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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 XXXVI marks the fifteenth 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 front and back cover and section divider art for this year’s edition of the *Rhodes Journal of Biological Sciences* was created by Julia Bergquist, an English Creative Writing and Literature major from Barrington, IL. The cover illustrates the subject for one of the featured articles in this year’s edition which researched, the Spotted Lanternfly, and the section divider is an artistic rendition of the molecular structure of COVID-19.

Editorial Staff

Annelise Swords '21 (Senior Editor) is a Biology major with a concentration in Biomedical Sciences and Music minor from Springfield, Missouri. On campus, she served as a Patient Experience and Rotations intern at Methodist University Hospital, an Audiovisual Fellow at the Mike Curb Institute of Music, and the Senior Panhellenic Delegate for her sorority, Alpha Omicron Pi. Annelise is a member of the Health Professions Society, and the Beta Beta Beta and Order of Omega honor societies. Off campus, she volunteers with Thistle and Bee and writes for *Triage Health Humanities*. Upon graduating, she will spend a year as a research assistant in the Pharmaceutical Science department at St. Jude. And after that, who knows?

Emma Root '22 (Senior Editor) is a Biology major with a Chemistry Minor from Barrington, IL. On campus, she serves as the president of the Rhodes College Equestrian Team and is a member of the Chi Omega sorority. She also is a member of honor societies including Omicron Delta Kappa and Mortar Board, among others. Off campus, Emma is an academic intern in the Memphis Zoo Research Department, specifically working under a Conservation Biologist. Her favorite zoo project to date has been assisting with the reproductive efforts of the endangered Louisiana Pinesnakes. During academic breaks, she works as a veterinary technician in a small animal vet hospital and is applying to veterinary school this year.

Khanh Ton '23 (Junior Editor) is an Environmental Sciences major from Hue, Vietnam. She is a GIS Lab Supervisor RSA and an intern at the Overton Park Conservancy. In the past year, she worked as an Animal Behavior and Conservation Fellow at the Memphis Zoo and was also involved with inorganic chemistry research in Professor Eckenhoff's lab. She is part of the first cohort of the Kappa Alpha Omicron honor society. Outside of the classroom, she is the Chapter President of Food Recovery Network, an Event Chair of All Students Interested in Asia (A.S.I.A.), and a member of Delta Delta Delta's Diversity, Equity, & Inclusion Committee. This summer, Khanh will continue her research at the Overton Park's Old Growth Forest as well as the Rhodes College Arboretum as a Midtown Urban Forestry Fellow.

Madeline Yde '23 (Junior Editor) is a Biology and Environmental Science major from Racine, Wisconsin. She has been involved in research at the Memphis Zoo since Spring of 2020, with experience in monitoring African elephant behavior as well as dusky gopher frog tadpole growth rates. Maddy had the opportunity to intern for the Racine Zoo in their Conservation Education Department in Summer of 2020, gaining animal handling and presenting knowledge. Maddy is a member of the women's soccer team and Kappa Alpha Omicron Environmental Honor Society at Rhodes. She enjoys working at her local veterinary clinic and gymnastics center when not on campus. In addition to her interests in sciences, Maddy hopes to have the opportunity to continue exploring studies in Spanish and Latin American and Latinx studies. After graduation, she intends to pursue veterinary medicine.

Izzy Wollfarth '24 (Junior Editor) is from New Orleans, Louisiana. She is a rising sophomore and hopes to major in Biology with a minor in English. With these concentrations, she hopes to pursue a career in heart surgery! She really enjoys writing papers, especially about different aspects of the world and science. Aside from being involved in RJBS, she is also a part of KD, HPA, and A.S.I.A at Rhodes. In her free time, she likes making art, watching movies, running, and listening to music. She hopes you enjoy reading this journal that she and some other great people have put together!

Understanding the Mystery of Peto's Paradox to Treat Human Cancer

Deja Walls

Cooperation is a necessary feature in the evolution of multicellularity. Cancer occurs when individual cells are no longer willing to cooperate and cheat the benefits of being a part of an organism. Because these cells essentially revert to a unicellular state, cancer is fundamentally the breakdown of multicellularity. Because cancer typically occurs as the result of mutations during genetic duplication, it would be expected that cancer incidence rates would increase as a function of cell divisions. However, that is not the case in nature. This observation led to the creation of Peto's Paradox, the idea that there is no observed correlation between cancer incidence and the size and age of animals across life. It is hypothesized that this is the result of evolved mechanisms to avoid and suppress cancer. Some organisms have managed to master these mechanisms. For example, the naked mole rat utilizes early contact inhibition to prevent their cells from becoming too crowded. Blind mole rats have evolved massive necrotic cell death in response to uncontrolled proliferative cell behavior. Lastly, elephants have evolved many copies of tumor suppressor proteins TP53 and LIF that both contribute to highly sensitive apoptotic response to tumor cells. Learning from these different routes of evolving cancer resistance can lead to the creation of derived, novel cancer innovations for human cancer.

Keywords: blind mole rat, cancer, elephant, naked mole rat, Peto's Paradox

Abbreviations

NMR – naked mole rat

HA - hyaluronan

INF-β – interferon-β

LIF – leukemia inhibitory factor

Introduction

The study of multicellularity is relevant when considering cancer because cancer is the evolutionary failure of multicellularity (Aktipis et al., 2015). One necessary feature of multicellularity is the ability for individual cells to cooperate in group-beneficial behaviors (Smith and Szathmary 1995). Complex multicellularity is reliant on five pillars of cooperation in order to be effective – proliferation inhibition, controlled cell death, maintenance of extracellular environment, division of labor, and resource allocation (Aktipis et al., 2015). When individual cells undergo these cooperative behaviors, the benefits are shared among the group. However, individual “cheater” cells disrupt one or more of the pillars of cooperation. Although research has shown that cheating is almost never an all-or-nothing situation (Madgwick & Wolf, 2020), cheater cells reap the benefits of being a part of the group while avoiding the costs of contribution. Cancer is the manifestation of the breakdown of these pillars, characterized by uncontrolled proliferation, inappropriate cell survival, environmental degradation, dysregulated differentiation, and resource monopolization (Aktipis et al., 2015; Hanahan & Weinberg, 2011). This occurs in nature, and specifically in cancer, at the expense of the organism. (Aktipis, C. et al., 2015). Thus, complex multicellular organisms must also evolve

mechanisms to maintain cooperation among cells and suppress the development of cancerous cells (Smith and Szathmary 1995).

It has been assumed that with an increase in size and time spent exposed to carcinogens (Peto R. et al., 1975) comes an increase in the risk of cancer (Nunney, 2018). Hypothetically, with every cell division comes the same risk of a somatic mutation resulting in cancer (Hanahan and Weinberg, 2011). Thus, one would expect the rate of cancer incidence should appear across life on earth as a function of lifetime cell divisions between species (Caulin et al., 2015). Theoretically, animals that live longer and/or grow larger in size should have higher cancer rates than other smaller, shorter-lived organisms. However, research shown that this is not the case for many mammals (Abegglen et al., 2015). This seemingly contradictory observation was deemed Peto's Paradox after Richard Peto, the British epidemiologist that noticed this trend (Peto, 2016; Peto et al., 1975; see figure 1).

Across life, the highest rates of cancer incidence are found in mammals (Effron et al., 1977). These rates also tend to also increase more with age, although age may not necessarily be the direct cause (Peto et al., 1975). More specifically, cancer is responsible for up to 46% of all multicellular organisms' deaths and 23% of all human deaths (Heron, 2012). Cancer rates for humans continue to increase at a striking rate as average human life expectancy increases (Albuquerque et al, 2018). The most common human cancers include skin carcinomas, lung cancers, breast cancer, and colorectal cancer (Albuquerque et al, 2018).

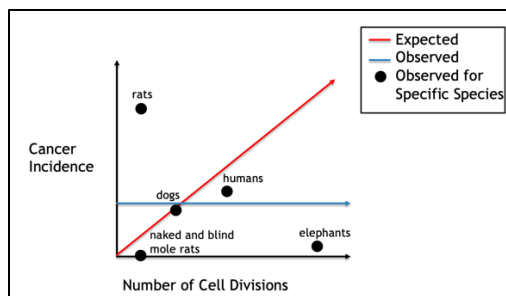


Figure 1. Peto's Paradox. Although cancer incidence rate would exist as a function of the number of cellular divisions, it is not observed in nature. It should be mentioned that the diagrammatic line representing the observed cancer rate has been dramatically simplified for the purpose of the figure. Actual observed cancer rates vary greatly based on the type of cancer, species, and the efficiency of evolved cancer avoidance and suppression mechanisms. (PEEL Therapeutics)

Many have asked how these large, long-lived organisms manage to avoid cancer despite their greater risk of cancer mutations. The answer to this question is that there is not simply one answer. Multiple routes of cancer resistance have naturally evolved over time. However, researchers have hypothesized some specific mechanisms of cancer suppression from examples of Peto's Paradox. How these mechanisms work and how to answer these questions is still under scrutiny. This article will work to discuss these mechanisms and their potential applications for human cancer in the case of three model organisms of Peto's Paradox – naked mole rats, blind mole rats, and elephants.

NAKED MOLE RATS

Heterocephalus glaber, or the naked mole rat (NMR), are vertebrates that can live up to 30 years, which is remarkable in contrast to the two to three-year lifespan of other similarly sized mice (Austad, 2010). In addition, NMRs only experience light changes in morphological and physiological characteristics including their fertility rates which stay consistent until the last decade of their (Buffenstein and Jarvis, 2002; Buffenstein, 2008). These animals are an ideal example of Peto's Paradox due to their long lifespan coupled with their highly successful mechanisms of cancer suppression. Very few instances have been found in which tumors or spontaneous neoplasia have developed in NMRs (Delaney et al., 2016; Taylor et al., 2016). These few cases must be considered with caution because the sample population consisted of descendants from a single pair of NMRs (Albuquerque et al, 2018). This

leaves a greater potential for inbreeding, which can lead to increases in rates of cancer for the offspring (Rudan et al., 2003).

One hypothesis for the NMR's efficient cancer suppression originates not from within the cells themselves, but from the extracellular matrix. They can produce a high-mass carbohydrate polymer called hyaluronan (HA) that works to maintain distance between cells (Tian et al., 2013). HA is an important player in maintaining extracellular matrix, one of the five pillars of cooperation that limit cancerous cheater cells as mentioned prior (Aktipis et al., 2015). This polymer is also found in other mammals, but the HA found in NMRs is much higher in molecular mass (Snetkov et al., 2020; Tian et al., 2013). Research has shown that this form as greater cytoprotective properties when compared to shorter HA polymers (Takasugi et al., 2020) and is caused by two mutations in genes for a hyaluronan synthase protein (Faulkes et al., 2015).

