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Evaluation and Modeling of the Different Material Properties of the Captive and Wild Diets of Carnivores

Jill den Outer

Bethany Janos

Elizabeth McCourt

William Molair

Director of Thesis: Dr. Abdel Bayoumi
Second Reader: Hope Holt

migalemechanica@gmail.com

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1. Introduction

1.1 Problem Definition

The black-footed ferret (BFF) is a carnivorous animal belonging to the Mustela family. BFFs once widely populated the North American Midwest region until the early 20th century. The species was driven to near-extinction as the population of their natural prey species, the prairie dog, declined due to overhunting. Additionally, the ferrets had a natural susceptibility to plague (Yersinia pestis) and canine distemper that caused further decline of the population. While the combination of these factors led to what many believed to be the extinction of the ferrets, a small population was found in Wyoming in 1981. A recovery effort was started in the late 1980s in an attempt to increase and stabilize the population[1].

With much of the BFF population housed in zoos and other captive facilities, a suitable diet had to be created that would match the nutritional requirements of the ferrets' wild diet. At the beginning of the Ferret Recovery Program a mink pellet-based diet, dubbed the 40/60 diet, was fed to most of the captive species. While this diet was regularly supplemented with hamsters and other small mammals, the nutritional value of the 40/60 diet was ultimately considered to be insufficient when compared to BFFs natural diet. In hopes of creating a diet that would meet the nutritional requirements of captive carnivorous animals, the Toronto Zoo diet was created and implemented in the early 2000s[1].

The Toronto Zoo diet is a ground horse-based diet supplemented with additional nutrients to better meet the nutritional needs of captive carnivores. While the Toronto Zoo diet is considered to be a better nutritional equivalent to the BFFs natural diet, concerns have been raised over the mechanical structure of the diet. Many zoos fear that introducing whole bones and tissues into captive carnivores' diets could impose a choking risk, so most food is kept simply as ground meat[4].

The soft nature of a ground meat-based diet compared to the natural diet of ferrets and other carnivorous species has been correlated with oral health problems such as calculus buildup and development of periodontal diseases[1]. Additionally, there is evidence to suggest that the soft captive diets might be related to cranial degradation among captive carnivores, which remains a large concern[2].

Specific to captive BFFs, the most significant changes in cranial morphology are related to an increase in zygomatic arch width. The zygomatic arch region is the area along which the masseter muscle (a critical muscle of mastication for carnivores) is stretched[3]. Mechanical stability of muscular tissues has been shown to be closely related to the structural integrity of the surrounding bones. In mice, mechanical unloading of the hind legs has been shown to induce muscular atrophy, which led to increased porosity and fragmentation in the femur and tibia[4]. As less force is required for a captive ferret to fully masticate softer diets, researchers have hypothesized that the relative "mechanical unloading" has led to an underdevelopment of the masseter muscle, which has in turn caused cranial morphological changes such as widening of the zygomatic arch[3].

1.2 Goals/Objectives

1.2.1. Goal 1: Make a Physical BFF Jaw

Objective 1: Produce a CAD image within 0.003 inches of the original cast.
The NextEngine 3D scanner allows control over how accurate the scan will be, and 0.003 inches is a fair estimate of the software. Any discrepancies will be analyzed to ensure accuracy. Whether the CAD image is used for the cast or not, it is necessary to be accurate since it will be used as a scale for other components of the project.

Objective 2: Build a physical model of the teeth within 10% error of the original cast.
Two methods will be used to create a cast for the teeth, both digital and material to ensure the most realistic one will be used for casting, as the sharpness of the teeth must reflect that of an actual ferret.

1.2.2. Goal 2: Evaluate the properties of BFF diets

Objective 1: Utilizing a force gauge, determine the amount of force needed for a BFF to fully masticate a prairie dog and the zoo diet.
A force gauge will allow for a constant upward rate when replicating BFF mastication while also outputting force against displacement, therefore making it the optimum solution. However, if a force gauge cannot be acquired a force sensor will still give force outputs.

Objective 2: Based on data provided by Dr. Hartstone-Rose determine if a significant difference in nutritional value exists between the soft zoo diet and the wild diet.
Comparing the nutritional properties of each diet will allow exclusion of nutritional properties as a source of muscle degradation. Therefore, if a determination of significance can be made solely based on mechanical properties this leads to the assumption that mechanical differences are responsible for muscle degradation and subsequent bone loss.

