Pharyngeal Jaws: An Explanation to the Rapid Diversification of Seven African Cichlids

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ABSTRACT

Understanding the evolution of species is very important because it allows us to know how animals interact with their environment over time. To better understand the evolution of a famous adaptive radiation of fish, I compared both the upper and lower pharyngeal jaws of seven cichlid species from lake Barombi Mbo in Cameroon. The goal of this research was to see if there was significant variation in the tooth density between jaws of different species. I was able to fully extract the jaws using tweezers, measure the standard length of each fish and take a photo of the specimen. Using an AmScope microscope, both lower and upper pharyngeal jaws were captured with high resolution so that the teeth could be counted and I could extrapolate the density (teeth per square millimeter) for the entire jaw. The standard length was plotted against teeth density to ensure that size did not affect my metric of interest. No slopes were greater than the absolute value of one so it was determined that standard length did not effect tooth density. Then tooth density was plotted for each species and a general lineal model was run to determine if the results were significant. For all 7 species with the exception of *Stomatepia mongo*, there were significant differences in the teeth per square millimeter in either the upper or lower pharyngeal jaws. These findings are an important contribution to our understanding of speciation as they occurred rapidly and without any clear geographic barriers.

KEYWORDS

INTRODUCTION

Africa is home to some of the largest and oldest freshwater lakes in the world (Odada et al. 2002), each hosting a variety of different fish species over millennia. Today, one family of fish in particular, thrives in these lakes, cichlids (CAS 2021). In many African lakes, cichlids have experienced adaptive radiations (Meier et al. 2017), rapidly diversifying into specialized species, all stemming from a few common ancestors. To understand how this family grew so quickly across the continent, it is crucial to look for evidence in a smaller, more manageable environment. A crater lake of Cameroon, Barombi Mbo, stands out as a perfect environment for studying adaptive radiations in cichlids. What makes this lake so applicable is the fact that it houses 11 species of endemic cichlids yet has a diameter of only 2.5km (Musilova et al. 2019). In such a small space, it is difficult to reason how one or two ancestral fish could diversify into eleven independently breeding species with unique diets (Galvez et al. 2021).

However, due to their specialized diets, part of cichlids' rapid diversification could be attributed to their unique set of pharyngeal jaws which aid in prey compression and digestion (Myer 1993). Unlike the main set of jaws, which help with prey capture (Shadwick and Lauder 2006), the pharyngeal jaws operate as a grinding plate to further break down food before it enters the stomach (Peterson and Müller 2018). While this element is not unique to cichlid fish, there are novel differences in structure from other pharyngeal jaw bearing fish. In particular, cichlids' lower pharyngeal jaw is fused as one ceratobranchial which gives it increased crushing ability (Hulsey et. al 2006). It is widely believed that this further modification of pharyngeal jaws has facilitated the explosion of cichlid radiations across African freshwater lakes (Ronco and Salzburger 2006).

Lake Barombi Mbo, however, differs from other locations that fostered cichlid radiations. Because of its symmetry and size, the existence of 11 closely related cichlids which all inhabit an almost uniform environment is very unlikely. It has long been thought that geographic isolation is a necessary component for speciation, however, lake Barombi Mbo provides little opportunity for its inhabitants to isolate themselves, so how has one species turned into 11?

I turned to the pharyngeal jaw as possible explanation to the diverse community of

cichlids living in sympatry in lake Barombi Mbo. I examined the diversity in pharyngeal jaw morphologies and assessed if there was a difference in teeth density for both the upper and lower jaws between species. My main question became; Is there is significant difference in the tooth morphology of endemic Barombi Mbo cichlid species? In order to answer this question, I focused my research on a few more feasible, testable, questions which are outlined as follows. 1.) What are the standard lengths of each individual fish being studied? And, 2.) How many teeth are present per square millimeter in in both the upper and lower pharyngeal jaws for each fish? I hypothesize that by answering these questions, I will find that there is indeed a significant difference in the tooth morphology of the seven Barombi Mbo cichlids.

METHODS

Measurements and Dissection

Measurements were taken from 26 individual fish from 7 species of endemic Barombi Mbo cichlids which included: *Konia eisentrauti*, *Pungu maclareni*, *Sarotherodon linnellii*, *Sarotherodon caroli*, *Stomatepia mariae*, *Stomatepia mongo* and *Stomatepia pindu* (Schliewen and Klee 2004). The first measurement taken was the standard length. This was calculated using calipers which extended from the tip of the jaw to the caudal peduncle (Kahn et. al 2004). After this metric was recorded, a photo was taken of the fish on a glass plate with a ruler, in case anything happened to my data or I needed to double check a measurement (Fig 1).

Figure 1. Stomatepia Mariae photographed with a ruler and label

The next part of my process involved removing the pharyngeal jaws from the fish. I began doing this my removing the head of the fish and taking the jaws out from the back of the

head. While this worked well, it made the decapitated specimens unusable for further research. To make the extraction more sustainable, I instead began lifting up the gill covers, removing gill rakers, and then using tweezers to detach the upper and lower pharyngeal jaws and pull them out through the gills. While this method made a clean extraction more difficult and time consuming, it kept the specimens intact and ensured their integrity for future studies.

