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About this Issue

Statement of Purpose

The Rhodes Journal of Biological Science is a student-edited publication that recognizes the scientific achievements of Rhodes students. Volume XXIX marks the eighth year since Mark Stratton and Dr. David Kesler brought the journal back into regular publication in 2006. Founded as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study among Rhodes College students. We hope that in reading the journal, other students will be encouraged to pursue scientific investigations and research.

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Image Credits

The cover is an illustration depicting the contrast between different sides of the brain and the different aspects of biology, with more hands-on, cellular and anatomical work on the left and the technological, data analysis aspect on the right. It was created by Allison Julien, aided in direction by Donya Ahmadian, both senior Neuroscience majors of '14. Allison balances research with both the neuroscience department and Memphis Zoo, and has maintained an interest in art since a young age. Post-graduation, she plans to pursue a career either in behavioral neuroscience research or opening her own tea and used book shop.

Editorial Staff

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Koshy George '16 is a Commerce and Business major aiming to focus in Medical Administration from Chennai, India. He is the captain of the Rhodes Crew Team and volunteer at the Regional Medical Center in the Elvis Presley Trauma Unit. Additionally, Koshy is a member of Sigma Nu and is also a resident assistant. His future plans consist of attending medical school with the hopes of practicing as a physician in the United States Army.

Alex Hooven '16 is a Biochemistry and Molecular Biology major from Santa Cruz, California. He is a board member of the Hispanic Organization of Languages and Activities (HOLA). In recent summers, he has broadened his perspective of healthcare in California and his hometown in Antigua, Guatemala. Growing up speaking Spanish, he spent last summer shadowing various doctors in an emergency room in Guatemala. He hopes to spend future time and summers volunteering and traveling to places like Germany, both to practice his German and to shadow doctors in Europe. He hopes to attend medical school after graduation.

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Rachel Nelson '16 is a Chemistry major from Ripley, Tennessee. She is a weekly volunteer at Lynx Club: a Special Olympics Memphis Program, assistant to the Vice President for the Chi Omega Fraternity, a member of the Campus Outreach program at Rhodes, and has formerly served as a tutor at the Soulsville Charter School. She plans on starting a research project at St. Jude Children's Research Hospital this summer as a part of the St. Jude Summer Plus Fellowship. After graduating from Rhodes, she plans to attend Pharmacy School to get a PharmD.

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MDMA as an Adjunct to Psychotherapy in the Treatment of PTSD

Peter W. Ketch

Although controversial because of its status as a schedule 1 drug, the use of 3,4-methylenedioxymethamphetamine (MDMA) as a pharmacological adjunct to behavioral psychotherapy is an effective treatment for Posttraumatic Stress Disorder (PTSD). Current FDA approved pharmacotherapies provide little to no relief from the severe, lifedisrupting symptoms associated with PTSD, creating a strong interest in more effective strategies. MDMA has several pharmacological mechanisms that can change patterns of brain activation and neurochemical activity that directly counter critical dysfunctions associated with PTSD. The effects of MDMA can also increase an individual's level of trust in his therapist and his comfort with the therapeutic process, critical elements of effective PTSD psychotherapy. Ultimately, based on its compensatory neurological effects, MDMA-assisted psychotherapy could improve the quality of life for PTSD patients for whom current, approved therapeutic strategies have provided no relief.

Introduction

Posttraumatic Stress Disorder (PTSD) is a severe anxiety disorder that causes affected individuals to experience frequent, crippling memories, dreams, and flashbacks of their trauma that can often interfere with many aspects of daily life (Lambert and Kinsley, 2011). The U.S. Department of Veterans Affairs reports that the lifetime prevalence of PTSD in adults is 6.8%, while increased rates are seen in specific populations like returning soldiers from Operation Iraqi Freedom, 15% of which are diagnosed (2011). Similarly, incidence rates are well over 20% for survivors of more personal traumatic experiences, such as torture, rape, and childhood abuse (Charuvastra and Cloitre, 2008).

Despite the high incidence rates of this disorder and its interference with everyday life in those affected, many current treatments fail to provide significant relief for PTSD sufferers. Currently, the FDA only approves two drugs for PTSD treatment, sertraline and paroxetine, both selective serotonin reuptake inhibitors (SSRIs) that require daily doses (Oehen, Traber, Widmer, and Schnyder, 2013). About 60% of PTSD patients show slight to moderate improvements in response to to SSRI treatment while only 20-30% are ever able to attain complete remission (Berger et al., 2009; Brady et al., 2000; Tucker et al., 2001). Furthermore, PTSD patients are likely to relapse if they terminate their SSRI treatments, suggesting that these drugs serve only to quiet the symptoms of this disorder, not to authentically treat it (Lambert and Kinsley, 2011). New approaches to pharmacological PTSD treatment have been investigated with hopes of developing clinical drugs that are more affordable, more effective, and have longer lasting results than SSRIs. Until it was classified as a Schedule 1 drug in 1985. 3,4-methlenedioxy-N-methylamphetamine (MDMA) was used as a psychotherapeutic adjunct in the United States. Despite its strict legal control,

MDMA is reemerging as a promising intervention for patients with chronic PTSD who have been unresponsive to existing forms of treatment (Oehen et al., 2013; Mithoefer M., et al., 2013).

Administration of MDMA under close supervision during only a few psychotherapy sessions resulted in 83% of participants no longer qualifying for a PTSD diagnosis. Furthermore, all but two patients maintained this status even 3.8 years later (Mithoefer M., et al., 2013). The recently reported effectiveness of MDMA for use in treating PTSD can be attributed to several important qualities of the drug. First, MDMA augments the effectiveness of psychotherapy by enhancing the level of trust that the patient establishes in the therapist, or the therapeutic bond (Bedi, Hyman, and de Wit, 2010; Hysek et al., 2013). Secondly, MDMA has been shown to instigate multiple changes to a patient's brain activity and neurochemical release that could improve his or her ability to confront and manage their traumatic experience (Carson, Guastella, Taylor, and McGregor, 2013; Hysek et al., 2013). These changes should allow the patient and therapist to work towards the extinction of his or her abnormal fear response. It is through these novel therapeutic characteristics of drug action that MDMA, although Schedule 1, should be seriously considered in the development of a more effective treatment for PTSD.

Brain Dysfunction in PTSD Sufferers

Several distinct neuroanatomical and neurochemical dysfunctions, mainly involving the amygdala and areas of the prefrontal cortex (PFC), have been demonstrated in PTSD patients that could explain their constantly elevated fear states and their perception of unrealistic threats in their environment (Johansen and Krebs, 2009). In a functional magnetic resonance imaging (fMRI) study in which the participants were shown happy vs. fearful facial expressions, the PTSD group displayed reduced activity in the medial prefrontal cortex (mPFC) and a reciprocally high level of activity in the amygdala compared to the control subjects (Shin et al., 2005). This finding suggests a neural basis for the exaggerated stress response to emotional stimuli seen in PTSD patients. The PTSD group also exhibited a weaker habituation to the fearful responses in their right amygdala across functional runs (Shin et al., 2005). The interesting design of this study, presenting the patients with a fearful stimulus unrelated to their traumatic experience, effectively addresses the fear and anxiety that individuals with PTSD may encounter in their everyday lives. The abnormal brain activity of the PTSD individuals, hyperactivity in the amygdala, which is responsible for registering the fearful stimuli, and hypoactivity in the PFC, which is responsible for responding appropriately, suggests a fundamental biological basis for their symptomology.

Compensatory Mechanisms of MDMA against PTSD

Interestingly, the effects of MDMA seem to exert precisely opposite effects in the human brain. At even relatively small doses, MDMA has been shown to increase activity in the ventromedial prefrontal cortex (vmPFC) and to simultaneously decrease activity in the amygdala (Johansen and Krebs, 2009). This finding suggests a clear neurobiological explanation for how MDMA may function as an effective treatment for PTSD, directly opposing the existing neural abnormalities that may be responsible for their exaggerated fear response and heightened levels of anxiety. In a clinical setting, these changes to the vmPFC and amygdala may improve emotional regulation, dampen the fear response, and decrease avoidance behaviors to augment the therapeutic experience.

The level of trust and openness between a PTSD patient and his or her therapist is extremely important. The strength of the therapeutic bond is directly correlated to recovery rates in PTSD patients (Charavastra and Cloitre, 2008). Similarly, individuals with PTSD often report a certain emotional disconnect with their family, friends, and therapists, which is certainly a perpetuating factor in their disorder (Charavastra and Cloitre, 2008). Ecstasy, a street drug containing MDMA, has been frequently reported by users to increase empathy and prosocial behaviors (Bedi, Hyman, and de Wit, 2010). One study showed that subjects treated with MDMA self-reported increased ratings of "friendliness" and "lovingness" compared to controls, while showing a reduced recognition of fearful facial expressions (Bedi et al., 2010). These subjective effects may be dangerous in social settings by increasing risk taking behaviors and instilling false senses of trust in others. However, used as an

adjunct to psychotherapy, these same effects could profoundly increase an individual's therapeutic experience, especially someone with a social dysfunction like PTSD.

Although the prosocial effects of MDMA have historically been considered mostly subjective, they have a strong biological basis related to the hormone oxytocin, demonstrated in both animal and human models (Forsling et al., 2002; Carson, Guastella, Taylor, and McGregor, 2013). Oxytocin is a mammalian hormone that is mainly associated with milk-letdown and parturition, but in humans it has also been shown to have profound effects on more complex psychological phenomena like memory, reward, and trust (Carson et al., 2013). In rats, MDMA injections to the isolated hypothalamus cause dose dependent increases of oxytocin release (Forsling et al., 2002). Similarly, a study on circulating hormone levels in humans after taking MDMA demonstrated increased plasma levels of prolactin, a marker of oxytocin activity in the body, which is also associated with prosocial behavior (Hysek et al., 2013). Although more studies should be conducted to illuminate the specific role of oxytocin in human prosocial behavior, the fact that MDMA is responsible for heightened oxytocin activity in mammals provides more evidence that it could strengthen the bond between patient and therapist, an encouraging benefit of MDMA treatment during psychotherapy for individuals with PTSD.

The ultimate goal of many PTSD psychotherapy strategies is to prompt extinction, a type of emotional learning, to successfully liberate the patient from his or her severe anxiety and elevated fear response triggered by unrelated stimuli (Foa, 2006). MDMA instigates a crucial chemical change in the brain that may enhance the rate of this emotional learning. Specifically, MDMA significantly increases the release of norepinepherine (NE) and cortisol (Green, Mechan, Elliott, OShea, Colado, 2003). Stressrelated release of NE and cortisol have been shown to critically facilitate multiple types of emotional learning, including extinction (Quirk and Mueller, 2008). Conversely, it has been suggested that pharmacological treatments aimed simply at reducing the anxiety and hyperactive stress responses associated with PTSD, like the current SSRI treatments sertraline and paroxetine, can actually be detrimental to emotional learning during therapy and can interfere with the extinction process (McNally, 2007). In this way, MDMA initiates another chemical change that could enhance the results of PTSD psychotherapy more so than the current FDA approved treatments.

Conclusion

The onset of PTSD is often related to trauma associated with the breach of a personal bond, through violence and betrayal rising from situations like combat or sexual abuse. To successfully engage in the emotional processing necessary during PTSD psychotherapy, the patients need to trust their therapist enough to share the most haunting details and memories of their traumatic experience. The most successful PTSD therapy sessions are ones in which the individual is extremely comfortable and firmly grounded in his or her present surroundings, while simultaneously delving into the traumatic experience and exploring it with the guidance of the therapist (Foa, 2006). Understanding the importance of the strength of the therapeutic bond and the multiple ways in which MDMA contributes to this interaction should provide a strong motivating basis for which to continue MDMA assisted psychotherapy research with hopes of its approval for PTSD treatment.

Furthermore, although there have been concerns with the safety of this drug and its potential neurotoxicity to 5-HT neurons in the brain, multiple studies have shown that two or three small doses over the course of several weeks led to no adverse psychological or physiological responses and that this acute neurotoxicity occurs mainly in subjects who use the drug chronically, in higher doses, and often in combination with other drugs (Bouso, Doblin, Farre, Alcazar, and Gomez-Jarabo, 2008; Mithoefer, M. et al., 2013; Ricaurte et al., 1988). Additionally, despite the common misconception that MDMA has high abuse potential, there are very few cases of true addiction to pure MDMA. Most of these cases involve the street drug ecstasy, which often contains other amphetamines and/or ketamine. Also, ecstasy is commonly used in tandem with other drugs like alcohol and cigarettes (Cole and Sumnall, 2003; Jansen, 1999). This complicated poly-drug usage can lead to uncharacterized and potentially dangerous responses that should not be associated with pure MDMA. Although more research on the safety and efficacy of MDMA should be conducted, control over research on MDMA-assisted psychotherapy should be loosened because it has a strong biochemical basis for combating the effects of PTSD. MDMA is safe in small, infrequent doses and has led to higher rates of recovery for longer periods of time than the current FDA approved SSRIs. MDMAassisted psychotherapy could lead to significant symptom relief and an improved quality of life for many PTSD patients.

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Location and Visual Features of Medulloblastoma Molecular Subgroups by MRI

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Abstract Medulloblastoma is the most common malignant brain tumor in children. There are four molecular subtypes of medulloblastoma. Research has uncovered the developmental origins of two of the four subtypes, Sonichedgehog and Wnt, but little is known about Groups 3 and 4. A previous study comparing Sonichedgehog and Wnt subtypes showed that these tumors exhibit different localization patterns in the posterior cranial fossa. This paper presents results of quantitative analysis of tumor localization in subtypes 3 and 4 based on landmark coordinates in a standard brain space. This analysis did not reveal any significant differences so a qualitative approach was taken to further analyze each subtype based on visual appearances of the tumors. Ongoing research is investigating the relationships between localization, molecular markers, and developmental origins using mouse models for Group 3 and 4 medulloblastomas.

Introduction

Medulloblastoma, the most common malignant brain tumor in children, is currently classified by four molecular subtypes: Sonic-hedgehog (SHH), WNT, Group 3, and Group 4. Each of these subtypes clusters based on a transcriptional profile and display different clinical prognosis and chromosomal aberrations. The development of these subgroups has progressed with the utilization of various techniques and compilations from multiple studies. Some of the landmark studies contributing to the establishment of these subgroups include Thompson (2006), Kool (2008), Cho (2010), and Northcott (2010) which are the studies summarized in Taylor (2012).

Thompson (2006) utilized unsupervised hierarchical clustering of gene expression profiles of primary medulloblastomas resulting in the identification of five subgroups. These subgroups displayed characteristic gene expression profiles indicating subgroup-specific genetic alterations. Real-time-PCR and IHC analysis confirmed the expression of WNT and SHH pathway members in two of the five subgroups. FISH analysis identified the source of WNT pathway gene expression to be mutations in CNNTB1 and SHH pathway gene expression was found to be driven by mutations in PTCH and SUFU. In this investigation, real-time-PCR analysis was also established as an accurate and rapid method for identifying tumors containing activation of SHH and WNT pathways.

Kool (2008) studied medulloblastoma at the mRNA expression level by Affymetrix Gene Chips and characterized genomic abnormalities by comparative genomic hybridization (CGH). Gene Ontology analysis suggested that cluster C and D tumors express genes marking a particular stage in neuronal differentiation or lineage with low cell cycle activity. Genes normally found in photoreceptor cells of the retina were significantly overexpressed in clusters D and E. CGH analysis revealed chromosomal aberrations specific to particular clusters which were also associated with significant differences in expression levels of the affected chromosomal domains. Clinical characteristics such as metastasis, age, and histology varied among the five clusters.

Cho (2010) utilized an unsupervised clustering algorithm based on NMF, a technique that identifies metagenes, to identify six subgroups of medulloblastoma. Identification of biologic pathways was performed by gene set enrichment analysis (GSEA) and revealed subclonal populations within subgroup c4 tumors contributing to their mixed gene expression signature. GISTIC analysis identified chromosomal aberrations present in each subgroup confirming many previous results. A unique finding in the study was the correlation of subgroup-specific miRNA profiles. Analysis by Kaplan-Meier revealed group c1 as having very poor prognosis as well as group c3/SHH tumors.

Northcott (2010) identified four medulloblastoma subgroups using unsupervised HCL of expression data and confirmed by prediction analysis of microarrays and consensus NMF. Patterns of genomic aberrations determined by GISTIC confirmed previous findings. Staining of medulloblastoma TMAs using antibodies for subgroup-specific signature genes, beta-catenin and GLI1 established a useful tool for subgrouping.

The Taylor (2012) consensus paper summarized the molecular subgrouping findings and established the four subgroups of medulloblastoma, represented here by **Figure 1** detailing the characteristics of each subgroup.



Figure 1 Diagram from Taylor (2012) summarizes clinical and genetic characteristics of the four medulloblastoma subtypes.

The development of mouse models applies this molecular and genomic data to create models of the disease that help identify driving mutations and other biological patterns. Research conducted by Gibson et al. led to the discovery of cellular origins of SHH and WNT subtypes through the development of mouse models and investigation of location-specific gene expression in the developing cerebellum. The effect of Cnntb1 mutation on ventricular zone cells and GNPCs was studied using the Blbp-Cre transgene and Atoh-Cre in conditional Ptch1 mice. Cnntb1 mutation was found to have no significant effect on the proliferation, apoptosis, cell-cycle, or differentiation control in GNPCs. In contrast to the SHH model, *Cnntb1* mutation resulted in the disruption of migration and differentiation of progenitor cells on the dorsal brainstem resulting in the accumulation of aberrant cell collections that persisted into adulthood. Mice with concurrent mutation of Cnntb1 and Tp53 resulted in tumors that displayed the immunoprofile and anatomical features of WNT tumors in humans. Analysis of patient MRIs was conducted to investigate location patterns based on molecular subtype. Six patients of each subtype, SHH and WNT, were analyzed by MRI based on tumor location (Supplemental Methods). Comparison of tumor/cavity distance from the brainstem showed that WNT tumors are closer to the brainstem than

SHH tumors. When comparing the position of the tumor/cavity relative to the midline, SHH tumors deviate from the midline more often than WNT tumors. These findings support the hypothesis that SHH tumors arise from the cerebellum and WNT tumors arise from cells of the dorsal brainstem.

