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Determining Dietary Niche in Primates Using Portable X-Ray Fluorescence

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Introduction

Within the scope of biological anthropology, many tools are used to gain information about the evolution of humans. Diet reconstructions provide a small window into an animal's life that shed light on things such as what it consumed during its life, what type of food sources were available, what the climate in the region was like, and explanations for extinction based on its surroundings. This information can then be used to piece together information about a specific area and time period, which can in turn give rise to new findings about the ways humans evolved. Unfortunately, many of these tools are destructive in nature, resulting in destroyed samples, which is not ideal because often, there are only very few samples of important specimens. For example, a jawbone may contain only a single tooth, which might be the missing link to our ancestral past.

These destructive methods have been used since the 1980's and have provided scientists with much of the information we possess concerning human evolution and past climates. However, there are non-destructive methods available that should be implemented on a greater scale. One of these is portable X-Ray Fluorescence or pXRF, which has been used for things such as identifying harmful elements in dishware, but which has not been frequently implemented in biological anthropology. In this study, I asses pXRF's potential as a tool for diet reconstructions by validating it as an accurate non-destructive method. I did this by analyzing six primate skulls housed at the University of Northern Colorado and comparing the pXRF results to a previous study done on the same samples that used a different, more established method (mesowear analysis) to infer diets of each specimen. By demonstrating the potential of pXRF to accurately indicate diets, I hope to prove that it has the potential to be used as a widespread tool in biological anthropology and other disciplines where samples are few and far between.

Background/Literature Review

Background: X-Ray Fluorescence

pXRF is a portable, non-destructive method employed for trace element analysis that uses a laser to bounce a low-level X-ray onto a surface (in this case, bone and tooth enamel) causing an electron to be knocked out of the inner shell of an atom. This causes the atom to briefly become unstable until another electron from another shell takes the place of the knocked-out electron. This process causes energy and the emission of a photon, leaving an elemental signature indicated by the resulting energy spectrum with distinguishable energy peaks for each element present. (Byrnes & Bush, 2016) The portable version is small enough to be stored in a handheld briefcase, is chargeable, and results in a much less expensive process than other techniques used for elemental analysis.

Previous research done using XRF in the scope of anthropology include a study done to explore the uses and validity of the portable XRF reader for forensic work. The researchers looked at the functionality of the pXRF reader through different thicknesses as well as through different layers of bone to determine possible changes in readings based on potential diagenesis. (Byrnes & Bush, 2016) Diagenesis is the replacement of bone by other chemicals and sediment.

More recently, an article by Sehrawat & Singh (2019) explored the use of XRF to extract trace elements to determine and analyze the sex and age of an unknown population. This provides a more variable application for XRF other than diet estimations. However, this study still crushed the teeth samples into a fine powder, making it destructive method. It is important to note that while the precision of destructive methods may be better than non-destructive methods, the accuracy of both was comparable. The non-destructive method provides the same overall conclusions as a destructive method, it just doesn't reproduce them down to the exact decimal.

Within the scope of anthropology, there have been few studies using X-ray fluorescence and even less using this method for non-destructive diet reconstructions. This study is one of few to use pXRF for a diet reconstruction and will therefore fill a gap in the current knowledge, laying a foundation for future diet reconstruction studies.

Background: Sr/Ca Ratios

This study uses strontium (Sr) and calcium (Ca) concentration ratios for the diet reconstruction because these elements are important indicators of diet. Strontium is a non-essential element that is found in the ground and is often taken up into plants via xylem transport (Eastham, Feranec, & Begun, 2017) as it looks atomically like calcium, which is an essential element for plant growth. Calcium plays a critical role in cellular structure and nutrient transport. Just like strontium, calcium is taken up through soil by the roots and transported through the xylem. In animals it is especially important for bone growth. There are different amounts of strontium and calcium found within the bones and teeth of animals depending on their diets. Since I wanted to compare the relation between strontium and calcium, this study categorized strontium and calcium into a ratio. This accomplished several things. First, pXRF has a differing level of sensitivity to both elements based on the density of the material (in this case teeth and bone). By putting these elements as a ratio, I controlled for the density of the material. Secondly, calcium has a higher raw amount than strontium and, since I needed to know which primate had more strontium relative to the others, it was necessary to use a ratio rather than the raw amounts.

