An Analysis of Disease-Causing Bacteria in Commercial Chicken Feed

Nat L. Shingler & Sabrina R. Brown

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

Background

Contamination of commercial chicken feed is common, whether it is the final product or simply an ingredient [1]. Most raw ingredients are stored in large, uncovered piles. These piles are easily accessible for animals like rats, mice, and birds. Each of these animals are known carriers of transmittable pathogens and can contaminate the feed through defecation. Due to this exposure making feed susceptible to be tainted, many within the community consider feed to be a fomite. Detection of the bacteria contaminating feed is extremely important, as many of the diseases caused by them are zoonotic (capable of being transmitted from animals to humans). Therefore, testing and analyzing chicken feed regularly is vital because the bacteria grown can quickly become lethal to poultry. Some of these bacteria are *Listeria monocytogenes, Salmonella, Escherichia coli,* and*Staphylococcus sp.*

Methods

Each of the commercial feeds was weighed (20 grams), soaked in deionized water, and then processed in a blender. Once blended the mixtures were plated on each of the four mediums using aseptic techniques. Samples were incubated at 37°C for 24-48 hours. Then, a sub-sample from each plate was gram stained. The presence, or lack thereof, of disease-causing bacteria was determined through an analysis of the stains and plates [2].

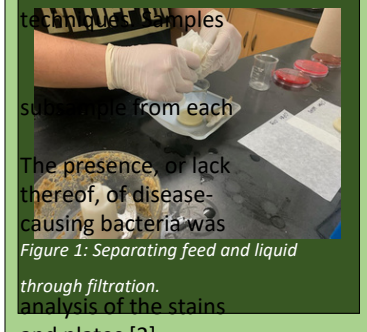


Figure 1: Separating feed and liquid through filtration.

Results

Over the course of six weeks, the commercial feed was processed and placed on four different media: MacConkey Agar, Blood Agar, Salmonella-Shigella Agar, and Mannitol Salt Agar. Each of the media was specifically chosen to grow certain bacteria. Each plating of feed on Mannitol Salt Agar produced growth of primarily yellow/white mucoid colonies with yellow zones. Salmonella-Shigella agar produced growth of a pink mucoid colony on the third plating of the DumorOrganic feed exposed to light during week six. Similarly, this feed was the only growth acquired for MacConkey agar during weeks two and three of the experiment, producing mucoid pink and rough cream colonies. Blood agar for each plating produced film-like rough white and yellow colonies with opaque black zones.

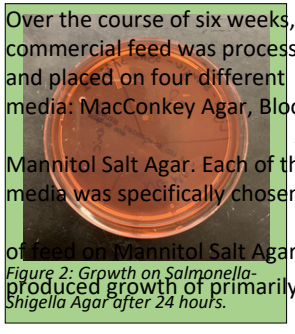


Figure 2: Growth on Salmonella-Shigella Agar after 24 hours.

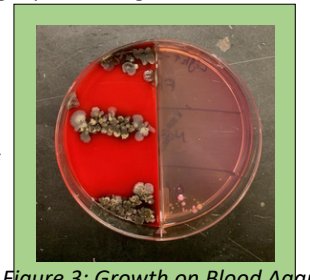
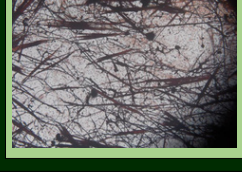


Figure 3: Growth on Blood Agar and MacConkey Agar after 24 hours.

Medium	Dates Plated	Feed Used	Storing Method	Potential Bacteria
Mannitol Salt Agar	2/22/22	Dumor Organic	Exposed to Light, Sealed	*Listeria monocytogenes*
Salmonella-Shigella Agar	3/2/22	Dumor NonGMO	Dark, Sealed	*Escherichia coli*
Blood Agar	3/23/22	Flock Party	Exposed to Light, Unsealed	*Staphylococcus sp.*
MacConkey Agar				*Salmonella*

Figure 4 (above): Table with basic information of the experiment conducted.

Figure 5 (below): Gram Stain of MacConkey Agar growth after 24 hours showing gram positive rods.