This HA provides NMRs with a highly sensitive form of contact inhibition that prevents the overcrowding of their cells faster than in other mammals (Seluanov et al. 2009). Researchers have shown that contact inhibition is an important cancer suppression mechanism and this ability lost in cancerous cells (Abercrombie 1979). HA functions in a signaling pathway inhibits cellular division by arresting the cell cycle in response to the cells reaching a high density. Although the p27 protein mediates this pathway in humans, mice, and NMRs, NMRs can utilize p16 to arrest the cell cycle earlier than p27 (Seluanov et al., 2009; see figure 2). In addition, NMR cells have a higher affinity for HA than mouse or human cells, which promote more sensitive hyaluronan signaling (Tian et al., 2013). It is hypothesized that these factors all contribute to the highly successful levels of cancer resistance in the NMR.

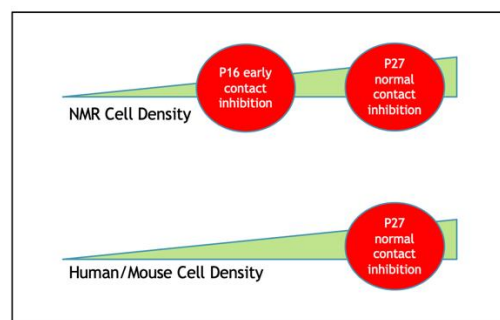


Figure 2. Difference between NMR and typical mammal mechanisms of contact inhibition. NMRs possess an additional, early ability to initiate cellular contact inhibition as compared to humans and mice. This provides one explanation for the highly successful anticancer capabilities of the NMR.

BLIND MOLE RATS

Organisms of the genus *Spalax*, or blind mole rats, also exhibit remarkable longevity. Their lifespan typically lasts 7 and 10 years longer than similar mouse species, sometimes reaching more than 20 years (Edrey et al., 2012). The BMR is also known to successfully utilize cancer suppression mechanisms, avoiding spontaneous and induced tumors (Manov et al., 2013).

BMRs are like NMRs in that both are able to produce high molecular weight HA (Faulkes et al., 2015). The difference lies in the mechanism of producing this molecule. NMR HA synthases possess two mutations that lead to the larger size HA, whereas BMR HA synthases do not have these mutations (Faulkes et al., 2015). It is understood that the BMRs primary mechanism of cancer resistance does not involve hypersensitive, early contact inhibition (Gorbunova et al., 2012).

The BMR's remarkable resistance to cancer is even more surprising considering the mutation found in its p53 gene. The p53 gene is known as a major tumor suppressor gene in many mammals and has been observed to contain mutations in most human cancers (Hollstein et al., 1991). The mutation in BMRs prevents p53 from inducing apoptosis, although it is still functional in other pathways (Avivi et al., 2007). It has been hypothesized that the BMR mediates cancer suppression by means of an immunoinflammatory response involving the interferon β 1 pathway (Gorbunova et al., 2012). When BMR cells are subjected to uncontrolled cell proliferation, the cells release INF- β to initiate massive levels of concerted cell death.

This concerted cell death functions to destroy any possibly malignant cells. BMRs are different from most mammals because their cells exhibit a preference for necrosis rather than apoptosis, despite its supposed lack of precision and disorganized results (Gorbunova et al., 2012). One explanation for why necrosis may be preferred to apoptosis is because necrosis destroys the entire microenvironment around the reactive tumor. This environment which necrosis destroys includes area that apoptosis might have missed (Mueller and Fusenig, 2004). The strikingly low rates of cancer incidents observed creates a case that the concerted necrotic cell death as a mechanism for cancer suppression is more beneficial than harmful for the BMR. Although this necrotic mechanism of concerted cell death is different from the controlled cell death pillar of cooperative behaviors mentioned prior (Aktipis, C. et al., 2015), it still functions to limit cancerous uncontrolled cell proliferation.

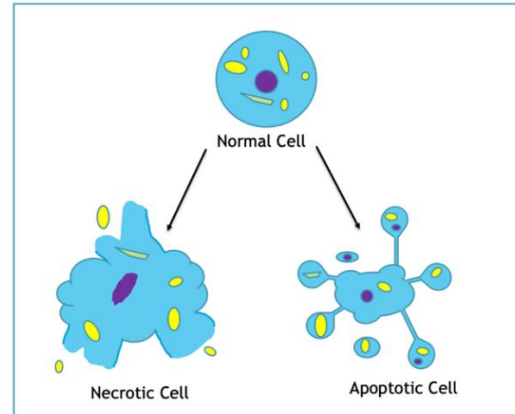


Figure 3. Difference between necrotic and apoptotic mechanisms of concerted cell death.

Necrotic cell death typically results in increases in cell volume, loss of plasma membrane integrity, and the dispersal of cellular contents to the extracellular matrix. Also, necrosis is an inflammatory response that often leads to premature cell death of neighboring cells. Apoptosis results in decreases in cell volume, the plasma membrane remains intact, and cellular contents are contained within apoptotic bodies. Apoptosis is typically considered the more controlled mechanism of cell death, because it is a programmed response that does not affect uninvolved, neighboring cells.

ELEPHANTS

Lastly, elephants are a great example of Peto's Paradox due to their combined longevity and large size. African elephants are the largest land mammal on earth (Howard 2017) and have a maximum lifespan of 74 years (Lee et al. 2012), while Asian elephants can live up to 80 years (Lahdenperä et al. 2014). Despite this longevity and large body size, elephants have a lower cancer incidence rate and mortality rate than that of humans (Abegglen et al., 2015).

The accepted hypothesis for the elephant's efficient form of cancer suppression involves multiple copies of a tumor suppressor gene called TP53 (Sulak et al., 2016; Abegglen et al., 2015). This gene encodes for the p53 protein, a protein that works as a transcription factor for many tumor suppressor target genes (Mandinova and Lee, 2011; see figure 4). TP53 gene family plays a major role in inhibiting the development of cancer. This supports the 100% decrease in cancer incident rates found in mice after knocking out this gene (Donehower et al., 1992) and the presence of mutations in this gene in almost all cancers (Nigro et al., 1989). The elephant genome contains a vast 20 different copies of this gene, in

contrast to the 1 copy found in humans (Abegglen et al., 2015). In addition, elephant cells exhibit twice the sensitivity to apoptosis induced by DNA damage than that of human cells. Apoptosis serves as a cheater suppression mechanism to control cell proliferation (Aktipis et al., 2015) and reduces ongoing mutation rate for the organism (Abegglen et al., 2015), thus explaining the elephant's success in resisting cancer.

An additional possible genetic source for the elephant's remarkably low cancer incidence rate may be due to another gene family called leukemia inhibitor factors (LIF). LIF proteins can function as either an oncogene or as a tumor suppressor depending on the situation (Vasquez et al., 2018). The elephant version of this gene, LIF6, functions as mitochondrial mediated apoptosis in response to DNA damage (Vasquez et al., 2018). LIF6 is upregulated by p53 (Vasquez et al., 2018). Elephants possess 11 additional copies of this gene than humans, which makes elephant cells more responsive to DNA damage.

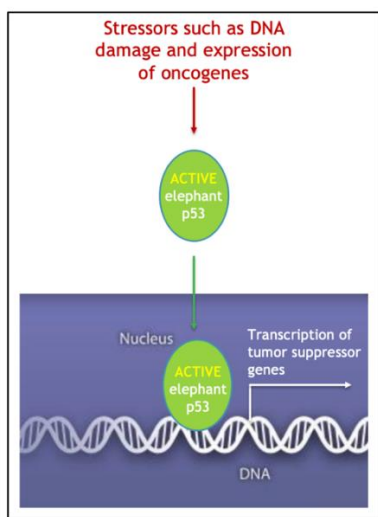


Figure 4. Function of the elephant TP53 protein. Elephant TP53 gene encodes for the p53 protein that responds to various stressors such as DNA damage and oncogene expression. It works as a transcription factor, promoting the transcription of various tumor suppressor proteins like LIF6. (Vasquez et al., 2018)

Applications in Human Cancer

The most promising evidence for the possibility of successfully applying these animal mechanisms of cancer resistance to preventing human cancer involves elephant TP53 genes. Joshua Schiffman, a pediatric oncologist at PEEL Therapeutics, believes that utilizing nature's pathways to cancer resistance should be considered when making new cancer drugs for patients.

Researchers at PEEL Therapeutics have already begun studying how to use the elephant TP53 gene to attack human cancers using nanoparticle drug delivery technology. This study is relatively new so only *in vitro* studies are currently underway. The fact that the elephant TP53 gene can be studied at such a context is compelling evidence for why studying model organisms of Peto's Paradox can fundamentally change the way novel cancer drugs are innovated.

On the other hand, it may be more difficult for research into the NMRs cancer resistance mechanism to provide new insights into human cancer prevention. The function of HA can differ based on its molecular weight, size, cell type in which it is found, and interactions with binding proteins (Liang et al., 2016). In contrast to HA in NMRs, human HA promotes and accelerates the growth of tumors in cancerous cells. These cancerous cells tend to accumulate low molecular weight HA, which blocks the interference of cancer drugs. One nanoparticle-based treatment involves degrading low molecular weight HA to promote the functionality of the drugs (Rankin and Frankel, 2016). However, anticancer drugs can also be delivered to cancer cells in the form of nanoparticle coated and covalently bound low molecular weight HA. HA has been found to be very useful as a delivery mechanism for humans (Choi et al., 2010) because of its tendency to accumulate in tumors (Lokeshwar et al., 2014).

It is even more difficult to apply the BMRs mechanisms of cancer suppression. There is much debate about whether the necrotic cell death response was due to the presence of cancerous cells or extensive stressors introduced to the cells by faulty methodology (Saey, 2012). In addition, necrotic cell death responses of human cancer cells are typically known to promote the aggressiveness and progression of tumors (Su et al., 2018). More research into the cellular mechanisms behind the BMRs necrotic cell death mechanism is necessary to determine if it is at all applicable to humans.

Conclusion

Cancer is essentially a disease of multicellularity due to cells' inability to perform beneficial behaviors collectively (Aktipis et al., 2015). Without successful cooperation, cancer cells reap the benefits of being a part of a group even to the detriment of the organism. Advances in human cancer prevention studies are critical to combat the ever-increasing cancer incident rates observed in humans. Peto's Paradox describes the unexpected lack of relation between cancer incidence and age or body size. This is because some animals have

managed to evolve various cancer resistance mechanisms to suppress cheaters. Although rodents such as mice and rats have historically served as important cancer models for humans (Albuquerque et al., 2018), the time has come to extrapolate data from model organisms of Peto's Paradox.