Unlike pXRF, employing strontium and calcium ratios to determine diet has been in common use for some time. In Sillen's study on *A. robustus*, his research team used Sr/Ca elements of *A. robustus* to reconstruct an omnivorous diet for the species. This research also mentioned the potential to use Sr/Ca ratios to identify carnivory. Sillen then looked at Sr/Ca ratios of modern animals, specifically the Western Cape food-web, the leaf-eating animals' steenbok (*Raphicerus* sp.), and duiker (*Sylvicapra grimmia*). They had relatively low Sr/Ca, and therefore were not distinguishable from carnivores. (Sillen, 1992) Sr/Ca values are normally reduced at higher trophic levels in food-webs yet, if some carnivorous animals are found to have similar Sr/Ca ratios to those of herbivores, it might mean that those carnivores are eating animals with a diet of high Sr/Ca. While I did not need to account for a carnivorous primate in my study, there are primates who are insectivores and opportunistic feeders.

Strontium and calcium ratios of modern animals can also be useful when attempting to determine the kinds of things their ancesters were eating. Researchers, Sponheimer, de Ruiter & Lee-Thorp, 2005, revisited old research about *Australopithecus/ Paranthropus,* which was done before the discovery that diagenesis was found to alter bone compounds. The researchers stated that in the time of the study virtually nothing about Sr/Ca in the enamel of modern African mammals was known, and much fewer fossil taxa. So, in order to address this gap, they studied Sr/Ca in tooth enamel from modern mammals in the greater Kruger National Park, in South Africa. The researchers used animals with different diet preferences, namely grazers, to identify Sr/Ca levels. (Sponheimer, de Ruiter & Lee-Thorp, 2005) Calcium and strontium are great for diet reconstructions because they can reveal an animal's diet during its life by the different amounts of each element found in the bone.

Finally, there is one source that uses both pXRF and Sr/Ca ratios. This research used pXRF to determine Sr/Ca ratios on fruits and leaves from the same tree. The pilot data from this study suggests that leaves have a much higher Sr concentration than fruit, suggesting that primates with a folivorous dietary preference have higher Sr/Ca ratios than frugivorous primates. (Hamilton, Drake & Nelson, 2019) Dietary niches for primates are typically either frugivores or folivores, with some insectivores. For this research, I attempted to group my findings into either frugivore, folivore, or mixed feeder (omnivore) for ambiguous results that fell somewhere in between the two. Since the validation of the pXRF is being assessed through this new methodology, I hypothesized that primates with low Sr/Ca ratios would have eaten a diet of fruits whereas, primates with high Sr/Ca ratios would have had a diet of leaves and stems.

Background: Mesowear

The primates used in this study were also used in a previous diet reconstruction. This was a directed study completed by Shelby Bundy in 2019, titled *Dental Ecology & Faunal Analysis*, which was able to determine the genus and age of the same primates. She was also able to identify diet via mesowear scores. Mesowear is another non-destructive method used to determine diet, which looks at the broad structures of teeth. By determining the shape of tooth cusp and occlusal relief (surface of teeth that are used to chew), or the sharpness/ dullness that can determine if an animal was eating hard or soft foods. A mesowear scoring system can go up to 6, with 6 being dull teeth typically belonging to grazer animals. A low mesowear score means the teeth were sharper, thus the animal was eating leaves and insects, whereas, a high mesowear score indicates a harder food diet, and therefore duller teeth. This method can be useful and can reach the same conclusions as pXRF. However, there is more room for human error as it relies solely on observation.

