12-2012

Phenotypic Plasticity of Oral Jaw Dentition in Archosargus Probatocephalus

Cynthia E. Worcester
Western Kentucky University, cynthia.worcester@wku.edu

PHENOTYPIC PLASTICITY OF ORAL JAW DENTITION IN *ARCHOSARGUS PROBATOCEPHALUS*

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirement for the Degree
Master of Science

By
Cynthia E. Worcester

December 2012

PHENOTYPIC PLASTICITY OF ORAL JAW DENTITION IN *ARCHOSARGUS PROBATOCEPHALUS*

Date Recommended _____

Steve Huskey, Director of Thesis

Philip Lienesch

Michael Collyer

12/13/12

Dean, Graduate Studies and Research Date

ACKNOWLEDGEMENTS

WKU:

I would like to give my sincerest thanks to my committee, Dr. Steve Huskey, Dr. Philip Lienesch, and Dr. Michael Collyer, for their continued support, comments, and guidance during this project. I could not have completed this project without the help from my wonderful committee. Every member of my committee went above and beyond what a graduate committee is required to do. I am forever grateful. Thank you.

I would also like to thank an honorary member of my committee, Dr. John Andersland, who was instrumental in this research. Finally, I would like to thank my dear friends at WKU, who listened, helped me when I needed it, and made my experience at WKU unforgettable.

Funding:

I would like to thank the Office of Graduate Studies and Research at Western Kentucky University for a grant in support of this research. I would like to also thank the Biology Department of Western Kentucky for the use of the equipment that was vital to this research. Finally, I would like to thank Dr. Jerry Gibbs for his generous donation to my research fund.

Family:

I would like to thank my family for their continuous support and encouragement through this long and trying process. There were times when others lost faith in the completion of this project, but my family's support never wavered. I would especially like to thank my child, Summer Moon, for her understanding while mommy was busy working for years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PHENOTYPIC PLASTICITY OF ORAL JAW DENTITION IN *ARCHOSARGUS PROBATOCEPHALUS*

Cynthia E. Worcester December 2012 38 Pages

Directed by: Dr. Steve Huskey, Dr. Philip Lienesch, and Dr. Michael Collyer

Department of Biology Western Kentucky University

Phenotypic plasticity, the capacity of a single genotype to exhibit variable phenotypes in different environments, is common in many species. A sample of wild caught *Archosargus probatocephalus*, also known as sheepshead, from Florida was randomly divided into two treatment groups: one group was fed soft prey, *Mercenaria* sp. muscle tissue, and the other group was fed hard prey, *Mercenaria* sp. in the shell, for 365 days. It was hypothesized that the sheepshead fed hard prey would have a thicker tooth enamel layer containing more calcium, and therefore be stronger than the tooth enamel layer of those fed soft prey items. Additionally, the mean functional jaw surface area, the percentage of tooth coverage of functional jaw surface, number of teeth per jaw, correlation between standard length and mean total tooth height, and the combined surface area of the teeth, when compared between the two treatments, should be greater in the hard prey treatment.

The seventeen jaws of two prey groups were acquired postmortem and each jaw was divided into four quadrants. The largest tooth in each quadrant was removed from the jaw, longitudinally sectioned, and examined using scanning electron microscopy (SEM) to measure the enamel and dentin layers. Using the SEM backscatter electron detector the elemental composition of the different layers was determined at multiple locations. Finally, data was analyzed using analyses of variance (ANOVA's) to compare mean tooth height, calcium content in enamel and dentin layers, mean functional jaw

number of teeth per jaw, and upper to lower jaw overall enamel and dentin thickness between each treatment.

Phenotypic plasticity was identified in three areas: percentage of jaw surface covered by teeth, a positive correlation between total tooth height and enamel height in hard prey treatment, and a positive correlation between total tooth height and soft prey treatment dentin height; but not in the other areas studied. It is apparent that phenotypic plasticity can increase an individual's ability to survive in a variable food resource environment by changing some aspects of tooth morphology, but the ability to change in response to stimuli was not found in all areas of tooth structure.

INTRODUCTION

Fish species have evolved various adaptations to jaws and teeth for mechanical breakdown of food into particles that allow for the separation of edible and inedible materials. This breakdown increases the surface area of the food particles exposed to the digestive enzymes found in the stomach and intestine (Schmitt & Holbrook, 1984; Schmidt-Nielsen, 1997; Evans, 1998). The ability of a species to acquire chemical compounds that supply energy and the essential compounds they cannot synthesize, such as essential amino acids, fatty acids, and vitamins, is vital to support all biological processes including reproduction, growth, and locomotion (Liem, 1980; Moyle & Cech, 2004).

To utilize food resources fish must first capture prey to acquire vital nutrients. There are three main categories of prey-capture by fish: ram feeding, inertial suction, and manipulation (Liem, 1980). In the ram feeding method a predator swims toward their prey, overtaking it through greater speed. Fish species that use ram feeding for prey-capture are among the highly derived teleost fishes and have specializations which include fast acceleration morphology, large gapes, and large gape to buccal cavity volume ratios (Norton, 1995; Evans, 1998).

Inertial suction is considered the most versatile type of aquatic prey-capture method among vertebrates and is found in most teleost fishes (Evans, 1998). During this method the fish expands its buccal cavity to create subambient pressure, which causes water along with the prey item to be drawn into the predator's mouth (Norton, 1995). The fish that use this method of prey-capture have specializations such as small gapes, agile locomotor morphology, and a small gape to buccal cavity ratio (Norton, 1995).

Manipulation is a prey-capture method that involves the actual use of dermal teeth or true teeth of the upper and lower jaw; this includes biting, clipping, scraping, and rasping (Liem, 1980). Fishes that use manipulation have highly varied morphologies. For example, fishes that bite prey have robust jaws with cutting teeth, restricted jaw mobility, and large adductor muscles, whereas fish that utilize macro algal diets in general have short blunt snouts, close set teeth that form a cropping edge, and highly kinetic jaws (Norton, 1995; Horn, Martin, & Chotkowski, 1999).

Fish may use a combination of these three prey-capture categories or modulate between them depending on food source availability (Liem, 1980; Ferry-Graham, Wainwright, & Bellwood, 2001). If a food resource in a habitat changes, any species that depends on that resource must change to utilize new resources, or perish. Change can occur at many different levels, including morphology, physiology, and/or behavior and each of these has been well documented in fishes (Liem, 1980; Sedberry, 1989; Norton, 1995; Hernandez & Motta, 1997; Clifton & Motta, 1998; Cutwa & Turingan, 2000; Price, Qvarnstrom, & Irwin, 2003).

