2 (M=5.50, SD=1.36) phase.


**Discussion**

This study was able to replicate past findings of the MCR paradigm and validated our use of a novel motorized mobile as a relevant stimulus to the babies. Similar to past results (Rovee & Rovee, 1969), the infants increased their kicking rate significantly from the Baseline to the Coupled phase and lowered their kicking rate back down to baseline rates during the Decoupled phase. The results also indicated that visual stimulation from the mobile alone, presented in Baseline 2, did not significantly increase the kicking rate in these infants from Baseline 1. In fact, the kicking rate decreased from Baseline 1 to Baseline 2. This decrease in kicking rate could be attributed to a surprise/interest reaction upon observing the mobile spin (Piaget, 1952; Sullivan & Lewis, 2003). As no increase was observed when the mobile moved in Baseline 2, the increased kicking rate in the Coupled phase could not just be due to a generalized state of arousal in response to the moving mobile. This indicates that the increased kicking rate during the Coupled phase was due to the infants learning the relationship between their movements and the spinning of the mobile.

The significant increase in kicking rate from the baseline to the Coupled phase hints at the emergence of agency in these infants as they realized they were in control of the mobile’s movements. Further analysis of infant movement may elucidate this issue. Validating the current novel motorized mobile in this experiment allows for further research into how the formation of agency in these infants during the Coupled phase depends on coordination between infant and mobile. In this study, and in other studies using the MCR paradigm, the movements of the mobile have not been measured, nor their coupling to the infant’s actions (for preliminary findings see Sloan, Jones & Kelso, 2020). Such analyses of movement between the infant and mobile should afford new insights into how agency forms as a result of organism–environment coupling (Kelso, 2016).

Overall, through the current novel motorized mobile the infants were able to learn that they were in control of the mobile’s movements and a sense of agency is hypothesized to have emerged in these infants while interacting with the mobile. The present preliminary findings allow for further analysis into how and when the sense of agency emerges, thus providing a scientific basis for the emergence of agency, currently lacking in the literature. Moreover, if the present work was extended, e.g. to include detailed measures of brain activity, it might allow even deeper insights into the workings of the infant brain and its relation to goal-directed behavior.

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**Table 1**

*Descriptive statistics for the mean number of kicks per minute in the Baseline 1, Baseline 2, Coupled, and Decoupled phase. N=15.*

<table>
<thead>
<tr>
<th>Phase</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 1</td>
<td>8.46</td>
<td>10.10</td>
</tr>
<tr>
<td>Minute 2</td>
<td>5.15</td>
<td>5.91</td>
</tr>
<tr>
<td><strong>Baseline 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 3</td>
<td>6.46</td>
<td>8.39</td>
</tr>
<tr>
<td>Minute 4</td>
<td>4.53</td>
<td>6.02</td>
</tr>
<tr>
<td><strong>Coupled</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 8</td>
<td>11.31</td>
<td>8.37</td>
</tr>
<tr>
<td>Minute 10</td>
<td>13.38</td>
<td>12.69</td>
</tr>
<tr>
<td><strong>Decoupled</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minute 12</td>
<td>8.58</td>
<td>7.78</td>
</tr>
<tr>
<td>Minute 13</td>
<td>8.23</td>
<td>6.25</td>
</tr>
</tbody>
</table>

*Note: Three infants did not participate in Baseline 2 and as a result they were not included in this sample.*
References


Sullivan & Young Children, 16(2), 120–142. doi: 10.1097/00001163-200304000-00005

A CONCERN FOR THE TEACHING PROFESSION: IMPACTS OF DEMORALIZATION

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Abstract

Within the United States public education system, many teachers encounter feelings of demoralization. They feel they can no longer access the inherent moral benefits that initially drew them to have the desire to teach in the first place. This study examined the contributing factors that cause demoralization feelings amongst public educators and the long-term implications for the institution and the students it serves. Factors such as the teaching environment, administrative support, and state-mandated curriculum and testing contribute to a loss of control and ability to serve their students and community best. Analysis of a teacher’s anecdote indicates dissatisfaction with the profession due to demoralization. A demoralizing teaching environment can cause teachers to leave the profession as a whole. Departure from a career as an educator damages the teacher and negatively affects the students and districts that must fill vacant classroom teaching positions. Analysis of literature creates recommendations for further research and open dialogue between teachers, colleagues, and supervisors.

Introduction

Educators in the teaching profession, particularly within the United States’ scope, face a variety of personal and contextual hurdles that risk teacher attrition, and increase turnover rates. An overwhelming and rising problem facing educators today is demoralization. Unlike teacher burnout, teacher demoralization occurs when educators feel they can no longer access the moral benefits and fulfill an educator’s moral duty while serving their students. Teacher burnout is addressed by managing self-care, which is quite different from addressing demoralization. Demoralization requires changes in the structure of the institution in which demoralization takes place. This study reviews the literature on demoralization to examine how demoralization ultimately affects teachers, school districts, and students. Overall, teacher demoralization puts highly qualified and credentialed teachers at risk of leaving their position or the profession as a whole.

Problem Statement

Public educators are experiencing they can no longer fulfill their reason for teaching and feel demoralized, putting them at risk for leaving their position or the profession altogether (Santoro, 2013, 2014; Woestman & Wasonga, 2015). This problem leads to a strain on the supply of educators and risks high quality and experienced teachers leaving. This is important because students are left educationally vulnerable. To understand the impacts of demoralization, we address three important questions. First, what are the most significant differences between feelings of burnout and demoralization among teachers, and what does this imply about the way teachers feel towards their job? Second, to what extent do teacher attrition and teacher turnover correlate with high feelings of demoralization within the profession? Finally, how is demoralization manifested in the teaching profession, and what actions should be taken to mitigate this phenomenon?

Distinguishing Demoralization from Burnout

It is imperative to differentiate demoralization from burnout to examine the effects of demoralization. Demoralization and burnout may both lead to frustration within the field, job dissatisfaction, and risk of teacher turnover, but they are fundamentally different in their effects on professional educators. The same can be said about the failure to recognize demoralization and a misdiagnosis by outsiders as burnout. Misdiagnosis can lead to failure of remedy. The critical difference between these two phenomena is their cause. According to Santoro (2013), burnout occurs when teachers fail to preserve themselves and their wellbeing. The resources in burnout are personal to the teacher and often lead to teacher stress.

In contrast, the resources in demoralization are the moral rewards that attract teachers to the profession in the first place. Rewards such as meaningful student connections, innovative lesson plan design, and bettering the lives of students and the community are essential to keeping teachers happy about their work and how they execute it. When school policies take these moral rewards away from educators, teachers encounter demoralization (Santoro 2013). In this framework, burnout is a personal problem having a unique individual-based solution. Demoralization is anything but personal since its cause can come from school policies. Hence, it cannot be addressed at an individual level as burnout can. Demoralization must be addressed at the institutional level where policy is made. Some institutional causes of demoralization among educators include destructive relationships with superiors, imposed policy and structural change, state-mandated curriculum and testing, and an overall loss of autonomy over their profession. To better understand demoralization and how the institution challenges the moral commitments that
teachers make to their students, colleagues, and communities, this study will review the literature that focuses on the contextual factors within schools, administration, and education policymaking.

**Methodology**

This research explores the literature and studies that survey the potential causes and implications of demoralized teachers. The rationale underpinning this methodology is to produce contextual knowledge of the impacts of demoralization on teachers. A search was conducted with teacher and demoralization as keywords between the late 1990s and present to ensure time relevancy. The studies selected were qualified further by only utilizing peer-reviewed literature within this timeframe. A thematic analysis was conducted within this qualitative methodology to examine and identify themes and patterns amongst the peer-reviewed literature, such as significant contributors to demoralization and teachers leaving the profession. This methodology is essential as it highlights the research which has already been conducted but has yet to identify significant action within the United States education system and provide any remedy.

**Review of Literature**

In a study published in a Teaching and Teacher Education Journal, Glazer (2020) interviewed twenty-five former public school teachers from a range of schools and outlined several competing theories used to study teacher attrition. One was an organizational theory that focused on the teacher environment and how organizations are structured and function. Another, called self-efficacy theory, focused on an individual’s “ability to execute behaviors in pursuit of a desired outcome” (Glazer 2020). We will use these two theories to establish the findings of this paper and the demoralization framework.

**Teaching Environment & Administrative Support**

Teachers find themselves demoralized because of their relationships with their school administrative faculty and leaders within education. A 2017 Educator Quality of Work Life Survey (EQWL) was made accessible to public educators and school staff through convenience sampling. Sampling was executed via email and social media as well as a random sample of AFT members. Results indicated that “respondents felt least respected by elected officials, the media, and US Secretary of Education Betsy DeVos” (2017). These results may be partly because leaders within education can often display destructive leadership behaviors (DLBs). DLBs are “actions that would be considered harmful and deviant toward followers and/or the organization” (Wasonga & Woestman 2015). Supervisors who display DLBs towards their subordinates are aware of their harm regardless of whether they are motivated to act destructively or do not feel helpful and constructive. An example of such behavior is when school leaders undermine good work and discount teacher concerns for their students. Using teacher interviews, Santoro concluded that DLBs cause teachers to feel they cannot conduct the work they believe they are set to do (Santoro 2018). Based on the self-efficacy theory, as teachers feel they cannot produce the desired outcome, their moral commitment to themselves and their students is challenged. This challenge leads to feelings of personal job dissatisfaction from loss of professionalism and productivity declines for themselves and their students. It is these feelings that align with demoralization.

**Deprofessionalization in the Age of Accountability**

Additionally, something that’s been destructive to the teaching profession and a substantial cause of teacher demoralization is the age of federal accountability and assessment policies, all of which contribute to an organization’s structure and evaluated function related to the organizational theory camp. From the passage of the No Child Left Behind Act (NCLB) in 2001, the American Recovery and Reinvestment Act in 2009, to the passage of the Every Student Succeeds Act (ESSA) in 2015, federal accountability is characterized through Statewide standards, standardized testing, educator evaluation and data systems (Urick & Woestman 2019). Effects of organizational changes such as forced testing and mandated curriculum correlate with the EQWL survey findings. The EQWL survey found that “40% of respondents reported having no influence or only minor influence in establishing curriculum at their schools,” and “62% of educators somewhat or strongly disagreed that their schools have good systems of evaluation” (2017). Analysis of this data reveals that lack of control over the curriculum and school procedures contributes to removing teachers’ creative liberties and sense of what they feel is right for their students.

Organizational changes that deprive teachers of the creative process in teaching again strip educators of what they view as teaching’s mission. Federal accountability policies help one discern the difference between successful teaching and good or quality teaching. Under Federal policy, successful teachers are merely competent in delivering a state-mandated curriculum for their students to pass their state-mandated tests. Simultaneously, good teachers also sustain the ethical and virtuous dimensions of teaching (Santoro 2011). More often than not, it is those “good” teachers who encounter demoralization due to a loss of autonomy in their work created by federal accountability policies. State-mandated testing and evaluation systems have taken away the educator’s ability to craft lesson plans in a way they feel caters best to their students. Removing any authority they have over their craft leaves educators feeling less productive and incapable of doing their job.

Santoro (2011) examined specific teacher’s accounts regarding state-mandated testing. For example, Stephanie, who was expected to follow Virginia’s Standards of Learning in the 1990s, found that the shift to a prioritization of testing over teaching made it so that the supplemental test prep materials she gave to her class now translated as the entire curriculum. More specifically, Stephanie described it as a “slap in the face to me as a
professional” and “dismissed her pedagogical knowledge, constrained her pedagogical judgment, and so tightly circumscribed her pedagogical authority” (Santoro 2011). This disregard for an educator’s pedagogical opinion and autonomy over class content falls into the self-efficacy theory because the teacher cannot work towards their desired outcome. In this case, state-mandated testing undermined Stephanie's desire to connect the student's learning with their experiences. This disconnect not only demoralizes teachers but is deprofessionalizing teaching as a whole. Another analysis of narratives from former teachers identified as “invested leavers,” certified teachers who made it past their new teacher phase and left schools entirely never to return, not even in the form as administrators or in nonprofit organizations. The overall themes for their resignation included “autonomy and imposed curricula, testing culture, and bureaucracy and job insecurity” (Glazer 2020). These themes hold when compared with data from the EQWL survey (2017). The results of this survey showed that teachers felt little to no control over their day to day classroom level decisions and that much stress does not come from students but comes from the flux of testing and other state requirements. Much like Stephanie had, many qualified and credentialed teachers grew dissatisfied with their job and the obstacles created by their supervisors and state mandates and left teaching because of prolonged demoralization. Much more than just teacher attrition or turnover, demoralization comes at a higher cost for both school districts and students.

Results

One of the most significant differences between feelings of burnout and demoralization among teachers and what this implies about teachers’ feelings towards their jobs is that burnout is a matter of a teacher’s self-care. In contrast, demoralization is a matter of situations the teacher cannot control and instead is forced upon them, circumstances such as state-mandated curriculum.

Demoralization is dangerous to the teaching profession as it relates to higher rates of attrition and difficulties in retaining teachers. This result answers the second research question by showing a positive association between teacher attrition and teacher turnover with high demoralization feelings within the profession. With qualified and seasoned educators leaving the profession or moving to different schools, students across affluent and low socioeconomic school communities are at risk. A thinning teacher force due to prematurely leaving the profession can lead school administrators to resort to what is known as out of field teaching. According to the National Education Association, out of field teaching is where teachers instruct subjects in which they have little to no qualifications. As it turns out, a third of high school math teachers have neither a major or minor in related disciplines, the same goes for a fourth of English teachers, and more than half of high school history students’ teachers lack a related major or minor (Ingersoll, 1998). With teacher demoralization threatening high attrition rates, difficulty filling teaching positions with quality and qualified teachers has made administrators “hire less qualified teachers, assign teachers trained in other fields or grade level to teach in the understaffed area and make extensive use of substitute teachers” (Ingersoll, 1998). More often than not, novice teachers are those put into out of field positions. High levels of out-of-field teaching and lack of experienced teachers leave students with limited opportunities educationally vulnerable to unqualified teachers.