Conclusions

Upon analyzing the plates and the subsequent gram stains performed with the bacteria grown, tentative declarations of the bacteria present were made. Mannitol Salt Agar grew what is presumed to be *Staphylococci sp.,* both pathogenic and nonpathogenic. MacConkey Agar grew what is tentatively thought to be *Staphylococci sp., Aerobacter a.,* and *E. coli* . Blood Agar growth is presumed to be *E. coli* and *Staphylococci sp.* Salmonella-Shigella agar growth is thought to be *E. coli* or *Enterobacter a.* Confirmation of these early assumptions will be made using additional analysis of the gram stains made from the bacterial growth.

In conclusion, each feed was indeed contaminated, but were found to have relatively the same level of contamination. Results determined that the Dumor Organic feed kept sealed, but with access to light was the only feed that had any changes to it. This is most likely due to the fact it was the feed with the rawest materials and that over the course of six weeks the storage conditions affected the feed itself.

Acknowledgements

My fellow senior capstone peers, Katelyn Smith and Brett Moravec. The Defiance College Molecular Biology Department for providing funding for this project.

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Diatom-Inferred Seasonal Fluctuations in a Rural Pond, Defiance Co. Ohio

Katelyn A. Smith & Sabrina R. Brown

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

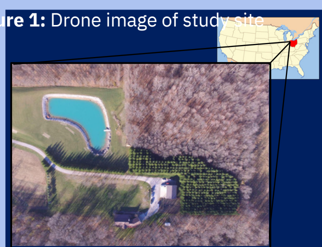
Background

Diatoms are a small, single celled algae that contain a silica cell wall. There are many different species of diatoms and other algae that can be found floating in the top layer, middle layer, or dwelling near the bottom of water bodies. For an organism to effectively be used as a biological water quality indicator, it must be sensitive to changes in both the abiotic and biotic factors in an environment and the responses they make should be predictable, which allows for researchers to make reliable inferences.[1] Identifying different diatom species can indicate the nutrients present (for example: nitrogen), the amount of sediment, and the overall quality of the water.2

Methods

The Smith's recreational pond (Figure 1) is a young pond (~3 years old) in rural Northwest Ohio. The pond gets treated regularly with copper sulfate and coloring. Pond water samples were collected every week from June to December using a plankton net.

Figure 1: Drone image of study site



Weather and pond conditions were recorded during each collection. The samples were kept refrigerated until processed in the lab using hydrogen peroxide. Processed samples were mounted on slides and were examined under the microscope to enumerate diatoms.

Figure 2: Field/Laboratory Work - Above are pictures of the pond during collection and counting diatoms on the microscope.

Results

FIGURE 3: Planktic and Benthic diatoms species (from left to right): *Discostella* (centric), *Actinocyclus* (centric), *Fragilaria* (araphid), *Navicula* (symmetric biraphid), and *Epithemia* (epithemioid).

Several diatom species were found and counted, the total number of valves can be found in Figure 7. Copper-sulfate crystals were also found (Figure 8). Copper sulfate is a treatment used in recreational ponds to control the amount of algae and diatoms in the water by killing them.3 In the graph, the lowest bars are associated with regular copper-sulfate treatments.

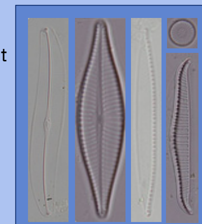


FIGURE 4: *Gyrosigma* (symmetric biraphid), *Navicula* (symmetric biraphid), *Nitzschia* (nitzchioid), *Discostella* (centric), and *Epithemia* (epithemioid).

The pond was treated with copper-sulfate three times during the collection period. These treatments coincide with the low presence of diatoms (July 13, September 22, November 3).

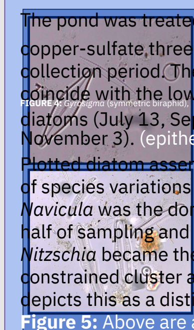


Figure 5: Above are assorted planktic and benthic diatoms from the Smith's recreational pond.