Objective 3: A two-sample t-test will be performed to determine whether there is a significant difference between the forces required to masticate the wild diet versus the captive diet and if there is a significant difference between the nutritional data of the wild and captive diets.
Using a p-value of 0.05, it can be determined whether there is a significant difference in observed mechanical quantities and nutritional data between captive and wild diets.

1.2.3. Goal 3: Predict bone degradation based off of a decreased bite force

Objective 1: Determine a correlation between the amount of force decreased and the amount of muscle unloading using current studies in small mammals.
Studies have evaluated muscle unloading in small mammals, such as mice or rats, however no evaluations have been done on BFFs. Therefore, it will be necessary to utilize the studies and corresponding methods that determined the values to gauge unique values for the BFF.

Objective 2: Determine a correlation between the amount of muscle unloading and the amount of bone degradation using current studies in small mammals.
Again, studies have shown this property in other small mammals, which will need to be applied to this project. Using the results from the previous objective, it will be possible to find a relationship

between muscle unloading, which will be determined by the bite force, and the occurrence of bone degradation.

2. Design

To determine whether the lack of mechanical similarities between captive and wild diets is the cause for the observed cranial morphological changes in captive BFFs, a procedure has been designed to evaluate the force required for a BFF to fully masticate various wild and captive diets that are available. There were initially two possible solutions to this problem: a physical model or a computational model. It was ultimately decided to create a physical model, as the properties of food are far too complex to feasibly replicate in computational form.

To physically model the BFF jaw, a metal, such as aluminum or silver, would be most ideal since they would be durable and easier to come across. However, the difficulty of replicating the complex and small geometry of the BFF jaw was not anticipated. This led to preparing two possible methods for creating the jaw replica. The first involved scanning a replica jaw and refining the scan using Geomagic. The second involved creating a physical mold of the jaw. It was determined that the CAD jaw will be acceptable for this project in terms of obtaining the necessary geometry, but the backup will be available if needed.

The jaws will then be applied to a force gauge to determine the mastication force of a BFF consuming various diets. Pairing this data with values in literature relating musculature unloading to bone degradation, it can be determined whether the mechanical differences of these foods are what is contributing to the bone degradation of not only captive BFFs, but other captive carnivores as well.

3. Methodology

3.1 Jaw Replica

First, a replica BFF jaw was scanned into the NextEngine 3D Surface Scanner to create a CAD image of the skull. The top of the skull was set on a platform covered in a dark cloth. The room was dark to ensure no artifacts were captured. The software was set to a 360° positioning, versus bracket or single positioning. It was set to perform 8 divisions, at a resolution of 10K points/in^2, which was indicative of a standard definition scan. These settings were ideal for a reasonable scan time that would operate within the processing capabilities of the computer. The target was set to “light” rather than “neutral” or “dark” to capture the light color of the skull against a dark background. The range was set to “macro” which allowed the skull to be an ideal distance of 6.5 inches from the scanner. Multiple scans were performed and aligned with one another to ensure an accurate image. This was then repeated with the bottom skull.

Post-processing was done using Geomagic software, as the scans had several shadows and artifacts that needed to be fixed. First, a general operation known as “Mesh Doctor” was run to improve the overall mesh quality. Next, the sandpaper tool was used to correct areas of mesh defects that were problematic for the general smoothing program. Finally, holes were filled and mesh defects on the teeth were removed to make the model more representative of the actual cast. After the post-processing the CAD design will be sent to Xometry, a 3D printing company, to be printed in aluminum.

In the case that the digital model of the BFF jaw does not work, a physical mold will be created of the BFF skull replica using molding putty, wax, and plaster. Both the top and bottom jaws of the BFF skull would be pressed into respective balls of putty. Next, wax will be melted and poured into the mold. Once this cast is formed, it will be surrounded by plaster. The plaster will then be subjected to heat after it hardened to melt the wax, creating a hollow mold. This mold will then be used to create aluminum casts of the jaw.

3.2 Force Analysis

Force analysis was performed using a Bose ElectroForce Mechanical Test Instrument which could measure up to 225 N of force. To achieve a graph displaying the force applied vs displacement, the load cell unit was raised with the food inside to almost the height of the moving arm. Next, a waveform compression was applied with a loading rate of 0.1 mm/s and an axial force of zero. The Instron stops compression upon fracture of the diets. Data from the compression tests were recorded in .csv files for later analysis.

