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Evaluating Muscle Fiber Architecture

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Evaluating Muscle Fiber Architecture

By

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Bachelor of Liberal Arts
Illinois Wesleyan University, 2013

Submitted in Partial Fulfillment of the Requirements

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DEDICATION

To Boston

ACKNOWLEDGEMENTS

I would like to thank the following for access to the deceased carnivores: University of South Carolina – Columbia, the Department of Cell Biology & Anatomy. I thank Dr. A. Hartstone-Rose for the use of laboratory space and materials. I thank the members of my committee: Dr. A. Hartstone-Rose, Dr. E. Blanck, and Dr. W. Ai. I would like to thank C. Leischner, A. Grant, and the other students in Dr. Hartstone-Rose's lab for their assistance and advice. I would like to thank Dr. A. Hartstone-Rose for helpful comments on drafts for this thesis. I would like to thank Dr. A. Hartstone-Rose, C. Leischner, and A. Grant for help in collecting anatomical data. This work was funded by Dr. Hartstone-Rose's Comparative Anatomy Research Lab.

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ABSTRACT

Mastication is the first step in the preparation of food for digestion. The masticatory anatomy of several families of Carnivorans (i.e., Family Canidae, Family Mustelidae, Family Hyaenidae, and Family Ursidae of the order Carnivora) will be compared in this study. The goal is to better understand masticatory adaptations through an examination of bite force and muscle fiber architecture in the various groups of carnivores, and to provide a proper protocol in acid dissection of fiber architecture.

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CHAPTER 1

INTRODUCTION

Mastication is an essential asset of most mammals. Mastication is the first step in the preparation of food for digestion. In the Hartstone-Rose lab, the masticatory anatomy of several families of carnivorans (members of the order Carnivora) is compared. The goal of this study is to better understand masticatory adaptations through an examination of bite force and muscle fiber architecture in the various groups of carnivores.

The order Carnivora appeared in the middle of the Paleocene Era about 60 million years ago (Radinsky, 1982). Around 40 mya, an evolutionary radiation occurred and the order Carnivora evolved into several extant families: Family Canidae (dogs), Family Mustelidae (weasels), Family Ursidae (bears), Family Viverridae (civets), and Family Felidae (cats). Around 25 mya, the Family Procyonidae (raccoons), Family Phocidae (seals), and Family Otariidae (sea lions), and around 25 mya, the Family Hyaenidae (hyenas) appeared (Radinsky, 1982). In some instances, an evolutionary radiation can occur “after the acquisition of morphological innovations of functional significance” (Radinsky, 1982). The morphological adaptations in the masticatory anatomy could have assisted in this evolutionary radiation that occurred.

Based upon comparative anatomical studies, Radinsky (1982) suggests that the modern carnivore family can be separated into two large groups. One group is the felids,

viverrid, and hyaenids while the other group is the canids, mustelids, procyonids, and ursids (Radinsky, 1982).

The carnivore order has the “largest ecological and body size diversity of any mammalian order” which allows us to examine a wide range of dietary adaptations (Christiansen, 2007). This order spans more than three orders of magnitude in body size (Christiansen, 2007), from 0.1 kg weasels to 800 kg brown bears (Lariviere, 1999; DeMaster & Stirling, 1981). The vast difference in body sizes “suggest that partitioning of prey resources by size may have been a factor in their initial radiation” (Radinsky 1982). By partitioning prey resources, species within the different families would be less likely to compete with one another for the same prey.

Specifically, evaluating the masticatory system, jaws, soft tissues, and dentition may have been influenced by natural selection and would have evolved differently (Gans et al., 1978). These differences could have resulted from the partitioning of prey resources.

In the Hartstone-Rose lab, the muscle fiber architecture has been evaluated. Muscle fiber architecture is an important determinant of a muscles’ function. Skeletal muscle is composed of numerous units called fascicles. Within each fascicle, there are smaller units called fibers. The fiber architecture is the makeup of the skeletal muscle fibers (Taylor 2009). The architectural elements of muscle function are the individual muscle fiber length and the physiologic cross-sectional area (PCSA) of a muscle. The force of a muscle is mostly determined by the PCSA, and the velocity of a muscle is determined by the muscle length (Eng 2008). Measuring the individual muscle fiber length as opposed to the length of an entire muscle provides better information about the

muscle (Taylor 2009). The muscle is composed of many fibers of which one fiber rarely spans the entire length of a muscle (Taylor 2009). The PCSA is measured by the division of muscle volume over fiber length and is the maximum strength of the muscle. These fibers are the determinants in the movements and forces during the action of these muscles.

CHAPTER 2

BACKGROUND

In the Hartstone-Rose lab, the muscle fiber architecture has been dissected and analyzed in order to further understand the functional anatomy of specific muscles or muscle group. There have been several leading scientists that have been examining the muscle fiber architecture that have been following similar protocols. These scientists have been able to apply this type of method in differing muscle groups and areas such as mastication, tail, and vertebral muscles. By studying the architectural design of these muscles we can better understand their function and performance.