Once removed, I then cleaned the jaws to the best of my ability, removing any particles on the teeth or excess tissue surrounding the jaws. The cleaned jaws went on to be dried with paper towels and placed under a microscope for high resolution imaging. By using the microscope, I was able to get clear enough images to count the number of teeth in a certain area. With image J. (Schneider et. al 2012), I traced a small square where the teeth were visibly the most dense and counted the number of teeth within that square (Figs 2 and 3).
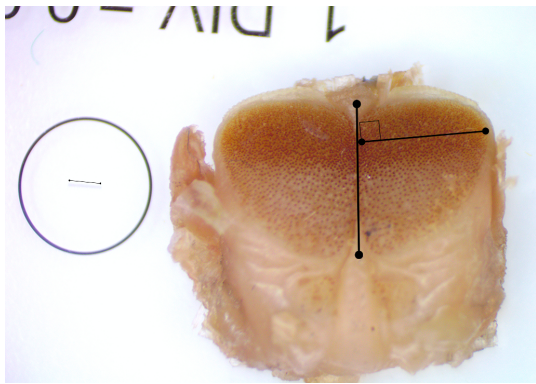

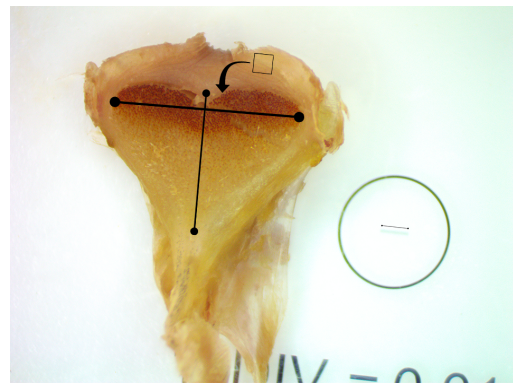**Figure 2**. Upper pharyngeal jaws with box showing location of tooth count

Figure 3. Lower pharyngeal jaws with box showing location of tooth count

I then used the segmented line tool to trace a calibration distance of .01 mm and used this calibration to convert the area of the square in pixels^2 to mm^2. The final metric, teeth per square millimeter was recorded for each fish using the following formula in excel.

Teeth/ Sq mm = (# of teeth in box) (calibrator length PIX) / [(Area of box, PIX) (calibrator length, mm)]

While tooth density was my main metric of interest, I also measured the overall size of each pharyngeal jaw. For the upper jaws, I again used ImageJ to trace the area of the right side of the pharyngeal jaw. The area being measured was standardized by only including the regions

which had teeth. This meant excluding any excess tissue and only focusing on the clearly defined borders of the toothed region. In some fish, there were 4 to 8 teeth sparsely situated outside of the toothed region. In these cases, those teeth were considered outliers and were not included in the area calculation. ImageJ provided an area in pixels squared which I converted to millimeters squared using the .01mm calibration line shown in figures 2 and 3 and multiplied by 2 in order to account for the other half of the pharyngeal jaws which were broken or damaged in some fish.

Taking the area of the lower jaws proved to be much more difficult. For some fish, like the *Pungu maclareni* shown in figure 3, there were small black teeth covering the bone at the very end of the jaw. Other fish, however, lacked these teeth and thus the area of tooth cover would be very different depending if the fish had them or not. In order to standardize my measurement method, I had to omit the small black teeth from my "area of tooth cover" measurement. In contrast to the upper jaw, I took the full area of tooth cover as opposed to half multiplied by two because there was no separation between sides in the lower jaws and in no case were the jaws broken or damaged on one side.

Statistical Analysis

In order to determine if body size played a role in determining tooth density, I used R to create a scatterplot by groups, comparing teeth/sq mm and standard length. Before running the code I decided that if the absolute value of any slope was below 1, it would mean that there is effectively no correlation between size and tooth density for that species.

Lastly, to compare the results of my data collection, I used R to create a Poisson distribution for the number of teeth per square millimeter for each species. This was done by coding for a general linear model using two variables from my dataset: Scientific Name and Teeth/sq mm. This test was conducted a total of two times, one test for the upper pharyngeal jaws and one for the lower pharyngeal jaws. P-values of less than 0.05 were determined to denote significance.

RESULTS

Average Tooth Density

I began the analysis of my data collection by finding the average tooth density on the UPJ and LPJ for each fish species (Table 1). I found that *Sarotherodon linelii* had the greatest average tooth density for both the UPJ and LPJ while *Sarotherodon caroli* had similar tooth density in the LPJ category (Table 1).