Phenotypic plasticity is defined as the ability to change a characteristic or expression of the genes of that individual that is not based on evolutionary change in genetic code (Stearns, 2009). Phenotypic plasticity is the ability of a single genotype to produce more than one change to an individual's behavior, physiological state, and/or morphology directly induced by different environmental stresses (West-Eberhard, 1989; Price, Qvarnstrom, & Irwin, 2003; Jong, 2004). An individual fish or any organism is restricted by its genetics, but has the ability to utilize different genes to exhibit a slightly different phenotype to survive in a given environment. This is an example of plasticity. "Plasticity is therefore shown by a genotype when its expression is able to be altered by

environmental influences" (Bradshaw, 1965). The mean change between different phenotypes, such as feeding mechanisms, in two different environments is a measure of phenotypic plasticity (Scheiner, 1993). The ease with which a species can change between variable environmental factors, such as different prey-resources, determines the number of different environments that species can utilize. "Phenotypic plasticity can provide increased environmental tolerance and is thus one solution to the problem of adaptation to heterogeneous environments" (Via, et al., 1995)

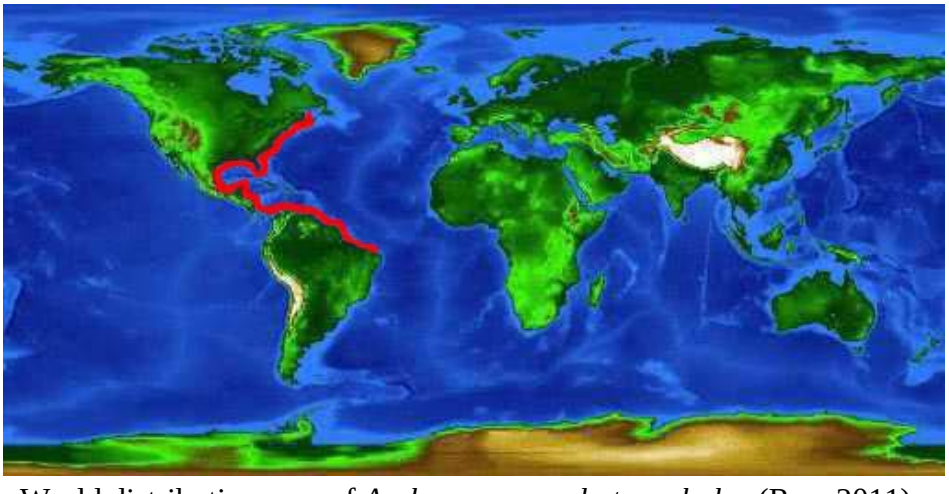


Figure 1. World distribution map of *Archosargus probatocephalus* (Ray, 2011).

Sheepshead, *Archosargus probatocephalus,* are found along the Atlantic Coast from Brazil to Maine (Figure 1; Ray, 2011). Sheepshead are omnivores and previous research indicates that individuals prefer a different diet depending on life stage (Hernandez & Motta, 1997). During the larval stage the diet is primary zooplankton such as copepods and amphipods, while juveniles less than 50 mm will consume any soft-bodied organism that might be in the seagrass including polychaete worms, bryozoans, and ostracods (Sedberry, 1989). When they reach more than 50 mm their diet changes to include more hard-shelled prey items such as barnacles, crabs, oysters, and clams (Hernandez & Motta, 1997). The sheepshead diet is varied during ontogeny because the

prey items they are able to utilize are determined by morphological factors such as: gape, jaw dimensions, and bite force. Simply, as sheepshead grow their bite force increases and this increase in the strength of the bite is responsible for the increased amount of hard prey items in their diet (Turingan & Huskey, 2000). This increase in durophagous feeding habits is likely to be accompanied by a concomitant increase in jaw robustness and dental resilience.

Prey-induced reaction norms are considered an adaptive reaction norm where an organism produces a phenotype that varies as a continuous function of the environmental signals, where the response to a specific environmental signal results in an improvement in survival, growth, or reproduction (Stearns, 1989; Via, et al., 1995). The specific environmental signal that causes this result could be experimental prey type manipulation, causing a phenotypic response such as a directional prey-induced change in jaw morphology and/or dentition.

The structure of the mouth of a fish is closely related to the feeding modes and habits of the fish, and is highly variable (Motta, 1987; Moyle & Cech, 2004). Meyer (1987) demonstrated that individual *Cichlasoma managuense* can change jaw morphology, including jaw shape and length, when fed different diets (e.g., hard versus soft prey) for eight months, called a reversible prey-induced reaction norm. “A reaction norm can be either inflexible, in which a characteristic once determined is never changed later in the organism's life, or they can be flexible, in which a characteristic can be altered more than once” (Stearns, 1989). In the Meyer study, half the fish that were fed soft prey after 8 months were placed in the hard prey group and fed hard prey for another 8 months. The result was another change in jaw morphology including jaw shape and

length. This revealed that this reaction norm was flexible and could be changed with a change in diet.

The phenotypic plasticity of *Archosargus probatocephalus'* jaw morphology in response to varied food resources has been well documented with respect to bone and muscle mass (Cutwa & Turingan, 2000), and bite strength (Hernandez & Motta 1997; Turingan & Huskey, 2000), but a study of potential tooth morphological differences and/or compositional changes in response to prey type has not been performed. Not only would the bite strength, bone mass, and the muscle mass have to increase to utilize a harder prey-resource, but likely so would the elemental composition of the enamel and the amount of enamel found in the molariform teeth. This study was an analysis of the phenotypic plasticity of molariform teeth in *Archosargus probatocephalus*, a change that occurs in response to experimentally-induced dietary differences.

The null hypotheses were *Archosargus probatocephalus* fed different prey items exhibited: no change in the enamel or dentin layer height, no difference in mean functional jaw surface, no difference in percent of tooth coverage of functional jaw surface, no difference in mean total tooth surface area, no difference in mean number of teeth per jaw, no correlation between standard length and mean tooth height, and that there will be no difference in the amount of calcium found in the enamel and dentin layers between the two treatments.

The alternative hypotheses were *Archosargus probatocephalus* that were fed different prey items exhibited: a thicker tooth enamel layer, a larger mean functional jaw surface in the hard prey treatment, a larger percentage of tooth coverage of functional jaw surface in the hard prey treatment, a larger mean total tooth surface area, more teeth in the hard prey jaws, a correlation between the standard length and mean total tooth height,

and more calcium in the hard prey, therefore will be stronger, than the *Archosargus probatocephalus* tooth enamel layer that were fed soft prey items.

MATERIALS AND METHODS

Specimen Collection

Sheepshead jaws were obtained from Dr. R. G. Turingan of the Florida Institute of Technology which were initially captured from one location in the wild, (Melbourne causeway) in the Indian River Lagoon (28˚05’03”N, 80˚35’30”W; Figure 2) using cast nets. Specimen collection started in September of 2007 and continued until December of the same year.

