Demonstrating Relationships between the Morphology of the Trigeminal System and Feeding Performance in the American Alligator: A New Tool for Understanding Feeding Evolution

By Kavita Jain

Personal Story

Ever since I was five years old I have always wanted to enter the medical field. Whenever someone asked me what I wanted to be when I grew up, the first and only profession I have answered it with was and is doctor. When entering high school, I realized I would finally have the opportunity to explore the research behind the medical field. The summer between ninth and tenth I took a science and research awareness seminar at Stony Brook University, which introduced me to various fields in science and medicine, some of which were hematology, cardiology and even robotic surgery. However, it wasn't until I was a part of my school's science and technology research class, where my dreams of research became reality. The summer following my sophomore year, I was able to start my research in evolutionary biology at Stony Brook University's esteemed department of Anatomical Sciences in the Medical School. Previously, a student in my school had conducted research in this field. Upon reading the student's paper, I became fascinated by the subject area and its implications to the world. Evolutionary biology has the potential to provide cures for diseases and conditions like AIDS and it sheds light upon Earth's past. While immense work has been done to build detailed phylogenies and learn more about the conditions of the Earth, including dominant species and clades; prevalent temperature; plant life etc., there is still much left to uncover. After researching more about this subject-area I contacted the professor and fortunately was able to start my research track. When I first learned that I would be research crocodilian evolution, I became especially intrigued because it was something completely new to me.

When first conducting this research, there was much I needed to learn. My first summer at SBU mainly involved reading background information, such as papers and dissertations, and becoming acclimatized with the different parts of the crocodilian and the software, Avizo 7.0, I would be using to study it.

Shortly after I had completed this project (about January of my junior year, 2012), I began working on my Intel research. In this research, I had to once again read up on background information, on similar topics, since previous work on this was never done before. However, I also had to review certain types of statistical analyses. I took an AP Statistics class in my school, however in addition to confidence intervals and R^2 values, I also had to learn about allometry, which is the rate at which one factor grows against another, and determine if my correlations were positively allometric, negatively allometric, or isometric. Statistical analyses were a large and integral part of the math involved in this work.

Doing this research project has only reinforced my love for math and science, and helped me envision my future vocation in science. Evolutionary Biology is an extremely diverse field, in which studies of evolution occur both on the anatomical and molecular level. The anatomical level of this incredible field includes research like mine, used to reveal more about Earth's past and creation, and to detail organism relationships using phylogenetic trees. This type of work reveals so much about the past that it is astounding. However, the other side of evolutionary biology—molecular evolution—is equally riveting. Molecular evolution also works to gain more insight about the past, but instead of working with an organism, it uses DNA. By doing research, I have been able to imagine potential paths for my future. In addition, I have always wanted to be a doctor, and studying the neuroanatomy of the American alligator added to my intrigue of neuroscience. Learning about the neuroanatomy of reptiles has helped me further develop my interest in neuroscience. Additionally, I have always been amazed by the pace of medical advances; the potential that evolutionary biology, whether that be at the anatomical and/or molecular level, provides for medicine is incredible.

This research experience reinforced my previous intentions of incorporating research throughout my education. I plan to continue doing research, and upon graduation from medical school, I would like to be able to start my own research lab in order to promote high school and college research along with a vocation as a neurosurgeon. I think it is very important for high school students who find science and math to be a passion to explore research. Although it may not be easy, it is definitely a beneficial and enriching experience. Research has never been a straightforward path for me. There are always factors slowing me down, pressuring me to stop and give up, yet it has taught me to persevere and overcome my obstacle. There were many chances in my research where I could have taken the easy way out, however I persisted in order to gain more knowledge and expertise in the area. I believe it is important for students to challenge themselves in order to realize their full potential. If I hadn't even thought about research I know that I would not be able to do half the things that I know I can do now, nor would I be where I am today. As a student who sees him/hereslf entering the scientific or mathematics field in the future you should do yourself a favor and start early. Not only will it help develop and further your passion, but also I believe it is crucial to advance your knowledge in an area that interests you in order to explore your opportunities in it. By integrating themselves in something you are passionate about, success is sure to follow.