Initial testing was performed using a flat compression plate to gain an understanding of the force necessary to crush several types of diets. In future testing, the BFF skull model will be connected to the machine to analyze how the sharp teeth of the BFF impact the force necessary to masticate the several diet types.

"Full mastication" will have different definitions depending on the diet type. For the wild diet, mice and rats will be dissected to isolate bones and tissue. Bones will be broken within the machine and tissue will be broken up into a particle size of 0.5 inches. An entire mouse and rat will also be placed into the machine to analyze the force required for the whole prey. For a captive diet, pellets will be broken into smaller particles and soft food will be formed into a cylinder and compressed.

3.3 Diet Comparison

Multiple captive diets and wild diets will be considered during testing. For the captive diets, pellets of multiple sizes and ground meat will be tested to encompass multiple food options. While the typical wild diet of the BFF is prairie dog, there is unfortunately extensive protection for the species, preventing any possible testing. Although to a lesser extent, smaller rodents are a part of the BFF diets and will be tested in place of prairie dogs. These multiple diets will be compared using a one-way ANOVA test with a p-value of less than 0.05 to find whether there is a significant difference between diets and, if found, a post-hoc test will be performed to determine the degree of those differences.

3.4 Nutritional Comparison

In conjunction with the force comparison, the nutritional values of the captive diets and wild diet will be compared. This comparison is just to ensure that the only difference between the diets are the mechanical properties, therefore allowing one to confidently correlate the cranial deformations to solely the mechanical properties. The nutritional data will be analyzed utilizing a one-way ANOVA, followed by a post-hoc test.

3.5 Correlated Muscular Atrophy and Bone Degradation
If the hypothesis is confirmed that the captive diet requires a significantly decreased bite force than that of the wild diet, then a correlation between the decreased bite force and muscular atrophy will be determined by utilizing the data and consulting literature that relates muscular atrophy to subsequent bone degradation.

4. Results

To replicate the BFF's bite force, a physical jaw model is required. Figure D shows the post-processed image of the BFF skull. The scan from the NextEngine 3D scanner had many artifacts, making post-processing with Geomagic necessary to fill any holes. Additionally, the scanner was unable to capture the complex sharp tooth geometry necessary for testing. Post-processing was not successful in correcting this and would need to be addressed after printing.

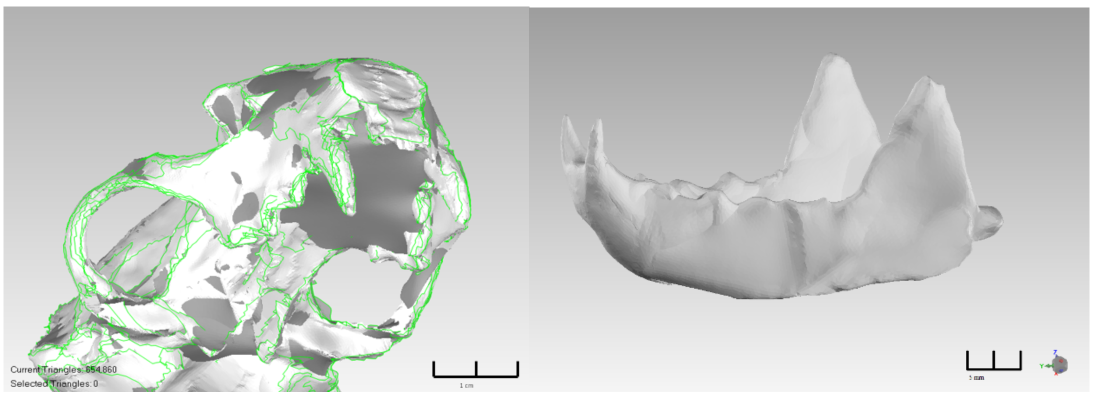

Figure 1. Final jaw models after correction. This figure shows the upper (left) and lower (right) jaws, along with the scale, which were used for printing.

Using a Bose ElectroForce force gauge with compression plates and a 25 N load cell, the captive diets of the BFF were analyzed by a compression test. The force found by compressing soft diets to 50% or hard diets until breakage is shown in figure 2.