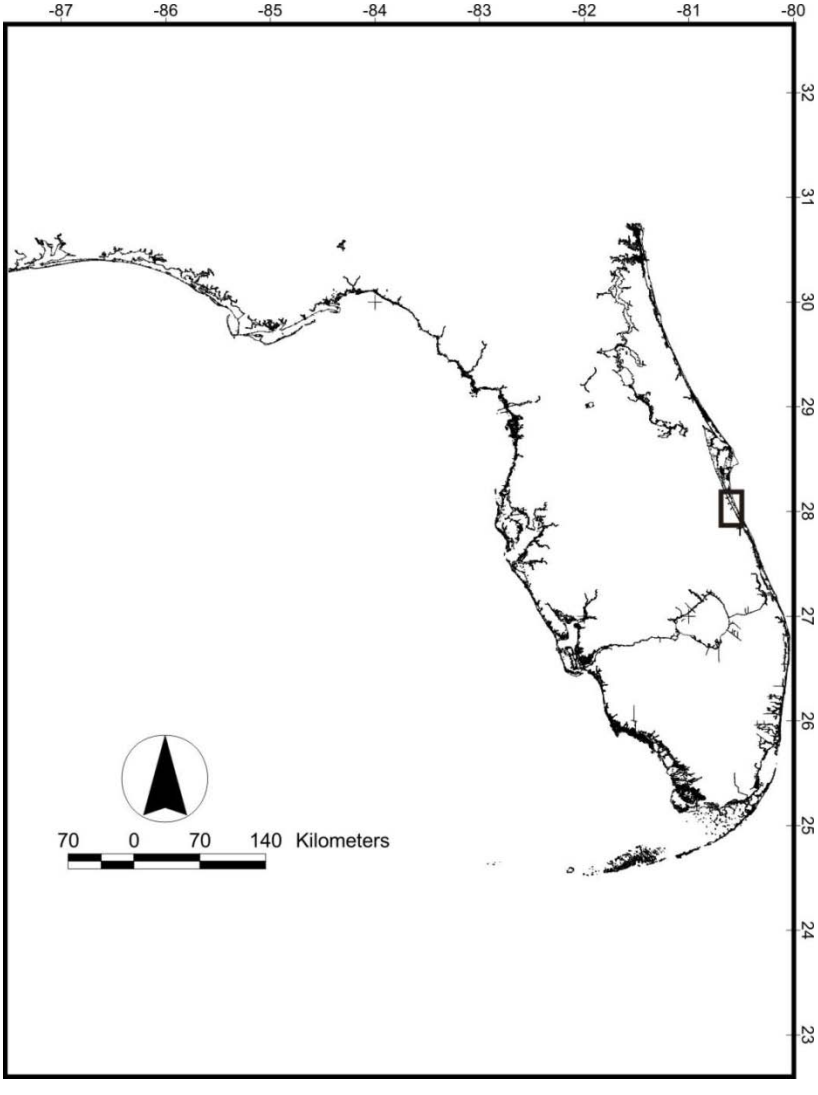


Figure 2. A map of Florida showing the Indian River Lagoon (open square), the site of *A. probatocephalus* field collections (Polohan-Maliao, 2010).

Thirty total fish were brought back to the lab and placed in a re-circulating tank, equipped with mechanical and biological filters, for 14 days where the salinity was maintained at 25-27 ppt and the temperature was 25-26˚C. After 14 days, each fish was transferred to an individual 10-gal tank for the duration of the study. During rearing, the series of tanks were equipped with mechanical as well as biological filters and with a flow through seawater system. The fish were randomly divided into two groups: hard prey vs. soft prey.

Experimental Design

Mercenaria sp., a saltwater clam found in the surf zone of Florida, was fed to both treatment groups until satiation one to two times a day. The hard prey treatment group was fed clams with the shells intact, while the soft prey treatment group was fed meat from *Mercenaria* but with the shell removed. Each group used *Mercenaria* muscle as a food source, while requiring a different level of prey-processing.

After 365 days the jaws of the two prey groups were acquired postmortem, preserved in 10% formalin, and sent to Dr. S. Huskey at WKU. Although there were equal numbers of sheepshead individuals in each prey group, 15 fish each when the study began, only six soft prey and eleven hard prey fish survived the year-long experiment. The decrease in individuals could have been due to disease, injury, stress, etc. but should not have been due to life expectancy, which is around 20 years (Liao, et al., 1991).

When the jaws were first received a photo of each jaw was taken using Leica MZ 16 Light Microscope and Auto-Montage Pro 5.02 beta software (Syntopics Ltd.) to document the features of each jaw, following the method described by Webb (2011). These photos were used to determine the mean functional jaw surface area and the percent of functional jaw covered by teeth using Image J 1.45s software (NIH). The mean

functional jaw surface area was determined by tracing the outermost bony ridge of the jaw and calculating the mean surface area for each treatment.

The total tooth surface area (mm^2) was calculated by measuring each individual tooth's surface area in each jaw, then combining these values for a total tooth surface area. This number was divided by the functional jaw surface area resulting in the percent of functional jaw covered by teeth (Figure 3).

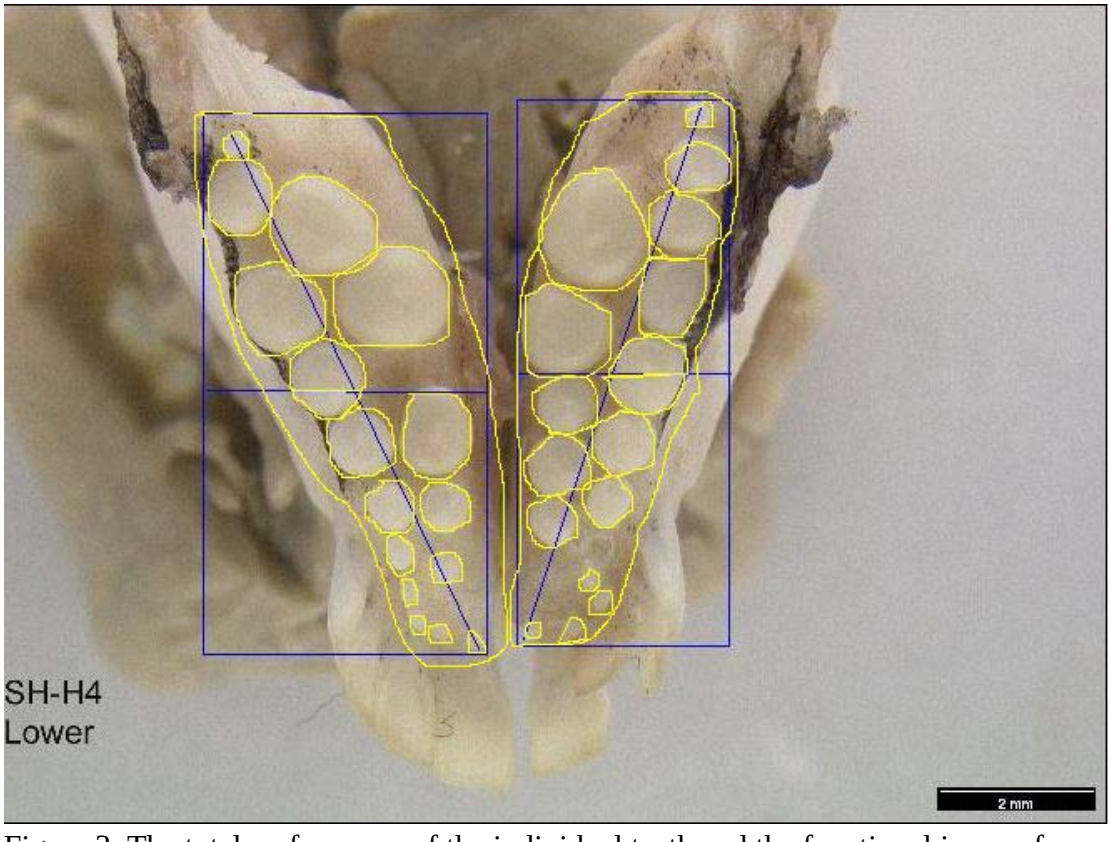


Figure 3. The total surface area of the individual teeth and the functional jaw surface area of SH-H4 Lower jaw measured using Image J.