On top of costing students a quality education, teacher attrition is expensive for school districts. In response to the second research question, how is demoralization manifested in the teaching profession, and what actions should be taken to mitigate this phenomenon? Research shows that there are many underlying costs when filling in teaching vacancies. These costs can be broken down into separation costs, replacement costs, and training costs for the newly hired. These categories are further explored in a study that explored calculating the cost of teacher turnover. Separation costs are associated with teachers who classify as the invested leavers described earlier, transferring schools, or retiring. Replacement costs cover travel for recruitment purposes (e.g., job advertisements, recruitment fairs, and incentives). Training costs classify as the cost for new teacher orientations and fees paid by the school district to attend professional development presentation sessions (Watlington et al., 2010). This same study uses these categories to calculate best the cost of teacher attrition in two Southeast Florida school districts. In the 2004-05 academic year, St. Lucie County School District’s cost per teacher replaced was $4,631, and in Broward County School District, it was $12,652 per teacher. This financial cost is just one of many considering the emotional and morally damaging cost for teachers who have left the profession or the cost to a student’s education.

Recommendations

Based on our analysis of the literature, we can make a couple of recommendations. It is evident that many considered burnout and demoralization to be intertwined and interchangeable. Based on teacher narratives, it is essential to differentiate the two as they are not the same, nor do they affect teachers the same. Burnout is a matter of a teacher’s self-care, while demoralization is a matter of school policy that exogenously challenges the teacher’s moral obligations. Because of this, we make two recommendations. First, we recommend there be further research into demoralization amongst public educators. Second, we recommend that school leaders facilitate discussions with teachers to promote constructive dialogue and teacher voice. In these discussions, we encourage teachers and educational leaders to take a step forward and have the difficult conversations to propose resolutions to the value and moral conflict that bring about demoralization. Additionally, this open dialogue should address which state mandates are negotiable, non-negotiable,
and which policies are already efficient and conducive to a healthy working environment. These steps will help alleviate and mitigate the moral roadblocks that teachers face within this career path.

Conclusion

Based on this literature review, we conclude that state education mandates and destructive leadership behaviors from school administrators and education professionals pose a threat to the teaching profession’s moral rewards. Both organizational and self-efficacy theory served as guides to understanding the different contributors to demoralization feelings amongst teachers. The changes in the structure of teaching fostered the loss of autonomy and feeling less efficacious for educators. These moral conflicts put highly qualified and credentialed teachers at risk of leaving their position or the profession as a whole. High teacher attrition and turnover rates have more than an emotional cost to our teachers’ wellbeing and status as a whole. They cause inefficiency in our educational system by leaving students educationally vulnerable to underqualified teachers and stressing school district budgets. New teachers must be hired and trained to fill vacant teaching positions.

References


The word "south" in American southern literature is often excluded into a common definition and heavily stigmatized. Many writers suggest that it is time to remove the imaginary borders and stigmas encompassing the South, such as those attached to slavery, the civil war, and religious devotion. One such border is the generalization that it is possessed by a singular faith, namely a protestant-centered faith. This stagnant faith hanging over the South is conflicted by the variety of faith found in contemporary southern fictional literature. This research elaborates on the definition of the South by introducing a new perspective of faith presented in American southern fiction. Particularly, this research analyzes how three short stories represent multiple versions of faith through the narration, character actions, and literary language used. The representations of faith outlined in this essay include faith in physical works, in the inherent goodness of humanity, and in personal redemption.

Abstract

The word "south" in American southern literature is often excluded into a common definition and heavily stigmatized. Many writers suggest that it is time to remove the imaginary borders and stigmas encompassing the South, such as those attached to slavery, the civil war, and religious devotion. One such border is the generalization that it is possessed by a singular faith, namely a protestant-centered faith. This stagnant faith hanging over the South is conflicted by the variety of faith found in contemporary southern fictional literature. This research elaborates on the definition of the South by introducing a new perspective of faith presented in American southern fiction. Particularly, this research analyzes how three short stories represent multiple versions of faith through the narration, character actions, and literary language used. The representations of faith outlined in this essay include faith in physical works, in the inherent goodness of humanity, and in personal redemption.

Essay

Southern Literature is a problematic enterprise when it comes to identifying key attributes of the genre. The value of exploring literature comes in the reader's ability to see language working as a combination of fictional, moral, linguistic, impractical, and normative expressions ( Eagleton, The Event of Literature 25). Such complexity suggests that trying to pin down a single definition of a genre, say southern literature, is not a straightforward enterprise. Thomas Haddox argues that the South should not be a term used to describe southern literature since readers may instantly think of abused slaves, the civil war, and a devoutly righteous people. Instead, the plural term, the "Souths", should be used when describing southern literature, to prevent it from being perceived from a stigmatized point of view (Haddox 250). Haddox demonstrates that there is no single work of literature that could stand for the absolute mother of all southern literature, but that each subculture within this southern culture has the opportunity to branch off into its' group that makes up the definition of the "Souths" (260; Mackethan). Therefore, the most reasonable attitude and approach one should have when discussing southern literature is to wrestle with the multitude of definitions and be open to discussion (Haddox 260). Ultimately, southern literature reflects the South as it is an ever-evolving subject that cannot be restrained to a single point of view. My research focuses on the analysis of three short stories written by contemporary American Southern writers and how diverse representations of faith can be understood through the narration, character actions and literary language found within southern fiction.

Susan Ketchin introduces the South as one that has been not Christ-centered but Christ-haunted. Southern writers regularly write on topics of religion and faith, being thoroughly concerned with the salvation of souls, loss or gain of eternity, and humankind's desire for God. However, an ever-changing society has posed a threat to the beloved southern values of family, community, and place (Ketchin, The Christ Haunted 15). Nevertheless, writers of the American South collaborate with this evolving region, fueled with nostalgia, by continuing to write about these cherished topics. For some writers, the most important aspect of producing southern fiction is to understand the complexities of faith, and for others, like Larry Brown, religious reference is a way to world-build in fiction (17). Still, religion holds tightly onto the writing of many literary artists, as a light to see the unfolding of the human experience (Ketchin, Religion in Twentieth 735). Ultimately, Ketchin depicts southern writers as individuals seeking to connect their southern origins to their understandings of spirituality and faith (736). Writers of southern literature reflect the shaping force religion has on the South by centering faith in their literary language. Many writers criticize the development that modernization has caused in the South, believing it has lost its sense of community that was not only tied to a faith-based, but also a societal force (Wilson 251). Writers of American southern literature are introducing a new view of faith in the American South through their writing.

Southern literature incorporates the effects that modernization has caused in the South and assists the reader in understanding what the South truly is. Micheal Bibler wants to "smash the Mason-Dixon line," meaning that he wants to remove the border that encompasses the South, inhibiting it from growing into the diverse society that it is. This invisible border promotes an exclusionary point of view of the South (Bibler 153). Depictions of faith in southern literature support the notion that faith is more expansive than the "protestant religion" that has blanketed the South for years. The South needs to be re-introduced through American literature and should be depicted in the light that the South is a diverse location that cannot be confined to standard definitions or ideals (154). Such as the standard definitions of faith in the South. Amazing southern literary artists and new ideas that are reshaping the South are dismissed because of the stigmas that have been associated with it (Ravenel). The audience of southern literature is limited as many readers believe in these stigmas and avoid the genre altogether. The genre of the "Souths" combines works based on their likeness but is upheld by the individual diversity of each; this shows how the many different southern groups
interact with their stained history and evolving society (Mackethan). American southern literature allows the reader to explore the differences between groups and acknowledge the diversity that makes up the South.

Furthermore, literary representations of southern faith may fail to capture the complexities of faith practices in the South. Faith is not confined to a single type of belief or religion. Contemporary southern literature points to the diversity of faith that is in the American South (Bibler 155). It is not limited to the Protestantism that haunts the South (Ketchin, The Christ Haunted 17). As a result, conceptions of faith in the South are not as narrow as one may think. The literary language used in southern fiction conveys this. Southern faith goes beyond Ketchin's landscape, which is evident in many works of American southern fictional literature. As it follows, the idea of a single stagnant way of belief hanging over the South is conflicted by multiple versions of faith presented in contemporary American southern short fiction.

When one reads a work of fictional literature, one can analyze the text in numerous ways by looking at how language functions through the various features that constitute the story. For example, both the narrative and character action of the story are two worthwhile qualities of literature to examine. The literary language used in the narrative and character actions of short fiction can position the reader to see different forms of faith. In “Zero Hour and I’m Taking Up Taxidermy” by Morris Collins, “Little Big Show” by Kathy Flann, and “SAB” by Ann Pancake one can see through the narration and character actions, how faith emerges in the form of physical works, a belief in humanity, and personal redemption. Character actions in fictional literature show the reader how the characters interact with the people and the world around them. In Flann’s “Little Big Show” Alexander is a man who has lost seemingly everything in life but still hangs onto the belief that humanity is ultimately good and that everything will be okay in the end. The protagonist in the Collins’ “Zero Hour” is losing his wife and has faith that acts of redemption will get her back. Lastly, the narrator in Pancake’s “SAB” has endured through pain and brokenness; nevertheless, she puts her faith in personal atonement. The prose in each of these stories suggests the various ways that the characters express belief in something other than themselves.

The narration incorporates literary language that demonstrates faith in the form of the character’s belief in physical works as a way to save his marriage. In the story, the narrator uses the first-person view inviting the reader into his life where he tries to win back his wife by resurrecting, through taxidermy, a dead bobcat he peeled from the road. The narrator described the state of their relationship, and presents a reason for picking up the bobcat, “my wife was heartbroken and almost gone, brittle as glass to the hammer of my dull touch” (Collins 125). This communicates to the reader that his wife is “almost gone,” yet the “almost” implies to the reader that there is still a thread of hope left to hang onto. Particularly, the protagonist of the story places his faith in acts of redemption and holds on to the hope that he will be able to win her heart back, believing that “sometimes love requires a gesture that transcends the usual ceremonies of marital discord.” (125). Consequently, the narrator poses the question, “what can you do when what you’ve already done is the problem?” responding that “Sometimes escalation is in order” leading to his dependence or faith in this last attempt to preserve unity (126). The narrative describes how the main character depends on this act of redemption as a way of maintaining faith that his marriage will work out in the end.

Furthermore, one can see how language enables the character’s actions to produce faith in physical works to win back his wife. In Collins’ “Zero Hour”, the main character interacts with the world around him as he peels the bug-eaten brittle bobcat off the pavement. Back at home, the narrator brings the reader and the bobcat down to the basement to begin work on the big cat. Hopefully, he declares, “And me? And now? Alone, yes. But not for long. A little spattered in gore, perhaps—but isn’t that the real stuff of the heart?” (130). He wishfully holds on to this idea that the cat could save his relationship, proclaiming that he is “alone, yes. But not for long” (130). The narrator trusts that healing and re-convention are possible, telling the audience, “where once this cat was mangled, I have made him whole. And where he was by man and tire rent, I have sutured him with bright neon stitching and, I should add, made him fatter than he ever was—for the flesh, like the heart, is more elastic than we think” (130). As he mends the dead, weathered, insect-chewed cat he hopes he can mend his own relationship. The declarations that he has “made him whole,” where once this cat was battered and bruised, and that he has “sutured him with bright neon” shows that the protagonist’s belief that there is still hope (130). He hopes that his attempt to reconstruct their marriage is bright enough, like neon, for hopefully his wife to notice. Additionally, the narrator indirectly interacts with his wife. Although hope is scarcer than food during a famine, when his wife “turn[s] and say[s], will you help me with these boxes?”, at a last desperate attempt to save his relationship with his wife, he helped her with the boxes (131). There is an implied faith in these acts of redemption. Faith that stuffing a dead animal as a present and helping with the moving boxes, that will take his disappearing wife away, will somehow bring back together their “trembling hands,” their “unfinished flesh” (131). The narrator is attempting acts of resurrection, like the physical resurrection of the deceased bobcat, as an allegory of his faith in the resurrection of his own marriage.

Comparatively, Flann’s “Little Big Show” is another piece of southern literature that utilizes narration in the third person view and character actions to portray faith in humanity. Alexander, the main character, has faith that humanity is ultimately good and that someone will come to his rescue. Consequently, he reaches out to his sister, girlfriend, parents, secretary, in faith that someone will be at the other end. At the beginning of the story, his sister's
girlfriend, parents, secretary, in faith that someone will be at the other end. At the beginning of the story, his sister's car is stolen along with Alexander's briefcase holding his precious proposal for work. Desperately, he thinks of all the people who could save him, thinking that "if his parents weren't trekking across the U.S.…. Maybe they'd have been excited to do him the favor since he rarely asks" (Flann 151). The narrative leads the reader to expect that Alexander's faith in his parents will result in his rescue. Through the bleak circumstances, he "realizes what it is, the thing he keeps wanting to know from everyone he loves: "Are you there?" he says" (159). Alexander has faith that someone he loves will respond. The continual hardships the narrator faces test his faith in humanity, especially in the face of impossible circumstances.

Additionally, the way the protagonist interacts with the world around him works to produce his faith in humanity. Throughout the story, the narration utilizes flashbacks in order to provide insight from the past that makes up Alexander's present. Through the instantaneous time travel trips, the reader learns that Alexander had endured some level of suffering. The reader can recognize this through the loss of his new-born infant. Alexander recalls the memory as he "blinks away the doctor's thick fingers, the way they worked tubes up the twins' noses," remembering how "they were perfect—too perfect to be alive and also too perfect to be dead" (157). Furthermore, soon after the death of their children, Alexander's wife, Sheryl, left him. Moreover, the once flourishing relationship he used to have with his sister began to dissipate as well. In an attempt to save this connection, Alexander invites his sister to go on a hike with him "as a way to win Marietta back," like a fearless warrior, "if that's what it took, he'd do it" (149). Coming down from the hike, they notice the car is not in sight. Someone stole it. Alexander struggles with the stolen car and the loss of his proposal. He sought rescue from his secretary and his parents, but neither was able to provide a helping hand. The narrator resorts to comfort as he looks for someone to sympathize with him. Eventually, he is able to contact Nichole, a newly made friend. Finally, someone shares his concern, "Oh no! That's awful!" she says. "Are you okay?" (159). Notwithstanding the reality of his life, Alexander has faith that someone will ultimately be there for him. His faith lies in the possibility that things will turn around for the good as he tries to mend the relationship with his sister or reaches out to someone for help.