Dr. Andrea Taylor of Duke University has been studying fiber architecture primarily focusing in primates but also in other mammals to better understand the masticatory system. In a 2006 study, Taylor and her colleagues examined the effects of dietary consistency of the masseter fiber architecture in post-weaning rabbits. In this study, they found that the rabbits with tougher diets had a larger superficial masseter PCSA due to an increased muscle mass with no changes to fiber length. In a 2009 study, Taylor and her colleagues compared the fiber architecture of the masseter and temporalis in primates by evaluating the fiber length, PCSA, and other variables. They found that the tree-gouging primates have a larger ratio of fiber length to muscle mass compared to non tree-gouging primates. The tree-gouging primates (marmosets) were also found to have a smaller relative PCSA and longer-fibered muscles. The longer fibers would aid in the larger jaw gapes exhibited. In a 2010 study, Vinyard and Taylor examined the jaw-

muscle architecture during chewing in primates. In this study, they showed how the arrangement of these masticatory muscles impacts on the function. In a 2013 study, Taylor and Vinyard collaborated again and examined the jaw-muscle fiber architecture of the masseter and temporalis muscle in extant apes and modern humans. They found that the PCSAs scale relatively isometrically in relation to jaw length with anthropoids but were positively allometric with humans. In addition, humans compared to extant apes have a reduction in masseter PCSA that may have resulted in a decrease in muscle force while chewing (Taylor and Vinyard 2013). By examining the fiber architecture of the masticatory muscles, Dr. Taylor has provided more information on the functions of these muscles.

Another researcher who uses the fiber architecture of muscles is Dr. Jason M. Organ of the Indiana University School of Medicine who primarily focuses on primates. In a 2009 study, Organ and his colleagues compared the fiber architecture of several vertebral muscles in primates by examining the fiber length, PCSA, and other variables in prehensile and non-prehensile tails of the Platyrrhini, a family in the primate order, and the Procyonidae, a family in the carnivore order. Prehensile tails have the ability to support the entire weight of an animal (Organ 2010). The prehensile tailed platyrrhines and procyonid genera were found to have higher PCSAs, which would allow them to generate a higher maximum muscle force than the non-prehensile taxa. However, no differences in the fiber lengths were found. In a 2014 study, Organ coauthored a study in which the forelimb muscle architecture in the groundhog (*Marmota monax*) was examined, specifically looking at the properties of the musculature. Scratch-digging mammals such as the groundhog are characterized as having large, powerful forelimb

muscles, which are necessary to generate enough force to excavate the earth (Rupert et al. 2014). It was found that the triceps brachii long head had the largest PCSA while the carpal and digital flexors had shorter fascicle lengths. Dr. Organ has been studying the fiber architecture in different areas of mammals (i.e. forearm and vertebral column) and contributed more information on the functions and abilities of these muscles.

Another scientist who studies the muscle fiber architecture is Dr. Samuel Ward at the University of California-San Diego. In a 2008 study, Ward coauthored a study in which the muscle architecture in rat hind limbs was examined. The anti-gravity muscles were found to have a greater PCSA and smaller fiber length to muscle length ratios, which would allow these muscles to generate a greater force than the non-anti-gravity muscles. The anti-gravity muscle supports an individual's weight against gravity. In addition, the hip extensors were found to have a longer fiber length than the hip flexor, which would allow the hip extensor to operate at two joints. Ward coauthored another study in 2008 in which the scapulothoracic and glenohumeral muscle architecture was examined in middle-aged individuals. They found that the shoulder abductor and adductor differed in PCSA but not in fiber length. In addition, the internal rotators were found to have larger fiber lengths and PCSA than external rotators. In a 2009 study, Ward and his colleagues studied the human lower extremity muscles specifically the muscle fiber length and the physiological cross-sectional area. The soleus, gluteus medius, and vastus lateralis were found to be the strongest muscles. Their findings will be able to help surgeons (Ward et al. 2009). In another 2009 study, Ward and his colleagues examined the musculature architecture of the multifidus muscle in order to further understand lumbar spine stability. The multifidus muscle was found to have a large PCSA and short

muscle fibers. In a 2013 study, Ward coauthored a study in which the rotator cuff muscles (supraspinatus, infraspinatus, subscapularis, and teres minor) architecture was compared among humans and several vertebrate species predominantly looking at the PCSA, muscle mass, and fiber length. The chimpanzees and the capuchins were found to be most like humans. Of the non-primates, smaller mammals' (mice, rats, and dogs) muscle architecture was more similar to humans than that of larger mammals (sheep, pigs, cows). Although primates provide the best representation, of the non-primates, the smaller mammals exhibit similar muscle architectural parameters than the larger mammals and may be better models in future studies involving the human rotator cuff (Mathewson et al. 2013). Dr. Ward utilizes fiber architecture in numerous organisms and differing muscle groups to further understand these muscles.