Teo (2013) investigated the validity of the conclusion from the Gibson study that WNT and SHH subtypes have distinct cellular origins by testing location correlation to subtype in a large cohort of pediatric patients. Investigation of tumor location revealed that most tumors followed the patterns observed by Gibson et al. However, this analysis also revealed that 8 out of 17 SHH tumors were midline suggesting that tumor location is not an accurate method of distinguishing between medulloblastoma subtypes. Correlation of SHH tumor location and age revealed that hemispheric medulloblastomas are more common in infants and children younger than 9 years of age and midline MBs are more common in adolescent patients. These findings may suggest differences across age groups within medulloblastoma subgroups. The results of this investigation show that the location of SHH and WNT tumors reflect the complexity of this disease.

In an effort to further investigate the location patterns of medulloblastoma subgroups and to clarify previous findings, a larger group of patient images were analyzed by MRI (SHH (n=27), WNT (n=18), Group 3 (n=15), and Group 4 (n=18)).

Methods

MRIs for each patient were spatially normalized into a standard stereotaxic space for quantitative comparison of tumor location (SPM8; www.fil.ion.ucl.ac.uk/spm). *Radiologists* masked to patient subtype determined the three dimensional location of the tumor or surgical cavity relative to pre-defined anatomical landmarks using Slicer 3D (http://www.slicer.org/). Using three-dimensional coordinates of the tumor center, graphs were generated by plotting data points overlaying a representative patient MRI in R (http://www.rproject.org/). Composite post-operative images were generated using the ImCalc tool in SPM8.

Results

The results of this analysis confirmed previous findings regarding location patterns of WNT and SHH tumors. Most SHH tumors were found in the cerebellar hemispheres and if midline, often distanced from the brainstem. WNT tumors were found to be generally localized to the fourth ventricle. Graphics generated using location data display the center of each tumor for SHH and WNT tumors overlaid on a representative WNT patient MRI.



Figure 2 SHH (red) and WNT (green) tumors overlaid on a representative WNT patient MRI **a**) coronal view **b**) sagittal view **c**) axial view.

These localization patterns were also confirmed by composite post-operative images shown in **Figure 3**. The SHH post-op composite image shows minimal damage to the fourth ventricle and minimal damage to the cerebellum, since SHH tumors are not localized to a single location in the cerebellum. The WNT post-op composite image reveals damage to the fourth ventricle, which is consistent with the localization pattern of this subtype. Even though a portion of SHH tumors are medial and may invade the fourth ventricle, the lack of damage to the fourth ventricle shows that these tumors do not cause significant damage compared to WNT tumors. Comparison to the normalization template shows the accuracy of this technique.





Since little is known about the etiology of Group 3 and Group 4 subtypes, a similar approach was taken to see if patterns in localization of these tumors might provide evidence for differences in developmental origins. Plots for Group 3 and Group 4 tumors shown in **Figure 4** reveal that these subtypes are both localized to the fourth ventricle and there are no patterns to distinguish one from the other.



Figure 4 Location data for Group 3 (red) and Group 4 (green) overlaying Group 4 patient MRI **a**) coronal view **b**) sagittal view **c**) axial view

A qualitative approach revealed some visual patterns for these tumors, which may serve as evidence for subtypes within the molecular subgroups. These patterns may be relevant for subtypes of Group 3 and 4 subgroups.

Discussion

This investigation of location patterns supports previous findings concerning medulloblastoma subgroups and demonstrates the location patterns for Group 3 and Group 4 tumors. The analysis of SHH and WNT tumor location patterns in a larger group of patients shows that these patterns are consistent and not due to bias in the small set of patients previously studied. Within the large group of patients, there was variability in the quality of pre-operative images since these images were done at various institutions. Due to the variability in quality of these images, there is some error present in the location data. Some of the variability in the location of non-tumor landmarks is due to the normalization of images where ventricles were enlarged and structures were displaced by large tumors. In order to show that differences between SHH and WNT tumors are significant and not due to variability in cerebellar landmarks, Figure 5 demonstrates the variability in the non-tumor landmarks used for analysis.



Figure 5 mean and standard deviation of non-tumor landmarks overlaying the template used for normalization of patient images **a**) axial view **b**) sagittal view **c**) coronal view, Apex = apex of the cerebellum, PostBS = posterior brainstem at the fourth ventricle, AQ = aqueduct, AC = anterior commissure, LatL/R= most lateral points of cerebellum, and InfL/R = most inferior points of cerebellum

As demonstrated in this study and Teo (2013), SHH tumors are not exclusively found in the cerebellar hemispheres and can be midline. These findings further emphasize the heterogeneity of medulloblastoma even within subgroups, providing challenges to treating this disease. Robinson's commentary on Teo (2013) raises important questions regarding clinical applications of medulloblastoma subtypes and highlights some potential effects of applying subtyping to treatment of medulloblastoma. Knowledge of subtype prior to surgical removal of the tumor could allow SHH inhibitor to be administered to improve resectability and less intense treatment may be beneficial for WNT patients.

Ongoing research is investigating the relationships between localization, molecular markers, and developmental origins using mouse models for Group 3 and 4 medulloblastomas. A recent study investigated the most aggressive subtype of the disease through the development of mouse models. Kawauchi (2012) established MYC amplification as the driving force behind the aggressiveness of these tumors by showing that transduced cells with *Mvc* formed tumors that killed mice faster than Mycn and displayed a consistent LCA phenotype similar to human medulloblastoma. These tumors were also shown to express markers for stem-cell-like progenitor cells and embryonic pluripotency as well as signature genes similar to those reported for human medulloblastoma MYCsubgroup tumors. Analysis by MRI may provide insight into subtypes of Group 3 tumors based on amplification of *Myc*. Localization studies contribute to this ongoing research by providing a way to visualize the development of these tumors and finding correlations between molecular subgroups and imaging patterns. Translational research is vital to finding means of discriminating between subgroups in order to better tailor treatment for medulloblastoma. The newly opened SJMB12 protocol incorporates these molecular subgroups by specifying treatment for patients based on molecular and genetic data and risk. This exciting advancement for treatment of medulloblastoma will provide further understanding of the disease and hopefully move towards a cure for the most aggressive form of the disease and diminishing side-effects or treatment.

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The Evolution of Plant-Pollinator Mutualisms and Effects on Angiosperm Diversity

Eden Johnson

Abstract Several recent studies aimed to determine the selective pressures driving angiosperm floral trait evolution. Results indicated that, although natural plant populations are subject to selection pressures from a variety of biotic sources (e.g., herbivores, pollinators), pollinator-mediated selection of floral traits consistently exerts the most selection pressure on plant systems. Floral traits may evolve in response to pollinator behaviors, such as flower constancy or trait preferences, or by exploiting pollinators' foraging or mate-seeking behaviors. Floral adaptations may emerge via coevolution (pollinator preferences evolve concurrently with floral traits), sequential evolution (one species has a pre-existing bias for an adaptation that arises later in another species), or convergent evolution (distantly-related plant lineages develop similar floral characters in response to the same pollinator). Ancestral character state reconstruction using phylogenetic analysis is the main method used to answer questions about the mode of pollinator-mediated floral trait selection. Pollinator specificity and attraction have strong chemical and genetic bases to the extent that phylogenetic analyses have confirmed that plant speciation is frequently associated with a change in pollinators and chemical compounds. Understanding the driving forces behind angiosperm biodiversity is critical to unraveling the proximate and ultimate explanations for the adaptiveness of specific floral characteristics and slight phenotypic alterations.

Questions regarding the evolution of angiosperm floral diversity prompt research that aims to uncover the selective effects of various pollinators. herbivores, and competitors on floral morphology and overall plant fitness. Floral signals, such as pigments, patterns, shapes, or volatile emissions, may attract suites of pollinators or herbivores, but the adaptive advantages of floral signals depend heavily on whether the benefits provided by pollinators (e.g., pollen transport, seed dispersal) outweigh the costs of attracting herbivores (e.g., physical damage, reduced reproductive success). Similarly, the benefits of producing and maintaining signals to attract pollinators must outweigh the costs of competition to individual plants, such as the increased energy required to outperform surrounding plants, or the higher likelihood of herbivory for more visible plants. In environments where floral signals that attract pollinators are adaptive, however, plants evolve mutualistic or deceptive relationships with species-specific pollinators.

Natural pollinators range from insects like bees, wasps, moths, and beetles to birds and bats, all of which utilize foraging strategies, such as flower constancy and social learning, to optimize the rewards received (e.g., pollen, nectar) from plants in random, heterogeneous environments (Gegear & Laverty 2005; Baude et al. 2008). Natural selection favors plants that have the ability to exploit pollinator strategies and preferences, making pollinatormediated selection of floral traits the dominant method of diversification in plant lineages (Parachnowitsch & Kessler 2010; Bartkowska & Johnston 2012). Though the extent of pollinator selection on individual plants may differ in natural environments where numerous selective pressures act on individuals, research shows that herbivory generally has minor selection effects on plants compared to pollinator selection (Bartkowska & Johnston 2012). The incredible diversity of extant plants raises important questions regarding the adaptiveness and incurred fitness of plants with specialized traits; in order to address questions regarding floral evolution, research is necessary to uncover how plant interactions with biotic environmental factors, such as animals or other plants, affect the relative rates of floral trait selection and overall plant fitness. Experimental and phylogenetic results allow researchers to more thoroughly understand the proximate and ultimate mechanisms of floral signal evolution, which are important to pollination biologists, botanists, insect biologists, and evolutionary biologists alike (Schiestl & Johnson 2013). The present review addresses the various strategies employed by pollinators to distinguish the most rewarding plants in an area, pollination syndromes and their role in floral evolution, plant exploitation of pollinator biases, as well as the emergence of deceptive species in multiple plant lineages.

Pollinator Foraging Strategies

Pollinators gather information about a plant's contents and refill rate from notable characteristics of previously-rewarding plants, such as location, color, flower shape, or scent; furthermore, different pollinators rely on distinct cues to differentiate between various flowers in an environment (Marshall et al. 2012). Bird-pollinated flowers, for example, generally have a tubular shape and bright colors,

whereas bat-pollinated flowers generally have large inflorescences that are not brightly colored and supply large amounts of nectar (Specht et al. 2012).

Foraging hummingbirds mostly rely on spatial cues to determine where a rewarding flower or patch of flowers is located, despite the fact that birds are also able to form connections between color and presence or absence of nectar (Dudash et al. 2011; Marshall et al. 2012). In experiments with rufous hummingbirds (Selasphorusrufus) where flower color in combination with spatial arrangement provided the most information about reward presence, concentration, and refill rate, the birds preferred to visit previously-rewarding locations over a flower color that had been rewarding in the past (Marshall et al. 2012). The finding that location is a more salient cue for hummingbirds than flower color is not surprising given the tendency of nectar-producing flowers to form clumped distributions (Marshall et al. 2012). Thus, a spatially-focused bird may be better adapted to find resources than a bird who exhibits alternative foraging behaviors (Marshall et al. 2012). Alternatively, it may be more adaptive for pollinators that travel long distances in search of adequate food sources-or if food sources are randomly-distributed throughout the environment-to search for specific flower colors and morphologies instead of one flower's location.

Most species of bees have limited abilities to uptake and store information about multiple plants that differ in several characteristics, such as a patch in which each flower differs from its neighbor in color, scent, and morphology (Gegear &Laverty 2005). In order to forage effectively, the bees develop trait preferences and flower constancy for certain plants, but flower preferences serve as individual rather than species-wide biases (Gegear & Laverty 2005). By consistently visiting flowers of a particular shape and color, such as yellow, radially-symmetrical flowers, the bee is able to become a specialist on one type of flower instead of wasting energy by testing every yellow flower or every radially-symmetrical flower for rewards (Gegear & Laverty 2005).

Bees also utilize social information from conspecifics (e.g., visual cues, scent marking) in novel flower distributions to locate rewarding flower sources, and/or to avoid non-rewarding plants (Cartar & Real 1997; Worden & Papaj 2005; Kawaguchi et al. 2006, 2007; Leadbeater & Chittka 2007; Baude et al. 2008). Bees that are unfamiliar with a particular area or predominant plant species tend to search for rewards where a conspecific demonstrator forages, which generally indicates a rewarding food source (Cartar & Real 1997; Worden & Papaj 2005; Kawaguchi et al. 2006, 2007; Leadbeater & Chittka 2007; Baude et al. 2008). Pursuing demonstrators that are knowledgeable about the area enables naïve bees to forage more effectively in novel environments because most nectar-producing plants grow in clumps rather than random distributions (Baude et al. 2008). In contrast, bees that are familiar with an area or particular flower type tend to forage in unoccupied areas without assistance from a demonstrator (Kawaguchi et al. 2007; Baude et al. 2008). Furthermore, bees frequently leave scent markings on flowers once the resources are depleted, which signals to subsequent bees that visiting the flower may not result in a substantial reward (Baude et al. 2008).

Each pollination strategy-utilizing environmental cues, flower constancy, or social information-is implicated in the evolution of floral diversity. For example, floral reproductive success increases with pollinator constancy and preference because the pollinator prefers to visit the same type of flower (Baude et al. 2008). Plants that reproduce by cross-pollination require a way to transport pollen to conspecifics, or else the pollen is wasted and the plant's reproductive success decreases. Thus, plants that pollinators are more likely to prefer and remember on each foraging trip are selected for in the next generation. Selection of preferred plant phenotypes establishes a plant-pollinator mutualism, whereby plants need a specific pollinator for successful reproduction, and pollinators benefit from increased numbers of rewarding plants in the environment. Contrastingly, nectar production and volume can vary temporally both between and within species, which may be a floral adaptation to decrease continuous visits by an individual pollinator and instead encourage cross-pollination on different individuals of the same plant species (Marshall et al. 2012).

The Evolution of Pollination Syndromes

A plant has a pollination syndrome when it possesses several floral traits associated with attracting a particular group of pollinators (Table 1). Because pollinators are attracted to a specific set of floral traits, recent research aims to uncover the method of trait acquisition in diverse plant lineages that are pollinated by the same species of animal (Lengvel et al. 2010; Smith 2010; Zhang et al. 2010; Schiestl & Dötterl 2012; Specht et al. 2012). As stated by Schiestl & Dötterl (2012), the two main methods of pollinator-mediated evolution of floral traits are coevolution and sequential evolution. Coevolution is the process by which plants acquire novel traits and pollinators develop preferences for the plants' newly-acquired traits, such that both species evolve in synchrony with each other (Smith 2010; Schiestl & Dötterl 2012). If coevolution occurs in a plant-pollinator mutualism, the most recent ancestor of either species should not have had the floral traits or pollinator preferences of the extant taxa. Sequential evolution, on the other hand, occurs in situations where flowers develop adaptations to pollinators' pre-existing sensory preferences (Smith 2010; Schiestl & Dötterl 2012); an example of sequential evolution may include convergent evolution, whereby distantly-related plant lineages acquire similar adaptations to attract the same pollinator (Schiestl & Johnson 2013).

Tab	Table 1. Description of floral traits associated with various pollination syndromes (Pollinator.org).							
	Type of Pollinator							
Trait	Bat	Bee	Beetle	Bird	Butterfly	Fly	Moth	Wind
Color	White, green or purple	Bright white, yellow, blue, or UV	White or green	Scarlet, orange, red or white	Bright red and purple	Pale,or dark brown, purple	Pale red, purple, pink or white	Pale green, brown, or colorless
Nectar guides	None	Present	None	None	Present	None	None	None
Odor	Strong and musty; emitted at night	Fresh, mild, pleasant	None to strongly fruity or foul	None	Faint but fresh	Putrid	Strong sweet; emitted at night	None
Nectar	Abundant; somewhat hidden	Usually present	Sometimes present	Ample; deeply hidden	Ample; deeply hidden	Usually absent	Ample; deeply hidden	None
Pollen	Ample	Limited; often sticky, scented	Ample	Limited	Limited	Limited	Limited	Abundant; small, smooth
Flower Shape	Bowl shaped; closed during day	Shallow; with landing platform; tubular	Large and bowl- shaped	Large, funnel -like; strong perch support	Narrow tube with spur; wide landing pad	Shallow; funnel- like or complex with trap	Regular; tubular without a lip	Regular and small
		****		P		*		

Schiestl & Dotterl (2012) used phylogenetic analysis to examine patterns of volatile organic compound (VOC) evolution between plants (Arceae) and pollinators (scarab beetles), as well as to determine whether these patterns support coevolution or sequential evolution. Scarab beetles exhibit strong responses to VOCs because the chemicals signify appropriate oviposition substrates, so plants that emit VOCs are adaptive in the population (Schiestl & Dötterl 2012). However, the evolution of VOC sensory bias in scarabs is significantly older (before angiosperm evolution) than plant VOC production. This finding suggests that floral trait evolution via a pre-existing olfactory bias, is thus a pattern of sequential evolution (Schiestl & Dötterl 2012). Similar patterns of floral scent compounds exist in several other plant systems that scarab beetles pollinate, such as Nymphaeaceae, Magnoliaceae, and Arecaceae, which provides evidence of convergent

evolution between the various plant lineages (Schiestl & Dötterl 2012).

Similarly, Specht et al. (2012) used phylogenetic analyses to determine how speciation rates within the

plant order Zingiberales correlate with shifts in pollination syndromes, which are characterized by further floral reproductive specialization on a new group of pollinators (e.g., shift from bee-to-bird or bird-to-bat pollination). Results show eight points in evolutionary history where significantly increased rates of evolution are correlated with simultaneous shifts from insect to bird pollination syndromes (Specht et al. 2012). Thus, new floral adaptations that result in pollinator specialization may increase effectiveness of out-crossing, which may then enable plants to colonize new areas and further speciate (Specht et al. 2012).

Seed dispersal by ants (myrmecochory) provides an additional example of convergent evolution in plant lineages (Lengyel et al. 2010). The fact that myrmecochory, a plant-animal mutualism, has evolved independently multiple times in several distantly-related taxa suggests that the selection pressures for myrmecochory are high, or that the floral structures necessary for seed dispersal by ants are energetically cheap to produce (Lengyel et al. 2010). There are numerous benefits of

myrmecochory: ants provide plants with routes for dispersal by transporting seeds back to the colony, the colony provides protection from seed predators, and the new site serves as a nutrient-rich habitat where seed survival is high (Lengyel et al. 2010). Plants that are adapted for dispersion and that easily colonize new areas may be able to exploit the new site's abundant resources or novel pollinators, and may also experience reduced kin and intraspecific competition (Lengyel et al. 2010). Furthermore, reproductive specification that results in pollinator shifts is frequently associated with plant diversification and speciation, as depicted in phylogenetic analyses (Smith 2010; Specht et al. 2012; Schiestl& Johnson 2013).