Lastly, Pancake's "SAB", depicts how narration and character actions work to show faith arising from personal redemption. The narration presents the story from the first-person point of view of the main character writing to her cousin, Sull. Both the protagonist and Sull have endured hardships induced by others and procured from themselves. The narrator writes to her cousin after she had come back to their hometown after 15 years of being away, wishfully writing that Sull had "returned to reach for what, after my own wreckage, I found" (Pancake 3). The main character addresses Sull multiple times, and sympathizes with her, "Sull, I feel the hurt of you" telling Sull about how she had faith that things could improve through personal redemption (1). Personal redemption came in the form of life from the nature around them and the life found within her cousin. The narrator reinvented herself, in a way, through nature as she "learned how the trees, if you move between them long enough, will eventually rub off your dirt" and learned how to "hear [the] unsingable song" (6:1). The narrator moves from telling Sull about the woods and the hills that "if you can open, will carry past your pain" to what was going on in her present and how she observed her cousin (1). The narration shows the reader how an interaction with nature led the protagonist to have faith in a personal redemption.

Faith in personal redemption is not only seen through the narration of "SAB", but also through the character actions within the short story. The narrator depicts her life as one marred with her troubles since life had taken her "son, husband, breasts, innocence, righteousness, [and] security" (6). To this end, she did not only have faith that nature was working for her reparation but also life. The narrator depicts the life sewn within her surroundings, "still the land sings" even though her whole world was collapsing, there is still some life in the dying body. The main character tells Sull that she finds life in her, "I saw it. The live coal of you, Sull. I smiled myself, then turned away. Partly to give you privacy, but part because I wasn't sure. I wanted to be for a while with the probably. That sab I took for me" (8). Sab is a term the narrator used to describe salve, which is a substance used to promote healing. The narrator's sab is seeing the "live coal" that was Sull, burning to feed the fire, the life within her. Altogether, nature does something for the narrator, but it is the healing and the life within her cousin, Sull, that ultimately gives the narrator faith in her redemption. The healing power of the sab manifested itself from the goodness the narrator saw within her cousin. The narrative leads the reader to believe that the narrator draws faith that her healing was possible by seeing the healing of her cousin, Sull.

Ultimately, literary language functions to imply faith through the characters' beliefs in something other than themselves. Such as in personal redemption, hope in humanity, or physical acts. As previously stated, this idea is depicted in Collins' "Zero Hour" by showing the main character's attempt to save his marriage through the physical reconstruction of the dead bobcat he picked up from the road. Similarly, faith in humanity is shown in Flann's "Little Big Show" as Alexander holds on to the hope that someone will be there for him even though everything has been taken away from him. Lastly, the protagonist in Pancake's "SAB" has faith that personal redemption will heal her brokenness. Nevertheless, as a reader, it is important to remember that the characters' form of faith in these short stories can be interpreted in many different ways than just the ones presented here. When analyzing a fictional work, specifically the narrative and character actions, it is important to look closely at how the language functions to create each effect. Through these pieces of southern short fiction, the reader can better notice the diversity of definitions for faith that
that constitute the South. This interpretation fits with the long-running conversation that the New Southern Studies are addressing of the fluidity of the South. Understanding the literary elements of short stories equips the reader to see the complex and diverse representations of faith across the full range of southern literature.

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OVERWINTERING POPULATION DYNAMICS OF CENTROPOMUS UNDECIMALIS IN ST. LUCIE RIVER

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Abstract

Sixty-one common snook caught in the Indian River Lagoon by the Florida Fish and Wildlife Conservation Commission (FWC) were measured, sexed, implanted with a Vemco acoustic transmitter, and released so their movement could be tracked. Snook were clustered based on movement between river systems during spawning and non-spawning seasons, resulting in four clusters: Resident, Spawning Transient, Overwintering Transient, and Traveler. Sex and length of individuals were compared between clusters using ANOVA to determine if these characteristics influenced movement strategy. Resident and Traveler snook had the most even sex ratio compared to other movement strategies. The majority of snook were categorized as Travelers but Spawning Transient snook had the highest mean length. It couldn’t be concluded that St. Lucie estuary serves as a significant overwintering habitat particularly for large, female snook, possibly due to small sample size.

Introduction

Fish move about their environment in response to a variety of ecological and physiological demands and environmental conditions. Many mobile organisms move with changing environmental conditions to stay within optimal salinity or follow the flow of similar salinity concentrations to allow their osmoregulatory processes to adjust before returning to their original location (Heupel and Simpfendorfer, 2008). Myliobatis californica, bat rays have been observed feeding in warmer, shallower waters then moving to cooler waters to lower energetic demands and decrease gastric evacuation rate during digestion (Matern, Cech, and Hopkins, 2000). Water temperature has been identified as a key regulator in the spawning runs of Atlantic salmon (Salmo salar L.; Byrne et al. 2004) and migration of European eels (Anguilla anguilla; White and Knights, 1997) once it reaches a certain threshold. Snook, like many other organisms, rely on environmental cues to coordinate spawning and other types of movement (Young et al., 2016).

Centropomus undecimalis (common snook, hereafter, snook) is a catadromous gamefish that contributes millions of dollars to Florida’s economy through recreational angler expenses (Fedler, 2009). Snook have been identified as a species of special concern due to the stock depletion in the late 1970’s (Bruger and Haddad, 1986) and Florida now enforces strict regulations on their harvest including specified open and closed seasons (Muller et al., 2015). Snook are protandric hermaphrodites, so they all mature initially as males and most transition to females as they age. Male snook reach sexual maturity at 169 to 222 mm in total length (TL) and under a year old while most transition to female between 246 and 876 mm TL at about 1 - 7 years of age, although some remain male throughout their life (Taylor et al. 2000).

Snook typically spawn between April 15th and October 15th in inlets along the coast (Taylor et al. 1998) in temperatures >22°C and salinities >27‰ (Peters, Matheson, and Taylor, 1998). Snook form spawning aggregations where they broadcast spawn, freely releasing their gametes into the water column in inlets (Taylor et al., 1998). Aggregations serve several purposes including ensuring availability of other spawners, larval distribution, and resource availability (Boucek et al. 2017). These aggregations are vulnerable to overexploitation from fishing due to their predictability of timing and high density of fish (Johannes, 1978).

Although snook are known to travel to river mouths and inlets prior to spawning to take part in aggregations, some overwinter in a different estuarine system from where they spawn. For example, in these data there were snook taking part in spawning aggregations in the Loxahatchee River in Jupiter, FL (Figure 1A) that overwintered in the St. Lucie River (Figure 1B) in Port St. Lucie, FL. Habitats serve different purposes in the life history of common snook possibly making them more important to a specific size or sex of snook. Snook are known to travel to several spawning sites outside of their home range and take part in multiple aggregations (Young et al., 2014). The Big Old Fat Fecund Female Fish (BOFFFF) hypothesis suggests that large females contribute proportionately larger than smaller females to spawning aggregations and larval recruitment by producing more and larger offspring (Hixon et al., 2014). Young et al. (2016) noted high site fidelity of snook to specific spawning sites. Snook were recorded in multiple spawning aggregations through the spawning season but were seen four times as often in their primary spawning sites. High site fidelity creates critical spawning habitats for returning and growing snook. In line with the BOFFFF hypothesis, these larger returning snook will contribute more to the population within those specific sites than other individuals.
The St. Lucie River, located on Florida’s east coast, is a brackish estuary that flows into the Indian River Lagoon. It has been hypothesized that this estuary is an important overwintering habitat for common snook, used during non-spawning seasons for foraging and development (Jud, 2014). This study focused on testing this hypothesis.

Effective regulations rely on identifying important snook habitats as well as the seasonal timing of their use. This study aims to identify the habitat use of St. Lucie River by large, female snook to inform proper regulations that will allow protection of the snook population.

Methods

Two hundred eighty-one snook were tagged by Florida Fish and Wildlife Conservation Commission (FWC) in St. Lucie (STL) and passively monitored using an array of acoustic receivers (Vemco). Snook were caught by FWC and implanted with a transmitter that emits a unique ID code every 60-180 seconds. Once a fish with a transmitter swims past an acoustic receiver, the receiver records the ID code, date, and time of a specific fish. Adult snook were collected by FWC researchers using a rod and reel, a seine, and a cast net (Young et al., 2016). They were measured for total length (TL; 602-1020 mm), sexed (33 Female, 17 Male, 11 Unknown) by examining the vent area (Lowerre-Barbieri et al., 2003), implanted with a transmitter (V16; 4-year tag life; Vemco), and released. Implantation was conducted by placing the fish ventral side up with the head and gills submerged in water and the surgical procedure was performed. Following implantation, fish were sutured and held in an aerated tank for observation post surgery for 30 minutes to ensure recovery before release (Young et al., 2016). The data used for this study were provided by the Florida Fish and Wildlife Commission’s Florida Wildlife Research Institute, Tequesta Florida. Tagging and data collection was carried out by FWC personnel from 2008-2015 as part of a larger FWC study. FAU personnel were not involved in the collection of the data or the handling of animals, only involved in the further analysis of the previously collected FWC data with the purpose of expanding our understanding of snook movement patterns.

Receiver data was downloaded by FWC every six months with our snook data ranging from 2008-2015. Snook were excluded from analysis if they had less than two seasons of spawning and non-spawning data or if they were tagged late in the season. Due to the exclusions, 61 snook (33F, 17M, 11U) ranging from 602-1020 mm in TL were used for this study. The proportion of days in St. Lucie was averaged over overall days of detections for non-spawning and spawning seasons to remove temporal bias. We used hierarchical clustering in R Studio (Version 1.2.1335) (agnes package) utilizing a Euclidean dissimilarity matrix and Ward’s linkage to cluster. Linkage methods were tested using the agglomerative coefficient, with Ward’s linkage being closest to 1, indicating the strongest linkage. Hierarchical clustering iteratively groups the two most similar data points until all data are merged, creating a dendrogram (Figure 2). The height of clustering represents the “distance” between those clusters, so it is a measure of similarity. The linkage method determines how the distance is calculated. Ward’s linkage bases the clustering on reducing the sum of squared distances of each datapoint from the average data in a cluster.

Results

Elbow and silhouette plots were used to determine that four clusters best fit the data which matched hypothesized potential behavior patterns. Using hierarchical clustering, the 61 snook were clustered into...
four categories: Resident, Traveler, Spawning Transient, and Overwintering Transient. Resident snook were observed in St Lucie estuary (STL) through the spawning and non-spawning seasons. Travelers did not have specific patterns to their movement and seemed to move in and out of STL freely. Spawning Transients overwintered in STL and spawned in other systems and Overwintering Transients spawned in STL and overwintered in another system.

Once the clusters were identified, we examined the mean number of days in STL for spawning season (SP) and non-spawning season (NSP). Residents averaged >87.5% days in STL during NSP and 100% days during SP. Travelers averaged <26.6% days in STL during NSP and <33.3% days during SP. Spawning Transient averaged >54.1% days in STL during NSP and <2.1% days during SP. Finally, Overwintering Transient averaged <46.4% days in STL during NSP and >50% days during SP. The majority of snook tagged in STL were classified as travelers (n = 31), while fourteen were resident, seven were overwintering transient, and nine were spawning transient (Figure 2).

Total length of resident snook had the largest range in total length (602-999 mm) but lowest average (799 mm). Travelers ranged in total length from 734 mm to 1005 mm and had the highest average of 869 mm. Overwintering Transient ranged from 753 mm to 1020 mm with an average of 848 mm and Spawning Transient had the smallest range of 763 mm to 922 mm and averaged at 858 mm (Figure 3). An ANOVA indicated no significant difference in length between clusters (p = 0.0729).

Resident (6F, 4M, 4U) and Traveler (16F, 10M, 5U) snook had more even sex ratios compared to both Overwintering Transient (5F, 2M, 0U) and Spawning Transient (6F, 1M, 2U) (Figure 4).
Discussion

If St. Lucie estuary serves as an overwintering habitat, more snook would be expected to spend time in St. Lucie during non-spawning season, i.e., spawning transient and resident movement types. In addition, if St. Lucie was a preferred overwintering habitat compared to available areas in Indian River Lagoon, large females should be present in higher numbers as they highly contribute to the spawning population and are able to outcompete other snook for that preferred habitat due to their larger size.

The large number of travelers through St Lucie estuary indicate that some snook could be either foraging or taking part in spawning aggregations, then moving on to other rivers. Many of the tagged snook could also be taking part in multiple aggregations, as much as 51% is seen in Young et al. (2016). Although there is a large range in length, Residents were smaller in average compared to the other three clusters. It could be due to higher risk of predation for smaller individuals once they travel through deeper waters for spawning or foraging. Travelers had the highest average total length; their larger size likely decreases risk of predation and increases their resilience to variable environmental conditions that they are more likely to encounter when traveling. Differences in size have been shown to influence movement strategy in fish (Trotter et al., 2012).

In previous studies, when examining snook spawning, residents were classified as snook that remained in the river and migrants were snook that migrated out of the river to spawning aggregations (Trotter et al., 2012). Based on that study, we would expect our transients and travelers to be larger and therefore female. Migrants in Trotter et al. (2012) were significantly larger than residents. Even though our results were insignificant, the trends are similar, and suggest that larger fish are more likely to move. We did not see a specific size or sex predictability of a category of movement. Movement could also be based on personality rather than size and sex, which will require future research to examine this parameter. We were not able to make a definitive conclusion that snook were moving because they were bigger and able to travel with less predation risk or they were growing larger due to traveling offering better foraging opportunities. To address this question, snook would need to be recaptured and measured throughout their life, which is a difficult feat for highly mobile fish in the wild. Size and sex as well as other factors could possibly affects movement or predict future movement, but these conclusions were confined by our sample size.