The Multi-Z Light Microsco Leica MZ 16 Light Microscope and Auto-Montage Pro 5.02 beta software (Syntopics Ltd.) photos were also used to determine how many teeth were found on each jaw in each prey treatment. This was performed to determine if

the number of teeth on each jaw could also be influenced by a difference in prey contraints.

Next, the jaws of both groups were analyzed using stratified sampling. Each jaw was divided into four quadrants where the center line of the hard palate was used to divide the jaws into two halves using Image J software (Figure 4). Those halves were divided again by measuring the total distance between the first and last molariform tooth in the jaw on each half. That number was then divided equally into two parts that resulted in four quadrants that was used to sample the jaws, again using Image J (Figure 5). For example, the line between the first and last molar tooth on the larger side of the soft prey jaw (Figure 4) was 5.2 mm; at 2.6 mm the purple line broke the jaw into two equal parts. On the smaller side of the jaw the line between the first and last molar was 4.25 mm; at 2.125 mm the purple line broke the jaw into two equal parts. This resulted in the jaws being divided into four quadrants that were used for this investigation.

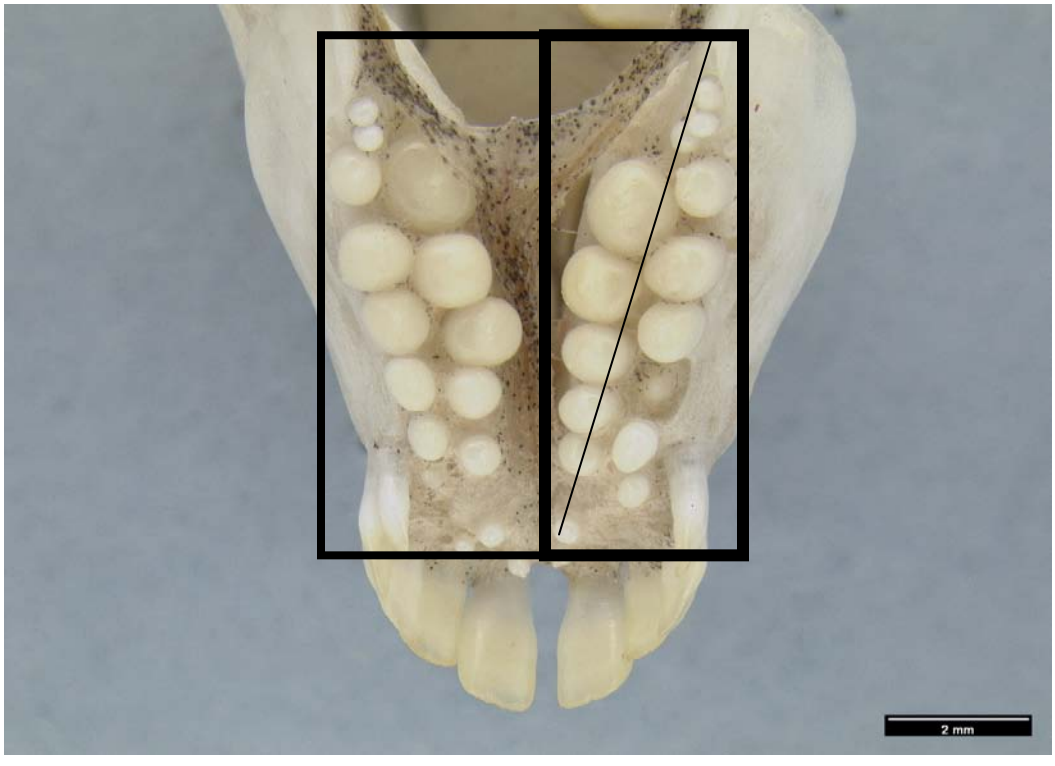


Figure 4. Sheepshead lower jaw (SH-H5) hard prey photo taken using Multi-Z Light Microscope. Black lines indicate separate halves of jaw and the black line is used to determine the distance between the first and last molar.