Background: Sample Specimens

The primate skulls were kept in a lockable cubby in the archaeology lab of the anthropology department at UNC for which a key is required to gain access. According to Bundy 2019, three out of the six skulls are juvenile. This means that none of these primates are young enough to be un-weaned. Strontium and calcium composition is different in mothers' milk, which is high in calcium and low in strontium. In a study done on deciduous (baby/milk) teeth using Sr/Ca ratios, a dietary shift was observed in one tooth that showed significant Sr/Ca differences when its diet was shifted from breast milk to formula. (Humphry, et al. 2007) Therefore, these primates are juveniles, rather than infants, and would likely have been eating a similar diet compositionally as the adults and therefore would not show a reading of a diet of milk.

Figure 1 shows the results of the mesowear study. By looking at the shape of each tooth cusp (canine, first, second, and third molars) and occlusal relief (the sharpness/dullness), Bundy was able to give each primate a mesowear score. If a tooth was sharp, it received a lower mesowear score, indicating a diet of leaves and insects. This is because leaves are tough but not hard and tough foods leave teeth with high and sharp cusps. A higher mesowear score indicates a harder food diet and duller teeth. Bundy (2019) found that all the primates were folivorous, with the exception of one. The *Macaca spp.* #07648 was found to be omnivorous due to its low mesowear score as well as the dietary category for its species. It is important to note that while most of the primates were found to fall into the same dietary category, they vary in age and sex.

Directed Studies: Dental Ecology & Faunal Analysis

Final Project: Primate Skull Identification

ID #	Genus	Species	Sex	Age	Diet	Post canine area (mm^2)	M1 area	M2 area	M3 area	Molar Tooth Row Measurement (mm)	Mesowear score
#07652 "Treese"	*Chlorocebus*	*Pygerythrus*	F	Adult	Folivore	81.5	27.83	28.14	25.53	15.82	2/3
#07645	*Colobus spp.*		F	Juvenile	Folivore	122.61	43.27	42.47	36.87	19.2	0/1
#07648	*Macaca spp.*		U	Juvenile	Omnivore	N/A	N/A	N/A	N/A	30.68	0
#07643	*Chlorocebus*	*Pygerythrus*	M	Adult	Folivore	71.58	24.13	28.48	18.97	16.92	2
#07653	*Chlorocebus*	*Pygerythrus*	M	Juvenile	Folivore	N/A	N/A	N/A	N/A	16.03	1
Unnumbered (crania in take-out container)	*Chlorocebus*	*Pygerythrus*	M	Adult	Folivore	75.12	24.94	29.75	20.43	15.3	4

Figure 1. Chart of Primate Skull Identification. Adapted from Directed Studies: Dental Ecology and Faunal Analysis by Shelby Bundy, 2019. Reprinted with permission. (Unnumbered crania in take-out container is primate # 0629 “food” in this study).

Research Question and Hypothesis

To what extent can we reconstruct the diets of an unknown primate sample using pXRF? I hypothesized that primates with low Sr/Ca ratios will have eaten a diet of fruits whereas, primates with high Sr/Ca ratios will have had a diet of leaves and stems. In order to address my hypothesis, I conducted a quantitative experiment using pXRF. I then compared my results to the data of the dental ecology study (Bundy 2019) to assess the accuracy of pXRF.

Methods

Teeth are better used for determining diet through elemental analysis than bone due to the potential for diagenesis (the physical and chemical changes that occur during the fossilization process). Although the skulls I worked with have not gone through diagenesis as they are not fossilized, I still used teeth as a precautionary measure. Using the teeth also solidified the experiment for a time when this methodology is applied to fossils. The directed study, Bundy (2019), used the canine, first, second, and third molars of the primates to determine mesowear. For the sake of continuity, I took measurements on the same teeth. However, for the pXRF to take a proper measurement, teeth should lay flat on the surface of the machine and since these skulls were quite small, I could not accomplish that with most of the molars. Also, some teeth were broken or missing from the skull. Therefore, I took measurements of an incisor, a canine, and/or a molar when possible as well as the occipital bone of each skull. I wanted to take measurements of the skull and the teeth to see if there would be a difference between tooth enamel and bone. These six skulls (pictured below) were donated to the University of Northern Colorado and are a completely unknown set of primates. However, they most likely came from a North American zoo, which has steady feedings and where animals are fed based on species-specific food preference. They have not needed to forage for food or become opportunistic feeders and because of this, it was a good way to further validate the accuracy of the pXRF unit. It also means that their diets would have been more regulated and therefore would yield better results as certain environmental variables are taken out of the picture.