Another scientist that uses fiber architecture in her studies is Dr. Sharlene Santana at the University of Washington. In a 2010 study, Santana and her colleagues examined the mechanics of bite force production and diet in bats. They found that their data supports the hypothesis by Nogueira and his colleagues that the masseter muscle is important in the production of bite force (Nogueira et al. 2009). The bite force variation among bats attributed to the masseter could be a result of the differing feeding behavior and ecology (Santana et al. 2010).

Another leading scientist, Dr. Jonathan Perry, uses fiber architecture in his studies. In a 2008 study, Perry evaluated the mastication architecture in extant strepsirrhines and Eocene adapines by dissecting and studying the fiber architecture. They found that folivorous strepsirrhines tended to have short fibers for masticatory adductor muscles compared to the frugivorous strepsirrhines. In another 2008 study, Dr.

Perry collaborated with Dr. Hartstone-Rose in analyzing the masticatory architecture and bite size in lemurs. They found that the fiber length of masticatory muscles appears to be correlated with bite gape. In addition, folivores were found to have smaller muscle fibers, which could be attributed to their dietary uptake of small foods. In a 2011 study, Perry and his colleagues studied the jaw adductor fiber architecture. They examined several hypotheses involving the influence of body size and diet on the masticatory muscles. In a 2013 study, Perry along with Dr. Hartstone-Rose and his students examined the unique masticatory of the *Daubentonia madagascariensis*, commonly known as the aye-aye. They found that the PCSA increases, as the aye-aye becomes an adult. This could be attributed to the increase foraging without any parental guidance (Perry et al. 2013).

Another scientist who has been studying fiber architecture is Dr. Adam Hartstone-Rose of USC-Columbia who has been primarily studying masticatory muscles in carnivores and primates. In a 2007 study, Hartstone-Rose and Perry examined the felid masticatory system. They found that individual muscle mass correlates with body size; thus the masticatory muscle mass can give a fairly accurate body weight estimate (Hartstone Rose & Perry 2007). In a 2012 study, Hartstone-Rose and his colleagues studied the muscles in the masticatory system in nine species of felids. They found that the species that predominantly preyed on small animals had short muscle fibers as opposed to those that preyed on large animals that had longer muscle fibers (Hartstone-Rose et al. 2012).

CHAPTER 3

DISSECTION PROCESS

The architectural variables within several families of carnivores have been compared (i.e., Family Canidae, Family Mustelidae, Family Hyaenidae, and Family Ursidae). The muscles of each species were analyzed from the Canidae (N=12), Mustelidae (N=10), Ursidae (N=5) and a few other (N=5) carnivorans from other families as shown in Table 3.1. In addition, specimens (N=10) from the Hartstone-Rose and his colleagues (2012) study will be included

Table 3.1: Sample, following Audet et al. 2002, Bekoff 1977, Clark et al. 1987, Collins & Harveson 2013, De Mello Beisiengel & Zuercher 2005, DeMaster & Stirling 1981, Dietz 1985, Fitzgerald & Krausman 2002, Fritzell & Haroldson 1982, Gompper & Decker 1998, Grassman et al. 2005, Hartstone et al. 2012, King 1983, Larivière 1998, Larivière 1999, Larivière 2002, Larivière 2003, Larivière & Pasitschniak-Arts 1996, Law 2004, McGrew 1979, Mulheisen & Allen 2002, Paradiso & Nowak 1972, Pasitschniak-Arts 1993, Pogluyen-Neuwall & Toweill 1988, Roberts & Gittleman 1984, Walton 2003, and Ward & Wurster-Hill 1990

Scientific Name	Common Name	Family	Body Mass (kg)	Condition of weight taken	Sex*
<i>Ailurus fulgens</i>	Red Panda	Ailuridae	4.95	Species Average	U
<i>Alopex lagopus</i>	Arctic Fox	Canidae	3.46	Species Average	U
<i>Canis latrans</i>	Coyote	Canidae	14	Species Average	M
<i>Canis mesomelas</i>	Black-Backed Jackal	Canidae	7.7	Female Average	F