The degree of floral specificity varies widely among angiosperms. Recent experimental studies show that the range of specialization may arise from dependence on multiple pollinators, which typically results in generalized floral morphologies, or a single pollinator, which often gives rise to sets of very specialized floral organs (Smith 2010). The main method employed by scientists to reconstruct ancestral character states in various plant lineages is phylogenetic analysis, which answers questions about how often floral adaptations correlate with pollinator switches, the mode of evolution in a given system (e.g., coevolution, sequential evolution, convergent evolution), and how the evolutionary transitions affect floral speciation and diversification rates (Smith 2010).

Exploitative Mimicry and Deception

Plants may mimic or exploit pre-existing pollinator preferences in order to increase reproductive success and individual fitness, such as through food-based or sexually-based deception, Batesian floral mimicry, or Müllerian floral mimicry (Roy & Widmer 1999; Schiestl & Johnson 2013). Although floral deception strategies have been widely studied, less evidence exists for the various forms of mimicry in plant systems because testing the adaptiveness of perceived similarities presents challenges (Roy & Widmer 1999; Schiestl & Johnson 2013). For example, scientists often categorize obvious visual similarities (e.g., color, morphology, patterning) as Batesian or Müllerian mimicry based on definitions from animal systems without actually testing the perceived mimic's incurred fitness benefits from resembling the model species (Roy & Widmer 1999). As it stands, a plant definitively mimics a model species when two or more species have overlapping distributions and phenology, share a pollinator that is required for both species' reproduction, and the similarities incur fitness benefits for both plants (Roy & Widmer 1999; Ellis

& Johnson 2010). Contrastingly, food-deceptive and sexually-deceptive plant species exploit pollinators' innate affinities for food, mating signals, or rewarding plant species through pollinator-mediated selection for deceptive traits that increase pollinator attraction and efficiency (Gumbert & Kunze 2001; Internicola et al. 2006; Peakall et al. 2010; Sletvold et al. 2010; Schiestl & Johnson 2013).

The mechanisms responsible for the evolution of Batesian mimicry are well-known and supported by empirical evidence, whereas the mechanisms involved in Müllerian mimicry in plants remain unresolved and understudied (Roy & Widmer 1999). An example of a well-supported case of floral Batesian mimicry is seen in Orchisboryi (Orchidaceae), a food-deceptive orchid that relies on insect pollination to reproduce (Gumbert & Kunze 2001). Because the bees that pollinate O. boryi do not specialize on particular flower traits (generalists), it is more likely that deception in O. boryi coevolved with neighboring rewarding plant species than as a result of the bees' preferences (Gumbert & Kunze 2001). Results from Gumbert & Kunze (2001) that nearly half of bee flower choices did not end in visitation suggest that more information is available to pollinators at close range that is not readily apparent from a distance, such as additional visual or olfactory cues. In general, non-rewarding mimics have lower volatile concentrations than rewarding plant species, which may be an adaptive deceptive plant strategy that discourages associative learning via salient olfactory cues in bees (Gumbert & Kunze 2001; Gegear & Laverty 2005). As mimicry models predict, reproductive success of O. boryi increases when it is rare in the population because the pollinator is unable to consistently distinguish the non-rewarding from rewarding species (Gumbert & Kunze 2001).

Sexually-deceptive plant systems are most wellknown from Orchidaceae examples, where extremely diverse flower morphologies mimic copulatory postures and pheromones of sexually-receptive females, which solicit pseudocopulations, and thus pollination, from sexually-receptive male wasps or bees (Peakall et al. 2010; Sletvold et al. 2010). There are very few confirmed reports of sexual deceit outside of Orchidaceae, but one study by Ellis & Johnson (2010) confirms sexual deceit in Gorteriadiffusa, a daisy in the Asteraceae family that is fly-pollinated. Petal spots on G. diffusa closely resemble the pollinator's body, and trichomes along the petal appear to act as tactile cues that attract male flies for pseudocopulations (Ellis & Johnson 2010). Deceptive plants that emit chemical compoundsusually compounds involved in insect communication-exhibit different strategies for attracting pollinators: although some individuals

produce and emit only one compound, others secrete multiple compounds, and still, other individuals release a unique blend of compounds to attract specific pollinators that are unique to a subset of individual plants in the population (Peakall et al. 2010).

It is important to note that plant systems that rely on food or sexual deception for pollinator visitation and out-crossing generally have more variable morphologies and chemical compositions than rewarding, non-deceptive species (Internicola et al. 2006; Peakall et al. 2010; Sletvold et al. 2010). Chemical variation, floral trait variation, and plant aggregation are critical to deceptive plant reproductive success because pollinators frequently exhibit associated and avoidance learning (Roy & Widmer 1999; Gumbert & Kunze 2001; Internicola et al. 2006; Sletvold et al. 2010). Thus, because pollinators avoid aggregations of non-rewarding flowers and distributions of plants where deceptive species are common, deceptive species benefit from 1) reproductive specialization to a specific pollinator, 2) chemical variation among individuals, which mediates alternative pollinator attraction to different members of the same species, and 3) floral trait variation, which promotes pollinator visits to multiple individuals of the same deceptive species and minimizes avoidance behavior (Gumbert & Kunze 2001; Internicola et al. 2006; Ellis & Johnson 2010; Peakall et al. 2010; Sletvold et al. 2010; Schiestl & Johnson 2013). Similarly, non-rewarding Batesian floral mimics, as opposed to rewarding Müllerian mimics, should exhibit substantial phenotypic variation because pollinators actively avoid mimetic phenotypes that are common in the environment, resulting in decreased fitness for both the model and the mimic (Roy & Widmer 1999).

Conclusion

A wealth of evidence supports pollinatormediated selection as the main biotic pressure driving angiosperm floral trait evolution, as in the case of sequential evolution of volatile organic compounds in scarab beetles and the Araceae plant lineage (Schiestl & Dötterl 2012); however, evidence also supports floral trait evolution via interspecific mimicry and pollinator deception, both of which exploit pollinator sensory biases in order to increase floral reproductive success. A commonality among plants that undergo pollinator-mediated selection and plants that utilize deceptive strategies to exploit pollinators is the fact that, in all cases, plant specification to novel pollinators stimulates increased rates of plant speciation and diversification, thereby contributing to the enormous diversity of extant plants (Lengyel et al. 2009; Ellis & Johnson 2010; Peakall et al. 2010;

Sletvold et al. 2010; Smith 2010; Zhang et al. 2010; Schiestl & Dötterl 2012; Specht et al. 2012; Schiestl & Johnson 2013). Although recent studies reveal a great deal about the evolution of floral diversity and factors that drive floral speciation, researchers know substantially less about how pollinators perceive their environments, particularly how olfactory cues influence pollinator decision-making. Considering the diverse ranges and concentrations of volatile signals in plants, pollinators may have a large array of olfactory receptors that are currently unknown. Research is needed to investigate the extent to which various pollinators utilize olfactory signals, as well as the effects of pollinator olfactory perception on floral trait evolution.

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A Comparison of Risk Factors and Incidence of Cancer in the United States and Denmark

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Abstract In an attempt to better understand the etiology of cancer, this study compared the risk factors and incidence rates of cancer in the United States and Denmark. It focused on three cancers: breast, cervical, and colorectal, which had higher incidence rates in Denmark compared to the United States, and attempted to relate these incidence trends to the relative rates of specific risk factors in each country. For breast cancer, the selected risk factors that increased risk were a stringent national screening protocol and being of white race. Having children prior to age 30 was selected as a factor that decreases the risk of breast cancer. American women are screened more thoroughly and have children younger, but fewer of them identify as white. The risk of developing cervical cancer is elevated by chlamydia infection and smoking, and lowered by HPV vaccination. More Americans are positive for chlamydia, but Danes have higher smoking and HPV vaccination rates. Colorectal cancer is characterized by risk factors that increase risk such as being of black ethnicity and consuming red meat increasing risk and eating fruits and vegetables. Exercising decreases risk. More Americans are black, but Danes have a less healthy diet, even though they exercise more.

Introduction

Cancer is a disease drawing increasing levels of global concern due to the almost 12.7 million new cases in 2008 (World Health Organization, 2013). The same year, specific types of cancer, such as breast and colorectal, also individually contributed to the health burden, with 1.38 million and 663 thousand new cases respectively (World Health Organization, 2013). Because of the enormous impact that cancer has across the globe, and because its epidemiology is so complex, any new information regarding the complexity of its cause is both relevant and valuable. Researchers are currently attempting to determine as many risk factors as possible, with varying degrees of success (American Cancer Society, 2013 a). For example, the correlation between cigarette smoking and lung cancer is strong enough to be considered causational, but only a few oncogenes and mutations have been linked with the development of brain cancer (American Cancer Society, 2013 a).

A new perspective can be gained by comparing cancer incidence in two seemingly similar industrialized, first world nations, the United States and Denmark. Although the incidence of many cancers in these countries is similar, there are others that differ significantly, such as breast, cervical, and colorectal (International Agency for Research on Cancer, 2008 a,b). This study seeks to assess possible correlations between risk factors and the difference in cancer outcome in the US and Denmark.

Method

The World Health Organization's (WHO) GLOBOCAN database was used to identify the cancer types with the 15 highest incidences per 100,000 people in populations that were agestandardized to the World Standard Population (ASR-W). This was done for both males and females in the United States and Denmark. Each type of cancer was defined using the WHO's 10th revision of the International Code of Disease (ICD-10), obtained from the GLOBOCAN database.

Table 1: ICD-10 for cancers with the 15 highest incidences in males and females in the United States and Denmark

Type of Cancer	ICD-10
Bladder	C67
Brain	C70-72
Breast	C50
Cervix uteri	C53
Colorectal	C18-21
Corpus uteri	C54
Kidney	C64-66
Leukemia	C91-95
Lip/Oral	C00-08
Liver	C22
Lung	C33-34
Melanoma	C43
Multiple myeloma	C88,C90
Non-Hodgkin's Lymphoma	C82-85,C96
Esophagus	C15
Ovarian	C56
Pancreatic	C25
Pharynx	C09-10,C12-14
Prostate	C61
Stomach	C16
Testis	C62
Thyroid	C73

The International Code of Disease is displayed for each of the types of cancer that have the 15 highest incidences for males and females per 100,000 of ASR-W population in the United States and Denmark. GLOBOCAN, 2008.

Age-standardized incidence rates (ASR-W) per 100,000 people were used, which allowed comparisons to be drawn between the United States and Denmark even though the populations of the two countries have different age distributions.

The data was separated by gender and the incidence values were compared using Microsoft Excel 2011. The difference in incidence was calculated by subtracting the incidence ASR-W in the United States from the incidence ASW-R in Denmark for each type of cancer. All cancers for which the difference in incidence was less than 5 per 100,000 ASR-W were excluded from the study.

The American Cancer Society (ACS) website was used to investigate known risk factors for each type of cancer with a difference in incidence of greater than 5 per 100,000 ASR-W. Three cancers that satisfied this criteria and which have known risk factors were selected for further investigation: breast, cervix uteri, and colorectal cancer.

The PubMed database and Google Scholar search engine were used to research earlier studies on the potential presence of each risk factor in the United States and Denmark.

Results

Incidence is reported as the number of new cases per 100,000 of the male (Figure 1) and female (Figure 2) ASR-W populations of the United States and Denmark. The cancers with the 15 highest incidences in each country are shown. For men in both the United States and Denmark, prostate, lung, and colorectal cancer have the first, second, and third highest incidences, respectively. Breast, lung, and colorectal cancers have the three highest incidences for women in the United States and Denmark. The red bars represent Denmark, and the blue bars represent the United States (Figures 1 and 2). Adopted from GLOBOCAN, 2008.







Figure 2- Incidence per 100,000 of the top 15 cancers in females, 2008

Incidence is measured as the number of new cases per 100,000 of the age-standardized male (Figure 3) and female (Figure 4) populations (ASR-W) of the United States and Denmark. The cancer types shown in blue have a difference in incidence between the United States and Denmark of >5 per 100,000 of the age-standardized population. Some types of cancer have a higher incidence in the United States, such as prostate and kidney cancer, but breast

and brain cancer have higher incidence rates in Denmark. The biggest differences in incidence are for breast cancer, which affects 76.0/100,000 ASR-W in the United States, compared with 101.1/100,000 ASR-W in Denmark and lung cancer, with an incidence of 83.8/100,000 ASR-W in the United States, which is much greater than the 72.5/100,000 ASR-W in Denmark. Adapted from GLOBOCAN, 2008.

Figure 3- Difference in incidence of top 15 cancers in males in the United States and Denmark, 2008.





Figure 4- Difference in incidence of top 15 cancers in females in the United States and Denmark, 2008.

Table 2-	Known	risk	factors	for	10	types	of	cancer
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	Known risk factors				
Type of cancer	Increases incidence	Decreases incidence			
Breast	Thorough screening protocol, gender: female, increased age, BRCA1/BRCA2 genes, race: white, high number of menstrual cycles, post-menopausal hormone therapy, high alcohol consumption, overweight	Having children before age 30, breastfeeding for 1.5-2 years, exercise			
Brain	Family history, radiation exposure	-			
Cervix uteri	Thorough screening protocol, smoking, oral contraceptives, HPV infection, Chlamydia infection, diet low in fruits and vegetables, obesity, first pregnancy prior to age 17, 3+ full-term pregnancies	Intrauterine devices, HPV vaccination			
Colorectal	Increased age, family history, race: black, diet high in red and processed meat, high alcohol consumption, overweight, smoking	Exercise			
Kidney	Family history, genes, obesity, smoking, asbestos exposure, high blood pressure	-			
Lung	Family history, smoking, asbestos and radon exposure, air pollution	-			
Melanoma	Gender: female, increased age, UV sun exposure, high number of moles, fair skin, light hair, freckling	-			
Prostate	Increased age, race: black, family history, genes, diet high in red meat and calcium, obesity, smoking	-			
Testis	Age 20-34, race: white, family history, HIV infection, undescended testicle	-			
Thyroid	Gender: female, increased age, family history, low-iodine diet	-			

Known risk factors for 10 types of cancer with a difference in incidence between the United States and Denmark >5 per 100,000 ASR-W are separated into factors that are known to increase incidence and those that decrease the incidence of each cancer. Some risk factors, such as smoking, increase the risk for numerous cancers, including cervical, colorectal, kidney, lung, and prostate cancer. Other risk factors, such as UV sun exposure and HIV infection only

affect the incidence of a single type of cancer, melanoma and prostate, respectively. For certain cancers, such as brain, only genetic changes have been implicated in causing tumors, but other types have strong associations with lifestyle related risk factors, such as poor diet and lack of physical activity increasing the risk of developing colorectal cancer. Obtained from the American Cancer Society, 2013.

Table 3- Known risk factors for breast, cervical, and colorectal cancers selected for further investigation

	Known risk factors				
Type of cancer	Increases incidence	Decreases incidence			
Breast	Thorough screening protocol, race: black	Having children before age 30			
Cervical	Chlamydia infection, smoking	HPV vaccination			
Colorectal	Race: black, diet high in red and processed meat	Diet high in fruits and vegetables, exercise			

Known risk factors for breast, cervical, and colorectal cancer separated by factors that increase incidence and those that decrease incidence of each cancer. Adapted from the American Cancer Society, 2013.

Discussion

The known risk factors for breast, cervical, and colorectal cancers were selected for further investigation because each of these types of cancer were characterized by a difference in incidence between the United States and Denmark of >5 per 100,000 ASR-W. Incidence was higher in Denmark for all three cancers, and only cancer in females was considered when further investigating the selected risk factors. Research on the selected risk factors was carried out to determine if variation in their prevalence was correlated with the difference in cancer incidence in the United States and Denmark.

Investigation of known risk factors by type of cancer:

Breast cancer:

One known risk factor that increases the incidence of breast cancer is a thorough screening protocol (American Cancer Society, 2013 b). Because the screen checks for early stage breast cancer, rather than pre-cancerous tumors, more rigorous screening guidelines should result in more cases of breast cancer being caught, and a higher incidence level. The American Cancer Society (ACS) recommends that women aged 20-40 have a clinical breast exam every 3 years and those older than 40 have a mammogram every year (American Cancer

Society, e). The Danish Cancer Society (DCS) is not as thorough, and does not start screening for breast cancer until women are 50 years old, and then only screens every other year until age 69 (Danish Cancer Society, 2013). American women are encouraged to be screened more than their Danish peers, so based on this factor alone, there should be a higher incidence of breast cancer in the United States than in Denmark, but this does not represent the whole picture. In Denmark, cancer screening falls under the national health insurance plan, so it is completely free for all women, and they are even assigned an appointment when they are due for one (Danish Cancer Society, 2013). One result is that Danish women do not face many of the barriers that prevent American women from adhering to the ACS's recommendations, such as cost and remembering to find a time to schedule an appointment every year. Additionally, since the Danish guidelines are less demanding on the patient, they are more likely to be followed. However, even though it is easier for Danish women to obey the DCS's protocol, there is much more negative publicity in Denmark concerning the negative mental effects of the high number of false positive diagnoses generated by mammograms (Danish Cancer Society, 2013). Unfortunately, it is presently not possible to obtain

data on the number of women who are actually screened for breast cancer each year in both the United States and Denmark.

Another risk factor that increases the incidence of breast cancer is being of white ethnicity. Although the reason is not presently understood, white women have a much higher risk of developing breast cancer than women with darker skin tones. The United States is characterized by significant racial diversity, with only 80% of the population identifying as white or Hispanic, which is much less than the nearly 99% in Denmark (Central Intelligence Agency, 2013 a,b). Therefore, based solely on this risk factor, it makes sense that there is a higher incidence of breast cancer among Danish women compared with American women. Unfortunately, although the racial composition of a country can change based on immigration and emigration, individuals cannot change their ethnicity, or their subsequent increased risk.

Additionally, high consumption of alcohol can also increase the incidence of breast cancer. The

drinking culture in Europe in general, and more specifically in Denmark, is much more relaxed than in the United States. There is no minimum age to consume alcoholic beverages, and 16-year-olds are legally able to buy beer (Denmark DK, 2013 b). In the United States, no one under the age of 21 can legally purchase or consume any type of alcohol. This difference in government protocol is reflected in drastically different drinking behavior between American and Danish females. For example, although women that do drink in each nation drink a similar amount, 8.45L of pure alcohol per year in America and 8.42L in Denmark, the amount of women that abstain from drinking, and do not consume any alcohol, is only 7.9% in Denmark, compared with 40.5% of American females (World Health Organization, 2011). This results in Danish females being at an increased risk of developing breast cancer when compared with American females.

Figure 5- Liters of pure alcohol consumed each year by females in the United States and Denmark, 2011.



The bars represent the average amount of pure liters of alcohol consumed each year by a female in the United States and Denmark that consumes alcohol (i.e. does not abstain). The average female in the United States consumes 8.45L of pure alcohol every year, and an average Danish female consumes 8.42L. Adopted from the WHO, 2011.