Table 1: Average teeth per millimeter in the UPJ or LPJ for 7 cichlid species

Species	Teeth per mm^2 UPJ	Teeth per mm^2 LPJ
Konia eisentrauti	25.926	19.545
Pungu maclareni	12.785	9.468
Sarotheridon linnelli	85.152	69.27
Sarotherodon caroli	61.536	63.925
Stomatepia mariae	19.221	12.291
Stomatepia mongo	21.748	15.916
Stomatepia pindu	25.789	11.645

Testing Significance of Teeth per Square Millimeter

To see if these averages were significantly different from each other, I ran a general lineal model in R. I found that, for the UPJs, almost all averages were significantly different (having at least one asterisk) with the exception of pindu and mongo, both members of the Stomatepia genus (Table 2, Figure 4).

Table 2: Poisson test for the difference in means across 7 species' UPJ

Species	Estimate	Std. Error	z value	p value	
Konia eisentrauti	3.255	0.098	33.151	<2e-16	***
Pungu maclareni	-0.707	0.132	-5.35	8.81e-08	***
Sarotherodon linnellii	1.189	0.106	11.177	<2e-16	***
Sarotherodon caroli	0.864	0.106	8.143	3.86e-16	***
Stomatepia mariae	-0.299	0.122	-2.456	0.014	*
Stomatepia mongo	-0.176	0.132	-1.336	0.182	
Stomatepia pindu	-0.005	0.116	-0.046	0.964	
Significance Codes:	***(0.001)	**(0.01)	*(0.05)		

Running a GLM in R showed that, for the upper pharyngeal jaws, there were significant differences, p-values less than 0.05, for species: *K. eisentrauti, P. maclareni, S. caroli, S. linnelii* and *St. mariae.* The results of this test coincide with the graph in figure 4 as *St. mongo* and *St. pindu*, which were deemed non-significant, display very similar average tooth density values and similar boxplot shapes.

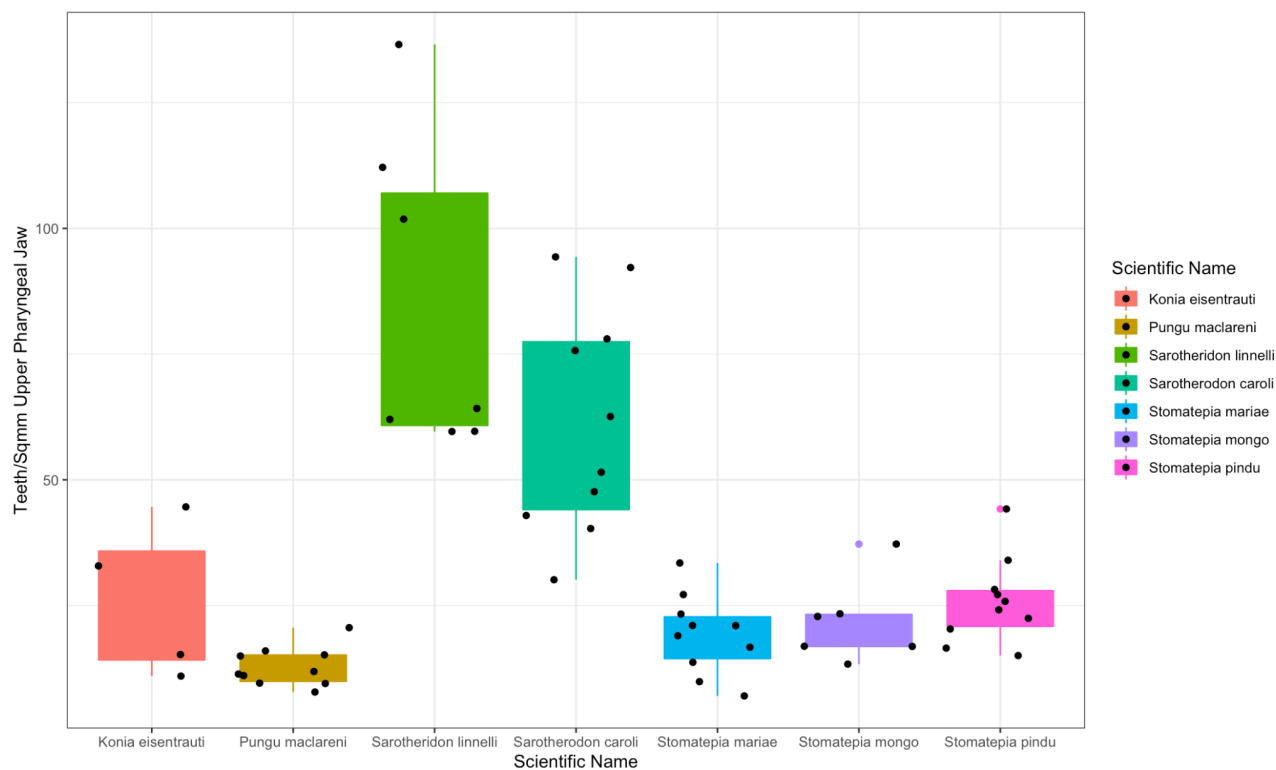**Teeth per Square Millimeter in the UPJ**

Figure 4. For each of the seven species being studied (x-axis), the number of teeth per square millimeter in their upper pharyngeal jaws is given on the y-axis.

