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BIOGRAPHY OF
DR. RONALD E. McNAIR

HE OVERCAME OBSTACLES.

Dr. Ronald Erwin McNair, Physicist & Astronaut, dared to dream. As an African-American growing up in a poor community in the South, he encountered discrimination early in his youth. Yet this did not stop him from pursuing his dream of becoming a scientist.

HE ACHIEVED ACADEMIC EXCELLENCE.

In 1971, he graduated magna cum laude from North Carolina AT&T State University with a B.S. degree in physics. Ronald McNair then enrolled in the Massachusetts Institute of Technology. In 1976, at the age of 26, he earned his Ph.D. degree in laser physics.

HE BECAME A LEADER IN HIS FIELD.

Dr. McNair soon became a recognized expert in laser physics while working as a staff physicist with Hughes Research Laboratory. He was selected by NASA for the space shuttle program in 1978 and was a mission specialist aboard the 1984 flight of the shuttle Challenger.

HE WAS RESPECTED AND COMMENDED.

For his achievements, Ronald McNair received three honorary doctorate degrees and many fellowships and commendations. These distinctions include: Presidential Scholar, 1967-71; Ford Foundation Fellow, 1971-74; National Fellowship Fund Fellow, 1974-75, Omega Psi Phi Scholar of the Year, 1975; Distinguished National Scientist, National Society of Black Professional Engineers, 1979; and the Friend of Freedom Award, 1981.

HE EXCELLED IN MANY ASPECTS OF LIFE.

Ronald McNair also held a fifth degree black belt in karate and was an accomplished jazz saxophonist. He was married and was the dedicated father of a daughter and a son.

After his death in the Challenger explosion in January 1986, members of Congress provided funding for the Ronald E. McNair Post-Baccalaureate Achievement Program to encourage college students with similar backgrounds to Dr. McNair to enroll in graduate studies. Thus, the program targets students of color and low income, first-generation college students. This program is dedicated to the high standards of achievement inspired by Dr. McNair's life.

Biography courtesy of the University of Nevada, Las Vegas

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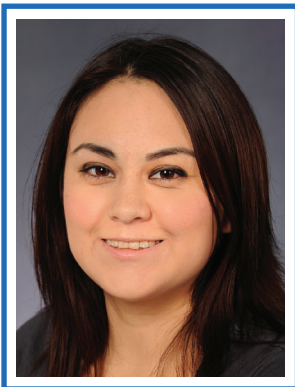
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2018 McNAIR RESEARCH ARTICLES

NEGOTIATION FOR MEANING: VIEWS ON SEXUAL CONSENT IN RELATIONSHIPS

by SHAKIRA BARNES

ABSTRACT

Sexual assault is common among female college students. One in five women in college experience sexual assault, and females ages 18 to 24 have the highest rate of rape and sexual assault victimizations compared to females in all other age groups. Two factors that seem to play a role in perception of consent are the relationship between perpetrator and victim and use of alcohol. 313 students from Texas A&M University - Corpus Christi participated in an online survey that questions their understanding of sexual consent based on descriptions of a date between a man and a woman. Participants completed measures of sexual attitudes, gender norms, and attitudes regarding alcohol, and responded to vignettes describing various romantic interactions. Research demonstrated that vignette actors who were in a relationship as opposed to casually dating were viewed as more consensual, as having a greater ability to say no to sex, and as more consenting unless a no was stated. The findings in this study was derived from cross sexual data, and the implications of these findings for future research is discussed.

Keywords: *perception of sexual consent, alcohol use and sexual consent, relationships and sexual consent, gender norms and sexual consent.*

MENTOR

Dr. Olga Berkout

Assistant Professor of Psychology
Department of Psychology
and Sociology
College of Liberal Arts

INTRODUCTION

Sexual assault victimization is common among female college students. According to data from the National Crime Victimization Survey, one in five women in college experience sexual assault (Sinozich & Langton, 2014; Krebs, Lindquist, Berzofsky, Shook-Sa & Peterson, 2016). In addition, females ages 18 to 24 have the highest rate of rape and sexual assault victimizations compared to females in all other age groups (Sinozich & Langton, 2014). A notable recent case of sexual assault was Brock Turner, a former Stanford swimmer who assaulted an unconscious woman, received a sentence of six months in jail, and was released after three months. Another case was three Michigan State football players who were cut from the team and expelled due to sexually assaulting a young woman. As in these cases, research indicates the victim knew the offender in 80% of rape and sexual assault victimizations, with 51% of

student rapes occurring when the victim was engaging in recreational activities away from home (Sinozich & Langton, 2014). Lastly, the victims perceived their offender to be under the influence of alcohol or drugs in more than half of rape incidents (Krebs et al., 2016).

What is Consent?

Sexual consent is a topic that often goes unnoticed by the public until there is a scandal; it plays a vital role in discussions and debates about sexual violence and is often the most defining characteristic of what is considered sexual assault or not (Beres, 2007; Hall 1998). However, we consider talking about sex to be taboo, therefore, we are unable to effectively communicate sexual consent based on the experiences with others (Beres, 2007). In addition, there is severely limited research examining what sexual consent is, how to define it, and how to communicate it (Beres, 2007; Jozkowski, Peterson, Sanders, Dennis, & Reece, 2014).

There is a consensus in our society that sexual consent represents agreeing to engage in sexual activity. The problem lies in how society defines sexual consent. Hickman and Muehlenhard (1999) explained according to legal definitions, consent is actively, verbally or nonverbally given. In contrast, many scholars do not define consent in their research, and those who do use varied definitions of consent (Beres, 2007; Hickman & Muehlenhard, 1999). Jozkowski et al. (2014) found that more than half of their college-aged sample defined consent as either an agreement to engaging in sex with 16% stating that consent indicated yes to sex. To combat the issue of various definitions of sexual consent floating around, researchers have begun investigating consent-indicating behaviors (Beres, 2007). Hickman and Muehlenhard (1999) argued considering consent to be a mental or physical act that does not always correspond with each other due to people engaging in token resistance, which is a stereotype indicating an individual saying no to sex when they really mean "yes" (Hall, 1998).

Recently, researchers have looked to physical behaviors to find signals indicative of consent, specifically verbal and nonverbal cues. In addition to Jozkowski et al. (2014) findings of how their college-aged sample defined consent, they also found they often use implicit consent signals rather than explicit or direct signals. The

majority of sexual consent signal cues are categorized as direct verbal, direct nonverbal, indirect verbal, and indirect nonverbal (Hickman & Muehlenhard, 1999; Abbey, 1987). While Hickman & Muehlenhard (1999) indicated students are more likely to report they use all these signals in some form to indicate their sexual consent, Jozkowski et al. (2014) found students are more likely to rely on nonverbal cues due to verbal cues being misleading. They state three reasons why college students are more likely to use nonverbal cues over verbal cues: a) Society endorses nonverbal cues and this influences college students' perception of sex communication, b) Students may feel uncomfortable asking for direct verbal consent, or c) There may be an expectation that consent is implied unless otherwise specified (Jozkowski et al., 2014).

In contrast, students indicated using verbal consent cues during sex and nonverbal consent cues for intimate touching for sex (Jozkowski et al., 2014). Both verbal and nonverbal cues come with a vital misinterpretation: token resistance (Lewin, 1985; Muehlenhard & Linton, 1987). When looking at how token resistance is related to consent, Jozkowski et al. (2014) stated men might interpret silence as consent to continue to engage in intimate behaviors or permission to engage in more intimate behaviors; thus, men may interpret a woman's legitimate and genuine refusal as token resistance, which ends with a man unintentionally having sex with an unwilling woman.

Relationships

Perception of consent is not a topic that is often discussed in the same context as relationships, yet it maintains the same level of importance as it does in cases of stranger sexual assault (Monson, Langhinrichsen-Rohling, & Binderup, 2000). Shotland and Goodstein (1992) found prior sexual involvement in a relationship important in determining how an incident of sexual violence is perceived. They gave an example that if a woman has as few as 10 prior consensual sexual interactions with her partner, her subsequent refusals will be seen as less legitimate and the violence will be seen as less serious or harmful, regardless if this matter becomes public or stays private. In addition, Monson et al. (2000) found participants were more rape-supportive and used more sex role stereotypical attributes about rape when

they were informed of a dating couple's past sexual history, but there was not a statistical difference in the participant's attributes when they were informed of a married couple's past sexual history.

The way society views sexual assault in relationships can be described by Humphreys' (2007) finding, experienced couples in terms of relationship length and sexual involvement were seen as more consensual, clearer in sexual consent, and more acceptable. Conversely, Humphreys (2007) described partners' assumptions and expectations of normative consent in more established relationships, meaning there is an unspoken knowledge that consent is always implied unless otherwise specified, and newer relationships require more explicit consent due to the unestablished normative consent expectation.

Shotland and Goodstein (1992) stated, "a sexual obligation may serve as a relationship-building and preserving function," and unintentionally found the existence of a sexual contract between men and women: consenting to unwanted sex can be seen as consensual on the grounds that if the situation would be reversed, as long as the situations and partners are equally accommodating to each other (p. 762). In their study, male participants were more likely than female participants to view actors of both genders to be more obligated to their partner, and they were more likely to feel like their partners with whom they have a sexual history are sexually obligated to them (Shotland & Goodstein, 1992). Male participants were also more likely than female participants to feel a sense of being wronged if their date refused sex (Shotland & Goodstein, 1992). Shotland and Goodstein (1992) mention, if a man finds himself in the situation where his female partner or date refuses sex, he can a) persuade her, b) use his superior status over her, or c) use force to make her have sex with him.

Gender Roles

Gender roles are a product of schemas and scripting, which represents the production of a behavior in everyday life (Simon & Gagnon, 1986). A behavior must occur on three different levels before it can be considered a script (Simon & Gagnon, 1986). Sexual scripts, in particular,

are not special on their own, nor do they possess a unique quality integral in human motivation (Simon & Gagnon, 1986). They gain meaning based on society or by the individual placing significance on it. Humphreys (2007) suggested gender socialization in sexual consent may be more important to woman, due to the traditional woman's sexual script including giving permission for sexual activity; however, he did not influence the perception of the relationship experience. Humphreys suggestion on gender socialization exemplifies how scripts create meaning and become important. According to Simon and Gagnon (1986), "Societal settings where the sexual takes on a strong meaning… should also be settings where sexual meanings play a correspondingly significant role in the intrapsychic lives of individuals" (p. 105).

Jozkowski et al. (2014) elaborate on Humphreys' (2007) and Simon and Gagnon's (1986) statements regarding the production and nature of gender roles as products of schemas and scripting, adding how consent is communicated or interpreted may differ among men and women. Society designates men to be the "initiators" of sex (O'Sullivan & Byers, 1992). O'Sullivan, Hoffman, Harrison, and Dolezal (2006) conducted a study looking at the extent to which young adults agree with behaviors and attitudes in line with traditional sexual scripts and found that male participants were more likely to agree with traditional gender roles. Male participants were also less likely to view sex as important in establishing intimacy, were less romantically involved in relationships, and engaged more often in sex. Researchers have hypothesized that men may not verbalize consent or refuse sexual opportunities because these actions would go against their traditional gender roles (Jozkowski et al., 2014; O'Sullivan et al., 2006; Shotland & Goodstein, 1992).

On the contrary, according to Simon and Gagnon (1986), "women [have rarely been] "selected" for sexual roles on the basis of their own interest in sexual pleasure" (p. 107). It is misunderstood that women rarely initiate sex, but as more women initiate sex, it becomes unclear what happens during consent exchanges (Beres, 2007). Jozkowski et al. (2014) conducted a study in which they surveyed 185 Midwestern U.S. college students to see how they define, communicate, and interpret sexual consent and non-consent, and they found that women

are more likely to rely on verbal cues, both direct and indirect, to signal consent. In addition, Jozkowski et al. (2014) hypothesized that women believe they should be asked for consent in order to directly accept or deny the offer, a belief that is rooted in their traditional sexual script as they are seen as the "gatekeepers" for sexual activity to occur (Simon & Gagnon, 1986; Clark, Shaver, & Abrahams, 1999; Werner and LaRussa, 1985; O'Sullivan & Byers, 1992).

The difference in which men and women communicate and interpret consent have implications on what is considered consensual or un-consensual sex. Research has found men are more likely to interpret interactions and signals as more sexually motivated than women, leading to inaccurate interpretations of a woman's consent and could lead to unwanted or coercive sex (Jozkowski et al., 2014; Hickman & Muehlenhard, 1999). Hickman and Muehlenhard (1999) conducted a study looking at how college undergraduates would interpret and communicate the consent of their own signals and their dates' signals, and they found several accounts of how male and female differences influence sexual consent. Firstly, men interpreted their dates' signals as more sexually consenting while women interpreted their dates' signals as similar to their own. Secondly, men interpreted females' verbal and nonverbal signals and intoxication statements as more indicative of their consent, which contrasts with how women rate their own consent signals. In addition, men rated their own signals and intoxication statements as more indicative of their own consent compared to how women rated them. Hickman and Muehlenhard's (1999) statements of both accounts show how much more important a man's perceptions and misconceptions are compared to a woman's.

Alcohol

Corcoran and Thomas (1991) identified scientific validity in the societal belief that sexual interest increases under the influence of alcohol. George, Cue, Lopez, Crowe, & Norris (1995) supported Corcoran and Thomas (1991), stating that sexuality is more accredited to the drinker rather than control over his or her mind and body. Continually, alcohol intoxication may not be defensible in legal cases against rape, but

can be sufficient to mitigate responsibility for the sexual violence (Gunby, Carline, Bellis, & Beynon, 2012).

Differences exist between how men and women interpret alcohol-related disinhibition. Considering that men are more likely to initiate sex than women, men are also less likely to engage in the belief that alcohol affects another's ability to consent to sex (Corcoran & Thomas, 1991; Gunby et al., 2012). In addition, men are less likely to be able to define consent and are more likely to describe situations where both parties are intoxicated as consensual (Gunby et al., 2012). Laurel Hustled and Cooper (1992) found men to drink more often and more heavily, resulting in more alcohol-related problems than women.

Abbey and Harnish (1995) conducted a study looking at the effects of rape supportive attitudes and alcohol consumption on perceptions of sexual intent, and they found 3 occurrences that describe the differences in men and women's interpretation of alcohol-related disinhibition. Firstly, college men were more likely to perceive sexual intent in both female and male vignette characters compared to college women and considered both targets at their most sexual when they were drinking. Secondly, men whose beliefs aligned with stereotypes about rape were more likely to interpret vague, obscure cues as signs of attraction. Lastly, men who agreed strongly to rape myths were more likely than men who agreed to rape myths at low or moderate rates to assume that women who are dressed up or who have kissed him have expressed interest in engaging in sex. In addition, George et al. (1995) found that men who had a high alcohol disinhibition expectancy, meaning that they believed in characteristics such as "alcohol makes one happy, outgoing, loud, sociable, and dominant," rated women who were drinking as more likely to engage in sex (p. 178).

When looking at women, research has found a negative relationship between traditional gender-role attitudes and the alcohol consumption of women, such that a woman who is seen drinking alcohol is viewed as more sexually available, more likely to engage in high and low intimacy behaviors, more likely to engage in sex, and less socially skilled than a woman who is drinking a non-alcoholic beverage (Huselid & Cooper, 1992; George et al., 1995). George et al. (1995) found more

reluctance in women to act on expectancies of alcohol-induced disinhibition in men than vice versa, describing that the reluctance may be due to less experience with alcohol or personally-held beliefs of how inebriated the man is.

While researchers found many gender differences in alcohol consumption and sexual consent, they found two instances of gender similarities. The first instance dictated that scenarios are more likely to be viewed as rape by both men and women when there is a higher difference in intoxication levels between both parties (Gunby et al., 2012). Secondly, Abbey & Harnish (1995) findings suggested that male and female college students believe that consuming alcohol in mixed-sex pairs is most appropriate, while a woman drinking alone is least appropriate. In addition, the researchers discussed that their participants viewed drinking as most appropriate when a companion was also drinking which demonstrated the social pressure placed on individuals who drink alone or are the only one drinking amongst a group.

Purpose of the Current Study

Through looking at relationships, traditional gender norms, and alcohol, the purpose of this project is to help understand how these factors influence perceived consent in sexual situations. Findings from this project may inform future interventions and strategies aimed at supporting consensual sexual encounters. We hypothesize that a) participants will be more likely to perceive sexual encounters as consensual when they occur in an established long term rather than casual relationship; b) more traditional gender norms will be associated with greater perception of consent; and c) descriptions of individuals who are drinking will be perceived as consenting more than those who are not drinking.

METHODS

Participants

313 undergraduate participants, 57 males, 201 females, and 55 missing, were selected from the College of Liberal Arts at Texas A&M University - Corpus Christi, specifically from Psychology classes during the Spring 2018 term. This gender difference may be partly accounted for by demographic differences in the College of Liberal Arts at this University (i.e., 38% male and 62% female; Texas A&M University - Corpus Christi, n.d.). The sample consisted of 38.1% freshman, 28% sophomores, 18.7% juniors, and 15.2% seniors. Approximately 47.9% of participants identified as Hispanic, 34.2% as Caucasian, 6.2% as African American, 5.1% as Asian, .4% as Native American, 4.3% as multiracial, and 1.9% as other. Participants ranged in age from 18 to 35 years of age, with a mean of 19.95 years (*SD* = 2.443).

Measures

Alcohol use. Participants completed the Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, De la Fuente, Grant, 1993) which is a 10-item questionnaire designed to assess alcohol consumption, drinking behaviors, and alcohol-related problems. The AUDIT is arranged in a Likert-type scale ranging from 0 to 4, however the meaning of each number is dependent on the question asked. The reliability and validity of the AUDIT has been established in research conducted by various researchers in various settings and nations (Saunders, Aasland, Babor, De la Fuente, Grant, 1993).

Perceptions of sexual activity. The participants completed three scales on sexual activity: The Sexual Consent Scale, Revised (SCS-R; Humphreys, 2007), the Double Standard Scale (DSS; Caron, Davis, Halteman, & Stickle, 1993), and the Sexual Risk Survey (SRS; adapted from Turchik & Garske, 2009).

Sexual Consent Scale, Revised (SCS-R; Humphreys, 2007). The SCS-R is a self-report questionnaire consisting of 40 items arranged in a 7-point ordinal scale that ranged from 1 (*Strongly Disagree*) to 7 (*Strongly Agree*). SCS-R provides six subscales: positive attitudes towards establishing consent (extent to which an individual believes they can vs. cannot influence a sexual situation, 9 items), lack of perceived behavioral control (extent to which an individual believes they are confident vs. not confident in asking for consent, 9 items), relationship length norms (extent to which consent should vs. should not be asked in a relationship, 5 items), assuming consent (meaning which situations warrant consent and how consent should vs. should not be communicated, 7 items), indirect behavioral approach (extent to which consent should vs. should not be communicated and interpreted, 6 items), and awareness of consent (extent

to which an individual has vs. has not discussed consent, 4 items). Internal reliability was found for all six sub scales with coefficients ranging from .63 to .71, and expectations were consistent with results obtained when compared to the Sexual Sensation Seeking Scale and Hurlbert's Index of Sexual Assertiveness (Humphreys, 2007).

Scoring for the SRS followed the directions of the authors (Turchik & Garske, 2009). Data was modified by creating a separate variable by hand to record numerical data from free response data: never, none, and not applicable (n/a) was recoded to 0; several times, a few, and multiple was recoded to 2; a range of variables (e.g., 2-5 times) was averaged with the smallest and largest number and rounded up; greater than or less than a variable was set at that variable (ex. 2+ was recoded as 2); and responses such as I don't know, too many to count, many times, every time, most of the time, and all fractions (i.e. half of the time or 3/8 of the time) were recoded as missing data.

Double Standard Scale (DSS; Caron, Davis, Halteman, & Stickle, 1993). The DSS is a self-report questionnaire consisting of 10 items arranged in a 5-point original scale that ranged from 1 (*Strongly Agree*) to 5 (*Strongly Disagree*). There were ten questions related to the sex roles of men and women, and each question reflects the participant's attitudes about the role of men and women in sexual activity. The Cronbach alpha for the DSS' reliability was 0.72, and expectations were consistent with results obtained by Caron et al (1993).

Sexual Risk Survey (SRS; adapted from Turchik & Garske, 2009). The SRS is a self-report questionnaire consisting of 23 items arranged in a free-response format. In this study, the SRS was adapted to consist of 11 items arranged in a free-response format. These eleven questions related to sexual risks that college students could most commonly engage in and the context in which it occurs. Each question reflects how risky the participant's sexual behavior is. According to Turchik and Garske (2009), the SRS demonstrated good internal consistency and test-retest reliability, as well as evidence of content, concurrent, and convergent validity.

Demographic Information Scale (DIS). Participants completed a demographic questionnaire to assess the participant's sex, age, college major, ethnicity, country

of origin, college classification, employment status, and marital status.

Vignettes (adapted from Humphrey, 2007). Participants saw eight vignettes describing a date situation between man and a woman. The vignettes were the same except for variation in relationship context (on their second date together or celebrating their 2-year dating anniversary), alcohol use (shared a dessert or had three drinks), and sexual activity (have or have not previously had sex). Participants answered questions that measured perceptions of sexual consent (female actor consent, perception female actor should have said no, assumed consent, and affirmed consent) adapted from Terry Humphrey's (2007) study. Participants rated their level of agreement on a 7-point scale that ranged from 1 (*Strongly Disagree*) to 7 (*Strongly Agree*).

Procedure

All study procedures were approved by the Texas A&M University - Corpus Christi Institutional Review Board. Participants who consented to participation after a brief introduction proceeded to take an anonymous online survey administered via Qualtrics. Participants provided demographic information, completed measures of alcohol use, agreement to traditional gender norms, sexual history in terms of sexual risks, perceived sexual consent, and responded to vignettes. Vignettes and survey measures were presented in counterbalanced order. Participants received course extra credit for their participation.

RESULTS

Data Cleaning

Univariate outliers were identified using standardized z scores greater than 3.29 (p < .001; Tabachnick & Fidel, 2007) and univariate outlier scores winsorized (such that their scores were within bounds). Using this criterion, ten participants were identified as univariate outliers and winsorized, thus maintaining 313 participants data for subsequent analyses. Multivariate outliers were identified using Mahalanobis distance greater than 49.728, p .01 (following Tabachnick & Fidel's 2007 suggestion). Using this criterion, zero participants were identified as multivariate outliers.

Bivariate Correlations

All analyses were conducted using SPSS version 24. Bivariate correlations among study variables were examined, which are presented in Table 1. Low double standard was negatively, and significantly, associated with a lack of perceived behavioral control and indirect behavioral approach. Alcohol consumption was positively associated with relationship length norms, assuming consent, and indirect behavior approach. Positive attitude toward establishing consent was significantly negatively associated with perceived behavioral control, relationship length norms, assuming consent, and indirect behavior approach. We found a number of significantly positive relationships between relationship length norms, assuming consent, and indirect behavioral approach. Sexual risk was positively associated with alcohol consumption, relationship length norms, assuming consent, indirect behavioral approach, and awareness of consent. No significant relationships were found between awareness of consent, low double standard, and alcohol consumption.

Participant Perceptions of Sexual Behavior

To assess the influence of relationship status, alcohol use, and whether the couple had prior sexual behavior along with the interactions of these variables on participant perception of sexual behavior, three-three-way repeated measure ANOVAs were conducted. All variables were assessed within subjects.

Perception of Female Actor Consent. An ANOVA was conducted examining the impact of relationship status, prior sexual behavior, and alcohol use and their interactions on perception of the extent to which the female actor described in vignettes consented. This analysis yielded significant main effect for relationship length ($F(1,205) = 8.852$, $p < .$, partial $\eta 2 = .041$), such that greater consent was perceived when the female actor was in a relationship rather than when she was casually dating the male actor. Additionally, there was a significant main effect for alcohol use ($F(1,205) = 8.852$, $p < .$, partial $\eta 2 = .039$), where eating a dessert rather than consuming alcohol was perceived as more consensual. Lastly, there was a significant main effect for prior sex ($F(1,205) = 8.852$, $p < .001$, partial $\eta 2 = .077$), where prior sexual involvement (versus no

prior involvement) was perceived as more consensual. No interactions were statistically significant.

Perception that Female Actor Should Have Said No. The analysis described above was repeated examining the extent to which participants believed the female actor should have said no. No main effects were statistically significant. This analysis yielded significant interaction for relationship length and prior sex ($F(1,205) = 4.153$, $p < .$, partial $\eta 2 = .020$), such that when the female actor was in a relationship, higher agreement was perceived if the female actor had prior sexual involvement with the male actor. In contrast, there was a minimal difference in agreement when the female actor was casually dating the male actor in regard to prior sexual history.

Assumed Consent. We examined participant perception of the extent to which consent was assumed (e.g. assume yes until you hear a no) in the context of the vignettes as described above. This analysis yielded significant main effect for relationship length ($F(1,205) = 11.881$, p 005, partial $\eta 2 = .055$), such that participants assumed greater consent when the female actor was in a relationship than when the female actor was casually dating the male actor. Additionally, there was a significant main effect for prior sex ($F(1,205) = 7.764$, p 01, partial $\eta 2 = .036$), where prior sexual involvement was perceived with greater assumed consent. Lastly, there was a significant interaction for relationship length, alcohol, and prior sex ($F(1,205) = 4.923$, p 5, partial $\eta 2 = .023$), such that for actors who were casually dating and did not consume alcohol, higher assumed consent was perceived for those who had prior sexual interaction versus who had not had prior sexual interaction. In contrast, for actors who were in a relationship and did consume alcohol, higher assumed consent was perceived for those who had prior sexual interaction.

Affirmative Consent. We examined participant perception of the need for affirmation of consent (e.g. assume no until yes) in the vignettes as influenced by varied vignette context (as described above). This analysis yielded significant main effect for relationship length ($F(1,205) = 8.257$, p 005, partial $\eta 2 = .052$), such that participants' perception of needed affirmed consent was greater when the female actor was casually dating

the male actor rather than when the female actor was in a relationship. Additionally, there was a significant main effect for prior sex ($F(1,205) = 11.264$, $p < .05$, partial $\eta^2 = .036$), where a lack of prior sexual involvement was perceived as needing more affirmed consent than when the actors had prior sexual involvement. Lastly, there was a significant interaction for relationship length and alcohol ($F(1,205) = 4.111$, $p < .05$, partial $\eta^2 = .020$), such that for actors who were casually dating, higher affirmed consent was perceived for those who had dessert as compared to alcohol. In contrast, for actors who were in a relationship, higher affirmed consent was perceived for those who did consume alcohol.

DISCUSSION

Assumed consent

Our results suggest that prior sexual involvement has an influence on when consent can or cannot be assumed. When the actors had prior sexual involvement, regardless of relationship length or alcohol consumption, their actions were perceived as more consensual despite the female actor's nonverbal cues stating otherwise. This result aligns with Shotland and Goodstein's (1992) sexual contract (consenting to unwanted sex can be seen as consensual on the grounds that on other occasions the situation would be reversed), meaning that participants assumed that a) there had been an underlying agreement about normative sexual behavior of the actors and b) the situations had been reversed prior to this situation.

Secondly, our results aligned with Jozkowski and colleagues (2014) findings of nonverbal cues and sexual consent. The actors in our vignettes used nonverbal cues to demonstrate both consent and non-consent, which were solely viewed as consenting by a majority of the participants. Our finding suggests that our participants relied on the actors' nonverbal cues to discern consent and may be due to our participants' perception of communicating sexual consent. Future research should analyze this notion in further detail to determine if students assume consent due to cultural perceptions of communication in sexual situations.

Coincidentally, our results aligned with the engagement in token resistance (Lewin, 1985; Muehlenhard & Linton, 1987). Keeping in mind that the female actor

used nonverbal cues to deny sexual intercourse, it can be assumed that our participants interpreted the female actor's behavior as contending to the social norm of women not wanting to be viewed as sexually promiscuous and wanting to maintain an air of sexual purity, thus viewing the sexual encounter as consensual.

Affirmed consent

Our results suggest that having a prior history with another individual influences the need for explicit consent. When the actors were in a relationship and had a history of prior sexual involvement, their actions were perceived as more consensual and expressing sexual consent clearly, as was found in Humphreys' (2007) study. Comparably, Shotland and Goodstein (1992) found that a woman's refusals of sex when there is a history of prior sexual involvement were seen as less legitimate, suggesting that even though the female actor refused, the participants believed her refusal was irrelevant in completing the overall goal of obtaining sexual intercourse. These findings align with Shotland and Goodstein's (1992) precedence theory (people are more likely to assume and expect normative sexual activity in more established relationships), meaning that our participants found situations in which there was prior history (sexual, relationship length, or both) to be more consensual and acceptable in today's society.

In addition, participants perceived more consent when the actors were either a) causally dating and eating a dessert and b) in a relationship and consuming alcohol. Gunby and colleagues (2012) found that rape scenarios were perceived when there was a difference in intoxication between both parties and that alcohol intoxication can mitigate responsibility for sexual violence. Our results suggest that due to both the male and female actor consuming the same amount of alcohol (three drinks each), our participants viewed those vignettes as a) the male and female actor acting according to the governing rules of society of mixed-sex pairs being the most appropriate grouping in which drinking can occur, and b) seen as consenting to sexual activity due to alcohol's ability to mitigate responsibility in sexual assault cases (Abbey & Harnish, 1995).

Double standard and lack of assumed consent

Our second hypothesis for this study was that participants who hold more traditional gender norms

may be more likely to perceive consent in sexual encounters compared to those with less traditional gender norms, based on the idea that men and women respond differently to sexual acts due to their sexual scripts (Jozkowski et al., 2014; Humphreys, 2007; Simon & Gagnon, 1986). However, our results failed to yield a significant relationship between assumed consent and the sexual double standard. One factor we believe led to this result could be that our sample was overwhelmingly female, making it unlikely that the disenfranchised group would self-identify with their own sexual oppression and could have unconsciously responded in a way that negated that effect. A second factor could be that the majority our sample was Hispanic, and it may be that cultural differences influenced findings. Future research should examine consent and the sexual double standard in Hispanic culture to try to determine potential explanations for this finding.

Limitations

Our study took place on a college campus with a college sample, therefore caution should be used when generalizing findings beyond this population. In addition, we used self-report measures in the survey, which may be prone to social desirability or other bias. While we collected students from psychology classes, we had a diverse range of students of different majors with Psychology and Nursing being the most common. Lastly, our sample was primarily female which may be due to the demographic differences in the College of Liberal Arts at this university being 38% male and 62% female (Texas A&M University - Corpus Christi, n.d.).

Suggestions for further research

Our findings on affirmed consent is intriguing in relation to Humphrey's (2007) study; we each found a similar result; despite their study occurring in Canada and ours occurring in the United States. Which begs the question if this finding is a norm for Western countries alone? In addition, our finding on assumed consent is revolutionary given the time of when the sexual contract was first discovered; 26 years have passed since Shotland and Goodstein's (1992) initial discovery with college students in Pennsylvania, and it is still relevant among college students in South Texas.

Future research should examine relationships in Eastern cultures to see how affirmed and assumed consent are constructed, and to see if the findings in this study and previous studies expand into a global construction of consent or if differences exist between Eastern and Western cultures. In addition, further research should identify if assumed consent correlates with a low or high double standard in other parts of the world.

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APPENDIX

TABLE 1.
Bivariate Correlations Between Variables of Interest

	2	3	4	5	6	7	8	9
1. Sexual Risk	.059	.526**	-.186**	-.023	.187**	.176**	.246**	.146*
2. Low Double Standard	-	-.062	.305**	-.461**	-.022	-.438	-.191**	-.038
3. Alcohol Consumption		-	-.159*	.073	.175**	.164*	.170**	.146
4. Positive Attitude Toward Establishing Consent			-	-.342**	-.212**	-.521**	-.284**	.053
5. (Lack of) Perceived Behavioral Control				-	.157*	.547**	.137*	-.019
6. Relationship Length Norms					-	.177**	.293**	-.049
7. (Pro) Assuming Consent						-	.485**	.083
8. Indirect Behavioral Approach							-	.096
9. Awareness of Consent								-

** $p \leq .01$
* $p \leq .05$

FIGURE 1.
Perception that Female Actor Should Have Said No
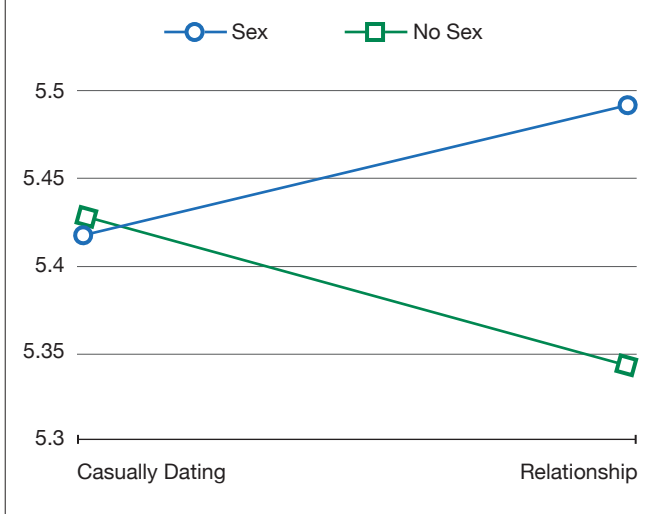
FIGURE 2.
Assumed Consent (Casually Dating)

○ Prior Sex   □ No Prior Sex

3.2

3.1

3

2.9

2.8

Dessert   Alcohol

Casually Dating

FIGURE 3.
Assumed Consent (Relationship)

○ Prior Sex   □ No Prior Sex

3.3

3.225

3.15

3.075

3

Dessert   Alcohol

Relationships

FIGURE 4.
Affirmed Consent

○ Dessert   □ Alcohol

5.55

5.4875

5.425

5.3625

5.3

Casually Dating   Relationship
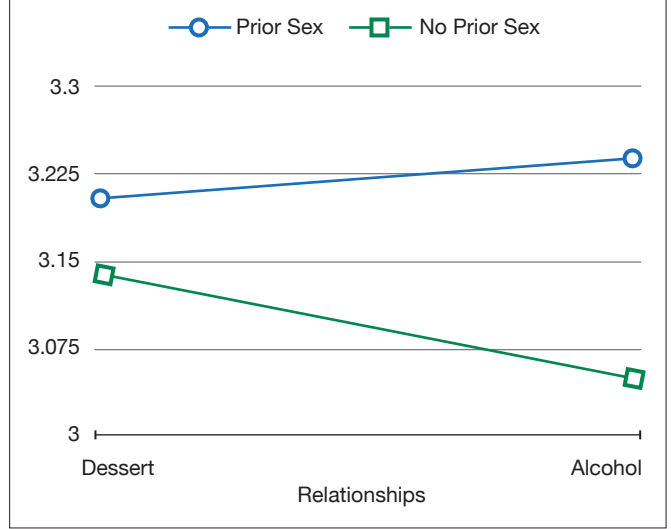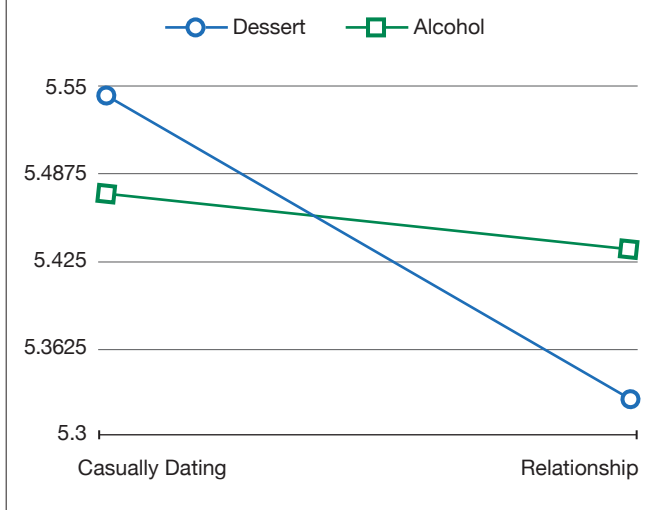
CHARACTERIZATION OF *VIBRIO VULNIFICUS* ISOLATES FROM THE COASTAL BEND REGION THROUGH THE USE OF POLYMERASE CHAIN REACTION (PCR) OF THE VIRULENCE GENE *vcgC*

by LARRESHIA BRUMFIELD

ABSTRACT

Vibrio vulnificus *is a Gram-negative bacterium found in estuarine and marine waters, as well as the tissues of shellfish and the skin of finfish.* Vibrio vulnificus *is known to cause diseases such as gastroenteritis, necrotizing fasciitis, and primary septicemia. When people are infected with the bacterium, mortality and morbidity are high.* Vibrio vulnificus *is known to contain different virulence loci, including* vcg*, whose function is not known. The* vcg *locus has been found to contain two different forms or alleles:* vcgC*, or clinical (disease-causing) isolate, and* vcgE*, or environmental isolate. Other virulence loci on* Vibrio vulnificus *strains are* vvhA *and* viuB*. This study tested the virulence-correlated gene locus to see if there was a correlation between several isolates of* Vibrio vulnificus *taken from local waters of the Coastal Bend region of south Texas. We predicted that all of the isolates that had a vcgC amplicon from previous experiments would still possess that same amplicon when run through the PCR process again.*

INTRODUCTION

Vibrio vulnificus is a halophilic, Gram-negative bacterium found in marine and estuarine waters, tissue of shellfish, and the mucous layer of thin fish. Infection from *Vibrio vulnificus* is associated with wounds exposed to water containing the bacterium and consumption of raw or undercooked seafood (Oliver, 2005). *Vibrio vulnificus* is known to be more prevalent during the warmer months. The incidence of infection is low (0.1 per 100,000), but the people most susceptible to becoming infected with *Vibrio vulnificus* are people who are immunocompromised, have diabetes, or have liver problems such as cirrhosis, or renal failure (Horseman and Surani, 2010; Vollberg and Herrera, 1997; Haq and Dayal, 2005). When infected, the morbidity and mortality of the infection is high (~60%) (Linkous and Oliver, 1999). The incubation period is normally 1-3 days, but may vary between eight hours to 21 days (Horseman and Surani, 2010).