Figure 6- Percentage of females in the United States and Denmark that abstain from consuming alcohol, 2011.

The bars represent the percentage of all females in the United States and Denmark that abstain from drinking completely and do not consume any alcohol. 40.5% of females in the United States abstain, compared with only 7.9% of Danish females. Adopted from the WHO, 2011. Risk factors do not always increase incidence; they can decrease it as well. One such example is that having children before age 30 lowers the risk of developing breast cancer later in life. The exact biology is presently unclear, but it is known that the hormonal changes that occur during pregnancy can protect against the development of malignant tumors (American Cancer Society, 2013 b). In the United States, the most common age group to have children is 25-29 years old, followed by 20-24 (United States Census Bureau, 2013). However, in Denmark, mothers tend to be older, with 37 % not giving birth until they are 30-34 years old (Statistics Denmark, 2012).





The age of the mother at the time of birth of their child is grouped into 7 age brackets: <20 years, 20-24 years, 25-29 years, 30-34 years, 35-39 years, 40-44 years, and 45-54 years. The bars represent the percentage of all births that occurred in each age bracket. The most common age brackets for American mothers are 20-24 years and 25-29 years, but the more Danish women wait until they are 30-34 years old to have children. The blue bars represent American mothers and the red bars represent Danish females. From US Census, 2008 and Statistics Denmark, 2008.

There is currently a lot of media attention devoted to the young age of many mothers in America. Teen pregnancy is often portrayed in a negative light, but in this case, these young mothers could actually be protecting themselves against breast cancer.

One explanation of why Danes tend to be older when they have children is related to the welfare system. Because the system relies on a large workforce to supply the taxes that fund the system, it is very common for both parents to work full-time jobs. Women might choose to delay having children when they are involved in a permanent career rather than only homemaking, which is more common in the United States. The fact that a greater proportion of Danish women are having children later in their life, specifically after the age of 30 helps to explain why their population has a higher incidence of breast cancer, because they are waiting longer to experience the protecting hormonal changes that occur during pregnancy.

Cervical cancer:

Cervical cancer is another cancer that is a major cause of disease in both the United States and

Denmark, but has a higher incidence in Denmark, due in part to a number of risk factors. The human papillomavirus (HPV) is known to cause approximately 70% of cervical cancer cases (Printz, 2013), but a vaccine to prevent the development of HPV has been created, which decreases the incidence of both HPV and cervical cancer (American Cancer Society. 2013 d). The vaccine is available and encouraged in both the United States and Denmark, but its use varies drastically between the two countries. In Denmark, one study found that 80% of girls born in 1996 had received the first dose of the vaccine, 75% received two of the doses, and 62% were administered all three doses (Anonymous, 2011). This is dramatically different from the United States, where it is estimated that 75% of eligible women had not received even the first dose (Printz, 2013). One explanation is that under the national healthcare system in Denmark, the vaccine is provided free of charge, but Americans are required to pay at least a portion of the cost through an insurance co-pay, or completely out of pocket if they are uninsured. Depending on their plan, this bill could be a significant financial burden. With more open access to vaccination in Denmark and a much

greater portion of the population being protected as a result, it does not make sense that cervical cancer incidence is higher than in the United States. However, the vaccine is a relatively new development and there is a time lapse such that the cervical cancer cases that are developing now are likely in women who contracted HPV prior to the vaccine being invented.

One risk factor that further increases the incidence of cervical cancer is another type of sexually transmitted disease (STD), chlamydia. Chlamydia is one of the most common STDs in Denmark, with more than 470 new cases per 100,000 ASR-W each year (World Health Organization, 2007). However, even though Denmark has developed a reputation for having a very relaxed sex culture, it is the United States that has a higher incidence of chlamydia, of almost 540 per 100,000 ASR-W (Center for Disease Control, 2011 a). This could be a result of an abstinence-only sex education in America failing to emphasize the importance of practicing safe sex, while Danish youth are taught extensively not only about STDs, but also about testing and treatment options (Fischer 2013). As a result, Danish women may contract fewer cases of chlamydia by using condoms and encouraging their partners to be tested. This is another risk factor that does not explain Denmark having a higher incidence of cervical cancer.



Figure 8- Incidence of chlamydia in females in the United States and Denmark, 2007

 560

 540

 520

 520

 500

 480

 460

 440

 420

 USA

The bars represent the incidence of chlamydia per 100,000 ASR-W for females in the United States and Denmark. Chlamydia incidence in the United States is 540/100.000 ASR-W, and 470/100.000 ASR-W in Denmark. World Health Organization 2007, Center for Disease Control 2011. Smoking is a final risk factor that increases the incidence of cervical cancer. Figure 9 shows that Denmark is known for having a high prevalence of smoking among women, with 29% currently smoking, compared with only 16.5% of American women (World Health Organization, 2000, Center for Disease Control, 2011 b).







It is also worth noting that in Denmark, the rate of smoking is the same today as it was in 1970, and although the prevalence of smoking in the United States has decreased, it has always been lower than in Denmark. It is the women who were smoking in the 1970s and before that are developing cancer now, so it makes sense that there is a higher incidence of cervical cancer among Danish women (World Health Organization, 2000).

Colorectal:

Consuming a diet high in red or processed meat is a risk factor that further increases the incidence of colorectal cancer, while eating fruits and vegetables can decrease the incidence (American Cancer Society 2013 d). Although it is difficult to directly compare the average diet of the two countries due to significant amounts of variation, it is still possible to observe trends. In the United States in 2000, 113.5 pounds per capita of red meat were ingested (United States Department of Agriculture, 2000). This is much lower than Denmark's consumption, which is the highest in the world, at 321.6 pounds per person, so it makes sense that there is a higher incidence of colorectal cancer among Danish women when compared with American women (Brown, F 2009). Adding to the trend, Americans consumed 428.3 pounds of vegetables in 2000, an amount that has been increasing steadily since the 1970s, and was already much higher than the only 254 pounds of vegetables consumed by Danes in 2012 (United States Department of Agriculture, 2000, European Food Information Council, 2012). Considering America's reputation for consuming large quantities of fast food, and having enormous portion sizes, it is surprising than Danes as a whole actually have poorer dietary habits concerning both meat and vegetable intake. This is due in part to traditional and typical meals, such as pork and potatoes and openfaced sandwiches, which often include sausage and liver (Denmark DK, 2013). Based on dietary factors, it makes sense that Denmark has a higher incidence of colorectal cancer when compared with the United States.

Along with a poor diet, physical inactivity can also increase the incidence of colorectal cancer. Danish women are more physically active than those of any other European country, with only 34% not exercising at all (NY Daily News 2013). This is due in part to the Danish economy, because the 180% tax on cars encourages alternate modes of transportation, including biking, which counts as physical activity. In America, the situation is much less positive, with over 50% of women failing to meet the guidelines for both aerobic and muscle-strengthening exercise in 2008 (National Center for Health Statistics 2012). The relatively high amount of physical activity among Danish women should protect them against colorectal cancer, but that is not the case, evidenced by a higher incidence rate than in the United States.

An additional risk factor, being of black ethnicity, can also increase the ethnicity of colorectal cancer. Since less than 1% of the Danish population identifies as black, many fewer Danes are at risk based on this factor than the 13% of Americans that are black (Central Intelligence Agency 2013 a,b). As with the opposite trend that being white increases the risk of developing breast cancer, race is not a factor that can be changed. The heightened incidence of colorectal cancer among Danish women is due to a characteristic other than their race.

Strengths

One strength of this study was that the same source of information was used for both countries whenever possible, which makes it less likely that unintentional variables confounded the results. For example, the GLOBOCAN registry was used to obtain all data on cancer incidence, racial data was from the CIA World Factbook, and the World Health Organization provided information on smoking and alcohol consumption for both the United States and Denmark. When it was not possible to use the same source, all efforts were made to obtain similar data. by using such related sources as the American Cancer Society and the Danish Cancer Society, or by using data from different sources collected the same year. This was the case for the statistics for chlamydia incidence, smoking rate, alcohol consumption, and age of mother at time of birth.

An additional strength was that all statistics were age-standardized to the World Standard Population, which makes it possible to compare data between the United States and Denmark.

Limitations

One limitation was that although all efforts were made, it was not always possible to get comparable data for both nations. For example, there is no record of the number of women who get screened for breast cancer in the United States, so there is no figure for the comparison to the Danish statistic. The same issue arose when comparing data on physical activity because each country has a different standard that constitutes being active. It was sometimes not possible to obtain data for only women; one case was the dietary habits of each nation. For alcohol consumption, only the amount of alcohol and the percentage of people who consumed it were compared, but adding additional variables, such as the type of alcohol or the timing of consumption would result in more meaningful findings. For the

incidence of chlamydia, it was only possible to compare known cases, but many cases are asymptomatic, and therefore undiagnosed. For smoking data, only the number of current smokers was used, rather than the quantity of cigarettes smoked by each individual and in each country. The most significant limitation was the time constraint that made it impossible to investigate all of the known risk factors for every type of cancer. Additionally, there are still many risk factors and causes of cancer that have yet to be discovered, including what triggers genetic changes, and until that is done, it is clearly not possible to determine their relative level in each country.

Ethics

The author declares not having any conflicting interests. This study is based on secondary register data and therefore does not require permission from any data inspection board.

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Signature Whistles of Bottlenose Dolphins (*Tursiops truncatus*) are Necessary to Maintain Group Social Structure

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Abstract: Bottlenose dolphins (Tursiops truncatus), like other odontocetes, maintain a strong, tight-knit, social group. To maintain their social group, they use a variety of vocalizations: echolocation, burst pulse sounds, and whistles. Of these vocalizations, whistles are the most effective for communication between individual dolphins. Signature whistles are unique to each dolphin and allow individuals to identify each other as well as communicate their location. Calves draw influences from other members of the community to form their signature whistle, giving each community of bottlenose dolphins a distinct pattern of signature whistles unique from other groups. Whistles are associated with specific behaviors and allow dolphins to communicate during feeding, milling, traveling, and socializing. They also allow separated individuals to rejoin each other by giving information about location. Whistles also are indicators of location of food, predators, and distress. It is the wide variety of whistles used between bottlenose dolphins that allow the dolphins to maintain such a close social group.

Introduction

Cetacea is the taxonomic order for whales (suborders Mysticeti and Odontoceti), dolphins (families Delphinidae, Iniidae, Lipotidae and Plantanistidae), and porpoises (family Phocoenidae), which are separated into baleen whales and odontocetes (Tyack 1986). Odontocetes is the taxonomic suborder for the closely related dolphins, porpoises, and toothed whales (Tyack 1986). While baleen whales have only brief social interactions, odontocetes function in much more stable groups that are coordinated and maintain contact (Tyack 1986; Connor et al. 1998). Larger groups are composed of smaller subgroups where the individuals remain together even if the larger group breaks apart; although in some species, such as the bottlenose dolphin (Tursiops truncatus), individuals change subgroups (Würsig 1978; Tyack 1986; Connor et al. 1998). This characteristic shows that there is a strong association between individuals in the odontocete suborder (Tyack 1986).

While females in the groups tend to stay with the main herd and foster a strong mother-calf relationship, males separate from the main group and join juvenile groups (Tyack 1986; Connor et al. 1998). In some species, juvenile groups consist of juvenile females as well (Tyack 1986). These groups are strongly invested in each other, and will care for an injured or distressed individual, even if it is a member of another odontocete species (Tyack 1986). When threatened by predators, adults of the groups will surround calves and their mothers in a tight group for protection (Tyack 1986). Specific species of the family Delphinidae also organize foraging and share information about locations of food, and even share the food itself (Conner & Norris 1982; Tyack 1986). There is evidence for relationships between individuals that are often fostered by reciprocal altruism (Conner & Norris 1982; Tyack 1986;

Connor et al. 1998). With these close relationships among individuals there has to be a strong level of communication to maintain the groups, which is done through sound vocalizations (Tyack 1986; Connor et al. 1998).

Odontocetes, specifically bottlenose dolphins, are known to produce three types of sounds: echolocation clicks, burst pulses, and whistles (Oswald et al. 2008). Echolocation allows odontocetes to sense the environment around them by producing a sound and listening to how it echoes off of the surrounding areas (Branstetter et al. 2012). Burst pulses are a series of clicks with a very short time interval between clicks; these clicks are so quick that humans can only hear the rate of the sound (Oswald et al. 2008). Individual dolphins have been observed using a variety of whistles, each one for a specific type of communication (Herzing 1996). Whistles are not formed from moving mouthparts so it is difficult to determine which dolphin made the sound in a group (Smolker et al. 1993). Variant whistles are not specific to individual dolphins and are used in a variety of social situations (May-Collado & Wartzok 2008). A signature whistle is the most often-used and specific whistle pattern observed from an individual while it is not around other dolphins, and is unique to each individual and used in a variety of situations (Watwood et al. 2005; May-Collado & Wartzok 2008).

There are variations in how frequently signature whistles are used. Hawkins & Gartside (2009) found variant whistles are used more frequently than signature whistles in social settings; whereas, Cook et al. (2004) found that signature whistles and variant whistles were each used about fifty percent of the time socially in wild free-ranging bottlenose dolphins. Signature whistles are often used for communication between mothers and calves to ensure they are not separated, as well as for communication between parents and other caretakers (Herzing 1996). Additionally, signature whistles are used during courtship, when the male is attempting to attract a female (Herzing 1996).

Understanding how social behaviors among intelligent and social mammals, such as bottlenose dolphins, can give insight into how these behaviors evolved (Connor et al. 1998). Knowing how such behaviors evolved could also give insight into the evolution of communication in other taxa, such as primates, which includes humans (Connor et al. 1998). Additionally, knowing how bottlenose dolphins communicate will minimize stress conditions in other experiments and maximize the information received from the study (Esch et al. 2008). This paper reviews how signature whistles are developed in individual dolphins, how different patterns of whistles are used, and under what circumstances signature whistles are used to communicate between individual dolphins.

How Signature Whistles Are Formed

The number of signature whistles a pregnant female makes is more than that of a non-pregnant female, and it increases as birth approaches (Mello & Amundin 2005). This increase in number of signature whistles could be due to increased restlessness, which is higher in primiparous females than it is in multiparous females (Mello & Amundin 2005). Additionally, the peak in number of whistles right before delivery could indicate the coming birth (Mello & Amundin 2005). Furthermore, it could be a method to introduce and familiarize the calf with its mother's signature whistle before the calf is even born (Mello & Amundin 2005).

Neonates produce their first whistles by an hour and a half after birth, and the characteristics of the whistles change throughout the day (Morisaka et al. 2005). Beginning frequency, end frequency, minimum frequency, and maximum frequency, as well as frequency range, increase by the hour until 8 hours after birth, but decrease by the hour from 14 hours after birth (Morisaka et al. 2005). Whistles vary among neonates and between neonates and adults (Morisaka et al. 2005). Frequency range and maximum frequency of the whistles are much higher in adults, meaning the whistles of the calves could change over time (Morisaka et al. 2005). Neonates cannot hold the same amount of air as adults; therefore, their whistles are shorter during dives, and the whistles increase in length as air capacity increases (Morisaka et al. 2005). Because all signature whistles are unique, calf whistles are not identical to the whistles of others dolphins, but are influenced by whistles of other adults they have heard (Fripp et al. 2005). Signature whistles of calves are most similar to the whistles of adult dolphins of their community, which shows that calves model their whistles off of the ones they hear most frequently (Fripp et al. 2005).

There is a stronger association between calves modeling whistles after females than after males (Fripp et al. 2005). Signature whistles are not similar to that of the calf's mother or the dolphins the calf interacts with the most frequently, but are from those adult dolphins with which they have fewer social interactions within their community (Fripp et al. 2005). Calves pull influence from an average of 6 other bottlenose dolphins to create their signature whistles (Fripp et al. 2005). Modeling whistles from various members ensures that the signature whistle is distinct to the calf, but ensures that the other dolphins in the community will be able to recognize the calf (Fripp et al. 2005; Harley 2008). Because calves model their signature whistles off adults' whistles, the signature whistles of community members are more similar to each other than to members of other communities (Fripp et al. 2005). Completely copying the signature whistle of another dolphin rarely occurs; whistles are usually a mixture of influences (Janik et al. 1998; Fripp et al. 2005). The same signature whistles have been observed being used by the same individual for more than 15 years, and the usage varies by time of day, as well as social behavior (Watwood et al. 2005).

Whistle Patterns

In a study of captive bottlenose dolphins, Therrin et al. (2012) found whistle frequency increased through the day and peaked in the late afternoon. Whistle type and frequency can vary between feeding sessions, but the number of surrounding dolphins during the individual's feeding does not have an effect on the number of whistles a dolphin emits (Hawkins & Gartside 2009). Number of surrounding dolphins also has no effect on the number of different whistle types from one individual (Hawkins & Gartside 2009). There is, however, a strong correlation between the rate of repetition of the whistle and the number of surrounding dolphins during feeding (Hawkins & Gartside 2009). Duration and volume of surrounding noise also have an effect on signature whistles (May-Collado & Wartzok 2008). More boats, or ambient noise, surrounding the bottlenose dolphins increased the frequency and duration of the signature whistle (May-Collado & Warzok 2008). In contrast, in captivity, dolphins show a reduced rate of whistles when ambient noise, such as scrubbing the sides of the enclosures, is louder than usual conditions (Therrien et al. 2012). This decrease in whistle rate could be because it is difficult to communicate over the noise, therefore

whistles do not occur, or the dolphins' activities during the cleaning do not require whistles for communication (Therrien et al. 2012).

Different whistles are associated with different behavioral activities (Hawkins & Gartside 2009). Bottlenose dolphins produce the highest number and diversity of whistles during milling behaviors and they produce the least diversity and number of whistles during pod separation (Hawkins & Gartside 2009). Because bottlenose dolphins stay in sight of each other during travelling, there is a lower frequency of signature whistles than during milling or socializing (Cook et al. 2004). The frequency of variant whistles is also lower during traveling than it is during socializing, milling, or feeding (Cook et al. 2004). Most variant whistles do not have a strong association with a typical behavior (Hawkins & Gartside 2009). There is, however, a correlation between type of variant whistle and foraging activities and social behavioral activities. There is also a difference in the repetition rate of the whistle with respect to location (Hawkins & Gartside 2009). In contrast, there is a high correlation between signature whistles and type of behavior, where socialization behavior has the highest number of whistles compared to other behaviors, showing that whistles are an important method of communication between dolphins during various social situations (Hawkins & Gartside 2009).