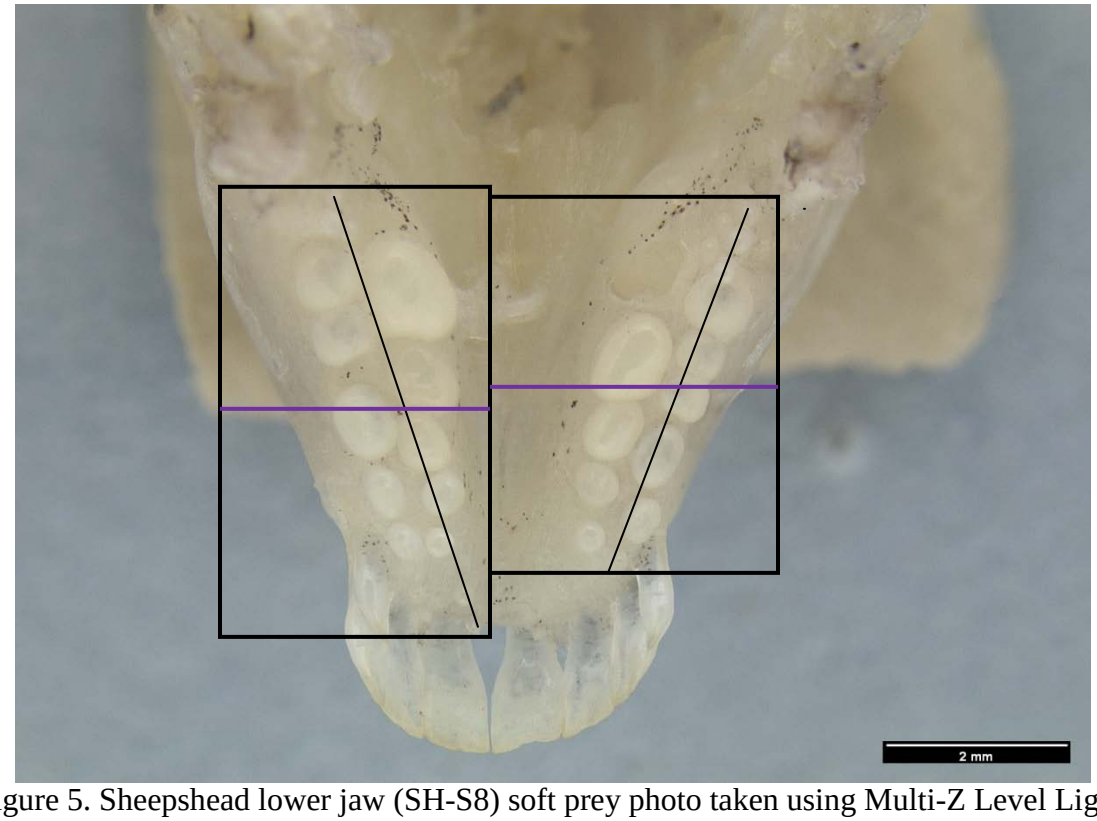
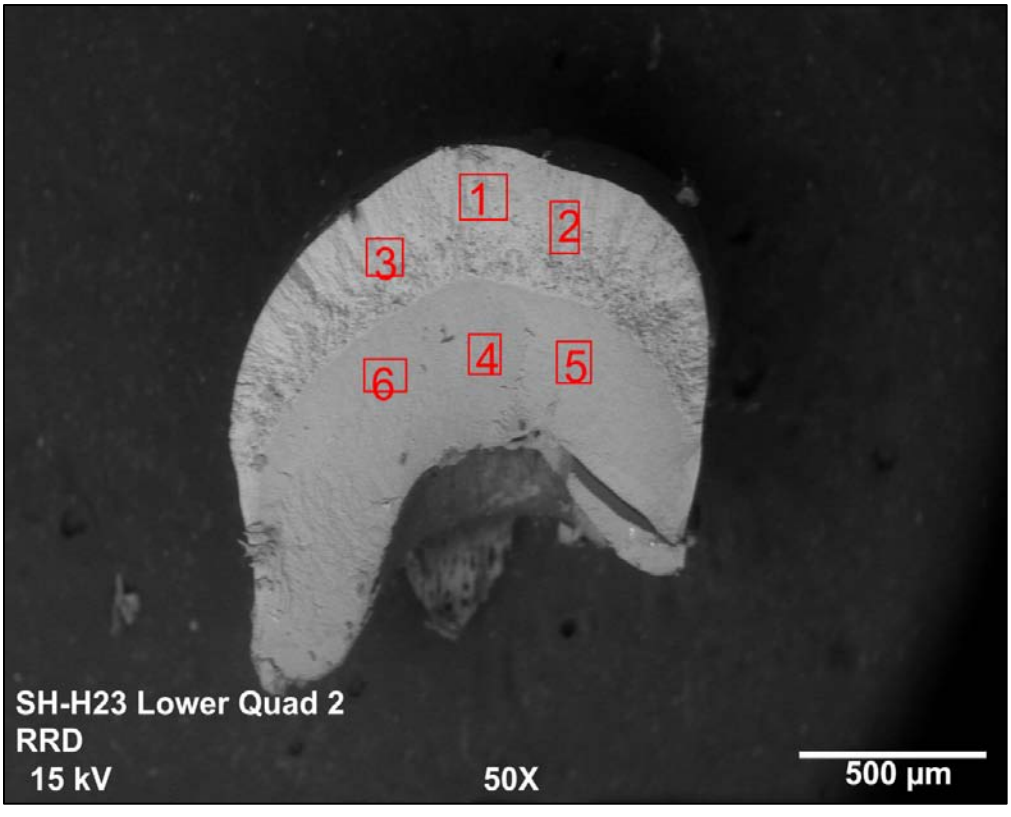


Figure 5. Sheepshead lower jaw (SH-S8) soft prey photo taken using Multi-Z Level Light Microscope. The boxes indicate the quadrants.

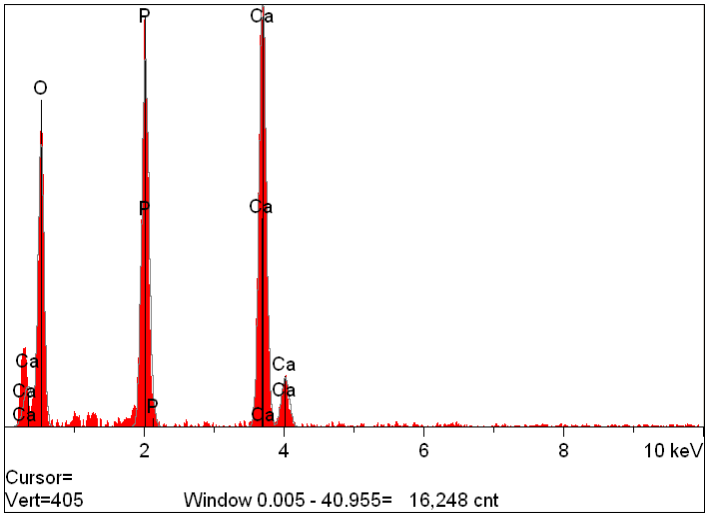
The largest tooth of each quadrant was extracted from the jaws for analysis using a metal probe, a compound microscope, and forceps. If there were two teeth that appeared to be the same size, the surface area of each tooth was measured to determine the largest tooth. A total of 88 hard prey fish teeth and 48 soft prey fish teeth were analyzed—four teeth from the top jaw and four from the lower jaw per fish.

Each extracted tooth was cut longitudinally using a heavy duty straight-razor and a compound microscope. The heavy duty straight-razor was placed at the midpoint of the tooth and in most cases this was where the enamel of the tooth came to a cusp-like point. The sectioned teeth were placed on a metal stub covered in double-sided tape with the cut surface placed up so it could be viewed using the JSM-5400LV SEM scanning electron microscope. Only one section from each quadrant was place on a labeled metal stub as to reduce error. The metal stub holder that is used in the JSM-5400L SEM holds four metal stubs at a time, therefore only one jaw's total four quadrants were being examined at a time. The images were acquired using the SEM in low vacuum mode and IXRF Systems Inc. 500 digital processing system and software. Images were analyzed using Image J software. The images were focused to the highest magnification while the maximum amount of tooth surface could still be observed; this varied from 50X to 150X.

The elemental compositions of selected areas were determined using the SEM's backscatter electron detector and computer analysis of selected sections of the tooth (Figure 6). In the SEM, the backscatter electrons that are produced are strongly dependent on the mean atomic number of the sample and are therefore thorough detectors of the presence of an element in a sample (Flegler, Heckman, & Klomparens, 1993).