MENTOR
Dr. Gregory Buck
Associate Professor
Department of Life Sciences
College of Science and Engineering

Vibrio vulnificus is divided into three different biotypes. Biotype 1 is most commonly associated with human infections. Biotype 2 is associated with an eel pathogen. Biotype 3 is associated with farmers handling tilapia in Israel (Kim and Cho, 2015). Biotype 1 can be divided into two genotypes, based on the presence of the virulence-correlated gene, *vcg* (Warner and Oliver, 2008). Clinical (disease-causing) isolates usually have the allele *vcgC*, while isolates of the environmental type usually have the allele *vcgE* (Kim and Cho, 2015).

In this study, *vcgC* will be observed to determine the genotype of 16 *Vibrio vulnificus* isolates. A PCR assay will be used to amplify the locus. The PCR products, or amplified DNA, will be run through agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light to determine the genotype of the locus tested.

BACKGROUND/REVIEW OF LITERATURE

Vibrio vulnificus has many virulence markers that have either the clinical or environmental genotype. V. vulnificus contains a locus with an unknown function called the virulence- correlated gene locus (*vcg*) (Rosche et al., 2005; Warner and Oliver, 2008). The clinical genotype is known as *vcgC* and the environmental genotype is known as *vcgE*. Most clinical isolates (90%) have the clinical genotype, while most of the environmental isolates (87%) have the environmental genotype at the *vcg* locus (Rosche et al., 2005; Bogard and Oliver, 2007; Warner and Oliver, 2008).

The *vvhA* gene encodes a hemolysin/cytolysin, *vvhA*, that destroys red and white blood cells. In a study done by Yokochi et al. (2013), the *vvhA* gene was observed. A previous experiment done by Senoh et al. (2005) showed that *vvhA* had two gene types, and Yokochi et al. (2013) observed a different primer for each gene type of *vvhA*. Senoh et al. (2005) and Yokochi et al. (2013) found that *vvhA* gene type 1 was associated with the clinical genotype, and *vvhA* gene type 2 was associated with the environmental genotype.

Another locus seen in *Vibrio vulnificus*, *viuB*, encodes a siderophore that allows *Vibrio vulnificus* to obtain iron, a necessary nutrient for bacterial growth and pathogenesis. When the *viuB* locus is present, the genotype is clinical. When it is absent, the genotype is

environmental (Yokochi et al., 2013). In a study done by Yokochi et al. (2013), four different virulence factors of *Vibrio vulnificus* were observed from three different bodies of water in Japan. The presence or absence of the *viuB* gene was noted and whether there was a correlation between the genotype expressed by the strain and the area from which the strains were obtained. Yokochi et al. (2013) found that most isolates had the *viuB* gene, and that certain areas within the bodies of water had a higher number of clinical *Vibrio vulnificus* isolates associated with infection. Other areas did not report high levels of infections; however, not enough data were available to determine if isolates were clinical or environmental.

The *rpoS* gene in *Vibrio vulnificus* encodes an alternative sigma factor that allows *Vibrio vulnificus* to survive and adapt to stress present in its environment, such as changes in temperature, osmotic shock, nutrient starvation, oxidative exposure, and stationary phase (Hulsmann et al., 2003). The *rpoS* gene is also known to aid in colonization and attachment to the host cell and control how certain virulence genes are expressed. In a study done by Hulsmann et al. (2003), an experiment tested the stress response of a wild-type and mutant of *rpoS*. The wild-type bacteria with *rpoS* had protection from stress, such as being in the presence of hydrogen peroxide, hyperosmolarity, and acidic conditions. Certain functions that were present in the wild strain were not expressed in the mutant strain. In addition, Planas-Costas (2011) showed that most Coastal Bend vibrio isolates tested possess the *rpoS* locus. The mutant *rpoS* strain was also less motile compared to the wild strain. Based on the results, it was found that *Vibrio vulnificus* needs the *rpoS* gene in order to survive the stress in its environment, and possibly plays a role in *Vibrio vulnificus* being virulent (Hulsmann et al., 2003).

In addition, work done by Gonzales and Imam (2007) showed that *Vibrio vulnificus* isolates from the Coastal Bend region were killed by 880 µM and 2000 µM peroxide. In the study done by Hulsmann et al. (2003) hydrogen peroxide was used to test the response of the *rpoS* mutant type and wild-type. They found that the *rpoS* mutant type strain was sensitive to exposure to hydrogen peroxide and its ability to survive was decreased compared to the *rpoS* wild-type strain (Hulsmann et al., 2003).

STATEMENT OF HYPOTHESIS

H_N—The hypothesis is that there will be no difference in the amplicon identified and tested by PCR for the vibrio isolates.

H_A—The hypothesis is that different isolates of *Vibrio vulnificus* will demonstrate different amplicons of the *vcg* gene.

MATERIALS AND METHODS

Bacterial Strains. Isolates were obtained from the Coastal Bend region by Dr. Joanna Mott (formerly of Texas A&M University – Corpus Christi, now at of California State University, Sacramento) and Mr. Gabriel Ramirez.

Incubation and Induction. Isolates were inoculated into Luria-Bertani Lennox agar with additional 2% NaCl, followed by aerated incubation for 18 hours at 37 degrees C. Five hundred microliters of saturated overnight culture were centrifuged at 13,200 xg, and frozen at -80°C until used. For DNA extraction, crude lysates were obtained from cultures via heat shock & flash cooled as described (Parvathi et al. 2005)

Sample Preparation. Isolates were resuspended in 500μL of 1X TE buffer, centrifuged at 10, 000 x g for 10 minutes and resuspended in 100μL of 1X TE buffer. The isolates were then put on the heating block for 10 minutes at 100°C and placed in the ice immediately after being removed. After centrifugation, 50μL of 1mM EDTA + 0.5% Triton X-100 solution was added to the pellet. Isolates were heated again for 10 minutes, centrifuged at 10, 000 x g for 3 minutes, and the supernatants were kept.

PCR. The master mix was made using dNTPs, forward and reverse primers, distilled water and Taq polymerase. Eighteen microliters of the master mix was added to each microtube followed by 2μL of the supernatant. The microtubes were placed in the thermocycler and allowed to run for 29 cycles.

PCR Protocol: 29 cycles

Denature: 94°C (40 seconds)

Anneal: 54°C (45 seconds)

Extension: 72°C (45 seconds)

Final extension: 72°C (10 minutes)

Soak cycle: 4°C (23hr. 59min.)

Gel Electrophoresis. PCR products were visualized in 2% Agarose in 1X TAE using 0.5 ug/mL ethidium bromide, run at 7.5 V/cm for 40-60 min. Gel was observed under UV light.

RESULTS

TABLE 1.
PCR results for experiment #26

Lane #	Isolate #	Isolate	Expt	Tube #	Band?
1	n/a	Roche Marker IV 4 uL			
2	1	ARA 0040407-39	16	4	Yes
3	10	BS 0607-31	23	2	Yes
4	11	BS 0607-5	25	2	Yes
5	12	BS 0607-9	24	3	Yes (faint)
6	13	BS 0906-13	24	4	Yes
7	14	BS 0906-130	24	5	Yes
8	15	BS 1006-273 (Tr)	23	5	Yes (faint)
9	16	BS 1106-92 (Tr)	24	6	Yes
10	17	CB 0407-15	23	7	Yes
11	18	CB 0407-18	25	4	Yes
12	20	CB 0407-5	23	9	Yes (faint)
13	43	RB0407-13	25	6	Yes
14	44	RB0906-57	25	8	Yes
15	143	ATCC 27562	14	2	Yes (faint)
16	143	ATCC 27562	25	10	Yes
17	n/a	DNA 70 ng 27562 Pos Control	25	12	Yes
18	n/a	DNA Ladder 5 uL			

FIGURE 1.
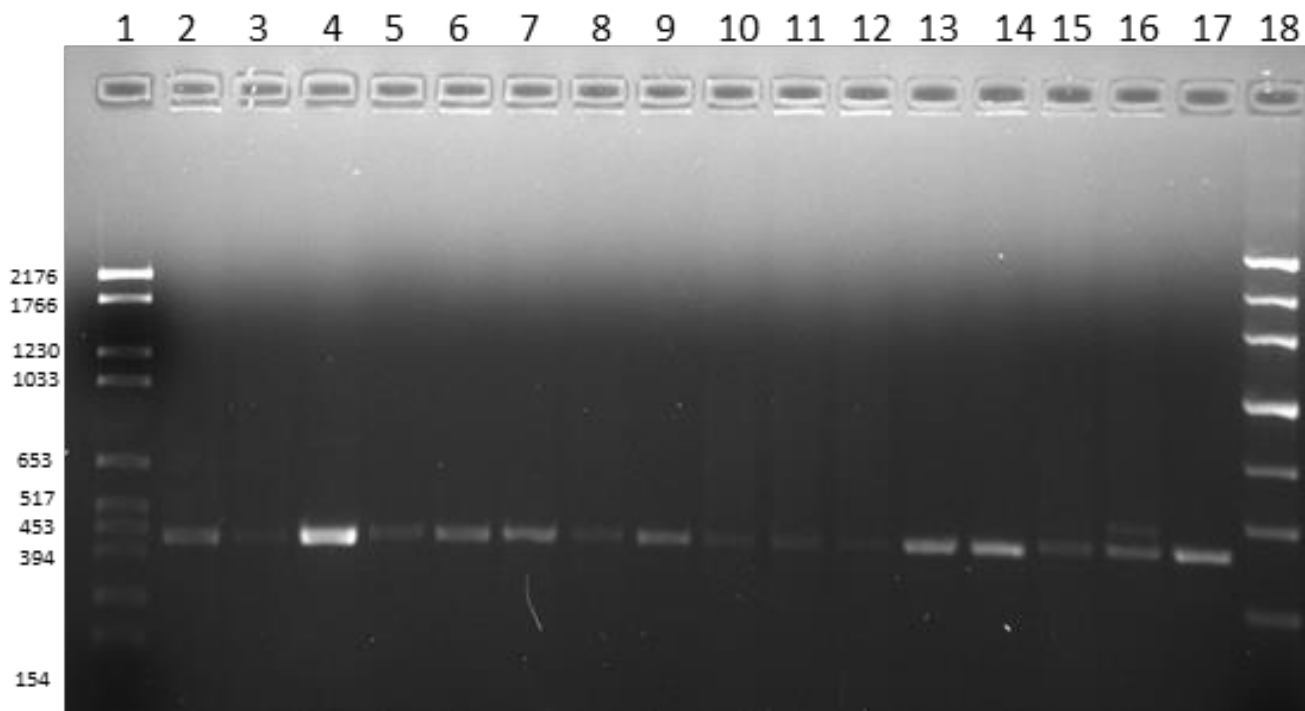
2% agarose gel in 1X Tris-acetate-EDTA, pH 8.3(TAE) of PCR products. Lane 1- Roche Marker VI (total 250 ng/µL); Lane 2- ARA 0040407-39 (Exp 16); Lane 3- BS 0607-31 (Exp 23); lane 4- BS 0607-5 (Exp 25); Lane 5- BS 0607-9 (Exp 24); Lane 6- BS 0906-13 (Exp 24); Lane 7- BS 0906-130 (Exp 24); Lane 8- BS 1006-273 (Exp 23); Lane 9- BS 1106-92 (Exp 24); Lane 10- CB 0407-15 (Exp 23); Lane 11- CB 0407-18 (Exp 25); Lane 12- CB 0407-5 (Exp 23); Lane 13- RB 0407-13 (Exp 25); Lane 14- RB 0906-57 (Exp 25); Lane 15- ATCC 27562 (Exp 14); Lane 16- ATCC 27562 (Exp 25); Lane 17- ATCC 27562 *Vibrio vulnificus* DNA (70 ng total) (Exp 25) positive control; Lane 18- DNA Ladder

DISCUSSION

Before the *Vibrio vulnificus* isolates could be run through the PCR process, the isolates had to be cultured. Nineteen isolates were inoculated in duplicates to grow in brain heart infusion broth with 2% sodium chloride, Marine Broth 2216, and Luria Bertani Lennox broth with 2% sodium chloride. Fourteen of the isolates did not grow in the brain heart infusion broth or the marine broth. Of the 19 isolates tested, only 16 isolates showed the amplicon for possessing *vcgC* in both duplicates. Thirteen of these isolates were run in the experiment. The other three were not included, because two isolates possessed the *vcgC* amplicon in one duplicate but not in the other, and the other possessed no *vcgC* amplicons in either duplicate.

In previous experiments, the *vcg* locus was observed using *vcgC* primers to determine whether the *Vibrio vulnificus* isolates contained the clinical, *vcg* allele.

In order for an isolate to be classified as possessing the allele, the isolates were lysed, and the lysate run through a PCR process and any amplicons observed under UV light. If an amplicon or band was observed at 428 bp, the isolate was said to possess the *vcgC* allele. Afterwards, isolates that contained the *vcgC* allele from the previous experiments were added to one gel and tested for consistency to confirm the presence of the amplicon at 428 bp. Figure 1 shows the results of this experiment. All the experiments run through the gel have been consistent in possessing the *vcgC* allele. Lane 1 contains the Roche marker VI. Lanes 2-17 possess an amplicon at 428 bp, which is consistent with earlier experiments. Thus, all the lanes possess the *vcgC* allele. Lane 16 possesses two amplicons, one at 428 bp and one around 453 bp. Lane 17 served as a positive control, so an amplicon at 428 bp should always be present. Lane 18 is a DNA Ladder used to serve as a

marker. Table 2 shows all *Vibrio vulnificus* isolates that were used, which experiment they were used from, and the results obtained from the gel after being observed under the UV light.

Future research will use the PCR process and *vcgE* primers to observe the amplicons expressed by the *Vibrio vulnificus* isolates. This will help to further identify the difference in the virulence correlated gene locus between environmental and clinical types of *Vibrio vulnificus* by making it easier to identify whether a strain is clinical or environmental.

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PERCEPTIONS AND ATTITUDES OF HIGH SCHOOL BASEBALL PLAYERS PARTICIPATING IN VISUAL SKILLS TRAINING

by MARY CARROLL

ABSTRACT

The purpose of this study was to investigate the attitudes and perceptions of high school baseball players participating in visual skills training. Eighty-five male subjects (age = 16.02 ± 1.05) completed a survey designed to assess their attitudes and perceptions regarding the effect of visual skills training on their baseball performance. Fifty-two of the subjects were from a high school in Texas and thirty-three from a high school in Oklahoma. Visual skills were assessed using the Vizual Edge Performance Trainer (VEPT), an internet-based software program designed to assess eye alignment, depth perception, convergence, divergence, visual recognition, and visual tracking. Individual subtest scores were used to generate a composite VEPT score. The participants averaged 3.35 training sessions per week for 16.32 minutes per session for a period of 10.86 weeks. Upon completion of training, subjects completed a twenty-five question Likert-scale survey, which examined the perceived effectiveness of VEPT. Results from the survey indicated that 82% percent of the subjects agreed or strongly agreed that visual skills play an important role in baseball performance, while 71% believed that VEPT actually enhanced those skills (20% were undecided, 5% disagree and 2% strongly disagree due to limited training time). In addition, 40% believed that VEPT improved their performance during this study and it took an average of 4.48 weeks for them to notice such improvements. As a group, 70% believed that VEPT increased their ability to "focus", 59% felt the visual practice improved their concentration, and 58% agreed the training augmented their ability to "see" the ball. In addition, 31% reported more consistency, 42% believed batting results increased, 39% felt their fielding improved, and 17% reported superior balance. Overall, the results of this survey indicate that a significant number of high school baseball players participating in VEPT believed their visual skills improved, which they felt translated into enhanced baseball performance.

INTRODUCTION

While the physiological components of visual acuity are well documented, the specific visual skills required for sport performance are much less understood. Tracking a rapidly moving object with vision takes skill and accuracy to be able to estimate the object's end result (Higuchi, Takatoshi, et. al, 2018). Researchers have found that visual skills are vital to sports performance

MENTOR
Dr. x
Professor of x
Department of xx
College of xxxs

(Alves, et al., 2015). According to Fischer and Whitney (2014), an important part of visual tracking is sensing significant variations in the setting. Several authors have reported a difference in visual skills when it comes to comparing athletes vs. non athletes (Alves, Spaniol, & Erichsen, 2015). Information processing through visual skills is necessary for athletes to respond quickly under complex situations (Takeuchi & Inomata, 2009). While a considerable amount of research has been published on professional baseball players, little research has been performed on high school baseball players. Thus, the purpose of this study is to investigate the attitudes and perceptions of high school baseball players that participated in Vizual Edge Performance Training (VEPT). The visual skills included in VEPT are designed to assess and train eye alignment, depth perception, convergence, divergence, visual recognition, and visual tracking.

LITERATURE REVIEW

Visual Skills

When vision and motor skills are combined, their involvement is known as visual-motor integration (a.k.a., oculomotor). Visual-motor integration is the changing of visual skills into a motor output which is a common technique that is used in all sports (Du Plessis, Coetzee, & Pienaar, 2015). This technique is an automatic occurrence between a person's reactions along with a combination of their visual and motor skills. According to Moschovakis (1994), visual skills are controlled on the cognitive side of the brain in the occipital lobe. Quick eye movement is referred to as saccadic and this motion can be used extensively during athletic participation. (Moschovakis, 1994).

Vision in Baseball

Vision is the main sense needed in baseball (Alfailakawi, 2016). Successful batting, catching, pitching, and throwing all require extensive use of multiple visual skills. Visual tracking plays an important role in baseball since pitching speeds often exceed 90-100 mph. Batters use early visual cues to guide their body positioning as they prepare to hit the ball (Muller, Lalović, Dempsey, Rosalie, & Harbaugh, 2014). If an athlete is unable to track the ball from the pitcher's release, they will not know the direction in which they should swing based on the ball path (Wimhurst, Sowden, & Cardinal, 2012). According to Wimshurst, Sowden, and Cardinal (2012) vision helps an athlete know where the ball is located as it is flying through the air in any sport. Notably, visual

tracking, and the ability to process visual information can be the difference of an elite vs. non-elite player (Wimshurst, Sowden, and Cardinal, 2012). Visual tracking is the ability to follow the ball in motion in order to catch, dodge, or hit it. Without visual tracking, playing competitive sports would be nearly impossible.

Vizual Edge Performance Trainer (VEPT)

VEPT is an internet-based software program designed to assess and train eye alignment, depth perception, convergence, visual recognition, and visual tracking. It was originally developed in 1989 by Dr. Barry L. Seiller, an ophthalmologist who founded the Visual Fitness Institute (Vizual Edge, 2017). VEPT includes a visual skills practice system that involves visual tracking exercises through the use of pre-programmed 3D games. VEPT has been used by Major League Baseball (MLB) Scouting Bureau to evaluate draft prospects, as well as with numerous other MLB organizations to aid in player development.

METHODS

Subjects

For this study, a total of 85 high school baseball players from high schools in Texas and Oklahoma were evaluated and questioned using a Likert scale survey. These athletes varied in age from 14-18 years old. During the 2017 baseball season, there were eighty-five male subjects (age = 16.02 ± 1.05), fifty-two of these subjects from a high school in Texas and thirty-three of these from a high school in Oklahoma. The majority of these athletes had their first experience with VEPT during this study. Athletes who participated in this investigation did it willingly and remained anonymous throughout. All subjects mentioned above signed a consent form, which was approved by the Texas A&M University-Corpus Christi Institutional Review Board (IRB).

Procedures

Visual skills were assessed using the VEPT program. Individual subtest scores were used to generate a composite VEPT score. After VEPT training was finalized, the participants completed a twenty-five question Likert-scale survey which examined the perceived effectiveness of VEPT. According to Barua, (2013) a Likert scale is a common psychological measurement scale that is used in a series of questions listed from strongly agree to strongly disagree. The survey included questions such as, "Do you feel that VEPT training

improved your performance?" and "Do visual skills play an important role in baseball performance?" (Table 1). The subjects answered the questions on a scale of Strongly Agree to Strongly Disagree. The participants averaged 3.35 training sessions per week for 16.32 minutes per session for a period of 10.86 weeks. Once the participants completed this 10.86-week experiment, they were required to fill out the Likert-scale survey.

The data presented in the tables was collected during the 2017 spring season. The surveys were analyzed for descriptive data (presented in tables 1 and 3), and Pre and Post VEPT scores listed in table 2. Comprehensive VEPT scores were calculated from pre and post training assessments.

Statistical Analysis

The twenty-five Likert-scale survey questions were analyzed on VEPT software using the 85 participants. These were determined based on two separate high school teams using VEPT Pre and Post scores, weeks of VEPT training, sessions per week, time of average session, how many weeks completed, and whether each athlete felt that VEPT improved their performance.

Microsoft Excel was used to calculate data for the means and standard deviations from the survey results.

RESULTS

The surveys indicated that 82% percent of the subjects agreed or strongly agreed that visual skills play an important role in baseball performance, while 71% believed that VEPT enhanced those skills. Of the remaining participants, 20% were undecided, 5% disagreed and 2% strongly disagreed due to limited training time. In addition, 40% believed that VEPT improved their performance during this study and it took an average of 4.48 weeks for them to notice such improvements. As a group, 70% believed that VEPT increased their ability to "focus", 59% felt the visual practice improved their concentration, and 58% agreed the training augmented their ability to "see" the ball. In addition, 31% reported more consistency, 42% believed batting results increased, 39% felt their fielding improved, and 17% reported superior balance. These results also found a positive trend between training frequency and the overall feelings of effectiveness.

TABLE 1. Percentages from Questions 10-25. Do you feel that VEPT training improved your:

Questions	Strongly Agree	Agree	Undecided	Disagree	Strongly Disagree
Visual Skills	13(15%)	48(56%)	17(20%)	4(5%)	2(2%)
Performance	3(4%)	31(36%)	41(48%)	9(11%)	1(1%)
Batting	6(7%)	30(35%)	36(42%)	12(14%)	1(1%)
Fielding	10(12%)	23(27%)	40(47%)	9(11%)	0
Pitching	4(5%)	14(16%)	29(34%)	12(14%)	3(4%)
Ability to "focus" on the ball	12(14%)	48(56%)	15(18%)	8(9%)	2(2%)
Ability to "see" the ball	10(12%)	39(46%)	14(16%)	18(21%)	2(2%)
Concentration	16(19%)	38(45%)	16(19%)	12(14%)	2(2%)
Balance	3(4%)	11(13%)	41(48%)	25(29%)	5(6%)
Timing	8(9%)	27(32%)	28(33%)	19(22%)	3(4%)
Consistency	8(9%)	19(22%)	39(46%)	16(19%)	2(2%)
Confidence	7(8%)	19(22%)	30(35%)	21(25%)	7(8%)
Reading Concentration	6(7%)	32(38%)	25(29%)	22(26%)	0
Reading Comprehension	4(5%)	18(21%)	35(41%)	25(29%)	3(4%)
Reading Speed	6(7%)	29(34%)	32(38%)	17(2%)	1(1%)
Academic Performance Positively	10(12%)	30(35%)	27(32%)	16(19%)	2(2%)

TABLE 2. Pre Scores from prior to using VEPT and Post Scores from after athletes participated in using VEPT.

Pre and Post scores	
VEPT Pre Score	73.68 ± 7.29
VEPT Post Score	80.84 ± 8.70

TABLE 3. Means and Standard Deviations from Questions 1-5

Questions	Answer
1. How many weeks did you participate in VEPT training?	10.86 ± 5.31
2. Approximately how many training sessions did you participate each week?	3.35 ± 4.50
3. Approximately how long was the length of each training session?	16.32 ± 3.79
4. Do you believe that VEPT training improved your performance?	Yes 69 No 16
5. If yes to # 4 how many weeks did it take for you to notice an improvement in your performance?	4.43 ± 2.81

DISCUSSION

The main findings of this study were that, of the 85 participating athletes, 79 (93%) agree or strongly agree that visual skills play an important role in baseball performance, while 61 (72%) agree that in using the VEPT training program their visual skills improved (Questions 1 & 2, Table 1). In addition, when comparing batting, fielding, and pitching; 36 (42%) of the athletes felt that VEPT improved their batting, 33 (39%) said VEPT helped improve their fielding, while 18 (21%) athletes felt VEPT helped improve their pitching. The results taken from the surveys indicate that VEPT helps with baseball performance, but when it comes to improving concentration and comprehension in the classroom, the athletes felt it made no significant difference.

Based on the responses received from the high school athletes that participated in this research, there is evidence to indicate that athletes believe VEPT can have a positive effect on their baseball performance. The surveys also allowed opportunities for comments, which found that many of the athletes wished they could have used Vizual Edge for a longer period of time. This indicates that prolonged use of Vizual Edge may have led to greater feelings of improved baseball performance by the athletes. Recommendations for future research would be to increase the length of time of the VEPT training to determine if it would have a significant impact on the survey results. Also, college and professional baseball players should be considered as possible samples.

The results of this survey indicate that a significant number of high school baseball players participating in VEPT training believed their visual skills improved, thus translating into enhanced baseball performance.

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PCR AMPLIFICATION OF *mcrA* GENE FROM ORGANIC RICH TERRESTRIAL RIVERINE SEDIMENTS

by CLAY CLARKSON

MENTOR
Dr. Brandi Kiel Reese

ABSTRACT

Methane does not only play a significant role in the evolution of Earth's climate, but also contributes to current greenhouse conditions. With the current state of atmospheric methane shifting towards biogenic signatures, there is a call to understand the underlying sources. Methanogens dwelling in habitats impacted by anthropogenic activity are potential sources of increased methane fluxes, and recently, the essential gene encoding for methanogenesis (mcrA) has been identified outside the Euryarchaeota *phylum. This study sets out to understand the phylogenetic diversity of methanogenic and* mcrA *communities in subsurface sediments from an organic rich riverine system using cultivation-dependent molecular techniques. Enriched cultures supplemented with a methanogenic media that satisfies multiple methanogenic pathways was used to amplify culturable communities. Methanogenic genes were then amplified via polymerase chain reactions (PCR) utilizing phyla specific* mcrA *gene primer sets targeting* Bathyarchaeota, Euryarchaeota, *and* Verstraetearchaeota. *This study confirms the presence and cultivation of* Euryarchaeota *and* Verstraetearchaeota *methanogens enriched from freshwater riverine sediments.*

INTRODUCTION

Over the past century, the concentration of methane has more than doubled (Dlugokencky, 2016; IPCC, 2013). It is evident that anthropogenic activity plays a major role in the increased rates of methane flux, but underlying sources are predominately driven by metabolisms of methanogens (Nisbet et al., 2016). Methanogens are abundant in industrial sectors including waterlogged agriculture, anaerobic landfills, and bioreactors. At such sites, methanogens perform methanogenesis under anaerobic conditions by reducing small molecular weight carbon substrates (e.g., carbon dioxide, methyl-amines/sulfides, and volatile fatty acids) into methane. This marks the ecological niche of methanogenesis as a terminal carbon pathway within the carbon cycle.

Methanogens are a cosmopolitan Archaea that thrive in anoxic environments including hydrothermal vents, marine subsurface sediments, estuaries, rumens of multicellular organisms, continental stream systems, and waterlogged wetlands (McKay et al., 2017; Vanwonterghem et. al, 2016; Welte, 2018). This global
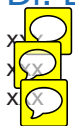
distribution further emphasizes the potential scale of influence these microbes have on the global methane flux. Not only are methanogens geographically diverse, but also phylogenetically diverse. Until recently, methanogens were classified among one phylum: *Euryarchaeota*. However, analysis of the small subunit ribosomal RNA (16S rRNA) gene and the alpha subunit of the methyl co-enzyme reductase gene (*mcrA*) has revealed that methanogens and essential core methanogenic genes are more phylogenetically diverse. Reconstructions of genomes obtained from Yellowstone hot springs and deep ground water revealed that *mcrA* is present among two additional phyla: *Verstraetearchaeota* and *Bathyarchaeota* (Evans et al., 2015; Vanwonterghem et al., 2016). The presence of *Verstraetearchaeota* and *Bathyarchaeota* methanogens has been explored among extreme and subsurface environments but it is possible *mcrA* genes and members of these candidate phyla are widespread like those belonging to *Euryarchaeota*.

Additionally, recent works has confirmed the use of phyla specific primer sets that target *mcrA* genes of *Euryarchaeota*/*Verstraetearchaeota* and *Bathyarchaeota* (McKay et al., 2017). Thus, with the use of the mentioned primer sets, this study attempts to cultivate and confirm the presence of *Verstraetearchaeota* and *Bathyarchaeota* methanogens within a mesophilic, organic rich riverine stream system. We present here a phyla evaluation of culturable subsurface riverine methanogens using anoxic enrichment cultivation techniques and amplify the *mcrA* gene using phyla specific primer sets by PCR to understand the distribution of cosmopolitan methanogens and candidate phyla members containing the *mcrA* gene.

MATERIALS AND METHODS

The focus of this study was to culture methanogens outside of the *Euryarchaeota* phylum to identify and evaluate the presence of *Bathyarchaeota* members containing the *mcrA* gene in organic rich riverine sediments. The approach to this study was enrichment cultivation followed by, *mcrA* amplification using primer specific primer sets (McKay et al., 2017).

Stock Media and Solutions

Media use for selective methanogenic enrichments were supplemented with multiple carbon substrates to enrich known methanogenic pathways. The supplemented substrates, carbon dioxide (CO_2, added to over pressurize culturing vials) and formate (60 mM), sodium acetate (85 mM), and methanol (125 mM) were added to satisfy hydrogenotrophy, acetotrophy, and methylotrophy pathways, respectively (Balch and Wolfe, 1976; Ferry, 1999; Long et al., 2017; Wolfe, 2011). CO_2 was supplemented to aliquots of stock media after addition of sediments and reduction with 2.5% sodium sulfide ($Na2S \cdot 9H_2O$) (w/v). Stock media was prepared under oxygenic conditions and was stored away from light at 8°C until enrichment of sediments were ready. All ingredients of the multi-pathway methanogen stock media with respective concentrations are listed in Supplemental Tables 1 and 2.

Sediment Core Sampling and Stream Characteristics

A total of three sediment core samples for enrichment cultures were obtained from Tranquitas Creek (TRC) near Kingsville, Texas (latitude 27°31'33", longitude 97°52'02"). The site of sampling was located under a roadway bridge and fed by a flood drainage outlet. Starting from the drainage-stream transition zone marked the 0 – meter site followed by three additional markers spaced 25 meters apart. These spaced marker sites were used for the assaying the stream chemical measurements. Though, at the time of sampling, the stream was very turbid which resulted in large uncertainties for measured parameters. Recorded averages of these parameters and respective uncertainties were as followed (n=4); dissolved oxygen 46.15 ± 26.06 %, total dissolved solids 2067.75 ± 218.59 ppm, pH 8.84 ± 0.33, and stream temperature 28.8 ± 0.4 ^{o}C. The values for total dissolved oxygen and solids were around twice as much than previous readings (data not shown). Other observations of the creek included a visible abundance of organic matter, human litter, and strong scents of sulfur compounds.

Location of the sediment cores used in this study were 75 meters downstream the drainage-creek transition zone. Creek sediments were sampled with a 1-meter acrylic core tube, capped with a black rubber stopper, and secured with plastic caps to minimize disturbance of the sediment profile and diffusion of gases. Two sediment cores were extracted along the stream flood bank and from the middle of the stream. Each core

varied from 8 – 30 cm in total depth with the stream core containing the least amount of sediments. All three of the cores were relatively similar in sediment composition and contained vascular plant roots and small pieces of plastic litter. The top 8 cm of each core consisted mostly of black fine silt and clay while the composition below the 8 cm mark was grey-black, compact, and sandy. The core samples were transported to a lab at Texas A&M University – Corpus Christi (TAMUCC) where they were immediately extruded.

Enrichments and Culture Transfers

Prior to core sectioning, culture serum vials (120 mL volume; Millville, NJ) for enrichments were each supplemented with 50 mL of multi-pathway methanogen media and 1 mL 2.5 % $Na2S \cdot 9H_2O$ (w/v) to reduce dissolved oxygen. Media in the serum vials were purged with nitrogen gas (N_2) for 3 min to establish anoxic conditions and to flush out excess H_2S (g). This was performed by connecting a 10.16 cm 16-gauge needle to a N_2 gas tank and lightly capping the vials with blue butyl stoppers (20 mm diameter, Chemglass; Vineland, NJ).

Sectioning of the core sample from Tranquitas Creek took place under oxygenic conditions. The sediment core was segmented into 2 cm sections where the 6 – 8 cm section was used for the enrichments. Each enrichment vial received approximately 4-grams of sediment. For cell counts, 1-gram sediment aliquots were placed into conical vials filled with 9 mL phosphate buffered saline (PBS) and 300 µL of formalin (37% formaldehyde). Additionally, serum vials lacking media also received 4-grams of the same sediment to serve as enrichment blanks. All culture vials containing sediment and media blanks were then capped with blue butyl stoppers and crimped with aluminum caps. The headspace of each vial was purged with N_2(g) for 1.5 min to maintain anoxic conditions. Two 1.5-inch 18-gauge needles (VWR ; United States) were used as an inflow outflow (Figure 1). Each vial also received 20 seconds of pressurization from a hydrogen and carbon dioxide gas mixture (H_2:CO_2, 80:20, Airgas; United States). The vials were placed onto an automated shaker at 80 rpm inside an incubator at 33°C.

Methanogenic activity of enriched cultures was determined by gas chromatographic (GC) connected to a flame ionization detector (FID) for methane and a thermal conductivity detector (TCD) for carbon dioxide (Trace 1310 – Thermo Scientific; Waltham, MA). Protocol for the gas analysis was performed by extracting 2 mL of headspace from culture vials and using laboratory air for background readings. Gas references were also measured by using 2 mL of 1% CH_4/CO_2 and 0.5% CO_2 calibration gases. Readout of raw data was stored on the instruments software data logger (Chromeleon - Thermo Scientific; Waltham, MA) and examined on Microsoft Excel® for graphical analysis.

Exponential rises in methane readings from enrichments were used to determine effective timing for transfers. Transfers were made by aliquoting 1-mL of enriched cultures into 50 mL of sterile anaerobic methanogenic media after approximately 188 hours of incubation. Each transfer was performed under anoxic conditions in an anoxic chamber filled with N_2 and H_2 gas.