All three model organisms discussed in this paper - naked mole rats, blind mole rats, and elephants - have incredibly low cancer incidence rates despite their increased risk from age or large body size. The naked mole rat does so via early contact inhibition between cells, mediated by hyaluronan. Blind mole rats resist cancer using a massive, necrotic cell death mechanism triggered by interferon- β when uncontrolled proliferative cells are detected. Elephants have an extensive number of copies of tumor suppressor genes, TP53 and LIF6, that makes elephant cells more sensitive to apoptosis induced by DNA damage.

The elephant TP53 gene for cancer resistance is already being studied for application to human cancer. Researchers are hopeful in finding novel cancer therapeutics using this protein delivered by nanoparticle technology. In the case of the naked mole rat, their version of HA is vastly different in function from humans. However, research into the similarities and differences between the other components of the hyaluronan signaling pathway could prove to be insightful. Is it possible to administer high molecular weight HA to humans? Could researchers modulate the effects of HA synthases so human cells could create high molecular weight HA on their own? It is important to investigate and answer questions like these to uncover the possibility of adapting the naked mole rat's early, sensitive contact inhibition to humans. The blind mole rat, however, is in need of more conclusive research in order to discover applications to human cancer. The same is true for other model organisms of Peto's Paradox like bats and bowhead whales (Callier, 2019; Albuquerque et al., 2018).

Currently being studied are other proposed cancer resistance mechanisms that stem from model organisms of Peto's Paradox not included in this article. Some of these include a low rate of low caloric intake, increased reproductive fitness with age, increased immune surveillance, decreased telomere length, and many more (Albuquerque et al., 2018). It is critical to perform genetic comparative analyses to determine if these efficient cancer resistance mechanisms may be translated to humans. Of course, this would also require extensive genomic sequencing studies of less-investigated Peto's Paradox model organisms. Investigating the mechanisms behind the experts of cancer resistance

will likely open new doors to cancer prevention and treatment in humans.

Literature Cited

- Abegglen, L. M., Caulin, A. F., Chan, A., Lee, K., Robinson, R., Campbell, M. S., Schiffman, J. D. (2015). Potential mechanisms for cancer resistance in elephants and comparative cellular response to DNA damage in humans. *JAMA*, 314(17), 1850–60. <https://doi.org/10.1001/jama.2015.13134>
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. (1990) CD44 is the principal cell surface receptor for hyaluronate. *Cell*; 61(7):1303–1313. [https://doi.org/10.1016/0092-8674\(90\)90694-A](https://doi.org/10.1016/0092-8674(90)90694-A)
- Aktipis, C. A., Boddy, A. M., Jansen, G., Hibner, U., Hochberg, M. E., Maley, C. C., & Wilkinson, G. S. (2015). Cancer across the tree of life: cooperation and cheating in multicellularity. *Philosophical Transactions: Biological Sciences*, 370(1673), 1–21.
- Albuquerque, T. A. F., Drummond do Val, L., Doherty, A., & de Magalhães, J. P. (2018). From humans to hydra: patterns of cancer across the tree of life. *Biological Reviews*, 93(3), 1715–1734. <https://doi.org/10.1111/brv.12415>
- Abercrombie M. (1979) Contact inhibition and malignancy. *Nature* 281:259–262.
- Austad, S. N. (2010). Methuselah's Zoo: how nature provides us with clues for extending human health span. *Journal of Comparative Pathology* 142, S10–S21.
- Avivi A, Ashur-Fabian O, Joel A, Trakhtenbrot L, Adamsky K, Goldstein I, Amariglio N, Rechavi G, Nevo E. P53 in blind subterranean mole rats--loss-of-function versus gain-of-function activities on newly cloned Spalax target genes. *Oncogene*. 2007 Apr 12;26(17):2507-12. doi: 10.1038/sj.onc.1210045. Epub 2006 Oct 16. PMID: 17043642.
- Buffenstein R, Jarvis JU (2002) The naked mole rat-a new record for the oldest living rodent *Sci Aging Knowledge Environ* 2002:pe7 2
- Buffenstein, R. (2008). Negligible senescence in the longest living rodent, the naked mole-rat: Insights from a successfully aging species. *Journal of Comparative Physiology.B, Biochemical, Systemic, and Environmental Physiology*, 178(4), 439-45. <https://doi.org/10.1007/s00360-007-0237-5>
- Callier V. (2019). Core Concept: Solving Peto's Paradox to better understand cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 116(6), 1825–1828. <https://doi.org/10.1073/pnas.1821517116>

- Caulin, A. F., & Maley, C. C. (2011). Peto's paradox: evolution's prescription for cancer prevention. *Trends in Ecology & Evolution*, 26(4), 175–82. <https://doi.org/10.1016/j.tree.2011.01.002>
- Caulin, A. F., Graham, T. A., Wang, L. S., & Maley, C. C. (2015). Solutions to peto's paradox revealed by mathematical modelling and cross-species cancer gene analysis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 370(1673). <https://doi.org/10.1098/rstb.2014.0222>
- Choi, K. Y., Chung, H., Min, K. H., Yoon, H. Y., Kim, K., Park, J. H., ... Jeong, S. Y. (2010). Self-assembled hyaluronic acid nanoparticles for active tumor targeting. *Biomaterials*, 31(1), 106–114. <https://doi.org/10.1016/j.biomaterials.2009.09.030>
- Delaney, M. A., Ward, J. M., Walsh, T. F., Chinnadurai, S. K., Kerns, K., Kinsel, M. J. & Treuting, P. M. (2016). Initial case reports of cancer in naked mole-rats (*Heterocephalus glaber*). *Veterinary Pathology* 53, 691–696.
- Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A. J., Butel, J. S., & Bradley, A. (1992). Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature*, 356(6366), 215–21.
- Edrey, Y. H., Casper, D., Huchon, D., Mele, J., Gelfond, J. A., Kristan, D. M., Buffenstein, R. (2012). Sustained high levels of neuregulin-1 in the longest-lived rodents; a key determinant of rodent longevity. *Aging Cell*, 11(2), 213–222. <https://doi.org/10.1111/j.1474-9726.2011.00772.x>
- Effron M, Griner L, Benirschke K (1977) Nature and rate of neoplasia found in captive wild mammals, birds, and reptiles at necropsy. *J Natl Cancer Inst* 59:185–198.
- Faulkes, C. G., Davies, K. T. J., Rossiter, S. J., & Bennett, N. C. (2015). Molecular evolution of the hyaluronan synthase 2 gene in mammals: implications for adaptations to the subterranean niche and cancer resistance. *Biology Letters*, 11(5). <https://doi.org/10.1098/rsbl.2015.0185>
- Gorbunova, V., Hine, C., Tian, X., Ablueva, J., Gudkov, A. V., Nevo, E., & Seluanov, A. (2012). Cancer resistance in the blind mole rat is mediated by concerted necrotic cell death mechanism. *Proceedings of the National Academy of Sciences of the United States of America*, 109(47), 19392–19396.
- Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646–74. <https://doi.org/10.1016/j.cell.2011.02.013>
- Heron M (2012) Deaths: Leading Causes for 2008, National Vital Statistics Reports 60(6):9–11. F Hollstein, M., Sidransky, D., Vogelstein, B., & Harris, C. C. (1991). P53 mutations in human cancers. *Science*, 253(5015), 49, 53.
- Howard, M. 2017. "Loxodonta africana" (On-line), Animal Diversity Web. Accessed November 12, 2020 at https://animaldiversity.org/accounts/Loxodonta_africana/
- Lahdenperä, M., Khyne, U. M., & Virpi, L. (2014). Reproductive cessation and post-reproductive lifespan in Asian elephants and pre-industrial humans. *Frontiers in Zoology*, 11(1), 54–54. <https://doi.org/10.1186/s12983-014-0054-0>
- Liang, J., Jiang, D., & Noble, P. W. (2016). Hyaluronan as a therapeutic target in human diseases. *Advanced Drug Delivery Reviews*, 97, 186–203. <https://doi.org/10.1016/j.addr.2015.10.017>
- Leroi, A. M., Koufopanou, V., & Burt, A. (2003). Cancer selection. *Nature Reviews. Cancer*, 3(3), 226–31. <https://doi.org/10.1038/nrc1016>
- Lokeshwar, V. B., Mirza, S., & Jordan, A. (2014). Targeting hyaluronic acid family for cancer chemoprevention and therapy. *Hyaluronan Signaling and Turnover*, 123, 35–65. <https://doi.org/10.1016/B978-0-12-800092-2.00002-2>
- Lee, P. C., Sayialel, S., Lindsay, W. K., & Moss, C. J. (2012). African elephant age determination from teeth: validation from known individuals. *African Journal of Ecology*, 50(1), 9–20. <https://doi.org/10.1111/j.1365-2028.2011.01286.x>
- Madgwick, P.G. & Wolf, J.B. (2020). Evolution of strategic cooperation. *Evolution Letters*, 4(2), 164–175. <https://doi.org/10.1002/evl3.164>
- Mandinova, A., & Lee, S. W. (2011). The p53 pathway as a target in cancer therapeutics: obstacles and promise. *Science translational medicine*, 3(64), 64rv1. <https://doi.org/10.1126/scitranslmed.3001366>
- Manov, I., Hirsh, M., Iancu, T. C., Malik, A., Sotnichenko, N., Band, M., Shams, I. (2013). Pronounced cancer resistance in a subterranean rodent, the blind mole-rat, spalax: in vivo and in vitro evidence. *Bmc Biology*, 11, 91–91. <https://doi.org/10.1186/1741-7007-11-91>
- Mueller, M. M., & Fusenig, N. E. (2004). Friends or foes - bipolar effects of the tumour stroma in cancer. *Nature Reviews. Cancer*, 4(11), 839–49.

- Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., et al. (1989). Mutations in the p53 gene occur in diverse human tumour types. *Nature*, *342*(6250), 705–8.
- Nunney, L. (2018). Size matters: height, cell number and a person's risk of cancer. *Proceedings Biological Sciences*, *285*(1889). <https://doi.org/10.1098/rspb.2018.1743>
- Orian-Rousseau V. CD44 Acts as a Signaling Platform Controlling Tumor Progression and Metastasis. *Frontiers in immunology*. 2015; 6:154.
- “PEEL Innovation.” *Peeltx*, www.peeltx.com/science
- Peto, R., Roe, F. J., Lee, P. N., Levy, L., & Clack, J. (1975). Cancer and ageing in mice and men. *British Journal of Cancer*, *32*(4), 411–26.
- Peto, R. (2016). Epidemiology, multistage models, and short-term mutagenicity tests. *International Journal of Epidemiology*, *45*(3), 621–37. <https://doi.org/10.1093/ije/dyv199>
- Pfeiffer, T., & Bonhoeffer, S. (2003). An evolutionary scenario for the transition to undifferentiated multicellularity. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(3), 1095–1098. <https://doi.org/10.1073/pnas.0335420100>
- Rankin KS, Frankel D (2016) Hyaluronan in cancer from the naked mole rat to nanoparticle therapy. *Soft Matter* *12*:3841–3848.
- Rudan, I., Rudan, D., Campbell, H., Carothers, A., Wright, A., Smolej-Narancic, N., Janicijevic, B., Jin, L., Chakraborty, R., Deka, R., & Rudan, P. (2003). Inbreeding and risk of late onset complex disease. *Journal of medical genetics*, *40*(12), 925–932. <https://doi.org/10.1136/jmg.40.12.925>
- Saey, Tina Hesman (5 November 2012). “Cancer cells self-destruct in blind mole rats.” *Science News*.
- Seluanov A, et al. (2009) Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat. *Proc Natl Acad Sci USA* *106*:19352–19357.
- Maynard Smith J, Szathmary E. 1995 The major transitions of life. New York, NY: WH Freeman.
- Snetkov, P., Zakharova, K., Morozkina, S., Olekhovich, R., & Uspenskaya, M. (2020). Hyaluronic Acid: The Influence of Molecular Weight on Structural, Physical, Physico-Chemical, and Degradable Properties of Biopolymer. *Polymers*, *12*(8), 1800. <https://doi.org/10.3390/polym12081800>
- Su, Y. L., Min, K. J., Hyun, M. J., Eui, K. J., Yig, J. L., Cho, H. K., et al. (2018). Regulation of tumor progression by programmed necrosis. *Oxidative Medicine and Cellular Longevity*, *2018*. <https://doi.org/10.1155/2018/3537471>
- Sulak, M., Fong, L., Mika, K., Chigurupati, S., Yon, L., Mongan, N. P., Lynch, V. J. (2016). Tp53 copy number expansion is associated with the evolution of increased body size and an enhanced dna damage response in elephants. *Elife*, *5*. <https://doi.org/10.7554/eLife.11994>
- Takasugi, M., Firsanov, D., Tomblin, G., Ning, H., Ablaeva, J., Seluanov, A., & Gorbunova, V. (2020). Naked mole-rat very-high-molecular-mass hyaluronan exhibits superior cytoprotective properties. *Nature Communications*, *11*(1), 2376. <https://doi.org/10.1038/s41467-020-16050-w>
- Taylor, K. R., Milone, N. A. & Rodriguez, C. E. (2016). Four cases of spontaneous neoplasia in the naked mole-rat (*Heterocephalus glaber*), a putative cancer-resistant species. *The Journal of Gerontology, Series A: Biological Sciences and Medical Sciences* *72*, 38–43.
- Tian X, et al. (2013) High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. *Nature* *499*:346–349.
- Vazquez, J. M., Sulak, M., Chigurupati, S., & Lynch, V. J. (2018). A zombie lif gene in elephants is upregulated by tp53 to induce apoptosis in response to dna damage. *Cell Reports*, *24*(7), 1765–1776. <https://doi.org/10.1016/j.celrep.2018.07.042>

Impact of a Colorful Enrichment item versus a White Enrichment item in *Rhinoptera bonasus* and *Dasyatis americana*

Meredith Bacue and Gretta Hotz

*Enrichment in zoos is a crucial part of ensuring physically and mentally healthy captive animals (who may not experience a varying daily schedule or varying environment) and avoiding and relieving issues such as stress, addiction, and stereotypic behaviors (Smith, 2016). The purpose of this research was to gain a better insight into how the color of an enrichment item affects stingray interaction with the enrichment item. The research was conducted at the Memphis Zoo's Stingray Bay touch pool. The subjects consist of 54 cownose stingrays (*Rhinoptera bonasus*) and 6 southern stingrays (*Dasyatis americana*). Two identically built enrichment items were designed, but one was colorful (orange, blue, green, purple) while the other was entirely white. Data for how many interactions each enrichment item experienced were collected using continuous recording and behavioral sampling, according to the ethogram for 5 minutes. We also collected data to see how many stingrays passed through the general area where the enrichment item had been floating. We found that the white enrichment item experienced more interaction ($t_8 = -2.597, p = .032$) and that Location A (by the waterfall) was preferred over Location B (opposite side of the waterfall) ($t_8 = 3.876, p = .005$). Understanding the impact of color in an enrichment item is key to creating more effective enrichment items.*

Key Words: captivity, color, *Dasyatis americana*, enrichment, interaction, *Rhinoptera bonasus*, stingrays

Introduction

Cownose stingrays, *Rhinoptera bonasus*, and southern stingrays, *Dasyatis americana*, are cartilaginous fish (Kittle, 2018). Both species are non-aggressive, however, they will use their venomous spine in self-defense when necessary (Kittle and Piercy, 2018). Sharks and other large fish are stingrays' main predators (Kittle, 2018; Passarelli and Piercy, 2018).

Cownose stingrays are found in shallow, brackish, and marine habitats, and tend to swim at the surface of the water (Kittle, 2018). These stingrays are known to school and complete long migrations and are considered oceanodromous (Kittle, 2018). Their diet commonly consists of nekton, zoobenthos, finfish, benthos crustaceans, mollusks, bony fish, crabs, lobsters, bivalves, and gastropods (Kittle, 2018). Cownose stingrays are very good at locating and obtaining deep-burrowing prey; they use mechano- or electro-receptive detection to seek out their meal (Kittle, 2018).

Southern stingrays' habitat consists of shallow or estuarine habitats and is usually found on the sandy floor (Passarelli and Piercy, 2018). Southern stingrays are not observed often in large groups, but more often alone or in pairs, unlike cownose stingrays (Passarelli and Piercy, 2018). Their diet consists of prey that lives on the sand at the bottom of their environment, stomatopods, mollusks, and annelids (Passarelli and Piercy, 2018). Southern stingrays also utilize electro-reception to catch their prey (Passarelli and Piercy, 2018).

In captivity, animals are exposed to a very predictable daily routine and a steady environment (Watters, 2009). To combat any issues that may arise from a constant lifestyle, using enrichment in zoos is a crucial aspect of establishing an environment that benefits the captive animal and enhances their brain function, in turn keeping captive animals both physically and mentally healthy (Smith, 2016). Enrichment can also promote natural time budgets of animals in a captive setting (Watters, 2009). To start, the addition of enrichment items gives animals the ability to control components of their environment, which in turn gives animals the authority to manage contingencies in their habitats (Watters, 2009). Furthermore, the addition of enrichment into the daily routine can help prevent and reverse effects of stress, addiction, and stereotypic, unnatural behaviors that may very well be a result of consistent routine and unchanging surroundings (Smith, 2016). The brain is stimulated as a result of the complexity that the enrichment item provides to the environment, especially if the object is novel, favorable to the one interacting with it, and if it increases both social contact as well as physical activity (Smith, 2016).

As of now, enrichment of aquatic animals is fairly basic and mainly entails variability in the tank. One easily achievable form of enrichment is structural enrichment, which can be used simply for shelter, but also for reducing aggression and providing sensory and cognitive stimulation (Näslund and Johnsson, 2014). Oftentimes, these structures include pipes, tiles, and non-buoyant plastic strips, or structures inhibiting maneuverability like entangled plastic strips or net structures (Näslund and Johnsson,

2014). Underwater feeding structures may also be used (Näslund and Johnsson, 2014). Other enrichment factors are natural food and predation stimulation (Näslund and Johnsson 2014). Using water as enrichment, such as varying the water current (perhaps with a waterfall) and depth, is also highly effective (Näslund and Johnsson 2014). Additionally, tank substrates should be considered, as they can provide the opportunity to perform burying behaviors and increase the serotonergic activity (as shown in crucian carp) (Näslund and Johnsson, 2014). Toys are also a form of enrichment, however, one study found that Atlantic cod did not interact much with the ball toys they were provided (Näslund and Johnsson, 2014).

Understanding the role of color in enrichment is important because some colors may be aversive to some species while others may be preferred. For example, some birds, monkeys, domestic chickens, and mice may avoid the color red (Wells, 2009). On the other hand, some moths, bumblebees, robins, bobwhites, and primates, may be attracted to the cooler colors (blues and greens) on the opposite end of the spectrum (Wells, 2009). It is heavily debated if stingrays are capable of seeing color at all, as elasmobranch vision has not been studied as much as their other senses (Theiss et al., 2006). It has been found that the retina of *Dasyatis kuhlii* (bluespotted stingray) does possess cone photoreceptors, meaning it is highly possible that they are capable of color vision (Theiss et al., 2006). Furthermore, the cone outer segments were found to have a short-wavelength (blue), a medium-wavelength (green), and a long-wavelength (red), however, the green cones were less abundant (Theiss et al., 2006).

Our research question was: How does the presence of color in an enrichment item impact the frequency of stingray interaction with the enrichment item? We hypothesized that the presence of color in an enrichment item increases the frequency of stingray interaction with the enrichment item. We predicted that the colorful enrichment item will experience more stingray interaction compared to the white enrichment item. Our study aims to help the Memphis Zoo design more effective enrichment items (specifically for the stingrays, which may be applied to other aquatic species), as enrichment for aquatic animals is not well understood or researched. It will also be beneficial towards the debate of whether or not stingrays are able to perceive color.

Methods

Study subjects and location

Our experiment took place at the Memphis Zoo's Stingray Bay. The touch pool was approximately

20,000 gallons and contained 54 cownose stingrays and 6 southern stingrays that cohabitated with 6 sharks (Figure 2). At the time of this study, enrichment at the Memphis Zoo's Stingray Bay consisted of a textured grass pad, a textured rope square hoop, a whiffle ball hurdle, and several sinking and floating food puzzles. Guest hand-feeding and touch could have also constituted as enrichment. The stingrays usually received enrichment only once a day, often in the mid-afternoon; however, whether or not they received enrichment and when they received enrichment depended on the density of visitors that day.

Data collection

In order to test how the presence of color affects enrichment item interaction, two enrichment items were designed; they were identical in build, but one was white and the other was colorful (it had green, purple, blue, and orange elements) (Figure 1). Each enrichment item featured a floating pool noodle bent into a circle and 6 floating balloon weights that are tethered to the pool noodle using about 5 feet of ribbon. Additionally, about 1.5 feet of the end of the ribbon without the balloon weight was allowed to flow freely in the water.



Figure 1: The white enrichment item (above), the colorful enrichment item (below)

Data were collected every Wednesday for 5 weeks from 1-4 PM using behavior sampling and continuous recording according to the ethogram (Table 1) for 5 minutes (Martin and Bateson 2007). For this experiment, interaction included swimming under, touching, and hovering (Table 1). The two enrichment items were introduced to the pool at the same time, one at Location A (by the waterfall) and the other at Location B (opposite side of the waterfall). One researcher recorded data for the white enrichment item at one location while the other researcher simultaneously recorded data for the colorful enrichment item at a different designated location (Figure 2).



