

Introduction

Amur Honeysuckle (*Lonicera maackii*) is an invasive plant predominantly found throughout the eastern and midwestern United States. This plant has negative impacts on native plant communities, such as reducing native plant richness and abundance, growth and survival of tree seedlings, and growth of forbs (Miller & Gorchoy 2004; Collier et al. 2002). However, the plant's impacts are more variable at higher trophic levels, with some species experiencing positive effects.

Some birds are found in greater densities in honeysuckle-invaded habitat during the breeding season (McCusker et al. 2010), but the reason behind this is not clear. One potential explanation for this could be an increase in the food source for birds: shrub-dwelling arthropods. However, no previous research has examined the relationship between birds and arthropods in honeysuckle-invaded habitat. Therefore, the goal of this study was to evaluate differences in the arthropod community hosted by Amur Honeysuckle and native shrubs at forest edges, as well as differences in the avian communities that forage in those shrubs.

The study was conducted at three forested areas in northwestern Ohio: Thoreau Wildlife Reserve, Oxbow Lake Wildlife Area, and Forrest Woods Nature Preserve. Birds were counted at 20 sites across these three areas using fixed-radius point counts. Arthropods were collected from shrubs at each site using the beat sheet method and were identified to the lowest taxonomic level possible in the lab (Figure 1). Abundance, species richness, and Shannon diversity were calculated for both birds and arthropods. Differences between invaded and native sites were compared using Linear Mixed Models (LMM), Nonmetric Multidimensional Scaling (NMDS), and

Analysis of Similarity (ANOSIM).



Fig. 1. Photography of the methodology used in this study.

Results

A total of 11 species of shrub-gleaning and foraging birds were observed with 104 individuals across the 20 sites (Table 1). The abundance, species richness, and Shannon diversity of birds were significantly higher in the honeysuckle-invaded habitat than in natively vegetated habitat (Table 2, Figure 2).

Acknowledgements

We would like to thank Defiance College for use of facilities. We would also like to thank the Black Swamp Conservancy and the Diehl Family Foundation for access to their properties. Funding for this project was provided by the Defiance College Summer Undergraduate Research Program and Title III Strengthening Institutions grant.

Both the NMDS (Figure 3) and ANOSIM (global $R=0.47$, $p=0.002$) showed that bird communities were significantly different between invaded and uninvaded sites. Similarly, the abundance, species richness, and Shannon diversity of arthropods were significantly higher in the honeysuckle-invaded habitat than in natively vegetated habitat (Table 2, Figure 4). However, while the NMDS indicated slight differences between invaded and uninvaded sites (Figure 5), there were no significant differences according to ANOSIM (global $R=0.05$, $p=0.16$).

Table 1. Maximum count and number of locations occupied by bird species in forest edge habitat surveyed through point counts.

Species	Common Name	Code	Max count	Number of locations
<i>Baeolophus atricristatus</i>	Tufted Titmouse	TUTI	1	5
<i>Catharus fuscescens</i>	Veery	VEER	1	2
<i>Dumetella carolinensis</i>	Gray Catbird	GRCA	3	8
<i>Geothlypis trichas</i>	Common Yellowthroat	COYE	2	9
<i>Poecile atricapillus</i>	Black-capped Chickadee	BCCH	3	6
<i>Setophaga pensylvanica</i>	Chestnut-sided Warbler	CSWA	1	3
<i>Setophaga petechia</i>	Yellow Warbler	YEWA	1	11
<i>Setophaga ruticilla</i>	American Redstart	AMRE	1	7
<i>Spinus tristis</i>	American Goldfinch	AMG	3	8
<i>Thryothorus ludovicianus</i>	Carolina Wren	O	1	2
<i>Turdus migratorius</i>	American Robin	CARW	2	14

AMRO

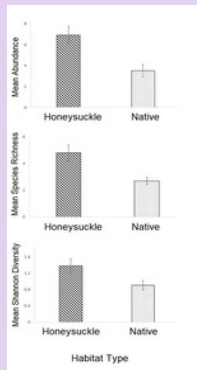


Fig. 2 Comparison of the abundance, species richness, and Shannon diversity of birds in honeysuckle-invaded and natively-vegetated habitats. Error bars represent standard error.

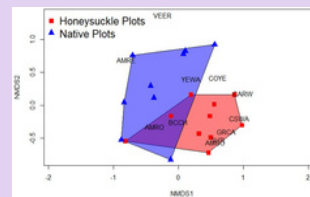


Fig. 3 Ordination of bird species composition in relation to honeysuckle invasion in forest edge habitat, using NMDS based on Bray-Curtis dissimilarity. Stress=0.14. Species codes are listed in Table 1.

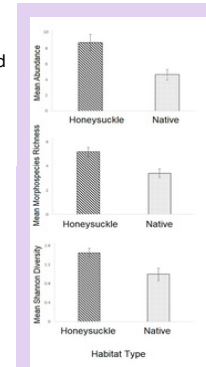


Fig. 4 Comparison of the abundance, species richness, and Shannon diversity of arthropods on invasive honeysuckle and native shrubs. Error bars represent standard error.

Table 2. Results of Linear Mixed Models for bird and arthropod abundance, richness, and Shannon diversity in relation to the presence or absence of Amur Honeysuckle.

Response variable	F-value	p-value
Bird Abundance	12.62	0.003
Bird Richness	10.91	0.005
Bird Shannon Diversity	5.09	0.04
Arthropod Abundance	11.52	0.004
Arthropod Richness	17.03	<0.001
Arthropod Shannon Diversity	9.13	0.008

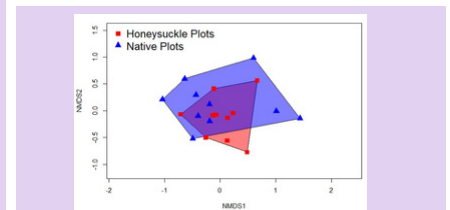


Fig. 5 Ordination of arthropod species composition in relation to honeysuckle invasion in forest edge habitat, using NMDS based on Bray-Curtis dissimilarity. Stress=0.17.

Discussion

Our study shows that Amur Honeysuckle hosts more abundant and more diverse arthropod communities than native shrubs, as well as more abundant and more diverse avian communities. However, not all birds benefited from the presence of honeysuckle. Dietary specialists and species that are more sensitive to disturbances such as the American Redstart and Yellow Warbler were associated with native sites, while omnivorous species and those less sensitive to human activity were associated with honeysuckle. These included species such as the American Goldfinch, Gray Catbird, and Tufted Titmouse. Therefore, we can conclude that Amur Honeysuckle must have some additional characteristics or impacts beyond its effects on arthropods that explain the patterns we observed in this study.

What limits the conclusions from our study is that we observed a somewhat low number of birds and our study covers a fairly small geographic area. In addition, the beat sheet method of arthropod collection is biased against flying insects, which could influence our results. Additional studies in other geographic regions and with additional arthropod collection methods are necessary to confirm the patterns we have observed here.

References

- Collier MH, Vankat JL, Hughes MR (2002) Diminished plant richness and abundance below *Lonicera maackii*, an invasive shrub. *Am Midl Nat* 147:60–71
- McCusker CE, Ward MP, Brawn JD (2010) Seasonal responses of avian communities to invasive bush honeysuckles (*Lonicera* spp.). *Biol Invasions* 12:2459–2470
- Miller KE, Gorchoy DL (2004) The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs. *Oecologia* 139:359–375

Environmental and Health Complications of Microplastics



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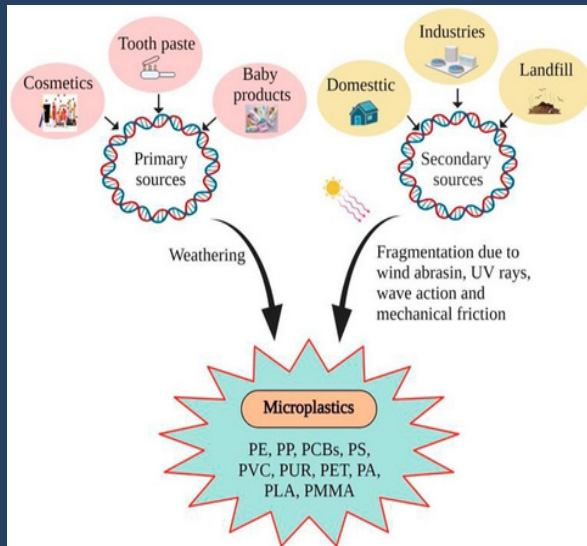
What are Microplastics?

Microplastics are particles of plastic substances which are less than 5 mm in diameter [8]. As global plastic use increases, the prevalence of microplastic particles does as well. Due to their small size, microplastics can easily go undetected and penetrate food supplies, water sources, and air consumed by the population, leaving humans exposed to harmful toxins through routes of ingestion, absorption, and inhalation. Therefore, microplastic pollution poses a critical threat to the environment, and to public health globally.



Environmental Concern

With demand for plasticising drastically and global plastic use exceeding 3 billion tons in 2016, plastic-related pollution is undeniably an increasing concern [8]. Consumer products, including but not limited to, pharmaceuticals, cosmetics, detergents, and dental hygiene supplies have all been identified as sources of microplastic pollution. While the majority of these products contain plastic particles of micrometer size, a significant amount of microplastics are derived from the erosion and breakdown of larger plastics. This breakdown occurs "during environmental exposure such as UV irradiation, mechanical abrasion, or microbial degradation [2]." Due to the minute size of the particles and the acuity of the concern, few environmental regulations and interventions are in place to alleviate the issue. Polyethylene terephthalate, PET, is among the most common sources of microplastic pollution, accounting for 80% of plastic used globally. According to Khairul Anuar et al. (2022) "PET is formed as a semi-aromatic polymer through polycondensation of terephthalic acid (TPA) with ethylene glycol (EG) or by the transesterification of dimethyl terephthalate with ethylene glycol." Due to its complexity, when recycled it is extremely difficult to recover the original components of terephthalic acid and ethylene glycol, and it is also non-biodegradable [5]. Given that the substance, along with other types of microplastics, is non-biodegradable and difficult to effectively recycle, it has ample opportunity to serve as an environmental pollutant and be ingested by humans.



Threat to Public Health

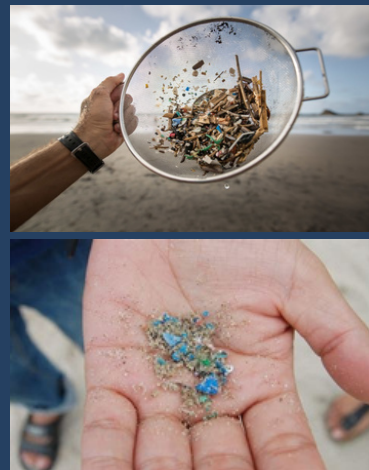
Given their prominence within the environment, microplastics are traceable not only in raw water, but also in treated water meant for human consumption by entering water supplies incidentally from run-off, directly through release from waste treatment facilities, leaching out of landfills, and a collection of other routes [6]. In fact, it is estimated that humans consume an average of 0.06 mg/kg/day of microplastic particles from drinking water alone. The presence of microplastics in water supplies ultimately leads to consumption of microplastics by wildlife and livestock, further contaminating humans by infiltrating food sources [2].

Between water and food contamination, microplastics such as polypropylene, polyethylene, and polyethylene terephthalate have been traced in salt, alcoholic beverages, canned goods, milk, and bottled water [8]. In fact, a study titled "Discovery and quantification of plastic particle pollution in human blood," provided evidence that microplastic pollutants are identifiable within the human bloodstream after sampling 22 individuals, with 17 of them having traceable concentrations of microplastics [9].

Rodent Studies

While the presence of microplastics in the human bloodstream is confirmed, the exact consequences to human pathology are unknown. However, studies on model organisms such as mice have exhibited concerning evidence across various organ systems following microplastic exposure, suggesting that humans are affected similarly. Studies conducted on mice have revealed negative neurological, gastrointestinal, hepatological, and inflammatory responses resulting from microplastic exposure [2]. Following exposure to microplastics, the brain showed evidence of oxidative stress and the kidneys exhibited glomerular necrosis [1].

Additionally, the spleen has been found to exhibit an impaired immune response due to decreased leukocytes in relation to an intraperitoneal microplastic delivery route. In fact, an immunological study addressing macrophages in mice and their response to microplastics was performed and produced significant findings [11]. After three days of exposure to microplastic exposure, the microplastics were noted to be consumed by the macrophages, however, instead of the macrophages ridding themselves of the waste, the microplastics stayed inside of the cells and phagocytosis was not occurring sufficiently. The inability for the microplastics to be degraded by the cell not only caused them to remain inside the macrophage, but also hindered cellular metabolism. Given the anatomical, immunological, and physiological homologies between mice and humans, it can justifiably be hypothesized that humans are affected similarly by microplastic exposure.



Conclusion

Microplastic pollution is an undeniable threat to the environment and public health at the global scale. As plastic demand and production increases alongside the human population, the issue will only worsen. While the effects of microplastic exposure in humans is unknown and little ability for experimentation exists due to ethics, the continuation of mice studies is vital to further understanding the potential risks and pathologies introduced by chronic microplastic exposure.

If evaluating histological and immunological responses to microplastic exposure in mice provides evidence that human comorbidities and health defects could be caused or exacerbated by accumulation of microplastics, then it is extremely relevant information to clinicians and the entire field of biomedicine. Thorough understanding of microplastic pollution sources and the risks they pose allows for efficient and informed development of clinical interventions and environmental precautions which should be carried out to address the issue of microplastics..

References:

1. Al Shoyab, Archie SR, KaramyanYV. Intra-peritoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? Pharm Res. 2019 Dec; 23:3711-12.
2. da Silva Brito, W. A., Muter, F., Wesde, K., Cecchi, A. L., Schmidt, A., & Bielecki, S. (2022, April 21). Consequences of nano and microplastic exposure in rodent models: The known and unknown. *particle and Fibre Toxicology*. BioMed Central.
3. Emerging contaminants: Should we be concerned about microplastics? *NADA Environmental*. (2019, April 24).
4. Jonathanodonell. (2020, March 20). The invisible, microscopic climate crisis—microplastic that we eat, drink, and breathe/Thrive Global.
5. Khairul AnuarNFS, Hyyoff, Us-Rehman G, Abdullah F, NormiYM, SabullahMK, Abdul Wahab R. An Overview into Polyethylene Terephthalate (PET) Hydrolases and Efforts in Tailoring Enzymes for Improved Plastic Degradation. *Int J Mol Sci*. 2022 Oct 20;23(20):16444.
6. Kim, H., & Homan, M. (2020). Evaluation of pharmaceuticals and personal care products (PPCPs) in drinking water originating from Lake Erie. *Journal of Great Lakes Research*, 46, 1321-1330.
7. Lamochinski, G., Acharya, R., Maratheja, R., Modi, B., Prasad, R., Adhikari, A., Rast, B. K., Aryal, S., & Parajuli, M. (2022, May 26). Microplastics in environment: Global concerns, challenges, and controlling measures. *International Journal of Environmental Science and Technology*. SpringerLink.
8. Lee S, Kang KJ, Sung SE, Choi JH, Sung H, SeongY, Lee J, Kang S, Yang SY, Lee S, Lee KR, SeoHS, Kim K. In Vivo Toxicity and Pharmacokinetics of Polyethylene Glycol Microplastics in ICR Mice. *Polymers (Basel)*. 2022May 30;13(5):2220.
9. Leslie HA, van VelzenKJM, Brandtsma SH, VethaasAD, Garcia-Valljo JI, LamoreePH. Discovery and quantification of plastic particle pollution in human blood. *Environ Int*. 2022 May;163:107199.
10. Magagnoli, M. E. A. L. T. H. (2019, November 21). Micro Plastics: an invisible danger to human health. *InnovHEALTHMagazine*. Retrieved April 14, 2023, from <https://www.healthmagazine.com/2019/10/21/issues/micro-plastics/>
11. Tripathy-Lang, A. (2021). Microplastics morph cell metabolism. *Ecol*, 102.



Kayla Boettger | Integrated SS Education & History

JAPAN'S REMEMBRANCE OF WWII

Dr. Don Buerk

Introduction

In the United States we often learn about major wars and events through an American or European perspective; however, understanding the perspectives and reasonings of other parts of the world can be useful to explaining why these events happened in the first place and how other countries feel about these events today. By analysing Japan's actions during and following World War II, it can be shown why memory of World War II in Japan is so complex.

One of the largest differences between the United States' perspective and Japan's of World War II, is that the Japanese have no single war to remember between the years of 1931 and 1945. Unlike the United States, Japan fought many overlapping wars with different names during this time period including the Manchurian Incident in China, activities in the Pacific such as Pearl Harbor, Korea, and later, the Soviet Union. The Japanese to this day struggle to give this time period a single name. In Japanese history, this time period is referred to as Greater East Asian War, the Fifteen Years' War, the Pacific War, the U.S.-Japanese War, and World War II. This small name disagreement is a part of a larger problem to collectively remember World War II in Japan.

Collective memory of World War II in Japan has been an ever evolving process due to a variety of factors. After the war and pressured by the Allies, individual Japanese were forced to figure out what this memory was. Were the Japanese the villains of eastern Asia who terrorized their fellow Asians in things such as Nanjing Massacre and Unit 731 experiments? Were they the victims of the war as the only to experience atomic bombs? Were they the heroes of Asia standing up to the imperial West? Through my research, I sought to show how Japanese remember their part in World War II today.

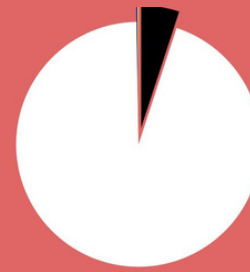


Historical Influences

After the end of World War II, the Japanese surrender, and the dropping of atomic bombs on Hiroshima and Nagasaki, the U.S. occupied Japan for over seven years. Due to this occupation and forced censorship, historians today grapple with biased public records from this time period. Despite being bitter enemies just months before, Japan was forced to figure out how to simply survive and move on with a U.S. presence. Emperor Hirohito asked his people to "accept the unacceptable" and "endure the unendurable." Unlike other Axis countries, Japan's leadership in Emperor Hirohito also was allowed to remain the same despite the Tokyo War Trials. Much of the responsibility for Japan's war crimes was placed on lower military leaders and officials. Imperial responsibility in the war was never truly addressed, and this adds to why responsibility of the war is not agreed upon in Japan today. In addition to U.S. Occupation, Japan's time to address the events of the war was also interrupted by a new constitution and later new threats from the Cold War. Japan's collective memory of World War II is greatly affected by the Cold War and the resulting relationship with the United States.