DNA Extraction, Amplification, and Taxonomic Identification via PCR

DNA was extracted from the 50-fold diluted enriched transfer with a FAST DNA kit and concentrated using Genomic DNA Clean & Concentrator -25 kit. Purity of extracted DNA was measured on an Eppendorf BioSpectrometer® and amplified via PCR containing *Euryarchaeota/Verstraetearchaeota* (mlas-mod-F, 5'-GGYGGTGTMGGDTTCACMCARTA-3'; mcrA-rev-R, 5'-CGTTCATBGCGTAGTTVGGRTAGT-3') and *Bathyarchaeota* (Bathy-mcrA-2/3F, 5'-GCTKGGRTTYTACATGAG-3'; Bathy-mcrA-2R, 5'-GGGTAGTTAAGGCCTCTC-3') *mcrA* gene primer sets for taxonomic identification (McKay et al 2017). The thermocycling program consisted of a 95°C 15 min initial hold, 95°C 20 sec denaturation, 48°C, 30 sec annealing (*Bathyarchaeota*) / 57°C 30 sec annealing (*Euryarchaeota/Verstraetearchaeota*), 72°C 45 sec extension, and a final 72°C 5 min hold. PCR products were eluted on a 1.5% agarose gel. The *mcrA* amplicons were confirmed based off size (*Euryarchaeota/Verstraetearchaeota* : 469 bp, *Bathyarchaeota* : 471 bp) in relation to a 100 – 1 kbp ladder.

RESULTS

Enrichment Cultures

Growth of enrichments and subsequent transfers were determined by production of methane as measured by GC techniques. Early exponential rises were observed among vials 2 and 3 at 188 hours of incubation (Figure 2). Enriched vial 2 was used for the initial transfer due to it yielding the greatest change in methane production. Vial 1 encountered an extended lag time but produced visible gas emulsions after about a week (data not shown). Both enriched vials 2 and 3 experienced exponential rises at ~215 hr. and vial 1 encountered a prolonged lag time (Figure 2). Enriched vial 2 was used for DNA amplification based off its polynomial trend over the course of incubation.

DNA Extraction, Amplification, and Taxonomic Identification via PCR

Enrichment culture DNA extracts measured on an (Eppendorf BioSpectrometer®) yielded an $Abs_{260/280}$ of 1.45. PCR products visualized and eluted on 1.5% agarose gel are displayed in Figure 3. Lanes 13, 15, and 17 confirm a high number of *Euryarchaeota/Verstraetearchaeota mcrA* gene PCR prodcuts near the 500 bp mark indicating successful amplification of the *mcrA* gene.

DISCUSSION

The initial transfer and exponential rise in methane production observed among enriched methanogen cultures at 188 hours is consistent with exponential growth time of other published data (Sowers et al 1984). The designed media used in this study can confirm the growth of methanogens; however, due to the lack of observable *Bathyarchaeota* amplification there could potentially be bias within the culturing or PCR methods. Specifically, with the medium, potential bias is possibly due to the presence of acetate which is a known substrate for the fast-growing acetotrophic methanogens (Methanosarcina spp).

Results of the *mcrA* amplicon separation via gel electrophoresis does not confidently show a presence of *Bathyarchaeota mcrA* amplification. Though, metagenomic studies have shown that aquifers and eutrophic freshwater lakes contain relatively high abundances of *Bathyarchaeota mcrA* genes (Evans et al 2015, Yang et al 2017). Our site, even though not chemically assayed yet, is organically rich and therefore a potential eutrophic site for *Bathyarchaeota* members to thrive. This site would require a sequencing assay on the small subunit ribosomal RNA (16S rRNA) gene to effectively rule out the presence. Additionally, since *Bathyarchaeota* members with the *mcrA* gene have been hypothesized to use methane and lactate as a source of electrons for metabolic function (Evans et al 2015), enrichment cultivation should be supplemented with the respective substrates to encourage selective growth. Repeating this study with the media modifications mentioned above and incorporating a 16S rRNA amplicon sequencing approach is necessary to determine the total putative methanogenic population present at Tranquitas Creek, Kingsville Texas.

In summary, this study confirms the use of a selective multi-carbon substrate media to successfully enrich *Euryarchaeota/Verstraetearchaeota* methanogens from freshwater surface sediments. It was also confirmed that the *mcrA* gene, specific to *Euryarchaeota* and *Verstraetearchaeota* members are present within surface sediments at Tranquitas Creek, Kingsville Texas. More studies will need to be employed to come to a confident conclusion about *Bathyarchaeota* members containing the *mcrA* gene at this site.

SUPPLEMENTARY DATA

TABLE 1. Multi-pathway Methanogen Medium

Chemical Formula	Concentration (mM)	Amount/L
NH_4Cl	11	0.60 g
$CaCl_2 \cdot H_2O$	1	0.14 g
$MgCl \cdot 6H_2O$	27	5.50 g
$NaH2PO_4$	4	5.76 g
$NaHCO_3$	50	4.24 g
NaCl*	20	24 g
KCl	10	0.80 g
CH_3COONa	85	7 g
HCOONa	50	4.10 g
CH_3OH	125	5.10 mL
2.5 % $Na_2S \cdot 9H_2O$ ♦	0.104	100 mL
Trace Element Solution	0.006	6 mL

List of chemical compounds in modified methanogen media.* ; NaCl is optional and was not used in this study. Total Na^+ without NaCl is 0.236 M. Addition of NaCl at 24 g/L gives the media stock a total Na^+ concentration of 0.647 M which can serve for disaggregation of methanogen colonies. ♦ ; Sodium sulfide is added to culture vials prior of inoculation and not supplemented in the stock solution.

TABLE 2. Trace Element Stock Solution

Chemical Formula	Concentration (mM)	Amount/L
Nitrilotriacetic acid*	0.15% (w/v)	1.5 g
$CoCl_2 \cdot 6H_2O$	1	130 mg
$FeSO_4 \cdot 7H_2O$	1	280 mg
$NiCl_2 \cdot 6H_2O$	1	130 mg
$Na_2MoO_4 \cdot 2H_2O$	1	280 mg
$NaSeO_3 \cdot 5H_2O$	1	280 mg
$ZnSO_4 \cdot 7H_2O$	1	300 mg

List of trace elements in the prepared stock solution.
* Nitrilotriacetic acid was used as a chelating agent to reduce unwanted redox reactions and formation of insoluble precipitates.
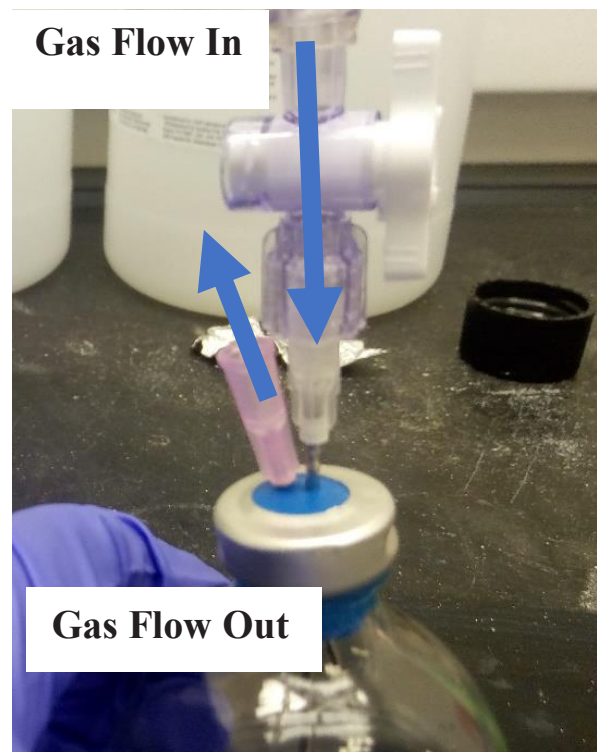
FIGURE 1. Setup for degassing culturing vials

FIGURE 2. Methane measurements from the headspace of enriched culture vials containing Tranquitas Creek sediments extracted on 05/12/2018 (TRC sed. 05/12).
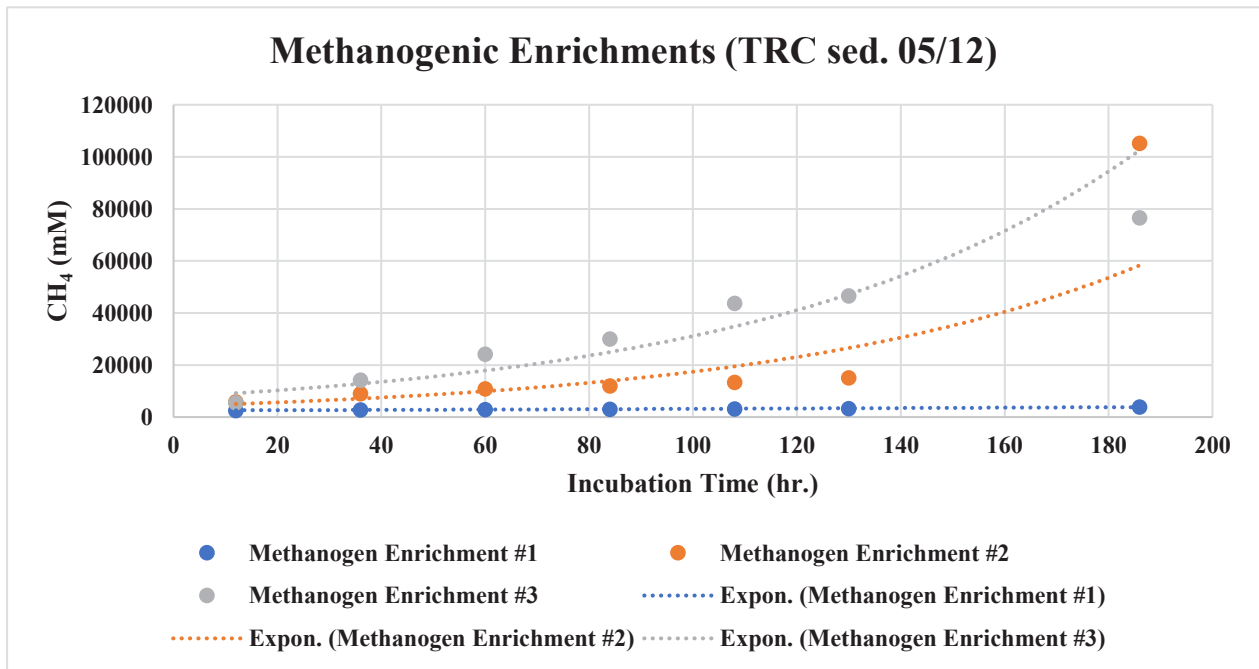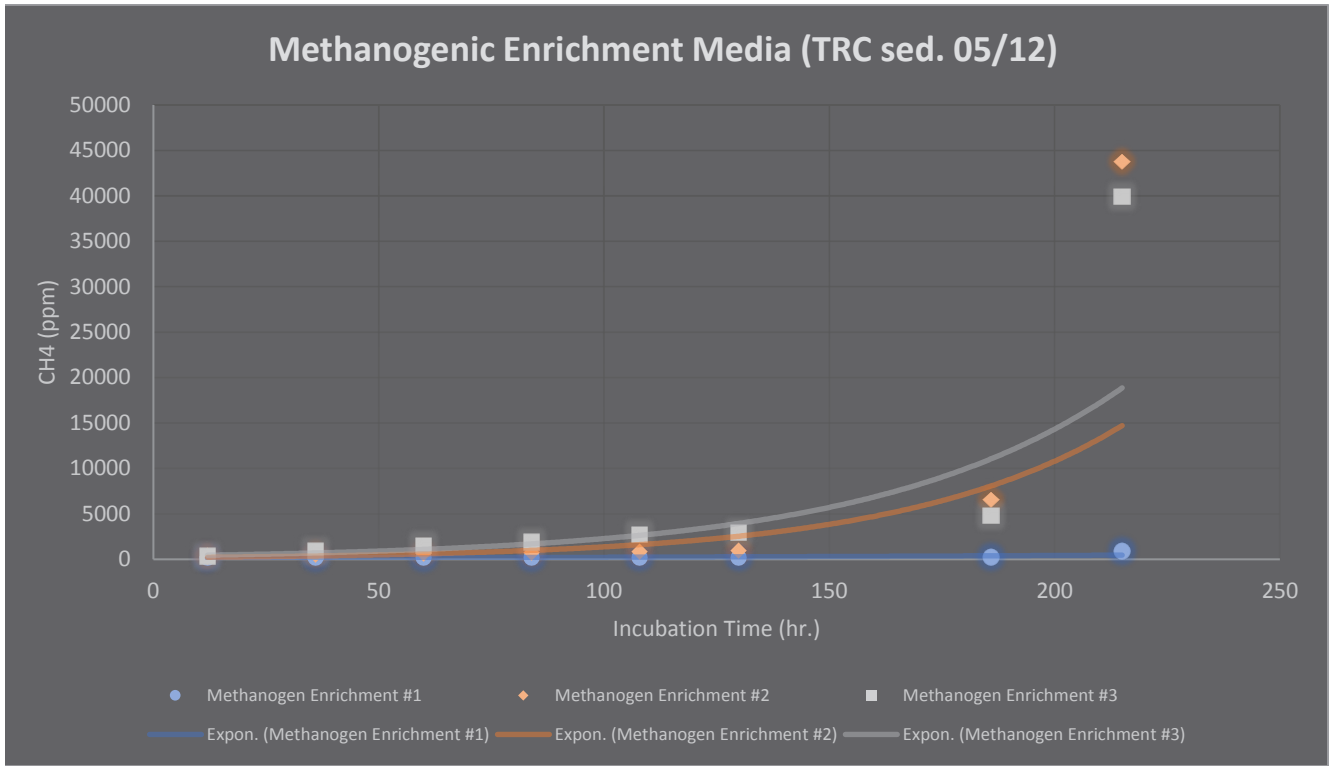
FIGURE 3. Methanogenic enrichment transfer methane production curve.
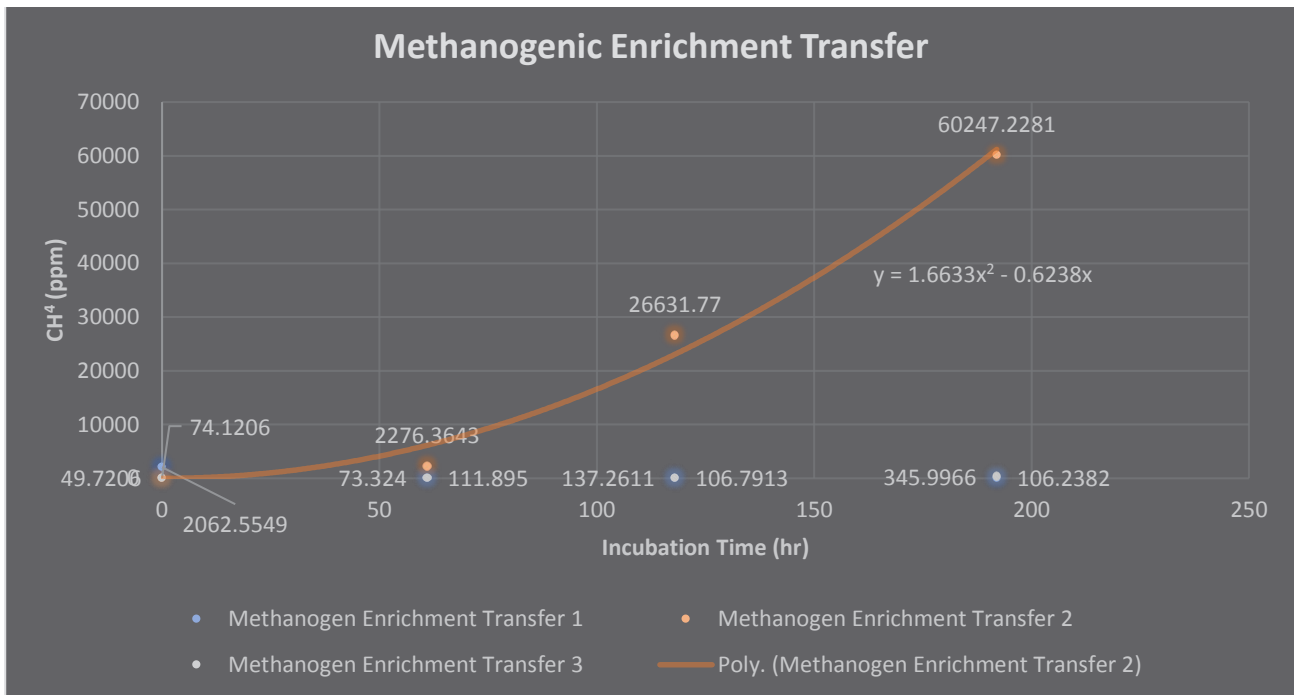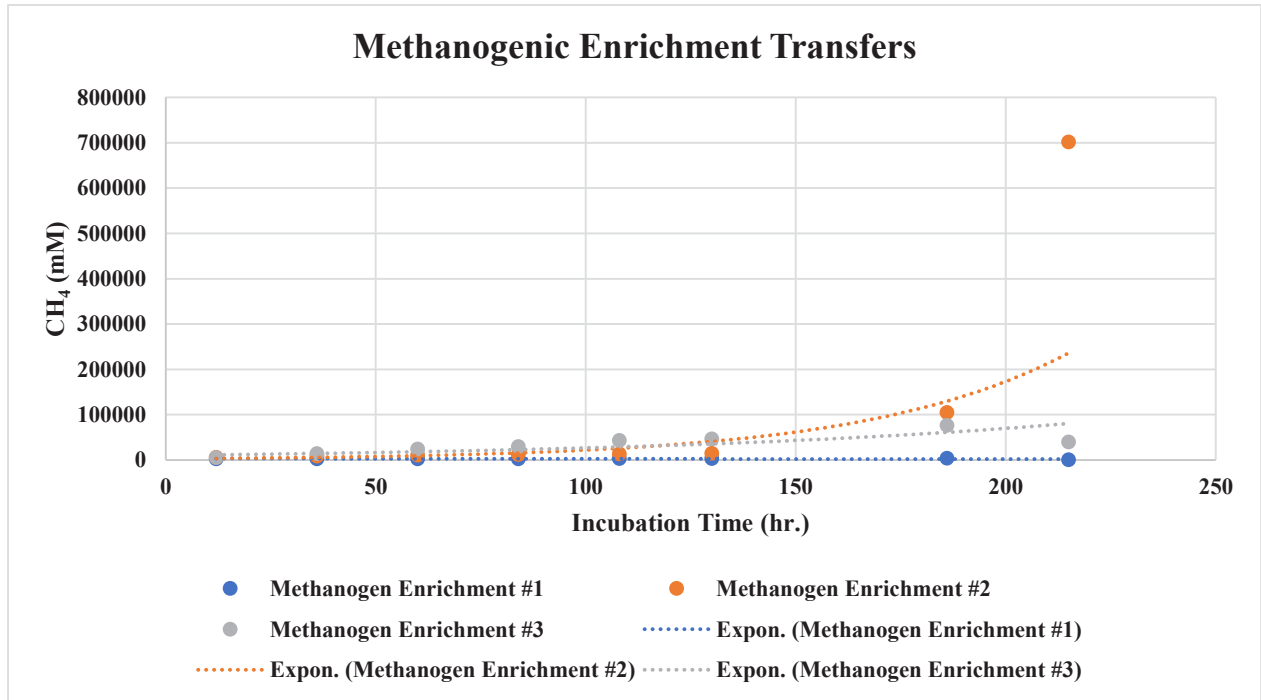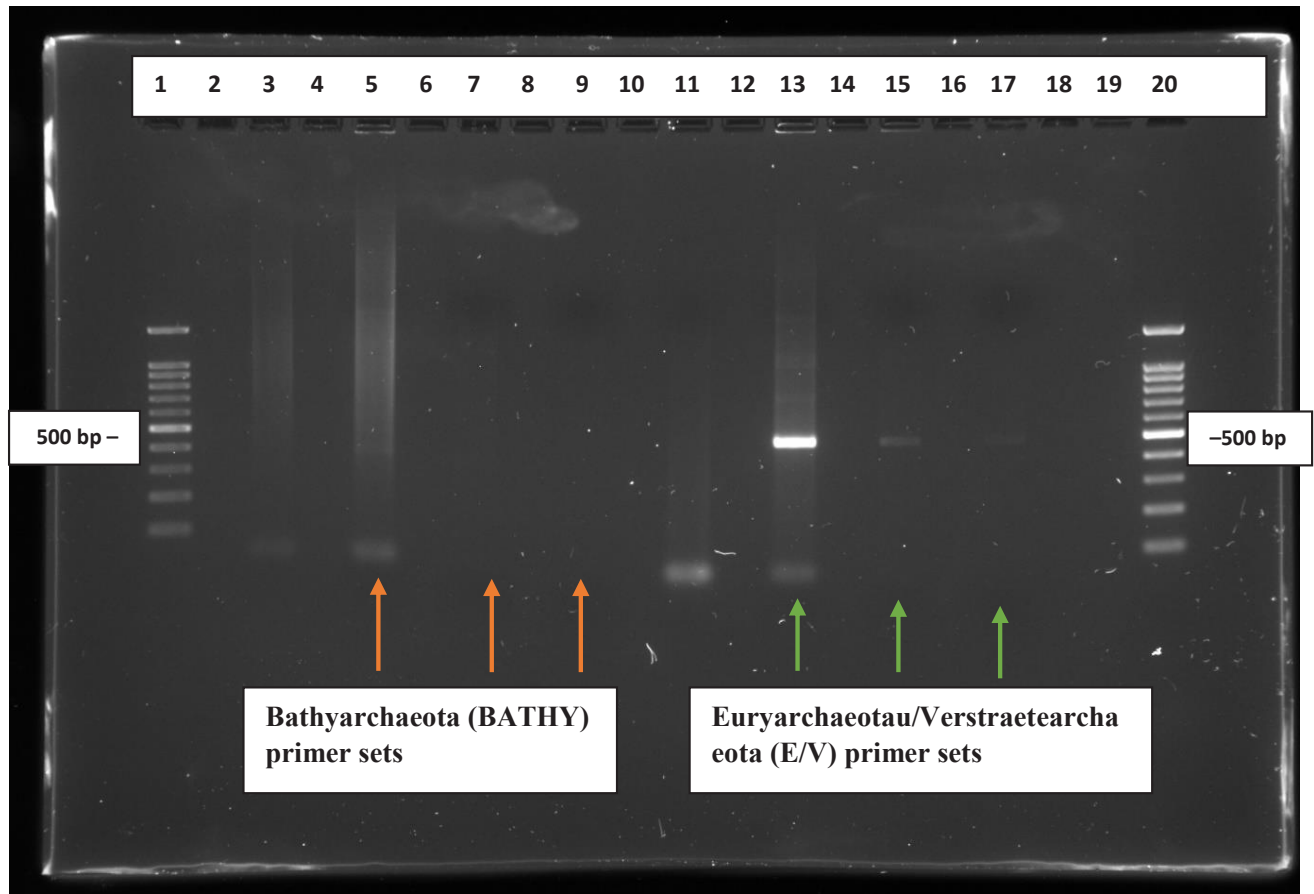Transfers of triplicate enrichments were performed after 212 hr of incubation.

FIGURE 4. The 20 lane agarose gel was loaded as followed: 1. 100-1000 BP LADDER; 3. BATHY NEGATIVE; 5. 1x BATHY; 7. 1:10 BATHY; 9. 1:100 BATHY; 11. E/V NEGATIVE; 13. 1X E/V; 15. 1:10 E/V; 17. 1:100 E/V; 20. 100-1000 BP LADDER.

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THE RELATIONSHIP BETWEEN CONVERGENCE, DIVERGENCE, RECOGNITION, TRACKING SKILLS AND READING COMPREHENSION OF ELEMENTARY SCHOOL STUDENTS

by MARIAH CLAY

ABSTRACT

The purpose of this study was to investigate the relationship between visual tracking skills and reading achievement of elementary school children. Seventeen elementary school students ranging from third to fifth grade were evaluated for visual skills and reading comprehension during the 2017-2018 school year. Visual skills were measured using the Vizual Edge Performance Trainer (VEPT), which is a 3-D computer – based program that is commonly used for training elite athletes. This program measures eye alignment, depth perception, convergence, divergence, visual recognition, and visual tracking. Scores for each tests and overall EDGE score were collected. Reading achievement scores were determined by the Gates-MacGinitie Reading Test 4th edition, Form S, which included scores from the Vocabulary and Reading Comprehension sections. Correlations were run for all variables. While the sample size was too small to show statistically significant correlations, results indicated a trend between visual skills and reading achievement for the population tested.

MENTORS
Dr. Frank Spaniol
Professor of X
Department of X
College of X

MENTORS
Dr. Corrine Valadez
Professor of X
Department of X
College of X

Relationship Between Visual Skills and Reading Achievement

Visual skills allow the human brain to track, react, and interpret actions that allow for enhancement in performance. Within visual skills, there are many components that pertain to different abilities within the visual scope. Many of these skills can be enhanced with different programs or tests to improve visual abilities. The ability to track, react, and interpret are often the

same skills necessary for successful readers. The purpose of this study was to determine the relationship between the visual skills and reading achievement of elementary students enrolled in third through fifth grade. Visual skills were tested using the *Vizual Edge Performance Trainer*, and reading achievement was tested by using the *Gates-MacGinitie Reading Test 4th edition, Form S*.

LITERATURE REVIEW

Children's learning environment is very important for early developmental skills. Having the proper foundation of education will be the basis of all future learning. One of the basic concepts to developmental learning is reading. Reading is involved in everyday life situations and provides the tools to understand and comprehend any subject that is introduced. This chapter is a review of literature that covers the components of reading, dependent reading skills, independent visual skills, and the use of technology in classrooms.

The Components of Reading

There are several components of reading that are involved with learning capabilities that include phonics, fluency, vocabulary, and comprehension. Reading is often overlooked as just comprehension, but according to Behruz (2016), it involves other aspects such as working memory, response inhibition, and time management. Reading is a complex, multilevel process that requires language, cognitive, and literacy skills; but also, more in-depth vocabulary, grammatical knowledge, word reading, and listening comprehension (Kim, 2017).

Dependent Reading Skills

While much is known about how to teach students how to read, there are many students still struggling with reading. Often students that have the capability to read the words of a text are unable to comprehend what they read. Although memorization is one of the components to reading, it should not be the only tool that the student uses when reading. Students must also have the desire to read or have teachers that stimulate students' interests in reading (Holloway, 2003). To master reading, students should develop skills and strategies that are effective in improving their reading comprehension in the classroom (Holloway, 2003).

Independent Visual Skills

Visual skills are critical when reading because each letter or symbol requires a differentiation or meaning to it. Having the correct visual skills will allow the student to have the ability to decode and comprehend the text. However, problems with visual skills could be caused by other problems with the eye such as visual acuity, visual fixation, accommodation, binocular fusion, convergence, field of vision, and perception (American Optometric Association, 2018). Having an awareness of any visual problems may help the student by allowing them to adapt.

The Use of Technology in Classrooms

In the beginning of the twenty-first century, technology emerged throughout the world and was used as a vital source for communication. In due time, technology found its way into classrooms with the intention to boost students' learning abilities. Technology such as virtual white boards, PowerPoints, and computers has grown to be faster, smarter, and more efficient than humans. While technology has provided many benefits to adaptive learning, the question remains: Is technology a good option when teaching young minds? Technology shows to enhance students' self-esteem due to the "increase competence they feel after mastering technology-based task and their awareness of the value placed upon technology" ("U.S. Department of Education," n.d.).

The Effectiveness of Visual Training Methods

The development and inclusion of technology into the curriculum has provided educators with another resource to improve visual skill and possibly improve reading achievement. One such example is the *Vizual Edge Performance Trainer* (VEPT). VEPT is a device used for a broad spectrum of people such as athletes, students, and children that are developing cognitive skills. Developing visual training methods will allow students to enhance depth perception, visual tracking response time, divergence, and convergence (Spaniol, n.d.).

METHODOLOGY

Participants

Potential participants were all students enrolled in third-, fourth-, and fifth-grade classrooms at a local elementary school. In addition, participants must be eligible to take the state exam, The State of Texas Assessments of Academic Readiness (STAAR). Students who were not eligible for the STAAR exam were not included in this study. Parental consent as well as student assent was obtained for each of the participants. Seventeen students completed the VEPT and GMRT tests measuring the visual tracking in comparison to reading comprehension. There were seven third grade, five fourth grade, and six fifth grade students that participated and obtained consent from their parents to be tested. Only one student was excluded from the data due to absence for part of the test.

Data

The researchers collected several pieces of data during the two tests that were issued to the students. The first test was conducted on the VEPT, commonly used on professional athletes for training purposes, which measured the visual processing. VEPT was a computer software program used in conjunction with 3-D glasses to measure the timing, decision-making, visual perception, and overall visual system ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). Within one session of testing on the VEPT, the participants were asked to complete five tests: Vizual Alignment, Depth Perception, Vizual Flexibility, Vizual Recognition, and Vizual Tracking.

The researchers also collected data using the *Gates-MacGinitie Reading Test* (GMRT) 4th edition, Form S. For the purpose of this study, the participants were only asked to complete the vocabulary and reading comprehension sections of the test. The GMRT is a paper test that "assess[es] student achievement in reading at the kindergarten through high school level" (Lee, 2016).

Data Analysis

The researchers used the data to answer the following question: What is the relationship between visual processing ability and reading comprehension scores among students enrolled in third through fifth grade?

By analyzing the results, the researchers were able to address the research question. The researchers coded each participant with a username to keep their identity confidential. The code was used as the participants' username when logging into VEPT. After the scoring was completed with the VEPT and GMRT, all scores were calculated and placed in a spreadsheet.

RESULTS

Vizual Edge Performance Trainer

The VEPT is a computerized program that allows you to train and improve visual skills. It is used commonly with professional athletes, but more importantly it is used to measure visual processing. For the purpose of this study, the VEPT consisted of five tests which were Vizual Alignment, Depth Perception, Vizual Flexibility, Vizual Recognition, and Vizual Tracking.

Depth perception. Depth Perception determines the ability to look at an object in the condition it is in, usually free space, and make the decision of which circle appears to be floating towards you. This test requires that both eyes are used simultaneously, while using the 3-D glasses that were provided to each student prior to starting the test ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). The Depth Perception grading scale determined the number of correct responses given by the test taker.

Grading for the Depth Perception test was on a scale of 0 to 4. The grading scale follows: 0-1 out of 4: the tester will have very limited depth perception, 2 out of 4: moderately limited depth perception, 3 out 4: mildly reduced depth perception, and 4 out of 4: perfect depth perception. For the purpose of this paper, the students who did excellently well and students who did not do well were included in the results (4 of each). In Table 1, the scores for all of the tests are included.

Vizual alignment. Vizual Alignment is the ability to track a moving object. The purpose of this test is catch the perception of the ball's location in free space and timing ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). This test can be critical to an athlete's performance, or in our case the students' performance because a slight imbalance could cause inconsistency in performance. For example, the student reading a simple sentence such as "Cats are running"

can be misinterpreted by false tracking; in this case the student could see "ats are running" due to the imbalance.

The grading for Vizual Alignment test was on two scales. The first scale determined where the student was on the target completely. Since this test consisted of putting an arrow inside a box, the test determined if the student either aimed correctly on the target (centered), aimed in front of the target (before), aimed behind the target (after), or aimed above the target (hyper). The second part of the grading for the Vizual Alignment test is the point scale that goes into more detail of how each aim relates to the eye muscle imbalance. If the student scores a 0-1: alignment deviation is normal, 0 is the peak score for an elite athlete. If the student scored a 1 before or after: a minor binocular eye muscle imbalance exists; 2 to 4 before or after: represents a moderate binocular eye muscle imbalance; 5 or greater before or after: represents a large binocular eye muscle imbalance. In Table 1, the scores for all the tests are included.

Vizual flexibility. Vizual Flexibility for Convergence and Divergence tested the eye's ability to gaze using both eyes from near to far and "the ability or recover from the effort required to move the eyes in each direction" ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). Convergence is the binocular eye movement used as targets move toward you, while Divergence is used to view targets at a distance. Since these two concepts are similar, they were tested in the same way. The tests consisted of a box with four quadrants and the purpose of the test was to determine in which quadrant the diamond was placed.

The grading for Vizual Flexibility for Convergence and Divergence were similar since both tests resulted in common scores. In both Convergence and Divergence, the maximum achievable station score is 77 with a 100% accuracy. However, between the two tests, there are different station score ranges within the 0 – 77 range. Convergence station scores ranges are as follows: 0 – 25 out of 77 is considered weak eye movements, 26 – 45/77 is considered to have a fair eye movement, 46 – 60/77 is considered good eye movements, and 61 – 77/77 displays excellent eye movements. Divergence station scores are as follows: 0 – 10 out of 77 is consider weak eye movements, 11 – 18/77 is considered to have fair eye movements, 19 – 24/77 is considered good

eye movement, and 25 – 40/77 displays excellent eye movements. In Table 1, the scores for all tests are included.

Vizual recognition. Vizual Recognition provides a series of simple visual targets that process information to respond with correct motor movement ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). This test is often compared to the video game *Dance, Dance, Revolution*. On the screen, three arrows will appear with each arrow presented in a different direction. The student must recognize the arrows presented to them and remember what direction and order that they are presented in. After the arrows are established, the student must repeat the order that was given into the VEPT in the correct order.

The recognition scoring is determined based on the reaction time that the student responded to the series of visual targets. The ideal percent to strive for is 90% or higher during the training. The grading scale will be completed in two components. The first component will be in the reaction time in seconds: .50-.75 seconds will establish excellent skills, 76- 1.00 seconds: good, 1.25 – 1.49: fair, 1.50 – 2.50 seconds: reduced, >2.51 seconds: limited. The seconds component will be percentage of visual targets answered correctly: 96-100% is excellent, 90-95 is good, and < 90 is reduced. In Table 1, the scores for all tests are included.

Vizual Tracking. Vizual Tracking test is a similar process to Vizual Recognition, however, this test only involves one arrow (target) in a random direction. This test required that you respond with the "correct motor movement" that the target displaced on the screen ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). This test determines how quickly the student will react to the target.

The recognition scoring is determined on the reaction time that the student responded to the series of visual targets. The ideal percent to strive for is 90% or higher during the training. The grading scale will be completed in two components. The first component will be in the reaction time in seconds: .20 - .44 seconds will establish excellent skills, .45 - .51 seconds: good, .52 - .55: fair, .56 - .59 seconds: reduced, >.60 seconds: limited. The seconds component will be percentage of visual targets answered correctly: 96-100% is excellent, 90-95 is

good, and < 90 is reduced. In Table 1, the scores for all the tests are included.

Vizual Edge Score. The Vizual Edge Score determines the overall categories within the VEPT and results it into a single number score ("Sports Vision Performance Training – Vizual Fitness by Vizual Edge", n.d.). This score will determine how well an athlete, or for the purpose of this paper a student, does on visual training skills. This score is used for individual purposes and should not be compared to other scores, because it is individually evaluated per person. In Table 1, the scores for all the tests are included.

Gates MacGinite

Gates MacGinite Reading Test norm referenced assessment. This test can include overall reading ability, specifically in the areas of decoding, phonemic awareness, phonics, vocabulary, and comprehension (Goltche, 2018), but for the purpose of this study the GMRT consisted of two tests: Comprehension and Vocabulary. Unlike the VEPT, these two tests were separate with categories of raw derived scores. Within the derived scores, it divides into two subtopics which are status scores (National Percentile Rank (NPR), Normal Curve Equivalent (NCE), and National Stanine (NS)) and growth scores (Extended Scale Score (ESS) and Grade Equivalent (GE)).

Raw score. The raw score is how many questions the student answered correctly within the entirety of the test given. This score will have no relation to the student's overall achievement, but better reveals where the student stands currently in their reading comprehension or vocabulary. This raw score should not be compared to other tests because it is not a test that looks at the overall score. Since it looks at correct percentage and not the overall score, the scores will be converted to derived scores. In Table 2, the scores for these tests are included.

Derived score. Derived scores determined "how well a student has done in comparison with the students in a norming group" and used to compare a student's achievement (MacGinitie, 2000). The Derived scores divide into two types: Status and Growth scores. Status score consists of ranked achievement that occurred within the same grade level and year of the students (MacGinitie, 2000). Status score also looks at the rank of achievement that the statistical normal curve lies on the continuum (MacGinitie, 2000). While the Status score looks at the present, the Growth score looks at the achievement across years. Since the Growth score looks at the achievement over years, Status scores compare more of the student's achievement in the present. In table 2, the scores for this test are included.