07643 07645 07648 07652 07653 0629 “food”

For my data collection, I set up the pXRF unit to remotely pull the trigger with a click of a button on the computer. I held each sample as flat as possible on the surface where the laser is expelled and took two different types of measurements: a 90-second quantitative measurement that calculates elemental concentration using the Bruker Nano "Mudrock" internal calibration, and a 60-second qualitative spectra. I took each measurement on three points of each primate skull (incisor, canine or molar, and the occipital bone of each skull). My data was stored in computer software called Artax, which is the basic software included with the pXRF unit. Using the portable X-ray fluorescence unit and the computer software to show the results, I was able to analyze the findings of the trace elemental reading.

Data Analysis

The qualitative spectrum allowed me to label each elemental energy peak. An example of this is shown in Figure 2. This also illustrates what the typical qualitative spectra looked like. A high calcium peak could be found around 4 kiloelectron volts (keV) whereas strontium could be found at 14 keV. A phosphorus peak could also be found on the primate skulls as well as iron which are typical indicators that there is dirt or dust on the sample.

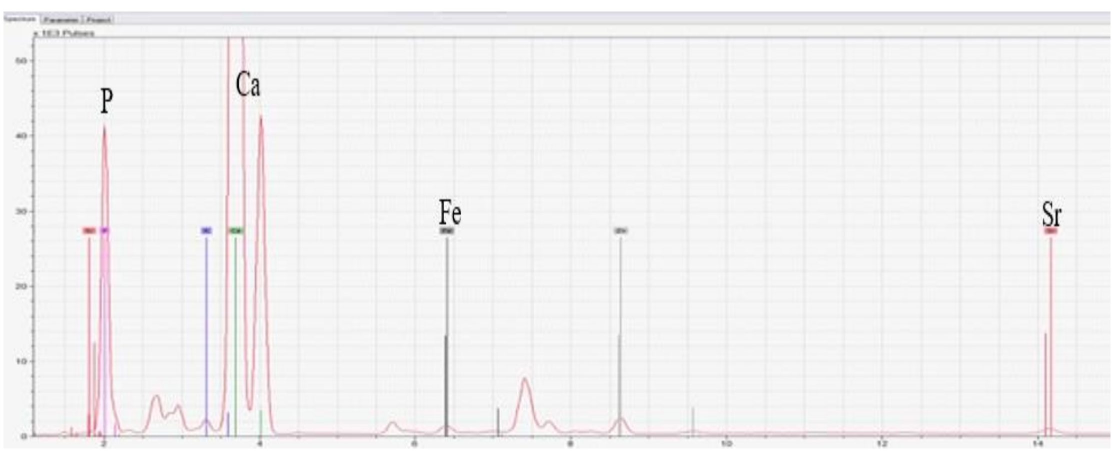

Figure 2. Qualitative spectra of 07648, right incisor.

To analyze the results, calcium and strontium needed to be calculated as a ratio, which I did in an Excel spreadsheet. To compare the primates to each other as a whole, I ran an ANOVA test to determine variation between the two groups to see if there were significant differences which would indicate a split in diet preference.

Although I compared the results of the pXRF to the results of the mesowear study, I was not sure if the pXRF results would match with the mesowear study. Mesowear is a more subjective tool than pXRF, which can allow for inter-observer differences in interpretation. There was also a possibility that the primates were mixed feeders, which could blur the data, or that they had a diet that we do not have a reference for, such as insectivory. To ensure quality control, a power analysis was done to ensure that enough measurements per primates were taken. The power analysis was taken with the consideration of sample size and amount of sample measurements. Three points of measurement were taken on each skull which allowed the data to pass all assumption checks.

Results

Figure 3’s boxplot shows the results of the ratios of each primate. The Y axis shows the values of Sr/Ca ratio. These ratios are all in line with a folivorous reading. The X axis indicates each primate (the 0 in front of each primate is missing in figures 3-5, but which are included in all other areas of this manuscript). Each black line indicates the result of the ratios of each measurement (tooth type and skull). A higher standard deviation indicates a larger diet breadth. This is shown in most of the primates especially 07648 which means it ate a variety of leaves.