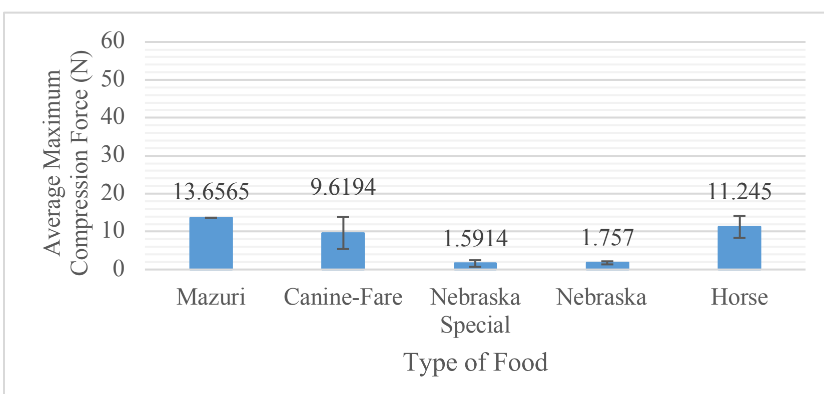

Figure 2. Average maximum compression force for each captive diet. These are compared to the BFF's maximum bite force, 48 N, indicated by the dotted line.

The Mazuri diet, consisting of pellets, was compressed until fracture. Samples of Canine-Fare, Nebraska Special Beef Feline diet (Nebraska Special), Nebraska Chopped Frozen Feline diet (Nebraska), and horse tenderloin (Horse) were compressed roughly 50% and did not contain a breakage point. Ten trials were performed for the Mazuri and Canine-Fare diets and five trials were performed for the rest.

A one-way ANOVA was performed to compare the average maximum compression force for each diet. A Tukey test was used as the post hoc test and found significant differences, with a p-value of 0.05, between each food except; the horse tenderloin and the Canine-Fare, the Mazuri diet and the horse tenderloin, and the Nebraska Special and the Nebraska Chopped diets. The results of this test can be seen in Table 1.

Table 1. One-way ANOVA comparing different captive diets. With a $p < 0.05$, this shows that significant differences are present in different captive diets.

Captive Diet Compared	P-value
Horse – Canine Fare	0.4345
Mazuri – Canine Fare	0.0028
Nebraska – Canine Fare	0.0008
Nebraska Special – Canine Fare	0.0006
Mazuri – Horse	0.5184
Nebraska – Horse	0.00007
Nebraska Special – Horse	0.00005
Nebraska – Mazuri	0.0000001
Nebraska Special – Mazuri	0.0000001

Rat femurs and tibias were tested with a three-point bend test. This test applied the force to the center of the bone while being supported at the edges. A maximum force of 25 N was applied to the bones. Unfortunately, the extra part on the top plate caused the Instron to max out around 13 N, even after zeroing out the plates. Figure 3 and Figure 4 show the data obtained from this testing.

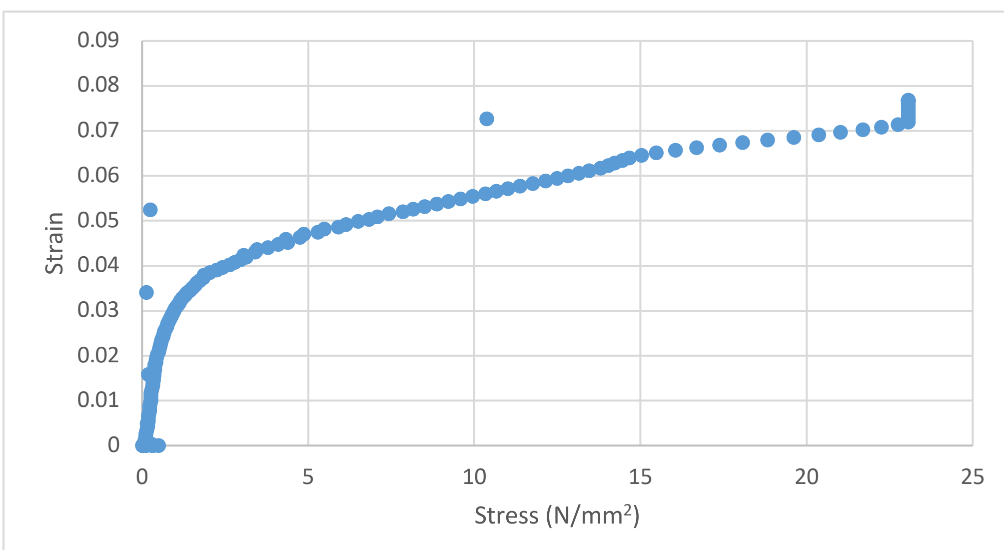

Figure 3. Stress vs strain of a three-point bend test for a single rat.