We recognized behavioral contingents, but due to a small sample size, no definitive conclusion could be met. With only seven overwintering transients and nine spawning transients, our hypothesis could not be supported. A more representative sample size would provide complete information on movement behavior contingents, wider range in sex and size distribution, and general snook population movement overall. This study provides us with important information as we observed behavioral contingents, but a larger sample size is necessary to provide the entire picture.

Potential bias in sampling methods may have skewed the results because telemetry data were initially collected for a different study. All sampled snook were at a size where they were assumed to be sexually mature. Tagged fish were sampled mainly from spawning aggregations and larger, female fish were specifically targeted. As a result of the methodology, the fish in this telemetry data may have preferentially sampled one movement contingent and the results would not be representative of the population's movement contingent distribution overall. The overall sex ratio (33F, 17M, 11U) as well as those for the contingents were heavily female-biased and do not reflect recorded population sex ratios (1.8M:1F) for snook in this region (Taylor et al., 2000). Even though the sex ratios were not representative, large, female snook are included in the movement data and were of the most interest in this study.

The BOFFFF hypothesis posits that larger females contribute proportionately more to the spawning population due to the larger body size and increased internal resources to contribute to spawning (Hixon et al., 2014). Managers concerned with preserving breeding populations would protect habitats that these fish highly utilize. In this study though, female snook were fairly evenly distributed between the behavioral contingents, indicating that large females are not all prioritizing one movement strategy. While St. Lucie is an important estuary for snook, providing a mosaic of habitats, our results suggest that large females are not seeking out this area as a primary overwintering habitat. Large female snook could not be protected in one habitat since they do not all congregate in the same place during the sensitive overwintering season. Therefore, it would not be possible under these conditions to protect the majority of the breeding female snook population in one place. Closed seasons and areas during overwintering (which are currently enacted), while not necessarily protecting the majority of large females, would likely protect enough large females to increase population resilience to disturbance events and recreational fishing.
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FASCIST SURREALISM: ARTISTIC DYNAMICS OF NATIONALIST ARTISTS IN THE SPANISH CIVIL WAR (1936-1939)

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Abstract

This research explores three artists during the Spanish Civil War (1936-1939) who utilized a surrealist aesthetic while promoting the causes of the Fascist, Nationalist front through their work. By focusing on artists Salvador Dalí, Alfonso Ponce de León, and José Caballero, the usage of "Fascist Surrealism" is explored to analyze its stylistic differences with "traditional Surrealism". Through paintings and variations in artistic techniques this research questions what factors led to the usage of Surrealism, a movement founded with a fundamentally anti-Fascist, left-wing, agenda, by artists who held vastly different political beliefs. This research examines prior political activities of the conventional Surrealist movement to illustrate their attempts at political change, and the factors that led to argumentation and disagreement among the group's members. By analyzing its foundational characteristics, Surrealism's ideological basis is compared with that of Fascism to identify their shared features, and the possible factors that led to their convergence.

Introduction

In 1936, a military coup led, mainly, by generals, Emilio Mola, José Sanjurjo, and Francisco Franco attempted to overthrow Spain's existing government, the Second Spanish Republic, subsequently sparking national polarization and the start of the Spanish Civil War (1936-1939). As Spain divided itself into the challenged Republican front and the rebel Nationalist front, Spanish artists began to assist in wartime efforts through the production of propaganda and politically charged art. Becoming increasingly aware of their potential to incite social change, Spanish avant-garde artists, previously focused on commercial success, created pieces to raise international awareness of wartime atrocities and their ideological goals.

This paper identifies the intersection of Fascism and Spanish Surrealism within the context of the SCW through the works of three artists. Beginning with a brief overview of the SCW and the Surrealist movement, this paper illustrates why the usage of surrealist aesthetics by Nationalist artists remains especially intriguing within the evolution of the conventional Surrealist movement. Prior work on Surrealism during the SCW, most notably, Robin Greeley's (2006) work Surrealism and the Spanish Civil War, has identified Surrealism's political ambiguity and associations with Fascism. However, specific connections between the ideologies of Fascism and Surrealism, and the factors that led for Spanish Surrealists to utilize Fascism, have yet to be fully explored. While Greeley has examined the difficulties Surrealism encountered whilst promoting progressive causes, this paper explores how the same characteristics that problematized the movement's progressive agenda ultimately led to its utilization by the Nationalist front during the SCW. Due to the significance of this usage, this paper will refer to the intersection between the two philosophies as "Fascist Surrealism" to illustrate how the Surrealist movement evolved over time and strayed from its original intentions within the context of Spain's political turmoil.

The body of this paper is broken down by artist. Section Three examines Alfonso Ponce de León's 1936 self-portrait to illustrate how a Falangist painter, perhaps unintentionally, adjusted Surrealism to fit a new cast. Section Four focuses on José Caballero's 1937 cover for the Nationalist publication Verticé, to clearly demonstrate Surrealism's ability to blend with Fascist aesthetics. Section Five explores Salvador Dalí's crucial role within Surrealism, his personal fascination with Fascism, and his overall disagreements with Surrealist leaders, within the context of his 1939 painting The Enigma of Hitler.

Through the exploration of these works, this study will illustrate how, and attempt to answer why, "traditional Surrealism", or the largely progressive/leftist movement headed by André Breton prior to the SCW, branched off into certain semblances of “Fascist Surrealism”. By identifying and exploring Surrealism's initial political context, this paper addresses which of the movements' foundational characteristics allowed for it to be rebranded into "Fascist Surrealism" while maintaining its overarching "Surrealist" identity. Essentially, through an analysis of the movements' central tenets and beliefs, this paper concludes that Surrealism and Fascism share many core principles. From these findings, and the subsequent rise of "Fascist Surrealism", the goals and motivations that defined André Breton's “traditional Surrealism” are contextualized within their own historical evolution. Ultimately, by identifying this evolution, as it occurred throughout the course of the SCW, this paper argues that artists' usage of "Fascist Surrealism" can be partially attributed to Surrealism's inherent fluidity and its philosophical similarities with Fascism.

1 This paper will refer to the Spanish Civil War (1936-1939) with the acronym "SCW".
2 See (Greeley 99-110) for previous insight into the connection between Surrealism and Fascism.
Spanish Fascism and the Surrealist Movement

Prior to the SCW, Spain was experiencing a cultural milieu from the dramatic cultural shift brought on by the formation of the Second Spanish Republic in 1931. Spain's new secular, progressive government led to the rise of avant-garde movements in the country: one such example being Surrealism. While disregarding traditional artistic technique, Surrealism began in France with revolutionary political goals and flirted with far-left causes. However, it was not until the SCW that Spanish Surrealism became politicized as artists used the aesthetic to criticizing the rise of Fascism and the Nationalist front (Greeley 99).

Through world renowned pieces such as Pablo Picasso's Guernica, Joan Miró's The Reaper, and Miro's Aidez L'Espagne (Help Spain), artists who sympathized with the Republican hoped to draw attention to the Nationalist's crimes and atrocities.  

Although there is no historical consensus on the definition of the term “Fascism”, the rise of the Nationalist front prior to and during the SCW contained clear Fascist undertones. Nearly half of Spain supported the Nationalists due to fears of social change surrounding the Second Spanish Republic's progressive agenda (Thomas 182). From its calls for a return to tradition, the Nationalists gained support from Conservatives, Monarchists, and Catholics by painting Progressives, Communists, and the Non-Religious, all represented in the Republican bloc, as a common enemy. Despite these early semblances of Fascist rhetoric, however, the Nationalist movement did not become overtly Fascist until it began identifying with the previously outlawed Falange party in 1937 (Casanova and Douche 286). In order to establish a concrete identity, and appeal to the sympathies of Italy and Germany, the Nationalists converged with the Falangist party, which, inspired by the Italian Fascist movement, aimed at revolution by targeting the Nationalists due to fears of social change surrounding Fascism (Brihuega 65).

The Nationalist side's violent, Fascist rhetoric led to most Spanish Surrealists allying themselves with the opposing Republican bloc. Despite the Republican's support from the Soviet Union, Mexico, and a cohort of foreign volunteers, the Surrealists encountered difficulties appealing to the Spanish working class, possibly, due to their elitist origins (Brigstocke 2). Originating in post-World War One France, Surrealism's founders were university educated artists and not, often, members of the working class (Greeley 99). Surrealisms' basis in prior avant-garde movements (Cubism, Dadaism, and Futurism), and their fundamental disregard for traditional artistic techniques, perhaps, made the group's experimental and nuanced approach to social protest particularly alienated from its target audience.

In 1924, André Breton, the founder of the Surrealist movement, published the First Surrealist Manifesto outlining the group's basic beliefs in an attempt to organize their efforts. Claiming that the “reign of logic” over life and art was based on “mediocrity and hate”, the Surrealists, and Breton, desired real social change (Breton 2-5). However, while Surrealists generally believed creative endeavors could function as a method of social revolt, the movement struggled to find common ground with other revolutionary causes. By focusing on the spiritual and metaphysical conditions of man, the Surrealist movement, from its inception, established a rift with Marxism and its focus on material and economic issues (Short 20).  

When Breton and five other Surrealists joined the French Communist party in 1927, most Surrealists did not share his political sensibilities and openly criticized his later decision to coauthor a manifesto with Leon Trotsky, Un Art révolutionnaire independent (Vaneigem 87). Met with severe scrutiny from the group's Communists and Non-Communists alike, the manifesto's proclamation that “the Communist Revolution is not afraid of art,” and denouncement of Fascism and Stalin, was criticized by many Surrealists including Pablo Picasso (Diego and Breton 3). Upset by the group's ideological differences, in the Second Surrealist Manifesto (1929), Breton specifically attacked members for their lack of collective action which led to further tension and division within the group (Queneau 7). As the movement spread to Spain, prominent Spanish artists began to see Surrealism as an instrument of personal expression and an opportunity to achieve commercial and financial success (Minik 178). Spanish Surrealists tended to deny any association with French Surrealists and sought to distance themselves from the ideological purity of the movement, instead, using the aesthetic as a kind of “floating reality” to advance ideologies (even that which it had initially rebuked such as Fascism) (Brihuega 65).

Alfonso Ponce de León and “Post-Surrealism’s” Fascist Sensibilities

Alfonso Ponce de León was born in 1906 to a family of minor nobility and strong military traditions. He started his artistic career at the School of Fine Arts in San Fernando and was introduced to artists Salvador Dalí, Maruja Mallo, Federico García Lorca, and his future wife Margarita Manso. After joining Lorca's theatre group, La Barraca, in 1933, Ponce moved to Madrid and drastically changed his societal and artistic outlook. Growing to detest the capitalist and bourgeois aspects of Republican Madrid, Ponce became increasingly radicalized and eventually joined the controversial Falange party. Ponce's involvement with the party led him to create caricatures which critiqued what he saw as a consumeristic and consumption driven culture through depictions of sleazy “fat cat” capitalists. Despite his early cartoonish work, Ponce's caricatures remain secondary to his later “Post-Surrealist style”.  

3 See Appendix Figures 1, 2, and 3

4 Marxism is a form of socioeconomic analysis that analyzes social conflict as a struggle of interests between socioeconomic classes (Marx 42).

5 For more information on Surrealism's internal politics, see the 1930 pamphlet Un Cadavre and The Second Surrealist Manifesto (1929).
While undoubtedly drawing influences from “traditional Surrealism”, Ponce developed “Post-Surrealism” as a more “serious” critique of society. Today Ponce’s style is considered “magic-realism,” however, this paper identifies the surrealist connotations in his work and believes it remains Ponce’s primary influence.

While in the middle of developing his “Post-Surrealist” style, Ponce’s life began to change drastically with the outbreak of the SCW. For their association with the Falange party, Ponce and his siblings became targets of the underground Republican police or Checas. Although initially remaining free to carry out his daily routine, and not a priority of the Checas, the Nationalist’s advances towards Madrid initiated widespread panic on the Republican side.

Ponce was eventually arrested and tortured before being shot and killed in 1936- months before General Franco stormed Madrid. Following his death, Ponce’s brother and father were executed by firing squads, his Mother later committed suicide, and his wife broke down and fled to Italy. Although he was posthumously found to be the leader of an ‘underground resistance’ to Republican forces in Madrid, Ponce’s role in the SCW was largely unrecognized until the late 1990s and his 2001 exhibition at the Reina Sofia Museum (Stradling 65).

Just months before his death at the hands of Republican soldiers in 1936, Ponce painted a grim self-portrait representing his unique style. Although not as experimental as conventional Surrealism, Ponce’s self-portrait, depicting the artist’s lifeless body, represents the artist’s “Post-Surrealist” style through an apparent foreshadowing of his own death. A homage to the absurdity of pre-Nationalist Spain, Ponce’s self-portrait includes several surreal elements. Interestingly enough, Ponce appears to utilize his own body for those elements: a plant growing out of the palm of his hand, the subtle flotation of his body, and a rock impaled in his eye. In contrast to the absurdity of his physical condition, the painting’s background appears as a more traditional conventional form of reality: representing a contrast with traditional Surrealism’s often immersive, dreamlike atmosphere. Displaying a car, some foliage, and a wooden sign, Ponce’s self-portrait juxtaposes the irrational with the rational- a subtle nod to the notion of transcendence. Appearing as absurd and strange, the surreal features of the artist’s body seem to rise above the material conditions of the physical world.

From his early use of caricatures, one can understand Ponce’s position as a staunch anti-capitalist and fervent proponent of revolution. It can be interpreted that Ponce’s decision to nitpick certain surrealist elements, while avoiding the usage of an overtly surrealist back drop, was a conscious move to avoid his bourgeois associations with Surrealism. Ponce’s early involvement in the theatre group La Barraca exposed the artist to a wide variety of avant-garde movements. It is possible that, since his initial exposure to Surrealism came through an elite avant-garde movement, Ponce associated Surrealism with his detest for the privileged class of Madrid.