Whereas a lower standard deviation like primate 09629 indicates a specialized diet and was likely only consuming one or two types of leaf species.

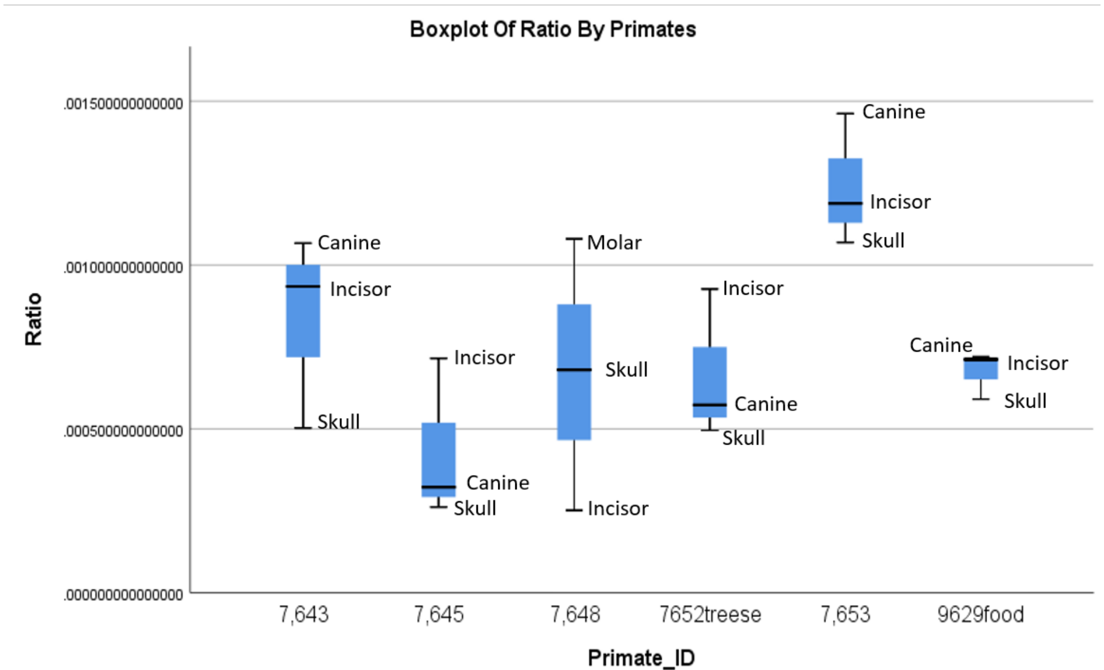

Figure 3. Boxplot of the ratio by primates by teeth.

The ANOVA test showed if there were any statistical differences between independent groups. In this case, it tested to see if any of the primates were statistically different from each other. This test shows that all but two primates are not statistically different, and they were all eating similar diets. This can be further explored in Figure 5.

ANOVA Table

			Sum of Squares	df	Mean Square	F	Sig.
Ratio * Primate_ID	Between Groups	(Combined)	.000	5	.000	3.157	.048
	Within Groups		.000	12	.000		
	Total		.000	17			

Figure 4. ANOVA Table. .048 significance.

7645	7643	-.000401955099231	.000215595441157	.465	-.001126123125662	.000322212927201
	7648	-.000237715000465	.000215595441157	.871	-.000961883026896	.000486453025967
	7652treese	-.000232389378384	.000215595441157	.881	-.000956557404815	.000491778648048
	7653	-.000806980970612*	.000215595441157	.026	-.001531148997043	-.000082812944180
	9629food	-.000241661328154	.000215595441157	.864	-.000965829354585	.000482506698278

Figure 5. Multiple Comparisons Table.

This figure demonstrates any significant statistical difference between the primates. The circled numbers highlight the two primates (07645 and 07653) which were significantly different from each other. This can be seen in Figure 3 as the highest data point on 07645 is below the lowest point of primate 07653.