TABLES AND GRAPHS

TABLE 1. Vizual Edge Scores

Name	Edge Score	Eye Align	Depth	Conv %	Conv Station	Div %	Div Station	Vizual Recog Resp Time	Vizual Recog % Correct	Vizual Tracking Resp Time	Vizual Tracking % Correct	Percentile Rank
Bear 1	49.01	3A	4/4	13%	0	19%	1	2.99	85%	0.94	75%	1st
Bear 2	44.08	2A	0/4	21%	1	94%	23	2.2	31%	1.04	63%	1st
Bear 3	55.48	1A	1/4	93%	22	91%	10	3.1	40%	1.01	68%	1st
Bear 4	55.08	6A	2/4	90%	15	90%	15	2.53	53%	0.72	51%	1st
Bear 5	54.49	1A	4/4	19%	1	86%	12	4.83	60%	1.16	69%	1st
Bear 6	66.53	2A	4/4	84%	23	86%	11	6.38	12%	0.99	95%	5th
Bear 7	64.66	0	4/4	95%	25	87%	19	1.84	5%	1.09	65%	5th
Bear 8	45.85	5A	1/4	73%	6	80%	8	3.92	63%	1.15	65%	1st
Bear 9	66.62	0	2/4	94%	38	94%	22	2.6	60%	0.94	76%	5th
Bear 10	69.5	0	4/4	90%	28	94%	19	1.95	66%	1.15	54%	5th
Bear 11	59.98	3A	2/4	80%	12	89%	15	3.58	83%	1.1	95%	1st
Bear 12	44.67	5A	1/4	20%	2	92%	32	3.72	33%	0.87	88%	1st
Bear 13	73.11	0	4/4	96%	40	97%	41	2.54	26%	0.99	85%	10th
Bear 14	65.39	2A	2/4	91%	24	91%	19	3.64	92%	1.11	100%	5th
Bear 15	51.52	2A	0/4	91%	30	90%	12	3.08	15%	0.79	72%	1st
Bear 16	42.12	1A	0/4	28%	1	69%	7	3.92	46%	0.97	96%	1st
Bear 17	43.86	2A	0/4	71%	16	59%	8	4.17	25%	0.97	84%	1st

TABLE 2. Gates MacGinite Scores

Name	Raw Voc	Raw Comp	Total Raw	NCE Voc	NPR Voc	NS Voc	GE Voc	ESS Voc	NCE Comp	NPR Comp	NS Comp	GE Comp	ESS Comp	NCE Total	NPR Total	NS Total	GE Total	ESS Total
Bear 1	39	37	76	68	80	7	6	510	58	64	6	4.5	489	63	73	6	5.1	497
Bear 2	8	18	26	7	2	1	1.4	380	26	13	3	2.3	428	13	4	2	2	407
Bear 3	20	24	44	32	20	3	2.5	434	38	29	4	2.8	447	34	22	3	2.6	440
Bear 4	31	22	53	50	50	5	3.8	472	32	20	3	2.5	438	41	34	4	3	455
Bear 5	27	24	51	44	38	4	3.2	458	37	27	4	2.8	448	39	30	4	2.9	451
Bear 6	10	16	26	10	3	1	1.7	392	22	9	2	2.2	420	13	4	2	2	407
Bear 7	25	21	46	44	38	4	4.1	478	36	26	4	3.5	467	39	30	4	3.7	472
Bear 8	22	19	41	38	29	4	3.5	467	33	21	3	3.2	461	35	24	4	3.4	464
Bear 9	21	20	41	36	26	4	3.4	436	34	23	4	3.3	464	35	24	4	3.4	464
Bear 10	27	31	58	47	45	5	4.4	485	51	52	5	5	497	49	48	5	4.6	490
Bear 11	28	35	63	48	47	5	4.6	488	57	63	6	5.8	509	53	56	5	5.1	498
Bear 12	23	25	48	46	42	5	5.1	499	43	37	4	4.8	495	45	40	5	5.1	497
Bear 13	34	45	79	65	76	6	8.2	535	90	97	9	12+	584	74	87	7	10	549
Bear 14	37	32	69	72	85	7	9.9	547	54	57	5	6.2	515	62	72	6	7.4	528
Bear 15	25	25	50	48	47	5	5.5	504	43	37	4	4.8	495	46	42	5	5.2	499
Bear 16	31	27	58	60	68	6	7.2	525	46	43	5	5.3	501	52	54	5	6	511
Bear 17	32	37	69	62	71	6	7.5	528	63	73	6	7.9	533	62	72	6	7.4	528

GRAPH 1.
Third Grade Gates-MacGinite Scores

Gates-MacGinite Scores
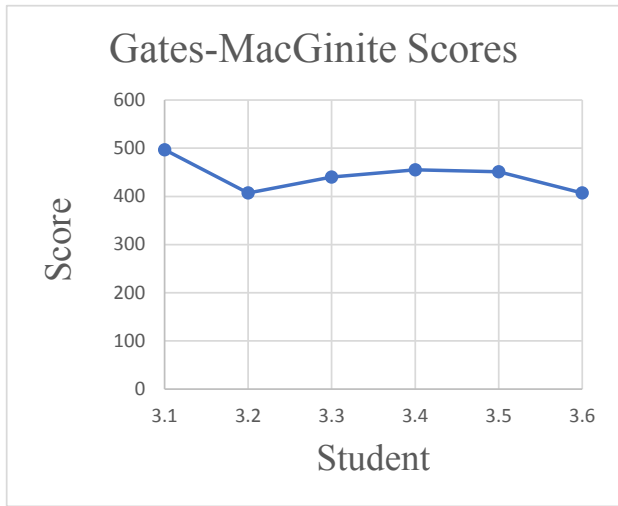
GRAPH 2.
Third Grade Vizual Edge Scores
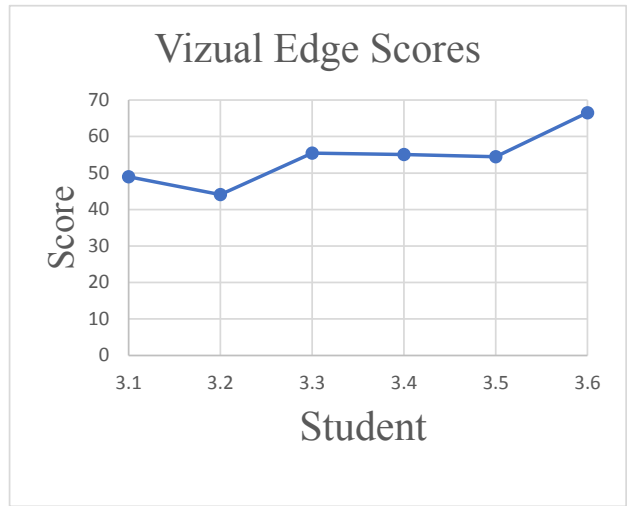
Vizual Edge Scores

GRAPH 3.
Fourth Grade Gates-MacGinite Scores
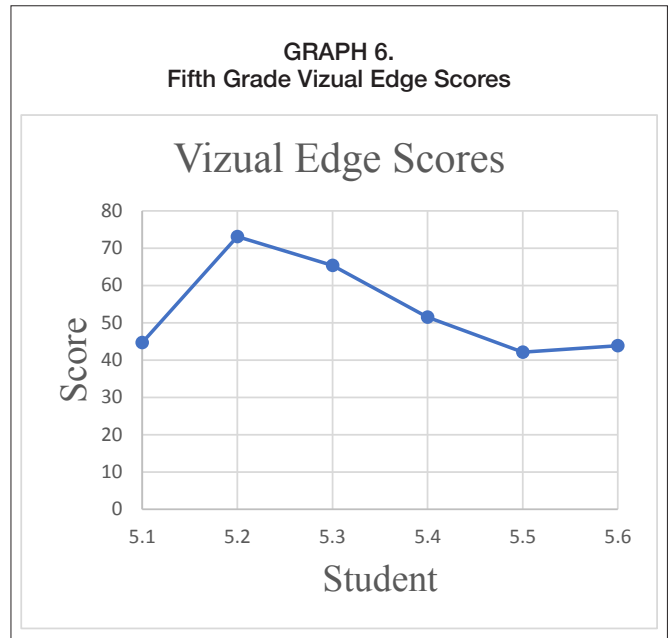
Gates-MacGinite Scores
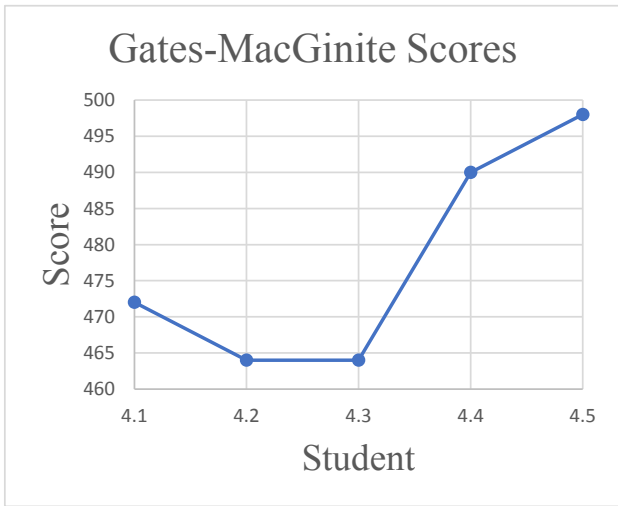
GRAPH 4.
Fourth Grade Vizual Edge Scores
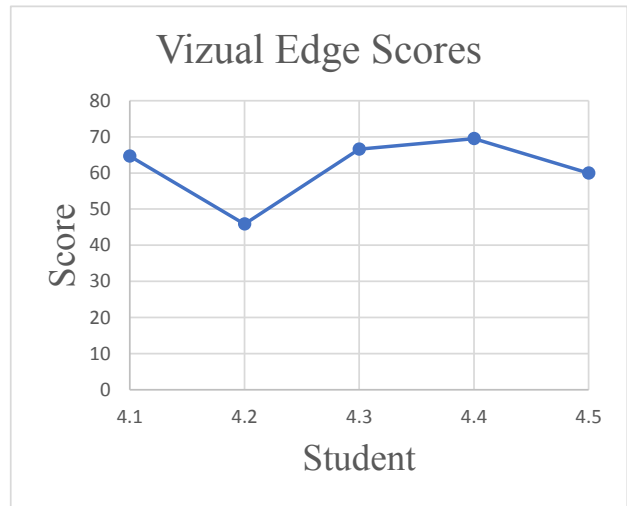
Vizual Edge Scores

GRAPH 5.
Fifth Grade Gates-MacGinite Scores
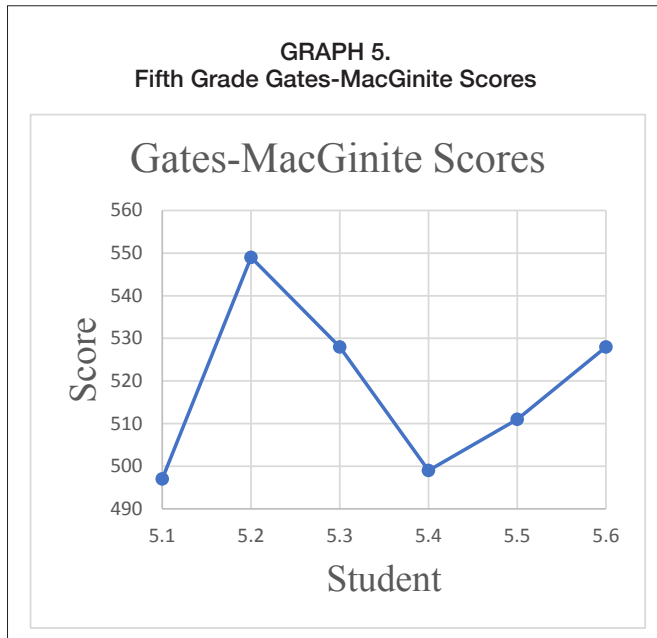
GRAPH 6.
Fifth Grade Vizual Edge Scores

DISCUSSION

The participants in this study used the VEPT and GMRT to determine the correlation between visual and reading comprehension skills. The sample size (n=17) was too small to establish a correlation. However, there were evident trends. The trend showed that the student with the highest VEPT score also had the highest GMRT score. Conversely, the student with the lowest VEPT score had a low GMRT score.

There were some observed factors that could have played a role in the students' VEPT scores. One factor was sound associated with VEPT. The sound may have been distracting for some participants. Participants could have worn headphones to block out any noise and therefor minimize any distractions. Another factor was that VEPT with not compatible with game controllers. Participants had to use the keyboard up, down, left, right arrows for VEPT. Participants were observed having difficulty using the keyboard. VEPT is a computer-based video program requiring participants to use arrow keys. Perhaps if participants could have controlled the games with a game controller similar to PlayStation or Xbox games controllers, results may have been different and a more accurate reflection of the participants visual skills.

CONCLUSION

In conclusion, this study was unable to determine if statistically significant correlations existed between visual skills and the reading achievement of third- through fifth- grade students. Further research with larger sample sizes and a software program utilizing gaming control devices should be considered.

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SWITCHABLE MAGNETS AS A POWER-EFFICIENT ALTERNATIVE FOR ELECTROMAGNETS IN A MOBILE ROBOTIC SYSTEM

by ANDREW GARCIA

ABSTRACT

The goal of this research is to investigate the power-efficiency of a switchable magnet, in place of an electromagnet, to pick up one-inch diameter ferromagnetic coins for a color coin sorting mobile robot. Recently, switchable magnets have been proven to be more energy efficient than an electromagnet in mobile robotic applications. This is because electromagnets require a constant state of power consumption where switchable magnets only require power to change its state on or off. Switchable magnets are used just like electromagnets for magnetic flux control to induce magnetic attraction to an object by changing its state. The switchable magnet's housing is fitted with a digital servo motor to rotate the top dynamic magnet 180 degrees using pulse width modulated signals to change its state to on or off. A switchable magnet and electromagnet are tested for their respective power consumption on the mobile systems battery pack by measuring the current and voltage over the time that it takes to pick up and drop off a single coin. The results show that the switchable magnet uses 59.8% less power, measured in Watts, while being able to hold 5 times more weight, measured in Newtons, than the electromagnet. Over the duration of 20 seconds, the time estimated to displace the coin, the switchable magnet will use 25 times less power, measured in milliAmp- hours, compared to the electromagnet; therefore, switchable magnets present a more power-efficient alternative for magnetically controlled applications, specifically on a mobile system.

Keywords: *Switchable Magnets, Electromagnets, Mobile Robots, Servo Motor, and Power Efficiency*

MENTOR

Dr. Mehrube Mehrubeoglu

Department of Engineering

INTRODUCTION

Single permanent magnets have limited applications because there is no control over such magnets' magnetism; permanent magnets are simply always magnetic [1]. The common solution for controlled
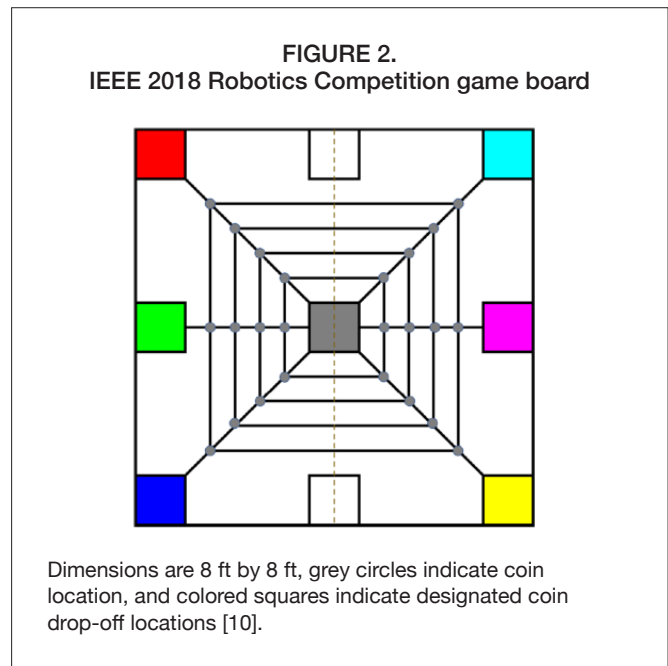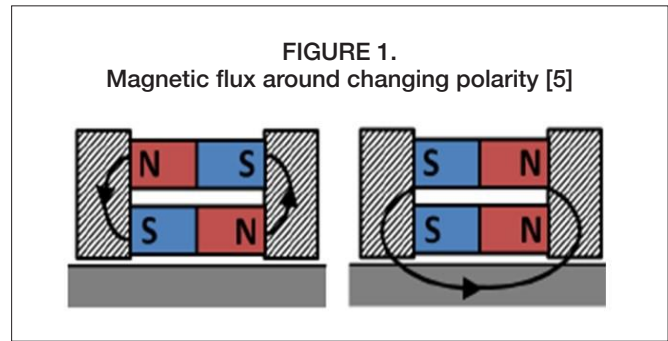
magnetism is an electromagnet which has been in use since the last century. An electromagnet is a device that generates a magnetic field around itself if current is provided [2][3]. When power is on, an electromagnet has magnetism. When power is off, an electromagnet has no magnetism [4]. The issue with electromagnet applications surfaces when powered on for long periods of time [5][6]. The longer the electromagnet is turned on, the more the current drawn from its power source. With the recent demand for mobile robots, battery power is always a major concern when it comes to the success of a mission [7][8]; therefore, alternate options must be investigated to prolong the life of batteries.

When it comes to picking up ferromagnetic objects, magnetic attraction is highly desirable because of its implicit reliability. A controlled magnetic device is necessary for sorting the ferromagnetic objects that need to be picked up and put back down. In the case of mobile robotic systems, the power source is provided by a battery and is limited by its supply of Watt-hours or Amp-hours. If at any point in time the battery is out of charge during operation, the electromagnet will turn off and lose the desired magnetic force. It is very important for a coin sorting robot not to lose any of its coins, and if power is cut to the electromagnet during the pick-up/sorting process, the coin will slip away and may become impossible to find again. To insure reliability and power efficiency, a switchable magnet (SM) is tested and compared to an electromagnet under the same conditions.

An H-type switchable magnet is tested for this research. The basic principle of switchable magnets relies on the ability of rotating one dynamic magnet 180 degrees over a permanent magnet, thus changing the orientation of the magnetic flux to either inside or outside its housing (Figure 1). The strength of the magnetic field grows as the angle of the dynamic magnet increases from 0 to 180 degrees reaching maximum magnetic strength at 180 degrees [9].

This experiment is meant to determine the option to improve battery efficiency on a colored ferromagnetic coin sorting mobile robot. The objective of this robot is to track the location of several unknown colored coins embedded in the floor, pick them up, sort them by color, and drop them off to their designated color

FIGURE 1.
Magnetic flux around changing polarity [5]

FIGURE 2.
IEEE 2018 Robotics Competition game board

Dimensions are 8 ft by 8 ft, grey circles indicate coin location, and colored squares indicate designated coin drop-off locations [10].

locations. Once a coin has been detected nearby, the robot will adjust its position until the coin is directly under the coin-lifting linear actuator. At the end of the coin-lifting linear actuator, resides the SM that will be turned on, attracting the ferromagnetic coin and lifting the coin out of its embedded hole (Figure 2). Once the coin is picked up, the coin sorting process begins. The experiment entails lifting the coin vertically upward and deploying a color sensor to analyze the coins' color components, or RGB (red, green, blue) values. After the coin's color content is determined, the correct color cup is placed underneath the SM to be released and therefore sorted. The last step, after all coins are collected, involves taking the coins out of their cups using the SM and placing them down in the matching color square (Figure 2).

RELATED WORK

A group of five research students in Portugal at the University of Coimbra explored the application and implementation of switchable magnets for adhesion in their climbing robots [11]. One of the robots being developed, the InchwormClimber [11], can scale 88 meters of ferromagnetic structures on a single charge with an overall weight of 1 kg. The method of climbing for the InchwormClimber relies in its biped structure which contains two switchable magnet units at each end for a higher adhesion force per mass unit. Magnetic attachment to the structure is highly desirable due to its inherent reliability [12]. This robot is a great example of what a controlled magnetic field can achieve.
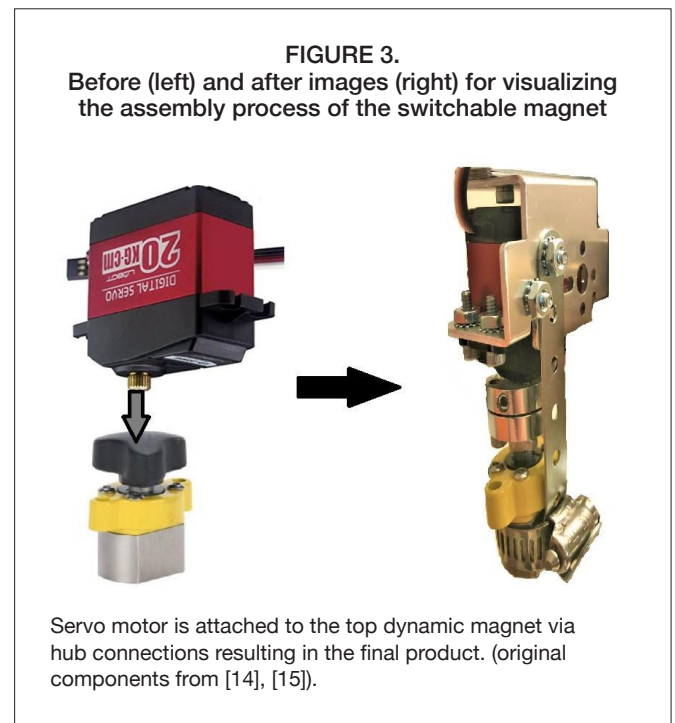
Another more advanced climbing robot from the University of Coimbra is called the OmniClimber. OmniClimber is an omni-directional climbing robot capable of scaling ferromagnetic structures and transitioning 90 degrees from one plane to the next [13]. The issues with electromagnets are stressed in this specific application of portable climbing robots, and the advantages of switchable magnets are described. The use of switchable magnets in robotics is rather new and few examples take advantage of this discovery. An H-type SM is composed of two cylindrical permanent magnets encircled in a ferromagnetic chamber. One magnet is fixed, and the other is moving. When rotating the moving magnet 180 degrees, the magnetic flux is closed between the magnets and turned off applying no adhesion force. This research group performed an extensive study using COSMOL Physics Simulator on the manufactured switchable magnets, testing magnet housing conditions. The researchers found that because the round ferromagnetic housing was flat on both sides, more magnetic flux was directed through the object it was trying to grasp compared to not having flat sides [13].

METHODS

Components and Setup

A switchable magnet MagJig from the manufacturer Magswitch was used for this research as the magnetic device. For autonomous control over the SM, a motor must be attached to the top dynamic magnet. The LewanSoul LD-20MG digital servo motor was chosen for the SM and integrated into the robot's systems (Figure 3). The servo motor is operational at 7 V with a maximum current of 300 mA and changes the state of the magnet in a tested 1.5 s. However, future work can improve upon the speed of the state change with all the same equipment. The electromagnet is driven by a power of 1 Watt transmitted through a constant voltage of 5 V at a max current of 1 A.

FIGURE 3.
Before (left) and after images (right) for visualizing the assembly process of the switchable magnet

Servo motor is attached to the top dynamic magnet via hub connections resulting in the final product. (original components from [14], [15]).

For the purpose of comparison, a 5 V electromagnet with a holding force of 50 N (Figure 4) is tested for its current draw under the same conditions as the switchable magnet.

Experimental Procedure

The servo motor and electromagnet are tested with the exact same procedure for current draw on a battery. A pulse width modulated (PWM) signal is sent to the servo motor so that its actuator rotates 180 degrees, thus turning the magnet on. A break is made on the black ground wire to connect a digital multimeter in series for testing servo motor's current draw from the battery (Figure 4). The current reading from the digital multimeter is recorded in units of Amps.

FIGURE 4.
A 5 V Electromagnet used to compare results of switchable magnet [16]

FIGURE 5.
Procedure used to measure current from the devices
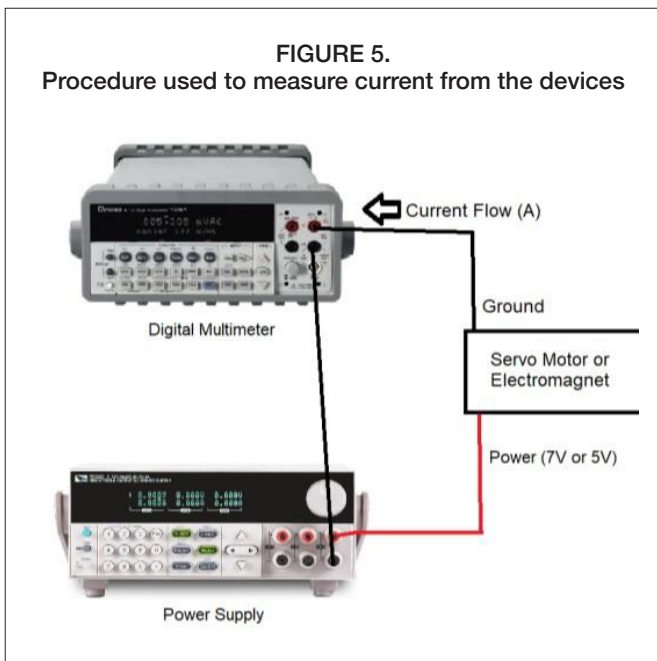
Equations

The power equation given below in Equation (1) is used for comparing the power consumption of the electromagnet and switchable magnet in this research:

$$P = I*V \qquad (1)$$

where, P is power (Watts), I is Current (Amps), and V is Voltage (V). Current and voltage are directly measurable during the experiments. Next, the energy equation given below in Equation (2) is used for understanding the amount of power consumed in a given time:

$$Wh = P*t \qquad (2)$$

where, Wh is Watt-hours, P is power (W), and t is time measured in hours. Since batteries are usually measured in the amount of amp-hours the can provide, a conversion equation is used to go from Watt-hours to milliAmp-hours, as in Equation (3)

$$mAh = (Wh/V)*1000 \qquad (3)$$

where, Wh is energy in Watt-hours and V is voltage in Volts.

RESULTS AND DISCUSSION

In this experiment, the coin sorting process took 20 seconds per coin to complete. This time interval included picking up a coin off the floor, analyzing its color, and moving the robot to the correct colored square, for the purpose of depositing the coin. The distance to the correct colored square varies depending on location. The electromagnet is required to be powered on for at least 20 seconds to complete this task. The SM with the servo motor only needs to be powered on for its two instances of switching the state of the magnet.

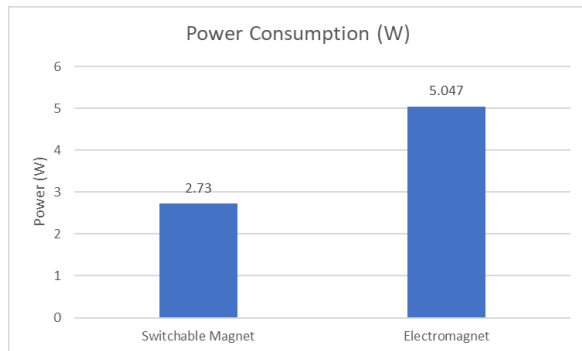The measurements from the experiment are used and compared in the following table:

TABLE 1. Comparison of SM and Electromagnet

Measurement	Switchable Magnet	Electromagnet
Holding Force (N)	266.89	50.00
Voltage (V)	7.00	5.00
Current (A)	0.26	0.72
Power (W)	2.73	5.05
milliAmp-hours (mAh)	0.16	4.01

Table 1 summarizes the specifications and performance of the two magnets over twenty seconds. Power measured for the electromagnet (5.05 W) is almost twice as much as the power drawn by the switchable magnet (2.73 W), also shown in Figure 6.

The table shows that during the 20-second experiment, the energy consumption for the electromagnet was over twenty times more (4.01 mAh) than the switchable magnet (0.16 mAh), demonstrating the advantages of using a switchable magnet over an electromagnet, since

FIGURE 6.
Bar graph comparison of the power
it takes to operate the two devices

FIGURE 7.
Bar graph comparison that shows the total power
consumed by the switchable magnet and electromagnet

The SM is shown here to be clearly advantageous because
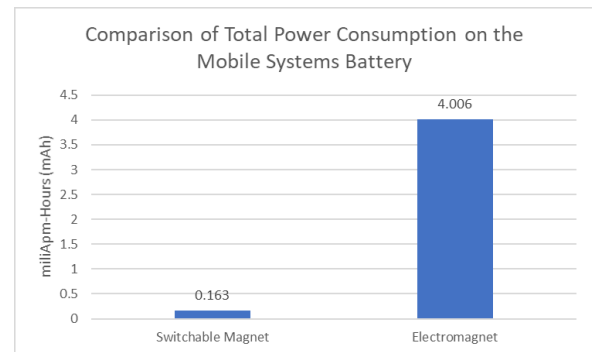of its small power usage.

the switchable magnet. A SM will allow the battery to last over twenty times longer, as also demonstrated in Figure 7.

The comparison in Figure 7 shows the advantage of using a switchable magnet over an electromagnet for long durations of controlled magnetism, for over 1.5 seconds in the reported experiments. Despite the SM operating at higher voltage levels, the SM uses less power. Furthermore, because of the SM's unique power feature, it consumes much less energy than the electromagnet, because the switchable magnet can be powered off while maintaining its current state of being on or off. The other parameters in Table I can be compared when considering which type of magnet to use. In addition, A SM can lift 5 times more weight and uses 60% less battery power than its electromagnet equivalent.

CONCLUSIONS

A switchable magnet is a power-efficient alternative that uses 60% less battery power than its electromagnet equivalent. This power efficiency stems from the SM's unique power feature that allows it to be turned off while maintaining its current state of magnetism as on or off. When using the SM in the presented case for sorting ferromagnetic coins, where the device must be turned on for 20 seconds, power consumption is reduced by a factor of 25 compared to that of an equivalent electromagnet. The SM is most favorable in situations where ferromagnetic objects must be attracted for periods of time lasting longer than 1.5

seconds. In the case of switchable magnets, after the magnet is turned on, is not spent on wasting battery power is not wasted to maintain SM's magnetized state. Power conservation is highly desirable in mobile robotic systems, where prolonged battery life is critical for the success of the mission, making switchable magnets an excellent alternative in such applications.

ACKNOWLEDGMENT

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PHYLOGENETIC RELATIONSHIPS IN THE GENUS *ERIMYZON* (FAMILY CATOSTOMIDAE) INFERRED FROM MITOCHONDRIAL AND NUCLEAR LOCI

by KAT HAMILTON

ABSTRACT

Erimyzon is a genus in the family Catostomidae that consists of four described species all native to North America; E. oblongus (eastern creek chubsucker), E. claviformis (western creek chubsucker), E. sucetta (lake chubsucker), and E. tenuis (sharpfin chubsucker). Most studies of catostomid phylogenetics focus on resolving relationships among genera and higher taxonomic groups. Due, in part, to the fact that catostomids are polyploid, previous studies have relied mainly on mitochondrial markers and inconsistencies between studies are likely due to the loci used and/or limited taxon sampling. While the genus Erimyzon is consistently recovered as monophyletic, in a recent study, E. oblongus and E. tenuis were each described as non-monophyletic; a result that could be due to misidentification, presence of cryptic lineages, incomplete lineage sorting, and/ or hybridization/introgression. Because the phylogenetic relationships among species in the genus Erimyzon have not been thoroughly examined, and because there may be unrecovered lineages in the genus, mitochondrial-encoded CO1 and nuclear-encoded IRBP2 sequences from multiple individuals of each of the four currently recognized species were used to construct a phylogenetic hypothesis for the genus.

MENTOR
Dr. x

x
Department of x
College of x

INTRODUCTION

Catostomidae, commonly known as suckers, is a family of freshwater fishes within the order Cypriniformes, the largest orders of fishes with more than 4,000 extant species (Chen and Mayden, 2012); excluding the order Perciforms (>10,000 extant species) which is likely a catchall for advanced fishes rather than a natural grouping (Nelson, 2016). Catostomid fishes first appear in the fossil record during the Eocene (Smith, 1992) and extant species (~78; Nelson et al. 2016) are primarily found in North American freshwater habitats but also can be found in Asia, mainly in China and Siberia, (Page and Burr, 2010; Smith, 1992). The family is currently organized into four subfamilies—Catostominae, Cycleptinae, Ictiobinae, and Myxocyprininae (Page and Burr, 2010; Nelson, et al., 2016). Suckers are characterized by large, thick lips; soft rays in the fins; no teeth on the bones of the jaws, but numerous molar-like or comb-like teeth on each pharyngeal arch; a single dorsal fin; nine or more dorsal

rays; abdominally-placed pelvic fins; anal fins toward the posterior of the body; and cycloid scales on the body, but no squamation on the head. The name sucker refers to their large lips and protrusible premaxillae that enable them to suck invertebrates from the benthos (Page and Burr, 2010).

To understand patterns of genetic variation within and among catostomid fishes, data has been collected from multiple mitochondrial-encoded genes, including cytochrome b (Cyt b; Sun et al., 2007), NADH dehydrogenase subunit 4 and 5 (ND4 and ND5; Doosey et al., 2010) and large and small subunit rRNA (LSU and SSU; Harris and Mayden, 2001). Because catostomids are polyploid (Chen and Mayden, 2012), fewer studies have used nuclear genes but both internal transcribed spacer 1 (ITS1; Sun et al., 2007) and interphotoreceptor retinoid-binding protein gene 2 (IRBP2; Chen and Mayden, 2012) have been used to build phylogenies. Further, morphological characteristics have been used to assess the phylogenetic relationships of catostomid fishes (Smith, 1992).

Smith (1992) conducted a morphological assessment of 64 catostomids and proposed two primitive (Cycleptinae and Ictiobinae) and two advanced (Eastern and Western Catostominae) clades. Cycleptinae included the genera *Cycleptus* and *Myxocyprinus* and Ictiobinae included the genera *Ictiobus* and *Carpiodes*. The Catostominae were divided into a western group that included the genera *Xyrauchen, Deltistes, Chasmistes*, and *Catostomus*, while the eastern group included the genera *Minytrema, Erimyzon, Moxostoma, Scartomyzon, Hypentelium* and *Thoburnia*. The groups are also recognized as tribes Catostomini and Moxostomatini, respectively.

Harris and Mayden (2001) examined 16 species of catostomids using sequence data from the mitochondrially-encoded LSU and SSU rRNA genes. In relation to Smith's (1992) hypothesis, they concluded that *Myxocyprinus* should be removed from the subfamily Cycleptinae and placed in its own subfamily (Myxocyprininae), with Cycleptinae restricted to the two species of *Cycleptus*. They also hypothesized that Catostominae should be divided further into tribes Catostomini (*Catostomus, Xyrauchen, Chasmistes, Deltistes*), Thorburniini (*Thoburnia, Hypentelium*), and Moxostomatini (*Moxostoma, Scartomyzon*), but were

unsure of the placement of the genera *Erimyzon* and *Minytrema.*

Sun et al. (2007) examined 17 species of catostomids using the nuclear-encoded internal transcribed spacer 1 (ITS1) and the mitochondrially-encoded cytochrome b (Cyt b). Three clades were resolved: Clade I (*Catostomus, Thoburnia, Xyrauchen, Chasmistes*, and *Deltistes*), Clade II (*Hypentelium, Moxostoma*, and *Scartomyzon*), and Clade III (*Ictiobinae: Ictiobus* and *Carpiodes*). Clade I resembled the Catostomini tribe of Harris and Mayden (2001) and Smith (1992), but included *Thoburnia*. Clade II was similar to the eastern group of Smith (1992) excluding *Erimyzon, Thoburnia* and *Minytrema*; whereas, Harris and Mayden (2001) had grouped the genera *Hypentelium* and *Thoburnia* together, placing *Scartomyzon* in the tribe Moxostomatini. The finding of a monophyletic Ictiobinae was consistent with both previous studies. The genera *Erimyzon* and *Minytrema* were of uncertain taxonomic placement.

Doosey et al. (2010) examined 60 catostomid species using the mitochondrially-encoded NADH dehydrogenase subunit 4 and 5 (ND4 and ND5). Four clades (subfamilies) were recovered: Catostominae, Cycleptinae, Myxocyprininae, and Ictiobinae. Catostominae included four tribes: Catostomini (*Catostomus, Chasmistes, Deltistes*, and *Xyrauchen*), Erimyzonini (*Erimyzon, Minytrema*), Thorburniini (*Hypentelium, Thoburnia*), and Moxostomatini (*Moxostoma*). Cycleptinae included *Cycleptus*, Myxocyprininae included *Myxocyprininae*, and Ictiobinae included the genera *Carpiodes* and *Ictiobus*.