Figure 2: The touch pool at Stingray Bay, with the locations where enrichment items were introduced and labeled

If an individual stingray interacted with the enrichment item multiple times - for example, it touched any part of the enrichment item, then looped back around and touched it again - both interactions were recorded separately. Also, if, at any point during data collection, the enrichment item moved locations in the pool due to the waves created by the waterfall, resulting in any of the behaviors listed in the ethogram, the interactions were counted. After the 5 minutes of data collection was completed, the enrichment items were removed.

Data were then collected, for 5 minutes, to see how many visits a stingray made in the area where the enrichment item had been, in order to see if they were simply following a predetermined path or coming to the area because of the enrichment item. The researchers then traded enrichment items (whatever enrichment item was in Location A will now go to Location B, and vice versa) but remained in their assigned location. The switching of the enrichment items was to determine whether or not the location of the enrichment item influences its interaction levels. Each week the researchers will switch locations to avoid researcher bias. Our first week consisted of only 4 trials, but every day after consisted of 8 trials, resulting in 36 trials total.

Table 1: Stingray Ethogram

Behavior	Description
Swimming underneath weighted objects	At least 50% of stingray body is underneath the area of the pool noodle (including hollow center), body is continuously moving forward, stingray may be touching objects
Touching enrichment item	Any part of the body touches any part of the enrichment item (includes touching while going under the object)
Hovering	50% of the body lacks movement in any direction for at least 5 seconds underneath area taken up by enrichment item

Data analysis

We ran a parametric T-test of the weekly averages (of all three interactions' values added together at the end of the week) for both the colorful enrichment item and the white enrichment item to analyze which enrichment item, colorful or white, was interacted with the most, on average, each week. We ran a second T-test of the weekly average visits to analyze their preferences of Location A or Location B. To determine whether or not there was a difference between the numbers of stingrays in the area when the enrichment item is present versus when it is not present, we ran two paired T-tests, one of every trial of interactions compared with every trial of visits in Location A, and one of every trial of interactions compared with every trial of visits in Location B.

Results

Swimming under was the most common interaction the stingrays performed, followed by touching, followed by hovering (Table 2).

Table 2: Averages of each interaction (swimming under, touching, hovering) of both the white enrichment item and the colorful enrichment item

Interactions	Average: White Enrichment item	Average: Colorful Enrichment item
Swimming Under	50.2	35.8
Touching	13.8	8.9
Hovering	.3	.2

The colorful enrichment item was interacted with less than the white enrichment item ($t_8 = -2.597$, $p =$

.032; Figure 3). Location A was preferred over Location B whether the enrichment item was there or not ($t_8=3.876$, $p=.005$) (Figure 5).

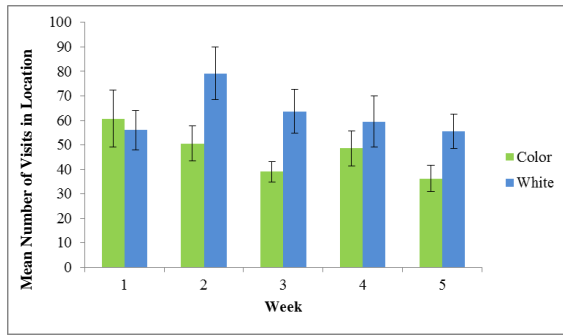


Figure 3: Mean number of stingray interactions with color enrichment item versus white enrichment item respectively (mean ± standard error) for week 1 (60.7 ± 11.66) (56 ± 7.93), week 2 (50.6 ± 7.21) (99.7 ± 10.77), week 3 (39 ± 4.20) (48.6 ± 8.88), week 4 (48.6 ± 7.19) (72.6 ± 10.40), and week 5 (36.3 ± 5.44) (47.8 ± 7.12).

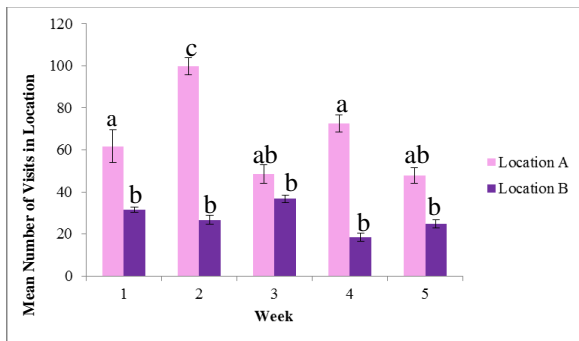


Figure 4: Mean number of visits (mean ± standard error) stingrays made in Location A and Location B respectively for week 1 (61.7 ± 7.85) (31.5 ± 1.19), week 2 (99.7 ± 4.06) (26.6 ± 2.13), week 3 (48.6 ± 4.36) (36.8 ± 1.75), week 4 (72.6 ± 4.13) (18.5 ± 2.03), and week 5 (47.8 ± 3.59) (24.7 ± 1.95).

As for the influence of the enrichment item in each location, there was no significant influence of the enrichment item in Location A ($t_{35}=.905$, $p=.371$), but in Location B, the enrichment item was influential in how many stingrays were in the area ($t_{35}=5.237$, $p<.001$), meaning the presence or lack of the enrichment item in Location A did not influence the number of stingrays in the area but in Location B, it *did* influence the number of stingrays in the area.

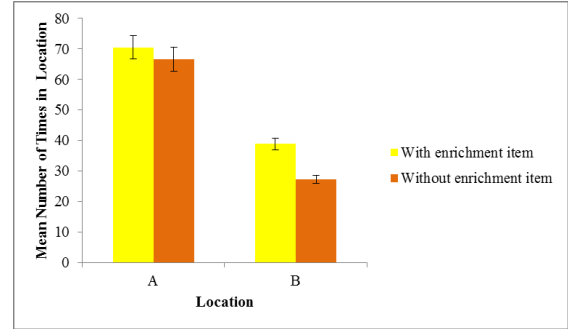


Figure 5: Mean number of visits (mean ± standard error) stingrays made in Location A with enrichment (70.5 ± 3.86), in Location A without enrichment (66.61 ± 3.88), in Location B with enrichment (38.89 ± 1.85), and in Location B without enrichment (27.22 ± 1.37).

Discussion

We found that the white enrichment item was preferred over the colorful enrichment item. Our hypothesis that the presence of color in an enrichment item increases the frequency of stingray interaction with the enrichment item was *not* supported, nor was our prediction that the color enrichment item will be interacted with more. It is possible that the bright orange pool noodle was a signal of danger or toxicity to the stingrays, so they interacted with it less than the white enrichment item; which is similar to different species avoiding red, which is on the same end of the spectrum as orange (Wells 2009). We know that the chances of them being able to see this orange color, as well as the purple and blue ribbons, is high because they have blue and red cones (Theiss et al. 2006). As for why the stingrays preferred the white enrichment item more, the stingrays' food (little cut-up fish and shrimp) and the cups that guests feed them out of are white, so the stingrays may have associated the white enrichment item with food. However, the relation of color can go both ways; the stingrays have many enrichment items that are colored, so you would expect them to see the colorful enrichment item and associate it with food as well. The fact that there was a significant difference in interaction with the colorful enrichment item and white enrichment item implies that stingrays *can* perceive color and are affected by color as well. The preference of Location A (by the waterfall) over Location B (opposite side of the waterfall) can be explained by the simple fact that the waterfall provides a form of enrichment on its own by creating waves and altering how stingrays swim. It is also possible that because of the waves, the enrichment item was less noticeable, and/or the stingrays did not care to interact with the enrichment

item because they were interacting with the waterfall. Because of this, the enrichment item would be more influential in Location B, as our results found.

Upon first starting this experiment week 1, we experienced a major problem: our enrichment item did not do what we had designed and expected it to do. We had designed our enrichment item so that the balloon weights would sink and hang in the water, but instead, they floated at the surface of the water. Since they floated instead of sinking, the ribbons were too short: the balloon weights stayed in close proximity to the noodle and oftentimes were touching the noodle. To make the balloon weights float further away from the noodle, we had to increase the ribbon length to allow the balls to float apart from the noodle and be more noticeable. Later on, these longer ribbons posed a problem: tangling. In a few instances, stingrays bit onto the ribbon (resulting in pulling the enrichment item a short distance, and/or becoming tangled) and some became tangled while trying to swim through the ribbons. The tangled stingrays may have been less likely and less willing to interact with the enrichment item in fear of becoming tangled again.

Another factor that influenced our results was guest presence. Some days had only a few visitors, and others had large groups. When there were visitors present, the stingrays were more focused on the visitors, especially if the guests had feeding cups, than they were focused on interacting with the enrichment items. The guests could be considered a form of enrichment, so we could have taken guest interaction into account in our experiment.

In the future, it would be interesting to see if there are specific individual colors that stingrays prefer. As stated previously, different animals prefer different ends of the color spectrum, so it would be interesting to see where stingrays fall. An experiment to test this would feature multiple enrichment items, each with its own single, solid color, or two different enrichment items, one with cool colors and one with warm colors.

Conclusions

- The white enrichment item had a higher mean interaction than did the colorful enrichment item.
- The enrichment item (whether colorful or white) was interacted with more while in Location A compared to when in Location B.
- Whether or not the enrichment item was in Location A (by the waterfall) did not influence the number of stingrays in the location, but in Location B (opposite side of the waterfall) it did influence the number of stingrays in the location.

Acknowledgements

We would like to thank the Memphis Zoo for allowing us to conduct research at Stingray Bay. We would also like to thank Jacob for giving us feedback regarding our enrichment item and being a great resource to answer any questions regarding the stingrays and their enrichment. Lastly, we would like to thank Dr. Boyle for helping us develop and carry out our experiment, understand and analyze our statistics, perfect our scientific paper, and, overall, for giving us this great opportunity.

Literature Cited

- Kittle K. 2018. *Rhinoptera bonasus*.
- Martin P, Bateson P. 2007. Measuring behavior: an introductory guide. 3rd Edition. Cambridge: Cambridge University Press. 187 p.
- Näslund J, Johnsson JI. 2014. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. *Fish and Fisheries*. 17(1):1-30.
- Passarelli N, Piercy A. 2018. *Dasyatis americana*.
- Smith, BL. 2016. The Benefits and Costs of Environmental Enrichment.
- Theiss SM, Lisney TJ, Collin SP, Hart NS. 2006. Colour vision and visual ecology of the blue-spotted maskray, *Dasyatis kuhlii* Muller & Henle, 1814. *Journal of Comparative Physiology*. 193(1):67-79.
- Watters, JV. 2009. Toward a Predictive Theory for Environmental Enrichment. *Zoo Biology*. 28(6):609-622.
- Wells, DL. 2009. Sensory Stimulation as Environmental Enrichment for Captive Animals: A Review. *Applied Animal Behavior Science*. 118(1-2):1-11.