Standardized Japanese Textbook (2017)



● 1931-1945 ● Japanese Textbook ● Nanjing Massacre ● Atomic Bombs

Bibliography

1. Bukh, Alexander. "Japan's History Textbooks Debate: National Identity in Narratives of Victimhood and Victimization." *Asian Survey* 47, no. 5 (September/October 2007), 683-704.
2. Cook, Haruko T. "Memories of Japan's Lost War." *The Journal of American-East Asian Relations* 11, no. 1/4 (Spring-Winter 2002), 25-40.
3. Dower, John W. "The Bombed: Hiroshimas and Nagasakis in Japanese Memory." *Oxford Journals* 19, no. 2 (Spring 1995), 275-295.
4. Koshiro, Yukiko. "Japan's World and World War II." *Oxford Journals* 25, no. 3 (Summer 2001), 425-441.
5. Saito, Hiro. "Reiterated Commemoration: Hiroshima as National Trauma." *American Sociological Association* 24, no. 4 (December 2006), 353-376.

Conclusions

Through my research, it can be shown that Japan has not agreed upon a collective memory of World War II and their role in it. This is because Japan's collective memory of the war was greatly affected by politics, their culture, and trauma. Instead, there has been multiple perspectives that individuals have taken in addressing their history. Although these are individual perspectives, my research has shown how the majority of the Japanese believe and use a variety of these perspectives still to this day.

1. Victim Perspective (emerged almost immediately following the atomic bombs in August of 1945)- The Japanese people lost over 100,000 citizens instantly following the atomic bombs at Hiroshima and Nagasaki. This doesn't account for the thousands of other lives lost during the war and in the months and years following the atomic bombings. Despite their war crimes across Asia, the dropping of the atomic bombs morally account for this feeling of being the victim amongst the Japanese.

2. "Sacred War" Perspective (emerged in 1937 as Emperor Hirohito led the Japanese to dominance in the Pacific)- This time period reflects Japan's efforts to create a greater society for the Japanese people and push for economic development. Japan was fighting to liberate themselves and all of Asia from the West's imperialism.

3. Anti-Military Perspective (emerged during U.S. occupation following WWII)- The Japanese were falsely led into a war against a militarily superior West. Military officials could no longer be trusted, and war was no longer the solution for the nation's problems. As anti-military sentiment grew, new emphasis was placed on peace and progressive reforms.

Japan's inability to collectively remember the war through one lens is shown in how they address the war in their educational systems. Following the dropping of the atomic bombs, there was a collective feeling among the leadership that Japan had lost to the enemy's science, technology, and critical thinking. As science and progressive reform became the main emphasis in their democratic postwar society, much of this time period's history was left out.



John Brancel

History

The British Raj

Advisor: Dr. Buerk

Introduction

The British Raj was the era of direct British rule over India from 1858 until Indian independence in 1947.

Methodology: In order to research this topic I used a variety of resources from prestigious organizations as well as museum and history databases.

Observations: While looking into this topic I discovered that the topic is much more in depth than at first glance. Hundreds of Historians and researchers have looked into the topic to better understand the impact of British rule on India as well as learning about how India became a free country.

Findings: This project helped me to learn a lot about the British Raj and India's struggle for independence while also helping me to solidify my knowledge of this topic from the classroom.

Themes: There are a few common themes which I have hinted at already when it comes to the discussion over the British Raj.

- The effects of both World Wars
- The decolonization period
- Gandhi's impact
- British treatment of the Indian population

Background

Before the British established a direct rule over India, they still had influence over the subcontinent with the British East India Company. The company was formed in 1600 to get involved with the spice trade in India but had instead grown powerful and with their personal armada, seized control over key regions in India.

After the Sepoy rebellion in 1857, which is also known as the First War of Indian Independence, the British decided to step in and rule over India directly by establishing the British Raj.

The British Raj was set up in 1858 to finish off the Sepoy rebellion and allow the British to use the wealth and resources of India for their own empire. The defeat of the Sepoys allowed the British to change command and establish a new order of government with both British officials and native Indians that were supportive of the crown.

These new offices also allowed many Indians to be voted into office by the people and help transform the country under the watchful eye of the British government. With their officials now in place and order restored in the region, the British could now focus on industrializing the area and gathering its resources.

British Rule

On August 2, 1858 the Government of India Act was passed which officially transferred power from the East India Company over to the crown. This change in power also came with a change in philosophy as the new British officials viewed developing India as the "white man's burden". In other words it was their duty as Europeans to help advance lesser nations and people. However, the British did have a level of respect for the Indian people at least initially as they granted religious freedom and allowed "free" elections. Of course the only people that appeared on the ballot were supportive of the British.

This level of respect was mainly just to preserve their control over the region since India was viewed as the "Jewel in the Crown". This term eluded to the fact that India was vital to the British Empire as they received tons of raw materials as well as generating a fortune for themselves. India was being exploited for its raw materials and extremely cheap labor but the British did treat the Indian people much more fair than some of their other colonial holdings.

The treatment of the Indian people began to worsen after the first World War when many Indians had served the British on both the Western Front and in Africa. They did this under the assumption that the British would allow India to become a free nation following the end of the conflict. However, this did not happen and began to create resentment against the British. This resentment only grew after the Massacre of Amritsar on April 13, 1919. The British had recently banned public assembly in response to the growing challenges to their rule and when 10,000 unarmed men and women gathered in a public square the British retaliated. British General Reginald Dyer ordered his 50 soldiers to fire on the crowd without warning killing 400 civilians and wounding another 1,200.

Figure #1



Independence

Following the massacre at Amritsar, many prominent Indian nationalists began to come to popularity. The most famous of these nationalists was Mohammed Gandhi who led many peaceful protests against the British and became famous for his non cooperation ideas. He did not want to fight the British with force but instead urged other Indians to boycott British goods and not to comply with the rules and regulations established by the British. Gandhi was also successful in his efforts to finally unite Hindus and Muslims with a common goal of independence.

The independence movement also gained more traction with the start of WWII when the British again asked for Indian support. This time the Indians were split on what to do after they were lied to following their efforts in the First World War. However, the Second World War was different since Japan had gotten involved and had swept across Asia and the Pacific. This meant that India itself was now under threat of attack and the people of India would fight to protect their homeland.

Following the Second World War the Muslim population of India no longer trusted either the Hindus or the British as talks had opened up to free India. These talks were more focused on the Hindus and created a rift between the two sides. This came as a bit of a surprise to the Muslims since they had been involved in the government for a long time as a part of the Muslim League. The British believed that the transfer of power would be much easier if they instead handed the country over to a single Indian government and this did not include the Muslim League. The lack of support for the Muslims led to them asking the British for their own land to govern freely in addition to the land being handed over to the Hindu Indians. These talks went on for months until increasing pressure from the international community finally saw the British give into their demands.

Figure #2



Conclusions

On the 14th of August 1947, the country of Pakistan was founded giving the Muslim population of India a new home. A day later on the 15th of August 1947, India was granted its independence. The nations were not given independence on the same day so that Lord Mountbatten could attend both of the ceremonies. Lord Mountbatten was a British officer who was the 1st Lord of Burma in India.

The British Raj had a huge role in History even though it is not one of the most well known historical topics. Studying the Raj allows you to see how both India and Pakistan gained their independence, how the British Empire began to crumble, and how important Indian soldiers were in both World Wars. The roughly 90 years of British control of the Indian subcontinent saw major developments in industry and in the Indian military. Even with all of the struggles and oppression of the Indian people, the British did help them bolster their infrastructure. However the Indians would have to learn and adjust to governing itself as well as transitioning from a purely exported territory to an economically sound nation.

Bibliography

1. "British Raj." Accessed March 20, 2023. https://www.newworldencyclopedia.org/entry/British_Raj.
2. Kaul, Dr Chandrika. "History - British History in Depth: From Empire to Independence: The British Raj in India 1858-1947." BBC. BBC, March 3, 2011. https://www.bbc.co.uk/history/british/modern/independence1947_01.shtml.
3. Masani, Zareer, Written by Zareer Masani, Wolfgang de Melo, Barnaby Crocroft, et al. "The British Raj: An Assessment." History Reclaimed, August 14, 2022. <https://historyreclaimed.co.uk/the-british-raj-an-assessment/>.
4. "Parliament and Empire - UK Parliament." Accessed March 20, 2023. <https://www.parliament.uk/about/living-heritage/evolutionofparliament/legislativescrutiny/parliament-and-empire/>.



Evaluating Athletic Training Policies for Individuals with Autism

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Abstract

There is a paucity of research related to best practices for athletic trainers treating athletes with autism spectrum disorder. This project provides information on the cognitive and physical differences between individuals with ASD and neurotypical individuals, as well as providing information on the type of classes, content and strategies for athletic trainers. Recommendations for best practices and educational preparation for athletic trainers are discussed.

Introduction

- According to the CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network, the prevalence of autism spectrum disorder (ASD) is 1 in 36 children.
- Given the high prevalence of children being diagnosed with autism spectrum disorder and the shift to make schools more inclusive, it is highly likely that athletic trainers will work with athletes who have ASD.
- Many athletic trainers do not feel equipped to provide services to people on the autism spectrum.

Motor Differences

- Individuals with autism are more likely to have gross-motor and fine-motor control difficulties.
- Gross-motor: movements between the left and right side of the body
 - Example: difficulty moving one's legs, which can lead to difficulties walking or running
- Fine-motor: small movements using the hands and wrists
- Some individuals may have low muscle tone and problems maintaining their posture or balance.
- Hand-eye coordination: in relation to sports, this can become difficult when participating in sporting events (example: catching a ball or imitating the movements of others).
- When athletic trainers work with athletes that have difficulties with gross-motor and fine-motor skills, different exercises and treatment plans may need to be looked at based on the athlete. Athletic trainers can also work with their athletes to increase these functions so the athlete is able to compete at their highest levels.

Cognitive Differences

Increased participation of autistic athletes

A 2010 report from the United States Government of Accountability Office was released stating students with disabilities were not being provided the same opportunities as their peers who did not have disabilities. School administrators were reminded of the legislation that supports equal access to all students. Schools in return began to see an increase in the number of students with disabilities who participated in activities.

Professional training

In a survey done by Alexander and Gardin (2020), sixty-six athletic training program directors were surveyed using multiple-choice and open-ended questions to determine what course content related to people with disabilities is currently included in their AT preparation curriculum.

- Seventeen percent of the directors reported including a course on providing care for people with disabilities. Ten schools reported it was a mandatory course
- Eighty-five programs included some content within a course
- Only 40% of the directors reported that ASD specific content was related in coursework
- Over 50% of the directors said that AT's will likely provide services to athletes with ASD

Recommendations

Physical therapists, along with athletic trainers, are specialized in human development, motor functions and specialized interventions that allow individuals to reach their potential and use their resources for maximum functionality (Rosenbaum, 2005). Athletic trainers can draw from physical therapy research to individualize the therapy sessions to meet the individual needs of athletes with ASD that they are working with.

- Individualized plans revolve around the injury that occurred, as well as types of exercises and equipment that each athlete knows how to use.
- The athletic trainer will also create the individualized plan to accommodate for sensory discomforts that the athlete may have

AT Preparation

Executive Function

Cognitive skills under executive function are necessary for deliberate reasoning, intentional action, emotion regulation, complex social functioning, and adaptation to changing circumstances (Ludyga et al., 2021).

- Inhibitory Control: the ability to comprehend a thought, action or feeling for a short period of time
- Working Memory: the amount of information that can be held in the mind
- Task Switching: enables goal-directed behaviors and leads to performing two tasks simultaneously (e.g., talking to someone and walking)

Social Comprehension

Theory of mind suggests autistic individuals have difficulty understanding others due to deficits in determining what others are thinking, feeling, and know.

The double empathy theory suggests that the social differences are due to a mismatch between 2 people that leads to faulty communication, both verbal and nonverbal.

- Regardless of the theory used to describe the breakdowns in communication, these disconnects can inhibit effective communication. For example, difficulty in reading the other person's facial expressions may stunt conversations between autistic and non-autistic people.

References

- Alexander, M. G., & Schwager, S. M. (2012). *Meeting the physical education needs of children with autism spectrum disorder*. National Association for Sport and Physical Education.
- Centers for Disease Control and Prevention. (2023, April 4). *Data & statistics on autism spectrum disorder*. Centers for Disease Control and Prevention. Retrieved April 12, 2023, from <https://www.cdc.gov/ncbddd/autism/data.htm>
- Duquesne, V., Richard, R., Andrieu, B., & Joncheray, H. (2022). Sports experiences of elite athletes with intellectual disabilities and/or autism spectrum disorders. *Sport in Society*, 1–15. <https://doi.org/10.1080/17430437.2022.2070480>
- Ludyga, Sebastian, et al. "Muscle Strength and Executive Function in Children and Adolescents with Autism Spectrum Disorder." *Autism Research*, vol. 14, no. 12, 5 Aug. 2021, pp. 2555–2563. <https://doi.org/https://doi.org/10.1002/aur.2587>
- Rosca, A. M., Rusu, M. R., Zavanescu, M., & Rusu, L. (2020). Current Intervention Possibilities for Autistic Children from the point of View of the Physical Therapist. *Journal of Sport and Kinetic Movement*, 11(36), 106–112.
- Melissa Alexander, and Frederick Gardin. "Preparing Athletic Trainers to Provide Services for People with Disabilities." *Palaestra*, vol. 34, no. 1, 2020.
- Miyake, A., & Friedman, N. P. (2013). The Nature and Organization of Individual Differences in Executive Functions: Four General Conclusions. *National Institute of Health*, 21(1), 8–14. <https://doi.org/doi:10.1177/0963721411429458>.
- Mouridsen, S. E., Rich, B., & Isager, T. (2016). Injury Patterns among Individuals Diagnosed with Infantile Autism during Childhood: A Case-Control Study. *Scandinavian Journal of Child and Adolescent Psychiatry and Psychology*, 42), 88–95.
- Schenkman, L. (2022, September 14). *Motor difficulties in autism, explained*. Spectrum. Retrieved April 13, 2023, from <https://www.spectrumnews.org/news/motor-difficulties-in-autism-explained/>
- Zamzow, Rachel. "Double Empathy, Explained." *Autism Research News*, 22 July 2021, pp. 1–4.



Along with creating individualized sessions, athletic trainers should also be prepared to accommodate for different needs that individuals with ASD may have. These can be in the form of helping with social interactions, understanding how their athletes communicate, staying consistent with times and length of treatment session, as well as being aware of sensory discomforts the individuals may have.

Alexander & Schwager (2012; see QR code) provide specific recommendations for accommodating the needs of individuals with hypersensitivity. Hypersensitivity is a common characteristic of autism spectrum disorder.

Colored Lighting's Effect on Stress and Anxiety in Zebrafish

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INTRODUCTION

Stress and anxiety-related disorders affect millions, around 33 percent of the population, costing nearly a trillion dollars per year. Environmental factors, such as color and lighting, are crucial in affecting these disorders. Different colors are perceived to have different effects on people's emotions. For example, in a study on the relationship between emotion regulation and color preference, both men and women thought brown and light blue created no feeling of joy, while light brown (sand) produced happiness (Sokolova et al., 2015). Women also had increased positive preferences for pink and violet, while men preferred orange (Sokolova et al., 2015). This suggests that being in positively correlated colored environments could help reduce stress. Studying the relationship between colored lighting and feelings or behaviors could alleviate the costs associated with treating individuals affected by stress and anxiety.

OBJECTIVES

The overall goal is to determine the effects of colored lighting on stress and anxiety in zebrafish (*Danio rerio*).

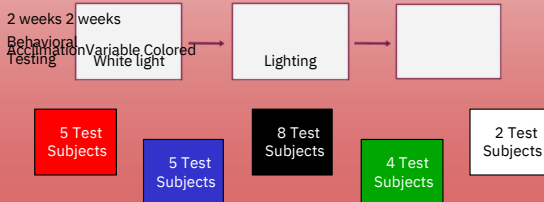
- Zebrafish were exposed to blue, green, red, white, or black (darkness) colored lighting
- Anxiety behavior was assessed using the Light/Dark test and the Novelty Tank Test

Hypothesis: Black, green, and red-colored lighting will be preferred over blue and white-colored lighting. Preferred colors will cause less anxiety for zebrafish with exposure to long-term colored lighting.

METHODS

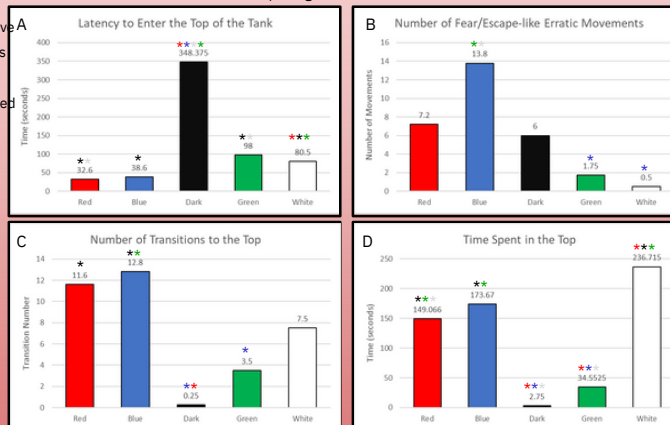
Wild-type adult *Danio rerio* of mixed genders were acclimated to the laboratory for 2 weeks before testing with a 12:12 hour light/dark cycle. Subjects were fed two-three times a week, and tanks were cleaned as needed. Subjects were randomly assigned to each group

for each colored lighting tank. The length of light exposure was 2 weeks with the colored light and testing for the 2 weeks and during the 12:12 light/dark cycle related behaviors were measured through the Novelty Tank Diving Test and the Light/Dark Tank Test. All behavioral trials were videotaped using a Google Pixel 6 Pro, to record data and lessen human error.