From his position as an underground resistance leader, a Falangist, and a social critic, Ponce despised the consumption culture associated with life in Madrid. Ponce’s “Post-Surrealist” style is, therefore, a stylistic adaptation which intentionally avoids elitist connotations of Surrealism while retaining its useful features. By painting himself as absurd and irrational in an otherwise plain reality, Ponce is expressing his own feelings of absurdity in a world blind to the causes he held dear. One can easily see how Ponce’s ongoing fears of persecution, hatred of capitalists, and revolutionary fervor pushed the artist to feel strange in Republican Spain. Fascism’s inherent irrationality and emotional undertones, perhaps, played into the artist’s depiction of “bodily transcendence” in his 1936 self-portrait. The painting, thus, does not appear as a prediction of his death, but as recognition of his own personal evolution. Through this interpretation, one can see Ponce’s painting as symbolic of the artist’s rebirth: the old Ponce is dead and has been forced to transcend material life for a revolutionary cause.

José Caballero: “Angelismo” and Artistic Desperation

In 1932, José Caballero enrolled in the San Fernando School of Fine Art working under Daniel Vázquez Díaz who introduced Caballero to the Spanish art scene. Caballero quickly befriended Federico García Lorca and, in 1934, was invited to join Lorca’s theatre group: La Barraca. Caballero’s association with La Barraca exposed him to several prominent avant-garde artists and introduced him to a unique blend of artistic styles. Although influenced by the styles of constructivism, expressionism and lyrical figuration, Caballero was most intrigued with Surrealism and began to utilize its aesthetic in 1934. Caballero, despite not even being aware of the French Surrealist movement, consistently worked under more experienced Surrealists such as Alfonso Ponce de León, Benjamín Palencia and Maruja Mallo until the outbreak of war.

The Spanish Civil War drastically changed the young artist’s life. In his own words the war became his life’s “axis” and permanently impacted him. At the outbreak of war, Caballero was visiting his hometown, Huelva, which was shortly captured by Francoist troops. In 1937, Caballero was recruited for his position as an artist and forced to work for the Nationalist front. Until the end of the war in 1939, Caballero produced illustrations for several propagandist publications including Verticé. Caballero was deeply pained by his tenure with the Nationalists, and in his own words described his emotional state, “everything was brutally silent and there was nothing left of that previous joy” (Caballero 353-354). Caballero’s growing cynicism would come to permanently alter the artist’s work and his life’s trajectory.

6 Biographical information is taken from (Stradling 198-200)
7 Figure 4 in appendix.
8 Biographical information on José Caballero from (González 1-20).
In 1937, Caballero drew his first war-time illustration for the propaganda magazine Vertíce. An early representation of Caballero’s new “Angelismo” style, one can clearly see the evidence of Caballero’s coverup (Madrigal Neira 204). However, upon first glance, the inclusion of an angel appears to accentuate the painting’s surrealist connotations instead of simply muddling them. It is important to note that José Caballero was aware Giménez Caballero had an avant-garde background, however, the everyday onlooker would not quite see the inclusion of angels as a “coverup”. It seems Caballero’s personal usage of angels to depict tragedy and despair cannot be understood through the piece itself. Instead, as angels (a traditional religious icon) are synthesized with seemingly miscellaneous elements in the painting (i.e., die, shells, scrolls) “Angelismo”, instead of appearing as a new style, demonstrates a new kind of Surrealism. As a dream-like desert overlays a combination of traditional religious symbolism and conventional surrealist elements, the painting appears as a homage to Francoist Spain and its Catholic foundations.

Following the conclusion of the SCW, Caballero returned to Madrid and immediately became distraught and disheveled upon experiencing how his country had changed. Influenced by these feelings, the artist completely abandoned the style of Surrealism, instead, opting to utilize an Expressionist aesthetic in the latter half of his life. Caballero felt as if Surrealism no longer expressed who he was, perhaps, due to the style’s newfound political connotations. Evidently, Caballero did not intend to create Fascist connotations with Surrealism as he resented the Nationalists and was coerced into producing Fascist propaganda. Nonetheless, Caballero through his “Angelismo” style blended the two ideologies together: effectively creating a form of Surrealism that carried with it the cultural foundations of the Nationalist side. A claim supported by Caballero’s refusal to adopt the style in his post-war career.

Salvador Dalí: “Paranoiac-Critic” and the Fascist Fantasy

Salvador Dalí joined the Surrealist movement in 1929 and quickly became involved with its leftist political causes (Gibson 34). However, despite his initial adherence to Surrealist Orthodoxy, from 1933 onwards Dalí began taking increasing apolitical stances. With his new “Paranoiac-Critic” method, Dalí regularly incorporated Fascist themes into his work while simultaneously refusing to denounce the ideology (Dalí and Haakon 145).

Starting in 1930, Dalí began to develop a new artistic method to solve issues arising from Psychic Automatism (Surrealism’s primary method). Labeled as “picturesque”, “cliché”, and lacking in revolutionary fervor, Psychic-Automatism disregarded any active role of the artist and, as a result, frequently carried elitist connotations (Greeley 120). The Paranoiac-Critic method, meanwhile, utilized the artist directly by denoting reality as a “construct” born out of subjective experience (Dalí and Haakon 233). Despite Paranoiac-Critic’s widespread acclaim, Dalí was heavily scrutinized for his tendency to portray Fascist material with the style.

Although not excommunicated, Dalí was put under trial by the Surrealists for including a swastika in his 1933 The Weaning of Furniture Nutrition. Following the trial, Dalí stood ideologically distant from the Surrealists—remaining so for the entirety of the SCW (Gibson 36). While remaining explicitly neutral, Dalí expressed his disapproval of the Surrealist’s interpretation of fascism. Labeling the Surrealists “reactionary” and arguing they were implicated in similar values, Dalí believed they missed an opportunity to understand Fascism’s origins (Greeley 134). Through his 1939 painting The Enigma of Hitler, Dalí believed that Fascism could be understood through the lens of sexual perversion and desire.12

The inclusion of the word “enigma” in the painting’s title, absent from any overt negative connotations, implies Dalí’s skepticism and perhaps fascination with Fascism. Reflective of this attitude, The Enigma of Hitler displays a wondrous amphitheater-like atmosphere of which Hitler is the epicenter. Appearing as a small photo, the likeness of Hitler is portrayed as closer to reality than anything else in the painting. The inclusion of a transparent umbrella, an aggrandized megaphone, and a wilted tree absent of a base, reflect a fundamental disconnect between the realistic image of the subject, Hitler, and his external world. It seems The Enigma of Hitler displays Hitler as the only constant in a subjective, plastic reality. In return, reality appears only as an amphitheater through which the individual can carry out his will.

9 Ernesto Giménez Caballero was a Falangist cultural administrator while José Caballero is the painter being referenced.
10 Figure 5 in appendix.
11 Figure 6 in appendix.
12 Figure 7 in appendix.
For Dalí, there is no underlying ideology of “Fascism”, but only the individual who has acted in accordance with his internal psychosexual struggles. This focus on the internal landscape of the individual (Hitler’s sexual perversions) allowed Dalí to appropriate surrealist ideas and justify the ideology. Instead of critiquing the ideological basis of Fascism, Dalí romanticizes the ideology by viewing it as an internal struggle between the individual and his desires.

After the victory of Francisco Franco in 1939, Dalí quickly praised the Falange movement and its respect for Catholic values (Gibson 67). Dalí’s appraisal of Franco, and previous creation of The Enigma of Hitler, was enough to finally have him expelled from the Surrealist movement. After his excommunication, Dalí was criticized by the Surrealists for his frequent commercialization and hunger for fame, but it is unlikely that Dalí’s association with the Francoist regime was solely for commercial purposes. It is possible that Dalí’s sister, Anna Maria Dalí, who was raped, tortured, and imprisoned by Republican soldiers, influenced his decision to advocate for the other side (Rubió Coromina, 76). However, it is more likely that Dalí was attracted to the Falangists because of their emotionality, irrationality, and overall “mystique”.

Conclusion

From the analysis of the three aforementioned pieces, the malleability and appropriation of the surrealist style is demonstrated by the ease at which it expresses Nationalist/Fascist causes. From Ponce’s “Post-Surrealist” style to Caballero’s “Angelismo” and Dalí’s “Paranoia Critic”, the three artist’s inclusion or omission of surrealist techniques varied according to personal sentiment.

The development of Ponce’s “Post-Surrealist” aesthetic illustrates the evolution and implications Surrealism held over time. An anti-capitalist and revolutionary himself, Ponce’s willingness to adopt semblances of Surrealism demonstrates that Fascist movements, based on radical and emotional appeals to revolution, could find commonality with Surrealism’s own irrationality and spiritual basis. Ponce’s decision to ground his self-portrait in reality, with surrealist elements, reveals the artist’s objections with the style and desire to circumvent its elitist connotations. As previously suggested, Surrealism’s basis in, oftentimes, elite avant-garde circles created obstacles for Republicans/leftists who intended to use the style for social change. Perhaps, paradoxically, as Fascists became attracted to the movement’s revolutionary fervor and blatant anti-capitalism, they too encountered difficulties juggling the movement’s elitist connotations.

Caballero’s development of his “Angelismo” style for the Falangist publication Verticé reflected his need to mask the Surrealist style with the inclusion of angelic imagery. However, through the analysis of his 1937 Verticé cover illustration, this paper stresses the inclusion of angels accentuates the work’s surrealist implications. The synthesis of angels (a traditional religious symbol) with random objects muddles the divide between surrealist and Falangist aesthetics-successfully promoting the Nationalist cause. Caballero’s inability to sufficiently distinguish the surrealist aesthetic reveals its inherent flexibility. Instead, it seems Surrealism’s spiritual and emotional basis, intended to serve leftist or anti-Fascist causes, allowed for its appropriation by revolutionary Fascist propaganda.

Dalí’s development of the “Paranoia Critic” method stemmed directly from Psychic Automatism’s inability to spark revolutionary fervor. The philosophy of Psychic Automatism, and the artists role as a mere medium in the technique, revealed that Surrealism’s groundbreaking methods were fundamentally removed from the goal of social change. The original elitist connotations of “Psychic Automatism” allowed for Dalí to develop “Paranoia Critic” and subsequently revealed Surrealism’s ability to romanticize Fascism. An ability, which, sprung from Surrealism’s original elitism and spiritual approach to reality.

Ultimately, Surrealism came to represent the very thing it sought to destroy. Grounded in hazy left-wing political goals, the nature of the Surrealist project, and its focus on the unconscious and dream realm, implicated the movement in elitist circles from the start. While desiring important societal change, the Surrealists themselves were oftentimes privileged and alienated from the working class. Although seemingly allied with the Marxists, paradoxically, Surrealism’s focus on the spirit, rather than material conditions of man, remains closer to Fascist sentiments. On the surface, Fascism’s emotional appeal, irrationality, and detest for greater bourgeois society appears ideologically compatible with Surrealism.

One could argue that Surrealism itself remains ideologically neutral and lies beyond the realm of political or social implications. However, while art does not need to carry political connotations, Surrealism’s appropriation by Fascist causes remained possible, perhaps, because of Surrealism’s lack of a foundational structure. The Surrealist project, based on a rejection of reason, capitalism, and contemporary society, loosely resembles many right and left-wing revolutionary movements of the 20th century, thus, allowing for its appropriation.
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Miró, Joan. The Reaper. 1937.


Appendix

Figure 1. Guernica


Figure 2. The Reaper

Miro, Joan. The Reaper. 1937.

Figure 3. Aidez l’Espagne (Help Spain)

Figure 4. Autorretrato (Self-Portrait)


Figure 5. Sin título (Untitled)


Figure 6. The Weaning of Furniture Nutrition


Figure 7. The Enigma of Hitler

CRISPR/CAS9-MEDIATED GUS GENE KNOCK-OUT IN THE TOBACCO PLANT

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Abstract

CRISPR/Cas9 technology facilitates gene editing by removing or adding nucleotides at specific DNA sequences. This results in a gene removal (knock-out) or insertion (knock-in), thus causing a cell to manipulate gene expression. Using Agrobacterium-mediated DNA delivery, CRISPR/Cas9 was utilized to genetically modify a transgenic RM-1 tobacco plant that expresses a reporter gene, β-Glucuronidase (GUS). The CRISPR/Cas9 integrated plants, named HaG, were regenerated, and the presence of Cas9 gene in these plants was confirmed by qPCR. The T1 and T2 progenies of the HaG plants were screened for effective gene editing by GUS staining assays. The GUS negative plants were selected and grown; the DNA was then extracted, sequenced, and compared to the original GUS gene sequence. Our results demonstrate that GUS expression had diminished in the CRISPR plants. DNA sequencing showed a deletion occurred in the expected coding region of the GUS gene, this resulted in a gene knock-down instead of a complete knock out.

Introduction

CRISPR/Cas systems were initially discovered in bacteria as the immune response to foreign DNA, such as bacteriophages or plasmids. It is facilitated by RNA-guided nucleases to target, edit, and degrade invading phage genomes. This natural mechanism can be used as an accurate gene editing system which targets and cleaves specific sites of nucleic acids [1].

The CRISPR/Cas9 system consists of guide RNA (gRNA), a 20-nucleotide targeting sequence; a 3-nucleotide protospacer adjacent motif (PAM), and a Cas9 enzyme. The Cas9 protein is an endonuclease commonly utilized in biotechnology that results in genome modification. The gRNA and Cas9 enzyme work in unison to find the specific gene of interest, the gRNA directs the Cas9 nuclease to the DNA sequence that is identical to the targeting and PAM sequences. Once the DNA is cleaved by Cas9, the double stranded DNA undergoes two forms of repair. One way of repairing is known as nonhomologous end joining (NHEJ), which is error-prone and results in an insertion or deletion of genes. The other form is homologous recombination (HR)-directed repair; in contrast to NHEJ, a DNA template is required. Results of HR-directed repair consist of a new gene knock-in or full gene restoration, depending on the template applied [1][2].