Plotted diatom assemblages (Figure 6) give an overview of species variations throughout sampling months. *Navicula* was the dominant genus (>45%) during the first half of sampling and near the end (November, December) *Nitzschia* became the dominant genus (55-95%). The constrained cluster analysis on the right of the figure depicts this as a distinct assemblage. The bottom cluster, containing a high abundance of *Nitzschia* and *Epithemia* is significantly different than the top cluster, which is dominated by *Navicula* and *Discostella.*

This large transition is likely the result of water input from a garden hose, Jul 07 Jul 21 which flowed into Aug 05 the pond from Aug 18 July 19 to Sep 01 October 25. *Navicula* prefer flowing water, Oct 06 Oct 20 while *Nitzschia* Nov 03 prefer standing w2Nov 17 ater. This transition could Percentagealso be related to a change in benthic habitat.2

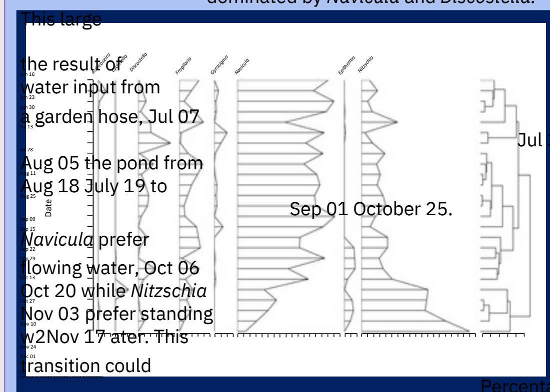


Figure 6: Diatom assemblages plotted by percentage, with a constrained cluster analysis on the right.

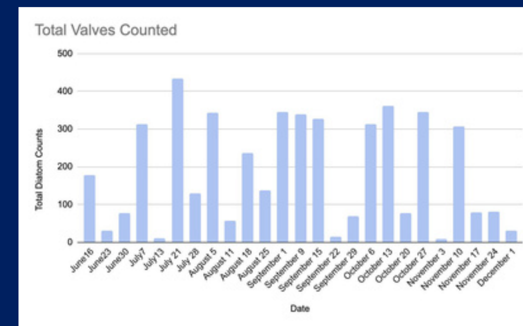
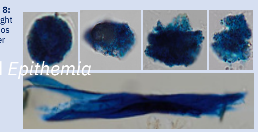


FIGURE 7: Total valves counted per slide by date

FIGURE 8: To the right are photos of copper sulfate crystals



Conclusions

After counting slides and compiling data, results show a significant difference between the diatoms found consistently throughout summer and early fall (July-October) versus the diatoms found in late fall (November and December). The diatoms found in summer through early fall were *Navicula* and *Discostella* and the diatoms found in late fall were *Nitzschia* and *Epithemia*. The assemblages at the end of collection reflects that the pond was full and flooding into the grass in some areas, where certain species prefer to dwell rather than in pond water. Copper sulfate was proven to be an effective treatment in killing off algae, including diatoms, in the pond. Counting diatom abundance would be a necessary addition to quantifying the impact copper sulfate had on the system.

Acknowledgements

I would like to thank my fellow Senior Capstone Peers, Nat Shingler and Brett Moravec. I would like to acknowledge the Defiance College Environmental Science Department for providing the funding for this project.

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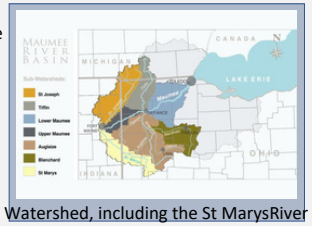
Reducing Nutrient Pollution in the Maumee River Watershed using Floating Wetland Platforms and Native Plant Species

Brett Moravec & Sabrina R. Brown

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

Eutrophication:

Eutrophication is the process of a water system acquiring high concentrations of nutrients over time. While this process normally takes centuries [4], it has been drastically accelerated to just a few decades in watersheds impacted by nutrient pollution in waterways through poor agricultural practices and industrial and urban dumping/runoff [1]. Anthropogenic eutrophication causes hazardous algal blooms that create dead-zones and deadly cyanotoxins that destroy local habitats [7]. The toxin produced by this alga causes severe illness, liver damage, and even death in humans and other organisms.