Distribution of Spotted Lanternfly (*Lycorma delicatula*) in Relation to Distribution of their Preferred Host Plant, Tree of Heaven (*Ailanthus altissima*)

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Introduction

The Spotted Lanternfly, *Lycorma delicatula*, is an invasive species native to China (National Invasive Species Info Center). It was first detected in the United States in 2014, although it is believed that it was around for 2-3 years before its detection (National Invasive Species Info Center). It was likely introduced through imported woody plants and general wood products (National Invasive Species Info Center). These Hemipterans (the order containing “true bugs”) pose a risk to viticulture, fruit trees, ornamentals, and timber, in turn severely affecting the U.S economy (National Invasive Species Info Center).

Another invasive species posing an issue in the United States is the Tree of Heaven (*Ailanthus altissima*). These trees are also from China but have been around for centuries (introduced in the late 1700s) longer than Spotted Lanternflies (National Invasive Species Info Center). They were purposely implemented for ornamental use, but quickly overpopulated (National Invasive Species Info Center). Tree of Heaven crowd out native species and damage anthropomorphic structures such as pavement and building foundations (National Invasive Species Info Center). They are also allelopathic, meaning they use chemicals as means of suppressing other tree species around them, allowing them to more easily take over an area (Department of Ecosystem Science and Management).

Understanding the relationship between Spotted Lanternflies and the Tree of Heaven is essential, as the Tree of Heaven is a preferred host of the Spotted Lanternfly. The Spotted Lanternfly can reproduce on many plants, however it has preference for Tree of Heaven (Department of Ecosystem Science and Management). Although controlling Tree of Heaven numbers and growth could be beneficial in slowing the spread of Spotted Lanternflies, these trees have a mechanism for ensuring their survival. If an individual stem is cut, the root system produces several sprouts, which makes the trees harder to simply cut down (Department of Ecosystem Science and Management).

With Tree of Heaven being so widespread across the United States, there is the risk of Lanternflies spreading further across the country rather than being contained around Pennsylvania,

which is currently the state facing the worst invasion. Considering Tree of Heaven has been around since the late 1700s, the opportunity to control this species has passed, however there is still time to combat the invasive Spotted Lanternfly. It is important to be able to predict the potential spread of Spotted Lanternfly so that their expansion may be contained before too much damage can occur.

Methods

Data were gathered from Global Biodiversity Information Facility (GBIF) for both Spotted Lanternfly sightings and Tree of Heaven sightings from the year 2014 until November 2020. ArcMap 10.7 was the software used for all the mapping. The excel files downloaded from GBIF were then converted into database tables in order to plot X and Y coordinates (latitude/longitude). Point density analyses were completed for Spotted Lanternflies as well as for Trees of Heaven, both using natural break (Jenks) classification. Given that Pennsylvania is the state facing the strongest outbreak in the country and had the most overall recorded sightings, the SLF map was concentrated on Pennsylvania.

Results

The hotspot for reported Tree of Heaven is contained within the hotspot for reported Spotted Lanternflies (Figures 1 and 2). The distribution of the Spotted Lanternfly aligns with where Tree of Heaven is prominent; the parts of Pennsylvania with very few recordings of Tree of Heaven have very few recorded Spotted Lanternflies. The range of Spotted Lanternfly is currently limited to where Tree of Heaven is present (Figure 3).

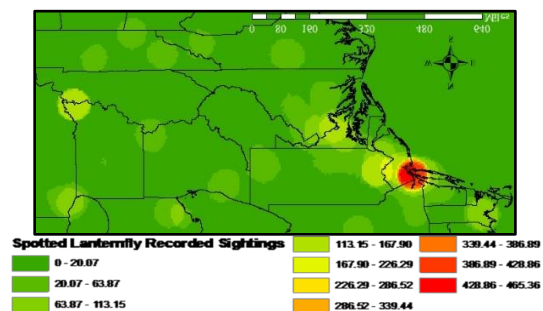


Figure 1: Kernel density of recorded Spotted Lanternfly sightings.

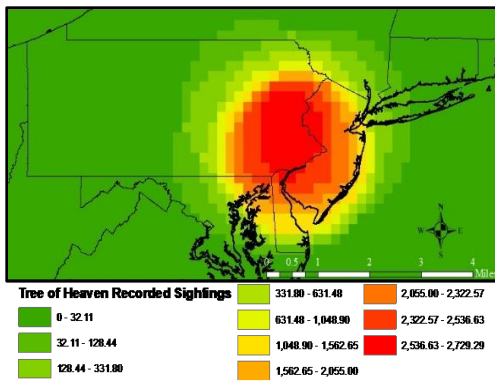


Figure 2: Kernel density of recorded Tree of Heaven sightings.

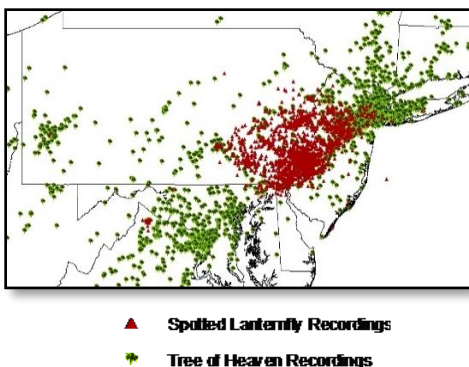


Figure 3: Points of each recorded Spotted Lanternfly sighting and each Tree of Heaven sighting.

Discussion

Based on the data, the Spotted Lanternfly's range closely corresponds to the Tree of Heaven's range. It can therefore be concluded that the further expansion of Spotted Lanternfly can be predicted based on Tree of Heaven sightings. Looking at the upper half of Virginia, where Tree of Heaven is rather heavily reported but there are minimal sightings (between 32 and 128 recordings) of Spotted

Lanternflies, it can be predicted that Virginia is likely at risk of a Spotted Lanternfly invasion in the future. It is important to note, however, that even if Tree of Heaven is not present in a given area, Spotted Lanternflies could still colonize through the use of a different host (ex. grape vines, *Prunus* trees, etc.) , but the presence of Tree of Heaven can be a good starting point to predict potential areas for infestation.

Literature Cited

Spotted Lanternfly | National Invasive Species Information Center. (n.d.). Retrieved from www.invasivespeciesinfo.gov website:
<https://www.invasivespeciesinfo.gov/terrestrial/invertebrates/spotted-lanternfly>

Tree-of-Heaven | National Invasive Species Information Center. (n.d.). Retrieved from www.invasivespeciesinfo.gov website:
<https://www.invasivespeciesinfo.gov/terrestrial/plants/tree-heaven>

Tree-of-heaven and the Spotted Lanternfly: Two Invasive Species to Watch. (2018, August 18). Retrieved November 29, 2020, from Department of Ecosystem Science and Management website:
<https://ecosystems.psu.edu/research/centers/private-forests/news/tree-of-heaven-and-the-spotted-lanternfly-two-invasive-species-to-watch>

GBIF.org (22 November 2020) GBIF Occurrence Download
<https://doi.org/10.15468/dl.q4rxnn>

GBIF.org (22 November 2020) GBIF Occurrence Download
<https://doi.org/10.15468/dl.pdbwqu>

The Future of Single Cell Sequencing in Cancer Research

Jake Friske

The world of cancer research is advancing at an exponential rate. Over the past decade new techniques and technologies have allowed for greater manipulation and observation of cancer cells in both *in vitro* and *in vivo* approaches. As these new techniques are developed, scientists find increasingly diverse ways in which to apply them for their personal experimental gain. This further helps perpetuate development of new techniques and technologies, creating a snowball effect that has allowed for major changes to scientific exploration in just a few short years. One of these new technologies that is helping to advance the research landscape is single cell sequencing. This new technology allows a more holistic overview of tumors themselves and allows for deeper investigation not only into the tumor itself, but the microenvironment in which it resides. Single cell sequencing has been growing exponentially over the past few years and new ways to apply this technology are being developed all of the time, leading to a platform that will allow for very precise applications in both basic laboratory and clinical settings.

In order to better understand the different ways that single cell sequencing can be applied, it is important for one to have a general understanding of how the process works as a whole. First, a machine (each company has their own device) is used to isolate single cells in a water in oil emulsion (10X and Mission Bio applications) or on a chip (Takara Bio applications). Once cells are isolated into their individual emulsion or well, they are lysed, exposing the genetic material. The genetic material in each emulsion or well is then tagged with beads to identify the cell in which they came from. Then, the genetic material is amplified and fragmented. Finally, next generation sequencing adaptors are ligated onto the ends of the fragments and a final amplification is conducted. The genetic material is now ready for sequencing, comprised of three distinct parts: first, the next generation sequencing adaptor; second, the genetic material of the cell itself; lastly, the oligo designating the individual cell the genetic material came from (Haque et al., 2017).

The above is just a general overview for the single cell sequencing pipeline. This can be changed in many different ways to adapt to the needs and wants of scientist. For example, Mission Bio specializes in targeted single cell sequencing. Most panels of this nature comprise of up to 200 genes which are deep sequencing to identify just a single cell that harbors a mutation in one of these genes.

This can be a very powerful tool for identifying small clones that are unable to be identified in a diagnosis sample, but that are prevalent in relapse samples. In addition to this kit, Mission Bio also offers an Antibody Oligo addition. This uses antibodies targeting specific proteins, both surface and cytoplasmic that have a specific known nucleotide oligo attached to the other end. This oligo then goes through the same protocol as the normal genetic material, but its unique sequence can be identified and matched during the bioinformatics process. This technique allows for combined targeted sequencing and protein expression on the single cell level, giving researchers tremendous power to identify and track disease progression from diagnosis through to remission, and in some unfortunate cases, relapse.

Another novel uses for single cell sequencing have been developed by 10X Genomics. This was one of the first companies to take single cell sequencing mainstream and have led the research and development of additional platform uses. These include whole exome sequencing to identify gene expression differences on a single cell level. This can give valuable information to the inner workings of the cell that may not have been able to be detected in a conventional sequencing application. Another sequencing that 10X has developed is Assay for Transposase-Accessible Chromatin (ATAC) sequencing. This allows for researchers to identify chromatin changes in tumor cells, which can give valuable insights into the mechanisms that may be causing aberrant gene expression. There are many types of single cell sequencing and the above are just a few recent innovations that have aided the data that can be obtained by these platforms.