Table 3.1, continued

<i>Canis rufus</i>	Red Wolf	Canidae	24.6	Species Average	U
<i>Chrysocyon brachyurus</i>	Maned Wolf	Canidae	23	Species Average	U
<i>Lycaon pictus</i>	African Wild Dog	Canidae	22.5	Species Average	U
<i>Nyctereutes procyonoides</i>	Raccoon Dog	Canidae	4.34	Female Average	F
<i>Speothos venaticus</i>	Bush Dog	Canidae	5.5	Species Average	U
<i>Urocyon cinereoargenteus</i>	Gray Fox	Canidae	5	Species Average	U
<i>Vulpes zerda</i>	Fennec Fox	Canidae	1.175	Species Average	M
<i>Vulpes macrotis</i>	Kit Fox	Canidae	1.9	Female Average	F
<i>Vulpes vulpes</i>	Red Fox	Canidae	5.78	Species Average	U
<i>Caracal caracal</i>	Caracal	Felidae	16.59	Weight of Specimen	U
<i>Leptailurus serval</i>	Serval	Felidae	13.90	Weight of Specimen	U
<i>Leopardus pardalis</i>	Ocelot	Felidae	11.59	Weight of Specimen	U
<i>Lynx rufus</i>	Bobcat	Felidae	15.50	Weight of Specimen	U
<i>Neofelis nebulosa</i>	Clouded Leopard	Felidae	20.87	Post Mortem Weight	U
<i>Panthera onca</i>	Jaguar	Felidae	100.00	Weight of Specimen	U
<i>Panthera pardus orientalis</i>	Amur Leopard	Felidae	47.1	Live Weight	M
<i>Panthera uncia</i>	Snow Leopard	Felidae	56.5	Post Mortem Weight	U
<i>Panthera tigris</i>	Tiger	Felidae	200.00	Weight of Specimen	U
<i>Puma yagouaroundi</i>	Jaguarundi	Felidae	7.1	Post Mortem Weight	M
<i>Crocuta crocuta</i>	Spotted Hyena	Hyaenidae	57.5	Species Average	U
<i>Gulo gulo</i>	Wolverine	Mustelidae	18.14	Post Mortem Weight	M

Table 3.1, continued

<i>Aonyx cinerea</i>	Asian Small-Clawed Otter	Mustelidae	3.1	Species Average	M
<i>Lontra canadensis</i>	North American River Otter	Mustelidae	8.31	Species Average	U
<i>Martes americana</i>	American Marten	Mustelidae	0.71	Species Average	U
<i>Martes pennanti</i>	Fisher	Mustelidae	4.1	Post Mortem Weight	M
<i>Mustela ermine</i>	Ermine	Mustelidae	0.131	Species Average	M
<i>Mustela vison</i>	American Mink	Mustelidae	0.852	Species Average	U
<i>Pteronura brasiliensis</i>	Giant Otter	Mustelidae	19	Live Weight	M
<i>Taxidea taxus</i>	American Badger	Mustelidae	7.65	Species Average	U
<i>Bassariscus astutus</i>	Ring-Tailed Cat	Procyonidae	0.985	Species Average	M
<i>Nasua narica</i>	Coati	Procyonidae	4.6	Species Average	M
<i>Melursus ursinus</i>	Sloth Bear	Ursidae	143.8	Live Weight	U
<i>Ursus americanus</i>	American Black Bear	Ursidae	113.4	Unspecified	U
<i>Ursus arctos</i>	Brown Bear	Ursidae	125.57	Species Average	U
<i>Ursus malayanus</i>	Sun Bear	Ursidae	45	Species Average	U
<i>Ursus maritimus</i>	Polar Bear	Ursidae	387.5	Species Average	U
<i>Arctictis binturong</i>	Binturong	Viverridae	15	Species Average	U

Based on previously published methods (Hartstone-Rose et al., 2012), the masticatory muscles were dissected from each specimens (Figure 3.1) including the superficial masseter (SM), deep masseter (DM), zygomatico-mandibularis (ZM),

zygomatic temporalis (ZT), superficial temporalis (ST), deep temporalis (DT), and medial pterygoid (MP).



Figure 3.1 Removal of masseter muscles in a *Bassariscus astutus* (Ring Tail Cat). The masticatory muscles were removed in each specimen. In this picture of a *Bassariscus astutus* (Ring Tail Cat) the masseter muscles were removed.

The muscles are split into two categories: jaw abductors and adductors. The masticatory muscles that close the jaws otherwise known as the jaw adductors are composed of three major groups: masseters, temporalis, and pterygoideus as shown in Figure 3.2.

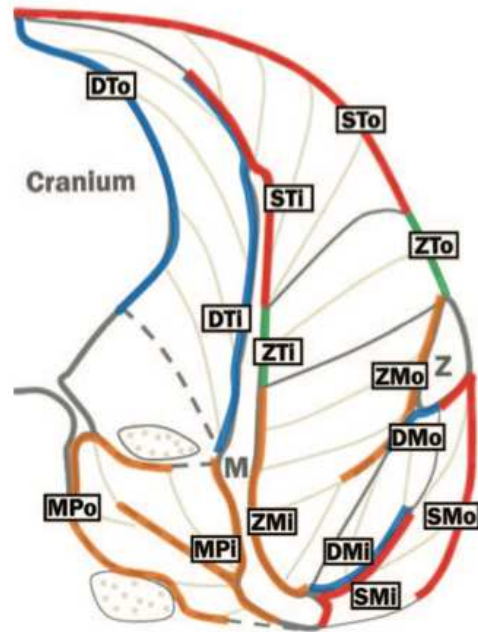


Figure 3.2 Coronal diagram of the mandibular adductor origins (o) and insertions (i). From Hartstone-Rose et al. 2012. Note that some fibers (e.g., most of those of Superficial Temporalis) attach on one or both ends to connective tissue and not directly onto the bony origin or insertion.