Five rat tibias and five rat femurs were tested. Out of these, four tibias and four femurs gave a stress-strain plot comparable to Figure 3. Utilizing the stress-strain plot up until the yield point, shown in Figure 4, the stiffness can be determined via Young's Modulus, which is the slope on the displayed trendline. The Young's Modulus for all eight specimens are shown in Table 2.

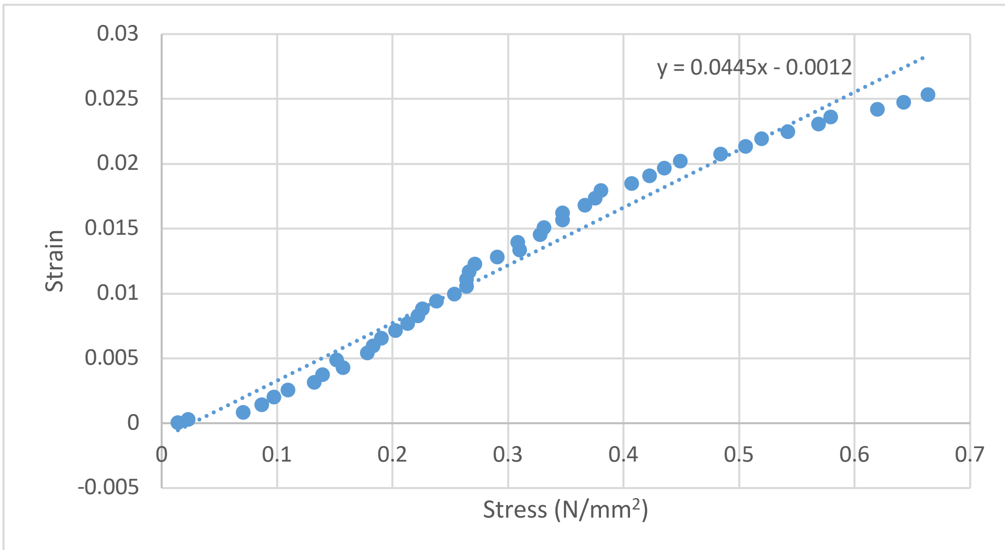

Figure 4. Stress versus strain until the yield point of the three-point bend test shown in Figure 3.

Table 2. Young's Modulus for each successful three-point bend test. This data was used to find the stiffness of rat bones.

	Rat 3 (Tibia)	Rat 4 (Femur)	Rat 5 (Tibia)	Rat 6 (Femur)	Rat 7 (Tibia)	Rat 8 (Femur)	Rat 9 (Tibia)	Rat 19 (Femur)
Young's Modulus (mPa)	0.0204	0.0112	0.0002	0.0221	0.0046	0.0034	0.0634	0.6635

To confirm that the nutritional content of captive diets do not negatively affect captive BFF health, nutritional data of 90 prairie dogs were analyzed, resulting in the average percent of protein, fat, and moisture contents. These results were compared to the breakdown of four captive diets: Mazuri, Canine-Fare, Nebraska Special, and Nebraska, the horse tenderloin was not included in this comparison because there was no nutritional breakdown provided by the supplier. Table 3 shows the different diets and their Z-scores in relation to the prairie dog content.

Table 3. Nutritional data of the captive diets related to the nutritional data of the prairie dogs. The following z-scores show how many standard deviations above or below the components are to the prairie dog. This shows no significant lack of nutrients in any captive diets.