Using some of the above techniques and others not mentioned, scientists can use this technology to probe many aspects of the tumor and tumor microenvironment. For example, researchers can use conventional single cell sequencing to identify clonal evolution after preclinical drug testing. This can help them to identify possible mutations that are markers of specific treatment resistance. Single cell sequencing has also been used by and large in the research setting in terms of looking at gene expression. One of the newest advances in basic laboratory research has been looking at gene expression profiles of tumors and how these relate to prognosis and treatment outcomes. There have been many meta-analyses over the past few years that have changed the way scientist are looking at diseases overall. For example, Nehme

et al. have shown that Acute Myeloid Leukemia and all of its subtypes share common deregulated genes compared to normal bone marrow, and 22 of these gene's expression profiles can be used to prognosticate treatment outcome and survival (Nehme et al. 2020). This study and others are helping researchers to better understand tumors and possible ways to treat them in a more precise and personalized fashion.

On the clinical side, things are very similar. In clinical practice, single cell sequencing has been used to try and understand why relapse occurs. In most cases, clinicians are finding that small clones (undetectable in bulk sequencing) that are resistant to therapy become the dominant clone at relapse (see figure 2)(McMahon et al. 2019). For example, in the research conducted by McMahon et al., the authors discovered that after treatment, a small NRAS-mutated clone became the dominant species which led to relapse of AML in certain patients. The clone was undetectable by bulk sequencing but was detectable after performing single cell sequencing. This shows the power of single cell sequencing and its potential for use at diagnosis to guide treatment, especially if there is a possibility for relapse that can be avoided. This technology is a powerful tool and is being used more and more in the clinical setting and could be set to redefine how cancer is diagnosed and treated.

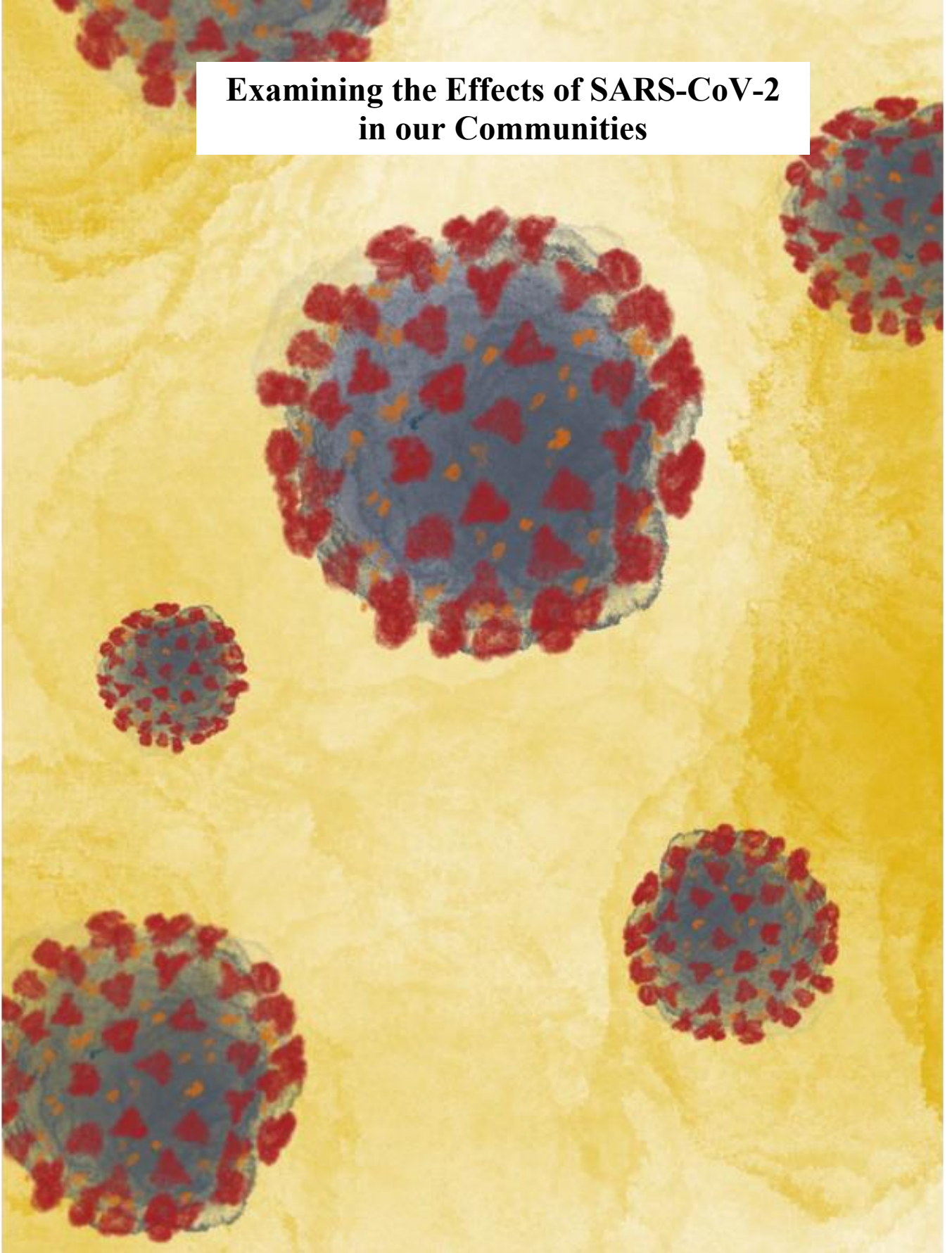
The future of single cell sequencing will be a constant evolution. Even in just a few short years it has evolved to fit the needs and wants of researchers and clinicians alike. Most importantly, the ability to isolate individual cells has allowed for untapped potential in downstream applications. There are not many applications for this technology that have not been developed yet, but there are other aspects that can be improved for the future. Most notably, currently the cost and time commitment of the protocol make this technology prohibitive for many

labs and uses. At this moment in time, a single sample costs about \$5,000 USD (not including the upfront cost of the machine) and two long days of bench work to prepare the sample from cells to a library that is ready to be sequenced. Then, sequencing and analysis can take another two weeks at least. This will need to be shortened and costs will need to go down for mainstream single cell research to take hold. But this will all change. At its inception, Sanger sequencing took a long time and was very expensive, but modern advances have driven cost and time down to a point where a sample can be sequenced and analyzed in just over a few hours and costs are minimal. It is only a matter of time until single cell sequencing follows suit. It will be exciting to see where single cell sequencing will go over the next few years and how it redefines the research and clinical landscape for the betterment of researchers, clinicians, and patients alike.

Literature Cited

- Haque A, Engel J, Teichmann SA, Lönnberg T. A practical guide to single-cell RNA-sequencing for biomedical research and clinical applications. *Genome Med.* 2017;9(1):75. doi:10.1186/s13073-017-0467-4
- McMahon CM, Ferng T, Canaani J, et al. Clonal selection with RAS pathway activation mediates secondary clinical resistance to selective FLT3 inhibition in acute myeloid leukemia. *Cancer discov.* 2019;9(8): 1050-1063. doi:10.1158/2159-8290.CD-18-13
- Nehme A, Dakik H, Picou F, et al. Horizontal meta-analysis identifies common deregulated genes across AML subgroups providing a robust prognostic signature. *Blood Adv.* 2020;4(20):5322-5335. doi:10.1182/bloodadvances.2020002042

**Examining the Effects of SARS-CoV-2
in our Communities**



The Impact of the Covid-19 Pandemic on Scientific Research in Memphis

Annelise Swords, Emma Root, Madeline Yde

Flipping through the pages of this year's issue of the Rhodes Journal of Biological Science, you may be surprised to find fewer research articles, experimental proceedings, and case studies than in the issues of years previous. Similar to many facets of our lives, the changes this year has brought to the scientific community in Memphis and beyond can be attributed to the COVID-19 pandemic. While we are certain that you are sick of hearing folks drivel on about "these unprecedented times", it may bring you hope to know that the pandemic has not stifled the efforts of the scientific community here in Memphis. Rather, it has showcased the creativity, fortitude, and dedication to research that this community possesses. We interviewed students, professors, and researchers in Memphis who have experienced working in a lab or in the field during this time of crisis with the hope of documenting the changes this pandemic has brought to the field of research, and celebrating the tenacity of scientists in this city we call home.

As a student, how has the pandemic impacted your undergraduate research experience?

"At first, it prevented me from being able to collect live data, and I had to rely on poor camera footage to collect data- data that was significantly less accurate than what I could have produced in person. I was less affected than other students given that I reside in Memphis and was able to resume in person research in the fall of 2020. Also, not all students were doing research that could be completed remotely like the work that I was doing. Had I been in a lab, I would have lost a lot more opportunities than I did as a researcher who could rely on remote camera footage."

- Anonymous student researcher, Rhodes College

"There are positives and negatives.

Negatives: It has made hands on lab time more difficult to schedule and partake in, because hands on experience and lab bench work is important for progressing in my field, the research experience does not feel as complete as it would have without this interruption. Skill set does not give developed as much in remote work.

Positive: I discovered a passion for computational chemistry that I may not have without the need for remote research opportunities (computational chemistry = using computer programs to explore theoretical chemical principles)."

- Mary Rose Rutledge, Rhodes College student researcher

As a researcher, how has the pandemic affected your research?

"The pandemic thankfully didn't limit very much of the progression of our research, but it did impact the amount of collaboration we could have and in part the workload that each person in our lab carried. Some research visits had to be cancelled or postponed, and for work trips the number of people was more limited- when usually more staff members are encouraged to go for trips. Thankfully, being able to meet virtually helped immensely and many interns in our lab have thankfully been able to continuing working with our lab by doing data analysis remotely."

- Allison Bogisich, MSc, Memphis Zoo Research Technician

"My research was affected in three main ways.

First: certain laboratory materials have either become more difficult to maintain or significantly more expensive.

Second: social distancing restrictions mean that my lab could only accommodate two people working at one time, and even then, accessing equipment could be tricky. Additionally, teaching some techniques from six feet away really limited my ability to accept and train new students.

Finally: I have international collaborators who were in and out of strict lockdowns in their country. This meant that it was challenging to send materials for collaborative experiments- sometimes they couldn't be present to receive them and their own work was so severely delayed that they had a lot to catch up on for their own studies once they had lab access again."

- Elaine Frawley, Ph.D., Rhodes College Assistant Professor of Biology

What precautions have you taken to ensure safety in the research environment?

“Once Covid-19 became a safety concern, our lab went fully remote. If we needed to go into the lab, only a limited number of students that could be in there at one time, and we maintained clear communication between our professor and the students. We also practiced social distancing when we were in the same indoor rooms.”

- Sarah Delahunt, Rhodes College student researcher

“For our lab, we try to ensure that everyone could feel as safe as possible while doing their work, which largely meant masking, sanitizing and social distancing whenever possible. However, since much of the work we do in the lab and in the field requires two or more people it means we need to trust that our lab mates are being diligent in their individual safety practices. If one of us were to get sick, it would likely cause the rest of us to get sick if proper safety precautions aren’t taken. For example, we would get Covid-19 tests if one of us met a group of people outside of our lab bubble, and then wouldn’t come into contact with other lab members until they got a negative result. We also are working remotely from home whenever possible to minimize risk.”