Agro bacterium-mediated infection is commonly implemented to deliver DNA construct into plant cells to produce genetic modification. It transfers bacterial plasmid DNA into host cells, allowing recombination to occur. The Agrobacterium exploits the factors of the host to transfer the gene of interest from the bacterium plasmid T-DNA into the host genome. In the natural world, gene transfer from Agrobacterium in plants can result in a crown gall formation, this tumor growth is due to the tumor-inducing characteristic of the Ti-plasmid. However, the Agrobacterium used in research has a modified Ti-plasmid possessing an absence in tumor formation expression. The Agrobacterium-mediated transformed cells spontaneously regenerate and produce transformants that carry cellular T-DNA sequences from Agrobacterium [3][4].

Within the T-DNA gene construct utilized for transformation, a hygromycin phosphotransferase gene (HPT) served as a selection marker. Derived from Streptomyces hygroscopicus, it expresses an enzyme that functions as a hygromycin B detoxifier by phosphorylation. Hygromycin B is known as an inhibitor of protein synthesis in ribosomal translocation and aminoacyl-tRNA recognition, in both prokaryotes and eukaryotes [5]. Successful transformants would display HPT gene expression, thus showing resistance to this inhibitor.

The T-DNA construct also contains the Cas9 gene and gRNA sequence to target the gene of interest, β-Glucuronidase (GUS enzyme), in order to produce GUS gene knock-out in this experiment. The GUS gene does not naturally occur in plants but is found in E. coli and other organisms. An RM-1 tobacco plant that was modified prior to this experiment served as the model organism due to its expression of GUS activity. In the presence of the X-Gluc substrate (5-bromo-4-chloro-3-indolyl--D glucuronide), cleavage occurs by the GUS enzyme. Following cleavage of glucuronide, oxidative dimerization arises that is stimulated by O2, resulting in a blue colored precipitate. In the application of a GUS staining assay, blue coloration indicates the presence of the GUS gene, the absence of blue indicates that GUS activity is not present [2]. If proper execution of CRISPR/Cas9-mediated GUS knock-out occurs, the transgenic progenies will express hygromycin resistance and an absence of blue precipitate in the GUS staining assays.
Methods and Materials

Gene construct

The cloning vector utilized in this experiment contained the genes of interest within the left and right border of the T DNA, which was then transferred into the plant cell, facilitated by Agrobacterium. The recombinant T-DNA construct in Figure 1, contained three critical genes: a hygromycin phosphotransferase gene (HPT) for hygromycin resistance, the Cas9 enzyme coded gene, and the gRNA GUS-targeted sequence. HPT served as a selection marker gene; it was used to detect and select the transformed cells. The GUS gene in the host plant coincided in serving as the knock-out target and a reporter gene to confirm the success of gene removal. The right border begins with the U6 promoter driving the gRNA, followed by the appropriate CaMV35S promoter that drives the Cas9 enzyme sequence, which is then tailed the NOS terminator. To the left of this terminator is the 35S promoter driving the HPT gene that is then concluded by the 35S terminator.

Agrobacterium infection

With the noteworthy capability to infect hosts through DNA transfer, an Agrobacterium tumefaciens bacterial infection was vital to the investigational transformation. To exploit this mechanism, a co-cultivation process was applied, infected explants were placed onto treated media; this allowed for proper T-DNA integration into the tobacco genome. A previously prepared overnight culture of A. tumefaciens that contained the T-DNA plasmid was grown, constantly aerated and incubated at a controlled environment of 28°C for 1-2 days. The culture was then transferred into a sterile 50 ml tube and centrifuged at 3,000 rpm for 10 minutes at 25°C. Excess liquid was properly discarded under a fume hood while keeping the pellet undisturbed. The pellet was resuspended with 5 ml of a previously prepared co-cultivation solution. Preparing the plant host to be infected, RM tobacco leaves were cut into ten 5x5 mm sections (per experimental sample) using a sterilized scalpel. The leaf discs were incubated in the bacterial solution for 15 minutes, and then were removed from the solution, blotted dry by sterile paper towel, and placed on the filter disk over the co-cultivation medium without hygromycin. The leaf sections were positioned with the abaxial surface facing upwards. The filter disk served to avert undesirable bacterial growth. The filter disks containing the infected leaf tissues were incubated for four days in a dark environment.

Transformation & regeneration

Following the four-day incubation, the filter paper-placed leaf sections were removed and replaced on selection/regeneration media containing both hygromycin B (30 mg/L) and timentin (500 mg/L). The application of timentin prevented any unwanted overgrowth of Agrobacterium on this media, the bacterial overgrowth would have potentially caused necrosis which would threaten proper transformation. About six shoots from each regeneration dish (lacking contamination) were removed from the callused tissues that showed HPT resistance-linked transformation and were inserted in prepared rooting media boxes containing hygromycin B (30 mg/L). Sterilized scalpel and forceps were applied in isolating the shoot from the surrounding callus. It is important to note that the shoots were not entirely submerged in the media to allow proper root formation and continuous growth. The boxes were placed and incubated in a controlled growth chamber with light and a temperature of 26°C for approximately five weeks.

Promising plantlets from these preliminary rooting boxes were aseptically transferred into new individual rooting boxes and placed in the equivalent environment for about two weeks. At significant root formation, small sections of leaf tissue were cut from each plantlet for DNA extraction and GUS knock-out analysis. Nineteen suitable transformant juvenile plants (T1 HaG) were transferred into potted soil to reach maturity.

Cultivation of T2 seeds and Mendelian segregation ratio

The seeds of the mature 19 T1 HaG lines (T2 HaG) were collected. The seeds were folded into fabric squares containing about 30 seeds each, labeled appropriately with the genotype of the parent T1 HaG plant line, and paperclipped closed. The compartmentalized seed packets were sterilized in a flask containing a solution of 10% bleach. The sterilized T2 HaG seed packets were rinsed with DI water and were inserted into seed germination media containing hygromycin (30 mg/L) (one Petri dish for each seed line) by sterile forceps. The dishes were properly labeled with the correlated T2 genotypes and placed inside of the growth chamber for a duration of approximately two weeks.

Following the growth period, the Petri dishes were surveyed for hygromycin resistance by determining which seedlings displayed resistance with dark green cotyledon and root initiation; as well as nonresistance with pale yellow cotyledon and no roots. The Mendelian ratio, involving resistance versus nonresistance, was determined. 12 out of the 19 T2 seed lines proliferated and were selected to be applied towards further analyses.

GUS staining assay of RM, T1 and T2 plants

To screen for GUS knockout in the experimental tobacco, GUS staining of the original, untransformed RM-1 plant served as a key visual reference and control. Small segments of leaf tissue were cut from the RM and T1 HaG plants, and immediately placed into individual microcentrifuge tubes. 100 μl of X-Gluc solution was added to each tube, ensuring complete submergence of the leaf tissues. The tube was incubated in a 37°C-water bath overnight. Following, the X-Gluc staining solution was removed. To increase the appearance of blue staining, a substantial amount of 70% ethanol was added to each tube, immersing the leaf sections for two days.
The leaf tissue samples were then observed and photographed under a low-power dissecting microscope. This GUS assay protocol was duplicated on the T2 HAG plants. It was noted that the T2 leaf tissues were inserted into 96-well plates in place of microcentrifuge tubes. Four leaf samples of the T2 plants (two HaG-LD2 and two HaG CT), that showed predominant GUS knockdown, were chosen to continue to the DNA extraction and sequencing phase of this research.

DNA Extraction and purification

A GenCatch protocol was executed towards an Epoch extraction kit for effective purification of genomic DNA, approximately 100 mg of fresh leaf sample from each T1, T2 and RM plant were ground to a fine powder by a small pestle with the assistance of liquid nitrogen in a microcentrifuge tube. Four hundred µl of PX1 buffer and 4 µl RNase A stock solution were added to each tube and vortexed to homogenize. Subsequent to the vortex and a 10-minute 65°C incubation period, 130 µl of PX2 buffer was added to the lysate of each sample. After another vortex, the samples incubated on ice for 5 minutes, the lysates were then added to shearing tubes (inserted in the collection tubes) to be centrifuged for 2 minutes at maximum speed. The flow through from each tube was carefully transferred to a new tube without disrupting the pellet nor obtaining unwanted cellular debris. Dependent on the amount of flow through for each tube, half of the flow through volumes worth of PX3 buffer and one volume of 100% ethanol were added to the clear lysate and mixed by pipetting. Six hundred and fifty µl of each sample were added to individual Plant Genomic DNA Mini Columns sitting in a collection tube. The tubes were closed and centrifuged at 10,000 rpm for 1 minute, the filtrate was properly discarded. Two washing periods consisting of 0.7 ml of WS buffer with 30 second centrifuge periods and immediate filtrate disposal were applied. Centrifuging the columns for an additional 2 minutes to allow all residual WS buffer to be removed from the columns; the columns were then transferred to new 1.5-ml tubes. Two hundred µl of warmed ddH2O was added to each tube, tubes were centrifuged for 1 minute and stored in -20°C. It is noted that the concentrations of the kit solutions were not disclosed.

DNA Analysis & qPCR of T1 and RM

DNA concentration was measured for each T1 extraction by Nanodrop spectrophotometer. Each sample was diluted to 2 ng/µl to achieve standardization. Applying the qPCR protocol, each sample was aliquoted into six wells; three wells paralleled to target Cas9, with the remainder three corresponding to EF-alpha (internal standard). Each well contained 11 µl of mastermix, this consisted of 2X fluorescent SYBR Green Dye, forward and reverse primers, and 9 µl of loaded sample. The qPCR ran for about two hours. The Cas9 relative gene dosage was quantified for each T1 sample with RM as reference.

PCR for GUS gene from T2 plants

The extracted and purified DNA from each T2 HaG-LD2 and HaG-CT leaf samples were thawed out along with the appropriate reagents. Each PCR tube, four in total, contained 25 µl of 2X PCR mastermix, 1 µl of plant DNA, 1 µl each of GUS forward and reverse primers and 22 µl of nuclease-free water. The tubes were centrifuged to ensure proper mixing of the template and reagents which were placed inside of the thermocycler. The PCR was initiated at 94°C for 2 minutes, followed by 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 25 seconds. After completion, the tubes were stored in -20°C.

Gel electrophoresis of T2 PCR products

Gel electrophoresis was performed on the T2 PCR products to purify the DNA prior to sequencing. The PCR tubes were taken out of storage and thawed. A 1.2% agarose gel was made using TBE (Tris borate-EDTA) buffer and the PCR samples were loaded. The electrophoresis was run at a constant 80V for an hour. The DNA bands were excised from the gel while on top of a UV light box and placed into the microcentrifuge tubes.

GUS DNA clean up and gel extraction

The PCR product (GUS DNA fragments) were extracted from the gel with the addition of 0.6 ml of GEX buffer. The tubes were incubated in a 50°C water bath for approximately 10 minutes while being inverted every 2 minutes until the gel completely dissolved. The dissolved gel solution containing the DNA and buffer were transferred into new individual columns and centrifuged for 30 seconds at 5,000 rpm, the filtrate was discarded. This step was repeated. The columns were washed by adding 0.5 ml of WN buffer and centrifuged for 30 seconds at 5,000 rpm, the flow-through was discarded. The columns were washed with 0.5 ml of ethanol containing WS buffer and centrifuged for 1 minute at 5,000 rpm, flow-through discarded. The columns were centrifuge at 12,000 rpm for an additional 3 minutes to remove residual ethanol to ensure DNA quality. The columns were placed into new labeled 1.5-ml centrifuge tubes and the DNA was eluted by applying 15 µl of elution buffer directly to the center of the column membranes. The columns remained undisturbed for 2 minutes, and then centrifuged for 1 minute at 12,000 rpm. Six µl of DNA from each tube were transferred into individual and labeled tubes that were sent out to be sequenced. The remainder of the tubes containing the gel extracted DNA were stored in -20°C for future use. Upon sequence completion, the edited GUS sequences were analyzed and compared to the GUS sequence of the original transgenic RM plant.
Discussion and Results

Nineteen transformant plants were created by implementing Agrobacterium mediated infection of T-DNA containing hygromycin resistance and a CRISPR/Cas9 system engineered to target the GUS sequence. The first generation (T1), labeled as the HaG genotype, were primary transformants derived from RM-1 tobacco prevent overgrowth of Agrobacterium; if this were to occur, it would have been detrimental to the potentially regenerated tissue by inducing necrosis. In the process of regeneration, the leaf sections grew ample in size, along with the formation of callused tissue; maintenance was mandatory to provide an optimal environment of this process (Figure 2.1C). Once green buds formed and shoots began to proliferate (Figure 2.11D), suitable shoots were removed from the callused tissues and inserted in rooting media boxes containing

Figure 9. Quantification of relative Cas9 gene dosage of RM-1 (-C) versus T1 HaG plants. hygromycin (Figure 2.2). Juvenile plants leaf tissues. Upon infection, the tissues were placed on media containing hygromycin to ensure that only the plant cells that express Figure 7. Sequence data analysis of CRISPR T2 HaG plants HPT resistance gene to flourish (Figure 2.1A). The plasticity of the plant tissue enabled successful regeneration of the selected tissues when being placed in regeneration medium consisting of hygromycin and timentin (Figure 2.1B). The addition of timentin was served to displaying strong root formation were removed from the rooting boxes and placed in potted soil to reach maturity (Figure 2.2). Seeds were harvested from the 19 mature, transgenic tobacco T1 HaG plants, which were then sterilized, and planted in petri dishes of seed germination media containing hygromycin to examine the segregation of HPT gene according to Mendelian law (Figure 4). These seeds gminated, rooted and grew into T2 seedlings. A Mendelian ratio was determined according to their sensitivity to hygromycin. The hygromycin sensitive seedlings were properly discarded, and the resistant seedlings were selected and utilized in further experimental analyses.