NOVELTY TANK TESTING

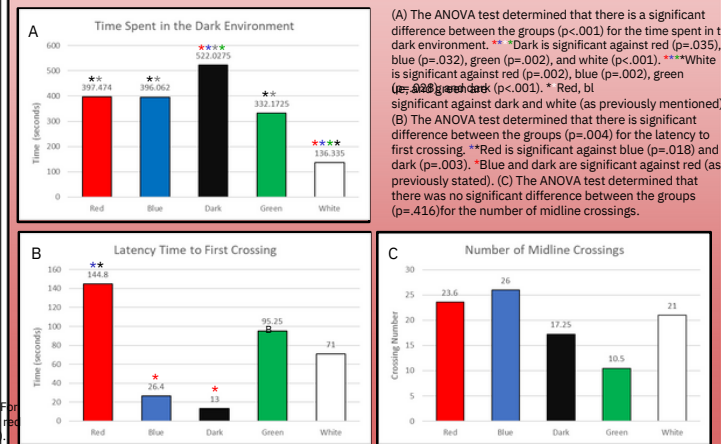
This test was performed by placing small groups of two to three zebrafish into a narrow tank (5" x 40" x 12") over a 6-minute interval. Each group was tested once in a quiet environment and recorded. The tank was split into two with a predetermined line halfway up in the tank in a horizontal direction. Parameters examined included the latency to enter the top, the number of transitions to the top, time spent in the top, and the number of fear/escape-like erratic movements. Previous studies suggest that zebrafish exhibiting fear and stress-like behaviors would spend more time at the bottom of the tank and less time exploring (Parker et al., 2012).



(A) The ANOVA test determined that there is significance between the groups ($p < .001$) for the latency to enter the top of the tank. For the black group, some subjects were given the max time (360 seconds) due to them never crossing. **Dark is significant against red ($p < .001$), blue ($p < .001$), white ($p < .001$), and green ($p < .001$). **White is significant against red ($p = .050$), dark, and green ($p = .008$). *Green and red are significant against white and dark (as previously mentioned). *Blue is significant against dark (as previously mentioned). (B) The ANOVA test determined that there is significance between the groups ($p = .016$) for the number of fear/escape-like movements. *Blue is significant against green ($p = .020$) and white ($p = .045$). Green and white are significant against blue (as previously mentioned). (C) The ANOVA test determined that there is significance between the groups ($p < .001$) for the number of transitions to the top of the tank. *Red is significant against dark ($p = .002$). *Blue is significant against dark ($p < .001$) and green ($p = .033$). *Dark is significant against red and blue (as previously stated). *Green is significant against blue (as previously stated). (D) The ANOVA test determined that there is significance between the groups ($p < .001$) for the time spent in the top of the tank. *Red is significant against dark ($p < .001$), green ($p < .001$), and white ($p = .005$). *Blue is significant against dark ($p < .001$) and green ($p < .001$). **White is significant against red, dark ($p < .001$), and green ($p < .001$). *Dark and green are significant against red, blue, and white (as previously mentioned).

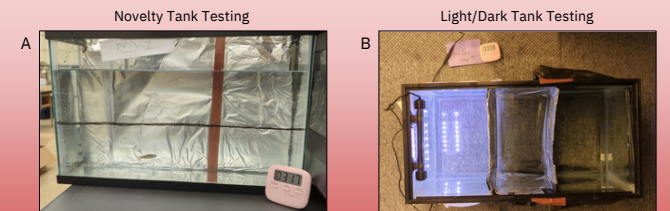
LIGHT/DARK TANK TESTING

The testing apparatus consisted of a half-white, half-black tank with a central starting area for the subjects, separated by a holding compartment within the middle. The white side had a light over the top and the dark side had no light. The subjects were placed into the control area for approximately 3 minutes before being allowed to explore for 10 minutes. The values recorded were the time spent in the dark environment, the latency time to first crossing, and the number of midline crossings. Zebrafish were tested in groups of 2-5 (based on survival), and each test was recorded and rewatched to ensure accuracy.



(A) The ANOVA test determined that there is a significant difference between the groups ($p < .001$) for the time spent in the dark environment. **Dark is significant against red ($p = .035$), blue ($p = .032$), green ($p = .002$), and white ($p < .001$). ***White is significant against red ($p = .002$), blue ($p = .002$), green ($p = .002$), and dark ($p < .001$). *Red, bl, significant against dark and white (as previously mentioned). (B) The ANOVA test determined that there is significant difference between the groups ($p = .004$) for the latency to first crossing. **Red is significant against blue ($p = .018$) and dark ($p = .003$). *Blue and dark are significant against red (as previously stated). (C) The ANOVA test determined that there was no significant difference between the groups ($p = .416$) for the number of midline crossings.

TESTING APPARATUS SETUP



(A) Shows the testing apparatus for the Novelty Tank Test, with the predetermined line separating the tank into top and bottom sections. (B) Shows the testing apparatus for the Light/Dark Tank Test, with the sectioning of light and dark.

SUMMARY

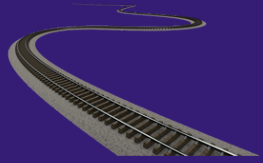
Based on the results of our experiments, the fish housed in darkness for two weeks exhibited the most stress and anxiety-related behaviors. During the novelty tank test they exhibited a significantly higher latency period to enter the top of the tank, made significantly fewer transitions to the top of the tank, and spent the least amount of time in the top of the tank compared to all other treatment groups. During the light/dark tank testing they spent the most time in the dark environment compared to all other treatment groups. The fish housed in white light did not exhibit many stress and anxiety-related behaviors, potentially because they were housed in the same lighting environment. These fish spent more time in the light during the light/dark tank test and spent a majority of their time in the top of the tank during the novelty tank test. Fish housed in the red and blue lighting exhibited normal exploratory behaviors during the novel tank test. However, fish housed in blue lighting had the greatest number of erratic movements during the novelty tank test, and the fish housed in white lighting took a significantly longer time to cross the midline during the light/dark tank test. Fish housed in green lighting exhibited cautious behavior during testing, due to fewer transitions into the top of the tank and a longer latency period to enter the top of the tank during the novelty tank test, and fewer midline crossing for the light/dark test.

REFERENCES

Sokolova, M. V., Fernández-Caballero, A., Ros, L., Latorre, J. M., & Serrano, J. P. (2015). Evaluation of Color Preference for Emotion Regulation. In J. M. Fernández Vicente, J. R. Álvarez-Sánchez, F. de la Paz López, Fco. J. Toledo-Moreo, & H. Adeli (Eds.), *Artificial Computation in Biology and Medicine* (Vol. 9107, pp. 479–487). Springer International Publishing. https://doi.org/10.1007/978-3-319-18914-7_50

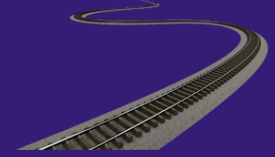
ACKNOWLEDGEMENTS

Thank you to Dr. Linda Tucker-Serniak for helping us understand how to use the statistical software and for housing the fish after the experiment was concluded. Thank you to Dr. Nathan Griggs for allowing us to use his lab space. I would also like to thank Lily Linke for feeding the fish. Thank you to the Department of Biology at Defiance College for funding the project.



Levi Dirig Steam Engines In Europe

FACULTY MEMBER: Donald Buerk



Abstract

Steam Engines are the cornerstone of the First Industrial Revolution, especially in Europe. They allow traveling to become affordable and common, as well as a revolutionary new venture in the field of technology. The steam engine became an important asset to allow people to transport more easily than ever before. Not only did it become available to ride commercially, but it also sets the stage for what sort of trains the world would see today.

What was Europe Like Before The Revolution?

Before the Industrial Revolution, most countries in Europe had to rely on agricultural substances like coal and timber. There were localized coal deposits so that people can work in the mines, but the conditions were quite dreary and wet, making earning materials substantially difficult for many people. On top of that, there was limited efficient technology available like cars, trains, and factories. In addition, the cost of purchasing handy materials and tools were too expensive for many people at the time.

How Steam Engines Emerged From the Industrial Revolution?

As said before, steam engines are the cornerstone of the First Industrial Revolution thanks to James Watt and his efficient innovation. However, there was another development that allowed steam engines to become an innovative powerhouse, and that is the production of high-quality steel. With increased steel production comes increased mechanization and with increased mechanization comes mass production and factories being built for increased efficiency and production of materials.

The First Industrial Revolution

This event in history occurred from the eighteenth and nineteenth centuries and it is considered extremely influential for the development of technologies and materials including steam engines, metal, and glass. It first spread across various parts of Europe like Great Britain, France, and Austria-Hungary. But it also spread through North America too. Not only is the revolution influential for technologies, but it changed agriculture and people's daily lives, both economically and socially.



Conclusion

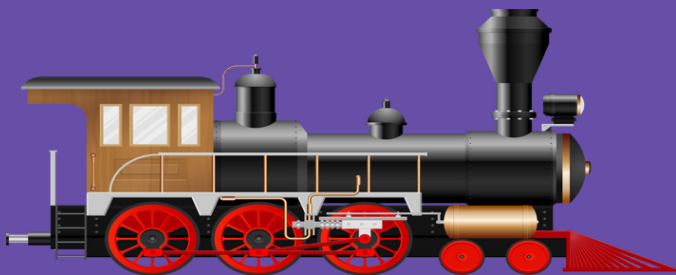
In summary, steam engines are some of the most important innovations in world history. In a time where there was limited technology, dreadful conditions, and scarce resources, this was valuable for many people. It became the most influential during the First Industrial Revolution and it changed the way people live and do things. James Watt's influence as well as the development and production of steel, made the engine more efficient and easier to travel than previously possible. It may not be as fast as a car, but it has got an amazing legacy that will live on for many generations.

Who Innovated the Steam Engine?

According to Levent Menga, the innovation of the steam engine was credited to James Watt. He did not invent the steam engine, but he did improve upon the foundations set by Scottish inventor: Thomas Newcomen. Previously, Newcomen's engine suffered from low efficiency. This was largely because of the lack of thermodynamic knowledge to early engineers at the time. However, that all changed when Watt added a separate condenser onto the train to greatly improve its efficiency and thus produced steam. This was considered a huge breakthrough in the development of the steam engine, as it has allowed them to travel more effectively and easily than ever before.

References

- Menga, Levent. "Industrial Revolution and the Birth of Modern Architecture." *Vision International Refereed Scientific Journal*, vol. 7, no. 1, Mar. 2022, pp. 105-122., <https://doi.org/10.55843/ivisum2271105m>.
- Spear, Brian. "James Watt: The Steam Engine and the Commercialization of Patents." *World Patent Information*, vol. 30, no. 1, Mar. 2008, pp. 53-58., <https://doi.org/10.1016/j.wpi.2007.05.009>.



Unit 731

Name: Branden Durkee

Mentor: Dr. Steven Bare



Unit 731 began in Harbin, China, in 1937 with General Ishii on the left having legitimate intentions beginning as an agency to promote public health. The unit was to conduct research that would help Japanese soldiers in ways such as learning the ways the human body can fight off disease, and stave off hunger and thirst. It also helped to understand how an injury affects an armed fighting force. In the early days of experimenting, it was limited to volunteers who signed consent waivers giving their permission. However, this would soon change into forced trials with brutality never seen..



References

Manila Star, Friday, May 4, 2018, Pg. 17

Roadside Struck, Japan's Disturbing Human Experiments Program During World War II, October 2, 2022.

Andrew Lenoir , *The Twisted Story Of Shiro Ishii, The Josef Mengele Of World War 2 Japan*, November 24, 2020.

Charlie White, *Japan's BW Group*, April 2020.



Method Development for Sulfite Determination in Wine

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INTRODUCTION

Sulfites are artificially added as a preservative in some foods as SO₃- or SO₂ to inhibit food decay, preserve food flavor and color, and keep medication in their active form (Chiaverini & Mortier, 2015). Along with being a helpful tool for preserving food, sulfites can cause asthmatic reactions. Sulfite allergies are rare, affecting about 1 in 100 people. Symptoms of these allergies include flushing, fast heartbeat, wheezing, hives, dizziness, upset stomach, diarrhea, difficulty swallowing, etc (Administrator, n.d.). If added to foods in excessive amounts, sulfites can cause an off-flavor in the product.

AIMS AND OBJECTIVES

The goal of this research project was to evaluate different methods for determining sulfite concentration, finding sulfite content in various items, and determining our standards accuracy.

- We used several different methods including HPLC (high performance liquid chromatography), Spectrophotometry, and Iodometry to determine sulfite concentration. In addition to evaluating the effectiveness of these methods, we also wanted to make slight variations to the methods to see if they could be improved upon.
- Detecting the accuracy of standards prepared during this project was important to determine the significance of our results. After preparing the stock solution and our standards, they were tested using sulfite testing strip analysis to ensure that the concentration was correct. Using these strips we were also able to evaluate sulfite solubility issues.

Hypothesis: Sulfite concentrations can be determined using HPLC, Spectrophotometry, and Iodometry.

HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)

Preparation:
The buffer was made by first measuring .96 grams of anhydrous Na₂SO₃ by analytical balance. Next a clean 1000 milliliter volumetric flask was filled with 1000 milliliters of H₂O. Using a micropipette .815 microliters of H₂SO₄ was added to the H₂O. This H₂O and H₂SO₄ mixture was then added in small quantities to the volumetric flask containing the anhydrous Na₂SO₃. This same volumetric flask was then filled with 500 milliliters of H₂O, this was added to the mixture, again, in small quantities. After calculating the volumes of the H₂O and stock solution, these columns were collected through micropipetting. These vials were refrigerated overnight.

To determine the pH of our standard stock solutions, clean and dry pH strips were quickly dipped into the stock solution and allowed fifteen seconds. After fifteen seconds the pH strips were properly color matched to the pH indicator color scale located on the side of the bottle. The pH of the stock solutions was found to be 2.3 (acidic).

Procedure:
Two large bottles were filled with our stock sample solution. This two bottles were hooked up to the HPLC Spectra System 1000. To run a sample on this system, click Run New Sample located under the menu tab of the computer. Click the drop down option and type 'External Non-manual'. Choose p 1000 for 1 milliliter per minute as a flow rate. Click UV 1000 to set the proper wavelengths for particular solution.

Before running the experiment, the connector to the left bottle should be turned on first. Let all the solution flow through the pump, then completely flush. Then turn the connector to the left bottle off and the connector to the right bottle on. Flush the system one more time and the system is ready for experimental run through.

Materials:
- Water
- Anhydrous Na₂SO₃
- 1000 mL volumetric flask
- Sulfuric Acid
- pH strips
- HPLC Spectra System 1000
- Two large wash bottles

Complications:
The HPLC machine was unable to properly flush any solution through the system due to calcification in the column. This did not allow for the stock solutions to run through the machine. Due to the HPLC age and limited time, it was not possible to replace the column in time to complete this method of our research.

SPECTROPHOTOMETRY

P preparation:

The phosphate buffer was prepared by adding 40.428 grams of sodium phosphate dibasic heptahydrate and 6.788 grams of sodium phosphate monobasic monohydrate to 800 milliliters of distilled water in a suitable container. The pH of the solution was then adjusted (a round 7.68) using HCl. Finally, more distilled water was added so the solution volume was one liter. Each sample solution was then created from the stock.

The wine sample was prepared through a dilution using deionized water. 0.25 mL of wine was diluted to 10 mL and also included 0.2 M phosphate buffer at a pH of 9.

The crude extract of anthocyanin had a more involved preparation. 30 bilberry extract capsules were opened and the weight of the powder inside was taken (7.4 g). The sample was then transferred to a round bottom flask and 200 mL of ethanol was added. After being mixed into solution, it was left to sit for 24 hours. The final concentration was 4 g anthocyanin per liter of ethanol.

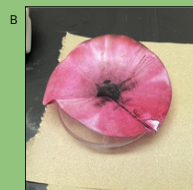
Procedure:

The phosphate buffer was prepared first using both the dibasic heptahydrate and the monobasic monohydrate. The pH had to be adjusted so that it was at 9 and then the diluted solutions were made from this stock. Ten different concentrations were prepared starting with 1000 mg/L and halved all the way to 1.95 mg/L. The crude extract was prepared next using the bilberry pills and mixing the powder into solution with ethanol. After letting this solution sit overnight, it was filtered and put through two rotary evaporators. The solid anthocyanin left in the flask was scraped out into a clean empty beaker.

Before testing on the wine sample, the standard solutions were tested to obtain base numbers. The absorbances on these solutions did not come out as expected so they were eventually remade. After the first trial of all dilutions, it was found that not all of the anthocyanin was binding, meaning that it wasn't completely soluble in water. The anthocyanin from the bilberry pills had a higher concentration than expected which means that all other expected values were also off.

After remaking the standard solutions they were tested again and again showed unexpected values. Along with values being inconsistent, the diode array spectrophotometer was not working as it should, so this protocol had to many technical difficulties to continue.

Materials:
- Water
- Sodium Phosphate Dibasic Heptahydrate
- Sodium Phosphate Monobasic Monohydrate
- Bilberry pills
- Qualitative paper
- Rotary evaporator
- Quartz cuvette
- Diode array spectrophotometer
- HCl or NaOH
- White wine
- KI₂
- H₂SO₄
- I₂
- Na₂SO₃
- HCl or NaOH



Sample Label Number	Anthocyanin Volume (mL)	Sulfite Volume (mL)	Q ₁ Water (mL)	Final Volume	Concentration (mg/g)	Absorbance @605
1	0.25mL	(210/10) = (21.0mL) X = 5.128	4.022	10 mL	2	.641
2	0.25mL	(42/10) = (7.12mL) X = 5.12	4.83	10 mL	4	.075
3	0.25mL	(84/10) = (7.812mL) X = 7.68	2.07	10 mL	6	.062
4	0.25mL	(84/10) = (15.625mL) X = 6.12	4.83	10 mL	8	.070
5	0.25mL	(105/10) = (15.025mL) X = 6.4	3.35	10 mL	10	.641
6	0.25mL	(126/10) = (15.025mL) X = 7.68	2.07	10 mL	12	Not measured



Figure 1. (A) Different stages in the process of preparing the crude extract of anthocyanin. The round bottom flask furthest to the left is being filtered so that the end solution doesn't contain any large particles from the Bilberry pills. The middle round bottom flask contains the solution that is being filtered. Once the majority of liquid in the funnel has emptied into the flask below it, more solution from the middle flask will be poured in, and so on. The furthest right beaker contains the ethanol being used to help filtration. (B) A filter paper after a completed filtration. The larger particles that weren't able to go into solution are shown piled in the middle of the filter paper. The ethanol has evaporated from the paper leaving behind a pink and purple dye. (C) Table showing volumes, concentration, and absorbance for samples one through six. Calculations for determining sulfite volume are also included. There is enough anthocyanin in the solution however the binding reached a saturation at 4 ppm. Therefore, it can be concluded from the chart that anthocyanin is not completely soluble in water at a concentration of 4g/L. More diluted samples were prepared after coming to this conclusion. (D) Label for the wine used in the samples.