Chen and Mayden (2012) examined 24 catostomid species using the interphotoreceptor retinoid-binding protein gene 2 (IRBP2). The four subfamilies defined as Catostominae, Ictiobinae (*Ictiobus, Carpiodes*), Cycleptinae (*Cycleptus*), and Myxocyprininae (*Myxocyprinus*) were again recovered. Four tribes were established within the subfamily Catostominae: Erimyzonini (*Erimyzon, Minytrema.*), Catostomini (*Catostomus, Deltistes, Xyrauchen, Chasmistes*), Thorburniini (*Thoburnia, Hypentelium*), and Moxostomatini (*Moxostoma*). The results of this study were almost identical to Doosey et al. (2010), with the exception that the genus *Chasmistes* was included. This study found samples from the species *Erimyzon oblongus*

and *Erimyzon tenuis* did not form monophyletic groups within each species.

Research into the phylogenetic relationships among catastomid fishes is quite broad with studies focused on relationships among genera and therefore only one (or few) representatives of each genera are present in the data. Little effort has been focused on understanding the relationships among species within the genera, and this area of research could change our understanding of species-level diversity within the family. It is important to investigate patterns of diversity at the species level because over- and under-differentiation of species is common in freshwater fishes. Over-differentiation occurs when a single species is broken into multiple species due to disjunct distributions and/or morphological plasticity across a species range. For example, molecular studies

have revealed that pairs of co-distributed, parasitic and non-parasitic lampreys sometimes represent single species with differing life histories rather than species pairs (Schreiber and Englehorn, 2009; April et al., 2011). By contrast, under-differentiation occurs when multiple species are lumped into a single species and is common in freshwater fishes because of conserved morphology across phylogenetically related species (April et al., 2011).

In this study, I examined the phylogenetic relationships of suckers in the genus *Erimyzon* (chubsuckers). In the genus *Erimyzon*, there are currently four described species—*Erimyzon sucetta* (lake chubsucker), *Erimyzon tenuis* (sharpfin chubsucker), *Erimyzon oblongus* (eastern lake chubsucker) and *Erimyzon claviformis* (western lake chubsucker). *Erimyzon sucetta* are found

FIGURE 1.
Erimyzon sucetta distribution within the United States
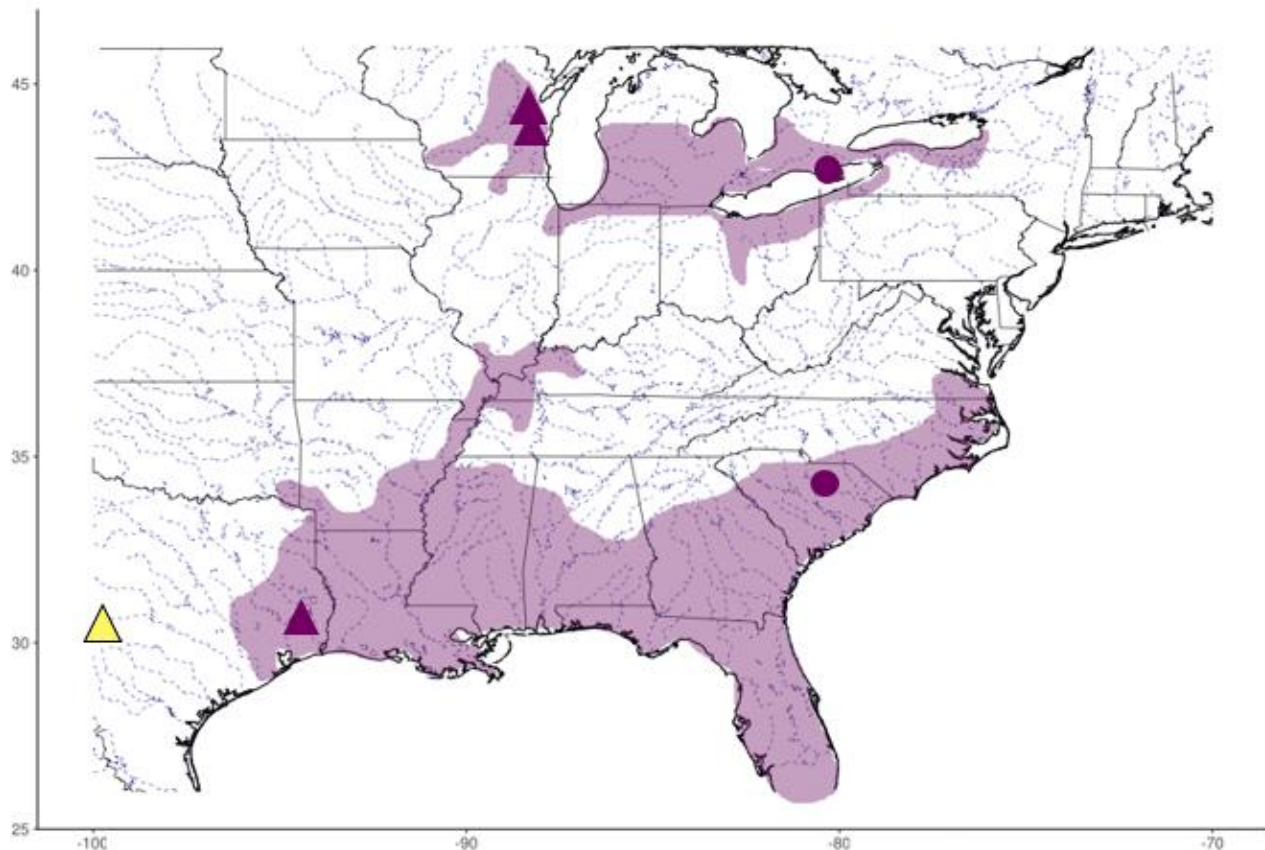
Triangles represent samples collected for this study, and circles represent samples retrieved from GenBank. The yellow triangle represents individuals sampled in the Guadalupe River in Kerrville, Texas.

FIGURE 2.
Erimyzon tenuis distribution within the United States

Triangles represent samples collected for this study, and circles represent samples retrieved from GenBank.
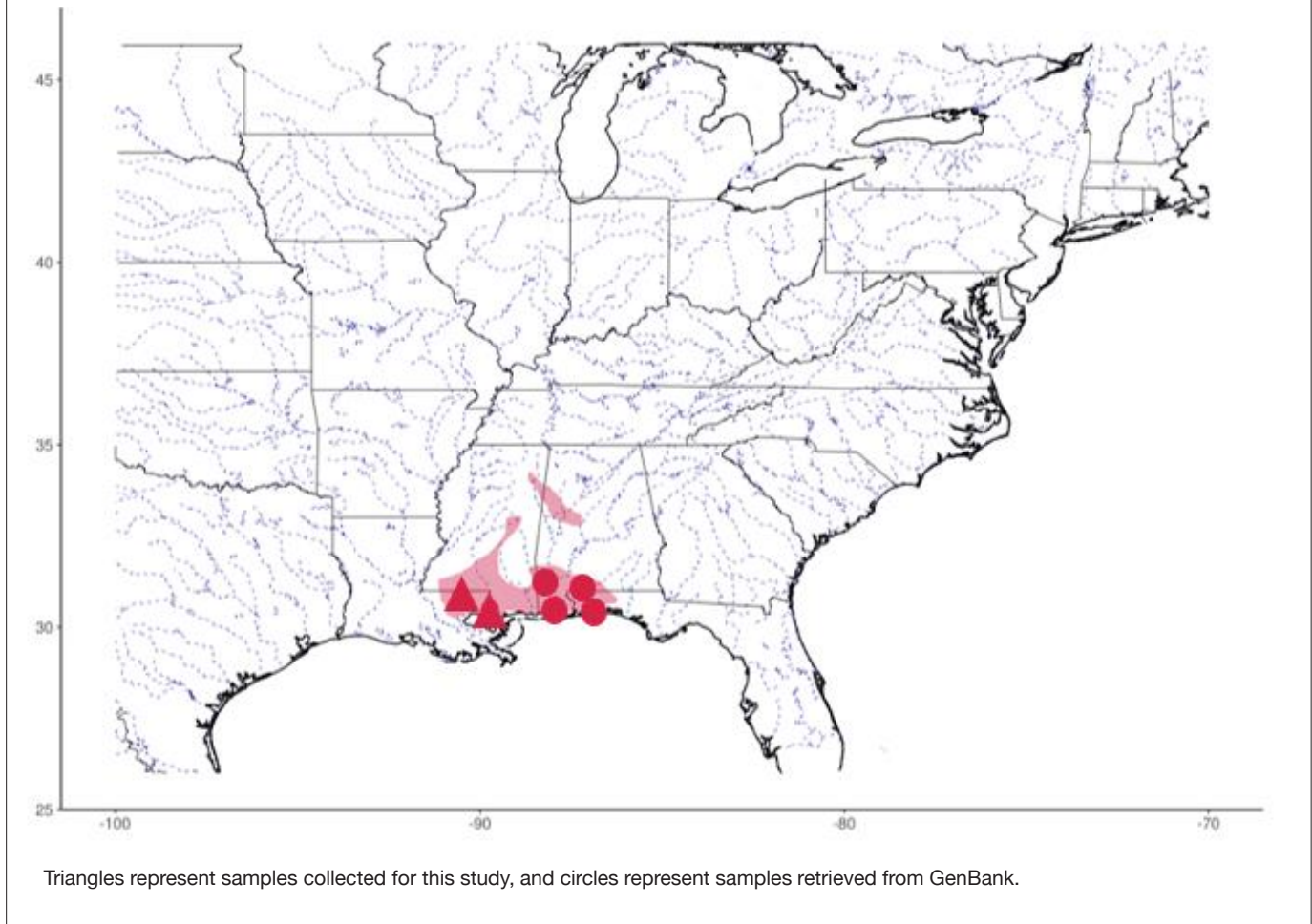
along the Gulf Coast, from eastern Texas to Florida; throughout the south-Atlantic, up to southern Virginia; and in semi-isolated populations in the Midwest, as far north as the Great Lakes and Ontario (Figure 1; Page and Burr, 2010). They are described as having a lateral stripe (Page and Burr, 2010). *E. tenuis* has the smallest distribution of the chub suckers, located in eastern Louisiana, southern Mississippi, Alabama, and into the Florida Panhandle (Figure 2; Page and Burr, 2010). *E. tenuis* has a pointed dorsal fin, hence the name "sharpfin" chubsucker; a lateral stripe; and black-edged dorsal and anal fins. *E. oblongus* is found throughout eastern states bordering the Atlantic Ocean, up to New York and southern New England (Figure 3; Page and Burr, 2010). *E. claviformis* is distributed from the Midwest down to eastern Texas and as far east as western Georgia (Figure 3; Page & Burr, 2010). *E. oblongus* and *E. claviformis* are almost identical in appearance, with their dark lateral blotches and rounded dorsal fin edge. The only defining characteristic, besides location, is dorsal ray count, with *E. claviformis* having 9-11 (10) dorsal rays and *E. oblongus* having 11-14 (12) dorsal rays (Page & Burr, 2010).

An isolated population of *Erimyzon* is found in the Guadalupe River drainage in Kerrville, Texas, shown in Figure 1. This isolated population is described in Peterson's Field Guide (Page and Burr, 2010) as *E. sucetta*, however, individuals collected from this population appear morphologically distinct from *E. sucetta*. Because of the isolation of this distinct population, I also sought to clarify the species designation for individuals sampled from the Guadalupe River.

FIGURE 3.
Erimyzon oblongus and *Erimyzon claviformis* distributions within the United States
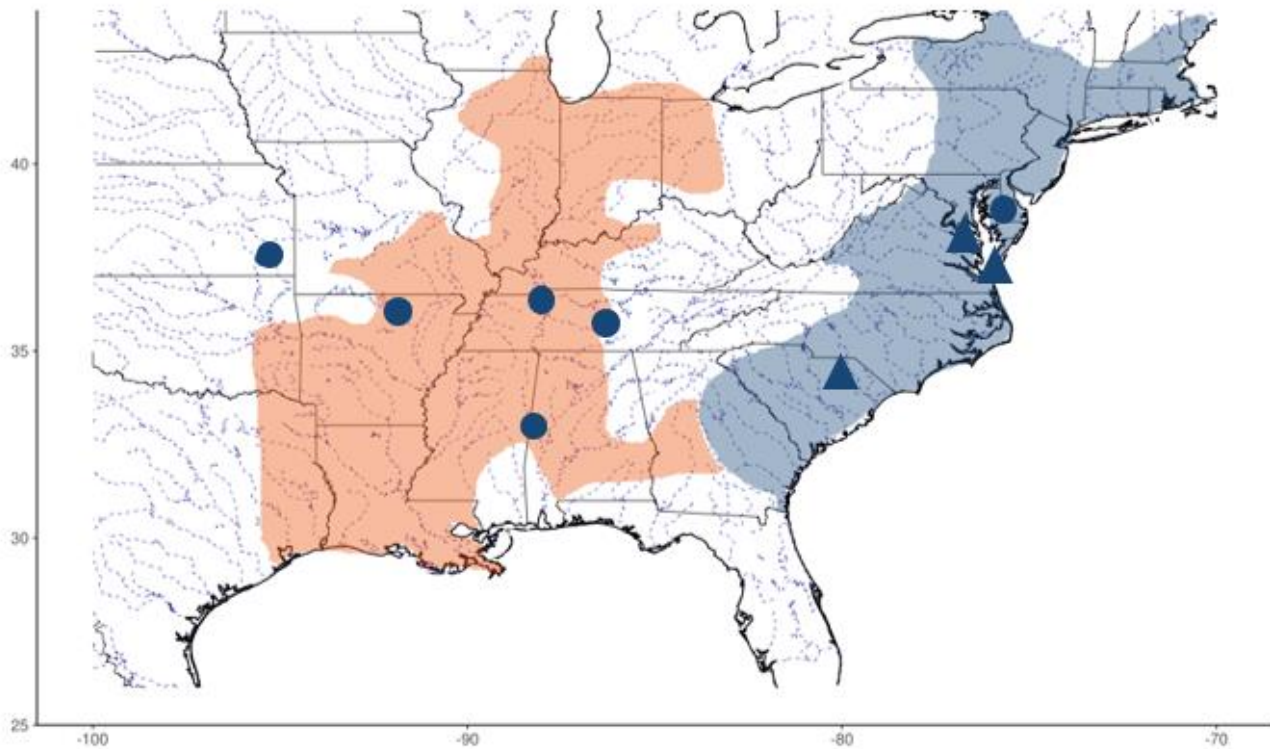
E. oblongus is shown in blue, and E. claviformis is shown in orange. Triangles represent samples collected for this study, and circles represent samples retrieved from GenBank.

METHODS AND MATERIALS

Samples were obtained from three species in the genus *Erimyzon*, three other catostomid fishes (as outgroups), as well as individuals in the genus *Erimyzon* collected in the upper Guadalupe drainage (Table 1). Tissues were stored in 95% ethanol or 20% DMSO buffer (Seutin et al., 1991) at room temperature until DNA extraction. DNA was extracted using a modified Chelex extraction protocol (Estoup et al., 1996). Briefly, tissues were placed in microcentrifuge tubes and submerged in 500 μl of 10% Chelex solution and 28 μl Proteinase K. Samples were vortexed and incubated at 60°C for 40 minutes, then vented and incubated at 95°C for 15 minutes. The supernatant, containing the DNA product, was moved to new tubes for storage and processing.

The mitochondrially-encoded, cytochrome c oxidase subunit 1 gene (CO1) was amplified using four universal fish primers (Ward et al., 2005). Each 30 μl reaction contained 1 X buffer, 1.5 mM $MgCl_2$, 0.20 mM each dNTPs, 0.04% Tween (0.1-1%), 0.25 μM forward and reverse primers each (F_1 and F_2, R_1 and R_2), 0.033 units/μl Taq polymerase and 1.0 μl of DNA template. PCR amplification was run with initial denaturing at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 50-55°C for 1 minute, and elongation at 72°C for 1 minute. A final round of elongation was run at 72°C for 10 minutes.

The nuclear-encoded, interphotoreceptor retinoid-binding protein 2 gene (IRBP2) was amplified using two specific primers designed for catostomid fishes (Chen et al., 2012). Each 20 μl reaction contained 1 X buffer, 2.5 mM $MgCl_2$, 0.40 mM dNTPs each, 0.20 μM IRBP2 forward primer and reverse primer each, 0.05 units/μl Taq polymerase and 2.0 μl of DNA template. PCR amplification was run with initial denaturing at 95°C

TABLE 1.
Identifications of samples used for sequencing, and appropriate primer used per sample,
X indicates successful sequencing of a locus

Sample ID	Family	Genus	Species	Location	CO1	IRBP2
Cca-01	Catostomidae	Carpidoes	carpio		X	
Cco-01	Catostomidae	Catostomus	commersoni		X	X
Eob-01	Catostomidae	Erimyzon	oblongus	Louisiana		
Eob-02	Catostomidae	Erimyzon	oblongus	Illinois		
Eob-03	Catostomidae	Erimyzon	oblongus	Illinois		
Eob-04	Catostomidae	Erimyzon	oblongus	South Carolina	X	X
Eob-05	Catostomidae	Erimyzon	oblongus	Virginia		X
Eob-07	Catostomidae	Erimyzon	oblongus	Virginia	X	X
Eob-09	Catostomidae	Erimyzon	oblongus	Virginia	X	X
Eob-11	Catostomidae	Erimyzon	oblongus	Virginia	X	X
Esu-01	Catostomidae	Erimyzon	sucetta	Wisconsin	X	
Esu-02	Catostomidae	Erimyzon	sucetta	Guadalupe River	X	X
Esu-03	Catostomidae	Erimyzon	sucetta	Guadalupe River	X	X
Esu-04	Catostomidae	Erimyzon	sucetta	Guadalupe River	X	
Esu-09	Catostomidae	Erimyzon	sucetta	East Texas	X	X
Esu-10	Catostomidae	Erimyzon	sucetta	East Texas	X	X
Esu-11	Catostomidae	Erimyzon	sucetta	East Texas	X	X
Esu-12	Catostomidae	Erimyzon	sucetta	East Texas	X	X
Esu-13	Catostomidae	Erimyzon	sucetta	East Texas	X	X
Ete-01	Catostomidae	Erimyzon	tenuis	Louisiana	X	X
Ete-02	Catostomidae	Erimyzon	tenuis	Louisiana	X	X
Mco-01	Catostomidae	Moxostoma	congestum		X	X
Mme-01	Catostomidae	Minytrema	melanops		X	X
Esp-01	Catostomidae	Erimyzon	unknown	Guadalupe River		X
Esp-02	Catostomidae	Erimyzon	unknown	Guadalupe River	X	X
Esp-03	Catostomidae	Erimyzon	unknown	Guadalupe River	X	X
Esp-04	Catostomidae	Erimyzon	unknown	Guadalupe River	X	X
Esp-05	Catostomidae	Erimyzon	unknown	Guadalupe River	X	
Esp-06	Catostomidae	Erimyzon	unknown	Guadalupe River	X	

TABLE 2.
Acquired GenBank samples for CO1 primer.
GenBank samples are associated with a circle
in species range maps.

Genus	Species	Accession No.	Location
Erimyzon	oblongus	AP011228	
Erimyzon	oblongus	HQ579034	Alabama
Erimyzon	oblongus	JN025433	Alabama
Erimyzon	oblongus	JN025434	Arkansas
Erimyzon	oblongus	JN025436	Arkansas
Erimyzon	oblongus	JN025437	Tennessee
Erimyzon	oblongus	JN025438	Tennessee
Erimyzon	oblongus	JN025439	Tennessee
Erimyzon	oblongus	JN025440	Tennessee
Erimyzon	oblongus	JN025441	Tennessee
Erimyzon	oblongus	JN025442	Maryland
Erimyzon	oblongus	KF929862	Maryland
Erimyzon	sucetta	EU524567	Canada
Erimyzon	sucetta	JN025445	South Carolina
Erimyzon	sucetta	JN025446	South Carolina
Erimyzon	sucetta	JN025447	South Carolina
Erimyzon	sucetta	JN025448	South Carolina
Erimyzon	sucetta	KX145352	Canada
Erimyzon	sucetta	KX144986	Canada
Erimyzon	sucetta	KX145318	Canada
Erimyzon	sucetta	KX145453	Canada
Erimyzon	sucetta	KX145104	Canada
Erimyzon	tenuis	JN025449	Florida
Erimyzon	tenuis	JN025450	Alabama
Erimyzon	tenuis	JN025451	Florida
Erimyzon	tenuis	JN025452	Alabama

for 2 minutes, following by 45 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and elongation at 72°C for 2 minutes. A final round of elongation was run at 72°C for 10 minutes. Amplicons were gel extracted and purified using the QIAGEN® QIAquick® Gel Extraction Kit and re-amplified through a second round of PCR as above.

Amplicons were cleaned using the QIAGEN® QIAquick® PCR Purification Kit and sequenced bidirectionally at the Genomic Core Lab at Texas A&M

University – Corpus Christi on an ABI 3730. Twenty-nine samples (Table 1) were sent for sequencing but some samples were not successfully sequenced and subsequently excluded from the study (5 COI, 8 IRBP2; Table 1). Chromatograms were trimmed and aligned using BioEdit (Hall, 1999). To increase the breadth of geographic sampling, additional sequences were downloaded from GenBank (26 COI, 0 IRBP2; Table 2).

Maximum-likelihood analysis were run using RAxML v8.02 (Stamatakis et al., 2014) for each gene using the GTR+GAMMA model. Node support was assessed with 700-1000 bootstrap replicates (Pattengale et al., 2009) for COI and IRBP2 respectively. Bayesian analyses were run using Beast v2.4 (Drummond and Rambaut, 2012). Site mutation models for each gene were determined using jModelTest 2 (Darriba et al., 2012) using decision-theoretic performance-based selection and the HKY+I model (Hasegawa et al., 1985) was selected for both genes. Bayesian runs consisted of 10 million steps, with trees samples every 1000 steps. Adequate run lengths and sample sizes were assessed in Tracer v1.6 (Rambaut et al., 2014) and a strict molecular clock was used. TreeAnnotator v.2.4 (part of Beast) was used to generate a maximum clade credibility tree with the first 10% of samples discarded as burn-in.

RESULTS AND DISCUSSION

Phylogenetic hypotheses generated were similar across methodologies and genes but differed slightly in the arrangement of external nodes between the Bayesian and maximum-likelihood trees. Bayesian trees had better node support compared to the maximum-likelihood trees. The end analysis for CO1 showed five groups (clades shown in Figures 4 and 6). Fewer groups were recovered using the IRBB2 data set (Figure 5 and 7), but less individuals were included due to a lack of sequences on GenBank.

Clade A contained samples of *E. sucetta* from East Texas, Wisconsin, and the Great Lakes Area (Table 1); some GenBank samples came from as far north as Ontario, Canada (Table 2). Individuals sampled on the Guadalupe River were also recovered in Clade A in both the CO1 and IRBP2 trees (Figures 4-7).

Clade B contained samples of *E. sucetta* from South Carolina (B2; Table 1) and *E. oblongus* from South

FIGURE 4.
Phylogenetic relationships of species in the genus *Erimyzon* inferred from
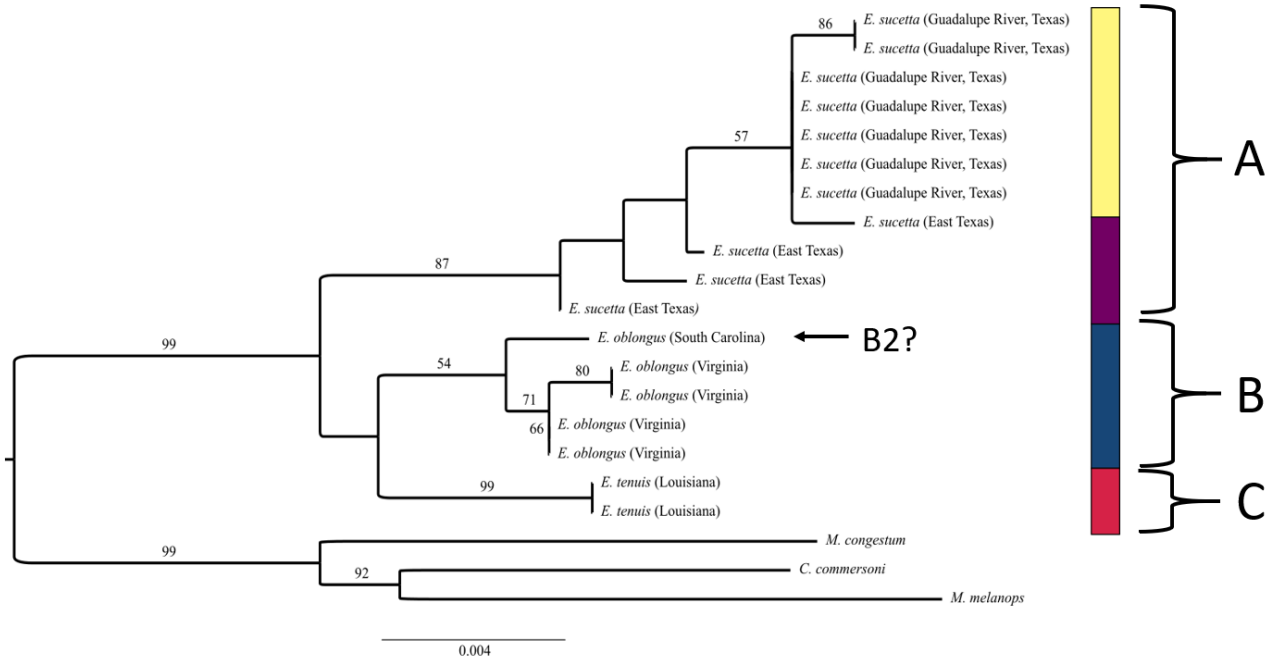mitochondrial-encoded cytochrome c oxidase subunit 1 gene (CO1) (Ward et al., 2005).
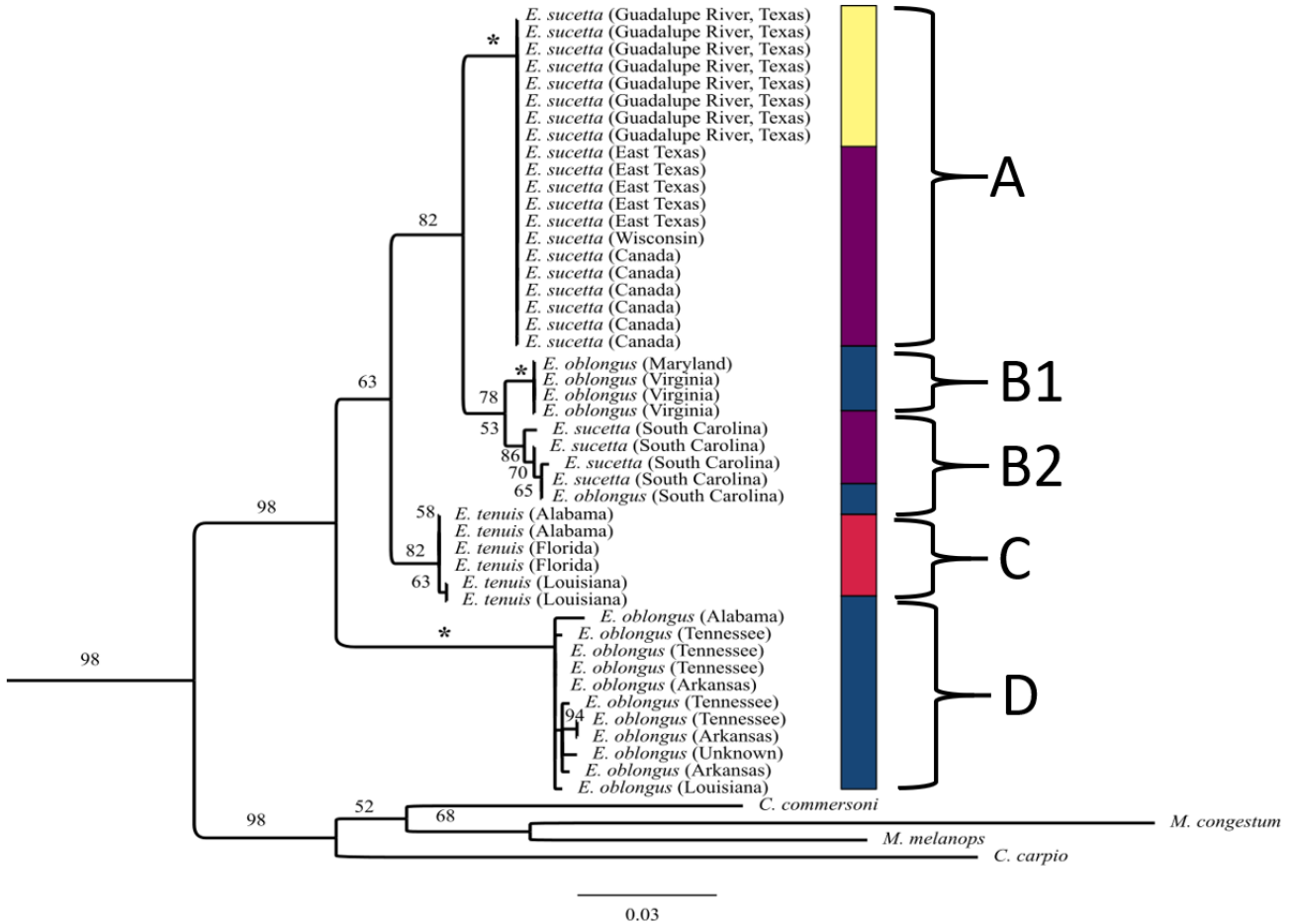
The maximum likelihood approach was implemented in RAxML v8.02 (Stamatakis et al., 2014). Yellow indicates the isolated samples from the Guadalupe River, Texas; purple indicates *E. sucetta* samples; blue indicates *E. oblongus* samples; and pink indicates *E. tenuis* samples.

Carolina (B2; Table 1), Maryland (B1; Table 1), and Virginia (B1; Table 1). Clade B1 contained only samples identified as *E. oblongus*, while B2 contained individuals identified as both *E. oblongus* and *E. sucetta*. This result suggests that either (1) the *E. oblongus* sample in clade B2 is misidentified and should be *E. sucetta*; (2) *E. sucetta* samples in clade B2 are misidentified and should be *E. oblongus*; or (3) *E. oblongus* samples in clade B1 and the *E. oblongus* sample in B2 are misidentified and should be *E. sucetta*. Scenario 1 would result in distinct B1 and B2 clades, representing *E. oblongus* and *E. sucetta*, respectively. Scenario 2 would result in a clade consisting of just *E. oblongus*, indicating that Clade B1

and B2 represent distinct evolutionary lineages within *E. oblongus* (i.e. populations or incipient species). Scenario 3 would have the same result as Scenario 2, but all samples would be *E. sucetta*. If Clade B or Clade B2 are *E. sucetta* then that would mean the species is present in both Clade B and Clade A. Because *E. sucetta* was originally described from specimens captured in the Carolinas, Clade A would represent a fifth species in the genus that needs to be described. Less samples were included in the IRBP2 trees (no appropriate sequences available from GenBank) making it impossible to assess for the presence of Clade B1 and B2 (Figures 5 and 7) though the South Carolina sample was sister to all

FIGURE 5.
Phylogenetic relationships of species in the genus *Erimyzon* inferred from
nuclear-encoded interphotoreceptor retinoid-binding protein (IRBP2) (Chen and Mayden, 2012).

The maximum likelihood approach was implemented in RAxML v8.02 (Stamatakis et al., 2014). Yellow indicates the isolated samples from the Guadalupe River, Texas; purple indicates *E. sucetta* samples; blue indicates *E. oblongus* samples; and pink indicates *E. tenuis* samples.

other *E. oblongus* samples in Clade B, suggesting that with sufficient sampling the same pattern might be recovered with nuclear DNA. Chen and Mayden (2012) described samples of *E. oblongus* as non-monophyletic, and though geographic locations of those samples were not reported, the result is consistent with results obtained here.

Clade C contained *E. tenuis* samples from Louisiana (Table 1); with GenBank samples from Florida and Alabama (Table 2). The recovery of a monophyletic *E. tenuis* in this study is inconsistent with the results of Chen and Mayden (2012), where the species was not monophyletic. Compared to the other three described species, *E. tenuis* has a relatively narrow distribution, found between Pensacola, Florida and far eastern Louisiana (Page and Burr, 2010). Sequences in this study came from individuals sampled across this range, so the discrepancy between studies is unlikely due to the geographic breadth of samples in this study. Unfortunately, no information on sample locations was

provided in Chen and Mayden (2012). There are several disjunct northern populations of *E. tenuis* in Alabama (Page and Burr, 2010) and individuals sampled from those locations should be included in future studies.

Clade D, shown in Figures 4 and 6, contained samples identified in GenBank as *E. oblongus* from Tennessee, Louisiana, Alabama, and Arkansas (Table 1). However, due to the collection location of the samples in Clade D and the fact that field-identified *E. oblongus* are in Clade B (Figure 3), we suggest that Clade D is likely *E. claviformis*. The geographic distributions of *E. oblongus* and *E. claviformis* overlap slightly and the species are very similar, differing only in the number of dorsal rays (Page and Burr, 2010), so misidentification is possible.

FIGURE 6.
Phylogenetic relationships of species in the genus *Erimyzon* inferred from mitochondrial-encoded cytochrome c oxidase subunit 1 gene (CO1) (Ward et al., 2005).
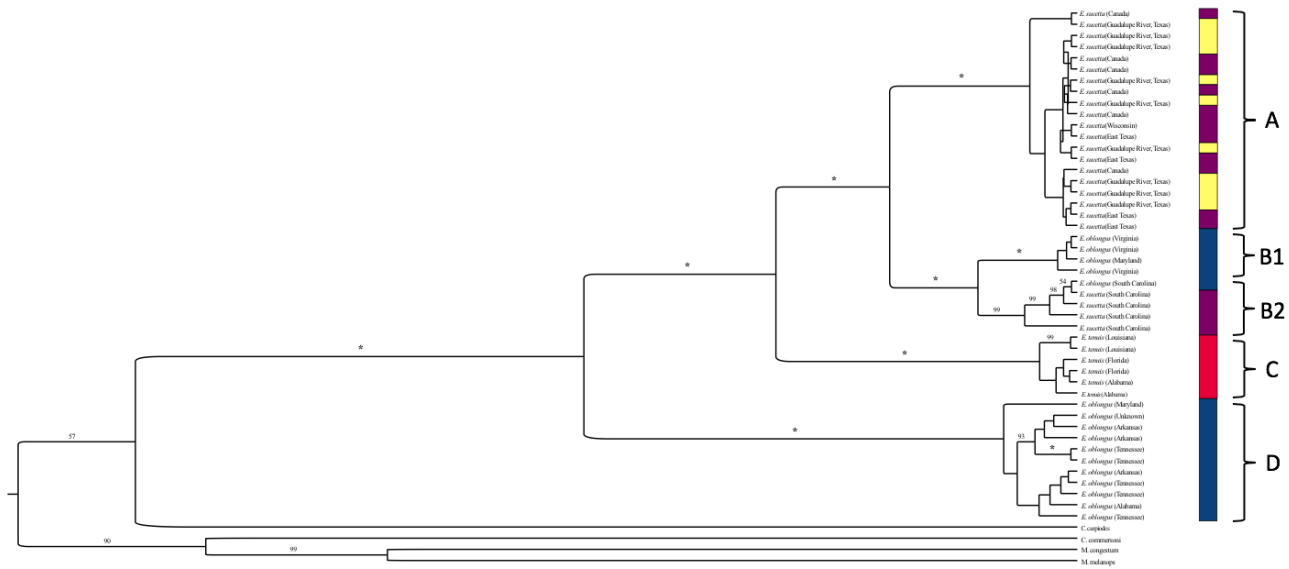
The Bayesian inference approach was implemented in Beast v2.4 (Drummond and Rambaut, 2012). Yellow indicates the isolated samples from the Guadalupe River, Texas; purple indicates *E. sucetta* samples; blue indicates *E. oblongus samples*; and pink indicates *E. tenuis* samples.