The boxplot in figure 4 shows an incredible range of tooth density in a single square millimeter which spans from 9 teeth in the upper jaws for *Konia eisentrauti* to 137 teeth for *Sarotherodon linelii*. Looking at this graph, it is clear that *S. linnelii* and S. *caroli* have the greatest number of teeth per square millimeter in their upper pharyngeal jaws. In addition to showing these two extremes, figure 4 allows us to view differences in sample size. For example, the 4 black points for Konia eisentrauti versus the 10 points for most other species. This graph allowed me to clearly see that S. linnellii and caroli are the largest outliers, having an average teeth per square millimeter much greater than the other species being studied. This is consistent with the results of the GLM as these two species had some of the lowest p-values, denoted by three asterisks in table 2.

I continued this analysis by running an identical GLM focused on the lower pharyngeal jaws (Table 3, Figure 5).

Table 3: Poisson test for the difference in means across 7 species' LPJ

Species	Estimate	Std. Error	z value	p value	
Konia eisentrauti	2.973	0.113	26.284	<2e-16	***
Pungu maclareni	-0.725	0.153	-4.743	2.11e-06	***
Sarotherodon linnellii	0.982	0.125	7.88	3.26e-15	***
Sarotherodon caroli	0.896	0.122	7.345	2.05e-13	***
Stomatepia mariae	-0.464	0.145	-3.206	1.34e-03	**
Stomatepia mongo	-0.205	0.153	-1.347	0.178	
Stomatepia pindu	-0.518	0.146	-3.542	3.98e-04	***
Significance Codes:	***(0.001)	**(0.01)	*(0.05)		

For almost all species, significance levels remained constant from the upper to lower pharyngeal jaws with the exception of *Stomatepia mongo*. Once again, I used a box and whisker plot to visualize the results of the GLM (Figure 5).
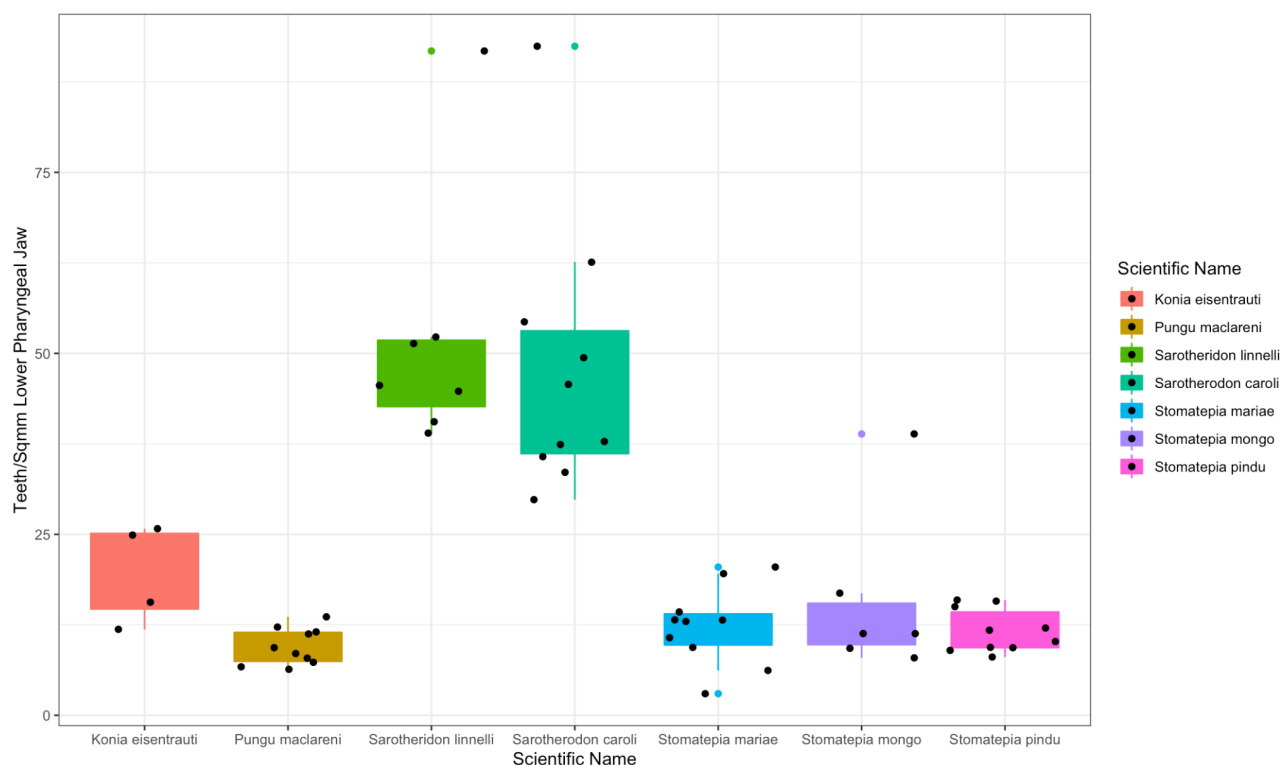
Teeth per Square Millimeter in the LPJ

Figure 5. For each of the seven species being studied (x-axis), the number of teeth per square millimeter in their lower pharyngeal jaws is given on the y-axis.