Percent	Prairie Dogs	Nebraska Special	Z-Scores	Nebraska	Z-Score	Mazuri	Z-Score	Canine-Fare	Z-Score
Protein	8.583	18	7.926	18	7.926	38	24.759	26	14.659
Fat	9.647	10	0.078	12	-0.522	20	2.295	11	0.3
Moisture	38.372	62	5.429	62	5.429	N/A	N/A	41	0.604

5. Conclusions

Due to the complex geometry and inefficient scanning of the top jaw was unable to be successfully printed to the degree necessary for accurate testing. The bottom jaw was printed utilizing both Xometry, an outside supplier, and the University of South Carolina's Mechanical Engineering department. A design was sent into Xometry in hopes to print the bottom jaw in stainless steel. Unfortunately, the geometry was too complex and had to instead be printed using their SLS process in a White Nylon. The university printed the bottom jaw in ABS plastic utilizing traditional 3D printing techniques. The bottom jaw was printed to the best of the university's ability, however the exact percent error between the original cast and the final print have yet to be determined.

The Bose system was utilized to derive mechanical properties from the captive diets. While the jaw may provide more accurate bite forces required to masticate food, the force gauge allows for mechanical testing of constitutive properties such as yield point and stiffness. Using this force gauge, different captive diets could be tested. The captive diets showed that minimal force was required either to compress 50% or bring to a breakage point. The highest force found for a captive diet was 13.66 N, where, from literature, a BFF's maximum bite force is 48 N[1]. This decrease in force indicates muscle unloading, which has been proven to lead to bone deformation. Since many of the diets had unique structures, they were tested in different ways, which could bring some error to these results. For example, the horse tenderloin was cubed where the ground diets were rolled into spheres. The captive diets were shown to be significantly different from one another, except for horse and Canine Fare, Mazuri diet and horse, and Nebraska and Nebraska special. This shows that certain diets may be better options for captive ferrets to prevent oral problems.

Utilizing the Bose system, stiffness values for rat femurs and tibias were derived from dissected rats. Initially data from mice and rats were to be used to extrapolate the force required to masticate prairie dogs. However, the mouse bones were too small for the three-point bend test. The data taken from the rat bones can provide a methodology for future groups to follow for the prairie dog samples, as well as comparative data for future studies. Additionally, 10% of the BFF wild diet consists of small rodents, providing some insight to its mechanical properties. While attempting a three-point bend test on the rat bones, there was an attachment on the top compression plate, which prevented the load cell from reaching its maximum force of 25 N and instead causing it to reach around 13 N. This affected data collection, as 13 N was not enough force to fracture the bone. It was, however, enough to bend the bone to provide insight for the actual force needed to crush through bone. Additionally, the stiffness of the bones was determined, utilizing a stress-strain curve and more specifically the curve up to the yield point. As there were no other bones to compare, it was difficult to determine meaning from the values. However, stiffness is a constitutive model that does not rely on area and therefore would be an accurate way to compare the mechanical properties of captive diets to the wild diets.

Nutritionally, all the diets were within one negative standard deviation for the measured quantities of moisture, crude fat, and crude protein. In fact, most of the diets were significantly more fortified in these areas than prairie dogs. This shows that it is unlikely that nutritional factors were a significant player in the dental health issues and subsequent cranial deformations of captive BFF populations.

This project has laid down a foundation by which future teams can build on a substantial base of knowledge and methods that are not only applicable to the Black Footed Ferret, but to numerous other captive carnivores as well.

7. Future Plans

As the prairie dogs could not be used during testing, the carcasses the that have been purchased will be passed on to Dr. Hartstone-Rose to be tested in the future with the methodology that has been designed. Additionally, an IACUC protocol will be created for use of prairie dogs so the only necessary steps in the future using the prairie dogs will be special training.

Since this project addresses a concern that affects not only the BFF but all captive carnivores, it is a natural step to adjust the protocol to utilize the tooth geometry of other species.

9. Finalized budget

Table 4 shows the budget for this project. Out of the $1000 provided, a little over half was spent. Initially, most of the budget was intended to go towards printing the jaws. However, due to complications in printing in stainless steel, that part of the budget remained unspent.

Table 4. Itemized budget for this project. As many of the higher priced items were loaned, the project came out well under the initial budget of $1000.

Item	Price
BFF skull	$99.95
Prairie dogs	$138.45
Casting materials	$36.15
Xometry 3D print	$57.74
Captive food	$150.00
Mechanical Engineering 3D print	$75.00
Instron	*Provided by Dr. Shazly*
3D scanner	*Provided by Dr. Hartstone-Rose*
Total Spent: $557.29	Budget remaining: $442.71

9. References

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