CONCLUSION

CO1 and IRBP2 sequences were used to determine the phylogenetic relationships of species within the genus *Erimyzon* and clarify the relationship of the samples from a small, isolated population in the Guadalupe River. The samples from the Guadalupe River were grouped with *E. sucetta* collected throughout the western part of its described range. However, South Carolina samples also identified as *E. sucetta* appeared elsewhere in the tree, sister to *E. oblongus* collected in the Mid-Atlantic. These inconsistencies suggest that there may be an additional species of *Erimyzon* beyond the four that are currently described. Additional molecular and morphological analyses incorporating further specimens collected from all four described species throughout their described ranges will be required to further clarify species designations and ranges.

ACKNOWLEDGMENTS

FIGURE 7.
Phylogenetic relationships of species in the genus *Erimyzon* inferred from
nuclear-encoded interphotoreceptor retinoid-binding protein (IRBP2) (Chen and Mayden, 2012).
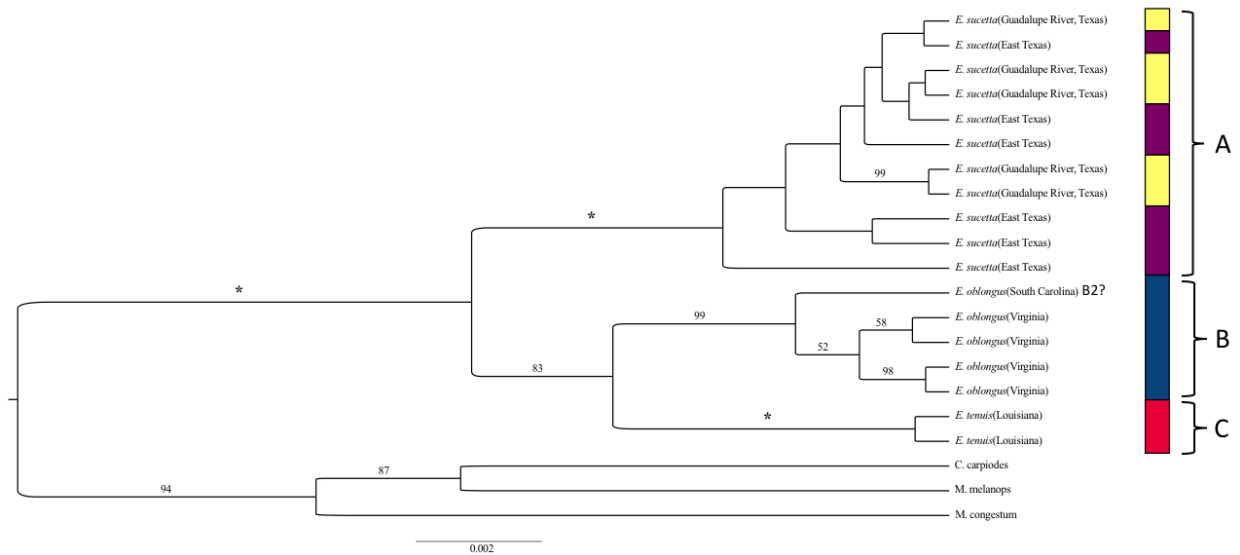
The Bayesian inference approach was implemented in Beast v2.4 (Drummond and Rambaut, 2012). Yellow indicates the isolated samples from the Guadalupe River, Texas; purple indicates *E. sucetta* samples; blue indicates *E. oblongus* samples; and pink indicates *E. tenuis* samples.

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AUTONOMOUS MAPPING OF CONTROLLED GAME ENVIRONMENT MATRIX UTILIZING POINT COMPARISON OF REAL AND IDEAL IMAGE REGIONS

by DOINA MORALES

ABSTRACT

Autonomous Navigation is paramount in robotics applications. An agent's ability to perform complex tasks in the absence of human supervision, enables surveying and interaction with environments that pose a high security risk or are not feasible for human exploration. A vision implementation is proposed to enhance an agent's traversal of the environment by matching the images collected from a camera configuration to a two-dimensional topological map. Utilizing the focal point of the camera as the origin, the images retrieved are matched to the expected pattern by performing a two-dimensional spatial transformation of the real coordinate space against the ideal topological frame of reference. This spatial transformation enables the autonomous agent to path plan accordingly. Once the match has been made within a low boundary of error, the agent will proceed to navigate the environment and detect predefined objects of interest. By mapping its environment, the agent has the freedom of following a first-detected, first-collected logic, without interfering with its ability to return to the starting point. In this research, novel implementation of topological mapping in conjunction with image processing methods has been achieved. The system successfully detects tokens as objects of interest in a predetermined game environment, employing a simplified version of the A* algorithm and probabilistically tying the agent's orientation to a priori knowledge of the game environment.

MENTOR
Dr. Ruby Mehrubeoglu
Department of Engineering

INTRODUCTION

Autonomous robotics places the emphasis on path-planning to navigate the proposed terrain. Path-planning can be achieved utilizing cameras, either in a monocular or stereo-vision set up, in a static or rotating camera configuration. By capturing frames through the camera and comparing areas of interest among them, the autonomous agent may determine its location and orientation in the environment and to plan its next steps accordingly. Due to the availability of small computers with strong processing power that can serve as the centralized control unit of the agent, powerful image recognition tools may be employed via complex libraries, such as OpenCv, to solve challenging machine vision problems. Setting up parameters and mapping does not have to be as rigid as in the past, and the agent does not necessarily have to follow a pre-ordained

sequence of motions to achieve successful autonomy. It can instead traverse a mapped environment keeping track of its location utilizing two to three distinguishable visual anchors. As described by Secuianu *et al.* [1], the Raspberry Pi computer board can perform advanced image-based mapping activities, employing vision methods available on the OpenCv library. The researchers successfully implemented a target detection and target locking algorithm for an autonomous service robot designed to water house plants. Throughout this research effort, the link between topological and metric map traversal was explored and emphasis was placed on a computationally effective way of traversing a game environment, where a high level of *a priori* information is known and flat topography is expected. In the work presented, high efficiency was achieved in mapping a known game environment at low computational expense, utilizing image processing techniques to detect items of interests, and gain information about the orientation of the agent.

POPULAR MAPPING METHODOLOGY SUITABLE FOR AUTONOMOUS AGENTS

Inverse perspective mapping, block matching, pure topological mapping, and visual odometry are well-known mapping techniques applied in path finding of autonomous agents. This section summarizes these methods from the literature as they relate to the described project.

Inverse Perspective Mapping

To remove the effect that perspective has on an image acquired from the camera, Bertozzi *et al.* propose a geometrical transformation known as inverse perspective mapping [2]. Considering the skewed angle at which a camera collects visual information in pixels and the top-down view of the same visual information, the geometric transformation known as Inverse Perspective Mapping (IPM) takes the 3D world space, W(x, y, z), and converts it into a 2D image space, I(u, v), on which the 3D image is projected. To map from 3D to 2D, it is necessary to know the camera view point constrained by length, depth, and height C(l, d, h), as well as the optical axis or viewing direction of the camera which is formed by two angles: the angle of the camera to the x-direction of the top down x-y plane

view (γ), and the angle formed between the x-axis and t h e optical axis, or the downward angling of the camera (θ). Furthermore, knowledge of the camera aperture and resolution are also necessary. Camera aperture is defined by the ratio of the focal length to the diameter of the entrance pupil. Image spatial resolution is the amount of detail displayed in an image and is measured in megapixels, namely the total area of pixels in the image in millions.

First, to simplify the operation, the environment is considered devoid of elevation with z(u, v) = 0, where z is the change in elevation or the z axis in 3D space. Values (x, y) are treated as functions of the location, (u, v), in the image, since in monocular applications, depth perception must be reconstructed. Thus, considering the height of the camera, the angle formed by the camera's trajectory to the ground, and the distance from the viewed area to the ground below the camera, the following trigonometric relationships are obtained where n is the total number of pixels considered:

$$x(u,v) = h * \cot\left[(\theta-\alpha) + u\,\frac{2\alpha}{n-1}\right] * \cos\left[(\gamma-\alpha)+v\,\frac{2\alpha}{n-1}\right] + l \quad (1)$$

$$y(u,v) = h * \cot\left[(\theta-\alpha) + u\,\frac{2\alpha}{n-1}\right] * \cos\left[(\gamma-\alpha)+v\,\frac{2\alpha}{n-1}\right] + d \quad (2)$$

The u, v values in the flattened image are thus extracted by:

$$u(x,y,0) = \frac{\tan^{-1}\left\{\frac{h*\sin\left[\tan^{-1}\left(\frac{y-d}{x-l}\right)\right]}{y-d}\right\} - (\theta-\alpha)}{\frac{2\alpha}{n-1}} \quad (3)$$

$$v(x,y,0) = \frac{\tan^{-1}\left(\frac{y-d}{x-l}\right) - (\gamma-\alpha)}{\frac{2\alpha}{n-1}} \quad (4)$$

This technique is powerful for mapping a course to very specific and detailed *a priori* information of the environment to be navigated since it removes the necessity of searching for predefined reference points. This technique keeps track of the angle of error, α, introduced by rotation of the robot as image frames are captured, as well as, taking into consideration the angle to the camera from the point of interest, θ, and the angle from the camera to the point of interest in the frame, γ.

Block Matching Technique

In the work of Yaakob *et al.* [3], motion estimation is utilized to achieve image matches among neighboring frames of video, where the motion vector is the

displacement between a block in the current frame and one in a corresponding frame. The frame is segmented into blocks of n^2 size, and p is defined as the search parameter. Generally, the larger the motion, the larger the search parameter, and the smaller the value of n the higher the computational complexity. Maximum correlation is sought through the application of a matching criterion of which Peak Signal to Noise Ratio (PSNR) is the most popular.

First the Mean Square Error (MSE) is measured where C is the current frame, R the frame C is compared to, and (i,j) are horizontal and vertical image dimensions. To further what is given in Yaakob *et. al* [3], the formula should be altered accordingly for frames that are rectangular rather than square.

$$MSE = \frac{1}{n^2}\sum_{i=0}^{n-1}\sum_{j=0}^{n-1}(C_{i,j} - R_{i,j})^2 \qquad (5)$$

Afterward, the MSE is plugged into the PSNR formula:

$$PSNR = 10\log_{10}\left(\frac{255^2}{MSE}\right) \qquad (6)$$

A high PSNR and low MSE is indicative of a good optical flow and a low error margin.

Pure Topological Mapping

Topological mapping focuses on the connectivity of an explored environment, rather than the metric accuracy of the map. A topological algorithm can be devised as a decision tree [4] where the agent reassesses the most efficient path at each step of the process. Discretized locations on a map are represented by vertices, while paths between points of interest are indicated by edges. The model described by the researchers [4] used a heuristic that places higher weight on exploring areas of the environment with reduced connectivity, which must be managed to avoid exponential growth of the exploration tree.

As illustrated by Amigoni *et al.* [5], geometric maps represent a composition of a grid map and a topological map. A topological map limits knowledge of the environment to identification of places of interest and the connections between them. Relevant information at a node could be the type of terrain to expect, a collected image while at the node, or alternatively an action to take once a node is reached.

Bloechliger *et al.* discuss the development of a mapping/navigation system optimized to work with the SLAM method outlined below [6]. This article emphasizes the benefit of segmenting the agent's environment into topological areas of interest to which a vertex is dedicated. The researchers investigate 3D image data and grow volumetric representations of the navigated environment.

Visual Odometry

Similar to wheel odometry, which estimates the location/motion of an agent based on an integration of its wheel rotations over time, visual odometry estimates location based on the visual changes introduced by motion into the camera feed. A feature-based or hybrid matching technique is utilized in the analysis of consecutive frames. As outlined by Scaramuzza *et al.*, since the path is computed incrementally because mapping is conducted strictly at the local level, error/drift reduction methods become necessary and can generally be alleviated by coupling with other motion tracking or orientation detecting sensors [7], [8]. The first part of the tutorial considers the relative advantages of employing a monocular or stereo camera system. Camera modeling and calibration are described geometrically from a linear, catabiotic, and spherical standpoint. The tutorial concludes that, based on prior research, a 2D to 2D method of feature correspondence is more effective for a monocular scheme than a 3D to 3D method because point triangulation is avoided. The authors emphasize that lines are harder to track than point features and that outlier removal is crucial in tracking motion using VO.

HEURISTIC PATH PLANNING ALGORITHMS FOR AUTONOMOUS AGENTS

Multiple heuristic path planning algorithms have been successfully implemented for autonomous agents. In this section, two algorithms related to this project are described from the literature.

A* Algorithm for Path Planning

The A* algorithm is well suited for problems where a local optimum is sufficient. In the case of a robotics competition in a controlled game environment, incremental decisions based on local maximums

are more appropriate, specifically when resolving a detection and collection optimization problem. The application of the A* algorithm for path-planning on a grid map is described by Duchon *et al.* [9]. The researchers found that the JPS (A*) algorithm finds a viable path in shortest time, yet the path is longer than the optimal one. This may work best when seeking a path in shortest time is of paramount importance. If computation time is not significant and length of path is paramount, then the Basic Theta* algorithm is more effective.

Potential Function Based-RRT (P-RRT*)*

The Rapidly Expanding Random Trees algorithm described by Qureshi *et al.* [10] generates random samples and connects them together to form a map. A tree graph is crated with every node connected to a single parent. The start location is seeded, and the algorithm grows from that location in all directions. The algorithm seeks a point in free space and then attempts to connect this random configuration to the nearest node in the tree. If the distance between the two nodes, delta, is less than its predefined value, the points are connected. If distance, delta, is greater than the same predefined value, the algorithm steps back in its iteration and conducts a new random number generation. Alternatively, a two-tree procedure may be employed where one tree begins at the seeded location and another at the goal state/goal location.

SENSORS USED AND THEIR PROPERTIES

The sensors used to solve the mapping problem are the Raspberry Pi Camera V 2.1 and the Berry IMUv2, an inertial measurement unit utilized to sense movement and orientation. The Raspberry Pi Camera has an aperture (*f*): 1.8 and a horizontal resolution of 1080p, 2592 × 1944 pixels for still pictures and supports 1080p30, 720p60 and 640x480p60/90, standard resolution for video recording.
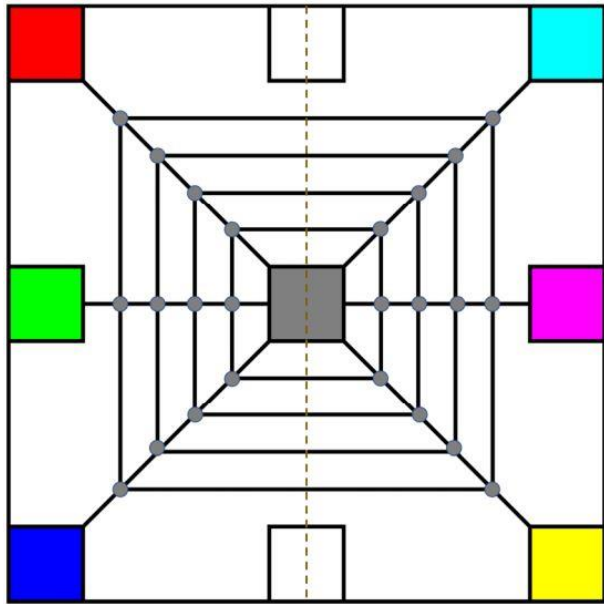
PROBLEM FORMULATION

The agent is to traverse a predetermined course (Figure 1) efficiently and to detect an object of interest (coin-like tokens), maintaining awareness of its own position and orientation in the mapped environment. Since the navigation problem is to be solved with *a priori* knowledge of the test environment, the problem of mapping the real environment to the ideal map while tracking the agent's location will be solved through topological mapping and a graph navigation algorithm, in conjunction with image processing techniques. As it scans the environment, the agent will be challenged to match its predefined topological map to the real map it is acquiring from the environment. Since the game field contains six solid color square-shaped drop off locations along the edges, it is proposed that the agent will utilize color detection to match the colors of the squares it recognizes in the environment to the *a priori* directional information it has of the mapped environment. To navigate the game environment, the agent will maintain information of its location by performing a probabilistic calculation of its orientation, location square color, and time. Map north will be assigned to the directional queues first observed by the autonomous agent.

The Naïve-Bayse equation is routinely used for probability measurement is robotics and will be augmented to fit the needs of this color-coded game environment.
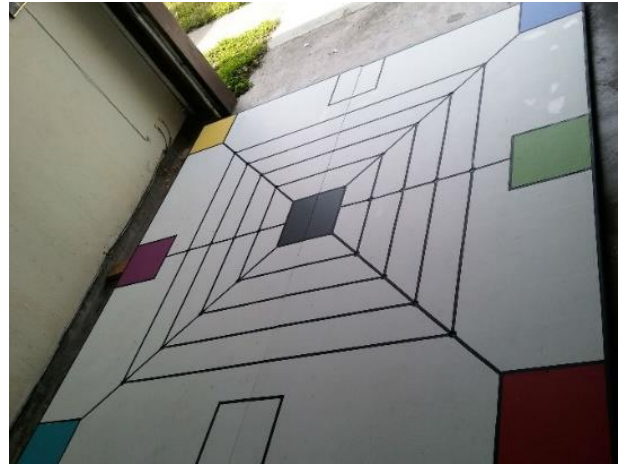
$$Square\left(\frac{Color}{Orientation\ Match}\right) = \frac{Square\left(\frac{Orientation\ Match}{Color}\right)*Squre(Color)}{Square(Orientation\ Match)} \quad (7)$$

The probability of a good color match within assigned respective color thresholds will be based on data acquired through mock field tests. Orientation probability will be based on the cardinal directions assigned to the field and the colors associated with these directions as seen in Figure1.

FIGURE 1.
A Priori Field Information.
(Source: 2018 IEEE Region 5 Robotics Competition)

FIGURE 2.
Mock Test Field Specifications
(Source: IEEE Region 5 Robotics Competition)

The agent will detect tokens on the mapped environment through the application of Hough circles, which will be detected in the environment and matched to the mapped environment. Each time it detects a Hough circle, the algorithm will compute its location in the mapped environment against the expected location of the object of interest and begin tracking the object by detecting the displacement of the object of interest in the image space between acquired frames.

Each time a token is detected and collected, the token total is decremented so that once all tokens have been detected/collected, the agent can begin the drop-off routine. Once the robot has completed the drop off routine of its algorithm, the agent will again determine its location in the environment utilizing color squares and travel back to the start position.

TABLE 1.
Agent Orientation Probability

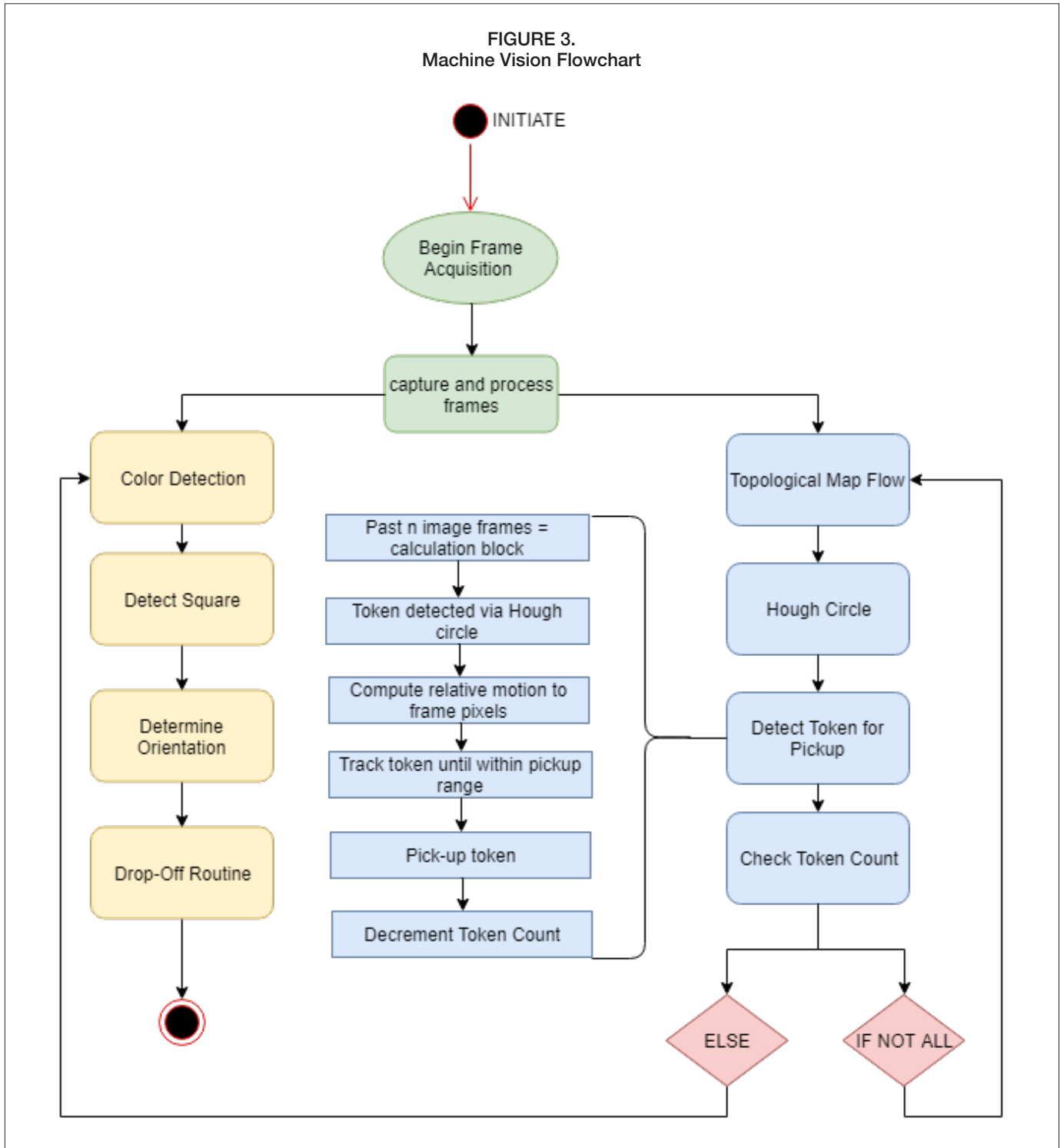| Color of Detected Square	Possible Orientation	Possible Rotation Angle
Red + Cyan	North	0, 180
Red	North	
Red + Green	West + North	
Green	West	90, 270
Green + Blue	West + South	
Blue	South	
Blue +Yellow	South	0, 180
Yellow	South	
Yellow + Magenta	East + South	
Magenta	East	90, 270
Magenta +Cyan	East + North	

The map will be treated as a topological environment with the origin at the starting location of the autonomous agent.

FLOWCHART

FIGURE 3.
Machine Vision Flowchart

ALGORITHMS

The following algorithms were devised to accomplish the tasks required for successful mapped environment traversal and to provide all the visual support functionality that the mapping algorithm required.

Algorithm 1: Square Recognition and Color Detection

Input: Frames, *f*, from Raspberry Pi Camera

Output: Boolean square detected and value (direction)

1: while f !=0 do

1:   Enhance image brightness level and contrast

2:   Extract RGB Channels

3:   Apply to-zero inverse thresholding (OpenCV lib.)

4: end while

5: return value (direction)

Algorithm 2: Hough Circle Detection

Input: Frames, f, from Raspberry Pi Camera, location

Output: Return Status detected/not detected

1: for f < = 5 do

1:   Enhance image brightness level and contrast

2:   Convert to Grayscale

3:   Detect Contours

4:   Detect Circles

4: end for

5: return location

Algorithm 3: Image Acquisition

Input: Frames, f, from Raspberry Pi Camera

Output: Return Images from Camera as sequence

1: for f < = 5 do

2:   Discard first few frames for camera adjustment/warmup

3:   Rapidly capture sequence of images, framerate = 30

4:   Collect Images in Array

5:   Return array of images

6:   Detect Circles

7: end for

8: return frames

Algorithm 4: Topological Mapping

Input: Total Node Count (n)

Output: Path Choice

1: Instance of the Graph Class is created

2: Nodes and Vertices are added according to the Topological Map of the Tokens

3: while tokens collected<n do

4:   Path choice according to shortest path to most distant node

5:   Detect Circles

6:   Collect tokens along the way and increment count collected

7: end while

8: Determine current orientation via color detection of nearest square
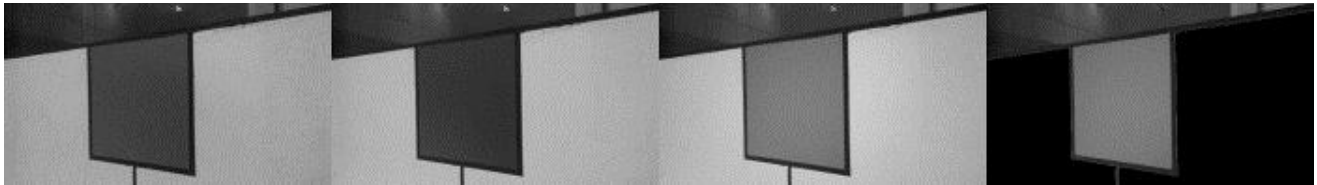
RESULTS

The following tests were conducted to enhance and perfect the algorithms described above. As the figures below show, the algorithms were implemented effectively and produced quality results.

Color Square Detection

FIGURE 4.
Original Images

FIGURE 5.
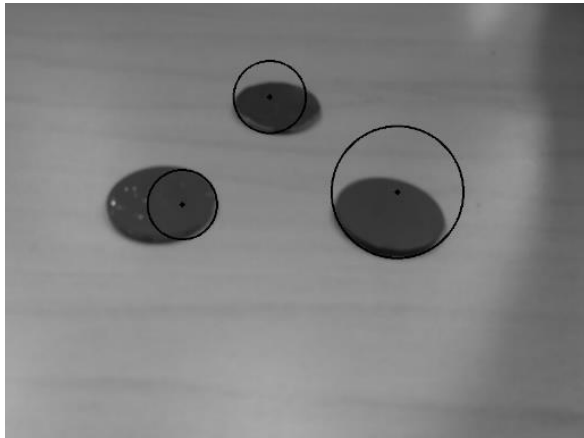**Red Square Color Channel to Zero Inverse Segmentation**

FIGURE 6.
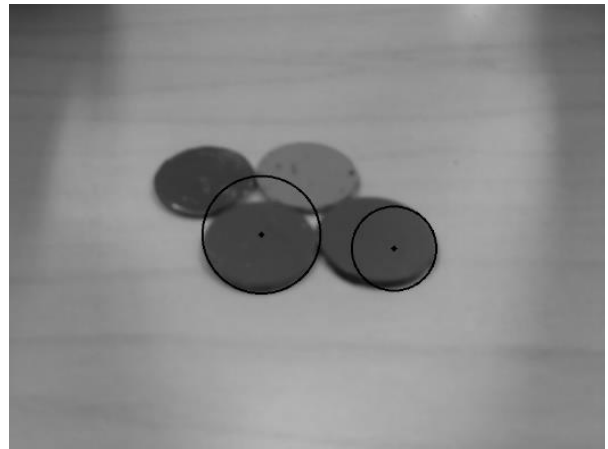**Green Square Color Channel to Zero Inverse Segmentation**

FIGURE 7.
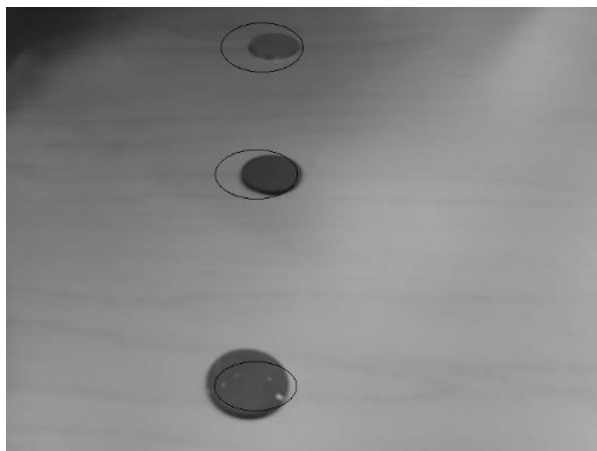**Yellow Square Color Channel to Zero Inverse Segmentation**

FIGURE 8.
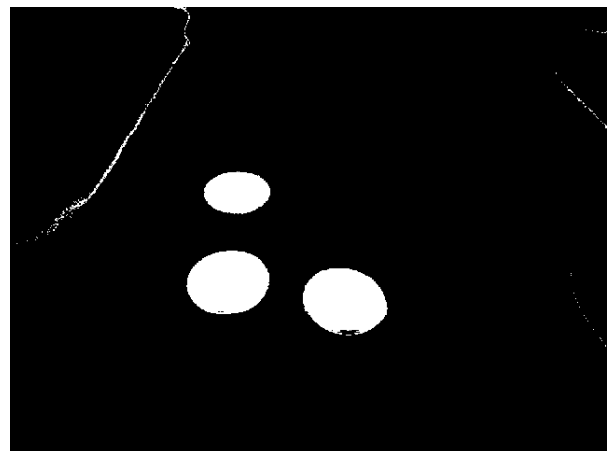Token Detection Hough Circles

FIGURE 9.
Token Detection Error Margin Test

FIGURE 10.
Hough Ellipse Depth Detection

FIGURE 11.
Masking for Token Detection and Color Recognition

Token Detection Tests

Various tests were employed to detect the tokens. The OpenCv library was used for optimized token detection. It was determined that having the camera 10 inches above the ground at an angle of 45 degrees was optimal for token detection for the existing hardware parameters.

Testing Topological Mapping

The agent detects the tokens in the mapped environment according to the logic of the mapping algorithm. Figures 10 and 11 show the original topological map. The two maps are topologically equivalent since they maintain the logical interconnections of the nodes according to the order and assignment of the vertices. Figures 12 to 15 show the traversal according to the shortest path to the most distant node method. The theory that the shortest path to the most distant node would be the most effective traversal was tested via multiple runs of the mapping algorithm. The lower the node repetition in the traversal, the more successful the graph logic. The most effective traversal was from 'Start' to node 'K', from node 'K' to node 'H' and again from 'Start' to 'L', then from 'L' to 'E'.

FIGURE 12.
Logical Conversion of Tokens into Nodes
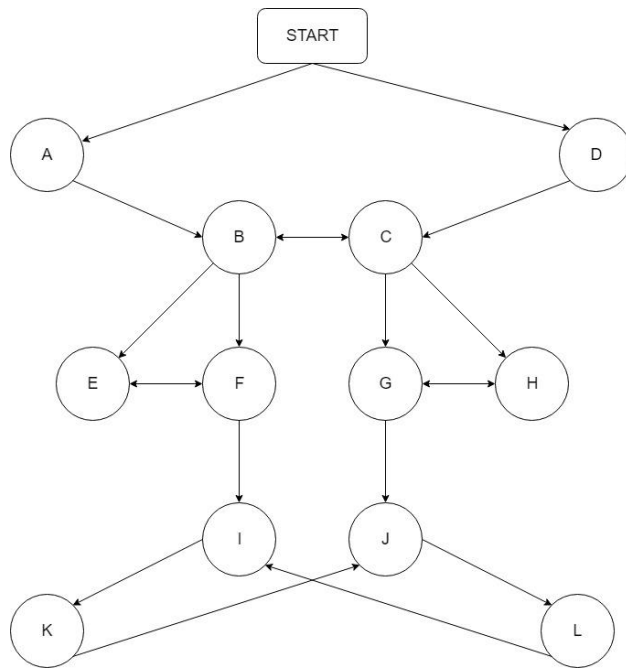
FIGURE 13.
Original Topological Map 1
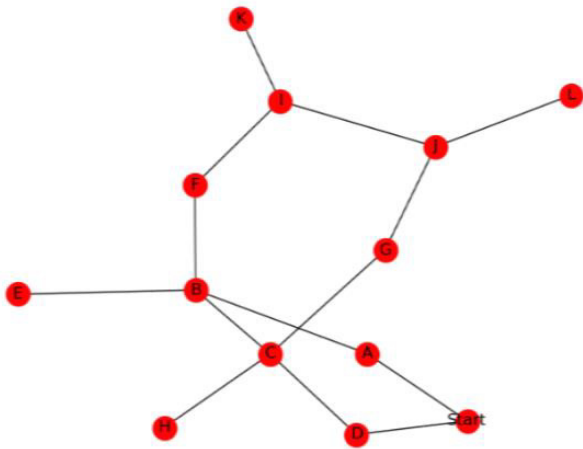
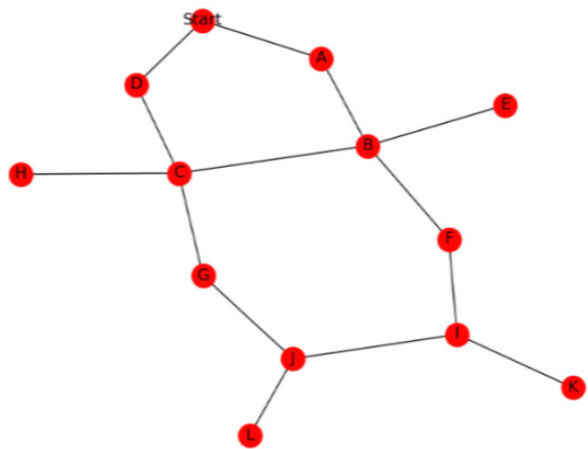FIGURE 14.
Original Topological Map 1a

FIGURE 15.
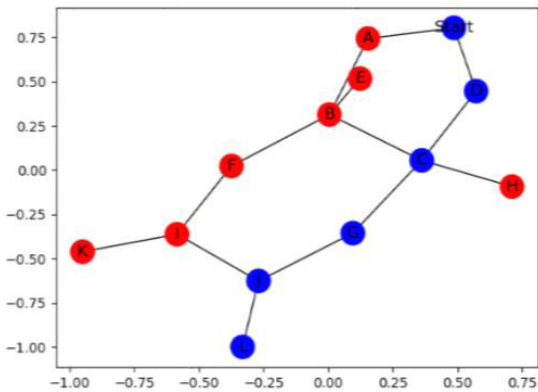Map Traversal from 'Start' to 'L'

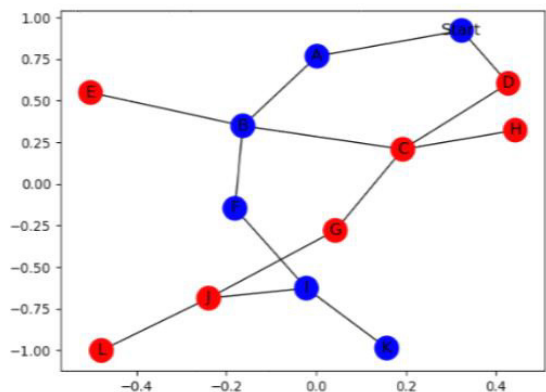FIGURE 16.
2 Map Traversal from 'Start' to 'K'

FIGURE 17.
Map Traversal 'K' to 'H'
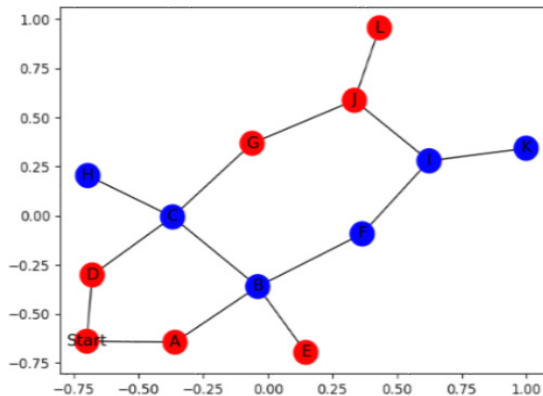
FIGURE 18.
Map Traversal 'L' to 'E'
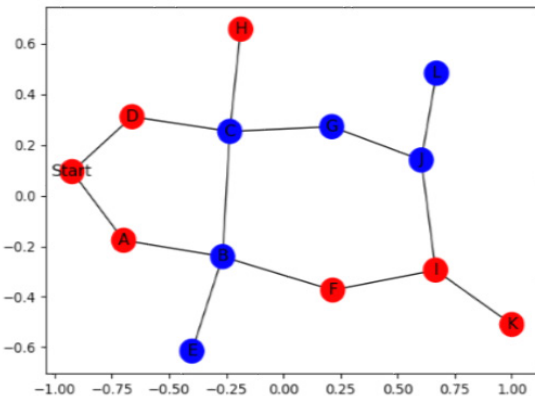
CONCLUSION

The proposed mapping system attempts to minimize path-planning error in a controlled game environment for a low computational power approach. While mapping the environment hinges on research in visual odometry, artificially intelligent algorithms, image processing and optical low strategies, the described method is novel in its implementation. Mapping is implemented via a graph with a simplified version of the traditional A* algorithm. To our knowledge, the use of color squares as markers for cardinal directions of the ideal map based on *a priori* information of the game environment, is unique to this implementation. Though the implementation is very specific to the environment it was implemented for, it has the potential of being adapted to more complex navigation problems.