Use of Signature Whistles as Contact Calls

Bottlenose dolphins can distinguish between the signature whistles of other dolphins and from which dolphin the whistle was produced, indicating that signature whistles can serve as a means for identifying other dolphins (Janik et al. 1998; Harley 2008). Because they are not specific to an individual, variant whistles do not serve as identifiers for other dolphins (Harley 2008). Bottlenose dolphins are able to track multiple whistles at a time while associating the whistles with the dolphins that produced them (Harley 2008). Watwood et al. (2005) found that some of the signature whistles used while the dolphin was restrained were also used while the dolphin was free ranging. Pairs of male bottlenose dolphins use fewer signature whistles than either single males, or a group of two males and a female (Watwood et al. 2005). Males separated from their partner were most likely to use signature whistles for communication, with a pair of males and other females producing fewer, but not as few whistles as two males (Watwood et al. 2005). In contrast, females separated from other groups of females use signature whistles as they reunite (Smolker et al. 1993).

Although calves typically do not produce signature whistles when with their mother, signature

whistles are used to reunite mothers and calves (Smolker et al. 1993; Mello & Amundin 2005). The frequency of signature whistles from the calf increases with distance and time the calf is separated from its mother, and the whistles ceases when the calf and mother are reunited: whistles are more likely to occur from the calf before, rather than after, reunion of the mother and calf (Smolker et al. 1993). The calf's whistles tend to occur towards the end of the separation, meaning they facilitate the reunion, and the mothers produce more whistles per minute than the calf after being reunited (Smolker et al. 1993; Mello & Amundin 2005). When forced separation between mothers and calves occurs, mothers whistle with a much higher frequency than when separated during free-ranging activity, which was also seen when mothers were separated from their calves by herding males (Smolker et al. 1993).

Smolker et al. (1993) found that the mothers whistle some during the separation, but not as frequently as do calves, whereas Mello & Amundin (2005) found mothers emit more whistles per minute than calves. Because there are variations on whistles produced by bottlenose dolphins of various communities (Fripp et al. 2005), the difference could be due to a difference in location. Furthermore, the bottlenose dolphins studied by Mello & Amundin (2005) were in captivity, whereas the bottlenose dolphins Smolker et al. (1993) studied were freeranging. During their first capture of capture-release experiments, some dolphins were still dependent calves, meaning their increased calls could be contact calls while they were trying to rejoin their mother (Smolker et al. 1993; Esch et al. 2009). Though these findings seem to indicate signature whistles can indicate stress level, the increase in signature whistles could be due to increased communication efforts between captured dolphins and free-swimming dolphins (Esch et al. 2009). Infants use signature whistles when separated from their mother to give the mother their location, to induce a response from the mother that might give her location, and to prompt cooperation from the mother, like waiting for the infant to rejoin her (Smolker et al. 1993). Overall, signature whistles are more likely to occur in a situation where the dolphin is separated and trying to rejoin the group, whether as a way of communication between individuals or as a way of expressing emotions, such as stress (Watwood et al. 2005; Harley 2008).

Whistles as Indicators of Stress

Because bottlenose dolphins are so often studied through capture-release, Esch et al. (2009) studied the stress response from the process. Although studying bottlenose dolphins in the wild is difficult

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methodologically, even short-term captivity can cause stress (Esch et al. 2009). The stress from captivity could alter signature whistles of bottlenose dolphins (Smolker et al. 1993). Vocalizations have been found to vary with the level of stress on the animal (Esch et al. 2009). In one study, Esch et al. (2009) found an increase in rate and number of loops of signature whistles during capture-release than during normal conditions. Whistle rates slowed through the duration of the capture-release, but were still not the same as in undisturbed conditions (Esch et al. 2009). In all, Esch et al. (2009) found signature whistles to be 90 percent of the vocalizations of captive bottlenose dolphins compared to the 50 percent of vocalizations for free-ranging bottlenose dolphins. Females had higher signature whistle rates than did males, which could be due to the fact that males do not travel in large groups in the way females do; therefore, they would not use contact calls as frequently (Watwood et al. 2005; Esch et al. 2009)

Additionally, Esch et al. (2009) found that whistle rate decreases with subsequent capturerelease experiences, suggesting that stress decreases as the capture-release process becomes more familiar. In addition, Esch et al. (2009) observed a decrease in whistle rate with increased age, which could mean that age, in addition to stress, changes signature whistles, which was also supported by the findings of Morisaka et al. (2005). During their first capture experience in the study, many of the dolphins were very young, so the change in whistle frequency and number of whistle loops in subsequent capture situations could also be a factor of maturity (Esch et al. 2009). Females with dependent calves also produced higher frequency whistles than females without dependent calves (Smolker et al. 1993; Esch et al. 2009). Overall, Esch et al. (2009) found that signature whistle rate is an indicator of stress in bottlenose dolphins. For example, the increased frequencies could be an attempt to communicate something other than usual social communications and identifications (Esch et al. 2009). Additionally, the decreases in frequency of signature whistles through the period of captivity could be due to fatigue, not adjustment to captivity (Esch et al. 2009). However, because during their first capture-release experience dolphins struggle and tangle themselves in the net, which is something they do not try on subsequent captures, there is an indication that they are under some stress (Esch et al. 2009).

Conclusion

Odontocetes, specifically bottlenose dolphins, have highly socialized groups, which require a strong level of communication among members (Tyack 1986). Of the three vocalizations used, signature

whistles are the most useful for distinguishing and identifying individual members (Watwood et al. 2005; May-Collado & Wartzok 2008). Signature whistles are modeled off of up to six other members of the community to ensure that no two whistles are the same (Fripp et al. 2005). Whistle use varies with behavioral activities, such as feeding, milling, traveling and socializing (Hawkins & Gartside 2009). They can also be used as contact calls between dolphins when they are separated to give one's location and identification (Smolker et al. 1993; Mello & Amundin 2005; Watwood et al. 2005). Signature whistles have also been identified as indicators of stress in capture-release research methods (Esch et al. 2009). Future research should examine signature whistles in even more behavioral situations. There are still types of communication among adults that are not fully understood, such as leaving and rejoining groups. More detail on signature whistles during foraging and mating would be useful in completely understanding how signature whistles are used in these tight social groups. Additionally, there is not an understanding of if and how bottlenose dolphins use signature whistles to communicate with other communities of bottlenose dolphins, or with groups of other odontocetes. Whistles provide bottlenose dolphins with a distinct way to communicate with each other in a variety of situations and allow them to maintain their close group social structure.

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Evaluation of the Simplexa Flu A/B & RSV Direct Assay[™], Prodessa Proflu^{+™} Indirect Assay, and Rapid Influenza Antigen Test for Detection of Influenza and RSV.

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The symptoms of influenza and respiratory syncytial virus (RSV) plague hundreds of thousands of Americans every year. Treating these sicknesses begins with a timely and accurate diagnosis. It is greatly disputed which diagnostic test maximizes accuracy of diagnosis and efficiency. The two most common types of tests used by molecular diagnostic laboratories to diagnose influenza and RSV include rapid influenza antigen tests (RIDT) and real time polymerase chain reaction tests (PCR). We measured the specificity, sensitivity, and inhibition of these tests by running over 200 clinical patient samples. We found that the sensitivity and specificity of two real time PCR tests was significantly greater than the rapid influenza antigen test. Among real time PCR diagnostic tests are direct and indirect assays. We found that an indirect real time PCR test suffered much more inhibition, as the direct assay was able to report results on 75% of the samples that the indirect assay was unable to diagnose. However, the direct real time PCR test also reported significantly lower sensitivity than the indirect real time PCR method on samples positive for flu A, flu B, and RSV. We discovered that by extracting the nucleic acid during the indirect assay, PCR was performed on a greater concentration of the desired target product, which resulted in greater sensitivity. In addition, an indirect assay provides more exposure to RNases causing greater inhibition. These findings reveal the strengths and weaknesses of three diagnostic tests. This allows laboratories to choose which test best suits their specific sample population.

Introduction

The United States experiences an average of approximately 200,000 hospitalizations and 28,000 deaths per year resulting from the influenza virus (CDC, 2009). Among those individuals most susceptible to infection are young children and elderly adults (Falsey et al., 2005). The influenza sickness stems from three immunologic types of RNA viruses (A, B, and C). The different core protein of each type of RNA virus accounts for the difference among the three types (SIRD; Focus Diagnostics, Cypress, CA). Specifically, influenza A and B are the RNA viruses characteristic of the dreaded seasonal flu. Another respiratory RNA virus, the respiratory syncytial virus (RSV), is the leading cause of hospitalization for infants. It is a lower tract respiratory infection that causes cold-like symptoms (SIRD; Focus Diagnostics, Cypress, CA).

Because of the widespread effect and severe symptoms of these respiratory viral infections, Children Hospital clinics devote much time, space, and effort to testing and diagnosing these infections. This study was conducted at Le Bonheur Children's Hospital in Memphis, Tennessee, where several molecular methods are used to test patients for RSV and Influenza A, B, and C. These tests include rapid influenza diagnostic tests (RIDT) and real-time PCR testing. Among real-time PCR methods, there is also variation. For example, direct assays are used, which perform PCR and detection directly from a sample. Indirect assays also exist, which first extract RNA from a sample of cells before amplification and detection with PCR.

The aim of this study was to compare both the sensitivity and specificity of an RIDT with that of two different real time PCR methods in the molecular diagnostic lab at Le Bonheur Children's Hospital. In addition, the purpose of this research is to contrast the sensitivity and inhibition of a direct real-time PCR test versus an indirect real-time PCR test. The direct PCR assay studied was Simplexa Flu A/B & RSV Direct (SIRD; Focus Diagnostics, Cypress, CA). The indirect PCR assay studied was the Prodesse Proflu+ (Proflu+; Hologic, Bedford, Ma).

Based off of a study done by Paxton (2013) where 12 RIDT's were shown to have inconsistent specificity and low sensitivity, we would hypothesize that the rapid influenza antigen test at the molecular diagnostic lab of Le Bonheur Children's Hospital would also have extremely low sensitivity and specificity compared to a real time PCR detection method. Thus, when compared to real time PCR methods, many of the RIDT's negative samples are expected to actually be false negatives due to low sensitivity, and many flu positives are expected to be false positives because of low specificity. In comparing the sensitivity and inhibition of the SIRD direct assay versus the Proflu+ Indirect Assay, we would predict the SIRD direct RT-PCR assay would have less inhibition, but also less sensitivity than the Proflu+ indirect PCR method. This hypothesis is made based off the fact that the sample input required for the Simplexa direct assay is only 50 microliters compared to 200 microliters for the Proflu+ assay. This volume discrepancy effectively means that the Simplexa Direct Assay inputs much less inhibitors but also less target RNA for amplification. We expect to observe the SIRD to have less internal control fails

Methods

In order to conduct this study, over 400 nasopharyngeal swab specimens in viral transport medium were collected from patients at Le Bonheur Children's Hospital. These specimens were collected from September 2012 to March 2013. Before receiving each sample, the sample first underwent a rapid influenza diagnostic test. This RIDT utilized a standard immunoassay, in which Flu A and B antigens were detected using a specific antibody. Results were reported after the reaction ran for 15 minutes. The specimens were then stored at 4 degrees Celsius for one week at which time PCR would be performed on the sample before being transferred to a -20 degree Celsius freezer. To perform the Prodesse Proflu+ indirect real time PCR assay, 200 microliters of nucleic acid of the sample was first extracted using the NucliSENSE easy MAG. Following extraction, the real time PCR reaction was performed on the Smart Cycler according to the package insert. For the SIRD, 50 microliters of specimen was added directly to the well of a PCR disk. Each PCR disk contains eight wells for eight specimens to undergo the PCR reaction per run. Following the completion of both types of real time PCR assays, the crossing thresholds were measured by utilizing the computer software as described in the package insert.

due to inhibition, but also to amplify at lower CT (crossing threshold) values due to less sensitivity.

Results

Specificity and Sensitivity of RIDT vs. RT-PCR methods:

Out of over 150 samples, the RIDT proved to have significantly lower specificity when compared to the real time PCR result. It was found that the rapid test reported 18 false positive flu results out of 34 total samples. Thus, only 16 of these samples that the rapid test determined to be positive were actually positive for the influenza virus. In addition, it was found that the RIDT suffered extremely low sensitivity when compared to the sensitivity of a real time PCR assay. Both the sensitivity and specificity of the rapid antigen test compared to RT PCR methods are shown in Tables 1 and 2 below. **Fig 1: Rapid Influenza Diagnostic Test Accuracy for Flu A**

Flu A +	Rapid Influenza Diagnostic Test (RIDT)			
SIRD/Prodesse RT-PCR	Positive	Negative	Total	
Positive	14	90	104	
Negative	4	48	52	
Total	18	138	156	

RIDT sensitivity for Flu A: 48 true negatives/138 negative results = 34.7% sensitivity

RIDT specificity for Flu A: 14 true positives/ 18 positive results = 77.8% specificity

Fig 2: Rapid Influenza Diagnostic Test Accuracy for Flu B

Flu B +	Rapid Influenza Diagnostic Test (RIDT)			
SIRD/Prodesse RT-PCR	Positive	Negative	Total	
Positive	2	22	24	
Negative	15	116	131	
Total	17	138	155	

RIDT sensitivity for Flu B: 116 true negatives/138 negative results = 84.0% sensitivity

RIDT specificity for Flu B: 2 true positives/ 17positive results = 11.8% specificity

Inhibition and Sensitivity of direct RT-PCR assay vs. indirect RT-PCR assay:

It was found that the SIRD direct assay suffered 75% less inhibition than the indirect Prflu+. Of the 36 samples that the ProFlu+ indirect PCR assay failed to perform PCR on because of inhibition, the Simplexa Direct assay reported PCR results on 27 of these samples (all negative). However, it was found that the SIRD direct assay also experienced low sensitivity compared to the ProFlu+ indirect assay. For these two PCR methods, viral detection sensitivity was measured in cycle threshold, known as CT value. This CT value represents the cycle of a PCR reaction, in which the target nucleic acid signal can be amplified. As seen in the table below, the ProFlu+ indirect PCR was able to amplify Flu A, Flu B, and RSV specimens at lower CT values, revealing higher sensitivity than the SIRD PCR reaction (table three).

Table 3: A comparison of CT values for Prodesse**RT-PCR** and **SIRD RT-PCR**

	Flu A Ct	Flu B Ct	RSV Ct
	average	average	average
Pro Flu	29.79	25.60	24.13
SIRD RT-PCR	32.51	31.35	27.75

The average crossing threshold for SIRD RT-PCR is lower than that of Prodesse RT-PCR for samples positive for Flu A, Flu B, and RSV. Note: 5 samples positive for Flu a by the ProFlu+ assay were negative for Flu A by SIRD. In addition, 1 sample positive for Flu B by the ProFlu+ assay was negative for Flu B by SIRD.

*Note: 5 samples positive for Flu a by the ProFlu+ assay were negative for Flu A by SIRD. In addition, 1 sample positive for Flu B by the ProFlu+ assay was reported negative for Flu B by SIRD.

Discussion

As seen in Tables 1 and 2 the rapid antigen test used by the Le Bonheur molecular diagnostics lab (Memphis, TN) supported the initial hypothesis of this study, claiming that a rapid antigen test would vield results with low sensitivity and specificity. The reason for this low sensitivity and specificity is cause for additional research, but these deficiencies likely result from one or more obstacles characteristic of electrochemical immunosensors in clinical settings. According to Wan, et al (2008) the low sensitivity observed could occur because of the failed orientation and immobilization of specific antibodies needed to detect the influenza antigen. If the antibody involved in the assay is not immobilized to the well with its antigen binding arms oriented correctly, there will be fewer interactions between the antibody and the antigen of interest. Without antibody-antigen binding, false negative results are reported. Figures 1 and 2 demonstrate this low sensitivity of the antigen test. While it is supposed that this low sensitivity stems from the failed orientation and immobilization of specific antibodies, that conclusion would take further study. In the case of the low specificity observed in the RIDT, non-specific antibody binding could be to blame. If an antibody binds to an antigen, it will remain bound and then an enzyme-conjugated antibody will bind to it. This secondary antibody that has been conjugated with an enzyme will give off a detectable signal. This signal indicates that the primary antibody has bound to the antigen it was designed to detect and thus, that the antigen is present for a positive test result. However, when the primary

antibody binds non-specifically to an antigen other than that which it was designed to detect, the secondary conjugated antibody still gives a detectable signal indicating a positive result. Thus, non-specific antibody binding yields false positive results, like those reported in Figures 1 and 2. According to a study published by Ahluwalia, Giusto, and Rossi (1995), non-specific antibody bonding occurs through a variety methods. The mode of non-specific antibody binding that is the most common and the hardest to prevent is that of protein-protein interactions, in which antibodies adsorb nonspecifically to other proteins by hydrophobic or hydrophilic interactions. Because these nonspecific interactions are so difficult to prevent, it is likely that this method of binding is giving rise to the high number of false positives reported in Tables 1 and 2. If the antibodies were binding non-specifically with other antigens than what they were designed to detect, false positive results ensued.

This study also gave information reporting the performance between two real time PCR methods. As hypothesized, the Proflu+ indirect assay performed with better sensitivity but also experienced greater inhibition than the SIRD. This can be observed in Figure 3 in which the ProFlu+ was shown to detect Flu A, Flu B, and RSV at lower CT values than the SIRD. In addition, the SIRD did not detect five samples found to be Flu A positive and one sample found to be Flu B positive by the ProFlu+ assay. This discrepancy in sensitivity was to be expected because the Proflu+ assay extracts nucleic acid for the PCR reaction from 200 microliters of specimen. In contrast, the SIRD performs PCR on only 50 microliters of actual specimen. Thus, the indirect ProFlu+ assay performs RT-PCR on a greater volume of nucleic acid allowing for earlier amplification and greater sensitivity. While having more sample volume for the PCR reaction does yield greater sensitivity, it also allows more inhibitors to be present during the reaction. For this reason, our results showed the SIRD to suffer from far less inhibition than the ProFlu+ assay. In addition, because the ProFlu+ assay is an indirect assay, it requires a step to extract the nucleic acid. This additional time allows for greater RNase accessibility. RNases are capable of degrading the nucleic acid present and thus inhibiting the PCR reaction.