The masseter group is comprised of the superficial masseter (SM), the deep masseter (DM), and the zygomatico-mandibularis (ZM). The temporalis group is comprised of the zygomatic temporalis (ZT), superficial temporalis (ST), and deep temporalis (DT). The smaller pterygoideus group is comprised of two muscles the medial pterygoid (MP) and the lateral pterygoid (Hartstone-Rose et al., 2012; Turnbull, 1970). Figure 3 shows all of the dissected muscles. The temporalis muscle group is the dominant muscle group, while the masseter group and the pterygoideus group act as accessories and aid the temporalis muscle (Turnball, 1970). The masticatory muscle that open the mouth (jaw abductors) is the digastric muscle (Dig). In addition, we also evaluated the lateral pterygoid (LP) muscle. In primates, the lateral pterygoid accounts for the anterior translation of the mandibular condyle in primates (Hartstone-Rose et al., 2012). However,

this muscle is very small and is most likely not utilized as a masticatory adductor muscle (Hartstone-Rose et al., 2012). Table 3.2 shows an overview of the masticatory muscles examined in this study and further information on the location of each muscle, which is shown in Figure 3.4.

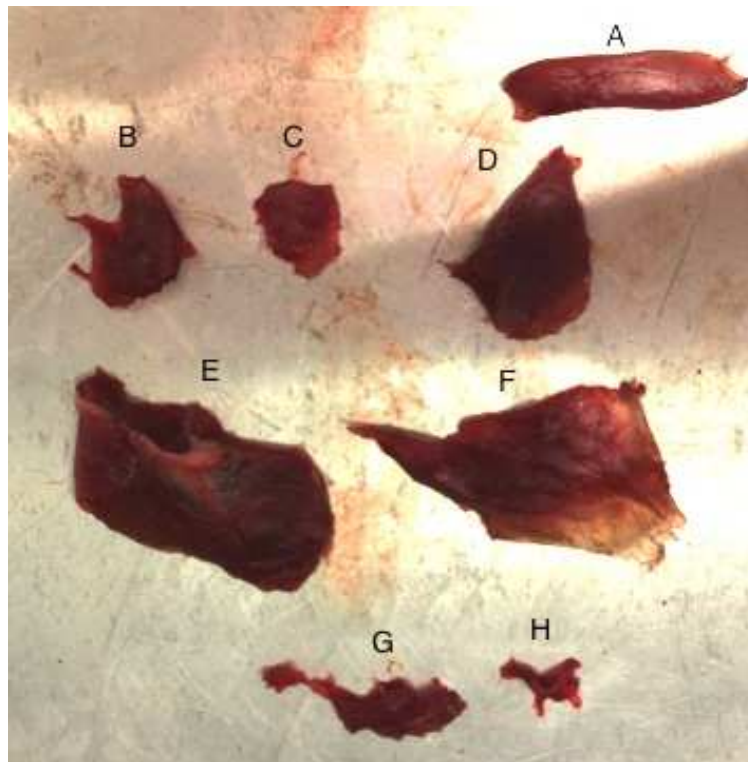


Figure 3.3 *Bassariscus astutus* (Ring Tail Cat) masticatory muscles removed. A: Digastric, B: Zygomatico-Mandibularis, C: Deep Masseter, D: Superficial Masseter; E: Deep Temporalis, F: Superficial Temporalis and Zygomatic Temporalis, G: Medial Pterygoid, H: Lateral Pterygoid

Table 3.2: Overview of the studied masticatory muscles in felids, canids, ursids, and mustelids; their origins; insertion; and functions, following Christiansen and Adolfssen, (2005); Druzinsky, Doherty & De Vree, (2011); and Turnbull (1970)

	Origin	Insertion	Function
Superficial Masseter (SM)	Zygomatic process (underneath origin of M. zygomaticus)	Mandibular ramus	Adduction

Table 3.2, continued

Deep Masseter (DM)	Lower ventro-lateral part of the zygomatic arch	Antero-dorso-lateral area of the mandibular ramus	Adduction
Zygomaticomandibularis (ZM)	Medial part of the zygomatic arch	Lateral surface of mandibular ramus	Adduction
Zygomatic temporalis (ZT)	Anterior upward edge of the rear buttress of zygomatic arch	Posteromedial edge of coronoid process	Adduction
Superficial temporalis (ST)	Frontal and temporal bone	Coronoid process	Adduction
Deep Temporalis (DT)	Sagittal crest, temporal bone	Coronoid process	Adduction
Medial pterygoid (MP)	Lateral edge of pterygoid, some palatal	Medial edge of angular process	Adduction
Digastric (Dig)	Ascending ramus	Foramen rotundum	Abduction
Lateral Pterygoid(LP)	Ventro-lateral surface of alisphenoid	Medial edge of mandibular condyle	Indeterminate, most likely not used as an adductor muscle