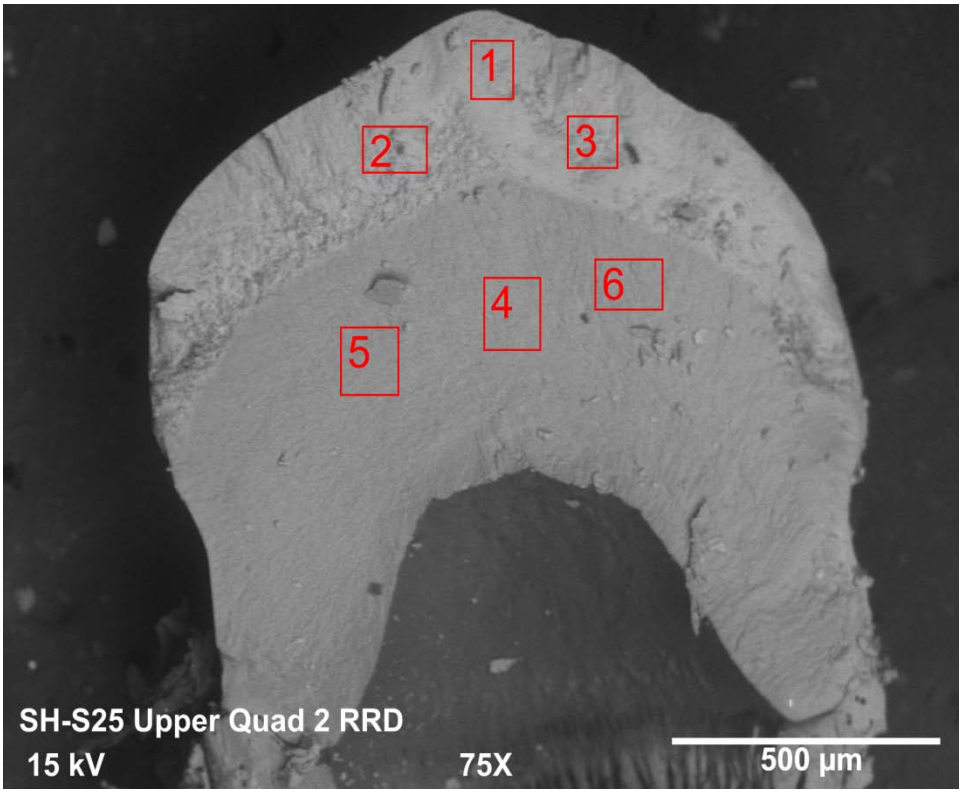
Analysis Report: Image11-4



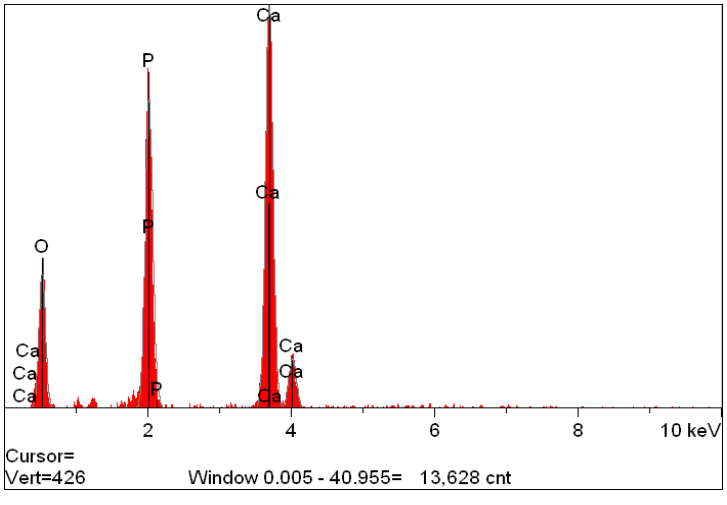
Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc	Units	
O	Ka	96.31	3.802	73.58	55.23	wt.%	
P	Ka	151.37	4.863	11.47	16.66	wt.%	
Ca	Ka	176.97	5.050	14.95	28.11	wt.%	
				100.00	100.00	wt.%	Total

Figure 6. SEM picture and elemental data from one of the six sample areas (e.g., box 5) of sheepshead hard prey molariform tooth section number 23.

Since the elemental composition of the highly mineralized tissue called enamel is $Ca_{10}(PO_4)_6(OH)_2$(*solid*) (Brown T. L., 2003) with trace contaminants (Na, Si, N, S) (Herold, Graver, & Christner, 1980; Nelson, Hildebrand, & Major, 2002) the layers of the teeth could be identified from the elemental analysis. The teeth with increased calcium levels in their elemental composition of enamel have greater microhardness of enamel (Davidson, Hoekstra, & Arends, 1974). For each of the tooth sections, there were six sections randomly selected for analysis: three in the enamel layer and three in the dentin layer (Figure 7).



Analysis Report: Image7-5



Elt.	Line	Intensity (c/s)	Error 2-sig	Atomic %	Conc	Units	
O	Ka	53.70	2.765	65.57	45.53	wt.%	
P	Ka	140.14	4.711	13.73	18.46	wt.%	
Ca	Ka	187.77	5.205	20.70	36.00	wt.%	
				100.00	100.00	wt.%	Total

Figure 7. SEM picture and elemental data from one of the six sample areas (e.g., box 5) sheepshead soft prey molariform tooth number 25 sectioned.

The enamel and dentin layers of each tooth section were distinct and easily identifiable (Underwood, Mitchell, & Veltkamp, 1999). The layers were measured for each sectioned tooth in multiple locations (Figure 8). The mean thickness of each layer in the tooth section was also determined using Image J software. Ten lengths were measured in the enamel and the dentin layers of each tooth section and recorded (Figure 9). The mean of these ten measurements was used as the mean thickness of each layer in that tooth. The four means of the four quadrants in each jaw were used to determine if there were any significant differences in the two treatments.

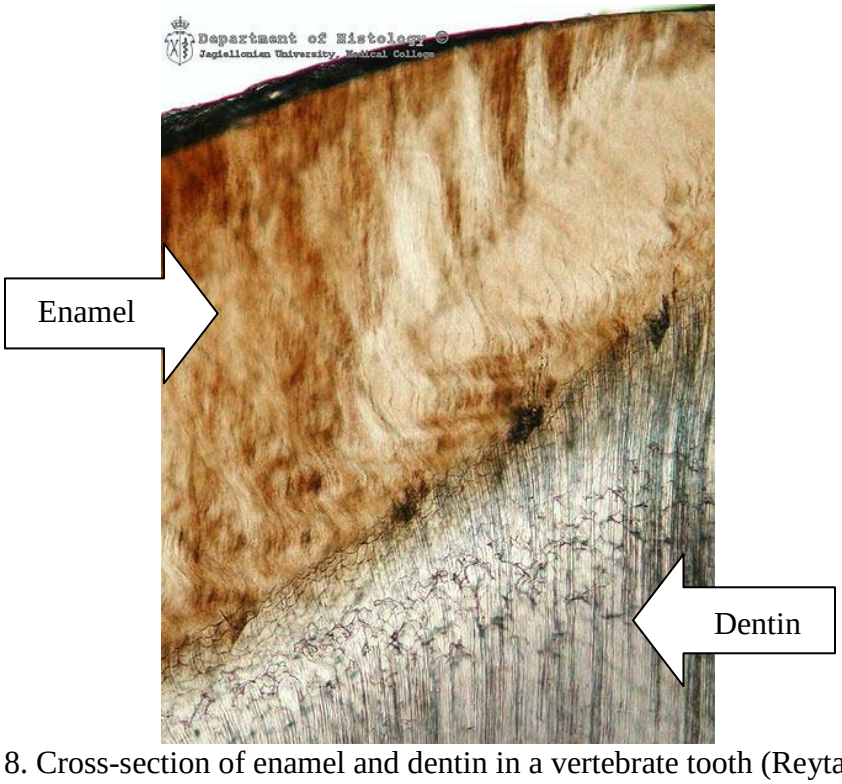


Figure 8. Cross-section of enamel and dentin in a vertebrate tooth (Reytan, 2006).