Once again, *Sarotherodon linnellii* and *caroli* show tooth densities much greater than the rest. However, one thing that this graph allowed us to see is the extreme outliers within *S. linnelii and caroli*. These two data points were revisited multiple times to ensure that they were not the result of data collection error. Another species with a visible outlier was *St. mongo*. There is one point that rises high above others and creates some ambiguity between the *mongo* distribution and that of the intercept, *Konia eisentraut*i. This could explain why *St. mogo* was the only species to not show a significant difference in tooth density for the LPJ (Table 3).

Effects of Standard Length

Next, I ran a regression to assess the relationship between standard length of each fish and its corresponding tooth density in both UPJ and LPJs (Figure 6). By doing this I was able to assess if size of the fish played a role in tooth density. In the context of this scatterplot, if the the

absolute value the regression equation (slope) is less than one, it means there is no relationship between tooth density and standard length. 6 species seem to reflect this, however, there is a notable negative relationship for *Sarotherodon linnelii*. Although it may seem like a large correlation visually, the slope was only -0.5.

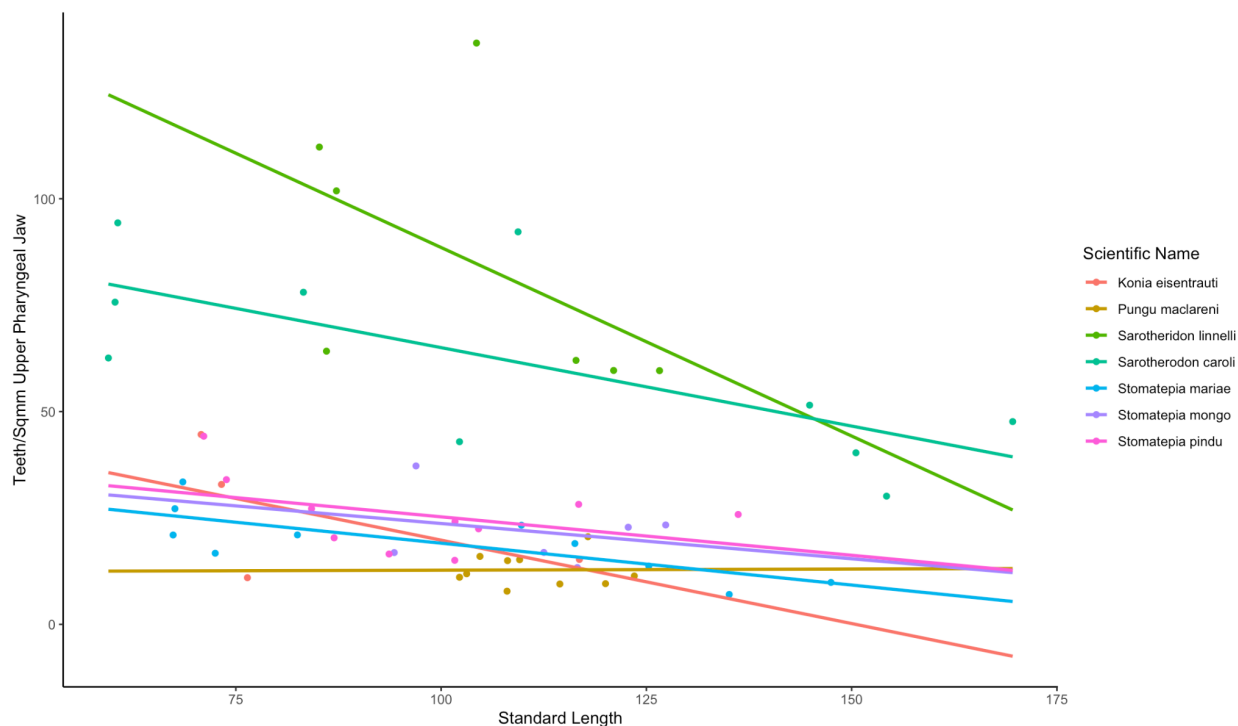**Teeth Per Square Millimeter (UPJ) Versus Standard Length**

Fig 6.Teeth/sq mm in upper pharyngeal jaws for each member of seven cichlid species versus the standard length of each individual fish.