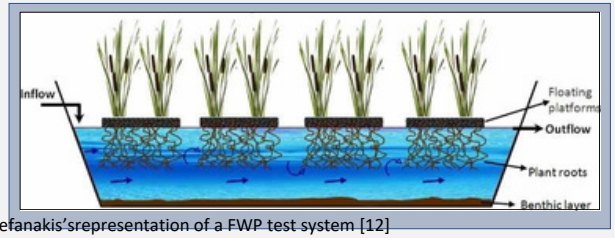
Eutrophication of Lake Erie is a problem that affects the tri-state (Ohio, Michigan, and Indiana) region. In 2011, Lake Erie experienced its largest harmful algal bloom on record; covering about 2,000 square miles of the lake in toxic blue-green *Microcystis*algae. The St. Marys River, which flows fromMaumee Watershed, including the St MarysRiver [5] Shelby County in Ohio northwesterly to meet the St. Joseph River in Fort Wayne, Indiana, is a tributary of the Maumee River and covers an area of roughly 795 sq. mi (508,618 acre). Elevated levels of nitrogen and phosphorus in the St. MarysRiver have been documented since 1996 [10]. Probable sources of contamination identified by the EPA are outflows of Combined Sewer Overflows (CSO's), non-point sources from agriculture fertilizer and livestock waste, and unspecified urban stormwater drainage into the river. According to a 2015 survey, the St. Marysexceeds the quality benchmarks by 65%-95%, depending on season [3].

Floating Wetland Platforms:

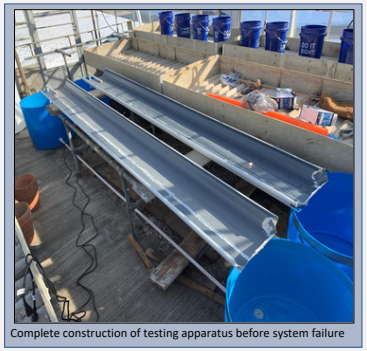
Floating Wetland Platforms (FWPs) are floating planters that allow plants to grow on top of the water and increase the amount of nutrients drawn out of the water by allowing their roots to grow out of the bottom. FWPs act as an artificial wetland to increase ecosystem services in areas that have had their wetlands degraded or destroyed. Aquatic and wetland plants can help remove nutrients from a water body and store them in their roots and stems [11]. Rush species (*Juncaceae*) have been shown to decrease the nitrogen levels in water systems while cattail species (*Typha*) have been shown to be better at reducing phosphorus concentrations [9][6]. Creating FWPs with a combination of these two species of plants have the potential to significantly reduce the nutrient load of the St. MarysRiver and, thus, help decrease the eutrophication of the St. Marysand connected watersheds.

Methods:

Two mock streams were constructed in the Defiance College greenhouse, one for the control and one for the treatment. The design of the stream was based on the design of C. Ryeret al.'s artificial stream forStefanakis'srepresentation of a FWP test system [12]



lotic invertebrates [8] and H. Carrick et al.'s affordable artificial streams [2]. Each river is an independent system that has its own reservoir, water pump, and plumbing. The channels were constructed from 5x120 inch rain gutters that had their inner wall bent down and flattened to create a plane. Two gutters were then sealed together using silicone to create a steam bed of 10 inches and a bank of 5 inches at a 45oangle. The downstream end of each river system was bent up in order to create a pool of water in order to create depth to the river system, and was supported by the frames of grow beds in the greenhouse. The downstream and upstream water reservoirs were constructed from a 50 gallon plastic drum. The two reservoirs were connected with ½ inch PVC tubing and sealed into the bottom of one downstream reservoir and the side of upstream reservoir. The downstream reservoir was elevated on large planting pots in order to facilitate gravity feeding from the downstream to upstream side. A small fountain pump attached to a PVC arm was inserted into the upstream reservoir in order to pump water up and into the stream system.



Complete construction of testing apparatus before system failure

Results:

The mock stream system was unable to function properly after 4 weeks of construction and time to complete the experiment had expired. The system failed to drain the downstream water reservoir back to the upstream reservoir fast enough in order to make sure the pumps did not run dry. After several attempts to correct the issue, a catastrophic failure occurred in the system and one of the river beds fell into the downstream reservoir and broke the plumbing underneath. This failure unfortunately halted the progress of the experiment.

Discussion:

Floating Wetland platforms can have many different designs, which should be selected to compliment the environment in which they will be utilized. The St. MarysRiver can have drastic fluctuations in flow rate and depth over the course of a year. Future designs for an FWP should take into account this variance and consider reinforcing the upstream side to protect it from damage via the large woody debris that is often present.