FUTURE WORK

The next research step is to integrate the vision, topological mapping, and orientation logic system into a Robotics Operating System (ROS) that can perform basic seek, find, retrieve, and return operations in a wide variety of environments and for a wide array of implementations. The ROS will achieve this proposed goal by taking assigned task as input and sweeping a given environment as instructed. The focus will be on lowering computational needs via clever algorithmic manipulation at compilation and execution time. Good error management techniques will be employed in the event of an unsuccessful sweep.

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NITRIFYING AND DENITRIFYING REACTIONS OF PLEUROTUS OSTREATUS, COPRINUS COMATUS, AND CORIOLOPSIS BYRSINA WITHIN AN AMMONIUM CHLORIDE SPIKED MODEL MARICULTURE GROWTH SYSTEM

by CODY PORTER

MENTORS
Dr. Xavier Gonzales

Professor of XXX
Department of Life Sciences
College of XXX

MENTORS
Dr. Anthony Siccardi

Professor of XXX
Department of Life Sciences
College of XXX

ABSTRACT

It is now abundantly clear in 2018 that the method's we have chosen to use for the management of the immense amounts of waste produced by every action we perform throughout the day are causing the one and only place in the universe we are able currently of inhabiting to become toxic, unstable and ultimately dangerous to live. To our credit there has been an effort by some to begin mitigating these disastrous conditions through the development of new innovations which allow us to decrease the amount of damage done from disturbing the natural ecosystems that persist around us and from the introduction of pollution in general. One area of waste management that is increasingly employed as a sustainable alternative is known as bioremediation which typically has employed plants and bacteria to perform transformation reactions to turn pollution into reusable resources. In this study we investigate mycofiltration as a bioremediation application involving the resiliency of fungi to perform infinitely diverse filtration methods to address the wastewater produced within a recirculating aquaculture farm as they grow marine organisms to be sold

at marketplaces which substitutes the need for overfishing of the naturally occurring communities of shrimp and fish. It was discovered here that fungi have the ability to perform nitrifying and denitrifying reactions at a rate suitable to reduce toxic nitrogenous compounds within recirculating aquaculture systems to levels low enough to allow for continuous use of a finite volume of water during production which reduces the discharge of wastewater into the environment and the need for continuously pumping in of new water which increases the budget for this form of farming and increases the negative impact we have on the local environment. Results from this preliminary study are promising as we saw the most efficient fungal species, Coprinus comatus, able to reduce ammonia-nitrogen and nitrite-nitrogen to a concentration of 0.453 mg/L which is acceptable for beneficial growth of most organisms currently produced. The other two species, Coriolopsis byrsina and Pleurotus ostreatus, were not far behind as they reduced levels of these toxic nitrogenous compounds to concentrations of 0.5926 mg/L and 0.547453 mg/L, respectively. While this is the greatest concern when operating an aquaculture farm, there are other concerns that must be addressed but were outside the scope of this study yet are likely susceptible to remediation using fungi which may ultimately prove to be a better answer to the filtration needs of aquaculture farms than the traditional bacterial based filtration systems employed within the current aquaculture systems of today.

INTRODUCTION

Mycofiltration, using mycelial infused substrates to filter environmentally toxic materials and microorganisms from aquatic environments, has only in recent years begun to be seriously considered (Stamets, 2005; Akpaja and Olorunfemi, 2014). Hyphae - the vegetative structure of the mushroom - join into a network (*mycelium*) which is the key structure in performing the biodegradation of substrates and nutrient absorption which allows the organism to acquire nutrients they require from its environment. Bioremediation mechanisms have been elucidated in studies on terrestrial sites and to a lesser degree in aquatic environments which have been contaminated with polyaromatic hydrocarbons, organophosphates, pentachlorophenols, heavy metals, synthetic dyes and surfactants, explosive munitions, pharmaceuticals, biological and radioactive waste, and excessive unabsorbed nutrients (fertilizers and feed products) (Steffen et al. 2002; Rasak and Mujic, 2007;

Dighton et al., 2008; Magan et al. 2010; Vasco et al., 2011; Molla and Fakhru'l-Razi, 2012; Tortella et al., 2013; Akpaja and Olorunfemi, 2014; Singh et al., 2014; Kuka et al., 2016;). Fungi maintain a dominate role in the environment as the most diverse and efficient degrader of organic and inorganic debris, this ability is supported by their ability to produce a wide array of non-specific extracellular enzymes and organic acids which imbibe fungi with the capacity for adaptive modifications to their metabolic systems which allow them an increased ability for exposure to a diverse array of substrates and facilitate their movement through environments composed of recalcitrant toxic compounds which would rapidly prove as prohibitive toxic barriers and even completely uninhabitable real estate for any and all other organisms besides the strongest and adaptively diverse members of the fungal kingdom (Vasco et al., 2011). Substrates in which fungi are cultured have been shown to induce the production of biochemically specific as well as generally consummate external digestive enzymes necessary for the bioaugmentation of structural and chemical compounds present in the environment which are required to reach the nutrients necessary to their development and it is this versatility which serves to accentuate the ability of most fungi to adapt and compete in unique environmental systems (Tortella et al., 2013; Steffen et al., 2002). So far studies have limited their investigations into projects which have their focus and practice centered around the separation of certain target enzymes & precursor molecules which are known to specialize in their affinity for certain contaminating compounds or which function dominantly within a particular chemical pathway where certain compounds they are attempting to target have been shown to be introduced as they move through the environment and interact with bioorganic milieu of the planet. Such compounds are being targeted due to either their slightly beneficial interactions with the local flora and fauna or more commonly to be removed as they are known to produce environmental conditions unsuitable to humans and antagonistic adverse agriculturally conditions. Most contaminant studies focusing on this area of research have developed their procedures in a manner which individually tests within a laboratory controlled setting which is lacking in the real world competition and variable physiochemical environments involved during the *in-situ* application

typically involved during the mycoremediation of these contaminants and can be seen to suffer from the anemic amount of real world large scale exhibitions of the truly unique and powerful way in which fungi can be applied for the reversal of many toxic spills, superfund sites, landfills, wastewater treatment plants, nuclear waste fallout and large scale human accidents which have left portions of our planet deadly for all life and which can then spreads into the parts of the world in which we all live our daily lives (Tortella et al., 2013). Data gleaned from these studies, however, does further enumerate and enrich the overall understanding of the necessary components involved in the infinitely diverse metabolic enzymatically driven biological processes within the environmental amalgamations of organisms which function in concert with organic acids and other chemical compounds to produce the successful machination of biodegradation for nutrient acquisition through which these fungal organisms conduct their idiosyncratic bioremediation propensities.

Extracellular enzymes secreted by all fungi contain and disseminate the reactive chemical energy which can be tapped by these organisms throughout their life cycle which allow fungi to tunnel through compact organic material while also contributing greatly to their enhanced bioremediation capabilities which were first demonstrated by Dr. Paul Stamets in his 2005 study where he demonstrated one used of these fungi to control contamination of oyster beds from bacterial pathogens from creek runoff into the ocean. Within this surfeit of enzymes, are a few highlights as bioremediation focal points from further investigation, such as manganese dependent-peroxidase (MnP), lignin peroxidase (LiP), laccase, cellulase, lipase, protease, lysozymes, and amylase (Ruggaber and Talley, 2006). Enzymes which catalyze the degradation of the substrate, through the reduction of the activation energy or energy needed to kickstart a chemical reaction, are integral to producing bioremediation results which can be increasingly more economical and sustainably more time conscious such that they encourage a process which can more completely degrade targeted toxic compounds from the environment of interest. In their 2006 study, Ruggaber and Talley demonstrate the complimentary enzymatic actions of MnP, LiP, and laccase are responsible for the degradation and transformation of the overwhelming

majority of xenobiotic substances encountered throughout the world. These compounds have been discovered to be produced in high concentrations by those fungal organisms classified within phylum Basidiomycota and in lower relative concentration by those classified within the phylum Ascomycota. (Verma et al., 2010). Lipase, protease, lysozymes, and amylase were shown also by Ruggaber and Talley (2006) to produce the antimicrobial functions exhibited in one of the first applications of mycoremediation on agricultural land for the control of *E. coli* (Taylor and Stamets, 2014). Each of these enzymes are inducible which means that the substrate and environmental conditions in which the fungi are growing has a direct deciding influence on whether certain enzymes are produced and in what concentration (Terashita et al., 1995).

Substrates are of pivotal concern when deciding physical and chemical characteristics necessary for addressing the application of fungi for bioremediation, particularly when it comes down to the cost since this component of any mycoremediation application must be provided in great quantities. Taylor and Stamets discovered in their 2014 study that different combinations of alder wood chips and straw created varying levels of hydraulic loading, porosity, and mycelium surface area available to perform bioremediation functions. Bertrand et al., investigated this further in their 2015 study which enumerated a myriad of various isoforms of laccase which they produced during the growth of *Trametes versicolor* which were induced by it growing on oak sawdust with various amendments of white wheat flour and bran flake medium, each of these isoforms have shown oxidative capabilities for a variety of xenobiotics. Supplementation of malt extract media with Mn(II) from MnCl2 during the initial stages of vegetative growth was proven by Steffen et al., during a 2002 study which induced an increased MnP production by the fungal colony throughout the rest of the organism's life cycle as a result of their exposure to these manganese-based compounds during early life development. Native microbial populations are also a factor when considering the ideal media as it has the potential to hinder a study, as was observed by Stamets et al. in their 2013 study which attempted to quantify the elimination of *E. coli* and thermotolerant coliforms but was plagued by false positives and were later

identified to be a result of contamination by the bacteria *Raoultella planticola* (native flora of straw) during the Coliscan MF assay. However, using a substrate which can encourage bacterial growth when challenged by particularly resilient mixtures of xenobiotics which may be more structurally or chemically complex, the synergistic degradative activity of mycelial associated bacterial biofilms can be utilized to fully degrade the compounds (Banitz et al., 2013; Cheng et al., 2016). Furthermore, a substrate that is comprised of a highly variable environment of xenobiotic concentrations and microbial communities exhibits the characteristics that are encountered when performing bioremediation in situ and as such must be the research environment in which these studies take place when bioremediation techniques are vetted.

During the course of this study we attempted to discover which of two basidiomycetes and one ascomycete fungal species could perform the most efficiently within one of four separate but similar model marine aquaculture systems which were dominantly used for the investigation of different methods of filtration which can be employed during the growth of mostly white shrimp in a system which uses the locally sourced seawater from its ideally situated location on the Gulf of Mexico coastline in Corpus Christi, Texas. We attempted to focus on the most important filtration aspect of a no exchange closed loop recirculating growth system which does not discharge wastewater but involves the nitrification and denitrification of nitrogenous waste compounds which are produced as ammonium or ammonia during the cultivation of stock organisms taking place *in-situ* inside a model hypersaline growth environment of warm gulf waters which is pumped into 8 growth tanks attached to a sump which makes up one of four individual systems which are setup in a slightly contained portable building which has been modified for temperature, light, oxygen exchange and water delivery for similar studies and which is will allow for a controlled exposure of our MarineMycoFiltersTM to all manner of real world microbes or other externally sourced antagonists which may challenge the effectiveness of these filters applied in such an application.

Gaps exist in the literature for such investigations which attempted to demonstrate the different applications of fungi contaminants in a diverse mixture of compounds and associated with competitor microorganisms (Tortella et al., 2013). There is also insufficient knowledge regarding the fungal species which would be distinctly suited to each application of mycoremediation or mycofiltration due to the profile of enzymes that the species produces, environment it can tolerate, and substrate on which it prefers to grow (Ruggaber and Talley, 2006). Additional research must also be performed to catalog the ideal substrate composition and potential supplementation by coenzymes and cofactors which will maximize the effectiveness of the bioremediation process overall (Steffen et al., 2002). Eliminating these gaps in the knowledge base will increase our ability to fashion an economically sustainable system with a high specificity for a desired xenobiotic consortium (Pointing, 2001).

In the scope of economic sustainability, it is necessary to reduce the steps between initial contamination and final remediation of the native flora and fauna (Stamets, La Dena., 2012). The filtered seawater from the Gulf of Mexico this study will utilize will be spiked with ~19.36 mg/L of ammonium chloride to better demonstrate the ability of each MarineMycoFilter™ to adequately perform the nitrifying or denitrifying reactions under hypersaline conditions of 30ppt necessary to adequately remove or reduce the levels of toxic nitrogenous compounds contained within such a system during the production of stock organisms by aquaculture farms. Filtration will take place in four configurations on three substrate mixtures containing three separate fungal species and one uninoculated control mixture which will contribute to the foundation of information available for reference during the implementation of mycofiltration.

HYPOTHESIS

$H_0 1$: There will be no significant reduction of un-ionized ammonia-nitrogen by basidiomycetes or ascomycete strains as a result of mycofiltration treatment.

$H_a 1$: Un-Ionized ammonia-nitrogen will be reduced by the basidiomycetes or ascomycetes to levels below those set by the Texas Commission for Environmental Quality prescribed limits.

$H_0 2$: Mycofiltration will not be effective using basidiomycetes or ascomycetes because the reactions do not happen fast enough or at a high enough rate to keep up with the production of the nitrogenous waste products produced by such a model marine aquaculture system.

$H_a 2$: Mycofiltration will prove to be an effective form of filtration using basidiomycetes or ascomycetes and should be further investigated to determine their levels of tolerance for the different stressors experienced within such systems and to discover the most efficient member of either species for such an application.

METHODS

Fungal Species

Fungal species were ordered from Fungi Perfecti LLC and American Type Culture Collection based on the preliminary advantages discovered in previous research that was found in the literature evidence and extolled their bioremediatory capabilities. Chosen were three species of fungi: Pleurotus ostreatus (Fungi Perfecti LLC), Coriolopsis byrsina (ATCC MYA-4557), and Coprinus comatus (Fungi Perfecti LLC), the first two basidiomycetes and the last was an exceptionally unique ascomycete.

Fungal Cultures

Fungal cultures were maintained on five separate types of media, three of which were designed to maintain healthy vigorous cultures and the other two were designed to condition the cultures to the future substrate. Supplementation was done with 3 grams of equal parts pasteurized compost, sawdust, casing, and straw which were used in the preparation of each MarineMycoFilter™. Potato-dextrose agar (PDA) and supplemented PDA were used to ensure healthy vigorous growth of cultures. Boyd & Kohlmeyer media (B&K) [glucose 10g; yeast extract 1g; peptone 2g; agar-agar 18g; seawater(15ppt) 1000ml] and supplemented B&K were used for culture conditioning and testing of species for their tolerance to increasing salt concentrations. Filtered seawater from the Gulf of Mexico was chlorine sterilized and combined with the other ingredients in the B & K media to stretch these tolerances and determine some expectations to aid the collection of data and configuration of the testing environment.

Malt extract agar (MEA) was also prepared to ensure continued viability and healthy vigorous growth of all fungal cultures. All media was sterilized at 121°C at 15psi for 30 minutes and kept at 4°C; media was brought back to room temperature before inoculation. Aseptic techniques were used to transfer excised hyphae to sterile media for incubation. Incubation took place at 25-29°C in darkness for 7-12 days or until media was thoroughly colonized by mycelium at which time they were sub-cultured to maintain viability and sterility.

Grain Spawn

Organic rye berries (1500g) were used for mycelial expansion inside autoclavable 1-gallon glass jars with lids fitted with 0.3micron filters to increase the rate of inoculation in the bulk substrate. Moisture content was brought up to 49-54% with DI water (1000ml), grain was then supplemented with gypsum (4g) and alkalinity [$CaCO_3$] (1.33g) and sterilized at 121°C, 15psi for 90 minutes in Tuttnauer automatic autoclave. Jars cooled inside a Forma Scientific biological safety cabinet for 8 hours and were then inoculated or placed in 4°C refrigerated storage to be used within 24 hours. Inoculation was performed through aseptic transfer of a colonized cube of supplemented media on a scalpel into the grain and lids were sealed with parafilm and autoclavable tape. Shaking and tapping the jars on a heavily padded office chair thoroughly mixed hyphae throughout the grain to initiate a higher rate of inoculation throughout the entire jar. On day 3 and 5 of incubation the grain was again thoroughly mixed as before, then were left to fully colonize every kernel of grain. Various time frames were seen for full colonization with P. ostreatus having the most vigorous growth followed closely by C. byrsina and C. comatus.

Aquaculture Growth Tank Systems

Each of the 8-Polystyrene tanks (18" x 26" x 15" x 14") were filled with ~28.36 gallons, a traditional sump (21" x 29" x 16") was filled with ~42.18 gallons, the new mycofilter sump (24" x 29" x 16") and with the water retained in the transfer pipes (18" x 72" x 16.8") which was ~0.54 gallons, each testing environment contained a total of ~269.63 gallons of filtered chlorine sterilized seawater pumped from the Laguna Madre in Corpus Christi, Texas per growth system which exists at a salt concentration of ~32 ppt. The measurements

notated above refer to rectangular tanks of dimensions length x width x height x water level and cylindrical pipes of dimensions diameter x length x water level, respectively. Four independent systems consisting of eight tanks, one sump, and enough PVC/rubber piping to transfer the waste water from the growth tanks to the filter and back again to the growth tanks at a rate of 146 gals/day and distributed into the sumps evenly through a trickle fed system before being recirculated back into each of the growth tanks. Each tank was equally aerated to increase the dissolved oxygen (DO) to a level (~5.75 mg/L) near the Texas Commission of Environmental Quality permit standard (6.0 mg/L) set for the Taiwan Shrimp Village in Arroyo, Texas which is located 165 miles south on the gulf coastline.

Substrate Inoculation

Bulk substrate used to create the mycofilters consisted of casing, compost, alder wood sawdust, 2-3in. alder woodchips, and wheat straw. Compost and casing material was delivered pre- pasteurized but was additionally autoclaved along with the sawdust and wood chips in a Tuttnauer automatic autoclave for 120 minutes at 121°C at 15psi before preparation of mycofilters. Before autoclaving experimental and control substrates were saturated and allowed to dry until ~60% moisture content was achieved. For each mycofilters, half of the bulk substrate was used to fill the buckets creating the base of the mycofilters. While the top half was placed in an autoclavable bag, sterilized as before, and placed along with the substrate containing buckets in storage at -4°C for 14-21 days until grain spawn was fully colonized. Before inoculation, bulk substrate was thawed and stabilized at room temperature (25°C).

To begin inoculation, wheat straw was pasteurized in a hot water bath at 74°C for 45 mins inside of a silk pillow case then spread out on sterile bench top to drain and cool for another 45 mins. Jars of grain spawn were shaken and tapped on the heavily padded office chair to break-up spawn creating a greater surface area of hyphae available to be spread through the bulk substrate. Grain was added to the autoclave bags containing the top portion of the bulk substrate and the

bag was shaken to thoroughly spread the mycelia-laden grain throughout the bulk substrate mixture. The

wheat straw was then added to the top of the substrate in the bucket and then covered with the inoculated bulk substrate mixture inside the autoclave bag. A lid was placed on to the bucket and buckets were placed into an incubator (25°C) for 3 days to facilitate the acclimation of hyphae from grain to substrate. On day 4, exponential growth was observed causing a generation of heat from the metabolic breakdown of the organic substrates through enzymatic digestion, substrate was moved from incubator to the lab where the temperature was cooler (21.67°C) until day 11. On day 12 the mycofilters substrate temperature stabilized enough to be placed back into incubator to continue mycelial running. After approximately 28 days full colonization of bulk substrate was achieved, mycofilters were then moved into the aquaculture room at Texas Agri-life to begin the mycofiltration process.

Introducing Ammonium Chloride Spike

Ammonium Chloride was added to each of the 8-tanks in the system at a total rate of 19.36 mg/L or in increments of 41.173g per tank in accordance with the amount a typical high intensity shrimp farm is predicted to produce in a twenty-four-hour period on the higher end for an evenly distributed concentration. Care was taken to introduce an even concentration of in each of the 8- tanks at the furthest point from the drain plug in each tank and each system was allowed to mix for a thirty-minute increment before the first sample was taken and followed by sampling every two to two and a half hours during the twenty-four hour test. Once ammonium chloride had thoroughly mixed throughout the entire system a timer was started and fifteen samples were taken over a twenty-four-hour period with some interpolation of data for times where the prohibitive access to the lab did not allow for direct sampling. This was repeated four different times for each of the four separate 8-tank growth systems which was then followed by the statistical analysis of data for the further determination of efficacy of the experimental MarineMycoFilter™.

Mycofiltration

Mycofilters were first submerged in sterilized distilled water and left to drain for fifteen minutes to ameliorate any physical variability of the filter media which would cause an uneven flow of water through the filter.

Concentration of ammonia-nitrogen in the water was tested before submersion of MarineMycoFilter™ (Table 4) and the effluent was also tested for the export of potential contaminants contained within the filters (Table 4). Bio-beads were then removed from each sump and mycofilters were inserted in their place, water was then trickle fed evenly across the top of the substrate. Enzymatic degradation of contaminants took place as the water traveled through the filter and filtrate was released from the base of the filter through ~1,500 holes of 1.5mm diameter at the base of the bucket.

Water Quality Testing

Baseline water quality analysis was performed on each of the four growth tank systems to determine the concentration of ammonium-nitrogen, nitrite-nitrogen and levels of other physiochemical parameters in the effluent water after a week of full efficient operation (Table 2). Water quality testing was then performed in two stages, the water that was discharged from the 8-tank growth system into the mycofilters was collected by grab sampling to be tested for the concentration of ammonia-nitrogen and second stage tested the water draining from the base of the mycofilters to determine the concentration of remaining ammonia-nitrogen, concentration of nitrite-nitrogen converted and levels of the other physiochemical growth parameter (Table 1,2). Each round of testing of influent and effluent was performed in the manner laid out in Ttable 1 from the study by Samocha et al., in 2004 and the permit issued to the Taiwan Shrimp Village Association Inc with a modification to standard testing methods which were replaced with HACH methodology.

Data Analysis

Quantitative data for nitrification, denitrification, and the other physiochemical growth parameters and where subjected to a one-way ANOVA test and student t-test to test for variance in efficiency of each species to perform nitrifying and denitrifying reactions in these model marine aquaculture systems. (Table 8, 9; Figure 4, 5). However, it must be said at the time of the submission of this article all the data presented here is based on theoretical extrapolation from known values of ammonia-nitrogen reduction which was to be the expected outcome from this study had the lab had not experienced multiple natural extreme weather events which resulted in the failure of already well used equipment. Further investigation is still being pursued to elucidated and contribute to the further knowledgebase for during the *in-situ* applications of mycofiltration techniques under extreme environmental conditions seen in industrial waste.

Contamination Control

Throughout this study, sporulation of the mushroom species being tested was a primary concern to avoid the contamination of the laboratory environment. Species chosen for testing were researched for their potential for human mycotoxicity and revealed no particular danger to humans. Mushrooms are known to produce billions of spores which are light enough to be distributed great distances by air. All initial subcultures on growth media were performed inside a biological vent hood equipped with a HEPA filter. During further experimental applications, all media producing mushroom sporulation were contained within autoclavable polypropylene bags. Passage of water from MarineMycoFilter™ to the drain collection basin will not necessitate additional control procedures as spores will be trapped in the aqueous solution restricting their movement. These methods will be sufficient to restrict escape of spores into lab environment. After inoculation, all bench tops were wiped down and sterilized with 10% bleach solution.

RESULTS

TABLE 1.
Water-quality parameters measured in influent and effluent study of two shrimp farms in South Texas and analytical methods used (Samocha et. al. 2004). *Methodology of APHA et al. (1992). Refractometer

Parameter	Analytical method
Dissolved oxygen (DO, mg · L^{-1})	Polarographic membrane electrode – Standard Method 4500-O G*
Hydrogen-ion concentration (pH, SU)	Electrometer method (pH meter) – Standard Method 4500-H1 B*
Ammonia-nitrogen (NH$_3$-N, mg · L^{-1})	Distillation/nesslerization – HACH Method 8038
Nitrate-nitrogen (NO$_3$-N, mg · L^{-1})	Cadmium reduction method – HACH method 8171
Salinity (g · L^{-1})	Refractometer

TABLE 2.
Effluent Limitations and Monitoring Requirements for Taiwan Shrimp Village, Arroyo City, Texas, based on permit, Issued by the Texas Commission of Environmental Quality (Samocha et. al. 2004).

Parameters	Daily Mean	Daily min.	Daily max.	Single Grab	Frequency and sample type
Flow (m^3 · day^{-1}) [MGD]	378500 [100]	N/A	180	N/A	1/day estimate
pH (-log[H$^+$])	N/A	6	9	N/A	1/day, grab
DO (mg · L^{-1})	6	N/A	N/A	N/A	1/day, 3 samples avg.
NH$_3$-N (mg · L^{-1})	1	N/A	2	3	3/week composite

TABLE 3.
Baseline Water Quality Daily Mean at Peak Efficiency of Traditional Biological Filter After a Month of Bacterial Growth.

Parameters	System 1	System 2	System 3	System 4
Temperature (°C)	27.4	27.2	27.6	28
pH (-log[H$^+$])	7.86	7.74	7.79	7.79
Salinity (g · L^{-1})	32	31.9	31.5	31.5
NH$_3$-N (mg · L^{-1})	13.05	13.05	13.01	13.08
NO$_3$-N (mg · L^{-1})	6.00	6.00	5.92	6.014
Flow (m3 · day^{-1})	3.785	3.785	3.785	3.785
DO (mg · L^{-1})	5.77	5.92	5.7	5.76

TABLE 4.
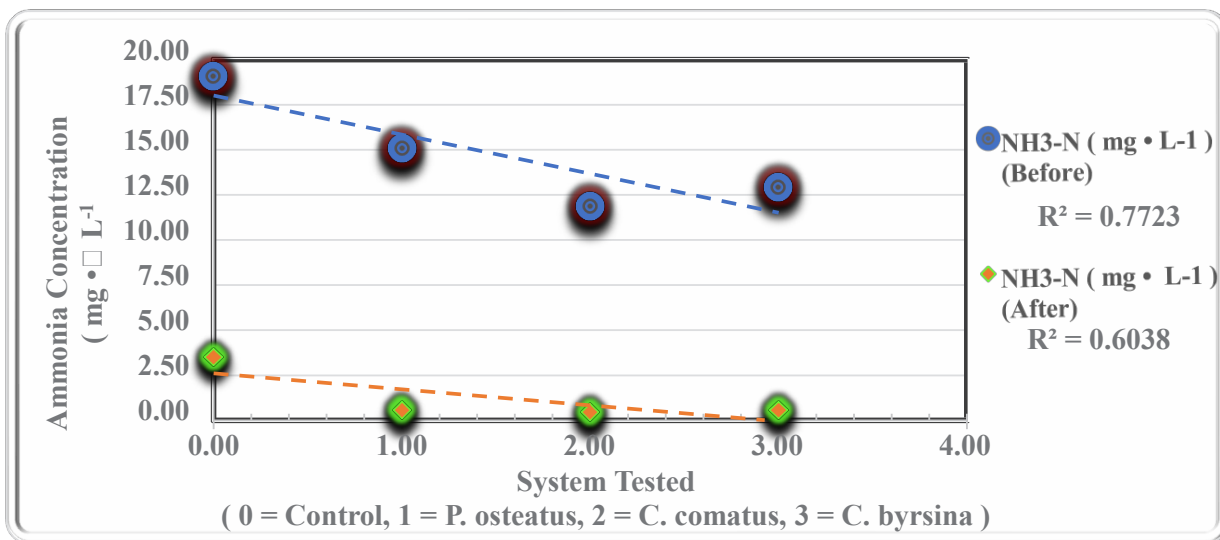**Average Water Quality Results During Timed Testing of Mycofilter Effluent for Pleurotus ostreatus.**

| Parameters	C	1	2	3
Temp (°C)	28	29	28	28
pH (-log[H$^+$])	7.16	7.19	7.72	7.07
Salinity (ppt)	21.2	13.7	11.1	17.125
NH$_{25}$-N (mg · L^{-1})	19.0905905	15.10851	11.9003	12.941471
NO$_3$-N (mg · L^{-1})	4.283292	4.148343	3.26747	3.5533398
Flow (m^3 · day^{-1})	23.075495	23.0755	23.0755	23.075495
Hydraulic Loading (GPD ▯ ft^2 ▯ ft-^{3})	0.79	0.62	0.22	0.34
DO (mg · L^{-1})	4.7875	4.7875	4.365	4.5525

TABLE 5.
Average Water Quality Results After Timed Testing
of Mycofilter Effluent for Coriolopsis byrsina.

Parameters	C	1	2	3
Temp (°C)	28	29	28	28
pH (-log[H^+])	7.16	7.19	7.72	7.07
Salinity (ppt)	21.2	13.7	11.1	17.125
NH_3-N (mg · L^{-1}	19.0905905	15.10851	11.9003	12.941471
NO_3-N (mg · L^{-1})	4.283292	4.148343	3.26747	3.5533398
Flow (m^3 · day^{-1})	23.075495	23.0755	23.0755	23.075495
Hydraulic Loading (GPD ▢ ft^2 ▢ ft-	0.79	0.62	0.22	0.34
³) DO (mg · L^{-1})	4.7875	4.7875	4.365	4.5525

FIGURE 1.
Average Water Quality Results During Timed Testing of Mycofilter Effluent for Coprinus comatus.

Legend:
● NH3-N (mg • L-1) (Before) $R^2 = 0.7723$
◆ NH3-N (mg • L-1) (After) $R^2 = 0.6038$

Y-axis: Ammonia Concentration (mg • ▢ L^{-1})
X-axis: System Tested (0 = Control, 1 = P. osteatus, 2 = C. comatus, 3 = C. byrsina)

FIGURE 2.
Average Water Quality Results During Timed Testing of Mycofilter Effluent for Control.
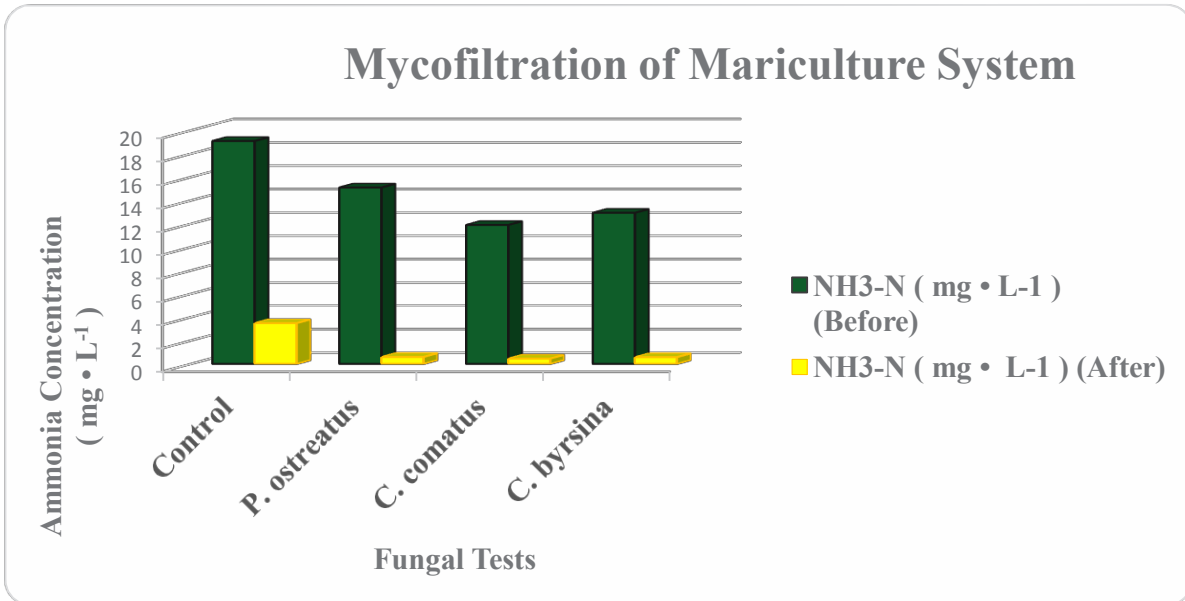
FIGURE 3.
Starting Relative Ammonia Concentration. Average Water Quality Values
Measured in The Pre-Soak Water Used to Initiate Each Filter Before Time Trials Began.
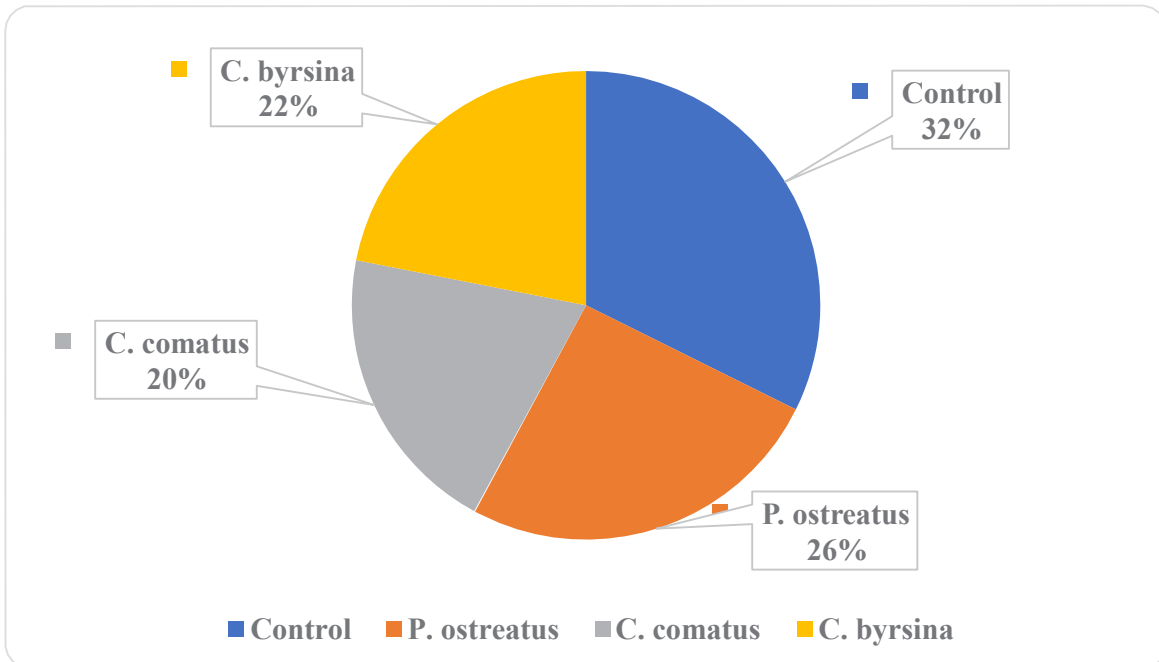
FIGURE 4.
Final Relative Ammonia Concentration. Average Water Quality Values
Measured in The Pre-Soak Water Used to Initiate Each Filter Before Time Trials Began.
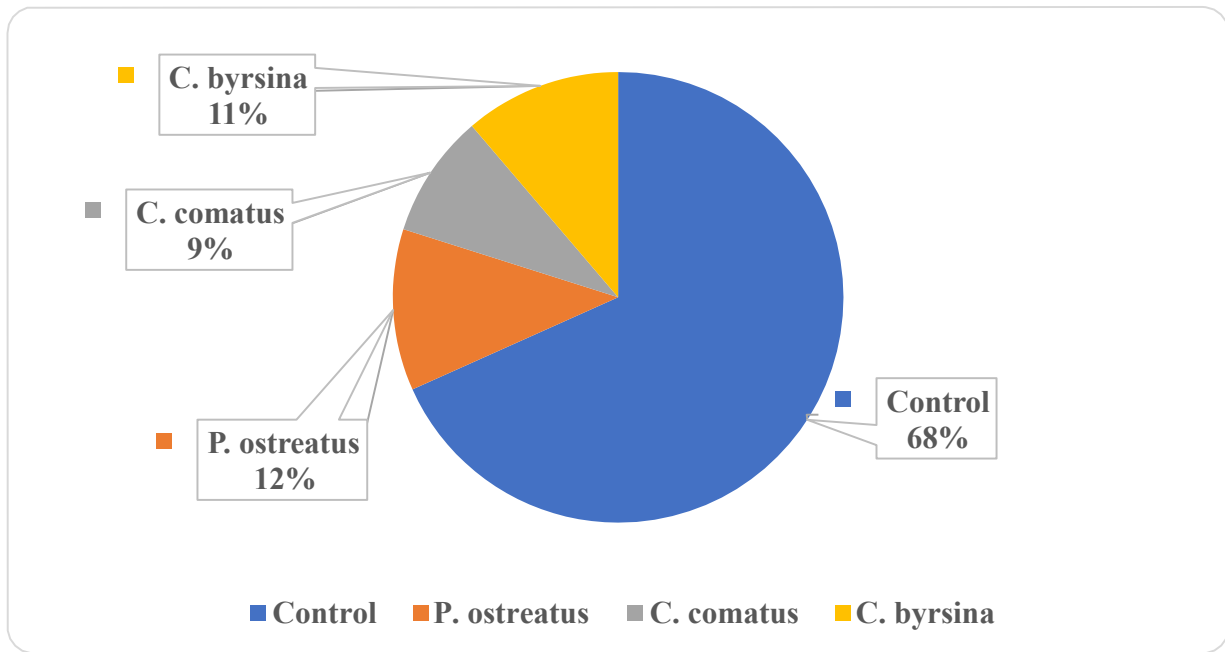
CONCLUSIONS

This study was performed to elucidate the dynamics involving mycofiltration within a marine aquaculture system modeled to represent concentrations of ammonia-nitrogen, nitrite- nitrogen and the conditions for key physiochemical parameters in a zero-water exchange high intensive shrimp mariculture growth system. A traditional biological filtration system containing nitrifying and denitrifying bacteria was tested on the same system which was used to establish the baseline values achievable by bacterial remediation which were used to compare the efficiency of mycofiltration in the removal of ammonia-nitrogen and nitrite-nitrogen under these particularly harsh environmental conditions while maintaining industry physiochemical

standards required for the cultivation of shrimp under these stressed conditions. ammonia- nitrogen and nitrite-nitrogen were ameliorated through the nitrifying and denitrifying reactions processes used by bacterial and fungal organisms during their metabolic consumption of these compounds as sources of chemical energy (Yoram Barak and Japp Van Rijn, 2000). As seen in Table 3 the bacteria in the traditional biofilter

maintained levels of un-ionized ammonia-nitrogen and nitrite-nitrogen below the prescribed TCEQ permit standards set for acceptable growth conditions for a high intensity shrimp marine aquaculture system as expected but were unable to match the levels during filtration as seen in comparison with any of the tested fungi as seen in Table 1.