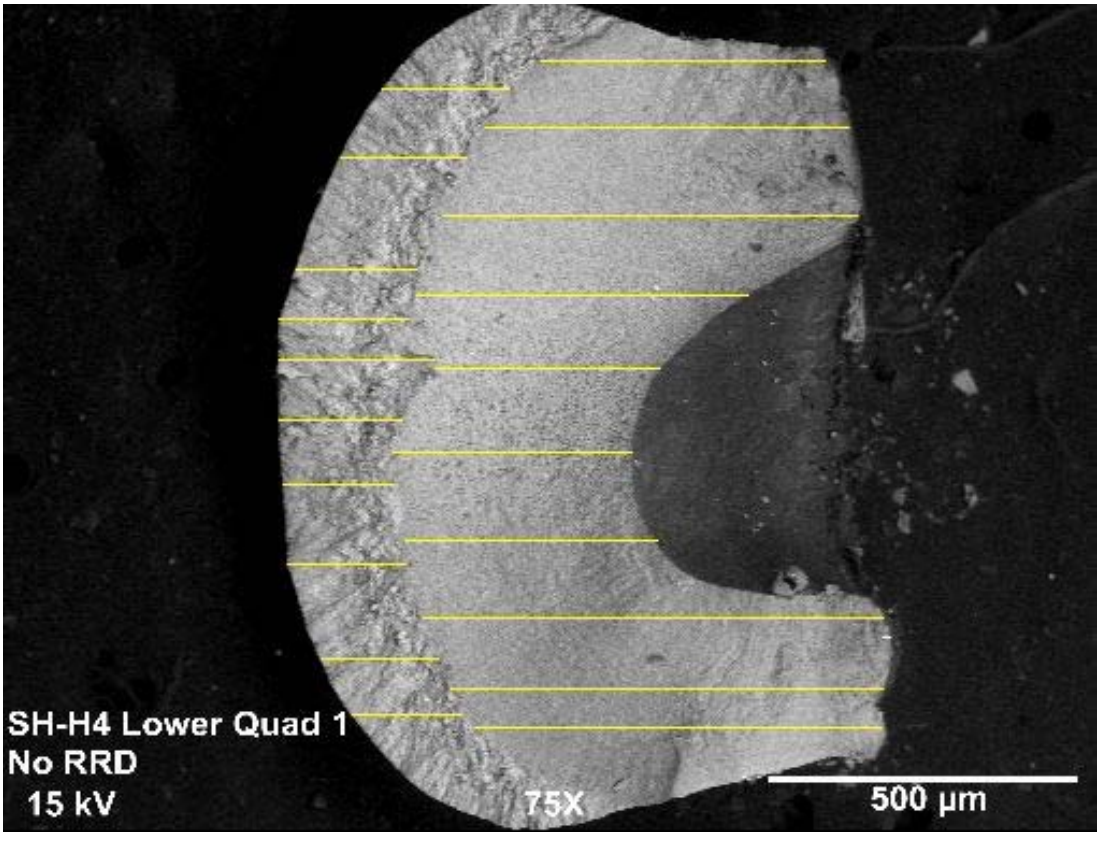


Figure 9. SEM photo where Image J produced ten lines used to measure the enamel and dentin layers of hard prey tooth from sheepshead number 4 from quadrant 1.

The data was analyzed using analyses of variance (ANOVA's) to compare the mean tooth height, calcium content in enamel and dentin layers, mean functional jaw surface area, percent of functional jaw surface covered by teeth, standard length, mean number of teeth per jaw, and upper to lower jaw overall enamel and dentin thickness between each treatment.

RESULTS

SEM analysis revealed a significant positive correlation between the thickness of the enamel layer in the hard prey group and the mean tooth height (Figure 10; $F_{1, 9}$=16.617, P=0.003; Table 1). As the tooth height increased the enamel of the teeth in the hard prey treatment increased at a greater rate than in the soft prey treatment, with a slope of 0.2844 and 0.1192, respectively.

There was no significant correlation found between the enamel layer of the soft prey treatment and mean tooth height ($F_{1,4}$=3.297, P=0.144; Table 1). There was also no significant correlation between the dentin layer of the hard prey treatment and mean tooth height ($F_{1,9}$=3.274, P=0.104).

There was a significant positive relationship found between the dentin layer of the soft prey group and mean tooth height (Figure 11: $F_{1, 4}$=9.154, P=0.039; Table 1). The dentin layer of the soft prey treatment increased at a faster rate than the hard prey treatment as the mean height of teeth increased, with a slope of 0.4263 and 0.217, respectively.

Table 1. ANOVA results for the regression between mean tooth height (HT) and the enamel and dentin layers of the sheepshead teeth in both treatments.

Variables	df	F	P	S.E.
Hard Prey Enamel/HT	1,9	16.617	**0.003**	0.024
Soft Prey Enamel/HT	1,4	3.297	0.144	0.022
Hard Prey Dentin/HT	1,9	3.274	0.104	0.041
Soft Prey Dentin/HT	1,4	9.154	**0.039**	0.046