A linear regression for the lower pharyngeal jaws yielded similar results with 7 species displaying very small negative slopes (Figure 7). The largest slope in this graph was achieved by *Stomatepia mongo* and was only -0.366.
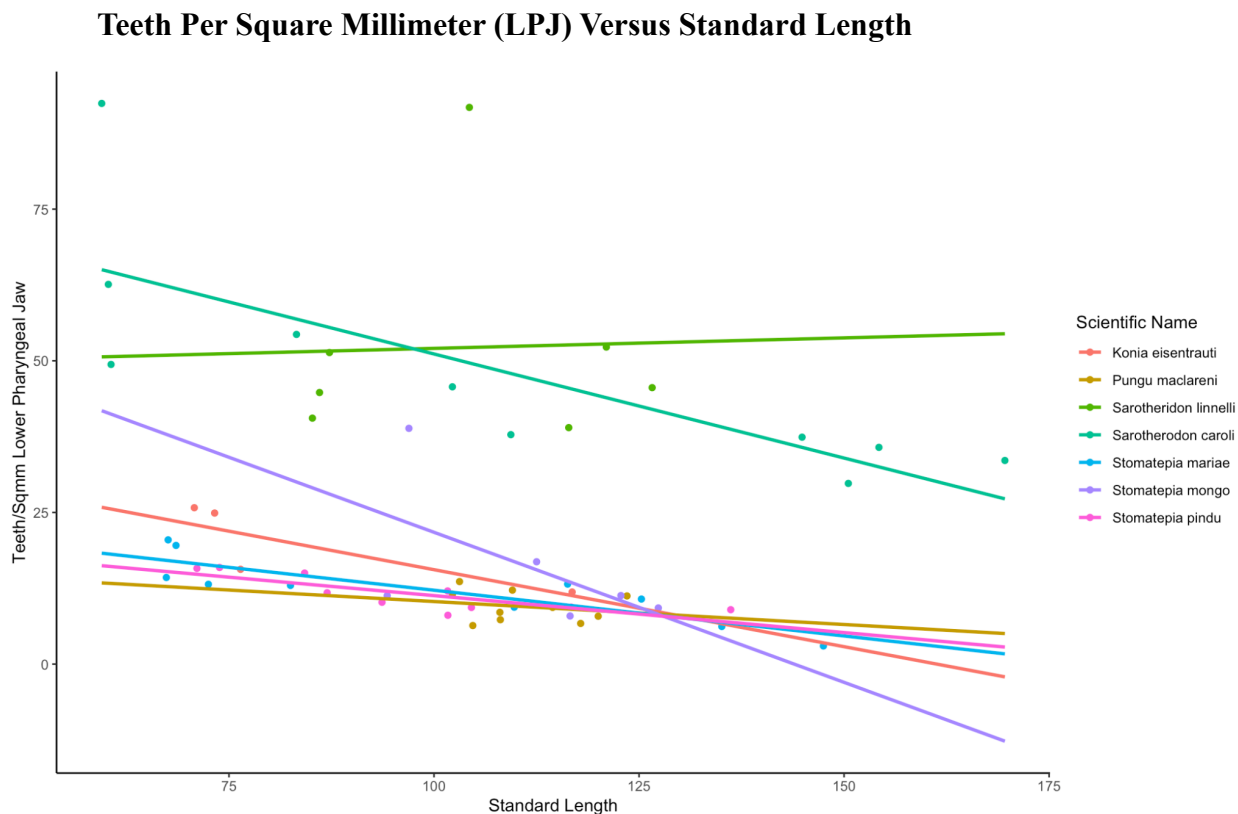
Teeth Per Square Millimeter (LPJ) Versus Standard Length

Fig 7. Teeth/sq mm in lower pharyngeal jaws for each member of seven cichlid species versus the standard length of each individual fish.

DISCUSSION

My results provide convincing evidence that the pharyngeal jaws of these seven species do differ from each other. I came to this conclusion based off the three metrics measured during the course of my study. These were, tooth density in the upper pharyngeal jaw (UPJ), tooth density in the lower pharyngeal jaw (LPJ) and standard length of the fish. Because each species specializes in a different diet item, it would make sense that they would have evolved unique pharyngeal jaws, the appendage crucial in breaking down food. My results show that tooth density does differ between species, giving each member of the crater lake community the ability to specialize on unique a diet item and thrive in an almost homogeneous environment.

Differences in Pharyngeal Jaw Tooth Density

Prior to conducting this research, I hypothesized that each species would have differences in tooth density due to their differing diets, and my results confirmed this, showing that members of the *Sarotherodon* genus has considerably denser teeth and every other species significantly differed from each other as well. I chose to examine the specific metric of tooth density because it plays a huge role in breaking down food. Denser tooth covering could be used for grinding down prey items while more sparse, yet larger, teeth, would likely be helpful for crushing food or shells (Deng et. al 2022). This is relevant because each of the seven species being studied occupies a different feeding niche, specializing on some specific prey item that other fish do not or cannot consume (Galvez et al. 2021). For this reason, diet seems to be a main source of divergence between cichlids in Barombi Mbo.