Mycofiltration proved to be a sustainably viable alternative for reducing concentrations of toxic un-ionized ammonia and nitrite-nitrogen species to levels below TCEQ's permit standards and maintaining the required physiochemical parameters which will allow successful production of healthy marine organisms within a zero-water exchange culturing conditions. These results from this study allowed for a rejection of the first null hypothesis for the alternate which projected that mycofiltration would produce a beneficial set of nitrifying and denitrifying reactions at a rate significant enough to maintain the necessary conversion of ammonia-nitrogen to nitrite-nitrogen and to maintain standard physiochemical parameters identified by the TCEQ as required for maintaining a beneficial growth environment. There was a

significant difference seen in the rate of remediation by mycofiltration delineated by the phylum and species of the fungi which were chosen for this investigation. The basidiomycete, Pleurotus ostreatus, exhibited vigorous growth through all stages of the culturing process and contributed to the resulting 91% conversion rate recorded during its implementation as a filtering agent in this aquaculture system. Pleurotus ostreatus oxidized and reduced ammonia-nitrogen and nitrite-nitrogen at a rate 33% better than the levels recorded in the traditional microbial biofilters (Table 5). A minimal reduction of the mycelial network of P. ostreatus was experienced resulting from the harsh environmental stresses produced by an increase in saline concentration of the substrate and exposure to toxic nitrogenous compounds. This rate was observed to be similar but slightly higher and at a slower rate than that experienced by the halophilic species C. byrsina species and was significantly higher than the concentration achieved by the ascomycete C. comatus. The basidiomycete, Coriolopsis byrsina, which was chosen for its natural affinity for halotrophic conditions, determined by its discovery as a result of isolation from a mangrove leaf found in India, was able to remove ammonia-nitrogen and nitrite-nitrogen at a rate of 33% which was relatively similar to the recorded baseline values achieved by the traditional microbial biofilter but with an increase of 92% (Table 6). Coriolopsis byrsina initially grew vigorously on supplemented B & K media prepared with a saline concentration 50% of that measured in the full-strength seawater sourced from the lower Laguna Madre in Corpus Christi which proved to be useful in initially identifying this organism as a suitable agent for mycofiltration under the harsh halotrophic conditions expected for the *in-situ* environment which was to be necessary during remediation processes of toxic nitrogenous based compounds. This ideal characteristic resulted in the C. byrsina experiencing only a slight inhibitory effect on the growth of its mycelial network during the mycofiltration process where it also achieved an ammonia-nitrogen & nitrite-nitrogen removal rate of 34% under these conditions which was the second best outcome achieved during this mycofiltration trial and was 93% better than that achieved by filtration by a traditional bacterial filter. The most surprising and encouraging results were obtained by these species C. comatus which experienced an ammonia-nitrogen

& nitrite-nitrogen removal rate of 43% which was a 105% increase from that obtained by the traditional biofilter. This encouraging set of results were unique as ascomycetes are not known for their overwhelming resiliency or higher rates of biochemical reactions compared to most basidiomycetes. Ultimately, it appears that the resultant increase in hydraulic loading rate due to the higher mycelial surface area was the main contributing factor responsible for this outcome in addition to this organism's affinity toward nitrogenous compounds as a result of its known natural habitat which can be found on the ground rather than upon a rotting log or tree where most basidiomycetes tend to be found. The potential of all species to tolerate the harsh environmental conditions suggested that further exploration of these organisms would be a beneficial endeavor and would be required to elucidate the native features facilitated by these fungi during their natural expansion processes. Additionally, beneficial characteristics to discover would be the ability of the species being used to also produce a fruiting body as a secondary product which would add additional value to the process, the amounts of extreme stress from temperature, pH change, elevated excessive concentrations of ammonia-nitrogen, extended exposure time to high salinity concentrations, and effect on efficacy when live organisms are introduced into the growth tanks which would produce other unique chemical compounds and general increases in total suspended solids which could cause clogging in the MarineMycoFilters™. The uninoculated control performed inadequate nitrifying or denitrifying reactions which produced only slightly reduced ammonia-nitrogen and nitrite-nitrogen concentration. The reduced levels observed can be contributed to the unaided reactions of natural chemical decay, energetic stimulation of chemical conversion from increased UV radiation and the oxidative reactions facilitated during exposure to the increased concentration 02 in the testing environment also referred to as aerial stripping. It was concluded from the observed values recorded in Table 4, that within the control filter the reduction of toxic nitrogenous compounds was not nearly effective enough to bring the effluent back within the TCEQ's prescribed growth parameters necessary to sustain the cultivation of marine organisms in this system and a synergistic complementation reaction was necessary

through the application of anyone of the tested fungal species which all showed the ability to bring levels down to within tolerable levels of ammonia-nitrogen and nitrite-nitrogen.

Physiochemical parameters experienced weak regulatory interactions influenced by their exposure to the processes of the remediation performed by these fungal agents while specifically pH exhibited a moderate regulation by these same interactions resulting in a typical net move of pH toward increasingly acidic conditions due to the production of organic acids by these fungi which attempt to modify their local environment to make it more suitable to their needs. The second null hypothesis was also rejected for the alternate which showed that the ascomycete performed better at this task than was experienced by the treatment of either of the basidiomycetes as a result from the exposure to remediation processes to reduce the levels of ammonia-nitrate and nitrite-nitrogen to levels lower than those produced by the traditional microbial biofilters and maintaining of stable physiochemical standard parameters in the model marine aquaculture environment.

Mycofiltration in this preliminary study has shown to potentially be an economical and sustainable supplement or replacement of the currently practiced methodology which has shown preferential reliance on the exploitation of nitrifying/denitrifying bacteria in biofilters of various design which are utilized in this industry as of the time of this study. However, an addition, a concern exists regarding denitrifying reactions which fungi have been observed to utilize with increased frequency especially in conditions of waste which exhibits increased concentration of nitrite-nitrogen and decreased concentration of DO which stimulates these reactions to produce anoxic conditions within the growth system. Under these conditions, fungal organisms have been shown to produce increased concentrations of the volatile end product nitrous oxide (N_2O). This compound warrants concern during any decision concerning the implementation of waste treatment processes as it has been observed in the literature compiled by regulatory agencies that these processes or the lack there of to be the largest sources of N_2O expression into the atmosphere. Further research has identified this increase of N_2O to be the cause of acute environmental damage to the stratosphere where it depletes the natural reserves of O_3 at a significantly higher rate than the damage seen from the same amount of carbon dioxide (CO_2) (Daniel, Russell James, 2012).

Further evaluation of the various physiochemical parameters of each MarineMycoFilter™ should be done to increase the understanding of the qualitative properties involved in the filtration process used by each fungal species. Identification of the enzymatic profiles produced by each species would also help identify which enzymes are responsible for the remediation action performed by these organisms and could determine which enzymes will be induced into higher rates of expression by the organism when exposed to the exclusive environmental conditions present within the untreated wastewater where they catalyze the necessary metabolic processes responsible for the degradation of the targeted chemical compounds. Investigation into the concentration of intercellular nitrogenous compounds and remaining extracellular concentrations within the substrate or released as gaseous by-products would elucidate the route of metabolic degradation exploited by each species of fungi during nitrification or denitrification of ammonia-nitrogen and nitrite-nitrogen. Overall, this preliminary study was successful in expanding the information available to researchers engaged in similar studies investigating the potential function of mycofiltration under extreme conditions and the methods fungi employ to overcome conditions which have prevented the successful remediation of recalcitrant sources of environmental contamination by traditional bacterial filtration practices.

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LITTLE LEARNERS, TOUGH TOPICS: THE USE OF BIBLIOTHERAPY IN PRIMARY GRADE CLASSROOMS

by RUTH YOUNGER

ABSTRACT

The purpose of this study is to discover how teachers are using bibliotherapy through the use of children's literature to address tough topics in primary grade classrooms. Participants involved in this study taught either kindergarten or first grade at a primary elementary school located in South Texas. An electronic survey was created to measure participants' use of bibliotherapy in the classroom. Seven participants responded to the electronic survey and two teachers participated in a one-on-one interview. After reviewing the results from the electronic survey and one-on-one interviews, the researcher discovered that teachers are not comfortable with approaching many sensitive topics in their classrooms. The results also show that teachers have received little professional development on bibliotherapy. With these research findings, school administrators and faculty who teach in teacher preparation

MENTOR
Dr. Bethanie Pletcher
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College of Education and Human Development

programs might consider ways to assist teacher candidates and in-service teachers with using bibliotherapy.

INTRODUCTION

Each day students enter the classroom with challenging situations of which their teachers may not be aware. Students may be dealing with the loss of a loved one, an abusive family member, adapting to a new sibling, or maybe they have just moved to a new school. Some students may feel uncomfortable in class because they are being bullied by a classmate. Teachers can play a vital role in helping those students navigate difficult situations. According to Forgan (2002), "Teachers can use children's literature to help students solve problems and generate alternative responses to their issues" (p. 75). The concept of using children's literature to help guide students is known as *bibliotherapy*.

The purpose of this study was to explore the ways in which primary teachers in one school use bibliotherapy in their classrooms and to what extent they are comfortable doing so. The research questions that guided this study are: What sensitive topics do kindergarten and first grade teachers at one school approach through the use of children's literature? What books do kindergarten and first grade teachers use to introduce and discuss these topics and how do they locate these materials? In what ways do they use texts to introduce and discuss sensitive topics?

LITERATURE REVIEW

Bibliotherapy

Bibliotherapy, the use of literature to help one's self through a challenging period, has been a practice since the time of ancient Greeks (Jones, 2006). This idea would eventually be given its name by Samuel Crothers, an author in 1916. This idea of using literature to better oneself moved further into the 1920s when a librarian, Sadie Peterson Delaney, began to use this technique to treat soldiers returning from war. Bibliotherapy then took its place as a common treatment in psychology and was additionally used by librarians in the selection of appropriate literature (Jones, 2006). This method is not strictly used for adults but can also be implemented in classrooms with young children. Although bibliotherapy is often sought out to help those with crises, it can also benefit children who struggle with the reading process. Bibliotherapy can also be comforting and motivational to others (Dajevskis, Cappiello, & Crain, 2016).

According to a classic article in *The Reading Teacher* by Shrodes (1955), bibliotherapy is comprised of three separate stages that the reader experiences. The first stage is comprised of the reader self-identifying with the main character in the story. The second stage is catharsis; at this point the reader is feeling motivated or enlightened. The final stage of bibliotherapy is insight or awareness; at this time the reader can use the motivation he or she has gained and apply it to their situation. Shrodes (1955) stated that bibliotherapy "offers a new frame of reference which extends the reader's awareness and enriches his understanding" (p. 25). The use of bibliotherapy is a resourceful tool that will not only be used in the classroom, but will also enhance students' views of their surroundings once they leave class.

The Use of Children's Literature for Bibliotherapy Purposes

Aside from the use of bibliotherapy by librarians and psychologists, educators may also use this technique in their classrooms. Bibliotherapy with the use of children's literature is one way to help children navigate through difficulties. According to Karlin and Bruneau (1985), "Books can be one avenue to make children who are suffering...realize that they are not alone, that someone else has experienced and understand their dilemma" (p. 8). Children's literature can be used as an impetus to the discussion of a variety of topics that a child may be encountering or that other children in the class may come across at one point (Forgan, 2002). Using children's literature can also bring awareness to issues that may not be openly spoken about, such as abuse, divorce, race, or death. The topics that are discussed allow children to have an open discussion and hear other peers' thoughts and experiences. Additionally, through bibliotherapy children may self-identify with characters in a book and see they are not alone in whichever situation they may be battling (Forgan, 2002). While in the classroom, teachers are using guided discussions based on the circumstances that are in the story line. Through these discussions, students can hear their peers' point of view and recognize problem solving strategies (Iaquinta & Hipsky, 2006). Teachers are also being active listeners and are able to recognize whether or not students are following the message from the story. For those students who may not recognize the underlying message, teachers are able to use different techniques for their students to express themselves. Drawing, journaling, or re-enacting the story can benefit other students in class and promote a deeper understanding of the story (Sullivan & Strang, 2002). Through the use of bibliotherapy, teachers are showing their students that literature can enhance their connections with their peers and foster positive problem-solving skills (Rozalski, Stewart, & Miller, 2010). Students are then able to take away from the various lessons and apply their new skills to their real-life circumstances.

Addressing the Social-Emotional Needs of Children

Students who have experienced or witnessed traumatic events enter our classrooms. These students may find difficulty in processing these emotions. The environments that they are coming from may also hinder their social-emotional skills in and outside of the classroom. They may lack coping strategies for the situations they are experiencing and decide to keep these emotions bottled up or react to these situations negatively (Dajevskis et al., 2016).

With the use of bibliotherapy, children, as a class or individually, are able to identify the problem in a text, discover how the main character finds a solution, and learn how well the solution worked for the character. Those students who need help coping are then able to

reflect and consider how their experiences relate to the main characters. Through bibliotherapy, even students who are not familiar with difficult situations learn about various struggles and become empathetic to others (Forgan, 2002). Teachers can promote healthy social-emotional skills in the classroom by selecting literature that entails helping others, sharing, making friends, and expressing emotions (Prater, Johnstun, Dyches, & Johnstun, 2006).

Literature Selection

When implementing bibliotherapy, it is important to ensure that the texts used are appropriate and relevant to the issue at hand. Selected literature should be linked to specific topics that one or more children are experiencing (Sridhar & Vaughn, 2000). Once literature has been selected, a teacher must be familiar with the storyline and the message it will give to his or her class (Sullivan & Strang, 2002). Teachers should consider the grade level, the problem the character in the book faces, whether the main character has a similar scenario to their students, and whether or not there is a solution that the main character configures at the end of the story (Rogalski et al., 2010). Teachers should also evaluate whether the illustrations support the text and how engaging they are. When selecting children's literature to use for bibliotherapy purposes, it is also important that the text is up to date and relatable. Not only is the text important when selecting a book, but the illustrations are also equally as important. Young children find the appeal of animal characters because they are diverse and are not always telling of gender or age (Nicholson, & Pearson, 2003). A follow-up discussion based on the literature should be included before, during, and after the reading so that children have opportunities for rich discussion. Using bibliotherapy as a therapeutic method may have been recognized by the ancient Greeks, but it is still a valuable classroom resource that should be utilized.

METHODS

In order to discover the ways in which teachers use bibliotherapy through children's literature, a sequential mixed methods approach was utilized. A survey was administered to the teacher participants first. Then interviews were held with two participants based on their survey responses.

Participants and Setting

The participating elementary school is located near the coast of South Texas. This school was chosen as the research site based on the willingness of the principal and teachers to participate in the study. According to the National Center for Educational Statistics, as of the 2015-2016 school year, the elementary served 403 students in prekindergarten through first grade. The school housed 23 teachers and a teacher to student ratio of one to seventeen. Out of the 403 students, 51% were males and 48% were females. The ethnicity makeup of the school is largely comprised of Hispanics (59%), Whites (36%), and other races (5%) including American Indian, Asian, Black, and mixed-race students.

Participants of the survey and interview were teachers who taught either kindergarten or first grade. Seventy-one percent (5) of the teachers who responded taught kindergarten and twenty-nine percent (2) were first-grade teachers.

Both teachers who participated in a one-on-one interview were first-grade teachers; one had taught for 20 years and the other had taught for 12 years. The teachers had taught various grades, from kindergarten through fifth grade.

Data Collection

Survey. Prior to the release of the survey, an electronic survey was distributed to three reading professors for review. They made several suggestions to improve the clarity and order of the questions, as well as the answer choices. Once the survey was reviewed and edited, a link to the survey was included in an email sent to kindergarten and first grade teachers at the school site (see Appendix A for survey). The electronic Qualtrics© survey consisted of 14 questions that were both open and close-ended. The items asked teachers to consider how familiar they were with bibliotherapy and if they had used this method in their classrooms. Teachers were also asked which sensitive topics they had approached with their students during this or in previous years and what sensitive topics the they did not approach. The survey also asked how teachers located materials to use for bibliotherapy purposes and whether they discussed bibliotherapy with their colleagues. Teachers who participated in the survey then indicated if they wanted to participate in an interview.

Interview. Two teachers participated in a 15- to 35-minute semi-structured interview to answer further questions about the use of bibliotherapy in their classrooms (see Appendix B for interview protocol). One interview was completed face-to-face, which was conducted over 34 minutes. The second interview was conducted in 15 minutes over the phone. The interviews were then transcribed by the researcher and used to support the data collected from the Qualtrics© survey.

Data Analysis

A list comprising of each question and responses from the Qualtrics© survey to discover the differences and similarities of responses from the kindergarten and first grade teachers. The survey information also displayed which sensitive topics were highly recognized by teachers and in which topics their students showed interest.

Interview transcripts were printed out and analyzed through two transcripts to get an overall sense of participants' responses. Open coding was then used to determine themes related to bibliotherapy use in the classroom. Themes that occurred during the interview are sensitive topics that students have spoken about to peers and their teacher, what topics they had covered, where they collect materials for the class, and how their life experiences play a role in choosing sensitive topics.

Trustworthiness

The survey for this research was vetted by three reading professors at Texas A&M University - Corpus Christi before being released to the kindergarten and first grade teachers at the school site. In addition to using the Qualtrics© survey to collect data, teacher interviews were conducted to enhance the information collected through the surveys. Member checking was also conducted with the two teachers who participated in interviews. Each teacher reviewed their interview transcription to ensure that their words were used accurately and within context.

Ethical Considerations

The principal of the elementary was contacted by mentor and was presented with a letter requesting to conduct this study with kindergarten and first-grade teachers. Upon approval, the principal of the elementary

dispersed an email to the teachers stating that the school would be participating in the research. The teachers who chose to participate in this survey were volunteers that gave consent through the Qualtrics© survey. As part of the survey, teachers were asked if they would consider volunteering for a one-on-one interview and contacted via email. The teachers who chose to participate in the one-on-one interview were given a consent form to sign prior to the interview.

FINDINGS

The Use of Bibliotherapy in the Classroom

Seventy-one percent of teachers surveyed answered that they do use bibliotherapy in their classrooms. The same percent of teachers used bibliotherapy in a whole group class setting. The remaining 29% of participants read to children individually or in small groups. All of the participants used bibliotherapy through the conduit of children's literature only when they felt it was necessary or when a particular topic was brought up.

Information gained through the two interviews indicated that the lack of familiarity with the term *bibliotherapy* was common. Both teachers interviewed declared that they were not familiar with the term, but that they understood the importance of children's literature and how it can play a role in students' ability to cope during a difficult period or when they are faced with new experiences.

Tough Topics

What tough topics emerge? Teachers encounter a variety of situations that their students face in and outside of the classroom. Survey participants were asked to select from a list of 26 topics which they have seen their students experience. From the list of topics, teachers marked Attention Deficit Hyperactivity Disorder/Attention Deficit Disorder (ADHD/ADD) and adoption/foster care as two topics that have come up in the classroom. One interviewee stated in her class, "children are noticing that not all of their peers are the same, some behave differently than others, and children are curious about their peers' differences, they want to know what makes them different." Bullying was the third-ranked topic that participants selected. During an interview with one teacher, she stated that cases of bullying were recurring in the classroom. To address these situations,

the teacher took time to locate children's literature related to bullying. Once the books were presented as a whole-group read-aloud, it helped students identify what bullying is and how to use problem solving skills when faced with a bullying situation. Reading as a group builds a safe environment for students and allows them to have a sense of belonging while opening up in discussions (Dajevskis et al., 2016).

What topics do teachers find comfortable and uncomfortable to approach? From the same list, the participants were asked to identify topics they felt most comfortable reading to their students from children's literature selections (see Table 1). The results showed that teachers are most comfortable using children's literature to discuss feelings, a new baby, self-esteem, and friendships. Sixty-six percent of those surveyed did not use particular children's books for bibliotherapy. However, one interview participant mentioned that she reads from the *Frog and Toad* series by Arnold Lobel for bibliotherapy purposes. This same teacher described how reading to her class about feelings was helpful when students were having a difficult time expressing to their peers the emotions they were experiencing. To incorporate the text the teacher said, "we read several of the books on the topic and use fact or opinion, I ask what character was right or wrong and how they would solve the problem." She additionally commented that there is not a lot of time that can be spent on special topics due to the fact that there are other content areas that need to be taken care of, but she recognizes that implementing and allowing students to listen and respond to their peers creates a healthy discussion in the classroom.

Using the same list of topics, teachers were also asked to identify which topics they did not feel comfortable addressing in their classroom. Among the list of topics teachers steered clear of were alcoholism, gender issues, and religion. One teacher noted on the survey that she would be willing to address any issue as long as it was to her students' benefit. Both teachers interviewed felt that hot-button topics should also be addressed with the families before being discussed with the students. They discussed how family support is welcomed and important when tough topics are being discussed in the classroom.

TABLE 1. Survey Responses to Topics Teachers Are Willing to Approach in the Classroom	
Topic	Responses ($n = 7$)
ADHD/ADD	5
Adoption/Foster Care	5
Bullying	4
Different kinds of families	3
Doctor/Dental Visits	3
Feelings	3
Friendships	3
Homelessness	3
Learning difficulties	3
Autism	2
Cultural diversity	2
Going to School	2
Moving	2
New baby	2
Nutrition	2
Abuse	1
Alcoholism	1
Death	1
Divorce	1
Drug use	1
Gender issues	1
Religion	1
Self-esteem	1
War	1
Refugee families	0
Other	0

Resources for Bibliotherapy: Where and How Teachers Locate Materials

This study found that several teachers at the studied school site are using bibliotherapy in their classroom and are talking to their colleagues about how to address tough topics in the classroom. One teacher discussed how when choosing a book, she often has to spend time looking online for more information about the tough topic and then find a book that corresponds with the topic.

This research has shown that some teachers currently in the field may lack the materials needed for this type of approach to using children's literature. One interview participant stated that the children's literature used for bibliotherapy in the classroom was purchased with her own money. Research conducted by Prater et al. (2006) determined that when implementing bibliotherapy, teachers can work together with more than just their colleagues; also, their school counselor, psychologist, and librarian.

Professional Development

Teachers' professional development experiences in bibliotherapy. All survey participants in this study declared that they had no training in bibliotherapy as part of their teacher preparation programs, nor had they received professional development on this topic since beginning their careers as teachers. Two of those surveyed did relay that even though they had no formal training in bibliotherapy, they sought information on their own. One teacher interviewed said, "there are often nights where I stay on the Internet finding children's literature that is meaningful and will be helpful to not only one child, but to the whole class." Fifty-seven percent of survey participants confirmed that they have at some point reached out to their colleagues to discuss children's literature about bibliotherapy and its implementation in the classroom.

The teacher participants described that there was a lack of support from their district administrators, along with a shortage of time to incorporate bibliotherapy into an already packed curriculum. Two participants stated that they did not have access to children's books that deal with sensitive topics. One commented, "It's easier to find children's literature on feelings and social skills, but more difficult for topics like death and mixed families." The teachers also had difficulty locating books that fit the appropriate age group of the students they teach. Additionally, interview participants felt that there was not a lot of support for reading about sensitive topics from parents. See Table 2 for a summary of these results.

DISCUSSION

With the growing variety of situations to which children are exposed within and outside of the classroom, there is evident need for teachers to experience formal training

TABLE 2.
Survey responses of Obstacles that Teachers find when Implementing Bibliotherapy

Obstacle	Response (*n* = 7)
Other: Lack of formal professional development	7
Lack of books to use for implementation	3
Lack of time	2
Lack of support from school and district	1
Lack of family support	1

in bibliotherapy through the use of children's literature. Sullivan and Strang (2002) noted that implementing bibliotherapy into a daily routine also aides with students' reading development. With the assistance of bibliotherapy, teachers might better understand how to approach sensitive topics, such as bullying, homelessness, and divorce in the classroom, as well as less sensitive topics, such as friendship and working well with others. Research conducted by Rozalski et al. (2010) discussed that the use of bibliotherapy can help students cope with these issues because their social and emotional skills are usually malleable.

Teachers and Their Approach to Sensitive Topics

According to the survey results, teachers are paying attention to just a few tough issues that their students are experiencing. Attention Deficit Hyperactivity Disorder/Attention Deficit Disorder (ADHD/ADD), foster care/adoption, and bullying were the three main topics included that the teachers surveyed said they addressed. Prater et. al. (2006) expressed that because society is changing rapidly, students face a variety of challenges at home and at school. Students who have these types of experiences also might be at risk for having more stress than their peers. With the added stress, they may be more likely to act out on their emotions in a negative way (Ableser, 2008).

While the teachers surveyed agreed that they would address any topic as needed, there were teachers who indicated they were uncomfortable with particular topics. For instance, alcohol, gender issues, and religion

were the top three hot-button issues that teachers did not feel comfortable approaching in the classroom. However, Mankiw and Strasser (2013) asserted that these types of hot-button topics are a part of students' lives and may be seen by some as topics that are too uncomfortable to talk about. Teachers may also find it difficult to approach certain topics because they simply do not know how. An approach such as bibliotherapy can help those teachers who experience discomfort with addressing sensitive topics related to what their students are going through (Mankiw & Strasser 2013).

Teachers' Use of Bibliotherapy in the Classroom

All surveyed and interviewed participants declared that they used some form of bibliotherapy in the classroom. Of those surveyed, 71% used bibliotherapy in a whole class setting, while the remaining 29% conducted bibliotherapy individually or in small groups. Sullivan and Strang (2002), however, found that when teachers used bibliotherapy in large settings, students at a young age were more distracted and that they actually preferred small group or individual settings. One teacher interviewed said, "It's easier to use bibliotherapy as a whole class, because it saves time, since schedules can be right on top of each other." This statement is consistent with the survey in that a quarter of teachers felt that time got in the way of using bibliotherapy in the classroom. Bibliotherapy does not have to occur at one specific time; teachers can choose to implement bibliotherapy any time during the school day. Bibliotherapy should be naturally implemented whether it occurs as part of a morning meeting, during guided reading, or at the end of the day (Sullivan & Strang, 2002).

Implications

Training. Teacher candidates should be exposed to what bibliotherapy is and ways to weave the concept into their lesson plans. Without the knowledge of bibliotherapy, teacher candidates like myself, will lack information that can help us to guide students through tough situations. Learning about the benefits of this approach might enhance their future classroom communities and relationships that they have with students and perhaps families. As such, this topic should be an objective that reading instructors include in teacher preparation courses.

After reviewing the findings from this study, it is noticeable that teachers are not experiencing professional learning in the area of bibliotherapy through their school workshops. It was also difficult to find evidence of teachers receiving this type of professional development in the literature related to bibliotherapy. However, the literature does refer to the librarian and school counselor as people who can help guide teachers to use bibliotherapy and help them locate materials, respectively (Prater et. al., 2006).

Resources in the school. School librarians have an important role to play in the implementation of bibliotherapy. Librarians can help teachers select books based on the students' needs, grade level, and content to be studied (Jones, 2006). More so, teachers should be able to confide in the librarian to discuss which children's literature might meet their whole classroom needs, before having to purchase materials on their own. Over half of the teachers surveyed said they usually gather literature from their personal collection. To help cut the cost of purchasing new children's literature, teachers can also visit local libraries to locate books that they may need for particular topics.

Furthermore, the school counselor is another resource with whom teachers can connect when they recognize that a student is dealing with a difficult situation or an issue arises in the classroom and the teacher is unsure how to deal with it. With the help of a counselor, a teacher can "brainstorm ideas for using bibliotherapy individually or collaboratively" according to Prater et al. (2006). Counselors can not only provide expertise about how to meet children's needs, but also help the teacher determine whether a student will do best with one-on-one bibliotherapy, small group, or whole group.

Families. I also discovered that teachers not only need the support of their colleagues and administration, but also support from families. When teachers are deciding on whether or not to discuss a sensitive or tough topic with students, they should be mindful of how comfortable (or not) families are with the topic (Sridhar & Vaughn, 2000). The backing from families can play an important role in which topics can be approached in the classroom. Teachers can also communicate with families and develop a plan to assist a child who may be having a difficult time in school. Additionally, the

teacher can send children's books home or make book recommendations that the family can read together to support the child. However, it is important to be mindful that sending home literature might feel accusatory or offensive (Prater et al., 2006).

Funding. The lack of funding for materials indicates that school and district administration may not be aware of the value of using bibliotherapy through children's literature in the classroom. Teachers can inform administrators about the strategy and how it can be beneficial to the students and families. For this initiative to be successful, teachers might research this topic further and make a list of topics that they have been witness to in their classrooms before approaching administrators and requesting money to purchase books.

Limitations

There were several limitations that may have affected the results of this study. The participants of the survey were employed at one school and were split between only two grade levels. Each grade level had a small number of teachers which limited the collection of data. The response rate was small, at 39%. Those who participated in the Qualtrics© survey self-reported their information, which may not always be accurate. Furthermore, only two teachers requested to meet for an interview. One of these interviews was brief, due to the teacher's time constraints.

Directions for Future Research

Upon completion of this study, there still exists a desire to learn why the teachers felt uncomfortable discussing topics in which their students had shown interest. Would teachers feel more comfortable with more support and resources for bibliotherapy? With more research we could discover what teachers are most interested in learning as part of their professional development about bibliotherapy through children's literature. Also, how much training should teacher candidates receive on bibliotherapy as it can be approached through children's literature? Would teachers welcome having a curated list of tough topics and corresponding children's literature? A discussion about bibliotherapy through children's literature with school librarians and school counselors would also be interesting in order to discover what they know about this topic and how

they can serve as a resource to teachers in this way. An expanded study involving more than one school, along with intermediate grades would assist in researching how other teachers implement bibliotherapy in their classrooms.

CONCLUSION

After interviewing and surveying primary teachers, I gathered that teachers are using bibliotherapy in their classrooms to address certain topics, even if they are unaware that what they are doing is labeled *bibliotherapy*. These teachers are using their own personal classroom libraries to approach sensitive topics and are discussing with their colleagues how to help students. Through the research, I discovered that teachers are not receiving professional development in the area of bibliotherapy and were not informed about bibliotherapy through during their teacher preparation programs. The findings from the survey concluded that there are topics teachers feel uncomfortable discussing in the classroom. However, during one-on-one interviews, teachers said that they have no problem addressing a tough topic if it will be beneficial to their students. This research has shown that several teachers in this school are using some bibliotherapy with their young students despite the lack of training or funding from their school. Teachers are providing students with ways to problem-solve, gain empathy, and cope through the use of bibliotherapy.

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APPENDIX A

Bibliotherapy Survey

- How many years have you taught?

- What grade level do you teach?

- What are some sensitive topics that have emerged in your classroom?

- Do you use bibliotherapy in the classroom to address sensitive topics?

- How often do you use bibliotherapy in the classroom to address these topics?

- Are the books read with individual students, small groups, whole class?

- Are there particular children's books you use to use for bibliotherapy purposes?

- Where do you locate books to use for these purposes?

- What hot-button issues are you comfortable discussing with your students?

- What hot-button issues are you not comfortable discussing with your students?

- What gets in the way of using bibliotherapy in the classroom to address sensitive topics?

- Do you talk with your colleagues about using books for these purposes in the classroom?

- Was bibliotherapy part of your preservice teacher training?

- Have you had any professional development for using bibliotherapy as an in-service teacher?

APPENDIX B

Individual Interviews

- What are some sensitive topics that have emerged in your classroom?

- In what ways do you use bibliotherapy in the classroom to address sensitive topics?

- What sometimes gets in the way of using bibliotherapy in the classroom to address sensitive topics?

- How often do you use bibliotherapy in the classroom to address these topics?

- How do you find appropriate books to address these topics?

- Do you use more than one book to discuss the sensitive topic?

- What hot-button issues are you comfortable discussing with your students?

- What hot-button issues are you not comfortable discussing with your students?

- Are there particular authors that write the kinds of children's books you might choose to use for bibliotherapeutic purposes?

- Do you make the books that you use to address these topics available to the students on a daily basis?

- Do you notice a change when students read or listen to books that provide them with a storyline that they are experiencing?

- Do you talk with your colleagues about bibliotherapy in the classroom?

- What topics do you feel should be more widely addressed to support young students?

- What are your future plans for using children's literature to discuss hot-button issues in your classroom?

TEXAS A&M UNIVERSITY-CORPUS CHRISTI
A BRIEF HISTORY

Texas A&M University–Corpus Christi began in 1947 as the University of Corpus Christi (UCC) which was affiliated with the Baptist General Convention of Texas. In 1970, Hurricane Celia causes more than $1,000,000 dollars in damage to the campus. In 1971, the Baptist General Convention of Texas sold UCC to the state of Texas and the Texas Legislature authorizes the Texas A&I University System to establish a state-supported upper-level institution of higher education in Corpus Christi. In 1973, Texas A&I University at Corpus Christi opens its doors on 4 September 1973 to 969 students as an upper-level institution of higher education. In 1977, the Texas Legislature changes the name of the University to Corpus Christi State University. In 1989, Corpus Christi State University joins the Texas A&M University System. In 1993, the Texas A&M University System Board of Regents renames the institution Texas A&M University–Corpus Christi and a year later it becomes a four-year comprehensive university and enrollment increases to 5,000 students. In 2004, the Board of Regents approves the College of Nursing and Health Sciences which opened in 2005. In 2005, Dr. Flavius Killebrew becomes President/CEO and initiates Momentum 2015, a ten year plan to establish Texas A&M University–Corpus Christi as the flagship university of South Texas. In 2016, Dr. Killebrew announced his retirement and former Provost and Vice President for Academic Affairs Kelly Quintanilla was appointed interim president. Today the University has over 12,000 students.

Today Texas A&M University–Corpus Christi is not only a proud member of the Texas A&M University System but it is also the premier public university in the region and is currently the only university in the United States to be situated on an island. Texas A&M University–Corpus Christi is currently a member of the Southland Conference under the NCAA division I.

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