IODOMETRY

Iodometry is the quantitative analysis of a solution of an oxidizing agent by adding an iodide which reacts to form iodine, which is then titrated.

Preparation:
Sodium sulfite (and sulfurous acid is unstable) in water used as standards with following concentrations: 1000ppm, 500ppm, 250ppm, 125ppm, 62.5ppm, 31.25ppm, 15.63ppm, 7.81ppm, 3.90ppm. Potassium Iodate, KI₂, was made at 0.1 N concentration. A Sulfuric Acid, H₂SO₄, solution was made at a concentration of 7.0 N. A Potassium Iodide, KI, solution was made at a .60 M concentration. A Sodium Thiosulfate, Na₂SO₃, solution was made at a concentration of .1 N. Finally a starch indicator, made with refined potato starch was made.

Procedure:

50.0 milliliters of standardized 0.1 N Potassium Iodate was added into a 250 milliliter Erlenmeyer flask. To that same Erlenmeyer flask, 25 milliliters of 7.0 N of Sulfuric Acid was added. To the Potassium Iodate and Sulfuric Acid mixture, 25 milliliters of 0.60 M Potassium Iodide was added. From the flask of solution, 25 milliliters was placed in a beaker. A 50 milliliter burette was filled with 0.1 N Sodium Thiosulfate. This was placed above the beaker containing the 25 milliliters of solution. The solution was titrated to a light yellow hay color. 5 milliliters of room temperature starch indicator was added to the solution, which created a royal blue color. This was then titrated again until the purple color disappeared and returned to the light yellow hay color. The buret was then measured to determine how much Sodium Thiosulfate was used. This process was completed again for each concentration standard Sulfite solutions. These results were then charted and a graph was created.

Materials:
- Buret
- Sodium Thiosulfate
- Potassium Iodate
- Potassium Iodide
- Sulfuric Acid
- Sodium Sulfite concentrations
- Graduated cylinder
- Starch indicator
- Wine

Standard Sulfite Concentration	Average Volume of Titrant
3.9 ppm	151.83 mL
15.7 ppm	140.23 mL
62.5 ppm	141.33 mL
250 ppm	135 mL
1000 ppm	110.42 mL
wine 147.92 mL	

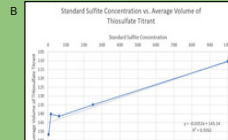
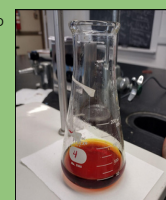
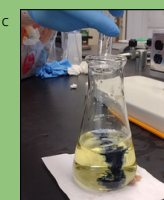


Figure 1. (A) Contains the volume of required titrant for each concentration of standard sulfite solution. There were three trials of each sulfite concentration completed, the average of each concentration was taken. (B) A graph comparing sulfite concentration, seen on the x-axis, and average volume of the titrant, seen on the y-axis. (C) The Erlenmeyer flask after the first titration while the 5 milliliters of starch indicator are being added to create the royal blue color. (D) The Erlenmeyer flask during the second titration, trying to return to the light yellow color seen in figure C.



SUMMARY AND FUTURE DIRECTIONS

Overall the most successful method of sulfite determination was the Iodometry. Due to complications involving the spectrophotometry method and anthocyanin these results are not consistent with sulfite solution concentrations. The HPLC method was unable to be completed due to complications involving the Spectra System 1000 and the column of the HPLC system.

If we were to continue our research, we would like to update the HPLC system and purchase a new HPLC column when given a larger period of time. We would also investigate a better way to both extract and purchase anthocyanins on the market. For the Iodometry, we would like to do more research involving commercially available Sulfite Testing Strips that are used for wine and other beverages.

REFERENCES

Administrator. (n.d.). Sulfite Sensitivity FAQ - Australasian Society of Clinical Immunology and Allergy (ASCIA). <https://www.allergy.org.au/patients/other-allergy/sulfite-sensitivity-faq#:~:text=to%20symptoms%20in,close%20up%2C%20last%20near%20the%20beast%20other%20than%20sulfites.>

Chiaverini, N., & Mortier, T. (2015). A Qualitative Analysis of Sulfite Ions in White Wine Based on Visible Color Changes. *Journal of Chemical Education*. <https://doi.org/10.1021/acs.jchemeduc.1c00813>

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Prenatal and perinatal experiences of individuals with autism pertaining to medical and mental healthcare

Logan Gray, Major: Social Work, Minor: Autism Studies

Dr. Clarissa Barnes

Background

Due to the stigma and stereotype of autism spectrum disorder, autistic individuals who want to start a family are overlooked. Individuals with ASD often struggle with pregnancy because there are few prenatal resources to accommodate the unique challenges associated with pregnancy and ASD. These challenges include finding prenatal resources, sensory issues, difficulties with social interactions, and adjusting to life after pregnancy.

Recommendations

Individuals with ASD experience increased stress, anxiety, and fear regarding the various stages of pregnancy. They experience sensory issues, transitioning to a new routine, and communication hardships with healthcare that most women do not experience. For this reason, here are some recommendations for helping break down these barriers.

- **Communication and respect:** healthcare workers can have written down information about what to expect during each appointment. As well as, respecting what the patient has expressed in their plans.
- **Birth plan:** include a detailed plan for all the healthcare workers. Ex: noting how the individual does not like to be touched or noting how it is helpful to explain every step.
- **Mental health screening:** there is an increased risk for postpartum depression, it is important for healthcare workers to be aware and perform frequent screenings
- **Research:** more research needs to be done to help individuals with ASD by providing resources to help accommodate their barriers to environmental stress. Becoming a parent is hard, but it is even harder when there is a lack of connection and communication.

Sensory differences

During each stage of the pregnancy, individuals with autism experience sensory issues; however, they experience a great deal during the perinatal stage of motherhood. Individuals may struggle with the environment at the hospital and are overstimulated. They may also experience struggles with breastfeeding which can lead to other mental health and self-degrading emotions.

- **The environment at the hospital**
 - Hampton et al. (2022) gathered qualitative information from new mothers and their experiences with childbirth. One mother noted how the contractions were not the worst pain. It was the sensory overload of everything in her environment, like loud noise, lights, and touching by the healthcare workers.
 - In the same study, a different mother with autism noted how the environment in the postnatal room was the hardest sensory-wise. It was challenging because of noises like babies crying, visitors talking, and background noise like music.
- **Challenges of breastfeeding**
 - Grant et al. (2022) conducted a qualitative study on the challenges that new mothers with autism face regarding breastfeeding and using formula. In the study, some women said they had no trouble with breastfeeding, which was a positive experience, while others had a negative and more challenging experience.
 - **Due to sensitivities, having the baby latch while breastfeeding resulted in extreme pain for new mothers.**
 - However, many felt misunderstood when healthcare workers told them they should not feel pain if they did it right.
 - One mother noted how all the touching (baby laying on her, latching, etc.) created a sensory overload. If mothers struggled with breastfeeding, the next option was bottle feeding with formula. This helped many with sensory issues; however, it created mental health struggles for women due to increased anxiety about preparing the bottle.

Adjustment to motherhood

Another challenge individuals with autism face involve their adjustment to motherhood. Many individuals who have an ASD diagnosis like to stick to a routine and a set schedule. This helps with stress and anxiety management; however, having a baby creates a new adjustment that can affect their mental health in both positive and negative ways.

- **Effects on mental health**
 - Hampton et al. (2022), evaluated experiences of two groups of new mothers and highlighted the differences they experienced during their pregnancy. One group of mothers had either been diagnosed with autism or were self-diagnosed, and the other group did not have an autism diagnosis. In the group of mothers with an autism diagnosis, for both depression and anxiety screenings, they had higher scores than individuals without a diagnosis of autism.
 - **There could be many reasons for this, but one possibility is the adjustment period and lack of support and resources.**
- **Life changes**
 - Each parent goes through ups and downs after having a baby and figuring out how to manage their day with a newborn. From various studies, main themes showed up, for example, how they had to adapt to not having as much time to themselves. Other challenges included adjusting to new sensory issues, the lack of routine, and constant exhaustion.
 - Dugdale et al. (2021), reported that some mothers initially felt love for their new child, and that feeling of love came easily to them. But other mothers felt awkward with their child and showed love differently, which made them adapt and change how they normally show love to their child.

Fears about ASD disclosure

Factors that may contribute to fear about disclosure include having their diagnosis overlooked, judged, or the possibility of child protective services being called on them.

- **Healthcare providers**
 - Dugdale et al. (2021) compared qualitative experiences from pregnant women who had an autism diagnosis. Even when they did disclose their diagnosis, the healthcare workers did not use any of their accommodations. On the other hand, some women had a more positive experience when their healthcare provider had training about autism, and they felt heard and not judged or overlooked.
- **Child protective services fears**
 - While disclosing the diagnosis may make the pregnancy process easier for the mother, they have to risk getting their child taken away. McDonnell & DeLucias in 2021 discussed how healthcare providers question new mothers with ASD about how they are as parents. They are also more likely to be reported to child protective services.
 - **Current research does not show how many of these CPS cases resulted in an opened investigation.**

References

1. Grant, A., Jones, S., Williams, K., Leigh, J., & Brown, A. (2022). Autistic women's views and experiences of infant feeding: A systematic review of qualitative evidence. *Autism: the international journal of research and practice*, 26(6), 1341-1352. <https://doi.org/10.1177/13623613221089374>
2. Hampton, S., Allison, C., Aydin, E., Baron-Cohen, S., & Holt, R. (2022). Autistic mothers' perinatal well-being and parenting styles. *Autism: the international journal of research and practice*, 26(7), 1805-1820. <https://doi.org/10.1177/13623613211065544>
3. Hampton, S., Man, J., Allison, C., Aydin, E., Baron-Cohen, S., & Holt, R. (2022). A qualitative exploration of autistic mothers' experiences II: Childbirth and postnatal experiences. *Autism: the international journal of research and practice*, 26(5), 1165-1175. <https://doi.org/10.1177/13623613211043701>
4. McDonnell, C. G., & DeLucias, E. A. (2021). Pregnancy and Parenthood Among Autistic Adults: Implications for Advancing Maternal Health and Parental Well-Being. *Autism in adulthood: challenges and management*, 3(1), 100-115. <https://doi.org/10.1089/aut.2020.0246>
5. Wilson, J. C., & Andriassy, B. (2021). Breastfeeding Experiences of Autistic Women. *MCH: The American journal of maternal child nursing*, 47(1), 19-24. <https://doi.org/10.1097/NMC.0000000000000779>

A Short-Term Water Quality Assessment of the Upper Maumee River Watershed Utilizing Diatom Frustules in Defiance, County, OH USA

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Background

The Maumee River is the main water source for the City of Defiance and its drainage basin, spanning more than 4.2 million acres (>6,500 mi²), is a major input into Lake Erie (Figure 1, MRBPLG). Anthropogenic eutrophication of the Maumee River is a significant concern due to the local impacts on Defiance



residents' drinking water and regional impacts of harmful algal blooms (HABs) in Lake Erie (Berardoet al. 2017). As such, monitoring the quality of the Maumee River is vital. Aquatic bioassessments are one method of monitoring water quality that utilizes knowledge about species-specific sensitivities to environmental conditions (temperature, nutrients, water clarity, water velocity) to assess water health (Angradiet al. 2009).

The purpose of this research is to assess water quality of rivers in and around Defiance, OH using bioassessment of golden-brown algae (diatoms).

Methods



Four locations (Figure 2) were sampled for bioassessment from May-June, 2021. Field methods included collecting diatom samples from the water column using plankton tows (Figure 3). All samples were returned to the lab where they were processed in 35% hydrogen peroxide to remove organic matter, rinsed thrice, and permanently mounted to slides. Diatom frustules were identified to genus level and enumerated under the microscope.

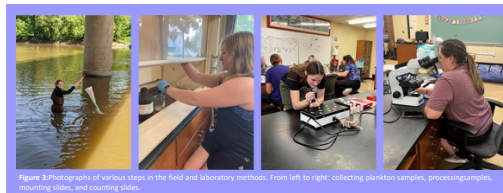
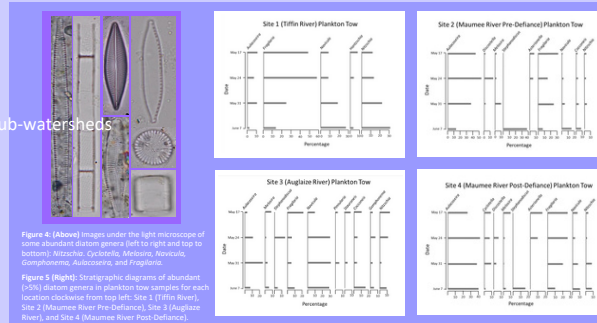


Figure 2: Photographs of various steps in the field and laboratory process. From left to right: collecting plankton samples, processing samples, mounting slides, and counting slides.

Results



In total, 37 diatom genera were identified, with 14 being abundant (>5%) in any given sample (Figures 4, 5). In general, planktic genera dominated samples, but benthic species were also present. Assemblages were dominated by *Fragilaria*, *Aulacoseira*, and *Navicula*. Results indicate that the Tiffin River site had the highest diversity in diatom communities with diatom species indicative of high nutrient availability. Plankton tows at the Auglaize River had a high percentage of benthic species present, indicating relatively turbid conditions. Both Maumee River sampling locations has similar species compositions, dominated by *Aulacoseira*, with an influx of *Stephanodiscus* indicative of nutrient runoff at the end of the sampling period. The presence of *Fragilaria* in high abundance (15-80%) early in the sampling season and higher abundance of *Aulacoseira* (>35%) later in the season is related to decreased nitrogen availability throughout the sampling period (Spaulding et al. 2022). Presence of *Nitzschia*, a genus common in soils, and increased abundance of benthic species (*Navicula* and *Cocconeis*) is likely indicative of increased runoff and/or higher flow rates during middle and late June.

The majority of samples have plankton:benthon ratios greater

1 (ex: Week 4 Tiffin River 2022) correspond with higher turbidity measurements (collected by a separate summer research team at Defiance College—see the Sorrell group poster) and we hypothesize

that increased influx of benthic taxa into plankton tow samples are a response to flooding events.

than 1. Ratios close to and less than

that increased influx of benthic taxa

Discussion

Aulacoseira and *Fragilaria* are the most abundant genera during this season of sampling on both the Maumee River and its tributaries. The diatom assemblages from the 2022 sampling season from all four locations are indicative of generally turbid conditions with moderate to high nutrients. Additionally, the diatom assemblages across sites generally indicate higher nitrogen early on and increasing flow and/or turbidity throughout the sampling period. Increased benthic species in plankton tows typically occurred after rain events or during active flooding.

Future Directions

This is an ongoing project focusing on providing a multi-year bioassessment of the surface waters in and around Defiance, Ohio. Our next steps include identifying diatoms to the species level and integrating chemical parameters into our interpretations. Additionally, we collected benthic grab samples in conjunction with the plankton tows presented here and will analyze those. We plan to test our hypothesis regarding the possible correlation of low plankton:benthon ratios in tow samples and flooding with high-resolution sampling before and after storm events. We plan to integrate analysis of spatial and temporal patterns of water quality in the Upper Maumee Watershed with continued sample collection over the next three years. We envision a more robust dataset with sampling throughout the agricultural season in Northwestern Ohio to better monitor nutrient conditions in the Upper Maumee Watershed throughout the year.

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- Photo credit: Mollie Sorrell

References

- Angradiet al. (2009). A bioassessment approach for mid-continent great rivers: the Upper Mississippi, Missouri, and Ohio (USA). Environmental Monitoring and Assessment.
- Berardoet al. (2017). Impact of land use activities in the Maumee River Watershed on Harmful Algal Blooms in Lake Erie. Case Studies in the Environment.
- Maumee River Basin Partnership of Local Governments (2022). Maumee River Basin Map. mrbplg.org.
- Ohio EPA (2022). Maumee Watershed Nutrient TMDL Project. epa.ohio.gov.
- Spaulding et al. (2022). Diatoms.org: supporting taxonomists, connecting communities. Diatom Research.

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INTRODUCTION

Over-the-counter (OTC) medications and Non-steroidal anti-inflammatory drugs (NSAIDs) are used by millions worldwide to mediate daily pain. More than 57% of health problems in the US are treated with non-prescription drugs (4). OTC pain medications and NSAIDs taken above the recommended dosage may lead to organ damage over time. Among all analgesic overdoses, 29% had used ibuprofen exclusively or combined with other analgesics, making ibuprofen the most common NSAID involved in overdose (3). Previous studies have shown significant adverse effects, such as heart failure, toxic hepatitis, and central nervous system depression when overusing these medicines. NSAIDs, such as ibuprofen and naproxen, are non-selective, which block COX-1 and COX-2 enzymes and inhibit prostaglandin synthesis. Gastric hypermotility and subsequent vascular disturbances are associated with a prostaglandin (PG) deficiency caused by COX-1 inhibition (8). COX-2 selective inhibitors have the potential for problems of fluid retention, edema, hypertension and congestive heart failure (6). Side effects of ibuprofen include induction of exocrine liver dysfunction, thrombocytopenia, and a higher incidence of occult bleeding (7). Massive Naproxen overdose can present with serious toxicity including seizures, altered mental status, metabolic acidosis, and renal failure (1).

OBJECTIVES

This study investigated the effects of high doses of over-the-counter medications on the myocardial and hepatic tissues in mice. Mice were administered large doses of Ibuprofen or Naproxen over the course of six weeks, to mimic an individual exceeding the recommended dose, and then assessed to see how their overall health was affected. The following parameters were examined:

- Red and white blood cell count (WBC)
- Weight
- Appearance discrepancies (weight-related, hair, and skin-related)
- Neurological function and Behavior

Hypothesis: Mice receiving Ibuprofen and Naproxen will weigh less than control mice and have an elevated white blood cell count.

METHODOLOGY

In order to properly administer the medication, a saline mixture was made to produce the desired amount of medication needed for the experiment. The mice were then spun and grabbed to be able to administer the solution intraperitoneally into the underside of the mice. Mice were placed back into the cage to acclimate to their environment. There were 3 different groups (Group 1, Group 2, and Group 3) with 5 mice in each group. These 15 mice were split into 5 control mice, 5 Naproxen administered mice, and 5 Ibuprofen administered mice. The experimental mice in Group 1 and Group 2 were administered drugs 2 times per week and the experimental mice in Group 3, the control group, were not administered medication at all. A 500mL saline solution and medication mixture was made to safely administer each medication to the mice. The solution was 70% Saline solution and 30% medication.