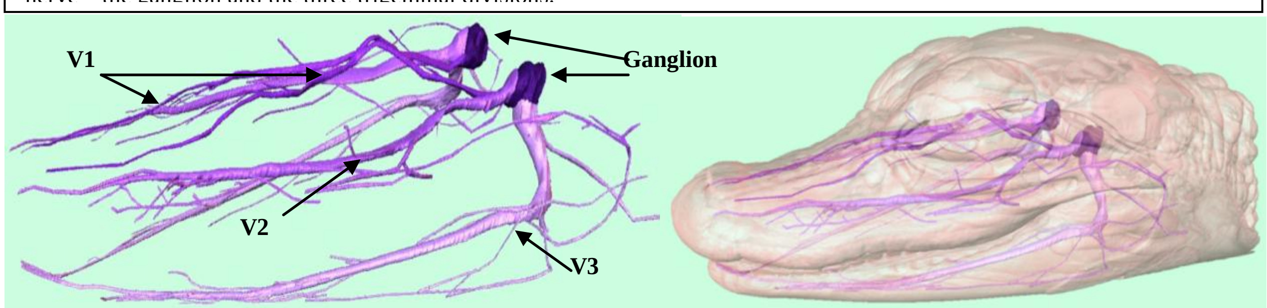
Research

Introduction

Archosaurs are an incredibly diverse and evolutionary highly successful group of organisms that include birds, crocodilians, and the now-extinct dinosaurs and pterosaurs (Brusatte, 2010). Their success as predators is particularly noteworthy. Not only were the long-extinct non-avian theropod dinosaurs the largest and most abundant terrestrial predators of the Mesozoic, but also during and since that period crocodilians have not only persisted but also dominated predatory aquatic and shore-line niches for more than 85 million years (Erickson et al., 2012). Like in many successful groups, feeding performance is a key reason for the evolutionary fitness of these animals (Erickson et al., 2003, 2012; Gignac, 2010).

The American alligator and its kin rely on the integration of their neuromuscular, skeletal, and dental systems to feed on and catch prey. Their postcranial anatomy (ascribed for movement) remains similar between crocodilians and different species, thus changes in feeding habits are thought to rely on developmental and evolutionary changes to the skull, jaws, and teeth, all of which affect performance (Erickson et al., 2012). Therefore, I studied four major components of feeding performance: body size, jaw muscles mass, bite force, and dome pressure receptor count. I compared these four factors to the morphology of the trigeminal nerve (length, cross-sectional area, mass). The trigeminal nerve (Figure 1), with its three major divisions—ophthalmic (V1), maxillary (V2), and mandibular (V3)—is not only the most complex and extensive cranial nerve in any vertebrate, but also its relationship to feeding performance has not yet been previously studied, making this a novel analysis.

Figure 1. This is a reconstruction of the trigeminal nerve and specimen head, displaying the basic anatomy of the trigeminal nerve—the ganglion and the three trigeminal divisions.



My work is broadly aimed at quantifying the relationship between the factors of feeding performance and the morphometrics of the trigeminal nerve in order to provide detailed neuroanatomical data on how crocodilians generate their impressive bite forces and, to then utilize this information to predict developmental and evolutionary changes to feeding performance in Archosaurs .

Materials and Methods

The alligator specimens were obtained from the Louisiana Department of Wildlife and Fisheries in Cameron County, Louisiana. Bite forces were directly measured by the principle investigator using protocols established in Erickson et al. (2003). In addition to bite force, standard body morphometrics were also taken. Body size (total length, snout-vent length, and body mass) was directly measured with a tape measure, digital calipers, and an electronic scale. After all experiments were completed and all measurements were taken, each specimen was euthanized, heads were removed and fixed in neutral buffered formalin for two weeks, and then placed in asolution of Lugol's iodine for iodine-enhanced mircoCT imaging. Specimens were stained with iodine in order to increase visualization of cranial tissue, including the trigeminal nerve. However, in order to ensure that the staining procedures didn't have any significant effects on mass, two specimens were dissected and various tissues were extracted; mass change in iodine was observed over a period of four weeks.