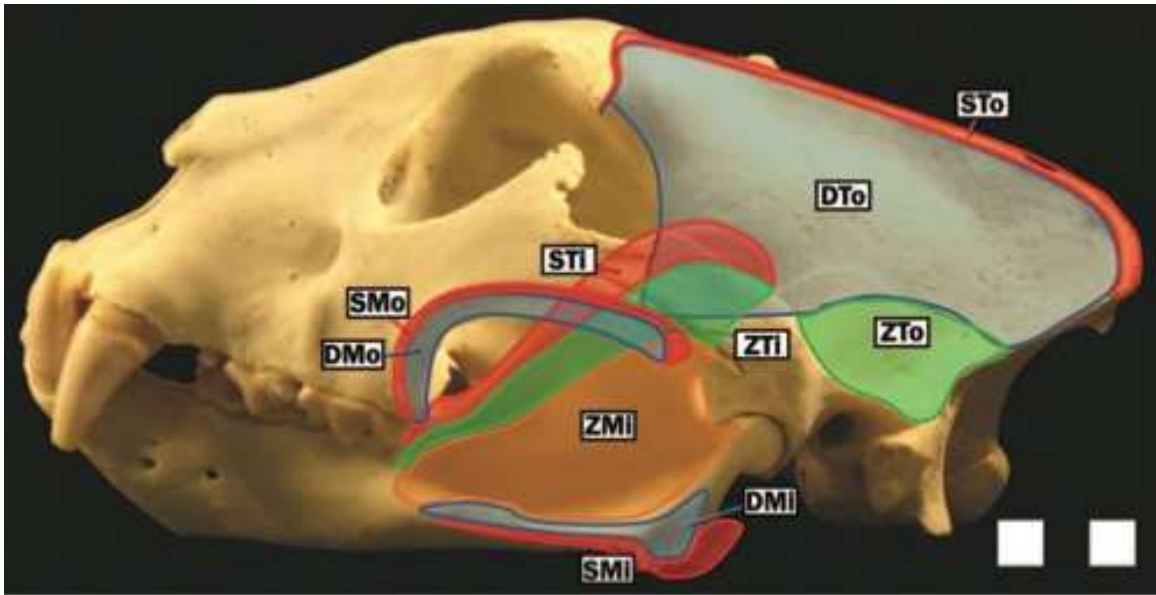


Figure 3.4. Masticatory muscle adductor origins (o) and insertions (i). From Hartstone-Rose et al. 2012

Each of the masticatory muscles was dissected and their masses and width were recorded as shown in Figure 3.5.

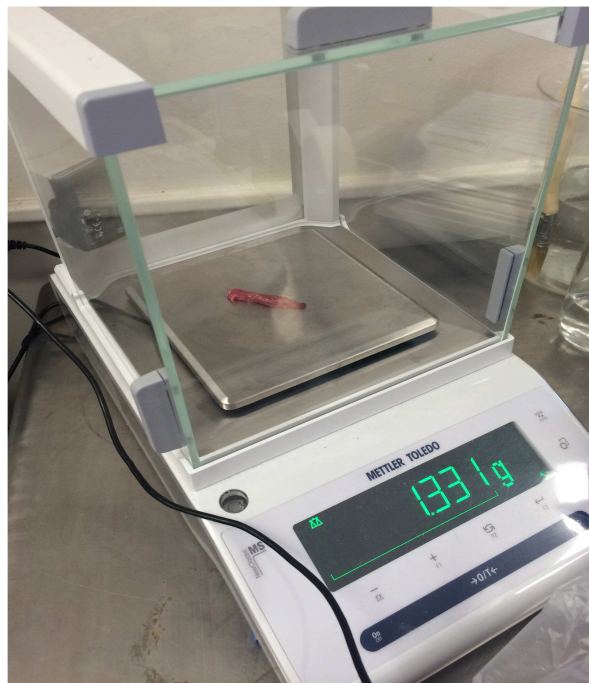


Figure 3.5 Masticatory muscle being weighed. The mass was recorded along with the width for each dissected muscle.

Muscle fibers can be removed and measured from formalin-preserved, ethanol-preserved or frozen muscles (Perry 2008). However, Perry concluded that the formalin-preserved muscles took a longer time to cook before extraction could occur (Perry 2008). The dissected muscle was either frozen or underwent a chemical dissection immediately.

In order to separate the fascicles without damaging them, each dissected muscle underwent a chemical dissection for a muscle architecture analysis following an established protocol (Perry and Wall, 2008; Perry et al., 2011; Hartstone-Rose et al., 2012) study involving felids. The chemical dissection removes the connective tissue that surrounds the muscle and holds the fascicles together. By removing this connective tissue, the fascicles can be separated easily. Each muscle was placed in a 10% sulfuric acid solution (Figure 3.66) and cooked at 70° C (Figure 7). Once sufficient connective tissue was dissolved, the muscle fascicles can be separated. The time to cook these muscles varied upon the size and the condition of the muscle (i.e. fresh, preserved, frozen) and requires constant monitoring to prevent over cooking.



Figure 3.6 Sulfuric acid (10%) solution is being added to the beaker of containing an individual masticatory muscle.



Figure 3.7 Masticatory muscles in 10% sulfuric acid solution and cooking at 70° C.