Monday	Tuesday	Wednesday	Thursday	Friday
Ibuprofen: Weight Testing	Ibuprofen: Injection Day 1	Ibuprofen: Observation/ Cleaning	Ibuprofen: Injection Day 2	Ibuprofen: Neurological Maze
Naproxen: Weight Testing	Naproxen: Injection Day 1	Naproxen: Observation/ Cleaning	Naproxen: Injection Day 2	Naproxen: Neurological Maze
Control: Weight Testing	Control: No Injection	Control: Observation/ Cleaning	Control: No Injection	Control: Neurological Maze

Figure 1. Shows a weekly schedule or research timeline for each of the three groups of mice used in the experiment. Monday consisted of weighing the mice in all three groups. Tuesdays and Thursdays were injection days for both Ibuprofen and Naproxen mice. Wednesday was an observation and cage cleaning day for all three groups. On Fridays all three groups of mice underwent Neurological maze testing.

BLOOD SMEAR HISTOLOGY

Methodology: Spleen tissue was observed within 1 hour of organ dissection. The spleens of each group were pooled and then homogenized with a tube. The mixture was smeared onto a microscope slide and stained with Wright-Giemsa stain. Slides were dried and then visualized beneath a light microscope.

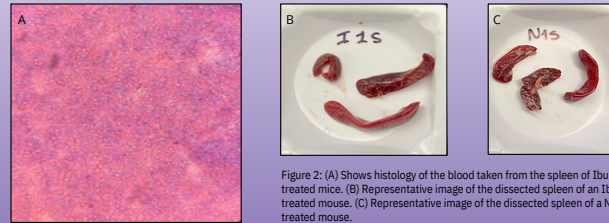


Figure 2: (A) Shows histology of the blood taken from the spleen of Ibuprofen treated mice. (B) Representative image of the dissected spleen of an Ibuprofen treated mouse. (C) Representative image of the dissected spleen of a Naproxen treated mouse.

BODY WEIGHT

Methodology: Small plastic containers were placed onto an analytical scaled and then zeroed out. Mice were individually placed into the container to be weighed and the number was recorded. Mice were weighed 5 times throughout the six week period (no weights were taken during the week of 2/6 - 2/10).

Weights of Mice						
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Control						
Mouse 1	19.18 g	21.59 g	24.79 g	25.26 g	25.26 g	25.26 g
Mouse 2	18.52 g	20.94 g	24.97 g	26.28 g	26.28 g	26.28 g
Mouse 3	16.10 g	21.88 g	24.44 g	26.88 g	26.88 g	26.88 g
Mouse 4	21.17 g	23.59 g	26.19 g	27.50 g	27.50 g	27.50 g
Mouse 5	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased
Ibuprofen						
Mouse 1	17.59 g	23.34 g	23.12 g	20.60 g	20.60 g	20.60 g
Mouse 2	15.89 g	17.12 g	21.96 g	19.20 g	19.20 g	19.20 g
Mouse 3	11.52 g	19.80 g	Deceased	Deceased	Deceased	Deceased
Mouse 4	12.28 g	14.85 g	22.25 g	24.35 g	24.35 g	24.35 g
Mouse 5	8.89 g	Deceased	Deceased	Deceased	Deceased	Deceased
Naproxen						
Mouse 1	15.26 g	Deceased	Deceased	Deceased	Deceased	Deceased
Mouse 2	21.04 g	20.35 g	24.36 g	22.70 g	22.70 g	22.70 g
Mouse 3	16.03 g	18.50 g	17.69 g	24.80 g	24.80 g	24.80 g
Mouse 4	13.14 g	Deceased	Deceased	Deceased	Deceased	Deceased
Mouse 5	18.14 g	20.02 g	18.89 g	26.00 g	26.00 g	26.00 g

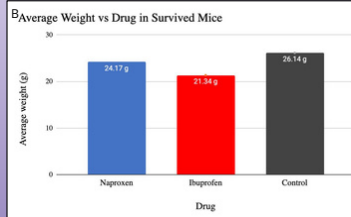


Figure 3: (A) Table shows the weekly weights recorded for each mouse used in the experiment over the course of six weeks. (B) Histogram shows the average body weight (grams) of survived mice from each group.

NEUROLOGICAL TESTING

Methodology: A wooden maze with an acrylic top was assembled with a short path for the mice to run. Mice were placed into a holding area at the beginning of the maze for a 30 second acclimation period. The barrier was lifted and the mice were released into the maze and timed to see how long it took for them to complete the maze. The timer was stopped when the mouse reached the end of the maze. Mice not completing the maze within 10 minutes were removed from the maze.

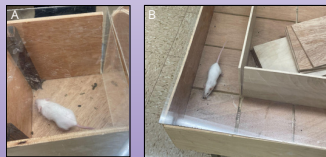
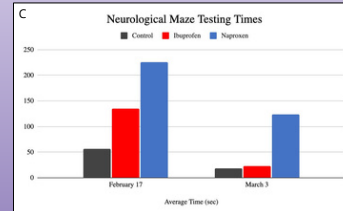


Figure 4: (A) Shows a mouse in the holding area for the 30 second acclimation period. (B) Shows an Ibuprofen mouse (♂) completing the maze. Histogram shows the average time in seconds for each group of mice to complete the maze. Mice were tested on two different dates. Naproxen treated mice took the longest time to complete the maze.



APPEARANCE DISCREPANCIES

Methodology: Small containers were placed onto an analytical balance and then zeroed out. Individual organs were then placed into the container to be weighed and the number recorded. The appearance of each mouse was noted before dissection.

Physical Characteristics of Mice	
Treatment Group	Appearance of Mouse
Control 1	Thin
Control 2	Normal
Control 3	Normal
Ibuprofen 1	Thin, Emaciated
Ibuprofen 2	Small growth on tail, Lesion growth on stomach
Ibuprofen 3	Thin hair, Large bladder
Naproxen 1	Normal
Naproxen 2	Normal
Naproxen 3	Broken tail end

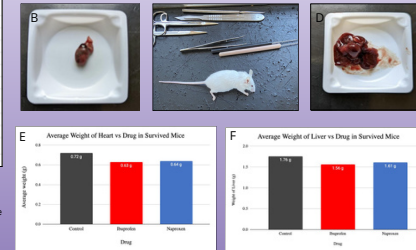


Figure 5: (A) Table shows characteristics noted from survived mice on dissection day. (B) Picture of heart from mouse. (C) Representative image of the mouse before dissection. (D) Picture of liver from mouse. (E) Histogram shows the average weight (grams) of hearts in survived mice from each group. (F) Histogram shows the average weight (grams) of livers in survived mice from each group.

DISCUSSION

The study's findings showed that the immune system, weight, neurological function, and appearance of internal organs and external features of the mice were affected by consistently high doses of Ibuprofen and Naproxen. As the weeks progressed, the experimental mice appeared more ill than the control group. There were similar weight loss and gain trends throughout the six weeks across the three groups. However, the experimental groups' average weights were lower than the control. A common side effect of NSAIDs such as Ibuprofen and Naproxen is severe indigestion and lower-bowel problems (5). The effects of GI problems and pain from the medications may explain the lower weights of experimental mice. The experimental mice, especially the Ibuprofen group, strongly desired water during the experiment, signaling they were dehydrated. Overconsumption of Ibuprofen may cause kidney impairment and dehydration (2). Injury to the kidney may generate swelling, edema, and skin abnormalities, which appeared in some experimental mice on the head, midsection, and tail. The study also showed spleen enlargement in experimental mice compared to control mice. An infection can cause splenomegaly that the mice may have been experiencing. Splenomegaly and a high WBC present in experimental mice suggest the mice's immune systems were fighting an infection before sacrifice. As the weeks progressed, experimental mice would quickly complete the maze run test, compared to earlier testing. There were also differences in the neurological function of the experimental mice versus the control group. On average, the Naproxen group took the longest to complete the maze. One of the main side effects of Naproxen is confusion and altered mental status. Not only did the medications impact the brain's function but the mice's mood and behavior. In both experimental groups, the mice were more aggressive and tried to bite and escape when they were handled. The control group was more docile and willing to be handled once moved to the larger enclosure. This suggests that the high levels of continuous medications do have an impact neurologically. Overall, this study provides evidence of the negative effects on the overall health of mice injected continuously with high doses of Ibuprofen and Naproxen.

REFERENCES

- [1] Al-Abri SA, Anderson IB, Pedram F, Colby JM, Olson KR. Massive naproxen overdose with serial serum levels. *J Med Toxicol*. 2015 Mar;11(1):102-5. doi: 10.1007/s13181-014-0396-1. PMID: 24756481; PMCID: PMC4371030.
- [2] Balestracci A, Esquer M, Elmo ME, Molini A, Torelli C, Torretti M, Toledo L. Ibuprofen-associated acute kidney injury in dehydrated children with acute gastroenteritis. *Pediatr Nephrol*. 2015 Oct;30(10):1873-8. doi: 10.1007/s00467-015-3105-7. Epub 2015 Apr 21. PMID: 25895445.
- [3] Ershad M, Ameer MA, Veerrier D. Ibuprofen Toxicity. Updated 2023 Feb 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK526078/>
- [4] Hong S. H., Spadaro, D., West, D., & Tak, S. H. (2005). Patient valuation of pharmacist services for self-care with OTC medications. *Journal of Clinical Pharmacy and Therapeutics*, 30(3), 193-199. <https://doi.org/10.1111/j.1365-2710.2005.00625.x>
- [5] Jones RH, Tait CL. Gastrointestinal side-effects of NSAIDs in the community. *Br J Clin Pract*. 1995 Mar-Apr;49(2):67-70. PMID: 7779646.
- [6] McCortegan P, Han P, Jones L, Whitaker D, Henry D. Selective COX-2 inhibitors, NSAIDs and congestive heart failure: differences between new and recurrent cases. *Br J Clin Pharmacol*. 2008 Jun;65(6):927-34. doi: 10.1111/j.1365-2125.2008.03121.x. Epub 2008 Apr 1. PMID: 18384444; PMCID: PMC2485215.
- [7] Mennander A, Järvinen O, Pajula J, Tarkka M. Long-term ibuprofen overdose may exacerbate the risk for acute hemorrhagic pericardial tamponade during myocardial infarction. *Interact Cardiovasc Thorac Surg*. 2003 Dec;2(4):529-31. doi: 10.1016/S1549-9293(03)00082-3. PMID: 17670113.
- [8] Takeuchi K. Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility. *World J Gastroenterol*. 2012 May 14;18(18):2147-60. doi: 10.3748/wjg.v18.i18.2147. PMID: 22611307; PMCID: PMC3351764.

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How High Doses of Tylenol and Aspirin Affect the Overall Health of Mice

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INTRODUCTION

Over-the-counter medications are used worldwide for daily pain management. Some well-known and commonly used over-the-counter medications include Tylenol, Aspirin, Aleve, and Ibuprofen. Based upon personal observations and a number of different studies, several people take more than the recommended daily dose of medication, which can lead to organ damage over time. More than 57% of health problems in the United States are treated with non-prescription drugs (2). Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used class of analgesic drugs, with approximately 30 million daily users worldwide and over 100 million prescriptions each year in the USA alone (1). Gastrointestinal (GI) toxicities from NSAID, both over-the-counter (OTC) and prescription, continue to be reported, increasingly in conjunction with cardiovascular, hepatic, and renal complications(3). More than 57% of health problems in the US are treated with non-prescription drugs (2). OTC pain medications and NSAIDs taken above the recommended dosage may lead to organ damage over time. Among all analgesic overdoses, 29% had used ibuprofen exclusively or combined with other analgesics, making ibuprofen the most common NSAID involved in overdose (3). Previous studies have shown significant adverse effects, such as heart failure, toxic hepatitis, and central nervous system depression when overusing these medicines.

OBJECTIVES

This study investigated the effects of high doses of over-the-counter medications on the myocardial and hepatic tissues in mice. Mice were administered large doses of Aspirin or Tylenol over the course of several weeks, to mimic an individual exceeding the recommended dose, and then assessed to see how their overall health was affected. The following parameters were examined:

- Body weight and physical appearance (hair, skin, tumors)
- Neurological changes (Maze Testing)
- Morphological changes in the heart and liver (weights, dimensions, appearance)

Hypothesis: Mice receiving Aspirin and Tylenol will weigh less than control mice and have smaller organs.

METHODOLOGY

In order to properly administer the medication, a saline mixture was made to produce the desired amount of medication needed for the experiment. The mice were then spun and grabbed to be able to administer the solution intraperitoneally into the underside of the mice. The mice were then placed back into the cage to acclimate back to their environment. There were 3 groups (Group 1, Group 2, and Group 3) with 5 mice in each group. These 15 mice were split into 5 control mice, 5 Tylenol administered mice, and 5 Aspirin administered mice. The experimental mice in Group 1 and Group 2 were administered drugs 2 times per week and the experimental mice in Group 3, the control group, were not administered medication at all. A 500mL saline solution and medication mixture was made to safely administer each medication to the mice. The solution was 70% Saline solution and 30% medications.

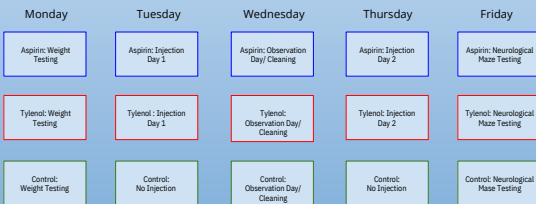


Figure 1. Shows a weekly schedule or research timeline for each of the three groups of mice being used in the experiment. Mondays consisted of weighing all three groups of mice. Tuesdays and Thursdays were injection days for the experimental Tylenol group and the experimental Aspirin group. Wednesdays were an observation and cage cleaning day for all three groups of mice. On Fridays all three groups of mice underwent Neurological maze testing.

TISSUE HISTOLOGY

Methodology: Spleen tissue was observed within 1 hour of organ dissection. Once the mice were dissected open, we removed the entire spleen from each mouse. Splens from each group were pooled and then homogenized with a tube and then placed onto a microscope slide. Slides were Gram stained and visualize the tissue.

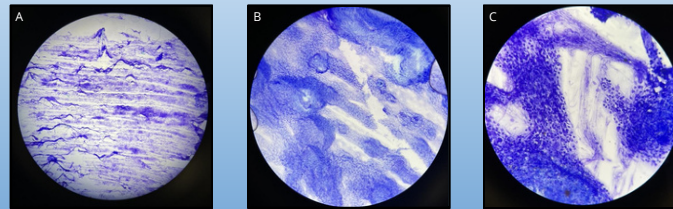


Figure 2. (A) Spleen tissue from Control mice stained with Gram stain. (B) Spleen tissue from Experimental Aspirin mice stained with Gram stain. (C) Spleen tissue from Experimental Tylenol mice stained with Gram stain.

NEUROLOGICAL TESTING

Methodology: A wooden maze with an acrylic top was assembled with a short path for the mice to run. Mice were placed into a holding area at the beginning of the maze for a five minute acclimation period. The barrier was lifted and the mice were released into the maze and timed to see how long it took for them to complete the maze. The timer was stopped when the mouse reached the end of the maze. Mice not completing the maze within 10 minutes were removed from the maze.

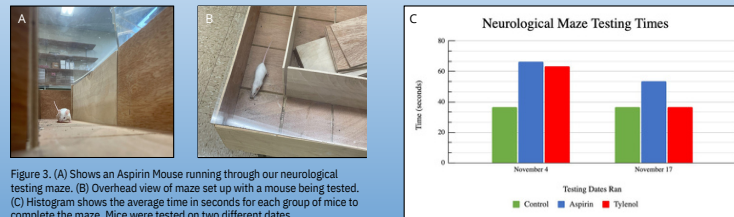


Figure 3. (A) Shows an Aspirin mouse running through our neurological testing maze. (B) Overhead view of maze set up with each group being tested. (C) Histogram shows the average time in seconds for each group of mice to complete the maze. Mice were tested on two different dates.

BODY WEIGHT

Methodology: Small plastic containers were placed onto an analytical scale and then zeroed out. Mice were individually placed into the container to be weighed and the number was recorded. Mice were weighed one time weekly, for a total of four weeks.

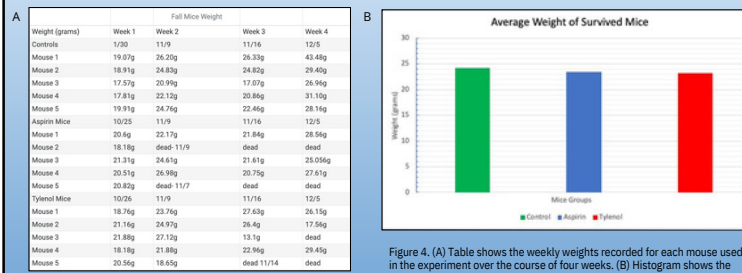


Figure 4. (A) Table shows the weekly weights recorded for each mouse used in the experiment over the course of four weeks. (B) Histogram shows the average body weight (grams) of survived mice from each group.

HEART AND LIVER MORPHOLOGY

Methodology: Small containers were placed onto an analytical balance and then zeroed out. Mice were then placed into the container to be weighed and the number recorded.

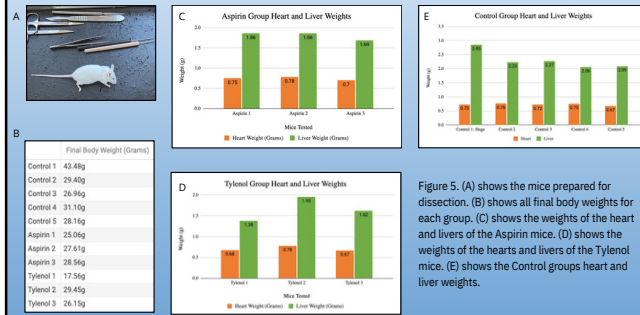


Figure 5. (A) shows the mice prepared for dissection. (B) shows all final body weights for each group. (C) shows the weights of the heart and livers of the Aspirin mice. (D) shows the weights of the hearts and livers of the Tylenol mice. (E) shows the Control groups heart and liver weights.