Using the iodine-enhanced microCT data, the trigeminal nerve of five specimens were constructed and the branches were measured for lengths, cross-sectional areas, and volumes through the software Avizo 7.0. Along with computer models of the trigeminal nerve, the five specimen heads were dissected using standard gross dissection techniques. The eight jaw adductor muscles were photographed and then extracted and weighed. In addition, high-resolution photographs provided a means for counting the dome pressure receptors.

Using Microsoft Excel 2007, the collected data were log transformed and regression analyses were constructed to test how the morphologies of the trigeminal nerve and ganglion change throughout growth and how trigeminal morphometrics scale against body mass and length, jaw adductor muscle mass, dome pressure receptor count, and bite force. The scaling relationships provide a means to see trigeminal nerve morphometrics (e.g. length, cross-sectional area, mass) changed compared to another biological factor (e.g. total body length, body mass) and how the growth of the structures compared to isometry (the hypothesized relationship).

Scaling relationships are determined by measuring the slopes of the best-fit line. Additionally, 95% confidence intervals (CI) were constructed around the lines of best fit to account for natural variation and measurement errors; they reveal if the isometric slope is statistically significant to the slope of the data. If the isometric slope falls within the CI, the data can be confidently claimed to be isometric. However, if it is higher than the upper bound of the CI, the data would be declared positively allometric (meaning the parameter increases in size at a rate faster than body size alone would predict). If it were lower than the lower bound of the CI, the data would be declared negatively allometric (meaning the parameter increases in size at a rate slower than body size alone would predict).

Results and Discussion

The first test performed was to verify that the iodine stain had a negligible effect on tissue mass, and it was found that the iodine did indeed have a negligible effect on tissue mass. As shown below (Figure 2), all changes have standard errors less than 5% of tissue mass, allowing accurate mass estimations, using Avizo 7.0, from already stained individuals.