After enough connective tissue has been removed, the muscles are removed from the oven. The 10% sulfuric acid solution is drained into a waste beaker. The muscle is rinsed to remove any remaining sulfuric acid and the remaining solution is drained into another waste beaker. If enough connective tissue has been removed, the muscle fibers were easily extracted. The muscles fascicles can be separated by the naked eye or may be separated under a microscope for smaller muscles. Typically, between 30-50 good, unbroken muscle fibers should be collected to ensure a good representation on the muscle collected. Afterwards, the mean fiber length should be calculated to be used in future equations.

CHAPTER 4

METHODS OF CHEMICAL DISSECTION

4.1 ACID PREPARATION

Muscle is composed of multiple bundles of fibers. Surrounding the entire group of fiber bundles is the epimysium. The Perimysium surrounds each fascicle, bundle of fibers, while the endomysium surrounds each individual fiber within a bundle. The epimysium, peimysium, and the endomysium are all connective tissues that serve to hold the muscle together. The muscles are placed in an acidic solution to dissolve the connective tissue. Once the connective tissue is dissolved, the muscle fibers can be easily extracted. (Ogilvie and Sawyer, 2015)

The types of acidic solutions can vary. In the Organ (2009) study, Organ and his colleagues used 30% HNO₃, while in a (2012) study, Hartstone-Rose and his colleagues used 10% sulfuric acid. In a 2013 study, Perry, Hartstone-Rose, and their students used a different protocol. The dissected muscles were cooked in acetic acid (as available in the field in Madagascar in the form of vinegar) instead of sulfuric acid.

4.2 CALIPER MEASUREMENT

During the dissection, the muscle is removed from its origin and insertion. However, many of the fibers do not span the entire length from the bony origin to bony insertion, but rather attach to tendinous sheets (Figure 3.6). In order to get a true representation of the muscle, we take the lengths of the muscle fibers. Once the

connective tissue has been removed, the muscle fibers are easy to separate. However, the unbroken muscle fibers should be the only ones measured.

To get the most accurate recording, a pair of digital calipers should be used and downloaded onto a spreadsheet. Digital calipers prevent most errors from occurring as opposed to handwriting. If handwriting the measurements, there are several locations where errors could occur. When taking the measurement from the calipers, one could miswrite the correct length. By miswriting a length, the average fiber length would become skewed which would lead to further miscalculations in which an equation used the average fiber length. Another error that could occur would be when one is typing the lengths into the spreadsheet; one could accidentally mistype a length. Thus, there is a greater probability of error occurring if one handwrites the lengths as opposed to using digital calipers and entering the data directly.

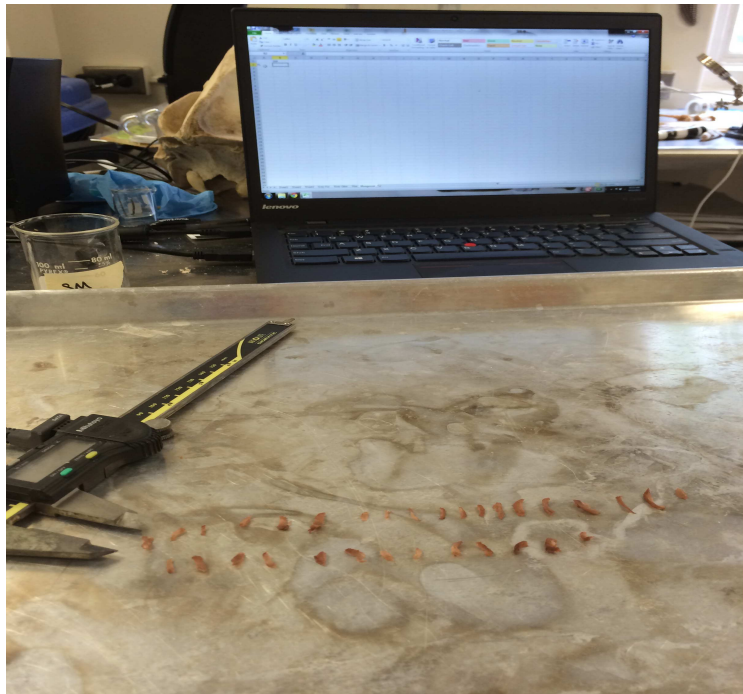


Figure 4.1 A pair of digital calipers measuring fiber lengths.

CHAPTER 5

STATISTICAL VARIABLES

5.1 TOTAL SPECIMEN BODY MASS

The total specimen body mass is the main independent variable. For the most accurate results, it is best to use a known individual specimen's body mass. However, sometimes the specimen's body mass is not known. This can occur when the shipper does not include the specimen's body mass or when just part of the specimen is shipped. When just part of the specimen is shipped, it is impossible to obtain the specimen's body mass. When the individual specimen's body mass is not known, the best option is to use the mean for the sex of that species. If the sex is not known, then the next alternative solution is to use the mean body mass for the species.