GUS stain assays were performed on the leaf tissues of the RM-1 and T1 plants. RM-1 served as the control which demonstrated a strong histochemical localization of GUS activity (Figure 3). The highly saturated blue precipitate signified the presence of the GUS gene due to enzyme-substrate cleavage activity. The T1 plant tissue (Figure 3, top right) displayed a great decrease in blue precipitate, this qualitatively represented a reduction in GUS activity. To ensure CRISPR/Cas9 editing process continued in the progenies of the T1 plant, GUS staining was also performed on the leaf tissue of T2 seedlings. Figure 3 (bottom) shows a visual reduction of GUS activity as well. It was noted that even though there was a drastic qualitative contrast between the RM and transformed progenies, there was still a residue of activity. This indicated there was not a complete silencing of the gene, but the Cas9 system did indeed successfully target and disrupt the gene of interest; leading to a significantly decrease in gene activity. Due to time constraints, only 14 out of the 19 T1 plants were utilized for qPCR analysis for Cas9. The forward and reverse primers used in the application of qPCR towards the T1 plants were designed to target the newly inserted Cas9 sequence in the host genome. RM-1 was also applied in this procedure and served as the negative control. In Figure 5, quantitative measurement of the relative Cas9 gene dosage was determined, which showed increased gene presence among the T1 plant lines versus the RM plant (-C). A presence of the inserted Cas9 gene was noted and inferred successful transformation, but this did not indicate whether Cas9 enzyme had edited the host genome.

Two HaG-CT and two HaG-LD2 plant lines were chosen for further analysis because they showed the greatest reduction in GUS staining. DNA was extracted from T2 leaf samples of these four plants. Instead of applying qPCR towards an end goal of quantitatively measuring gene dosage, it was directed towards the GUS sequence. The forward and reverse primers used in this application were engineered to target and amplify the GUS gene sequence flanking the CRISPR editing site. GUS sequence in RM 1 served as reference in order to determine differences between sequences. Following the completion of PCR, the amplified DNA of each sample was extracted from the gel and sequenced. Comparison of the GUS sequence from the HaG plants with the sequence from RM-1 revealed a “T” (thymine) nucleotide deletion in the boxed target site, illustrated in Figure 6. This edit resulted in a frameshift mutation of “L,” leucine, the 318th amino acid of a 603 amino acid sequence of the GUS protein to a stop codon. As a result, the GUS protein was truncated at the middle of the protein, resulting in the GUS enzyme inactivation. The reason why traces of GUS stains remained in the plant tissues is that since gene editing is the result of both Cas9 enzyme activity at the specific site and DNA repair process in the host cells. Not all the cells in the plant undergo the editing process at the same rate and to the same extent. The cells that have not yet completed CRISPR mediated editing will retain the GUS activity, thus showing blue when stained. Since our HaG plants possess the CRISPR/Cas9 gene system permanently, we expect that editing for GUS gene will continue during plants growth and development until the targeting site is exhaustibly edited.

Overall, the transgenic tobacco plants served as an achievable exemplification of biotechnology and genetic engineering through the exploitation of the CRISPR/Cas9 system. Regardless of the resulting data and correlating figures not necessarily displaying a complete removal and absence of the GUS gene, it was confirmed that CRISPR/Cas9 effectively targeted and disrupted the sequence of interest. The histochemical stain assay visually exhibited a robust blue coloration of the unedited RM-1 control plant, allied with active gene expression; whereas the T1 and T2 HaG plant tissues displayed a marked inhibition of blue precipitation which
correlated to GUS gene inactivity. qPCR analysis of the T1 HaG plants quantitatively solidified transformation by showing a predominant increase of Cas9 gene dosage but not indicating functionality. The sequence analysis of T2 HaG plants showed a thymine deletion resulting in the abolishment of leucine at the 318th amino acid of the GUS sequence, which resulted in a creation of a stop codon from the frameshift mutation. The mutation inactivated the gene and has led to GUS gene knock-down in place of a total knock-out due to the possibility of the RM-1 plant possessing multiple gene copies of GUS. This is a plausible explanation to why there was not a complete execution in targeting every GUS gene.

The applications for this technology are enormous as it can be used as a possible way to genetically modify plants in order to be resistant to various diseases and stresses that cause crop shortages around the globe. By creating more plants that are genetically resistant to top abiotic and biotic pressures, it is possible to prevent food shortages in poorer regions including a reduced loss of capital in the field of agriculture.
Figure 5. Quantification of relative Cas9 gene dosage of RM-1 (-C) versus T1 HaG plants.

Figure 6. GUS sequence analysis of T2 HaG plants versus RM-1 displaying a thymine deletion indicated by arrows, resulting in a frameshift mutation. *Deletion of leucine.
References


IDENTIFIABILITY ANALYSIS OF THE HUMAN H1N1 INFLUENZA VIRUS

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Harriet L. Wilkes Honors College, & Charles E. Schmidt College of Science

Abstract

Influenza is a significant source of morbidity and mortality both worldwide and also in the United States. In the U.S., the Center for Disease Control (CDC) estimates over 490,000 hospitalizations and 34,000 deaths during the 2018-2019 influenza season [2]. The objective of this research is to determine the epidemiologically important parameters of the H1N1 influenza virus such as the infection and recovery rates using mathematical modeling. Publicly available influenza incidence data from the CDC webpage was used to validate the mathematical model. The spread of the H1N1 influenza virus is modeled using the Susceptible-Infected-Recovered (SIR) compartmental model. To account for vaccination and treatment of the virus, SIVR and SITR models are considered. The models were run on the computer software MATLAB to compare the predictions of the model to the CDC data. To ensure the model’s precision, the parameters were manipulated so that the model predictions could mirror the data. It was found that the 2018-2019 season H1N1 influenza infection rate is 0.2567 per day and the recovery rate is 0.1774 per day. Finally, the identifiability of the models was analyzed through Monte Carlo Simulations, which were performed on MATLAB. The results show that the average relative errors of all the model parameters remained lower than the measurement errors. Thus, these results validate the identifiability of the epidemiological models considered in this study and the reliability of the parameter estimates.

Keywords: influenza virus; mathematical modeling; Kermack-McKendrick Model; parameter estimation; ordinary differential equations; epidemiology; Monte Carlo Simulation

Introduction

Background

Epidemiology is the field of science concerned with the diffusion of disease throughout human populations. Epidemiology is inherently associated to mathematics, using mathematical principles to model the manner by which infectious diseases may spread throughout a population. Infectious diseases are diseases that transmit between individuals and are caused by pathogenic microorganisms (e.g. virus or bacteria). Some examples of infectious diseases include chickenpox, HIV, West Nile virus, malaria, coronavirus, and influenza. When an infectious disease is present in a population, chance is that any contact between 2 individuals (one infected and one susceptible) could result in transmission of the disease. For example, the H1N1 influenza virus spreads through respiratory droplets that are transmitted from person to person when someone sneezes or coughs [3]. The CDC carries the epidemiological records of many infectious diseases, such as a recorded number of incidences, hospitalizations, and disease-induced deaths that resulted from the particular disease. The data collected by the CDC can be used to predict how infectious diseases will affect the population in future years, using epidemiological modeling techniques. Federal agencies and academic partners of the CDC can effectively use mathematical models, such as the Kermack-McKendrick model, to analyze the incidence data from the CDC and publish reports on the yearly activity of the influenza virus [4]. Because the CDC itself does not always conduct modeling of the data that they collect, there is a gap in knowledge and a need for predictive measures so that the public can understand how to prepare for future seasons of infectious diseases. Mathematical models, such as the Kermack-McKendrick SIR model, define parameters based on the epidemiological characteristics of infectious diseases and simulate how an infectious disease will diffuse across a group of individuals. By modifying a typical SIR epidemiological model, one can implement possible vaccination and treatment scenarios in the representation of the disease spread. In this research, various mathematical models were used to accurately represent the weekly new influenza incidences during the 2018-2019 season as reported by CDC. Furthermore, this project sought to estimate the parameter values of the systems of ordinary differential equations (Kermack-McKendrick models) that represented the spread of the H1N1 influenza virus during the 2018-2019 season and determine the identifiability of these parameter estimations.

Mathematical models have been used as an important tool in designing prevention strategies and control measures for infectious diseases. In such studies, the data reported by government health agencies is linked to mathematical models through parameter estimation. Parameters are estimated by minimizing the differences between the model predictions and the data. However, it is crucial to first analyze whether the parameter estimation problem is well posed [7]. That is, it needs to be understood whether it is possible to uniquely determine the parameters of the model from the reported data. Lack of such identifiability analysis might result in incorrect parameter values and, as a consequence, misleading strategies for prevention and control. In this research, identifiability analysis is
performed on the H1N1 influenza models (SIR, SIVR, and SITR) using Monte Carlo Simulations.

<table>
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<tr>
<th>Variable</th>
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<tr>
<td>S(t)</td>
<td>Number of susceptible individuals at time t</td>
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<tr>
<td>I(t)</td>
<td>Number of infected individuals at time t</td>
</tr>
<tr>
<td>R(t)</td>
<td>Number of recovered individuals at time t</td>
</tr>
<tr>
<td>V(t)</td>
<td>Number of vaccinated individuals at time t</td>
</tr>
<tr>
<td>T(t)</td>
<td>Number of treated individuals at time t</td>
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Table 1: Definition of variables in the epidemiological outbreak models (1), (2), and (3)

Kermack-McKendrick (SIR) Model

The Kermack-McKendrick Model is one of the earliest mathematical models of infectious diseases, from 1927, representing the spread of a typical infectious disease in a constant population [5]. The typical Kermack-McKendrick Model assumes a population that consists of individuals who are susceptible (S), infected (I), and recovered (R). Let N denote the total population size, then . The epidemiological model consists of Ordinary Differential Equations (ODEs) which describes the dynamics of each class, S(t), I(t), and R(t). The derivative of the susceptible class is equal to the number of individuals who are getting infected per unit of time, multiplied by -1 since the size of the susceptible class decreases as more people become infected. Incidences are defined as the number of people who are infected per unit of time. To model incidences, first a single individual is considered. If c represents the number of contacts one infected person makes per unit of time and p represents the probability that a contact with a susceptible individual will result in transmission of the disease, then pcS/N will represent the number of new infections per unit of time by one infected individual. Using β, an epidemiological parameter representing the rate of transmission, in place of pc, the expression is rewritten as βSI/N to represent the number of new infections per unit of time. Thus, the transmission rate β is the product of the number of contacts per unit time and the probability that this contact results in transmission of the infection. Assuming that during an influenza season the total population remains constant, the model takes the following form, where β represents the transmission rate and α represents chance of recovery.

\[ S' = -\beta SI/N \]
\[ I' = \beta SI/N - \alpha I \]
\[ R' = \alpha I \]

From the model, it is observed that \( N' = S' + I' + R' = 0 \). This total population N(t) is constant, and that constant is denoted as \( N(0) = N \). The flowchart representing the SIR model is presented in Figure 1.

Figure 1. SIR Model – Flowchart: A methodological flowchart that demonstrates the movement of individuals between the 3 classes of the SIR model is above, where β is the transmission rate and α is the recovery rate.

SIVR Model

Many people choose to take precautions in order to avoid contracting infectious diseases such as the influenza virus. One example of such precaution is vaccination. Vaccination can be incorporated in the epidemiological model by adding a vaccinated class of individuals to the SIR Model, and thus our model becomes the Susceptible-Infected-Vaccinated-Recovered (SIVR) model [8]. Susceptible individuals get vaccinated at rate Ψ and move to the vaccinated class. Individuals in the vaccinated class become infected at a reduced infection rate of δ1 where \( 0 \leq \delta 1 \leq 1 \). Let V(t) denote the number of vaccinated individuals, and then the final vaccination model becomes the following.

\[ S' = -\beta SI/N - \psi S \]
\[ V' = \psi S - \delta_1 V/N \]
\[ I' = \beta SI/N + \delta_1 V/N - \alpha I \]
\[ R' = \alpha I \]

Table 2: Definition of parameters in the epidemiological outbreak models (1), (2), and (3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
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<tbody>
<tr>
<td>β</td>
<td>Rate of infection</td>
</tr>
<tr>
<td>α</td>
<td>Rate of recovery</td>
</tr>
<tr>
<td>Ψ</td>
<td>Number of newly vaccinated individuals per unit of time</td>
</tr>
<tr>
<td>δ</td>
<td>Reduced rate of infection for individuals in the vaccinated class</td>
</tr>
<tr>
<td>δ1</td>
<td>Reduced rate of transmission for individuals in the treated class</td>
</tr>
<tr>
<td>γ</td>
<td>Percent of infected individuals who undergo treatment</td>
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</tbody>
</table>

Figure 2. SIVR Model – Flowchart: A methodological flowchart showing the movement of individuals between the 4 classes of the SIVR model (Susceptible, Infected, Vaccinated, and Recovered).
Reproduction Number

A secondary epidemiological parameter that can be determined by estimating the values of the parameters in the SIR model is the reproduction number. The reproduction number of an infectious disease, also denoted as R0, is used to represent the amount of consequent infections one infected individual will cause in a fully susceptible population during his/her infectiousness period. In this type of epidemiological modeling, the reproduction number can be given by the formula $R_0 = \frac{\beta}{\gamma}$. The value of the reproduction number suggests certain characteristics about the disease, such as whether the disease will die out quickly or remain endemic in the population. If the reproduction number is less than one, $R_0 < 1$, then the disease dies out in the population. If the reproduction number is greater than one, $R_0 > 1$, then the outbreak occurs.

Methods

For this project, publicly available H1N1 incidence data is obtained from the CDC website [4]. There was no physical data collection in this project. The data used in this project consists of the number of influenza-positive tests reported to the CDC each week during the 2018-2019 season, for a whole year (52 weeks) starting from September 28th. This incidence data does not include personal information such as names. The computer software (MATLAB) is used to simulate the SIR, SIVR, and SITR models and to compare the predictions of the model to the CDC data. The incidence data was compared to the predictions of the model to match the values of the model to the data as closely as possible.