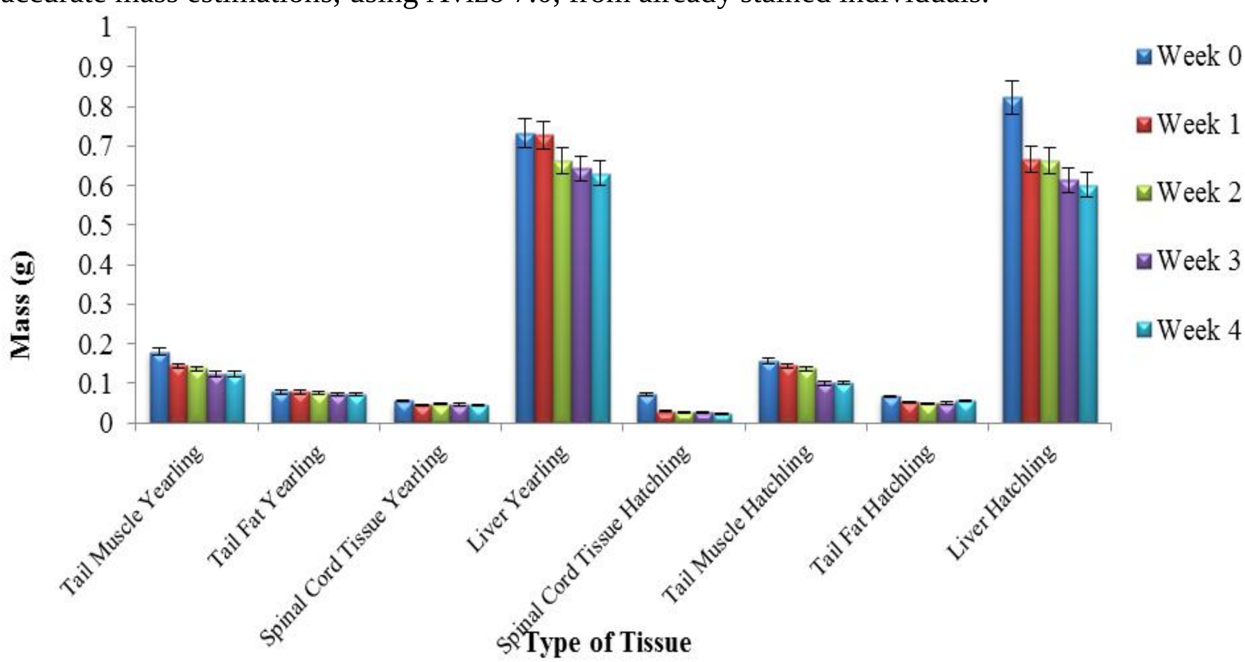


Figure 2: The effect of Iodine solution on the tissue mass of yearling and hatchling *Alligator mississipiensis*. This graph shows that all mass changes were negligible with iodine staining under standard error of 5%. All tissues underwent some level of mass change, as many "wisp-like" pieces detached from the main body. Each set of bars represents the change in mass for each type of tissue. Blue represents Week 0, red represents Week 1, green represents Week 2, purple represents Week 3 and light blue represents Week 4.

Mass estimates were plotted against total body mass, jaw adductor muscles mass, dome pressure receptor count, and bite force (Figure 3).

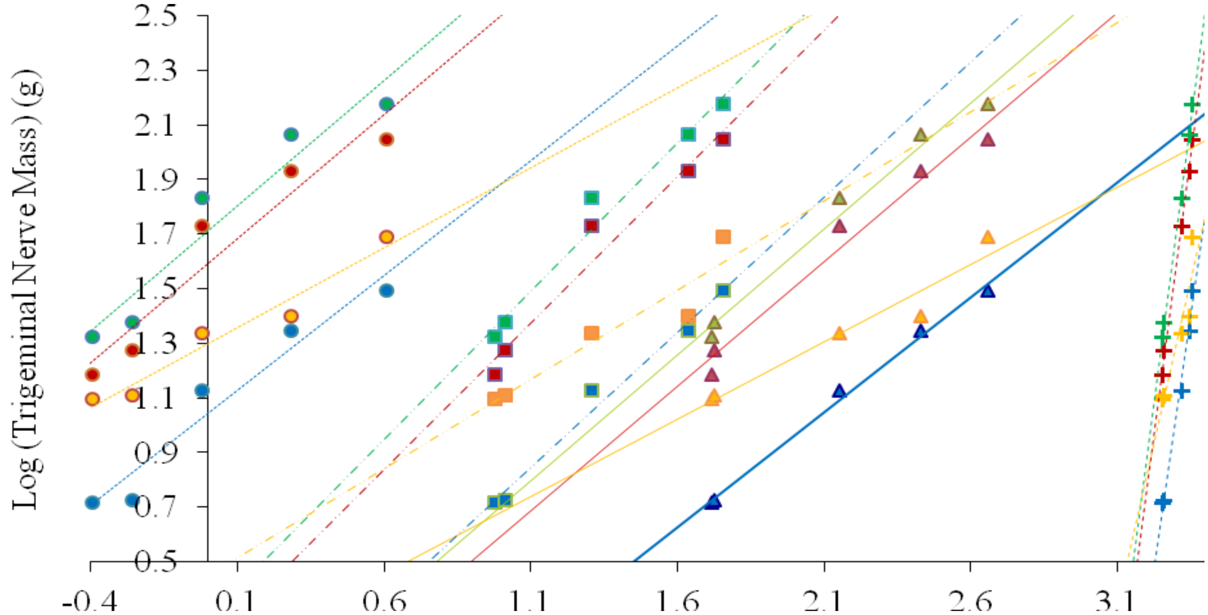


Figure 3: The relationship between trigeminal and ganglion mass and various biological factors. The four sets of regression lines (left to right) demonstrate: 1) trigeminal mass vs. jaw muscles mass, 2) trigeminal mass vs. bite-force, 3) trigeminal mass vs. body mass, and 4) trigeminal mass vs. dome pressure receptor count. The circles, squares, triangles, and pluses represent the above mentioned respectively. The blue lines represents V1, red represents V2, green represents V3, and orange represents the ganglion.