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Localization of Behavior in the Common Vampire Bat Desmodus rotundus

Allison Julien and Matthew Roberts

We collected altruistic, aggressive, and sexual behavioral data on the common vampire bat (Desmodus rotundus) population at the Memphis Zoo to determine 1) the influence of location on the expression of these behaviors and 2) whether or not captive behavior paralleled behaviors of wild vampires. In the wild, vampire bats display altruistic behaviors rarely found in other bat species and are among the only bats that are capable of agile, coordinated terrestrial movements in order to better compete for resources in the wild. When captive Memphis Zoo bats were terrestrial, a proportion of 0.006 ± 0.013 of behavioral scans were of aggressive behavior, while values were 0.0006 ± 0.0002 when the vampires were perched. Proportions of altruistic behavior represented 0.003 ± 0.018 of terrestrial behavior and 0.097 ± 0.009 of perched behavior. Sexual behavior represented a proportion of $0.004 \pm 0.0083 \pm 0.022$ when terrestrial and 0.796 ± 0.011 when perched. In conclusion, our findings suggest that in captivity, natural vampire bat behaviors are retained.

Introduction

There are three species of vampire bat: the white winged vampire bat *Diaemus youngi*, the hairy legged vampire bat *Diphylla ecaudata*, and the common vampire bat *Desmodus rotundus* (McNab, 973). *Desmodus rotundus* uniquely displays strong social bonds and some of the most diverse social interactions among bats, including the other two species of vampire bats (Carter et al., 2012). These interactions include neutral, sexual, altruistic, and agonistic behaviors (DeNault and McFarlane, 1995). Exclusive to vampire bats, many of these behaviors can be displayed while perched or when located terrestrially due to advanced locomotor abilities that are not found in other bat species (Riskin et al., 2005; 2006; Schutt et al., 1997).

Vampire bats have been observed to hang in groups of mixed sexes and to breed freely, both in captivity (Greenhall, 1965) and in the wild (Wilkinson, 1990). In these groups bats frequently groomed one another regardless of sex, and mating behavior was generally preceded by grooming (Greenhall, 1965). Some studies report a defined breeding season for vampire bats (DelPietro and Russo, 2001) while others claim that vampire bats breed freely year-round (Greenhall, 1965). When mating, males will mount the females while perched upside down (Greenhall, 1965). Other males may approach to compete for the female (Greenhall, 1965). In the wild, mating occurred most frequently in day roosts in trees than on the ground to decrease the risk of predation (Wilkinson, 1985). In addition to coitus, genital licking and masturbation have also been observed in vampire bats between both sexes (Greenhall, 1965).

In captivity and in the wild vampire bats are also known to exhibit altruistic behaviors, both reciprocal and one-sided. One of the vampire bat's most common reciprocal altruistic behaviors is blood regurgitation, which the bats use to feed members of

roosting groups who were unable to locate and ingest blood for the night (Wilkinson, 1986, Wilkinson 1990). Blood sharing is considered to be altruistic because it requires allocation of time and energy towards non-relatives, thereby potentially decreasing the blood donor's own fitness and increasing the recipient's fitness (DeNault and McFarlane, 1995). This finding is in accordance with Stephens'(1996) criteria for reciprocal altruism, which states that 1) the donor's fitness is reduced, 2) the recipient's fitness is increased, 3) the benefit for the donor is not immediate, 4) donation can be returned within the lifetime of the donor, and 5) that there are methods to detect recipients who do not reciprocate the donation at another time. Carter and Wilkinson (2013), however, argued that blood regurgitation is one-way altruism and that these blood donors may in fact be directly increasing their own fitness by eliciting reciprocation of blood sharing. Vampire bats are also capable of detecting individuals who do not reciprocate blood sharing, and may punish these individuals (Wilkinson, 1986).

Reciprocal blood sharing most often occurs between females that are related or roost together and have strong social bonds, rather than all members of the given wild or captive population (Voigt et al., 2011; Carter and Wilkinson, 2013). Captive males have also been observed exhibiting blood regurgitation towards one another, although this behavior has not been observed in the wild (Carter and Wilkinson, 2013; DeNault and McFarlane, 1995). While food sharing behaviors are more often directed towards relatives, the vampire bat commonly exhibits other altruistic behavior independent of relatedness that is thought to be a tool for social bonding such as grooming or huddling (Wilkinson, 1985: Wilkinson, 1988b). Several studies claim that blood sharing and other altruistic behavior cannot simply be explained by sexual selection or kin selection alone (Carter and Wilkinson, 2013; DeNault and McFarlane, 1995; Wilkinson 1986; 1988a). Blood regurgitation has been shown to be preceded by social grooming or nuzzling of the bat receiving the blood and the bat regurgitating it (Wilkinson, 1986). This finding suggests that nuzzling and grooming are used to assess the amount of blood in the recipient's stomach by feeling the stomach for bloating and are one-way altruistic behaviors (Wilkinson, 1986). It is posited that this prevalence of altruistic behaviors between males is a result of stronger social bonds males tend to develop in captivity where they roost together for longer periods of time than in the wild where male social relationships are much shorter (Carter and Wilkinson, 2013). A study by Ancillioto et al. (2012) found that interactions between newborn vampire bats lead to social bonds and interactions that persist well into adulthood.

The social bonds and examples of altruistic behaviors that vampire bats exhibit are not meant to suggest that they do not display agonistic behaviors; roosting groups contain dominance hierarchies and both sexes of bats have been shown to display agonistic behavior when competing for resources or mates (DeNault and McFarlane, 1995). Male/male aggression has been found most often when males are competing for mates (Greenhall, 1965). However, in larger roosts with a surplus of females, studies have found relatively harmonious and even altruistic relationships between males (DelPietro and Russo, 2001) and several recorded instances of male/female and female/female agonistic behaviors suggest that male/female aggression is more common than male/male aggression (DeNault and McFarlane, 1995). Agonistic behavior can vary from chasing to biting to kicking; lasting anywhere from a few seconds to several minutes (Wilkinson, 1988b). Although male/male aggression is more common when competing for mates, mates are only one source of competition. Vampire bats also compete for food when both in the wild and in captivity.

In the wild, vampire bats rarely feed from the same host animal, although host sharing has been observed (Wilkinson, 1988b). In a possible attempt to reduce feeding time and exposure to predators, vampire bats may cooperate and feed consecutively from the same host animal (Wilkinson, 1988b). Simultaneous feeding, however, is rare and may lead to agonistic competitive behavior in both sexes (Wilkinson, 1988b). In captive bats, due to limited individual sources of food, simultaneous feeding is much more common and thus can lead to many more agonistic encounters. Captive vampire bats tend to consume less food than wild vampires, and a study by Wimsatt and Guerrier (1962) stated that this may be due to the levels of competition that vampire bats exhibit in captivity compared to in the wild. Crowding and agonistic competitive behaviors were observed when the vampires were fed in captivity and the vampires that had to compete for blood consumed less than bats in isolation (Wimsatt and Guerrier, 1962). In addition, captive vampire bats do not expend large amounts of energy each night flying in search of prey. It is possible that reduction in blood intake occurs due to the lower energy expenditure of captive vampires. When feeding in the wild, vampire bats must fly great distances and exhibit agile terrestrial behavior in order to stealthily approach prey on the ground, run away if the prey attacks, or chase the prev if it moves (Riskin et al., 2006). Vampire bats' quick ground movements are limited to short bursts of running or hopping, as they quickly tire (Riskin et al., 2006). Reduced blood intake may also be due to competition that is not often present during feeding for wild vampires (Wimsatt and Guerrier, 1962). Despite competition for blood, vampire bats are commonly non-aggressive towards one another overall (DeNault and McFarlane, 1995).

For our behavioral study, we were interested in whether or not wild sexual, altruistic, and agonistic behaviors were retained by captive vampire bats in a zoo enclosure, as zoos, unlike laboratory settings, seek to imitate natural habitats. We hypothesized that physical location is correlated with type of behavior exhibited. We predicted that altruistic behavior would be more common when the bats were perched, as most altruistic behaviors occur in the wild while the vampire bat is roosting (DeNault and McFarlane, 1995; Wilkinson, 1986), not while it's seeking prey on the ground. Given that in captivity vampire bats are all fed together on the ground and simultaneous elicits greater prevalence of violent encounters in the wild (Wilkinson, 1988b), we predicted that agonistic behavior would be more common when the bat was on the ground due to competition for resources such as food. We also predicted that sexual behavior would be more common when the bats were perched, given that in the wild the ground would be too dangerous for sexual behavior due to the possibility of predation and competition for resources. While competitive behaviors may still be present in captivity, predatory behaviors are absent. Thus, a greater frequency of sexual behavior found when perched may be an example of a holdover behavior from wild animals.

The majority of studies on vampire bats involve wild populations (Wilkinson, 1985; 1990; Wilkinson, 1986; Voigt et al. 2011) or laboratory populations (Carter and Wilkinson, 2013; Riskin et al. 2006; DeNault and McFarlane, 1995; DelPietro and Russo, 2001), yet there have yet to be studies involving observation of a captive population in a zoo habitat which simulates wild conditions. In addition, to the best of the authors' knowledge, no research exists quantitatively comparing behaviors between wild and captive vampire bats and this study contains the first activity budget created for captive vampire bats, as well as the first qualitative evidence of whether or not captive bats retain localizations of behavior as seen in the wild. Knowing the proportion of time the vampire bats spend engaging in each activity can help contribute to the care of the bats, and any recorded abnormalities in behavior can aid the zoo in changes to the exhibit to better encourage natural behaviors.

Methods

Study subjects and location

There were approximately 76 individuals in the enclosure during the study, but due to the layout of the enclosure, only 25-60 bats were visible at a given time. The exhibit for the vampire bats at the Memphis Zoo is 2.7m high, 1.8m wide and 1.7m deep (Cooper et al., 2012). The enclosure has a glass front for viewing. Although the full depth and width are visible through the glass front, only about 1.75m of the overall height is visible. The remaining out of view height is a recessed area above the rest of the exhibit that allows the bats to hide and to have 24hour near-darkness with a red heat light that runs the length of the exhibit at the top of the visible area (Cooper et al., 2012), making it impossible to monitor all of the bats at once. In addition, the bats are allowed to breed *ad libidum* and it is impossible to accurately identify every individual. The Animals of the Night exhibit is on a reverse lighting schedule, meaning that all recordings took place during the subjects' "nighttime", and thus active hours. The bats received warm bovine blood with an added anticoagulant in three to five cat food bowls on the floor at 7 A.M. and 3:30 P.M. daily (Cooper et al., 2012).

The bats then feed *ad libidum* until there is no more food remaining.

Data collection

The researchers employed scan sampling at 3minute intervals (Martin and Bateson, 2007). The 3minute interval allowed for greater ease of recording the large and often variable number of individuals observed per collection. The researchers reduced the risk of inter-observer uniformity errors by alternating which observer recorded ground behaviors and which observer recorded perched behaviors each session. Researchers conducted observations during October of 2012 from 1:45 - 3:45 P.M on Mondays and January to April of 2013 on Mondays and Wednesdays from 10:00-11:00 A.M. and Tuesdays and Thursdays from 2:00-4:00 P.M. Observing the bats in the afternoon allowed researchers to collect data during feeding, when the bats were more likely to exhibit agonistic behaviors. Researchers recorded the observed behaviors based on an ethogram (Table 1) divided into agonistic behavior, altruistic behavior, sexual behavior, and neutral behavior.

Analysis

Once researchers had collected the data, they compressed the data into their respective behavioral categories for each day to form the activity budgets for the bats. Researchers then converted the frequencies of ground and perched behaviors to proportions and performed a chi-square goodness of fit test using Microsoft® Excel to determine if correlations existed between location, (i.e. ground, perched) and behavior (i.e. agonistic, sexual, altruistic, neutral). Recordings of neutral behaviors served to determine an accurate activity budget as they accounted for the majority of vampire bat behaviors.

Behavior	Code	Description
Agonistic Behaviors		
Chasing	CH	One bat chases another, either on the ground or in the air
Hitting/Kicking	KK	One bat kicks or hits another (but not reciprocated)
Fighting	FT	Bi-directional kicking, hitting, or biting
Altruistic Behaviors		
Grooming	GR	One bat licks another's non-genital fur
Clinging	CL	One bat holds on to one other bat (front to back)
Nuzzling	XO	Bi-directional clinging between two bats (front to front)
Sexual Behaviors		
Copulation Attempt	CA	One bat mounts another to attempt copulation
Sniffing	SN	One bat sniffs the genitals of another
Oral Sex	OS	One bat licks the genitals of another
Neutral Behaviors		
Stationary	IM	Motionless and awake

Table 1. Vampire bat (Desmodus rotundus) ethogram

Sleeping	ZZ	Stationary and eyes are closed
Flying	FL	In flight or taking off
Stretching	ST	Stretches out wings then returns to stationary position
Auto-Grooming	AG	Licks own fur
Feeding	FD	Feeds on blood in food bowl
Crawling	CR	Slowly creeps across ground on all fours
Group Clinging	GC	Clinging in which more than two bats hold on to each other

Results

Of the approximate 76 individuals in the exhibit, on average only 63.95 ± 1.35 (standard error) individuals were visible at any given point in time. Overall, the proportion of behaviors that occurred on the ground was 0.096 ± 0.007 , and the proportion of behaviors observed while perched was 0.904 ± 0.066 . An Independence of Classification Chi-Square indicated that the proportion of perched bats and bats on the ground that displayed altruistic behavior differed significantly, with a higher proportion of perched bats having displayed altruistic behavior compared to bats on the ground [X²(1)=289.63, p<0.001]. Another Independence of Classification Chi-Square indicated that the proportion of perched bats and bats on the ground that displayed aggressive behavior differed significantly, with a higher proportion of bats on the ground having displayed aggressive behavior compared to perched bats $[X^2(1)=2474.81, p<0.001.]$ Finally, a third Independence of Classification Chi-Square indicated that the proportion of perched bats and bats on the ground that engaged in sexual behavior differed significantly, with a higher proportion of perched bats having engaged in sexual behavior compared to bats on the ground $[X^2(1)=351.75, p<0.001.]$.



Figure 1. Activity budget for *Desmodu rotundus*, Mean proportion of vampire bat behaviors (\pm SE). Agonistic behavior: ground=0.006 \pm 0.013, perched=0.0006 \pm 0.0002. Altruistic behavior: ground=0.003 \pm 0.018, perched=0.097 \pm 0.009. Sexual behavior: ground=0.004 \pm 0.006, perched=0.009 \pm 0.002. Neutral behavior: ground=0.083 \pm 0.022, perched=0.796 \pm 0.011. Asterisks (*) denote statistical significance at the 0.001 level.



Figure 2. Activity budget for *Desmodus rotundus*, Mean proportion of vampire bat behaviors (\pm SE), excluding neutral behavior for easier comparison of aggressive, altruistic, and sexual behaviors. Agonistic behavior: ground=0.006± 0.013, perched=0.0006± 0.0002. Altruistic behavior: ground=0.003± 0.018, perched=0.097± 0.009. Sexual behavior: ground=0.004± 0.006, perched=0.009± 0.002. Asterisks (*) denote statistical significance at the 0.001 level.



Figure 3. Mean proportion of behaviors in each location (±SE).

Discussion

Each of our three predictions were supported by our results, as individuals located on ground displayed significantly higher proportions of agonistic behavior than those who were perched, and perched individuals displayed significantly higher proportions of sexual and altruistic behavior than those on the ground. While the proportion of bats exhibiting sexual behavior when perched compared to terrestrial was very significant, these proportions were the closest of all of the other behaviors. Originally we proposed that vampire bats would not exhibit sexual behavior on the ground in the wild due to the threat of predation. Captivity, however, removes this threat which may have led to the observed terrestrial sexual behavior. The findings of the current study are in line with studies conducted by Wilkinson (1985; 1986; 1988b) on vampire bats in the wild, which found that mating behavior most often occurred in roosts, agonistic behavior occurred most often on the ground where competition for resources was the greatest, and altruistic behavior occurred most often when perched. Our study therefore suggests that wild behavioral tendencies are retained in captivity. This result could also be taken to suggest that the enclosure design at the Memphis Zoo is adequate in its accommodations to afford a natural localization of behaviors. The researchers did not observe the presence of blood regurgitation (Wilkinson, 1986), although this is likely due to the bats' constant and unlimited access to blood. With constant access in close proximity, the bats are able to feed ad libidum and do not need to travel far for a meal. This lack of blood regurgitation and frequency of aggressive, competitive encounters on the ground may suggest that there are limitations to the Memphis Zoo enclosure. These could also be consequences of captivity in general and the closer proximities experienced therein.

Previous studies have also shown that aggression occurs between males due to competition for resources and mates (DeNault and McFarlane, 1995). This study was not able to assess whether the aggression occurred between females or males, but aggressive encounters increased with proximity to food bowls, which may be consistent with Wilkinson's (1985; 1986; 1988b) previous findings of competition for resources in captivity. Additionally, there were instances wherein sexual behavior between more than two individuals was followed by agonistic behavior, possibly consistent with the mate competition findings by DeNault and McFarlane (1995).

Despite significant results, the current study was limited in that the collection time was short. When data collection began, observation only took place from 1:45-3:45 P.M. Thus, the observed amount of agonistic behavior may not be representative of the entire day. The bats are fed around 3:30 P.M. each day, and we were able to observe the bats' behavior during this time. Around 4:00 P.M., however, food begins to run out leading to a possible increase in competition for food, and therefore, increased levels of agonistic terrestrial behavior. It is also possible however, that once all the food is gone, the frequency of agonistic behavior observed on the ground would drop to levels equal to that of perched bats, as there would be no more food to compete for. Moreover, only one hour's worth of data were collected during the bats' "nighttime" hours in each recording session. Because vampire bats are nocturnal, it is likely that the observed proportions of behaviors during the

bats' activity budget throughout a 24-hour period. In order to account for our lack of more observational data, in January of 2013 observation times and data collection increased to four times a week at various hours, during both the bats' "daytime" and "night time" hours for a period of four months. This gave researchers a larger window of time to observe the bats and thus the opportunity to capture more behaviors that the earlier observation times did not allow.

This study includes the first ethogram for captive vampire bat behavior and can be used as a reference point for the Memphis Zoo. It would be beneficial for further studies to compare the captive behavior of the vampire bats at the Memphis Zoo and captive vampire bat population in other zoos in order to look at disparities and correlations. By collecting behavioral data from vampire bats in other zoos and comparing them to the vampire bats at the Memphis Zoo, it would be possible to determine whether there are behavioral differences between the different populations. If similar future studies were to be conducted in other zoos with different environments (i.e. more room for flight, different spatial layouts, more individuals), researchers would be able to tell if any differences in behavior could be attributed to the physical setup of the exhibit. During the present study, the researchers considered whether or not the vampire bat population at the Memphis Zoo exhibited any stereotypic behavior as a result of their small enclosure space. Without information regarding the activity budgets of other populations of captive bats, however, it is difficult to conclude whether or not stereotypic behaviors exist and what behaviors would be classified as stereotypic. By comparing behavioral data from the Memphis Zoo population and populations in zoos with larger enclosures, researchers could look for behaviors that may be considered stereotypic, such as excessive stretching in response to the limiting size of the enclosure. This information would be very useful for the Memphis Zoo in order to determine whether or not their population is properly provided for in terms of space and resources.