Future Research:

Further refinement of the mock river system would allow for simulation of the river condition in and around Defiance, which could create a valuable tool for testing the implementation of FWPs and other hypotheses related to river conditions. Increasing the diameter of the return pipe from the downstream to upstream reservoir is one improvement that was discovered by this experiment. Other improvements could be made to the stream bed including the addition of sediment to more accurately mimic the condition in a real stream, the use of local river water collected from a sampling site to test current river conditions, or even the addition of a bank and floodplain to the system to increase the amount of variables the system could simulate.

Additional research into mitigation techniques for river eutrophication should be expanded upon, especially the use of native plants and FWPs. Additional native plant species to test would include *Scirpoides holoschoenus*(Bulrush), *Schoenoplectusacutus*(Hard Stem Bulrush), *Schoenoplectustabernaemontani*(Soft Stem Bulrush), *Carexstenoptila* (Riverbank Sedge), *Carexlacustris*(Lake Bank Sedge), *Pontederia cordata* (Pickerel Weed), *Iris versicolor* (Blue Flag Iris), *Hibiscus moscheutos* (Swamp Rose Mallow), *Justicia americana* (Water Willow), *Peltandra virginica* (Arrow Arum), and *Sagittariacuneata* (Arrowhead). Maximizing phosphate and nitrate uptake while also increasing carbon storage capabilities by growing more plants on a river would help address the concerns of anthropogenic climate change.

Acknowledgments:

Defiance College Environmental Science Department, Senior Capstone Peers Katelyn Smith & Nat Shingler, KayeCJones & Eric Ummelat the Fort Wayne Parks and Recreations Department

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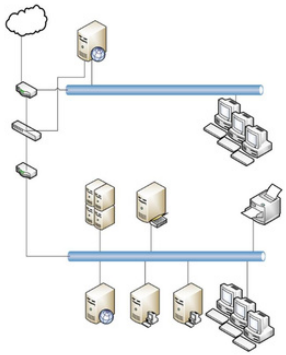
David Andrew
Virtualizing the Lab
Professor Naparíala

Introduction

Goal -The goal of this project is to create a virtual environment to help in the teaching of the cybersecurity minor and allow for students to use virtual environments for tasks that may seem dangerous or unwieldy for a normal machine.

Preparation-Some of the tasks completed before the project started was setting up the two servers (Dell PowerEdge R640). First Professor Naparíala and the IT department installed the two servers in Defiance Hall making sure to connect them to the network going to Dana Hall. The network cable going to Dana Hall is connected to a switch at the other end which has all the computers and servers in Dana connected allowing for communication between them. Below is a graphic showing how it is set up currently starting from Defiance Hall (the cloud) going to the switch and the PC's located in Dana 29.



Once the servers were set up, VMware ESXi was installed on them allowing for virtual environments to be deployed. This was key for setting up the virtual computers and labs that can be used in future classes.

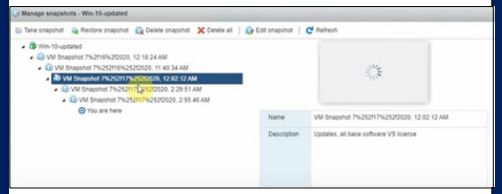
Key Terms

Virtual Machine-System created through software instead through a physical machine. Used to emulate a physical machine.
Snapshots-A way to backup and restore virtual machines. They take the current virtual machine and store all its properties to be used for a later time.
MultiCasting-A quicker way to send data to multiple devices. Instead of sending data to each device individually. Data is sent to all devices at the same time allowing for quicker transmission through eliminating the repetition of the data transfer.
ISO-A single file that represents an entire DVD or CD. Used to store useful tools that may be needed on many virtual machines.

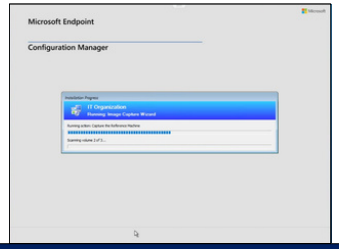
Updating

The task assigned to me was the updating of the lab computers and this is where the bulk of my research and work went into. While this may seem simple to do as computer updates are pretty easy to do. This becomes complicated when there are 20 computers. While feasible to update each individual computer it is also a good learning experience to learn the ways that major corporations do updates.