DISCUSSION

Our findings suggest that the excessive use of Tylenol and Aspirin negatively impacted the myocardial and hepatic tissues of experimental mice. This study also showed that the higher the dosage of medication the mice endured, the bigger the impact on the tests. Irregular feeding did not seem to impact the weight of the mice during the experiment. Both control and experimental mice exhibited a similar trend in weight gain and loss over the four-week experiment. As the weeks progressed, experimental mice would quickly complete the maze run test, compared to earlier testing. Conversely, control mice would take longer to complete the maze due to the expansion of their living quarters. During injections, the experimental Tylenol mice showed signs of skin abnormalities throughout the experiment, decreasing in size. Aspirin mice, however, had abnormalities that stayed the same or increased in size. In both groups, the experimental mice were more aggressive and tried to bite and escape when they were handled. The control mice were more docile and willing to be handled once they were housed in the larger enclosure. This suggests that chronic or continuous exposure to high doses of medication impact the heart and liver of mice, as well as their neurological abilities.

REFERENCES

- [1] Angiolillo, D. J., & Weisman, S. M. (2017). Clinical Pharmacology and Cardiovascular Safety of Naproxen. American journal of cardiovascular drugs : drugs, devices, and other interventions, 17(2), 97–107. <https://doi.org/10.1007/s40256-016-0200-5>
- [2] Hong, S. H., Spadaro, D., West, D., & Tak, S. H. (2005). Patient valuation of pharmacist services for self-care with OTC medications. Journal of Clinical Pharmacy and Therapeutics, 30(3), 193–199. <https://doi.org/10.1111/j.1365-2710.2005.00625.x>
- [3] Ershad M, Ameer MA, Veerrier D. Ibuprofen Toxicity. [Updated 2023 Feb 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK526078>

ACKNOWLEDGEMENTS

Thank you Dr. Mollie Sorrell and Dr. Sabrina Brown for helping us with the dissections, allowing us to use certain chemicals and for storing the organs after the experiment was concluded. Thank you Dr. Nathan Griggs for allowing us to use his lab space. I would also like to thank Lauren Criblez for feeding the mice. Thank you to the Department of Biology at Defiance College for funding the project.



Mental Health of Student Athletes: A Baseline Evaluation of Current Defiance College Student Athlete Mental Health

Student Researcher: Abbeigail Rank
Faculty Supervisor: Olivia Lozar, Ph.D.

Current Study: We are working with the coaches and student athletes on Defiance College's campus to collect data by survey. This data will provide a baseline of our student athletes' mental health which can lead to advocacy for athlete centered mental health resources.

Purpose: The purpose of this study is to assess Defiance College's student athletes' current state of mental health. We suspect that these student-athletes need more accessible and specialized mental health resources. The data from this study, pertaining to Defiance College student athletes' current state of mental health will provide us with the information necessary to advocate for these services. We aim to improve the mental health of student athletes at Defiance College and ensure that the specialized mental health resources are available to them.

THIS STUDY IS STILL IN PROGRESS

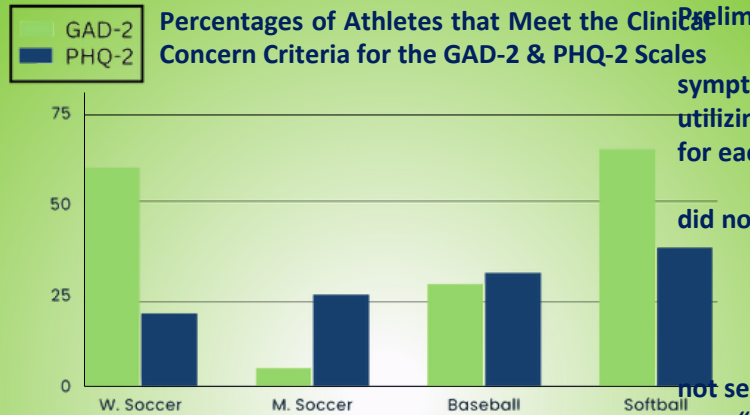
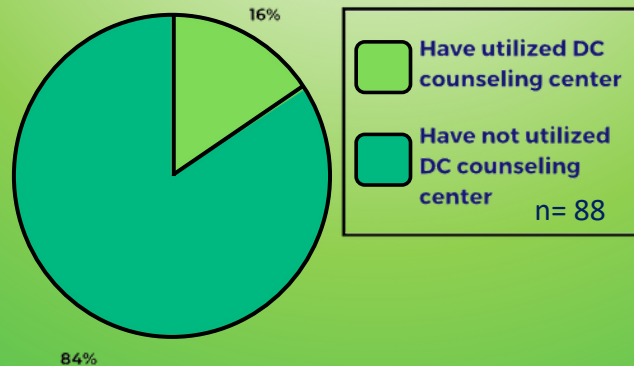


Figure 1: PHQ-2 & GAD-2 Scales: This graph gives a visual representation of individual athlete scores from sports teams that are considered a score of clinical concern (n=88). The GAD-2 measures generalized anxiety and the PHQ-2 measured depression levels. Both scales are composed of two questions which use responses that have point ranges from 1-3 and have a score of 3 as the clinical concern score.

Have you Utilized the Defiance College Counseling Center on Campus?



Preliminary Results: Although our research shows that athletes on campus may be experiencing symptoms of mental health issues, they are not utilizing the resources on campus. The reasoning for each person varied, but there are the general themes we observed. 25% of the surveyed athletes did not even know how to access our counseling services on campus and 40% of the student athletes participate in outside counseling services. It is important to note that 15% of the respondents did not know how to access counseling services and are not seeking outside help. One question we asked was, "why have you not used resources on campus?" Common themes observed were that they feel fear or embarrassment, there are limited counselors on campus to talk to, they don't feel like they (counselors) would understand, and they feel like they can handle it on their own or have other support to help them through the tough times.

Our goals are to:

- Continue collecting data among all sports teams
- Spread awareness for current counseling services on campus
- Raise awareness on the prevalence of mental health struggles in student athletes
- Work in coordination with the athletic department to provide training for coaches and staff to help with the day-to-day struggles
- Advocate for a sport psychologist to be hired on campus

Capture and Evaluation of Free-Floating Microplastics in the Upper Maumee Watershed

Autumn M. Saddler & Sabrina R. Brown

Division of Natural Science, Applied Science, & Mathematics, Defiance College, Defiance, OH

Background

The Upper Maumee watershed comprises the Maumee River and several of its tributaries, including the Auglaize and Tiffin Rivers (UMWP, 2022). The Maumee flows from Fort Wayne, Indiana, northeast towards Toledo, Ohio before it empties into Lake Erie. As such, the Maumee River watershed contains both heavily-farmed land and densely-populated industrial areas. Worldwide, plastic pollution is a major environmental issue. Microplastics resulting from the mechanical breakdown and improper production management are becoming an even larger problem that is seemingly going unnoticed because of their size. Microplastics are especially dangerous for many aquatic ecosystems because of the ease of their transfer (physically) and throughout the food chain.

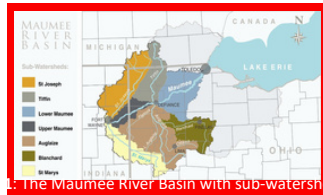
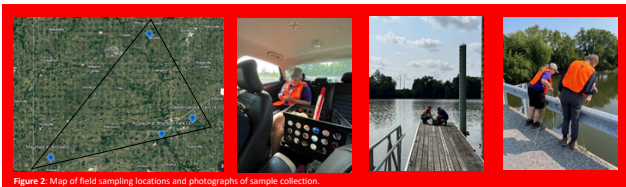


Figure 1: The Maumee River Basin with sub-watersheds

The purpose of this research is to assess microplastic contamination of the Upper Maumee River Watershed in and around Defiance, Ohio using free floating microplastic capture and quantification.

Methods



Four locations were sampled on September 15, 2022 in the Maumee River Watershed (Figure 2). Four samples per location were collected via a weighted plankton net. In the lab (Figure 3), samples were chemically digested and filtered using a sieve system to isolate microplastics for ease of identification and quantification per 100mL of sample. Microplastics were identified and verified using a hot needle. Plastic melts slightly and curls around the needle while other matter such as organics or rock pieces would not melt. Confirmed microplastics were tallied and recorded.



Figure 3: Photographs of field and laboratory sampling equipment.

Results

Examples of identified microplastics are shown in Figure 4. Concentration was calculated using microplastic particle counts for a 100 mL sample and recorded flow from nearby USGS gauging stations. Site 1 (the Tiffin at Stryker) had the highest concentration of microplastic pieces at around 124 pieces per 100 mL sample per (cubic meter per second) –1 unit of stream discharge. This is significantly higher than the other sites, which had averages of 10.00 particles/mL/m³/s (Site 2 –Maumee River Pre-Defiance), 21.65 particles/mL/m³/s (Site 3 – Auglaize River), and 3.94 particles/mL/m³/s (Site 4 –Maumee River Post-Defiance) per sample per unit of discharge. Considering this study only captured free floating microplastics, it is likely microplastics were also deposited in sediments along the riverbed.



Figure 4: Examples of hot needling and microplastics under a dissecting scope.

Table 1: Microplastic particle tallies, recorded flow, and microplastic concentration for each sample. Average concentration by sampling location is also provided.

Sample	particles / 100 mL	flow (cubic ft/sec)	flow (cubic m/sec)	conc. / 100 mL / cubic foot / sec	conc. / 100 mL / cubic meter / sec
1A	95	46.9	1.33	0.19	42.11
1B	40	696	19.95	0.01	3.29
1C	54	281	7.99	0.21	7.31
1D	95	1200	33.96	0.06	2
2A	183	46.9	1.33	3.9	137.89
2B	233	696	19.95	0.36	12.94
2C	201	281	7.99	0.77	27.2
2D	155	1200	33.96	0.13	4.90
3A	149	46.9	1.33	3.18	112.03
3B	252	696	19.95	0.36	13.96
3C	159	281	7.99	0.42	14.78
3D	179	1200	33.96	0.15	5.27
4A	271	46.9	1.33	6.78	203.76
4B	198	696	19.95	0.18	10.00
4C	270	281	7.99	1.08	37.38
4D	270	281	7.99	3.61	123.87
Avg 1				0.28	9
Avg 2				0.62	21.65
Avg 3				0.11	3.94

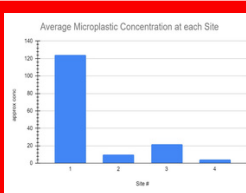


Figure 5: Average microplastic concentration by sampling location.

Figure 5 compares the average concentrations of free-floating microplastics per site. Averages were close among sites 2, 3, and 4. Site 1 had a significantly higher concentration of free-floating microplastics. This high concentration of particles corresponds with the lowest flow (1.33 m³/s) during the sampling period.

Discussion

Site 1 is on the Tiffin River near to the small town of Stryker, Ohio. The Tiffin River is a low order, slow flow stream that mostly travels through farmland. The highest concentration of free-floating microplastics were found here where flow and discharge were relatively slow. It is also possible that more resuspended sediment was collected in the plankton net samples at Site 1 due to the slower flow rate of the Tiffin. Other faster flowing sites may have microplastics more diluted or deposited in sediment, which is more difficult to capture with the methods deployed in this study. This indicates that we should further explore microplastic concentrations in the Maumee River Watershed, with a specific focus on collecting sediment samples in addition to water sample. Future studies would benefit from additional seasonal/weekly sampling or a sampling strategy focused on storm events.

Conclusion

This study aimed to assess the approximate concentration of free-floating microplastics in the Upper Maumee. This project utilizes methods for a low budget approximate free-floating microplastic concentration. This study provides preliminary data about free-floating microplastics in the Maumee Watershed and additional studies will need to be conducted to better understand the movement, timing, and amount of microplastics in the Maumee River. Evidence of microplastics, as determined from this study, is of particular concern for communities, like the City of Defiance, who rely on the Maumee River for drinking water. and the natural ecosystems of the area.

Acknowledgements

- Defiance College Summer Undergraduate Research
- Program Defiance College Department of Biology
- Field and Laboratory Assistance: Kaitlyn Smith
- Photo credit: Sabrina Brown and Kaitlyn Smith

References

- Surfriider Foundation Europe (Director). (2021, February 1). *How to sample & analyse microplastics in rivers*. <https://www.youtube.com/watch?v=jXFqwsHUq-4>
- *Upper Maumee Watershed Partnership*. (2022). <https://uppermaumeewatershed.org/>
- Wang, W., & Wang, J. (2018). Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TRAC Trends in Analytical Chemistry*, 108. <https://doi.org/10.1016/j.trac.2018.08.026>

DEFIANCE COLLEGE
STEM





Annual Assessment of Diatom Genera in Upper Maumee River Watershed Tributaries

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Division of Natural Science, Applied Science, & Mathematics, Defiance College, Defiance, Ohio, USA 43512



Background

- The Maumee River is the main water source in Defiance, OH
- Agricultural Upper Maumee watershed results in excess nutrients from field and stormwater runoff into tributaries and into the Maumee River
- Watershed monitoring and sampling helps predict when water quality may decline due to environmental events
- Diatoms are single-celled algae that contain a silica cell wall
- Diatoms are bioindicator species that can help assess water quality
- By comparing the numerical data from water sampling and diatom assemblages, the city of Defiance can learn more about the quality of the Maumee River and better understand the surrounding environment, the nutrients and amount of sediment in the water.

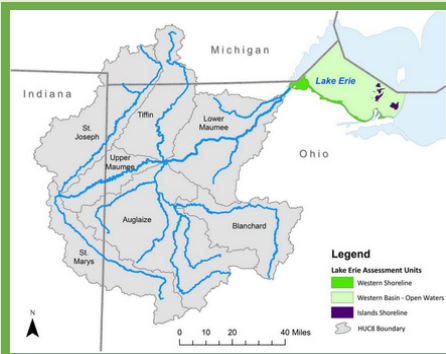


Figure 1: Map of the Upper Maumee Watershed

Methods

The City of Defiance collected weekly samples from mid-March to mid-November from four tributaries (Zuber, Cecil, Bend and Gordon Creek(313)) that flow into the Maumee River. Data was recorded on the weather and water conditions while sampling, along with data collected from a probe in the field. The Water Pollution Control analyzed a water sample in their lab after collection and another water sample was collected and brought to the Defiance College science laboratory for processing. In the lab at Defiance College, the sample settled for 24 hours and after was processed under a fume hood using H2O2 and a hot plate. After the samples were rinsed thrice, slides were made and mounted to be examined under a microscope to enumerate diatoms, to a count of 300.2

Results

Below in Figure 2 the cluster data splits the sampling season into two zones; Zone 1 is early spring into summer and Zone 2 is peak summer into the fall. Water temperature plays a role in this transition that happens during the peak summer months of July and August. When the water is warm, shallow plankton genera move into the streams and rivers, including *Cyclotella*, *Stephanodiscus* and *Aulacoseira*. In the fall when temperatures drop another transition occurs and ammonia decreases resulting in a rise of *Navicula*, *Nitzschia* and *Coconies*. The species that prefer cooler temperatures are present throughout the data but have higher abundances in the spring and fall as compared to the summer when the plankton species like *Cyclotella* are dominating.

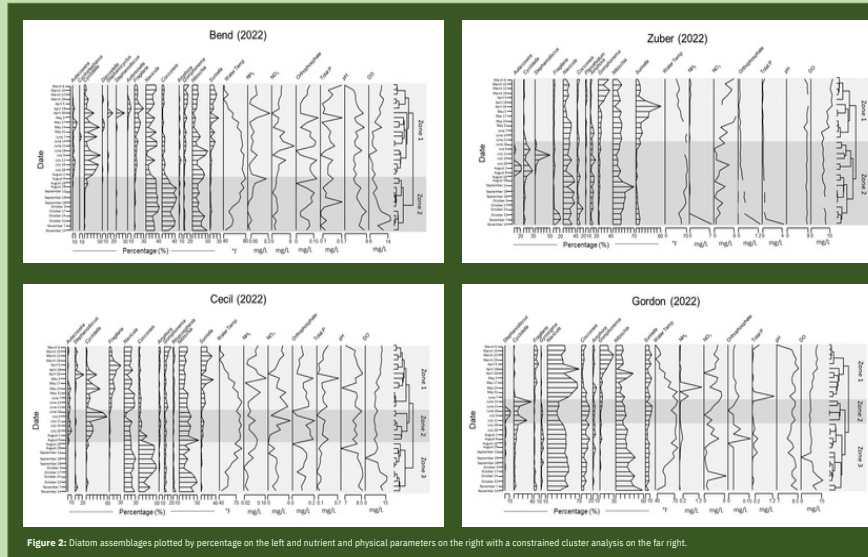


Figure 2: Diatom assemblages plotted by percentage on the left and nutrient and physical parameters on the right with a constrained cluster analysis on the far right.

In the early spring months of sampling, *Surirellais* consistently present in all four sites, however, it is more prominent in Zuber and Gordon (313). *Surirella* prefers a high DO (dissolved oxygen) while also following the trends of ammonia, nitrate, orthophosphate and phosphorus. *Gomphonema* also follows this trend by being present throughout the whole sampling season but is most abundant in the springtime, when DO is high and temperatures are low.

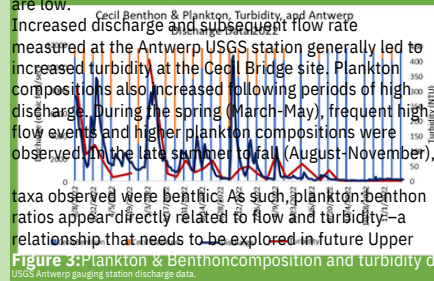


Figure 3: Plankton & benthon composition and turbidity data of the Maumee River at Cecil Bridge vs. Maumee research. USGS Antwerp gauging station discharge data.

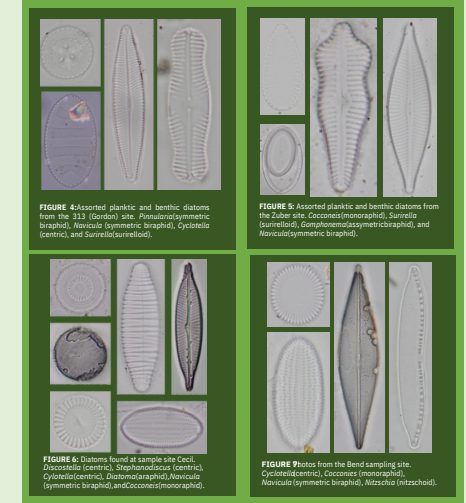


FIGURE 4: Assorted planktic and benthic diatoms from the 313 (Gordon) site. Pinnularid/symmetric biraphid), Navicula (symmetric biraphid), Cyclotella (centric), and Surirella (surirellid).