Discussion

As expected, based on the comparative study, all samples have ratios more in line with folivory than frugivory. Primate 07648 had the widest variation (standard deviation of .00414400072203) in samples indicating an omnivorous diet, which also concurs with the mesowear study. Primate 09629 "food" had the least variation (standard deviation of .00007254118921) indicating a more specialized diet. Since all data points were close together in their ratio, this primate was likely eating one type of food within the folivorous category. In the mesowear study, this primate received a score of 4 which was the highest score out of all the primates. High mesowear scores indicate duller teeth that could be for eating a specific food item. As this individual was an adult, it follows that its enamel would have been more worn down from use than the other primates. The other primates determined to be the same species (07643, 07653 & 07652 "treese") that had different mesowear scores and ratios is likely because they may have been housed separately, had a difference in age and health or perhaps, like humans, could have been a picky eater.

In all but one primate, the occipital bone had a lower Sr/Ca ratio than enamel. This is related to instrumental measurement capacity as much as it is to physiology. Because bone is less dense than enamel, the pXRF can measure more light elements, such as calcium, than it can in denser material like enamel. Additionally, bones hold and store calcium not only in the form of hydroxyapatite, which is the hard part of the bone that gives it structure, but also in the matrix of collagen proteins that make up the living part of the bone, which is the part that grows and repairs bone. Enamel is purely hydroxyapatite, without the added contribution of calcium in the collagen matrix. Since enamel is harder and denser than bone, there is generally less Sr/Ca packed into bone than enamel.

Conclusion

Limitations and Recommendations

There are some limitations to this research. Within the scope of a McNair research project, the resources are somewhat limited. A sample size of six skulls is not enough to provide significant results. It is also well-known in anthropological research that samples are difficult to come by. This is why it is so important to use non-destructive methods.

Secondly, there is no provenience sample, which is not ideal to validate pXRF if we are not sure what the sample is and we are not exactly sure where the primate skulls came from. I did not have a reference collection for insectivory, which might have helped discriminate between readings that may lie somewhere between frugivory and folivory.

Lastly, this study would have been best if the primates had a 100 percent known diet. I only had a sample with a presumptive diet. These primate skulls were initially stored in a cabinet in the University of Northern Colorado's biology department museum with only ID tags. There was no information on them such as where they came from, when they were donated, etc. The only thing I had to go on was Bundy's Chart of Primate Skull Identification. If there was a higher sample size, a known provenience, and a reference collection for insectivory, there would have been more significant results with more conclusions upon which future research could be built.

However, the results of this research do show that pXRF is a viable method for testing various things related to skeletal artifacts. With this tool, we can see the kind of diet ancient animals were eating. This study was not able to test its original hypothesis due to the lack of frugivore samples, but these results show that pXRF analysis can indicate dietary breadth (omnivore vs. specialization) and should be implemented on a greater scale.

Future Directions

I would like to do a microwear analysis on these primates. This would involve taking a mold of the teeth and analyzing them under a microscope. Microwear is another nondestructive way of diet estimation that looks at the microscopic lines on teeth that are left on teeth. Through the characteristics of these lines (pitting, depth, etc.), I could estimate which types of food the primates were eating up to two weeks before death.

I would also like to implement pXRF on a greater scale with more samples and on our ancestral bipeds. It is important to determine the capability for pXRF when there's a potential for more variables. There are more complex diets in the wild and the next steps should look for more variable diets like insectivores, frugivores, and folivores.

Final Notes

This research shows the potential for portable X-Ray fluorescence within the scope of biological anthropology, which is tremendously important: the key to knowledge about the future is to look at the past. By determining an extinct species' diet, it is possible to many paleo-biological attributes of life for that species. This method can and should be applied to ancient hominins so we can gain a better understanding of what went right and wrong for them in order to better our lives today. This type of research should be done using non-destructive methods such as pXRF, which can be as accurate as destructive methods yet leaves the sample intact so future research can be implemented upon it. The results of this research have opened many possibilities for diet reconstructions and more using portable X-Ray fluorescence.

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