The effect of Standard Length on pharyngeal jaw morphology

While tooth density was chosen as an unbiased metric, there was still a chance that it could have been effected by the size of the fish itself. Metrics like pharyngeal jaw size and area were not graphed or taken into account during the course of my study because of the many possible confounding variables that could have effected them. For example, a larger fish like Pungu maclareni, is thought to have wider pharyngeal jaws to allow for greater force to crush their prey. However, the integrity of this data could be easily confounded if you were to compare a small Pungu to a large Konia eisentrauti. To avoid this issue, I chose to take the tooth density of the each pharyngeal jaw, a metric which would likely remain constant even as the size of the fish changed. The standard length was graphed over tooth density in a multi-variable regression plot and showed that for all species, the standard length had virtually no effect on tooth density. This confirmed the validity of my measurement choice, that tooth density is a good, unbiased metric to use for fish of varying sizes and to simultaneously reflect differences in dietary patterns.

Phylogenetic Relationships

While there were very clear differences in pharyngeal tooth density for all the studied fish, there didn't tend to be much correlation between relatedness of fish species and tooth density. This hinted that the pharyngeal jaw morphology of these fish may be a very plastic trait that responds to dietary changes rapidly. Jaw plasticity was further assumed by the physical similarity of some fish like *St. Marei* and *St. Pindu*, yet the clear differences in PJ tooth morphology (Figures 4 and 5). Other fish, like *Sarotherodon carolli* and *eisentrauti*, who are very recently diverged, did have considerably denser teeth than all the other species being studied, however, they still differed from each other as well. This goes to show that even in the most recently diverged species, the pharyngeal jaw seems to be one of the first functional apparatuses to alter. What is even more likely is that the UPJ and LPJs begin to change even before speciation occurs and is ultimately what drives the divergence of one species into two. This could occur via disruptive selection where natural selection favors two phenotypic extremes within a population and over time, they separate. What makes disruptive selection so powerful in this case is the fact that it is working on the pharyngeal jaw, an integral eating tool. When one part of a species starts to feed where another cannot find their preferred food, isolation on a micro scale occurs. Fish of similar phenotypes spend more time together foraging and less and less interactions between the two morphs occur, making disruptive selection all the more powerful. This differs from if disruptive selection would act on something like fin size for predator avoidance. While two different fin sizes could be preferred, it would not stop members of different phenotypes from interacting and interbreeding would still occur, thus causing a much slower speciation process.

Alternative Hypotheses

While all of the fish used in this experiment were lab raised, some F0 specimens were available and showed morphological differences in the pharyngeal jaw when compared to their F1 counterparts. An important aspect of the specimens being used in this research is that all of them were second generation specimens raised in a lab and fed the same food for the entirety of their life. This is known as a common garden experiment and is used to protect the results from

biases caused by phenotypic plasticity. While this is beneficial in the context of the research being done, it does raise some questions on the effects that a few F0 specimens could have had on the results of the experiment. F0 individuals would create complications as they are wild caught from Barombi Mbo, meaning that they are raised on the diet they would get in the wild which can vary greatly for each species. Differing diets between fish could cause differences in the pharyngeal jaw morphology. This happens when the food consumed changes the shape of the jaw over time and works with genetics to create the final pharyngeal jaw form in a wild adult fish. When raised in a lab to only eat pellets, no opportunity is given to the captive fish to show phenotypic plasticity and their jaws can only reflect genetic differences in morphology. In order to assess if the pharyngeal jaws of barombi mbo cichlids were plastic, I would have separated F1 and F0 specimens during data analysis to show that there were significant differences in tooth density between the two and to avoid confounding the results of the study. However to make data analysis less complicated, I did not include any F0 specimens in my study.

Limitations and Future Directions

I hypothesize that pharyngeal jaw evolution drove the speciation of these seven Barombi Mbo cichlids however I cannot directly prove that pharyngeal jaws were some of the first things to evolve when speciation occurs. While I can prove that pharyngeal jaws differ, even between species like *S. carolii* and *linelii* which are almost identical, externally, I cannot distinctly say that tooth density in the pharyngeal jaw is the main source of divergence. This limitation arises because I was not able to measure every aspect of the specimens being studied and cannot rule out any other factors, only explain the significance of one. Additionally, convergent evolution cannot be ruled out as a reason why these fish look so similar today and it could be obscuring any extinct characteristics that were truly responsible for the divergence of the species.

Another limitation I faced in the process of my study was the influence of Leims Paradox (Cosandey-Godin et al. 2008). This is when species with very specialized morphologies have heavily overlapping diets. In other words, some of the items for which a species has specifically evolved to consume, accounts for little to no part of their actual diet. It is true that a lot of the fish being studied during the course of this research have more generalist diets yet the tooth

densities of their pharyngeal jaws are very unique when compared to others. An explanation for this counterintuition is that species evolve specific traits during periods of intense competition, when they are are forced to diversify their diet in order to survive. During this time, species diverge and competition lessens as each group begins to occupy different niches. In response to this, the easiest general prey items become available again and certain fish are left with incredibly specialized jaws which are now rarely needed. This makes my hypothesis difficult to prove because, while diet may have played a huge role in species divergence, it is difficult to assess the magnitude of its influence by looking at modern day specimens whos current diets may not reflect the true purpose of their distinct pharyngeal jaws.