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Oxidative Stress: A Neurobiological Overview and its Role in Autism and Major Depressive Disorder

Megan Ververis

Oxidative stress occurs in the brain when there is an imbalance between the production of damaging free radicals, reactive oxidative species, and neutralizing antioxidants. These antioxidants detoxify and counteract the damaging effects of oxidation. Neurons are very susceptible to such damage, and oxidative stress has been found to facilitate both the onset and the increased progression of neurodegenerative diseases, as well as psychiatric disorders including Autism and Major Depressive Disorder (MDD). It has been found in the brains of autistic individuals there are increased markers of lipid peroxidation and neuroinflammation and decreased levels of antioxidants than the general population, and therefore, their brains undergo higher levels of oxidative stress. Patients suffering from MDD have been found to have antioxidant deficiencies as well, and antidepressant treatment mechanisms include reducing cytokines in neuroinflammatory pathways along with stimulating growth factors that decrease oxidative stress.

Oxidative stress is the effect of the brain's imbalance between the production and clearance of reactive oxygen species (ROS), such as free radicals, and the manufacturing of antioxidant defenses. Homeostasis aims to create a dynamic equilibrium between the production of ROS and sufficient antioxidants to maintain damaging free radicals. The brain is extremely vulnerable to oxidative stress: it naturally does not produce enough antioxidants to fight ROS and the abundant membrane phospholipids are prime targets of ROS and thus vulnerable to oxidative stress (Chauhan and Chauhan 2006). Additionally, neurons have a higher energy requirement than most cells in the body and they have a limited capacity to detoxify ROS, making the brain extremely susceptible to oxidative stress (Chauhan and Chauhan 2006). When the body cannot control toxic levels of ROS, cellular damage to the neurons can ensue; it begins with lipid peroxidation, and continues with nuclear membrane degradation, production of free radicals, reduction of GABA receptors, decreased production of antioxidants, increased inflammatory responses, and ATP depletion via damage to mitochondria, all of which trigger to apoptotic cascades. These widespread apoptotic incidences lead to structural damage in the hippocampus and prefrontal cortex (Kubera 2011). Oxidative stress, or the overproduction of ROS in the brain, can occur in response to external stressors, such as negative life events, early life stress, tobacco use, alcohol intake, overly strenuous exercise, etc. (Kubera 2011, Chauhan and Chauhan 2006). Oxidative stress has been found to both facilitate the onset and increases the progression of neurodegenerative disorders, such as Parkinson's disease, Huntington's disease and Alzheimer's disease, as well as psychiatric disorders such as Autism and Major Depressive Disorder, which will be discussed here.

Autism is a neurodevelopmental disorder with an onset before three years of age. Autism is characterized by impairments in social interactions, difficulty expressing and comprehending emotions, and decreased language functions. The scientific community and general public hotly debate the cause of Autism, and there is likely not one trigger to its onset. Autism is considered a multi-risk factor disorder that is influenced by genetic, environmental, and immunological factors, and an autistic brain has an increased vulnerability to oxidative stress (Chauhan and Chauhan 2006). Glutathione is an enzyme whose job is to eliminate ROS; the brain has naturally low levels of this enzyme during conception through infancy, therefore children are more susceptible than adults to oxidative stress (Chauhan and Chauhan 2006). When a young, developing child that is predisposed to Autism undergoes high levels of oxidative stress, the neurodevelopmental disorder's chances of developing increase. Malonyldialdehyde (MD) is an end product and marker of lipid peroxidation in the brain. MDA levels are higher in 87% of autistic patients compared to developmentally normal children (Chauhan and Chauhan 2006). Ceruloplasmin, an inhibitor of peroxidation by metal ions, and transferrin, which reduces ferrous ions, are vital protective antioxidant proteins that are synthesized in the brain. Levels of these antioxidants in the serum of children is much lower in autistic children when compared to their healthy siblings, which supports the theory that there is a lack of regulation of these antioxidants in autistic brains. Additionally, the levels of ceruloplasmin and transferrin are even greatly reduced in nonverbal Autistic children, suggesting that these antioxidants may be protective against severe Autism (Chauhan and Chauhan 2006). A number of studies have also implicated that oxidative stress leads to detrimental neuroinflammatory responses in Autistic children.

against oxidative stress because they can treat many

Cytokines, byproducts of neuroinflammation, such as TNF- α and IL-1 β , are known to initiate production of free radicals (Chauhan and Chauhan 2006). In addition to this effect, transferrin is decreased by inflammation, hence lowering antioxidants levels. Overall, increased oxidative stress in Autistic patients is supported through the findings of decreased production of natural antioxidants and high levels of damaging cytokines (Chauhan and Chauhan 2006).

Major depressive disorder (MDD) is a mental disorder that is characterized by chronic sadness, low self-esteem and a lack of motivation. Like Autism, it is a multi-risk-factor disorder in which there are genetic and neurochemical bases, along with an abnormal reaction to external stressors, or oxidative stress. Treatment is usually a combination of counseling and antidepressants medications, which aim to increase monoamines, or the neurotransmitters serotonin, norepinephrine, and dopamine (Kubera 2011). Research shows that antidepressants reduce cytokines in inflammatory pathways, including TNF- α and IL-1 β , and the drug treatments stimulate synaptic plasticity by increasing brain-derived neurotrophic factor (BDNF), thus decreasing oxidative stress effects (Kubera 2011). Human studies have shown reduced levels of antioxidants, such as vitamins C and E, result from the depression itself, rather than from dietary insufficiencies (Ng et al. 2008). In one study, researchers chronically administered an SSRI antidepressant to rats and found that inflammatory cytokines were reduced while IL-10, an anti-inflammatory cytokine that reduces neuroinflammation, was increased (Kubera 2011). The knowledge that cytokines are produced in response to external stressors enforces the notion that depression is likely intensified and further is a result of increased levels of pro-inflammatory cytokines. In fact, cytokine-based immunotherapy for cancer and hepatitis C patients often leads to clinical depression (Kubera 2011). Animal models have shown that by exciting inflammatory pathways, the increased cytokines lead to lethargy, anxiety, helplessness, weight loss, and lack of concentration, all key symptoms in MDD.

BDNF is a neurotrophic factor that supports synaptic plasticity and axonal transport, therefore fighting neurodegeneration. Expression of BDNF in the hippocampus is reduced in depressed patients. The oxidative stress in depression leads to decreased BDNF levels and eventual apoptosis focusing in the hippocampus. BDNF production can be initiated through positive stress, such as exercise, or antidepressants (Kubera 2011). Damage to the hippocampus may be reduced by antidepressant use or BDNF-producing workouts prior to an external stressor. Antidepressants are useful in the battle aspects of the neuronal damage in MDD patients. Chronic antidepressant use leads to reduction of inflammatory pathways, direct suppression of cytokines such as TNF- α and IL-1 β while increasing the production of IL-10, and stimulation of neuronal differentiation, thus reducing apoptosis in depressed patients (Kubera 2011). Studies have shown that the severity of the depression is directly related to how advanced the oxidative stress damage is in the brain (Ng et al. 2008). For clinical practice, physicians should not only prescribe oxidative-stress reducing antidepressants, but also strongly emphasize the importance of reducing external stressors in the patient's daily life. Modern society demands a hectic lifestyle, such as long hours and performance stress at work, unemployment fears, raising families, excelling in academics, while simultaneously underrating the value of relaxation and a reduction of stressors. In addition, it would be beneficial for physicians to assert the importance of regular exercise, which leads to production of BDNF and other neurotrophic factors.

As the average person does not actively investigate research concerning oxidative stress in Autism and MDD, the majority of people will learn about brain health via the media. In 2011, an article in Time Magazine highlighted the recent study illustrating antidepressant use by the mother in the year before giving birth leads to doubling the risk of developing Autism in young children (Park 2011). One might conclude that since Autism is a genetic disorder that is related to depression, the children are developing Autism through their genetic makeup; or these findings could have been due to the postpartum depression which might lead to less interaction and bonding, therefore contributing to the lack of emotional understanding often seen in autistic children. To separate the contributing factors and isolate the drug treatment, another study was performed, which showed no increased risk of Autism associated with maternal depression or anxiety (Park 2011). If a pregnant woman battling MDD had read this article, she might have felt compelled to not treat her depression for the health of her baby. It is important that these findings should facilitate education about finding alternative methods for fighting depression, such as exercise, antioxidant rich diet, and counseling to help cope with external stressors. The researchers who conducted this study admit that there are many unknown underlying factors for Autism and MDD that can be influenced by antidepressants and oxidative stress (Park 2011). Future research hopes to focus on genetics and risk factors for MDD and Autism (Park 2011). Hopefully, these findings will lead to physicians and expectant

mothers having more in-depth conversations about depression and all-encompassing treatment options and their associated risks for the baby.

Oxidative stress is an important factor associated with MDD and Autism, along with many other neurodegenerative disorders. The balance between ROS and antioxidants can be maintained through lifestyle choices and can facilitate preventative action against neurological diseases. While most may know that stress can lead to early aging and higher risk for

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heart disease or stroke, they often do not comprehend the detrimental apoptotic cascades in the brain during periods of extreme or chronic stress. The scientific and healthcare communities alike need to vigorously emphasize the importance of stress management through exercise, healthy coping mechanisms, and antioxidant rich diets, as well as stress-reducing activities for children, such as recess, physical education and sports, art, and play time.

Red Panda (*Ailurus fulgens*) Behaviors and Exhibit Use at the Memphis Zoo

Maitland Frilot and Elizabeth Medved

Abstract: Studying the differences in red panda behavior between captivity and the wild is important because there is a huge decrease in red panda populations in the wild. Red pandas raised in captivity to be released into the wild need to have the behaviors typical of wild red pandas to survive. The purpose of this study was to investigate how the red pandas at the Memphis Zoo use their exhibit, which could later be compared to how red pandas use their natural habitat. We hypothesized that red pandas perform behaviors in location-specific areas. We used scan sampling to test our hypothesis. Lucile, the female red panda at the Memphis zoo, spent 50.35% of her time in the indoor enclosure and Justin, the male red panda, spent 57.04% of his time in the indoor enclosure as well. Outside, they spent 68.84% of their time on the ground, where they mostly were moving 60.58% of their time on the ground. They spent 43.79% of their time in the trees eating. Additionally, there is a pattern where eating and resting behaviors occur in the trees and moving and agonistic behaviors occur on the ground. Overall, there was a difference between captivity and wild red pandas with respect to how much time was spent on the ground or in the trees. Although the actual behaviors that occur in each location are the same as locations in the wild, due to a small sample size, this difference was not statistically tested. Keywords: Behavior, Captivity, Exhibit Use, Red Pandas.

Introduction

Red pandas, Ailurus fulgens, are carnivores closely related to raccoons (Flynn et al., 2000). Slightly larger than a house cat, 5.4 to 9 kilograms, red pandas have shorter front legs than back legs which gives them a waddle similar to their relative the giant panda (Ailuropoda melanoleuca) (Flynn et al., 2000; Wei et al., 2000). Their primary food source is bamboo, but they also eat other berries and plants (Wei et al., 2000). The red pandas' natural habitat extends along the Himalayas through Nepal, India, and China. Due to the higher elevation the climate in these areas tends to be cooler than climates in the United States, where this study was performed (Wei et al., 2000). The habitat of the red panda tends to be moist temperate forests with dense canopies and undergrowth (Mallick, 2010). The red panda's population distribution has decreased drastically over the last century (Wei et al., 1999; Wang et al., 2013). due to population isolation and a gradual loss of natural habitat (Wei et al., 2000) Because of the population decline, many conservation efforts have begun to restore the red panda population (Wei et al., 1999; Wang et al. 2013). In general, more than 85% of red panda cubs in the wild die before reaching adulthood (Wei et al., 2000).

In the wild, red pandas are only active 36.5% of the day, but their activity level does not vary by month or season (Johnson et al., 1988). In the wild they tend to rest for approximately 15 hours a day, broken up into less than 4 hour blocks (Johnson et al., 1988). Their rest sites are predominantly elevated off the ground, whether it is a log, bush, or tree (Johnson et al., 1988). Red pandas also prefer eating bamboo

grown at higher elevations because there are fewer interactions with other species (Wei et al., 2000). In some cases, trees have also been found to be the preferred site for defecation (Pradhan et al., 2001). The forest floor may be used more during winter (which is the red panda's mating season), and during the monsoon season, because bamboo falls to the ground during storms (Pradhan et al., 2001). However, wild red pandas spend 81.5% of the time in trees, most likely because the elevation offers more protection and safety (Pradhan et al. 2001). Red pandas often prefer areas with many tree stumps and fallen logs because it allows them better access to bamboo leaves (Johnson et al., 1988; Wei et al., 2000).

There are many differences between red pandas' natural habitat and exhibits in captivity, such as temperature and elevation (Wei et al., 1999). Additionally, red pandas are accustomed to forests with higher density of trees and bamboo, enabling them to have more areas to climb in the wild (Wei et al., 1999). Also, not all types of forests are preferable to red pandas due to a combination of density and species of trees (Wei et al., 1999), meaning their environment in captivity might be different than their preferred habitat, thus changing their behavior. Furthermore, due to their scarcity in the wild there are fewer opportunities for interspecies interactions (Pradhan et al., 2001). Zoos usually have multiple red panda individuals in close proximity, which could cause a difference in behavior.

Studying the differences in red panda behavior between captivity and the wild is important because there is a huge decrease in red panda populations in

the wild (Wei et al., 2000); therefore, conservation efforts to rerelease individuals need to be certain that the red pandas will have the necessary behaviors to survive once released. Knowing what differences exist between captive and wild behavior will allow zoos to change conditions to coax typical wild behavior in preparation for release attempts.

Differences between red pandas' natural habitat and captivity, such as temperature, forest type, and number of individuals, lead to questions of how captive red pandas interact with each other in captivity and how their behaviors differ from red pandas in the wild. The purpose of this study was to discover how the red pandas at the Memphis Zoo use their exhibit. Is there a correlation between specific locations and specific behaviors performed by red pandas? We hypothesized that red pandas perform behaviors in location-specific areas. Therefore, we predicted that resting and eating behavior would occur more in trees while moving and agonistic behavior would occur more on the ground. Additionally, the change in temperature between their natural habitat and the United States may have an effect on activity level. A second hypothesis was that temperature affects exhibit usage of red pandas. We predicted that there would be a higher activity level during colder months, though we did not test this hypothesis. A null hypothesis is that red pandas do not perform specific behaviors in location-specific areas. Therefore there all behaviors would occur the same amount of time in each of the locations. We chose to test the first and the null hypotheses to investigate captive behavior that could be used by others to compare to wild behavior.

Methods

Study Subjects and Location

The red panda enclosure at the Memphis Zoo had a large amount of ground space, with multiple trees and a moat of water at the front of the enclosures. One of the trees was a large oak tree with a platform and metal wrapped around it towards the top of the enclosure to prevent the red pandas from climbing too high. Another tree had a platform about halfway off the ground that held a water dish and a large supply of bamboo that the red pandas have access to use freely. There were a few other trees and bushes throughout the exhibit as well (Fig 1). Additionally, the red pandas had access to leaves and other food both in the trees and on the ground. There was also an indoor enclosure with air conditioning that was accessible to the red pandas during the hotter afternoon hours, and the entrance was obscured by large boulders that also obstructed the view of the red pandas in that area of the exhibit. In the enclosure were the two juvenile red pandas, Lucile (female, born June 2012) and Justin (male, born July 2012). Additionally there was a female Reeve's muntjac (Muntiacus reevesi), named Bamboozle that shared the exhibit.



Figure 1. Map of the red panda exhibit at the Memphis Zoo with specific locations marked.

Data Collection

To collect data we used behavior sampling where we recorded the particular behaviors of each of the red pandas as well as their interactions with each other and with the Reeve's muntjac (Martin and Bateson, 2007). Data collection occurred on September 30th, October 7th, October 21st, and October 28th, 2013. The data were collected every Monday afternoon for a four-week period in September and October for two hours and twenty minutes. We referenced each behavior from our ethogram (Table 1). We recorded behavior listed in the ethogram performed at the time of the scan, using intervals of two minutes (Martin and Bateson, 2007). Recording each behavior at two-minute intervals allowed us to formulate an activity budget to determine the percentage of time the red pandas spent performing specific behaviors. Additionally, we recorded the location in the exhibit where each specific behavior of the red pandas occurred using scan sampling (Martin and Bateson, 2007). These data allowed us to determine if there is an association between a particular behavior and a specific location. We focused on where eating, agonistic behavior, moving, and resting occurred.

Behavior	Code	Definition
Scent Marking	SM	Rub or drag anogential region along ground
Eating	Е	Consumption or handling of food
Climbing	С	Climbing up or down tree
Moving	Μ	Running or walking
Excretion	EX	Eliminating waste through defecation or urination
Sniffing	S	Inspecting objects by smelling
Out of Sight	OS	Out of sight, behind rocks, or in inside enclosure
Agonistic Behavior	А	Jumping on or chasing each other or Reeve's muntjac
Resting	R	Sleeping, sitting, lying down, not moving around

 Table 1. Ethogram of red panda behaviors

Analysis

We compared the activity budgets and locations of each individual to compare location and specific behaviors. Due to a small sample size, only descriptive statistics were used, instead of statistical analysis. Analyzing time spent by each red panda in each activity was performed by averaging the number of occurrences of each behavior over the four observation days. The total number of behaviors that occurred in the trees and that occurred on the ground were totaled to compare overall preference for arboreal or terrestrial locations.

Results

The red pandas (Lucile; Justin) spent most of their time (mean percent \pm standard error) out of sight (50.35 \pm 9.05; 57.04 \pm 16.59) (Fig 2.). Next, they spent the most of their time moving (26.76 \pm 11.35; 21.47 \pm 9.27), followed by eating (8.09 \pm 4.95; 6.33 \pm 3.37), climbing (4.57 \pm 2.39; 4.57 \pm 2.32), and scent marking (4.57 \pm 2.77; 1.76 \pm 0.67) (Fig 2.). They spent the smallest part of their time sitting $(1.40 \pm 0.57; 3.16 \pm 2.25)$, sniffing $(2.11 \pm 1.67; 2.46 \pm 1.66)$, performing agonistic behavior $(2.11 \pm 0.90; 2.11 \pm 0.90)$, and excreting $(0.00 \pm 0.00; 0.70 \pm 0.40)$ (Fig. 2).