5.2 MUSCLE MASS

The muscle mass is the raw variable used as an independent variable. Immediately after a muscle is dissected, the muscle was measured to determine the weight of the muscle before it underwent a chemical dissection. The muscle mass is used in calculation of the PCSA in concert with the fascicle length.

5.3 FIBER LENGTH

After the muscle has been cooked in an acidic solution, the muscle fibers should be extracted. The fibers are individually measured to determine the average fiber length.

The average FL was calculated using the following formula (from Hartstone-Rose et al. 2012). This formula may be applied to other muscle groups.

$$FL_x = \left(\frac{(FL_{MS} m_{MS}) + (FL_{TMP} m_{TMP}) + (FL_{PT} m_{PT})}{m_{MS} + m_{TMP} + m_{PT}} \right)$$

In this equation, the FL_x is the average FL. FL_{MS} , FL_{TMP} , and FL_{PT} are the average fascicle lengths of the masseter, temporalis, and medial pterygoid, respectively. While, m_{MS} , m_{TMP} , and m_{PT} are the muscle masses for the masseter, temporalis and medial pterygoid respectively.

5.4 PHYSIOLOGICAL CROSS-SECTIONAL AREA (PCSA)

The PCSA data were obtained from the muscle tissue to determine the muscle force produced (Close, 1972; Weijs and Hilen, 1985; O'Conner et al., 2005; Anapol et al., 2008; Hartstone-Rose et al., 2012). Physiological cross-sectional areas (PCSA) are measured by the division of muscle volume over fiber length and are the maximum strength of the muscle. The PCSA was calculated using a formula from Schumacher (1961):

$$q = m/l\rho$$

In this equation, q is the PCSA, m is the muscle mass, l is the fascicle length, and ρ is the density of the muscle (Hartstone-Rose et al., 2012).

CHAPTER 6

DISCUSSION

The regressions of the statistical variables allow us to further learn about the related adaptations that have been made in fiber architecture. The statistical variables that can be used are the muscle mass, body mass, jaw length, fascicle length, total PCSA, and bite force. These regressions will tell us how they correlate with one another. In this particular study, data was gathered on jaw adductor dimensions and data on moment arms from species in multiple carnivore families to determine if they were isometric or positively allometric to body mass. If an isometric relationship were to result, for example the adductor muscle mass and the body mass relationship would be equal for both small and large species. However if they scale with positive allometry, larger species would be expected to have larger adductor muscles.

These applications of studying the fiber architecture can be used in many muscle groups in a specific family or even across families and orders. In the Hartstone-Rose lab, we are further studying the fiber architecture of different families in the order Carnivora. Regressions between the adductor muscle mass against the body mass will be performed. The Hartstone-Rose et al. 2012 felid study found that larger cats have relatively larger masticatory muscles than do smaller cats. Further analysis will be done to determine if similar trends exist in other carnivoran families as well.

Regressions between the fascicle lengths against the prey size will be executed. This is to determine if fascicle length has been adopted towards differing prey size.

CHAPTER 7

BROADER IMPLICATIONS

The goal of this methodology is to better understand masticatory adaptations through an examination of bite force and muscle fiber architecture in the various groups of carnivores. By understanding these in extant animals, we will be able to apply these methods and findings to extinct species as well as humans in the biomedical field. Here, they can be applied to better understand how chewing architecture is adapted to variation in dietary requirements.

Carnivorans need to have competent skull morphology, jaw mechanics, and dentition in order to capture, kill, and consume prey and, in some cases, vegetation. Evaluation of the masticatory system (jaws, soft tissues, and dentition) may show how these structures have been influenced by natural selection and have evolved differently (Gans et al., 1978). These differences can provide insights into adaptations for specific diets and mastication processes. In our research, we examine species in the order Carnivora that have diverse diets: herbivores, omnivores, piscivores, insectivores and true carnivores that specialize in the consumption of vertebrate flesh. By having such a diverse sample size, the results of this study can be applied to a many areas of specializations.

By examining the skull and jaw morphology in the order Carnivora, we can apply these findings to future studies involving humans. As in the order Carnivora, specialization in the skull and jaw morphology has been seen in the different feeding

habits in the order Primates (Radinsky, 1981). These differences can provide insights in specific diets and mastication processes. By understanding the applications shown in the order Carnivora, we should be able to apply these findings in masticatory studies in humans to provide further insights in human masticatory specialization.

These findings can be applied to aging of muscles in humans. As our muscles age, they begin to lose the elasticity, and strength (REF). Future studies can be executed to learn more about the individual fibers within these muscles. The variation of fiber lengths and the possible effects of these changes would be valuable information. For example, what diets are best suited for these changes.

Another future study, that would be valuable, would be the study on the variation based on dental work. Humans are the only species that have consistent dental work performed on their teeth. Braces shift teeth in a specific alignment, root canals drill holes in teeth, and other procedures change the dental structure. What are the consequences of these procedures on the masticatory muscle architecture? By understanding the manmade procedures done to our teeth will provide further insights in the human masticatory system.

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