In compact form the epidemiological models (1), (2), and (3) can be rewritten as

$$x'(t) = f(x, p) \quad x(0) = x_0$$

where $x(t)$ is a vector of state variables, $(x_0, p) \in \mathbb{R}^+ \times \mathbb{R}^+$ is the vector of parameters and $p = \frac{\beta}{\gamma}$ is the vector of observed parameters. For example, for model (1),

$$x(t) = (S(t), I(t), R(t)), f(x, p) = (-\beta S(t)/N, \beta S(t)/N - \alpha I - \alpha R)$$

The observations, the data collected by CDC, are functions of the state variables. Hence let $y(t)$ denote the observation, then

$$y(t) = g(x(t), p)$$

For model (1), the observations are the incidence rate, then

$$y(t) = g_1(x(t), p) = \beta S(t)/N$$

For model (2),

$$y(t) = g_2(x(t), p) = \beta S(t)/N + \beta \delta V(t)/N$$

For model (3),

$$y(t) = g_3(x(t), p) = \gamma I$$

Clearly, while collecting the data, the observations are contaminated with error which is called measurement errors. That means that the data do not fall on the smooth curve given by the observations $y(t)$ above, and deviates from it. The statistical model is written as

$$y(t) = g(x(t), p) + E_i,$$

where $E_i$ are the measurement errors [5]. Then, to curve-fit the epidemiological models (1), (2) and (3) to the CDC data, the sum of the squared differences between the measurements and the model predictions is minimized. That is, the following optimization problem is solved.

$$p = \frac{\min_p}{\sum_{i=1}^n \sum_{j=1}^m} (y_i - g(x(t), p))^2$$

(4)

The computer software MATLAB is used to minimize (4) with the function fminsearchbnd, which is an optimization function directed to minimize this parameter estimation problem [6]. Each system of ODEs is solved using the built-in function ode15s to solve the epidemiological models (1), (2), and (3). The total population is fixed to 215,000. After fitting the mathematical model to the data, the parameter values that resulted in the best fit were recorded in results. This fitting process was repeated for each of the mathematical models (SIR, SIVR, and SITR).

The second part of this project is to analyze the identifiability of the models used to represent the spread of the H1N1 influenza virus using Monte Carlo simulations. The objective is to study whether the parameter estimation problem is well posed, in the sense that the solution is continuous. That is, if the data is varied, measuring how much the estimated parameter values will change. To achieve that, error was introduced to the model predictions at the data points gradually 1,000 times. After introducing error, the model was re-fitted 1,000 times to the data with error to observe the effect on the parameter estimates, which determines whether the initial parameter estimates are reliable. The process of Monte Carlo Simulations was executed for each of the three models, to observe the average relative errors of all the epidemiologically important parameters in all three models and measure the identifiability of the models [10]. The total average relative errors for each parameter in the respective models are also recorded as results. The Monte Carlo simulations executed in this project is itemized in detail as following.

Monte Carlo Simulation

After curve-fitting the model to the incidences data, the next step is to analyze the identifiability of the model by performing Monte Carlo Simulations. The purpose of Monte Carlo simulations is to introduce error into the incidence data to see how the parameter values of $\beta$ and $\gamma$ react [9]. To observe how the parameters change with error at each noise level, the model is re-fitted 1,000 times for each error level to conclude whether the parameter estimations are reliable. In this project, noise levels of 1%, 5%, 10%, and 20% were introduced as part of the Monte Carlo Simulations. Monte Carlo Simulations were performed in this project in the following steps.
1. Find the solutions to the epidemiological models using the true parameters $\beta$ and $\gamma$ and the output vector $g(x(t), \hat{p})$ at the data time points (each week).

2. Produce 1000 synthetic data sets from the statistical model that contains a set measurement error in the data to obtain the following statistical model where $E_i$ is the average relative error introduced into the data:

$$y_i = g(x(t), \hat{p}) + E_i$$

3. Fit the system of ODE model according to each of the 1000 synthetic datasets created in the previous step to estimate the parameters $p_j = [\beta, \gamma]$ for $j = 1, 2, \ldots, 1000$.

4. Find the average relative errors (AREs) in parameter estimates in the set $p$ as below:

$$\text{ARE}(\hat{p}^{(j)}) = 100\% \frac{1}{M} \sum_{j=1}^{M} \left| \frac{\hat{p}_j^{(k)} - \bar{p}_j}{\bar{p}_j} \right|$$

where $p_j^{(k)}$ is the $k$th parameter in the set $p$, $\bar{p}_j$ is the $j$th parameter in the set $\bar{p}$ and $\hat{p}_j^{(k)}$ is the $k$th parameter in the set $\hat{p}$ of true parameters.

The 4 steps of the Monte Carlo simulations are repeated with each increasing level of noise and executed for each of the 3 epidemiological models (SIR, SIVR, and SITR).

**Results**

This project found success in utilizing accurate epidemiological models to represent the spread of the H1N1 influenza virus during the 2018-2019 season according to data from the CDC [4]. The value of $N$ was fixed to 215,000 in each model to account for individuals who are infected with the disease but are not recorded by the CDC. Figures 4, 5, and 6, show the curve-fitting of the mathematical model to the incidence data. The blue line represents the respective mathematical model prediction while the orange bars represent the weekly influenza incidence data. The parameters that produced the best fit to the CDC data in each model (SIR, SIVR, and SITR) are summarized in Table 3, 4, and 5 below, respectively. For example, when curve-fitting the CDC data to the standard SIR model, the estimated transmission rate ($\beta$) was 0.2567 per day and the recovery rate ($\gamma$) was 0.1774 per day. These parameters represent conditions of the population and characteristics of influenza that produce a similar pattern of incidences compared to CDC data from the 2018-2019 H1N1 season. From the parameter values listed in the tables above, the secondary epidemiological parameter $R_0$, or the reproduction number, can be determined. Using the values of $\beta$ and $\gamma$ estimated from the SIR Model, the value of $R_0$ was measured to be approximately equal to $0.2567$ divided by $0.1774$, or about 1.447. Because the value of $R_0$ is greater than 1 for the 2018-19 season of H1N1 influenza, this suggests that the disease will remain endemic in the population, which is consistent with the knowledge that the influenza virus seasonally returns to the population each year.

The results of the Monte Carlo Simulations are summarized in Table 6, 7, and 8 below. As higher noise levels were presented into the data, the average relative error in the parameter estimates slowly increased as well. However, there were no extreme average relative errors, suggesting that the models have strong identifiability and that the parameter estimates found in this study for all 3 models are reliable estimates of the actual parameter values in real life.

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**Tables**

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**Figure 4** Graph demonstrating curve-fitting results of the SIR Model (1)

**Figure 5** Graph demonstrating curve-fitting results of the SIVR Model (2)

**Figure 6** Graph demonstrating curve-fitting results of the SITR Model (3)
Discussion

The results show successful modeling of the reported incidences of the H1N1 influenza virus to the CDC using systems of Ordinary Differential Equation models. When running the model on the computer software, a fitted curve was achieved that corresponded to the official incidence data. Furthermore, the sum of the squared differences between the model predictions and the official data at each week was minimized with respect to the parameters of interest, meaning that the model is as close as possible to the collected data values reported by the CDC. After running Monte Carlo simulations on the models, the total average relative error found for each model was moderately low. Thus, the models have strong identifiability and the parameter estimates are reliably dependable. The epidemiological model applied in this project can be efficiently used to model the spread of various infectious diseases among a population.

This type of epidemiological modeling can be used to predict the long-term behavior of infectious diseases, such as whether a disease will die out after one season or remain in the population and become an endemic. This knowledge equips health officials to prepare for upcoming infectious disease seasons by preparing vaccines and treatments in advance, as the model can predict the number of weekly incidences of the disease. Furthermore, manipulating different parameters in the epidemiological model demonstrates how different factors influence the dynamics of the spread of the influenza virus. For example, Figure 7 (shown below) demonstrates how manipulating the vaccination rate affects the epidemiological model after the other parameters is fixed to the value obtained in this project.

In this project, the vaccination rate was estimated to be 0.0793 or approximately 0.08 per day. Multiplying 0.08 times S (number of susceptible individuals in the population) will provide the number of people vaccinated per day. In Figure 4, the purple curve matches the vaccination rate that was estimated in this project and exhibits a number of incidences similar to the data reported by the CDC. The different curves in Figure 4 demonstrate how different vaccination rates could affect the number of influenza incidences over time. As seen in Figure 4, when the vaccination rate in the model is lowered to 0.01, the number of influenza incidences rapidly increases and reaches its peak at almost 15,000 incidences per day at about 25th day after the start of the influenza season. When the vaccination rate is increased to 0.2, it is observed that the curve has been flattened (see light blue curve). It reaches its peak at a later day, approximately 175th day after the start of the influenza season and the peak is at a lower value, 1000 incidences per day. This demonstrates how the dynamics of the epidemiological model greatly depends on the values of the various parameters. If the vaccination rate is low, then there will be a lot of new infections per day. The peak of incidences occurs later for higher vaccination rates. By showing how if less people get vaccinated, the number of incidences rapidly increases demonstrates the importance of vaccination in controlling the spread of an infectious disease.
REFERENCES


Aaron Mencia & Charlene Fournier

Aaron Mencia is a recent graduate from Florida Atlantic University (FAU). He finished with a Bachelor of Science in Biological Sciences. His interest in primatology and research led him to meet Dr. Kate Detwiler to work in her lab. The primatology lab at FAU works on a variety of projects from genetic analysis to ecological surveys and primate behavior. Aaron started inputting data from recent camera trap surveys in the Democratic Republic of the Congo, which sparked his interest in investigating polyspecific associations. During his time as an undergraduate, Aaron presented preliminary results at two conferences (South Florida Primatology Group & OURI symposium at FAU). He has plans to pursue graduate school studying primatology.

Charlene Fournier is a recent graduate from the Master of Science program at FAU majoring in Biological Sciences. During her time as a graduate student, Charlene studied the recently discovered species: Cercopithecus lomamiensis (lesula) through camera trap surveys collected in the field. Her thesis covered several aspects of lesula behavioral ecology; terrestriality, activity pattern, birth seasonality, group size, social system, and the impact of hunting on lesula group size. She presented her thesis work at several conferences (International Primatological Society, (IPS) American Association of Physical Anthropology (AAPA), South Florida Primatology Group, and the GPSA symposium at FAU) and is now looking to publish her work. She is continuing her education at FAU where she was accepted into the Integrative Biology PhD program.

Amy Argueta

Amy Argueta is a high school senior who graduated from Florida Atlantic University Highschool in the Spring of 2020. Starting in the Fall of 2020 she will be enrolled at the University of Southern California where she plans to complete her bachelor's degree in international relations. She also plans to continue pursuing undergraduate research.

Catalina Sampayo

Catalina Sampayo is a first year undergraduate student from Orlando, Florida majoring in Secondary English Education at Florida Atlantic University. Intrigued to further research Demoralization was found after a discussion held in my Intro to Diversity for Educators course. Special thanks to Professor Leichtman for introducing me to this phenomenon and aiding in the preparation of this manuscript.

Daniella Hernandez

Daniella Hernandez is a recent graduate from Florida Atlantic University High School. She is pursuing a major in neuroscience and behavior as well as a major in psychology. Throughout her time as a dual-enrollment student at FAU, she has been thoroughly involved in research. Recently, she had the opportunity to present research she conducted at the Infant Cognition Lab at FAU at the FAU undergraduate research symposium. Although she has done research in the college of science, she is thoroughly interested in writing and literature studies. Daniella believes that interdisciplinary studies will increase her knowledge base and prepare her for her future career. Her current concentration in this field focuses on analyzing literary language in southern fictional literature.

Delaney Frazier

Delaney Frazier is working under Lauren Kircher and Dr. John Baldwin studying population dynamics of snook in the St. Lucie river in partnership with Dr. Joy Young FWC-Tequesta. As well as that, she is working with Brent McKenna and Dr. John Baldwin in measurement of snook otoliths and an additional project with Katherine Galloway and Dr. Marianne Porter in the Biomechanics lab studying facial spines of the red lionfish. She is currently a co-author on an article that is currently under review with Katherine Galloway.
Kenneth Kalczuk

Kenneth Kalczuk is a senior at the Wilkes Honors College pursuing a Bachelors of Science in Economics with a minor in Spanish Literature. He first became interested in studying the history and culture of Spain after spending a semester abroad in Madrid under faculty advisor Dr. Carmen Cañete Quesada. The Spanish Civil War (1936–1939), and its political and cultural connotations, has since fascinated Kenneth as he continues researching and learning about the subject. His current paper on “Fascist Surrealism” aims to examine how the artistic and cultural movement, Surrealism, evolved within the context of the Spanish Civil War. As a student of history, he believes that, by studying the past, society can learn its future.

Rose Weinstein

Rose Weinstein is a senior at Florida Atlantic University who is majoring in Biology and completing a certificate in Biotechnology. Rose has been working under the guidance and mentorship of Dr. Xing-Hai Zhang through directed independent research in his plant biotechnology lab since the Fall 2019 term; and has presented a poster at the OURI's tenth annual Undergraduate Research Symposium. Following graduating with a Bachelor of Science in Biology, she aspires to attend graduate school and continue research in a master's program in Biomolecular Engineering.

Linda Do is currently a senior majoring in biology who plans on graduating in the spring of 2020. Currently, she is working in Dr Jia's lab working on C. elegans. During the fall of 2019, she had the opportunity to be in Dr Zhang's plant biotechnology lab. Upon graduation, she will be attending podiatry school at Barry University.

Annalise Wellman is a first year Biology major at Florida Atlantic University. In addition to being part of the National Science Foundation's Learning Environment and Academic Research Network program, she has also been part of Dr. Xing-Hai Zhang's lab since the Spring 2020 term. In the future, she hopes to continue plant biotechnology research and obtain a career as a molecular phytopathologist.

Vivek Sreejithkumar

Vivek Sreejithkumar is a dual-enrolled high school student at Florida Atlantic University High School, taking a full-time course load of undergraduate courses at Florida Atlantic University. He is majoring in Neuroscience and Behavior with a minor in Mathematical Sciences with an ambition to attend medical school in the future. He became actively involved with research during his first year as a full-time undergraduate student at FAU, conducting research in the department of mathematical sciences with Dr. Tuncer and as an undergraduate research assistant in the MPCR Lab. Vivek serves as the 2020-2021 Co-Director of Research for the American Medical Student Association, guiding fellow undergraduates to become involved with research and loves to involve himself with other extracurricular activities on campus.
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