The red pandas (Lucile; Justin) spent most of their time (mean percent \pm standard error) in the hidden area and indoor enclosure (49.29 \pm 18.61; 57.39 \pm 16.41) followed by the central trees and platform (8.45 \pm 5.75; 11.97 \pm 4.62), the back ground (11.27 \pm 3.94; 9.86 \pm 4.26), the left ground (9.86 \pm 5.81; 4.58 \pm 2.40), the front ground (10.56 \pm 4.09; 8.80 \pm 3.37), the right ground (8.80 \pm 5.09; 6.69 \pm 4.16), the back tree (1.76 \pm 1.76; 0.35 \pm 0.35), and the rocks (0.00 \pm 0.00; 0.35 \pm 0.35) (Fig. 3).

The red pandas spent 68.84% of time (\pm 4.81) on the ground and 31.16% (\pm 4.81) in the trees (Fig. 4), with Justin spending 64.03% on the ground and 35.97% in the trees and Lucile spending 73.65% on the ground and 26.35% in the trees.



Figure 2. Activity budgets (mean percent ± standard error) for the Memphis Zoo red pandas.



Figure 3. Percent of time (mean \pm standard error) spent in each location for the Memphis Zoo red pandas. The blue bars represent Lucile and the red bars represent Justin.



Figure 4. Percentage of time (mean \pm standard error) red pandas spent on the ground versus in the trees in the outdoor enclosure.



Figure 5. Percentage of time (mean \pm standard error) in the trees and on the ground spent eating, in agonistic behavior, resting, and moving.

Moving behavior occurred 60.58 (\pm 11.93) on the ground and 8.86% (\pm 3.56) in the trees (Fig. 5). Additionally, agonistic behavior occurred on the ground 2.38% (\pm 0.70) and 1.00% (\pm 0.35) in the trees, while eating occurred in the trees 43.79% (\pm 0.89) and on the ground 4.71% (\pm 1.87) (Fig. 5). Resting behavior took place 40.55% (\pm 4.16) in the trees and 3.60 (\pm 0.85) on the ground (Fig. 5).

Discussion

Justin and Lucile spent the majority of their time out of sight in their indoor enclosure. This meant they had a preference for the conditions inside, not for their outdoor habitat (Fig. 2). Because we did not have access to the indoor enclosure there is no way of knowing how similar the enclosure is to wild conditions or the behaviors that occur indoor. Justin and Lucile spent more time overall on the ground then they did in the trees (Fig. 3), which is important because in the wild, red pandas spend the majority of their time in trees to avoid predators and find available food (Johnson et al., 1988; Wei et al., 2000). In the wild, red pandas spent 81.5% of their time in the trees, compared to 31.16% of their time that we observed. Therefore, it is possible that captivity has changed their natural behavior. The difference could be due to the lack of predators and the availability of food on the ground, though red pandas do eat predominantly in the trees (Fig. 4). Additionally, this could be due to the fact that Justin and Lucile are juveniles and have not gained the courage to spend as much time in the trees.

The fact that the majority of their time spent resting occurred in the trees (Fig. 4) does show that they are comfortable in the trees, and that it is not a fear of trees that led them to remain on the ground so frequently. The preference for resting in trees is consistent with behavior in the wild (Johnson et al., 1988). Because Lucile only spent 1.4% of observed time resting and Justin only spent 3.2% of time observed resting, their rest time in captivity is either drastically lower than in the wild, or they are much more active during the afternoons than at other points of the day (Johnson et al., 1988). Red pandas' preference for moving on the ground (Fig. 4) shows that once in the trees they did not shift location as often as they do on the ground. Because so much of their time in the wild is spent in the trees and much of their days are spent resting (Johnson et al., 1988; Wei et al., 2000), as well as the fact that there is not much documentation of their behaviors in the wild, it is hard to tell if this large percentage of movement on the ground compared to in the trees is typical or atypical in the wild. Agonistic behavior occurred mostly on the ground (Fig. 4) due to the fact that it was often targeted towards Bamboozle, the Reeve's muntjac, who is unable to climb the trees. Because there is such a small population in the wild that there are rarely interspecies interactions and because red pandas prefer elevation to avoid interactions with others, it is difficult to tell if agonistic behavior occurs in the wild (Wei et al., 2000). Therefore it is difficult to tell if the preference for agonistic behavior on the ground is altered by captivity.

Our hypothesis that red pandas perform specific behaviors in specific locations was supported in the data. Specifically, our predictions that there would be correlation between moving and agonistic behavior

and ground activity and eating and resting behavior in the trees was reflected in the descriptive statistics, but cannot be confirmed without actual statistical analysis. One difficulty that arose in this study was the small sample size. With only two individuals it was difficult to run any statistics; therefore, this experiment would best be replicated with a larger sample size. Another unforeseen variable was the abundance of time spent in the indoor enclosure where behavior could not be recorded. A way to prevent this complication in the future would be to perform the study in the colder months when the air conditioning is not necessary, to prevent the red pandas from entering the indoor enclosure, or to record in the indoor enclosure as well. Future research could look at red panda activity in the morning hours, especially because temperatures tend to be cooler, and it is the time of day when red pandas most active in the wild (Johnson et al., 1988). Additionally, since they also tend to be active at night in the wild (Johnson et al., 1988), future research could observe their behaviors at night.

Conclusions

1. Red pandas in captivity at the Memphis Zoo spend the majority of their time in their indoor enclosure and moving throughout the ground of their outdoor enclosure.

2. The majority of the time that the red pandas at the Memphis Zoo were in their outdoor enclosure was spent on the ground instead of in the trees as they do in the wild.

3. There is a pattern of eating and resting behavior of Red Pandas occurring in the trees and moving and agonistic behavior occurring on the ground at the Memphis Zoo.

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The Detrimental Effects and adaptations of Invasive Lionfish (*Pterois volitans*) on the Southeastern Atlantic Ecosystem

Erin Lowrance

Abstract: Lionfish (Pterois volitans) are strongly adapted to surviving in any tropical or sub-tropical environment that has an abundance of prey. Recently, lionfish were introduced into the southeastern Atlantic coastal ecosystems and have been voracious consumers concerning herbivorous fishes and shrimp. These organisms are on the decline and this situation threatens to destroy the current coral ecosystems off of the coast of Florida and the Caribbean. Without human involvement or the establishment of a lionfish predator, the lionfish will continue to exhaust the coral reefs and their fauna until their resources are depleted.

Introduction:

The lionfish (Pterois volitans) is a species of fish native to the coastal Indo-Pacific region that has recently been introduced into the southeastern Atlantic coastal area (Akins et al. 2012). This introduction has shown the existing adaptations the lionfish have exhibited within their native region and how this transition has allowed lionfish to easily transition into the Atlantic coral environment (Akins et al. 2012). Lionfish are equipped with venomous spines, bright colors, and unique mechanisms. These adaptations have proven to be extremely advantageous to the lionfish in their new Atlantic ecosystem (Akins et al. 2012). The southeastern Atlantic and the Indo-Pacific climates are both classified as sub-tropical to tropical regions; therefore, the lionfish did not have to adapt to any major environmental changes (Hare et al. 2004). As long as the lionfish does not stray too far north during cold winter months, it is expected the lionfish will have a high survival rate. In this new ecosystem, the only major change was the new potential predator and new prey relationships; however, this concern found that the new predators and prey did not exhibit any constraint on the lionfish development (Benkwitt et al. 2012). This introduction and the lionfish ease of adapting has presented a dangerous problem to the coral ecosystem because a lot of the organisms within this region are being negatively impacted (Carleton Ray et al. 2002). It is important to observe the reasons and specific adaptations the lionfish use in order to thrive in this environment so scientists can find a solution to the growing lionfish population (Arias-González et al. 2011). This paper will explore the physical attributes of lionfish, their hunting behavior, and range of diet that allows them to thrive in the southeastern Atlantic. Along with the lionfish adaptations, this paper will discuss the impact these predators have on the ecosystem and how humans are directly involved in this ecosystem's struggle. Hopefully a solution can be found in time to prevent a wide-scale ecosystem collapse before it is too late.

Physical Appearance:

Lionfish are one of the most unique and ostentatious fish native to the Indo-Pacific coastal region. The lionfish have alternating red, white, and black zebra stripes and sport up to eighteen spines on their pectoral and dorsal fins (Matheson et al. 2006). These spines are exclusively used for defense against any natural predators within their natural habitat and contain venom (Matheson et al. 2006). While this venom can be detrimental and/or toxic to other fish or sea faring mammals, it rarely causes fatalities within humans (Matheson et al. 2006). Along with their distinguishable coloration, the size of the lionfish is another identifying factor when observing them in their habitat. Lionfish range from 30 cm to 38 cm in length and have an average wet weight of 1.2 kg (Carleton Ray et al. 2002). These fish over the years have adapted to thrive within their native environment, and these particular physical features allow them to have a significant advantage over their prey.

Hunting techniques and behaviors:

Lionfish are very efficient predators because their hunting techniques have been adapted over the years to become a dominant force within their native environment. The most common method lionfish use in both their invasive and native habitat is the use of herding and cornering prey (Benkwitt et al. 2012). As previously mentioned, lionfish have bright and elaborately designed fins, so when these fins are presented to the prey fish, the prey grow confused and begin to retreat (Akins et al. 2011). By flashing their pectoral fins, slowly moving their fins back and forth, and slowly moving towards their prey, lionfish are able to corner their prey into a location that is easy for them to consume the organism headfirst (Benkwitt et al. 2012). The reason lionfish desire to ingest their prey headfirst is because when they consume larger fish, some of the defense mechanisms, such as armored protection, could cause personal harm if positioned in an incorrect angle (Akins et al. 2011).

A technique that appears more frequently in lionfish that reside in the Pacific is water blowing (Benkwitt et al. 2012). Water blowing is where lionfish will direct a blast of water at their prey in order to disorient their prey and increase the probability that the fish will end up facing the lionfish head first (Albins, M.A. & Lyons, P.J. 2012). This technique occurs more often in the Indo-Pacific because lionfish need to use alternative methods of hunting in order to capture their native prey (Benkwitt et al. 2012). Lionfish are not novel predators in the Indo-Pacific so they have to try harder to successfully capture prey (Benkwitt et al. 2012). However, in the southeastern Atlantic, the native fish are naïve to lionfish and have not experienced a predator that hunts in the same manner as the lionfish. The Atlantic prey fish do not have an inherent adaptation to counter the ambush behavior of the lionfish so they are at a great disadvantage when it comes to avoiding predation (Akins et al. 2011). Since these prey fish are already confused by the cornering behavior of the lionfish, it would be inefficient to use the water blowing technique due to the high energy cost for maintaining this ability (Albins, M.A. & Lyons, P.J. 2012). If the prey fish are already affected by the rounding up technique, the water blowing technique is not effective or efficient for the lionfish (Albins, M.A. & Lyons, P.J. 2012).

Along with this adapted techniques, lionfish typically hunt for their prey during the crepuscular periods of the day, also known as dawn and dusk (Akins et al. 2011). The reason lionfish tend to hunt during these times is because their prey rely on sight to avoid predators (Akins et al. 2011). Lionfish have a perfect opportunity to take full advantage and easily capture their prey in this setting of low light (Akins et al. 2011). Comparing this new data with other studies performed in the past, the previous studies of lionfish consumption numbers could be greatly underestimated (Akins et al. 2011; Arias-González et al. 2011).

One aspect of lionfish behavioral patterns is their tendency to stay relatively sedentary and not migrate far from region to region (Hare et al. 2004). Hare et al. discusses some of the consequences the lionfish face because of their sedentary lifestyle farther up the United States coastline (2004). When temperatures reach below 16°C, lionfish grow very lethargic and can no longer feed to sustain themselves (Hare et al. 2004; Matheson et al. 2006). Specifically, when temperatures reach below 10°C, lionfish are no longer able to properly move or perform adaptive behaviors, and quickly die (Hare et al. 2004; Matheson et al. 2006). This finding shows that because of lionfish's lack of migration, any lionfish remaining around the coastline of New York or New Jersey around winter cannot not survive until the warmer months (Hare et al. 2004). The temperature changes act as an efficient bio control in areas where the water reaches temperatures cold enough to kill off lionfish (Hare et al. 2004; Matheson et al. 2006).

Lionfish Diet:

The diet of invasive lionfish consists of a wide range of organisms including crabs, shrimp, herbivorous and piscivorous fish (McCleery 2011). The majority of the larval and young lionfish eat shrimp for their nutritional value and for its easy availability and capture within the southeastern Atlantic coastline (Albins, M.A. & Hixon, M.A. 2008). On the other hand, the larger lionfish usually eat other fish because they have become more efficient predators and require larger prey to satisfy their necessary nutritional levels (Akins et al. 2012). The invasive lionfish diet in the southeastern Atlantic differs from their native diet in the Indo-Pacific because the availability of prey fish is significantly larger and their prey does not recognize lionfish as a potential predator (Benkwitt et al. 2012). In the Caribbean and the southeastern United States coasts, the lionfish remains very novel, so the majority of potential predators and prey are not cognizant of the role lionfish play within their ecosystem (Benkwitt et al. 2012). This lack of knowledge can be very dangerous to the ecosystem. If one tier of the ecosystem is severely disrupted, then the entire ecosystem could potentially on itself (Arias-González et al. 2011).

Ecosystem collapse and consequences:

Lionfish are causing an imminent collapse in the coral reef ecosystem if their population growth and rate of predation continue as they have been (Arias-González et al. 2011). There are a few areas of the current ecosystem that if left unchecked for an extended period of time, could cause major problems (Arias-González et al. 2011). Different species of parrotfish, coral, and grouper are genera that are seriously affected by the introduction of lionfish into the southeastern Atlantic (Arias-González et al. 2011). The parrotfish is a family of species that are a consistent prey option for lionfish (Benkwitt et. al. 2012). These small herbivorous fish serve as a deterrent for seaweed growth around coral (Benkwitt et al. 2012). With the steady decline of parrotfish populations, seaweed has begun to have more room to grow and expand beyond its previous range (Benkwitt et al. 2012). This expansion has seaweed beginning to encroach near and onto coral reefs (Benkwitt et al. 2012). Once seaweed begins to grow on top of coral, the coral is destroyed (Benkwitt et al. 2012). The seaweed uses the coral as nourishment

until both the coral and the seaweed die. Seaweed and coral are a major food source for herbivores and also serve as a natural habitat for a wide range of species; therefore, reduction in seaweed and coral take a large hit on the ecosystem. This environmental change forces the remaining organisms to adapt very quickly in order to survive.

Grouper are a good example of a genus of fish that have not been able to adapt to the drastically changing environment around them (Brumbaugh et al. 2011). Grouper are an overfished organism that is quickly on the decline within the southeastern United States and the Caribbean coastline (Brumbaugh et al. 2011). Grouper are a potential solution to this intense overpopulation of lionfish because it is one of the few predators that have been recorded to prey upon lionfish (Brumbaugh et al. 2011). With their help, the lionfish population could be kept in check and possibly cause a decrease (Arias-González et al. 2011). However, now that groupers are on the decline, there is little stopping the rapid growth of the lionfish and the subsequent deterioration of the environment it resides (Arias-González et al. 2011). Without human intervention within these ecosystems, this progress will continue until the habitat collapses (Arias-González et al. 2011).

Human Involvement:

The original cause of the lionfish introduction in the Caribbean and the southeastern coast of the United States is a result of human intentional or unintentional aquarium release into the Atlantic Ocean (Carleton Ray et al. 2002). There are many theories in how lionfish were introduced, such as hurricane Andrew or mismanaged aquariums, but all scientists generally agree that it was because of human involvement that lionfish emerged into the southeastern Atlantic (Arias-González et al. 2011; Carleton Ray et al. 2002; Brumbaugh et al. 2011). Now that lionfish are populating the southeastern United States and thriving, it is important to identify the aiding effects humans have on the growing lionfish populations. The demand for groupers within the continental United States has been a rising trend and the fishing levels exhibited by humans exemplify this correlation. The overfishing of grouper, along with sharks, allows the lionfish to remain unchecked because grouper and sharks are the only observed potential predator to the lionfish (Albins, M.A. 2013). While research is still being conducted to observe the effect of grouper on the lionfish population, this group of piscivores is one of the very small collections of potential predators to the lionfish within the Caribbean and southeastern United States coastline (Brumbaugh et al. 2011).

The lionfish problem has alerted the scientific community and small steps have been taken to try and hinder the growth of these detrimental fish. Some groups of volunteers and scientists S.C.U.B.A. dive in attempts to catch lionfish and either euthanize them or bring them into labs for further research (Matheson et al. 2006). In areas where this has occurred, the population of lionfish seems to remain in check; however, the lionfish population is still too large to completely eradicate the detrimental effects that are occurring (Arias-González et al. 2011). While only some places have been attempting to fix this problem, the majority of people along the coastline areplagued by the lionfish. It has only been recently that research has begun to pick up and to try and find a way to slow the progress of the lionfish and eradicate them from these areas. However, projections have shown this to be impossible (Arias-González et al. 2011).

Conclusion:

Many adaptations, such as ambush style, water blowing, and coloration, give the lionfish a great advantage over their native predators as well as the invasive southeastern Atlantic habitat.

A problem that occurred throughout the studies, except Akins et al. (2011), was the timing of data collection. All of the other articles used in this essay explained that they took data collection throughout the day but did not continue observations into the dusk or even nighttime. According to Akins et al. (2011), the feeding activity in lionfish spiked during the crepuscular periods of the day; however, there was never any data collected concerning lionfish behavior during the nocturnal hours. An experiment could be recorded where the lionfish are tagged and subsequently monitored for their movements and traveling distances. Lionfish remain sedentary during the day when they are not feeding, so tracking the movement amongst the lionfish has the potential to be correlated to feeding patterns (Akins et al. 2011).

Another problem observed with these studies is the lack of research concerning the potential role of sharks as a bio control for lionfish. Lionfish are large piscivores that have a reputation for eating a wide range of organisms. There could be the possibility that sharks could be eating the lionfish and controlling the population and scientists are just unaware of it. The best way to try and determine the feeding patterns of the sharks around the infested reefs is to take a few S.C.U.B.A. trained divers to observe their feeding during the dusk and dawn hours of the day. This future study could show how sharks react with a large collection of lionfish and if they favor other fish above lionfish. With these changes in lionfish research, the scientific community could have a better understanding of how to control this growing population.

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