FIGURE 5: Assorted planktic and benthic diatoms from the Zuber site. Coconies (monoraphid), Surirella (surirellid), Gomphonema (symmetric biraphid), and Navicula (symmetric biraphid).

FIGURE 6: Diatoms found at sample site Cecil. Discofella (centric), Stephanodiscus (centric), Cyclotella (centric), Stephanodiscus (centric), Navicula (symmetric biraphid), and Coconies (monoraphid).

FIGURE 6: Photos from the Bend sampling site. Cyclotella (centric), Coconies (monoraphid), Navicula (symmetric biraphid), Nitzschia (Nitzschid).

Conclusions

After counting slides and compiling data, the results show a significant difference between the diatoms found in Zone 1, early spring into summer and Zone 2, summer into fall. In Zone 1 diatoms that prefer higher temperatures are abundant while diatoms in Zone 2 prefer cooler temperatures and a higher Dissolved Oxygen (DO). Species like *Gomphonema* and *Surirellia* were abundant early on but as the seasons changed centric diatoms like *Aulacoseira* and *Cyclotella* dominated. Comparing the data recorded by Water Pollution Control to the diatom counts has given a better data record of the tributaries that flow into the Maumee River. The data also provides a baseline of water quality for a whole sampling season, from March to December and will create a reference for future research and sampling.

Acknowledgements

Thanks to all the workers at the Defiance Water Treatment Plant and the Waste Water Treatment Plant, especially Kevin Connor, for collaborating and providing samples and data. This research was supported by grant funds through the Department of Education (P1162220107).



References

- Spaulding et al. 2022. Diatoms.org: supporting taxonomists, connecting communities. Diatom Research. DOI:10.1080/0269248X.2021.2006790.
- Battarbee W. 2003. Diatom analysis. In: *Handbook of Holocene paleoenvironmental reconstruction* (Ed. by B. Berglund & R. Rasmussen), pp. 527-570. Blackwell Press, Malden, NJ.
- J. Patrick Kociolek et al. 2015. Chapter 15 - Centric and Achnanthes Diatoms. Editor(s): John D. Warty, Robert G. Shaath, J. Patrick Kociolek, In: *Aquatic Ecology, Freshwater Algae of North America* (Second Edition), Academic Press, pp. 653-708. ISBN: 9780123858764. <https://doi.org/10.1016/B978-0-12-385876-4.00015-3>.

Raising Awareness of RED-S, Relative Energy Deficiency in Sports
Defiance College Exercise Science Program



Student Researcher: Brianna Snider
Faculty Supervisor: Olivia Lozar, Ph.D.

Background: RED-S, Relative Energy Deficiency in Sport, is a condition caused by energy deficiency that affects both male and female athletes. It is characterized by disordered eating, irregular menstruation, bone loss, low activity levels, and a decline in athletic performance. As a result of inadequate food intake coupled with excessive training, athletes have an energy imbalance that causes intense fatigue and decreased athletic performance. RED-S is an evolved diagnosis (2014) as it used to be called Female Athlete Triad. The evolution of the name developed as case studies showed males to present the same symptoms. It is important to bring awareness to this condition as the long-term effects of untreated RED-S can be detrimental to the athlete's health.

Purpose: To raise awareness of RED-S and provide coaches information on how to spot the signs and symptoms. This will allow coaches to spot RED-S quickly and help the athlete seek treatment. Early resolution of RED-S will allow the athletes to return to play and compete at their highest level.

Why it Matters and Treatment: RED-S matters because if it is left untreated it can impair reproductive health, bone health, and immunity to sickness. It is treated by an increase of dietary intake, a reduction in exercise, and energy-rich supplemental drinks.

Signs and Symptoms: Fatigue, rapid weight loss, missed periods or delayed puberty (females), low libido (males), hair loss, trouble focusing, trouble staying warm, and depression.

Findings: The article, "Knowledge of the female Athlete Triad and Relative Energy Deficiency in Sports Among Female Cross-Country Athletes and Support Staff", assessed knowledge on athletes, coach, and athletics trainers have on RED-S at the collegiate level. They had found that, "Knowledge, confidence, and educational impact score regarding Triad or Red-s were lowest in female cross-country athletes and highest in ATs" (Lodge pg.385). Everyone who was studied in this article was apart of NCAA athletics. In another article, females who participate in high impact sports were found to be more at risk for urinary incontinence which controls for low energy availability and menstrual dysfunction. The final finding was that in the research, "Markers of Low-iron Status are Associated with Female Athlete Triad Risk Factors", athletes who reported to compete in lean/endurance sports, 15.5% had low-iron status.

Take-Away Message: More education should be given to athletes so they can understand what RED-S is and catch it if they begin showing symptoms. Early detection and treatment is vital because if RED-S is left untreated, it can harm the athlete's development, mood, immunity, bone health, metabolism, cardiovascular function, and athletic performance. It is also necessary to talk more about urinary incontinence since it can be a sign of RED-S and may be beneficial to adjust how some contact sports are played. Finally, with low iron, it is necessary to educate athletes on the importance of eating a balanced diet and possibly creating a plan for safe eating habits to stay energized.

References:

1. Finn, E., Tenforde A., Fredericson, M., Golden, N., Carson, T., Karvonen-Gutierrez, C., & Carlson, J. (2021). Markers of Low-Iron Status Are Associated with Female Athlete Triad Risk Factors. *Medicine & Science in Sports & Exercise*, 53 (9), 1969-1974. DOI: 10.1249/MSS.0000000000002660
2. Whitney, K., Holtzman, B., Bauer, S., Maffazioli, G., Parziale, A., & Ackerman, K. (2021). Low energy availability and impact sport participation as risk factors for urinary incontinence in female athletes. *Journal of Pediatric Urology*, 17(3), 290-290. DOI: 10.1016/J.JPUROL.2021.01.041
3. Lodge, M., Ackerman, K., & Garay, J. (2022). Knowledge of the female Athlete Triad and Relative Energy Deficiency in Sports Among Female Cross Country Athletes and Support Staff. *Journal of Athletic Training*, 57(4), 385-392. DOI:10.4085/1062-6050-0175.21

Establishing a Long-Term Assessment Study on Water Quality of the Rivers Around Defiance, Ohio USA

Riley Alcorn, Lauren Criblez, Jordan Nighswander, Seth Pearson and Dr. Mollie R. Sorrell

Department of Biology, Defiance College, Defiance, OH

INTRODUCTION

The Maumee River begins in Fort Wayne, Indiana and flows through several large cities, including Defiance, Ohio, before draining into Lake Erie (Ohio EPA, 2014). The Maumee River watershed covers more than 6,500 mi² and two of its largest tributaries, the Tiffin and Auglaize Rivers enter the Maumee River in Defiance, Ohio (Ohio EPA, 2014). As such, the Maumee River serves as the main source of drinking water for the city of Defiance and is heavily impacted by human activities (Ohio Lake Erie Commission, 2015). Since water quality can impact human health as well as aquatic ecosystems, it is crucial to track changes in water quality over an extended period of time (Bridgeman et al., 2012). The purpose of this study is to establish a long-term assessment program of the water quality in the rivers around Defiance, Ohio using chemical analysis, streamflow, and bioassessment of golden-brown algae and macroinvertebrates.

SITE SELECTION

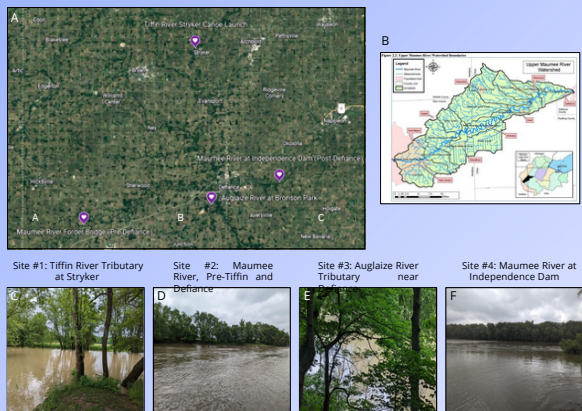


Figure 1. (A) Satellite image of study site with sampling locations indicated by purple hearts. (B) Map showing the boundaries of the Maumee River watershed. (C) Image of sampling site #1, the Tiffin River, a tributary of the Maumee, located in Stryker. (D) Image of sampling site #2, located on the Maumee River near Antwerp, before the confluence of the Tiffin and pre-Defiance. (E) Image of sampling site #3, tributary on the Auglaize River near Defiance. (F) Image of sampling site #4, along the Maumee River past the confluence of the Maumee and Auglaize Rivers, and downstream of the Independence Dam.

METHODS

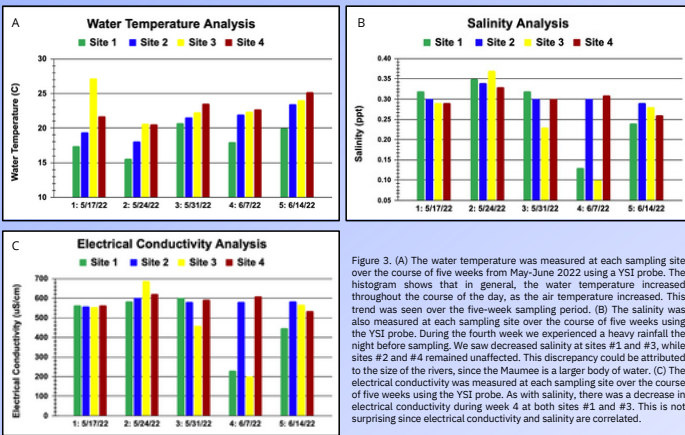
Four locations (Figure 1) were sampled from May-June 2022. Field tests were used to assess dissolved oxygen, electrical conductivity, salinity, temperature, and the turbidity of water at each site (Figure 2). Samples were collected and returned to the lab where chemical tests were used to assess nitrates and nitrites, phosphates, pH, ammonia, and alkalinity levels in the water from each site.



Figure 2. Field and Laboratory Work

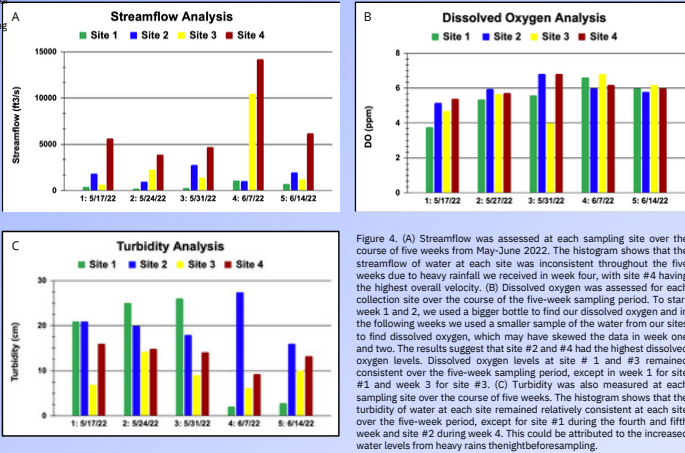
TEMPERATURE, SALINITY AND ELECTRICAL CONDUCTIVITY

Methodology: Water temperature, salinity, and electrical conductivity were measured and recorded at each sampling site over the course of five weeks using a calibrated YSI probe placed directly in the river.



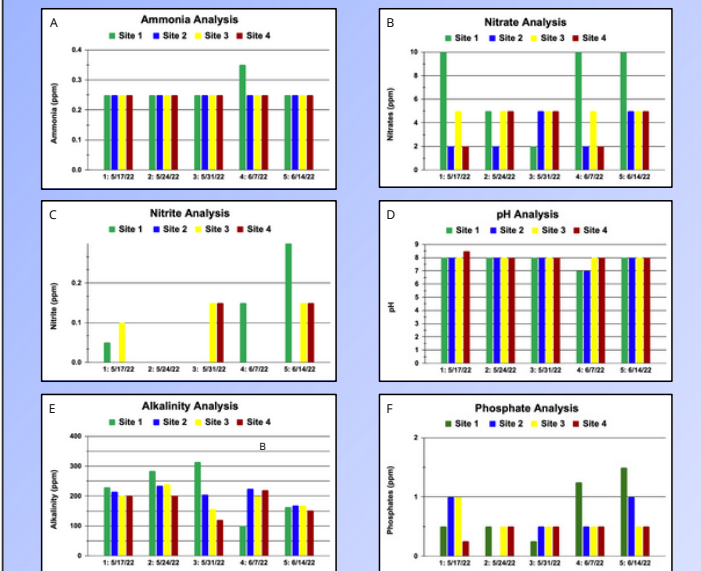
STREAMFLOW, TURBIDITY, AND DISSOLVED OXYGEN

Methodology: The streamflow of water was measured using the USGS website. A transparency tube was used to measure the turbidity of the water at each sampling site over the course of the five-week sampling period. Turbidity was impacted heavily by the amount of rainfall we received the night before sampling. Water samples were also collected at each sampling site for a dissolved oxygen analysis. Samples were fixed in the field, and then returned to the laboratory for chemical analysis.



CHEMICAL ANALYSES

Methodology: Water samples were collected at each sampling site and labeled by site number and date. Samples were returned to the laboratory and stored in a refrigerator until chemical analyses for ammonia, nitrates, nitrites, pH, alkalinity, and phosphate were performed the following day.



SUMMARY AND FUTURE DIRECTIONS

After collection and analysis of water samples over a five-week period we were able to establish a baseline for the chemical parameters of our four collection sites. This is an ongoing study focused on establishing a long-term assessment program of the water quality in the rivers around Defiance, Ohio using four major assessment tools: chemical analysis, streamflow, and the bioassessment of macroinvertebrates and diatoms. Our second five-week sampling period will begin in May 2023, and we hope to expand this study to include soil sampling and collection of macroinvertebrates at each of our collection sites.

REFERENCES

- Bridgeman et al. (2012). From River to Lake: Phosphorus Partitioning and Algal Community Compositional Changes in Western Lake Erie. *Journal of Great Lakes Research*, 38: 90-97.
- Ohio EPA (2014). Biological and Water Quality Study of the Maumee River and Auglaize River. epa.ohio.gov.
- Ohio EPA (2022). Maumee Watershed Nutrient TMDL Project. epa.ohio.gov
- Ohio Lake Erie Commission (2015). Western Lake Erie Tributary Water Monitoring Summary. lakeerie.ohio.gov.

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- Field and Laboratory Assistance: Soane Livingston, Michaela Hunt, Autumn Saddler, and Dr. Sabrina Brown
- Photo credit: Dr. Sabrina Brown and Dr. Mollie Sorrell



The Impact of Prescribed Fire on Native and Non-Native Vegetation: Marshall County, Indiana

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Background

Wildfires are considered natural disasters that occur when plants and the soil are too dry. This causes major damage to human health, the economy, and the environment. In California alone, citizens are seeing an overwhelming amount of wildfires and damage from them (Kramer et al, 2019). The wildland-urban interface –where wildlife and urban areas are starting to intermix and become increasingly more urbanized as years continue –is of particular importance for wildfire damage. Using fire management practices such as a controlled burn can decrease the damage that wildfires cause. Controlled burns have many benefits including decreasing the amount of debris on forest floors, destroying and stopping the spread of invasive plants, increasing biomass and density of species, increasing the amount of sunlight to smaller plants, and helping propagate plant seeds (Bottero et al. 2017).

The objective of this project is to determine whether or not using a controlled burning method in Northwestern Indiana would increase the overall health of a 4.04 acre plot. We hypothesize that prescribing a controlled burn to a specific piece of land will increase the native plant species biomass, decrease the amount of invasive species, and increase the amount of nutrients in the soil to create a healthier environment.

Methods

August 2nd, 2022

- Till the border of the burn site, creating a fire barrier
- Identify native and non-native plant species, determined to focus specifically on *Sericea lespedeza* (Chinese Bushclover)
- Let till area dry for five days

August 7th, 2022

- Call Marshall County Dispatch Center about large prescription burn start time and approximate end time
- Mix a 50/50 ratio of gas and diesel to a drip torch, ignite burn site
- Monitored the burn progress and take precautions to not ignite any other areas
- Call Marshall County Dispatch Center and inform them of the end time



Figure 1: Photographs from the burn on August 7th, 2022.

Results



Figure 2: Aerial photograph of the 4.04 acre burn area.

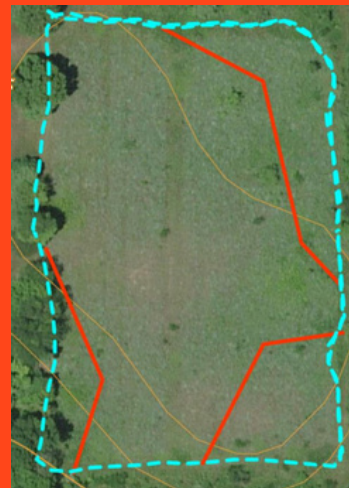


Figure 3: Aerial photograph of total area cover post burn on April 2nd, 2023.

The field was first observed on August 25, 2022. Majority of the undergrowth and plant debris had burned extremely well, and the healthy plants were still growing. It was obvious that the previous rain had helped keep the majority of the grasses alive. On September 27, 2022 the observation stayed quite similar to the previous. Lush, bright green patches of grass were now observable due to the burnt underbrush now being out of the way. However, *Sericea lespedeza* (Chinese Bushclover), an invasive species, was spotted in its new growth phase. There was very little observation of this plant across the 4.04 acres that was burned, not enough to be significant data. Similar observations were made on November 9th, however plant species were starting to dry out. On April 2, 2023 the last set of data was taken for this particular controlled burn. Due to unforeseen weather conditions and late freezing temperatures, plant shootings were not growing. So with the week of March 26th through April 2nd, 2023 there were warmer temperatures encouraging the plants to sprout. After observing the field, about 30% of the area was still covered in thorny shrubs, and 15% of the field was re-sprouting the Chinese Bushclover. The thorny shrubs are located in the Northeast and Southwest corner of the photo, and the Chinese Bushclover is located in the Southeast corner (Figure 3). Whereas the rest of the field had very little vegetation after the last freeze, noting that some of the plants may need a little bit of warmer weather.



after photos of the burn site on August 7th, 2022.