Broader Implications / Conclusions

Overall, the results of my study can help answer important questions surrounding adaptive radiations in sympatry and what causes them. In a small lake like Barombi Mbo, which leaves little opportunity for geographic isolation, adaptive radiations are a puzzling occurrence. However, given the results of my research, small structures, like the pharyngeal jaw, could be key in explaining how they take place. Dietary specialization is one way that a species can rapidly diversify in a new environment and because pharyngeal jaws play a vital role in breaking down food, they could be the trait that facilitated sympatric speciation in these cichlids.

In addition to this, my study can help inform future research on species interactions. When local extinctions are becoming more prevalent (Engelhardt et al. 2011), there is a growing need to understand how certain fish can quickly occupy new niches and survive amongst competition. The pharyngeal jaw could be the reason that african cichlids have been able to speciate to such a great extent and rule African freshwater lakes.

research over the past year. I would like to thank Dr. Chris Martin for guiding me through this project and always being passionate about the work being done. Additionally, his feedback was essential in keeping me on the right track and ensuring that my data was valid and meaningful. Huge thanks to Jacquelin Galvez for teaching me how to to use R and make the necessary tables and graphs to best represent the data being collected. I would also like to thank her for her previous research on these fish as information on their diets played a crucial part in my study. Kenna Clawson, Tyson Tonokawa and Ranga Bhardadwaj helped in every aspect of this project during fall semester. With their help, the dissection and measurement process was extremely expedited and through them I learned the skills necessary to complete the rest of the data collection on my own. I would like to thank Patina Mendez and Jessie Moravek for their feedback and time during office hours which helped me combine all the create all the individual parts of this paper and finally convert them into a finished product. I also greatly appreciated the support from Jenna Krugler and David Zhong who provided me with peer reviews during the course of this project, pushing me to write in a cohesive manner and put my research into words.

REFERENCES

"California Academy of Sciences." CAS - Eschmeyer's Catalog of Fishes - Genera/Species by Family/Subfamily, researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp.

Cosandey-Godin, A., S. A. Binning and L. J. Chapman. 2008. Specialized Morphology for a Non-Specialized Diet: Liem's Paradox in an African Cichlid Fish. Journal of Fish Biology. 7:1683–99.

Engelhardt, M. J., and R. C. Anderson. 2011. Phenological Niche Separation from Native Species Increases Reproductive Success of an Invasive Species: Alliaria Petiolata (Brassicaceae) – garlic mustard. The Journal of the Torrey Botanical Society. 138:418–433.

Galvez, J. R., K. McLean, C. D. Touokong, L. N. Gonwouo, and C. H. Martin "Trophic Specialization on Unique Resources in One of the Most Celebrated Examples of Sympatric SPECIATION, Barombi MBO Crater Lake Cichlids." 2021, doi:10.1101/2021.08.04.455125.

Hulsey, C. D., F. J. Garcíaa de León, and R. Rodiles-Hernández. "Micro and

Macroevolutionary Decoupling of Cichlid Jaws: A Test of Liem's Key Innovation Hypothesis." *Evolution* 60.10 (2006): 2096–2109. Web.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L.Excoffier and O. Seehausen. "Ancient Hybridization Fuels Rapid Cichlid Fish Adaptive Radiations." Nature Communications, vol. 8, no. 1, Nature Publishing Group, 2017, pp. 14363–14363, doi:10.1038/ncomms14363.

Musilova, Z., A. Indermaur, A. R. Bitja-Nyom, D. Omelchenko, M. Kłodawska, L. Albergati, K. Remišová and W. Salzburger. Evolution of the visual sensory system in cichlid fishes from crater lake Barombi Mbo in Cameroon. Mol Ecol. 2019; 28: 5010– 5031. https://doi-org.libproxy.berkeley.edu/10.1111/mec.15217

Odada, E. O., and D. O. Olago. The East African [i.e. African] Great Lakes : Limnology, Palaeolimnology, and Biodiversity . Kluwer Academic Publishers, 2002.

Peterson, T., and G. B. Müller. "Developmental Finite Element Analysis of Cichlid Pharyngeal Jaws: Quantifying the Generation of a Key Innovation." *PloS one* 13.1 (2018): e0189985–e0189985. Web.

Ronco, F., and W. Salzburger. "Tracing Evolutionary Decoupling of Oral and Pharyngeal Jaws in Cichlid Fishes." *Evolution letters* 5.6 (2021): 625–635. Web.

Shadwick, R. E., and G. V. Lauder. *Fish Biomechanics*. Amsterdam ;: Academic Press, 2006. Print.