- Allison Bogisich, MSc, Memphis Zoo Research Technician

“Once I was back at the zoo, I ran into many safety concerns. While I wore a mask and maintained my distance, zoo guests were not required to wear a mask at that time (since the zoo is outdoors. This policy has since changed). Maskless individuals would approach me with questions, and I was put into an awkward predicament between trying to keep myself and others safe while remaining respectful and trying to answer their questions. It just wasn’t a comfortable situation. I ended up moving back to remote research out of concern for my safety. The zoo itself provided Covid-19 training modules and required the passing of this course to do in person research. The zoo also required all staff, volunteers, and interns to wear masks at all times. There is also sanitizing stations and I would avoid touching any of the railings or high traffic areas. Additionally, the professor I worked with was incredibly understanding and wanted to ensure we were safe. She regularly checked in and tried to make sure we stayed healthy.”

- Anonymous student researcher, Rhodes College

“This semester we’ve been double masking and also wearing face shields to protect our masks from being contaminated with pathogenic bacteria. We’ve also been working one person to a bench to maintain distance. Meetings have been conducted through Zoom.”

- Elaine Frawley, Ph.D., Rhodes College Assistant Professor of Biology

How do you see scientific research continuing in the post-pandemic world? What will be the same, what will be different?

“I think that for the most part scientific research will go back to normal, more or less. Research almost always needs some aspect of hands on experiment, there will always be a need for work that can’t be done entirely remotely. However, I do think that COVID-19 set a precedent for the aspects of research that can be done remotely such as analyzing data and writing or drafting proposals and papers. In general, I think the pandemic created a need for flexibility and creative solutions within the scientific community and the obstacles we face.”

- Sarah Delahunt, Rhodes College student researcher

“I see research going back to normalcy in all honesty. I also see the possibility of continuing remote work as it is able to supplement some valuable areas of research.”

- Mary Rose Rutledge, Rhodes College student researcher

“I hope that scientific research in a post-pandemic world will allow for more virtual opportunities to be involved and collaborate whenever possible. This would help make research more accessible and equitable for many people who for one reason or another can’t easily travel to do things like take samples, attend conferences and workshops, have interviews or give lectures. While that might look different from what many aspects of research looked like previously, I think that what will continue to be the same is the nature and spirit of collaborative research and the desire to share ideas and get feedback from others- it might just look slightly different in practice now.”

- Allison Bogisich, MSc, Memphis Zoo Research Technician

“This question is a great one. Already people have seen how the pandemic has impacted scientists differently for a number of reasons. Some people who have impacted the most are the primary caregivers to children or older parents, or scientists whose research relies on having access to particular lab facilities, or scientists who have had to postpone their field studies. For many scientists (and truthfully, people in general) the pandemic has been very challenging. But, there have also been some positive aspects when it comes to having more people being able to participate in virtual conferences (because they tend to be lower-cost, you do not have to arrange for leaving family behind or missing work, etc.). As for the future, I think that there will be more opportunities for a greater number of people to engage in virtual conferences and workshops, which is a positive change that has occurred. I think the pandemic has put a spotlight on science and scientific research, and I hope that students will find ways to pursue their interests in science.”

- Sarah Boyle, Ph.D., Rhodes College Associate Professor of Biology

What do you think the scientific community in Memphis and beyond will remember most about the pandemic?

“There are so many facets of the scientific community in Memphis, so I can’t speak for the entire community. I think personally that the pandemic has been an important reminder that the community still has a lot to work on in terms of developing science literacy and highlighting the ways we need to improve as science communicators.”

- Allison Bogisich, MSc, Memphis Zoo Research Technician

Quarantine's Psychological Effects

Khanh Ton and Isabella Wollfarth

On March 19, 2020, California became the first US state to issue a stay-at-home order, which required residents to not leave the house unless they either were essential workers or needed to shop for essential needs. “Stay-at-home,” “lockdown,” and “quarantine” became very familiar words to society, as people continued to live, work, and study from the comfort of their homes. Quarantine, in fact, has been used as a response to the spread of different contagious diseases since its first imposition in the fifteenth century against the plague in the United Kingdom. Quarantine during SARS, Ebola, or H1N1 influenza pandemics was, however, much smaller in scale as it was only defined as “the separation and restriction of movement of people who have potentially been exposed to a contagious disease” (Centers for Disease Control and Prevention, 2017). In other words, its targeted people were only those infected, not the entire population. Regardless of scale and population, quarantine is mostly associated with negative psychological effects such as anger, anxiety, fear, and sadness (Brooks et al., 2020).

A 2008 study of the SARS quarantine experience reveals that over 20% of the quarantined sample reported feelings of fear, 18% sadness, and 10% guilt, as opposed to the 5% who reported happiness and 4% relief (Reynolds et al.). Another study following the Middle East Respiratory Syndrome (MERS), nevertheless, shows that such distresses could go away with time: 7% of the sample reported anxiety and 17% anger during quarantine and those numbers fell to 3% and 6% respectively after 4-6 months (Jeong et al., 2016). Certain populations seem to be more deeply affected than others, as three years after the SARS outbreak, 9% of the health worker sample showed high depressive symptoms. Likewise, 60% had been quarantined in that subgroup in comparison to 15% who ended up with lower depressive symptoms (Liu et al., 2012).

Stressors during quarantine include the duration of quarantine, fear of infection, inadequate information, inadequate supplies, and boredom (Brooks et al., 2020). These are all apparent during COVID-19, specifically from the frequent lockdowns, empty supermarkets, and “Zoom fatigue.” Some stressors post quarantine are finances and stigma (Brooks et al., 2020). The problem involving stigma is that what seems to be a health issue could easily turn into a political one: during the Ebola pandemic, in Liberia, stigma resulted in the disenfranchisement of minority groups under quarantine as they were deemed different hence

dangerous. This stigma led minorities to avoid reporting and seeking help for non-Ebola treatable diseases (Pellecchia et al., 2015).

Similarly, and often to a much greater extent, the COVID-19 quarantine has had extensive and detrimental psychological effects on the community as more people go under lockdown for longer periods of time. In a study conducted in China, 1210 participants in 194 different cities were asked about their mental health during the pandemic: 29% reported moderate to severe anxiety symptoms; and 17% reported moderate to severe depressive symptoms (Cullen et al., 2020). Some groups, however, are more severely influenced by anxiety and depression as well as fear of death than others, such as the elderly, which makes sense as 80% of COVID-19 deaths occur in people over age 65, and more than 95% of COVID-19 deaths occur in people older than 45. In addition, many minor racial and ethnic groups are put at risk by long-standing health inequalities (CDC 2021).

Health professionals have always been considered essential to pandemics but “essential workers”, first termed in the “Guidance on the Essential Critical Infrastructure Workforce” on March 19, 2020, include everyone who “conduct a range of operations and services in industries that are essential to ensure the continuity of critical functions” (CISA). Essential workers put their health, both physical and mental, on the line when going to work. In order to protect their mental health in the workplace, the CDC seeks to prevent trauma by encouraging them to be “[aware] of symptoms, taking breaks from work, engaging in self-care,” and overall asking for help (Cullen et al., 2020). An opposite issue also occurs since only certain workers are deemed essential, people fear their sudden losses of jobs and steady incomes as they continue to stay at home. Within the first month of lockdown, over 16.5 million claims of unemployment flooded government offices and such number has been increasing with new claims arriving at a rate of 6-7 million per week (Coibion et al., 2020). The high unemployment rate eventually turns the unemployed into “discouraged workers” as they stop looking for work. (Coibion et al., 2020). Having “discouraged workers” in the economy only continues to threaten the possibility of a stable economy and decreases the morale of society as a whole. About twenty million jobs had been lost before the COVID-19 crisis and would only continue to decline to a total of 16% unemployed citizens (Coibion et al., 2020).

In conclusion, quarantine generally amplifies severe anxiety, depression, fear of death, and confusion in communities where it is imposed upon. Quarantine during the COVID-19 pandemic, however, takes it to the next level as more people are ordered to stay at home for longer periods of time to prevent mass infection in society. This has resulted in other socio-economic issues such as a higher level of unemployment, which has discouraged people of working age from finding jobs.

Work Cited

- Brooks, S. K., Webster, R. K., Smith, L. E., Woodland, L., Wessely, S., Greenberg, N., & Rubin, G. J. (2020). The psychological impact of quarantine and how to reduce it: rapid review of the evidence. *The Lancet*, *395*(10227), 912-920.
[https://doi.org/10.1016/S0140-6736\(20\)30460-8](https://doi.org/10.1016/S0140-6736(20)30460-8)
- Centers for Disease Control and Prevention. (2021, March 29). "Certain Medical Conditions and Risk for Severe COVID-19" Illness.
www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html.
- Centers for Disease Control and Prevention. (2017, September 29). *Quarantine and isolation*.
<https://www.cdc.gov/quarantine/index.html>
- CISA. (2020, March 19). *Guidance on the essential critical infrastructure workforce*.
<https://www.cisa.gov/publication/guidance-essential-critical-infrastructure-workforce>
- Coibion, Olivier, Gorodnichenko, Y., Weber, M. (2020, April 20) "Labor Markets During the COVID-19 Crisis: A Preliminary View." *NBER*,
www.nber.org/papers/w27017.
- Cullen, W, Gulati, G., Kelly B., (2020, March 30) "Mental Health in the COVID-19 Pandemic." *OUP Academic*,
academic.oup.com/qjmed/article/113/5/311/5813733?login=true.
- Jeong, H., Yim, H. W., Song, Y. J., Ki, M., Min, J. A., Cho, J., & Chae, J. H. (2016). Mental health status of people isolated due to Middle East respiratory syndrome. *Epidemiol Health*, *38*.
<https://doi.org/10.4178/epih.e2016048>
- Liu, X., Kakade, M., Fuller, C. J., Fan, B., Fang, Y., Kong, J., Guan, Z., & Wu, P. (2012). Depression after exposure to stressful events: lessons learned from the severe acute respiratory syndrome epidemic. *Comprehensive Psychiatry*, *53*(1), 15-23.

<https://doi.org/10.1016/j.comppsy.2011.02.003>

- Pellecchia, U., Crestani, R., Decroo, T., Van den Bergh, R., & Al-Kourdi, Y. (2015). Social consequences of Ebola containment measures in Liberia. *PLOS ONE*, *10*(12), e0143036.
<https://doi.org/10.1371/journal.pone.0143036>
- Reynolds, D., Garay, J., Deamond, S., Moran, M., Gold, W., & Styra, R. (2008). Understanding, compliance and psychological impact of the SARS quarantine experience. *Epidemiology and Infection*, *136*(7), 997-1007.
<https://doi.org/10.1017/s0950268807009156>

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