Similar to trigeminal mass, trigeminal division cross-sectional area was correlated to total body mass, jaw adductor mass, dome pressure receptor count, and bite force (Figure 4).

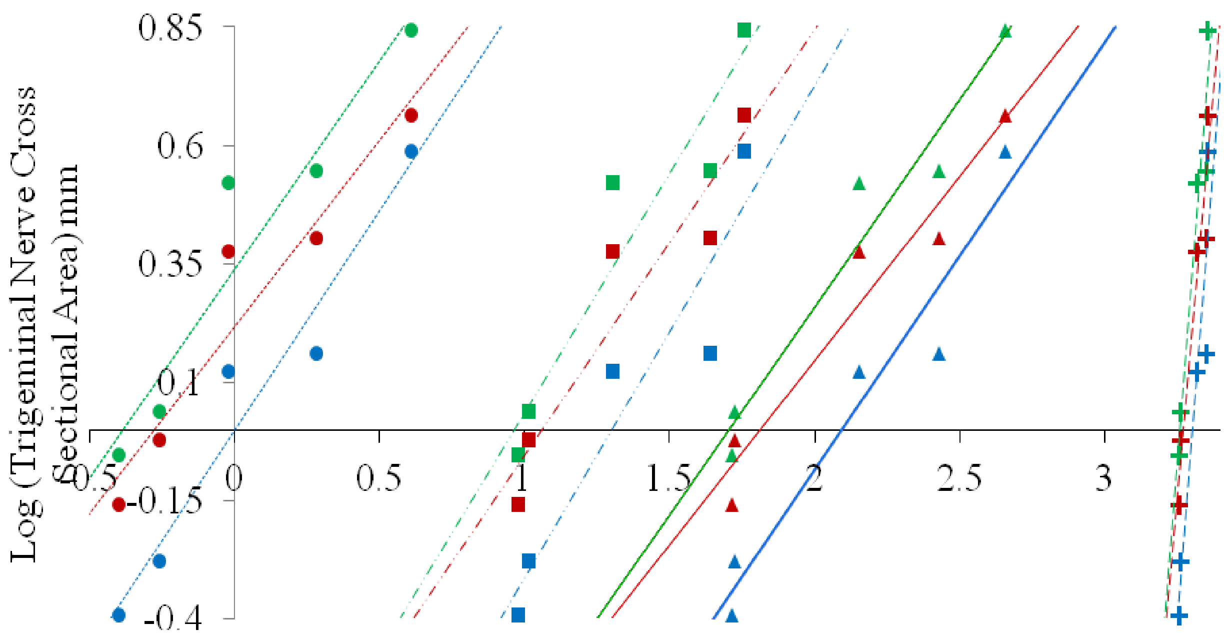


Figure 4: The relationship between trigeminal area and various biological factors. The four sets of regression lines (left to right) demonstrate: 1) trigeminal area vs. jaw muscles mass, 2) trigeminal area vs. bite-force, 3) trigeminal area vs. body mass and, 4) trigeminal area vs. dome pressure receptor count. The circles, squares, triangles, and pluses represent the above mentioned respectively. The blue lines represent V1, red represents V2, and green represents V3.

Furthermore, trigeminal division length was scaled against snout-vent length, jaw adductor muscles mass, dome pressure receptor count, and bite force (Figure 5). Snout-vent length does not include the length of the tail, which can vary greatly from individual to individual and negates the effects of natural variation in length among the specimens (including the loss of distal tail during intraspecific combat, which is common among crocodilians).