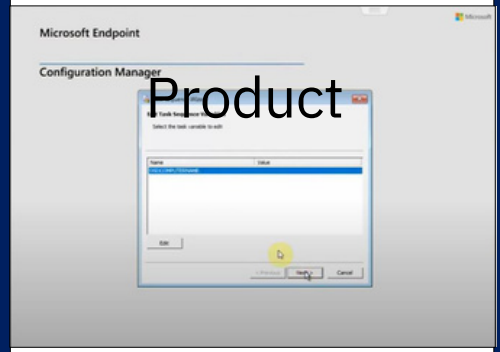
Step 1 -Snapshots:To start out you need to get a virtual machine to a point of where you want the lab computers at. So, if you want the lab computers to just have Windows 10 then there is not much work to do. However, for our lab environment we wanted specific things added to the Windows 10 machine I.E Arduino 1.8.15, Wireshark 3.4.7, Visual Studio 16.10.4, etc... To do so I started from a base snapshot of Windows 10 and did Windows updates and added the programs. While doing so I would take a snapshot every so often just incase a certain program or update that I did broke the virtual machine. However, there was no problems that I ran across doing the snapshots. Below is a picture of the old snapshots before I started work. The green arrow on the Win-10-updated representing what point the virtual machine is currently at (base Windows 10).



Step 2 -SCCM: SCCM (System Center Configuration Manager) is a management software used to manage large groups of computers running many different operating systems. SCCM is key to the distribution of our master snapshot. However, first we must upload our snapshot to the SCCM server. To do so we mount an ISO to the virtual machine that has the image capture wizard and enter the destination of your SCCM servers images and a .wim file will be created there. Once the installation progress finishes you add the .wim file to Operating System Images under the Software Library making sure to put it into a distribution point. Below is a picture of the Installation Progress for turning the snapshot into a image.



Step 3 -Deployment: This step is the easiest of the steps, however, it is the most tedious. Once we get the image onto our distribution point I went into the lab and started work on re imaging the computers. To do so I needed to restart all 18 computers and go into boot options (f12) and go into a network boot. Type in the administrator password and select the correct image name. "win 10 updated FA21" for our lab. Once that is done just name the computer and do it 17 more times. After a few hours each computer will have all the updates and applications we put onto the snapshot from step 1. Taking only a few hours is actually quite fast to deploy Windows 10 onto a machine and especially over network to many machines. The way we sped up the process was using multicasting with the SCCM server sending the information once but that information goes to all 18 computers at once rather than each computer making a connection to the server. There was a few problems with two computers losing internet connection, however, I just restarted the process for those and got them going. Once all the lab computers were completed I logged onto each one of them adding the chrome remote desktop extension and setting it up for each computer to allow remote access to the lab.



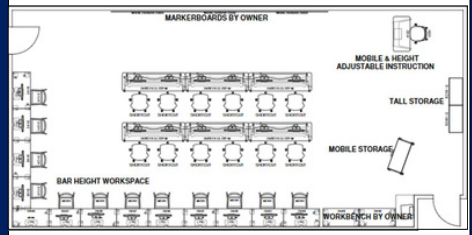
Experiences

One of the bigger problems that came from the project was during the image capturing process. I briefly remember Professor Naparíala mentioning the user profile causing problems with the capture, however, I completely forgot to do anything about it. This caused problems with the image as it did not want to capture. To fix the problem you need to remove all user profiles under systems properties as you want the user profiles to be generated on the machines you deploy the image onto. Once the user profiles are deleted you then want to do the image capture without pressing the windows start menu as the start menu is what creates the user profiles. This could be avoided if you never press the windows start menu button for the entire snapshot step but that is quite hard to do.

Product

The finished product is 18 lab computers with windows updates done and programs added for the fall and spring semesters. Plus on top of that the cyber-range was finished allowing for all computers to remote into a virtual environment, even the lab computers, for testing and access of machines we may not normally have access to I.E Unix machines.

Future plans for the cyber-range are still in place as currently we are not using it at its fullest capacity. Our plans over the summer were thwarted due to shipping. One of the plans was swapping out the old switches with Ubiquiti switches, however, they did not show up till the very end of the summer. Plus, we did not have any of the computers or furniture for Dana 28 which is what the cyber-range was built for as Dana 28 is the actual lab for it. The current plan is to take the computers out of Dana 29 and put them in the new lab as they will be older and slower. Since cybersecurity will only be using the computers to connect to the cyber-range they don't need much processing power. Then, the newer faster computers will be installed in Dana 29. Below is the planned setup for Dana 28.



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