Conclusions

In conclusion, the prescription fire did the majority of what it needed to do. It decreased the total overall percentage of *Sericea lespedeza*, and re-opened essential habitat for native grasses. Unfortunately, the burn was not done in the correct time frame. Ideally, professionals prefer the late fall and early spring, when the majority of the plants are drying. The controlled burn that was done, happened in the middle of a wet season in Northern Indiana, contributing to the observation that the shrubs and Chinese Bushclover were re-sprouting. As stated before, the burn did open up critical habitat for native grassland species but more controlled fires would be needed in order to clear the shrubs and non-natives fully to rehabilitate the area.

Acknowledgements

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- Defiance College Biology Department
- Site Management: Jeff Umbaugh, Marc Eads, Hunter Stettner, Kyle Umbaugh, Terry Houin, and Jimmy Stoag
- Peers: Autumn Saddler

References

- Bottero, A., D'Amato, A. W., Palik, B. J., Kern, C. C., Bradford, J. B., & Scherer, S. S. (2017). Influence of Repeated Prescribed Fire on Tree Growth and Mortality in *Pinus resinosa* Forests, Northern Minnesota. *Forest Science*, 63(1), 94–100. <https://doi.org/10.5849/forsci.16-035>
- Kramer Heather Anu, MockrinMiranda H., Alexandre Patricia M., RadeloffVolker C. (2019) High wildfire damage in interface communities in California. *International Journal of Wildland Fire*28, 641-650.

Analyzing diatom communities across different coastal environments of Lake Erie

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Background

- Diatoms are eukaryotic, golden-brown algae that form intricate silica casings¹
- Diatoms can serve as bioindicators given species tolerance to chemical or physical stressors²
- Common pollution-tolerant diatoms observed in Lake Erie include *Aulacoseira granulata*, *Stephanodiscus binderanus*, and *Actinocyclus normanii*³
- Pollution sensitive diatom species include *Fragilaria crotonensis* and *Cyclotella comensis*³

Hypotheses

- More pollution-tolerant species will be abundant at T1 and T6 due to urban and agricultural land-use.
- There will be more pollution sensitive species and a more diverse community at T3 due to proximal coastal wetland habitat.
- Higher species diversity will be found at T3 due to proximal coastal wetland habitat.

Diatom Flora

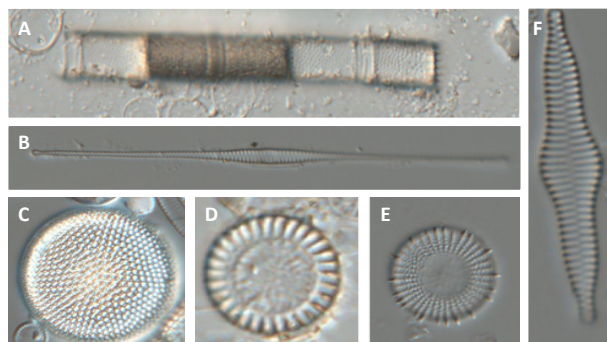


Figure 1. Diatom taxonomy from the Western Basin of Lake Erie. A. *Aulacoseira granulata*, B. *Fragilaria crotonensis*, C. *Actinocyclus normanii*, D. *Cyclotella meneghiniana*, E. *Stephanodiscus parvus*, & F. *Fragilaria polygonata*

Methods

- Sampled sites 1, 3, and 6 of the OSG coastal microbiomes project from the months of May, July, and September
- Collected diatom samples with bleached 10 micron tow net
- Digested samples with 30% H₂O₂, rinsed four times
- Made diatom slides with naphrax dissolved in toluene
- Identified to species level and counted to at least 300 valves with a 1000x light microscope

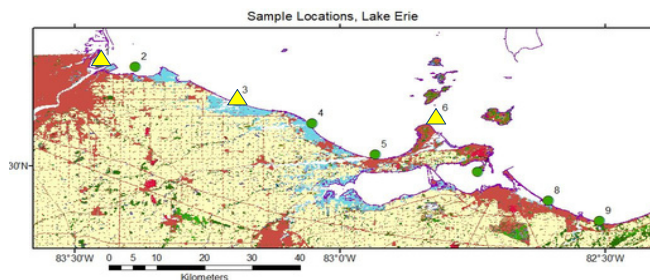
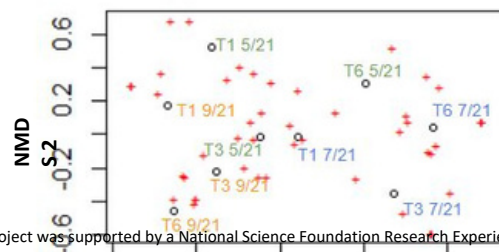


Figure 2. Ohio Sea Grant sites along the coast of the Western Basin of Lake Erie. Sites analyzed in this study are marked by a yellow triangle.

Results



This project was supported by a National Science Foundation Research Experience

Sustainable Solutions at the Lake Erie Land-Lake Interface (DBI-1852245) to the University of Toledo's Lake Erie Center, led by Dr. Jeanine Refsnyder and Dr. Kevin

NMDS1Czajkowski and the Ohio Sea Grant College Program subaward 60074861 led by Dr.

Figure 3. Nonmetric multidimensional scaling of samples and species. Spanbauer. We thank Rachel Lohner, Cameron McMillan, Garrett Moots, and Tom Bridgeman for logistical support.

REFERENCES 1. Spaulding et al. 2021. Diatoms.org: supporting taxonomists, connecting communities. *Diatom Research* 36(4): 291-304. 2. Lobo, E. A., Heinrich, C. G., Schuch, M., Wetzel, C. E., & Ector, L. (2016). Diatoms as bioindicators in rivers. *River algae*. (pp. 245-271). 3. Hazuková, V., Johansen, J. R., Sgro, G. V. (2019). Validation of a diatom-based index of water quality confirms its utility in monitoring of the Lake Erie's nearshore area. *Journal of Great Lakes Research*, 45(1).

Results Cont.

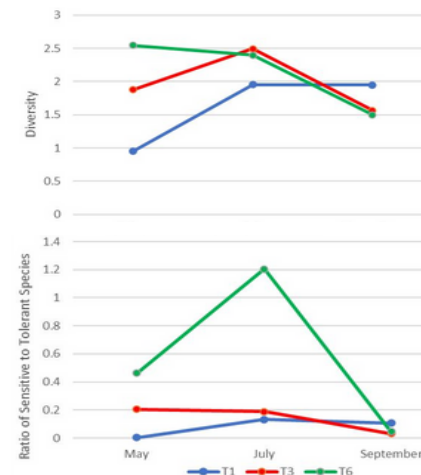


Figure 4. Upper panel, Shannon Diversity Index scores for each site. Lower panel, Absolute ratios of sensitive to tolerant diatom species.

Conclusion

- T6 had the highest average ratio of pollution-sensitive to pollution-tolerant diatom species
- T6 had the highest average species diversity
- NMDS shows clustering of all July 2021 communities

Acknowledgements

for Undergraduates Site Grant, Addressing Environmental Challenges and Proposing

Soil Analysis of Defiance College Campus and Athletic Fields

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Background

Turf needs about 16 different nutrients for good growth and development¹. Of these, nitrogen, phosphorus, and potassium are needed in high quantities (macronutrients)¹. These 3 nutrients are responsible for but are not limited to development, growth, and tolerances to many issues¹. Fertilizers are often used to improve the nutrient amounts.

pH affects the solubility of these nutrients in the soil. If the pH is too acidic, ammonification may occur which is harmful to plant life². If the pH is too alkaline, then it may contain more sodium than necessary and is also harmful to plant life³. A pH between 6.5-7.5 is considered neutral and is the aim of our study.

Lead is known as a carcinogenic substance⁴ that affects soil health and the health of those in contact with the contaminated soil⁵. Lead in soil is seen to prohibit growth from germination to transpiration and protein synthesis⁶. There are 12 different types of soil texture; each one has a unique water capacity, nutrient retention rate, and drainage capacity which affects plant growth⁷.

This project aims to conduct an overall assessment of soil health on the campus of Defiance College.

- Does the John Mansville plant impact soil pH on campus?
- What pH is more supportive of plant life?
- How do lead levels vary between athletic fields and the academic campus?
- Does the history of farming on the athletic fields impact the nitrogen levels found there compared to the academic campus?
- Are there any drastic differences between soil texture and color on the academic campus vs the athletic fields? Does this impact the other previous questions?

Methods

Students collected soil samples in a grid layout, obtaining one sample per square acre. A total of 26 samples were collected, 18 from the academic campus and 8 from the athletic fields, all within 24 hours and from approximately the same depth (8cm). Students tested soil pH, Nitrogen, Phosphorus, Potassium, Lead, color, and field texture which was corroborated by laboratory texture analysis.

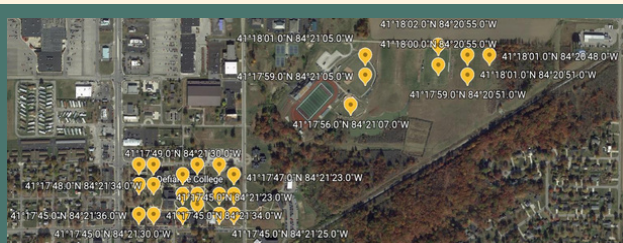
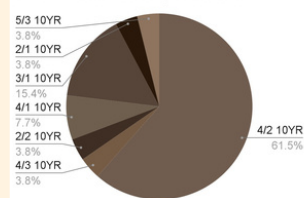


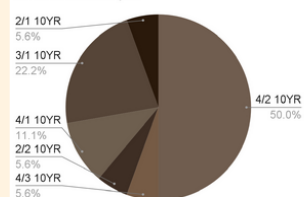
Figure 1: Map of sample locations and their coordinates on Defiance College Campus.

Results

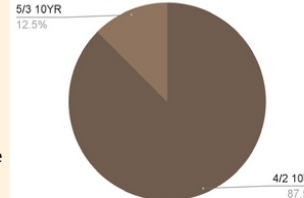
2a: Munsell Color Total Sample Assessment



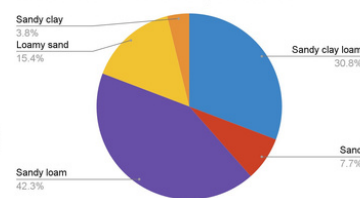
2b: Academic Campus



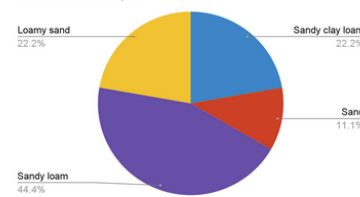
2c: Athletic Complex



3a: Count of Lab Texture Total Sample Assessment



3b: Academic Campus



3c: Athletic Complex

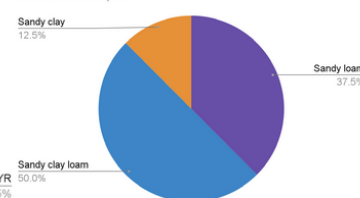


Figure 2a-c: Munsell soil color composition of a) total samples, b) academic campus samples, and c) athletic complex samples. Figure 3a-c: Laboratory texture compositions of a) total samples, b) academic campus samples, and c) athletic complex samples.

	Phosphorus	Nitrogen	pH
Academic Campus Average	13.33	30.42	7.22
Academic Campus Range	90	30	2
Athletic Fields Average	51.25	16.88	6.44
Athletic Field Range	40	90	1.5
Overall Average	25	26.25	6.981
Overall Range	90	90	2

Figure 4: Average sample quantity and range of phosphorus, nitrogen, and pH among total samples, academic campus samples, and athletic complex samples. Potassium and lead readings were not included in this figure because neither test detected substantial quantities of either element.

Discussion

Numerous limitations were presented by the study with one major flaw being that soil samples were not all collected on the same day or processed on the same day. Secondly, Potassium results were not specific, given that threshold had to exceed 100 lbs per acre in order to be quantified, leading all samples to be deemed as "trace" although having varying amounts of precipitate. Lastly, samples contained organic material which caused lab texture findings to be altered due to the presence of grass, roots, and other entities. In future studies, it is recommended that samples be obtained and processed in one day and collected from a depth which will not be impacted by organic materials. Also, tests of increased sensitivity would be of benefit.

Conclusions

- Using an unpaired t-test, this study determined statistical significance ($p < 0.01$) between increased proximity to John Mansville and soil acidity.
- Results portrayed campus has ample opportunity to support plant life with the average pH falling within optimum.
- There is no detectable amount of lead from samples obtained or difference in lead concentration between athletic fields and academic campus.
- There is an obvious relationship between athletic fields and higher Nitrogen content, however, further study is required to deem if this is actually a result of farming history or current fertilizer use.
- Drastically different texture results between academic and athletic complex, but color is mainly shared amongst.

Acknowledgements

- Terry Ranes, Defiance College Athletic Facilities Manager
- Funding provided by Defiance College Environmental Science Program

References

1. *Turfgrass fertilization: A basic guide for professional turfgrass managers*. Penn State Extension. (2016, November 10). Retrieved October 19, 2022, from <https://extension.psu.edu/turfgrass-fertilization-a-basic-guide-for-professional-turfgrass-managers>
2. Britto, D. T., & Kronzucker, H. J. (2002). NH_4^+ toxicity in higher plants: A critical review. *Journal of Plant Physiology*, 159(6), 567-584. <https://doi.org/10.1078/0176-1617-0774>
3. Patterson, S. (2021, July 6). *What is alkaline soil: Information and plants for Sweet Soil*. Gardening Know How. Retrieved October 21, 2022, from <https://www.gardeningknowhow.com/garden-how-to/soil-fertilizers/alkaline-soil-plants.htm>
4. *Lead -toxfaqs-agency for toxic substances and disease registry*. Lead -TOXFAQs. (2020, August). Retrieved October 21, 2022, from <https://www.atsdr.cdc.gov/toxfaqs/tfacts13.pdf>
5. Centers for Disease Control and Prevention. (2022, May 2). *Lead in soil*. Centers for Disease Control and Prevention. Retrieved October 21, 2022, from <https://www.cdc.gov/lead/prevention/sources/soil.html#:~:text=Lead%20contaminated%20soil%20continues%20to,of%20lead%20in%20the%20soil>
6. Pourrut, B., Shahid, M., Dumat, C., Winterton, P., & Pinelli, E. (2011). *Lead uptake, toxicity, and detoxification in plants*. *Reviews of Environmental Contamination and Toxicology*, 113-136. https://doi.org/10.1007/978-1-4419-9860-6_4
7. University of Hawaii. (2007). *Soil texture and soil structure*. Soil Management. Retrieved October 21, 2022, from https://www.ctahr.hawaii.edu/mauisoil/a_factor_ts.aspx
8. Queensland; (2013, September 24). *Soil ph*. Queensland Government. Retrieved October 21, 2022, from <https://www.qld.gov.au/environment/land/management/soil/soil-properties/ph-levels>



History of Nintendo

Arik Huffman
Defiance College
Dr. Buerk
HIST 350

Introduction

Founded by Fusajiro Yamauchi in 1889, Nintendo was originally a trading card company based in Kyoto. They sold hanafuda, which are a type of Japanese playing cards and continue to sell them in Japan to this day.

Unlike in 1889, Nintendo today is known worldwide for their gaming systems such as the Nintendo Switch, N64, Wii, and their massively popular games like Legend of Zelda, Mario, and Donkey Kong to name just a few. The company has grown exponentially since it was founded and has adapted to new technologies to flourish.

Formation

Fusajiro Yamauchi founded Nintendo in 1889. It was a small business that sold hanafuda playing cards in Kyoto, Japan. The business gained popularity in Japan, and eventually branched into other markets in the 1950's.

Adaptation

From 1889 to 1977, Nintendo was a playing card business selling hanafuda and other games. In 1959 they began to adapt to Western and worldwide markets. They began to sell hanafuda with Disney characters on them. However, after nearly 100 years of business Nintendo sold its first video game in Japan in 1977 called TV Game 15, and TV Game 6.

From this point Nintendo shifted into selling video games. In 1981 Nintendo of America Inc. was created, and they released the arcade game Donkey Kong in the same year. It wasn't until 1985 that they released the NES, or Famicom system as it was known as in Japan. The NES saw the release of many hit titles, such as Super Mario Bros and Legend of Zelda. The NES was a success, and as a result Nintendo expanded into the European market in 1986. A few years later in 1989 the Gameboy was released, which came with Tetris. Nintendo continued to innovate and in 1991, released the Super Nintendo, then in 1996 the Nintendo 64.

Starting as a small playing card business, Nintendo made a huge shift and has adapted from selling primarily cards and games, to selling game consoles and video games.

Hanafuda Cards

Pictured below are hanafuda, or "flower cards" in Japanese. Originating in Japan in the 1500's, there are several variations of the card game. In one game, Koi-Koi, the goal is to match cards faster than the opponent to gain a point. After 12 rounds, the person with the highest points wins.



Historical Context

During the late 19th and into the early 20th century, Japan went through a series of reforms and rapid modernization during the Meiji Restoration. The founding of Nintendo by Fusajiro Yamauchi fits right into this, as there were governmental reforms allowing for businesses and other markets to be created and flourish. Before Emperor Meiji, business and commerce was greatly restricted under the rule of the Shogun. In fact, merchants were the lowest social class under the rule of the Shogun, even lower than peasants.

1889
Founding of Nintendo
1933
Yamauchi Nintendo & Co.
1951
Company name changed to Nintendo Playing Card Co. Ltd.
1959
Sold cards with Walt Disney characters
1962
Listed stock on second Osaka Stock Exchange & Kyoto Stock Exchange
1963
Name changed to Nintendo Co., Ltd.
1963
Produced games & cards
1973
laser clay shooting system
1975
EVR videogame system sold in Japan
1977
First home videogames sold
1980
Nintendo of America Inc.
1981
Donkey Kong
1985
Famicom system, AKA the NES
1986
Expanded into European market
1989
Gameboy
1991
Super Nintendo
1996
Nintendo 64
1998
Release of Pokémon

References

"About Nintendo." Nintendo, n.d. <https://www.nintendo.com/about/>. "Nintendo History." Nintendo, 2016. <https://www.nintendo.co.uk/Hardware/Nintendo-History/Nintendo-History-625945.html>. Richert, Marcus. *KoikoiSet-Up 1*. n.d. https://upload.wikimedia.org/wikipedia/commons/thumb/1/15/Hanafuda_Koi-Koi_Setup.jpg/1280px-Hanafuda_Koi-Koi_Setup.jpg.