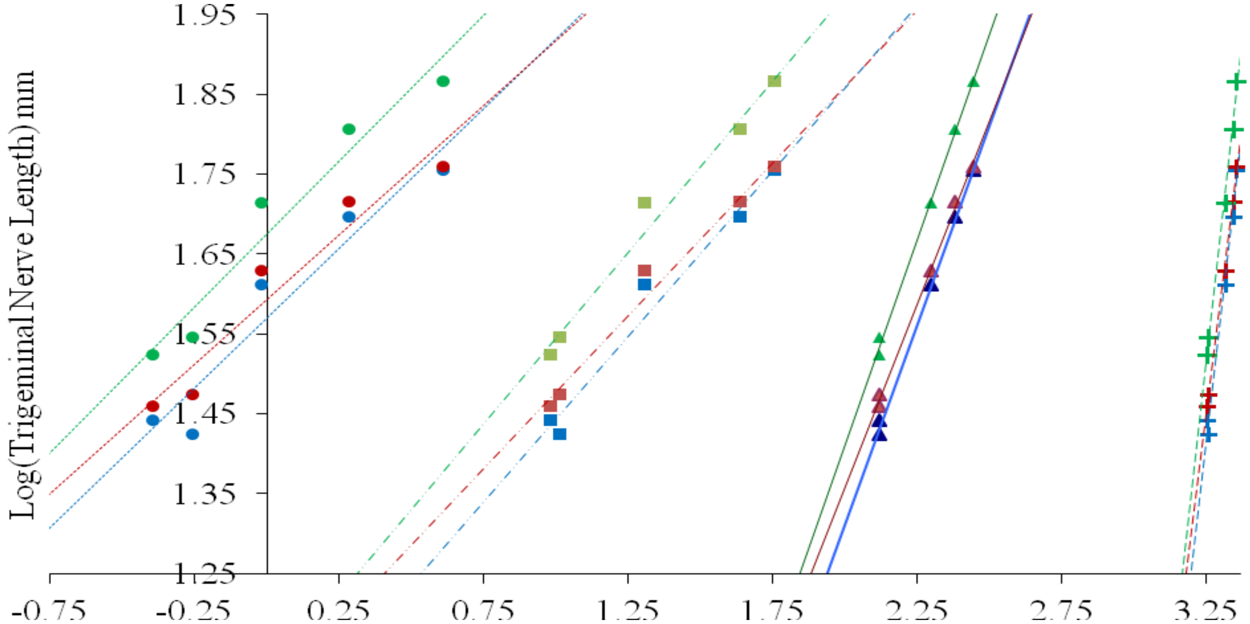


Figure 5: The relationship between trigeminal length and various biological factors. The four sets of regression lines (left to right) demonstrate: 1) trigeminal length vs. jaw muscles mass, 2) trigeminal length vs. bite-force, 3) trigeminal length vs. body mass and, 4) trigeminal length vs. dome pressure receptor count. The circles, squares, triangles, and pluses represent the above mentioned respectively. The blue lines represent V1, red represents V2, and green represents V3.

When scaling the morphometrics of the trigeminal nerve against various biological factors, the results showed that the trigeminal nerve strongly correlates to overall body size measurements as well as size of the jaw-closing musculature and bite-force performance with all correlation coefficients (R^2) having values ≥ 0.90 (Table 1).

Table 1: This table shows the different scaling relationships between trigeminal morphometrics (TM = trigeminal mass, TL = trigeminal length and TA = trigeminal area) and various biological parameters. This shows the slope of the regression line and the confidence interval. V1 = ophthalmic division V2 = maxillary division V3 = mandibular division

TM vs. Body Mass	TM vs. Jaw Muscles Mass	TM vs. Bite-force	TM vs. Dome Pressure Receptors
V1: .842 ± .108	V1: .841 ± .385	V1: .991 ± .241	V1: 7.432 ± 1.541
V2: .913 ± .275	V2: .909 ± .506	V2: 1.073 u .398	V2: 8.168 ± 1.168
V3: .922 ± .228	V3: .918 ± .487	V3: 1.087 ± .33	V3: 8.228 ± .877
Ganglion: .567 ± .258	Ganglion: .586 ± .238	Ganglion: .654 ± .412	Ganglion: 4.77 ± 3.682
TL vs. Snout-vent Length	TL vs. Jaw Muscles Mass	TL vs. Bite-force	TL vs. Dome Pressur Receptors
V1: .998 ± .097	V1: .349 ± .193	V1: .414 ± .131	V1: 3.125 ± .685
V2: .918 ± .087.	V2: .324 ± .46	V2: .383 ± .101	V2: 2.884 ± .399
V3: 1.028 ± .091	V3: .365 ± .163	V3: .4295 ± .108	V3: .429 ± .108
TA vs. Body Mass	TA vs. Jaw Muscles Mass	TA vs. Bite-force	TA vs. Dome Pressure Receptors
V1: .903 ± .417	V1: .929 ± .412	V1: 1.04 ± .668	V1: 7.734 ± 5.281
V2: .778 ± .36	V2: .788 ± .455	V2: .9 ± .558	V2: 6.57 ± 3.435
V3: .877 ± .373	V3: .882 ± .511	V3: 1.012 ± .615	V3: 7.716 ± 3.7

In addition to constructing the twelve logarithmic regressions, the relationships were also characterized as either isometric, positively allometric, or negatively allometric. By comparing the constructed correlations to the ideal correlation the relationship should show, it was deemed that four of the relationships were negatively allometric, six were positively allometric, and two were isometric (Table 2). While a relationship may be characterized as other than isometric, it does not negate the fact that there is still a strong relationship between the two factors.

Table 2. Trigeminal Nerve Morphometrics vs. Biological Factors This table shows the scaling relationships for the correlations between the trigeminal nerve morphometrics (mass, length, cross-sectional area) and various biological factors (body mass, jaw muscles mass, bite force, Dome Pressure Receptor (DPR) Count

Negative Allometry	Isometry	Positive Allometry
Mass vs. Jaw Muscles Mass Mass vs. Body Mass Length vs. Bite force Area vs. Bite force	Length vs. Jaw Muscles Mass Length vs. Snout-vent Length	Mass vs. Bite force Mass vs. DPR Count Length vs. DPR Count Area vs. Jaw Muscles Mass Area vs. Body Mass Area vs. DPR Count

Trigeminal length vs. jaw adductor muscles mass and trigeminal length vs. snout-vent length, were the only two relationships to scale isometric, meaning that the correlations found were close to ideal/expected. This also indicates that jaw muscles mass and snout-vent length can quite accurately be predicted by trigeminal length. Trigeminal length vs. bite force scale negatively allometric and trigeminal length vs. dome pressure receptor count scaled positively allometric indicating that these parameters are not well related functionally or developmentally.

Trigeminal mass vs. jaw adductor muscles mass and trigeminal mass vs. body mass scale negatively allometric; this shows the important role the trigeminal nerve plays in young alligators, which weigh less than adult alligators, particularly through its dual function of activating the jaw muscles and returning crucial sensory information about prey location. On the other hand, trigeminal mass vs. bite force and trigeminal mass vs. dome pressure receptor count scale positively allometric. Dome pressure receptors and bite force also seem to grow along similar trajectories in accordance to the trigeminal nerve therefore hinting at the developmental integration of these systems. Bite force and dom e pressure receptors arise from the skeletal and dermal embryonic layers respectively, and although they come from different tissue layers, they may actually have developmental integration, suggesting that dome pressure receptors evolved from the bite force system.

The last aspect of the trigeminal nerve that I studied was its cross-sectional area. Cross-sectional area scales positively allometric against body mass, jaw muscles mass, and dome pressure receptor count implying that the trigeminal nerve is accommodating an increasing number of axons as it innervates a greater volume of head tissue. In addition, although cross-sectional area scales negatively allometric against bite force, it just means that other factors other than muscle contraction, which are not directly influenced by the trigeminal nerve, affect bite force.

This work is an important, first step directed towards providing detailed neuroanatomical and musculoskeletal data on how crocodilians generate their impressive bite forces and then can be utilized to draw conclusions about the choices of prey and feeding preferences in both living and extinct archosaurs. Understanding how the trigeminal nerve relates to the structures it innervates is crucial for understanding how this most extensive nerve of the head can inform us about the paleobiology of fossil archosaurs.

Information gleaned from this study could be compared to available information in the literature for all other extant crocodilian species in order to draw inferences about life history and feeding ecology in living and fossil forms. From these data and results, I hope to describe the anatomical and functional aspects of a major neuromuscular system in a highly successful group of living vertebrates. In the future, this will aid in further study of the evolution of this system and its predictive potential to address similar questions in other living crocodilians and in their fossilized kin, dinosaurs.

Summary, Conclusions, and Future Work

Iodine staining paired with 3-D digital reconstructions are a relatively new, yet highly effective means of analyzing delicate anatomical structures that may have critical functions in the life history and evolution of vertebrates. Such is the case for the feeding functional morphology and evolutionary history of crocodilians. The i-e µCT staining allows for complete and much more detailed visualizations of the alligator trigeminal system. Using Avizo, the 3-D computer visualizing software, i-e µCT scans were rendered to quantify the size and distribution of the trigeminal nerve in order to understand the developmental changes to trigeminal morphometrics. These results were compared to overall body dimensions, jaw muscle sizes, bite-force performance and dome pressure receptor count for the corresponding individuals of

American alligators. Results showed that the trigeminal nerve development is predictably highly correlated to the growth of the tissues it innervates. This research represents an innovative and integrated approach to studying the development of feeding performance in any vertebrate group.

Crocodilians are a highly successful group with an 85 million year reign. Studying their living descendants provides a great deal of information on the lifestyles of their fossil precursors. These strong relationships between the anatomical structures and performance variables sampled suggest that in the future paleontologists could use this research to make predictions about the mass of the jaw-closing muscles, in fossil crocodilians and other Archosaurs, and their bite-force capacities.

One way to do this would be to use the various cranial foramina provided by the bones of the extinct organism. The trigeminal nerve exits the brain cavity through several foramina that capture the cross-sectional areas of its three major branches (Holliday and Witmer, 2007). Using the area of the various foramina, such as the foramen ovale, the trigeminal cross-sectional area relationships could be applied to the extinct specimens, substituting the trigeminal area with foramen area. Once the relationships are applied, bite force, dome pressure receptor count, jaw muscles mass and body mass can be found for extinct specimens.

Demonstrating that such relationships exist in a living crocodilian system strengthens our ability to assess similar relationships in other ecologically comparable and evolutionarily related taxa. The strongest inferences in paleontology are built in the most well established biology of living analogues, and with these new imaging methods we stand to make even greater strides in addressing the paleobiology of fossil vertebrates.

Aligned with these goals, I plan to expand these findings into a broader evolutionary context and utilize the methodologies discussed here to explore the archosaur fossil record and further uncover how the jaw system, bite-force performance, and feeding ecologies of these impressive organisms helped them become dominant predators for more than 200 million years. Moreover, by learning more about the feeding evolution of archosaurs, we can learn more about the Earth’s past and what life was like on Earth. By being able to predict feeding performance—heavily influenced by body mass, bite force, jaw musculature mass, and dome pressure receptors in crocodilians—we can also learn about changes on the Earth—what types of animals were present and what type of environments were prevalent on Earth.

References

Brusatte, S. L., Benton, M. J., Desojo, J. B., & Langer, M. C. (2010). *The Higher-Level Phylogeny of Archosauria (Tetrapoda: Diapsida).* Journal of Systematic Palaeontology, 8(1), 3-47.

Erickson, G. M., Gignac, P. M., Steppan, S. J., Lappin, K., Vliet, K. A., Brueggen, J. D., Inouye, B.D., Kledzik, D., Webb, G. J. W. (2012). *Insights into the Ecology and Evolutionary Success of Crocodilians Revealed through Bite-Force and Tooth-Pressure Experimentation.* PLoS ONE, 7(3).

Erickson, G. M., Lappin, A. K., & Vliet, K. A. (2003). *The ontogeny of bite-force performance in American alligator (Alligator mississippiensis)*. Journal of Zoology, 260(3), 317-327.

Gignac, P. M. (2010). *Biomechanics and the ontogeny of feeding in the American alligator (Alligator mississippiensis): reconciling factors contributing to intraspecific niche differentiation in a large-bodied vertebrate* (Unpublished doctoral dissertation). The Florida State University, FL.

Holliday, C. M., & Witmer, L. M. (2007). *Archosaur Adductor Chamber Evolution: Integration of Musculoskeletal and Topological Criteria in Jaw Muscle Homology*. Journal of Morphology, 268(6), 457-484.