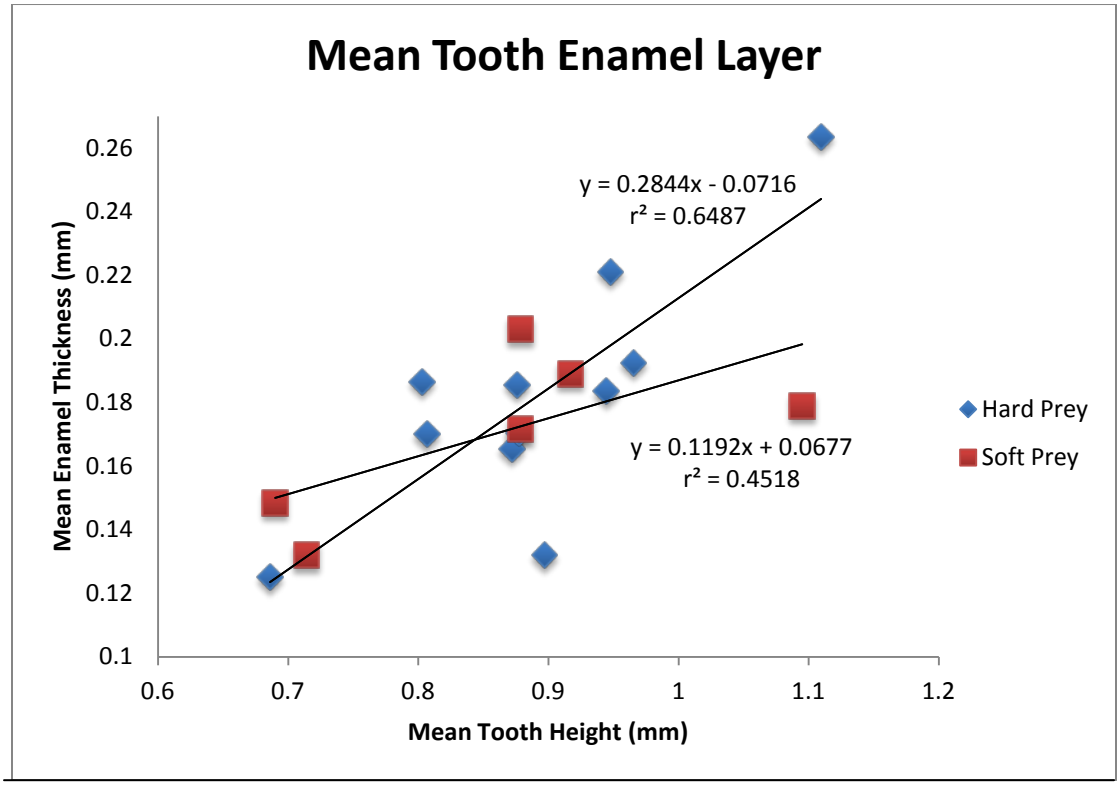


Figure 10. The relationship between the mean tooth height and mean enamel layer in sheepshead after 365 days of soft and hard prey treatments.

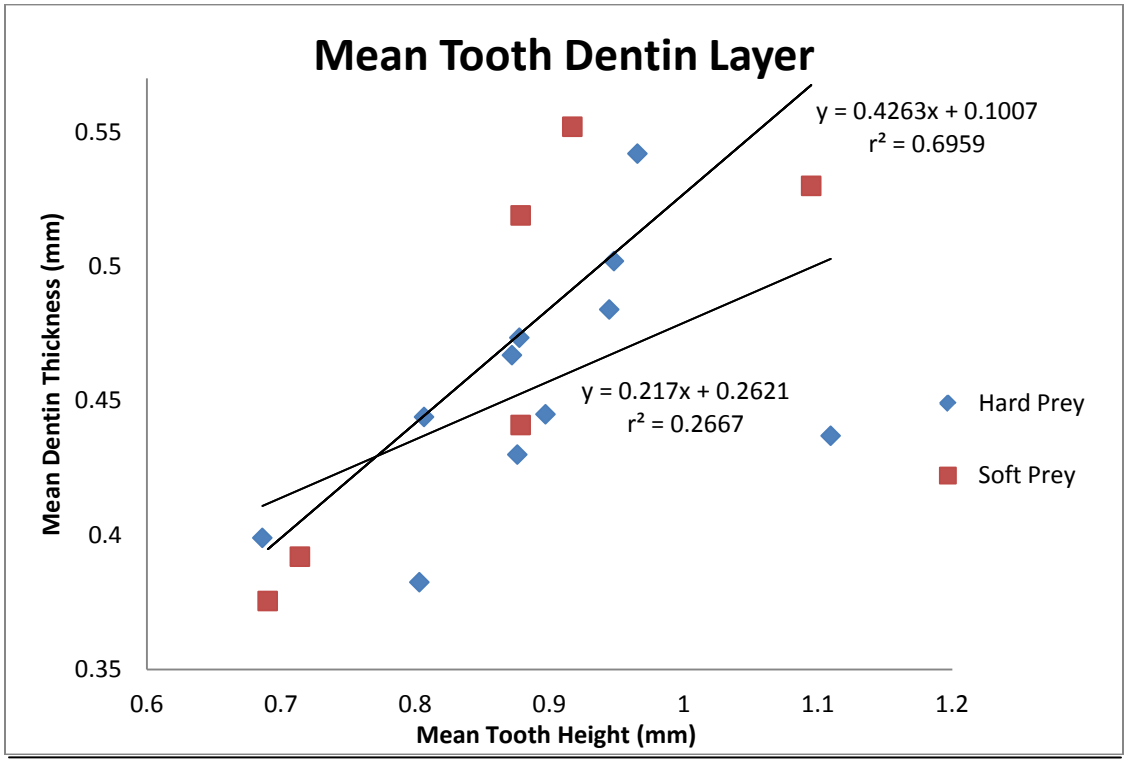
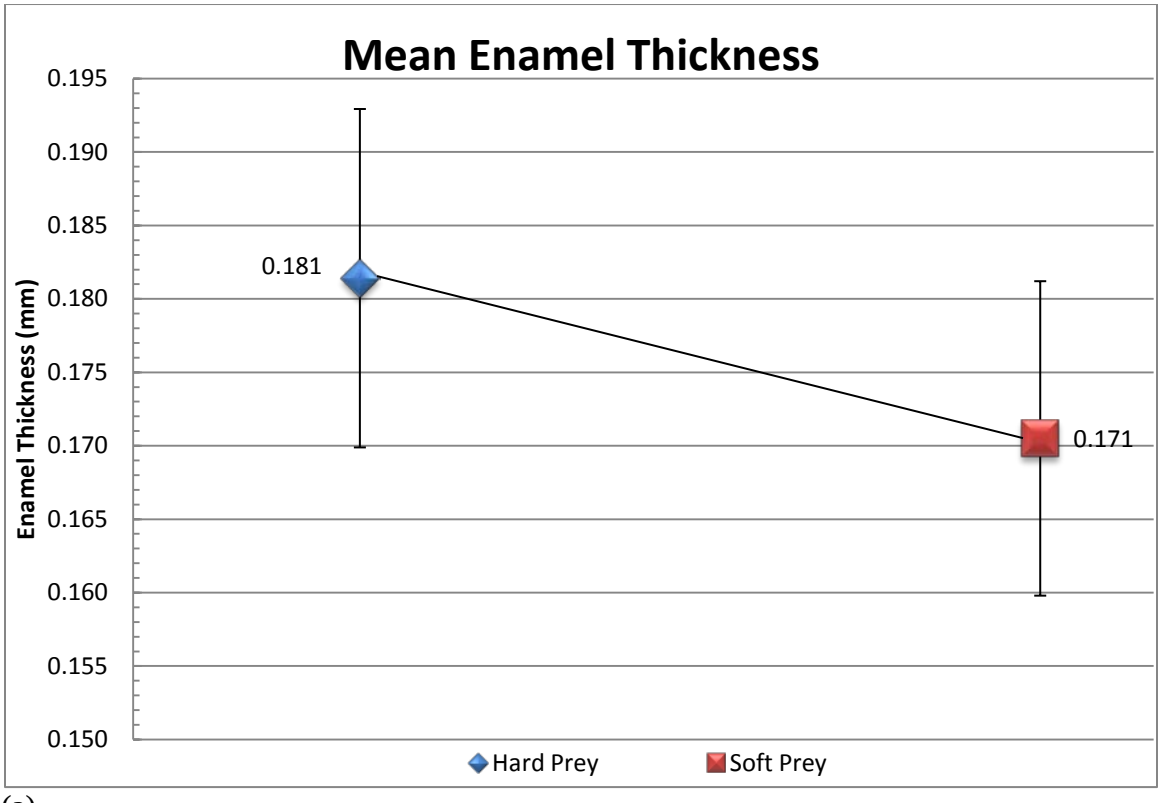
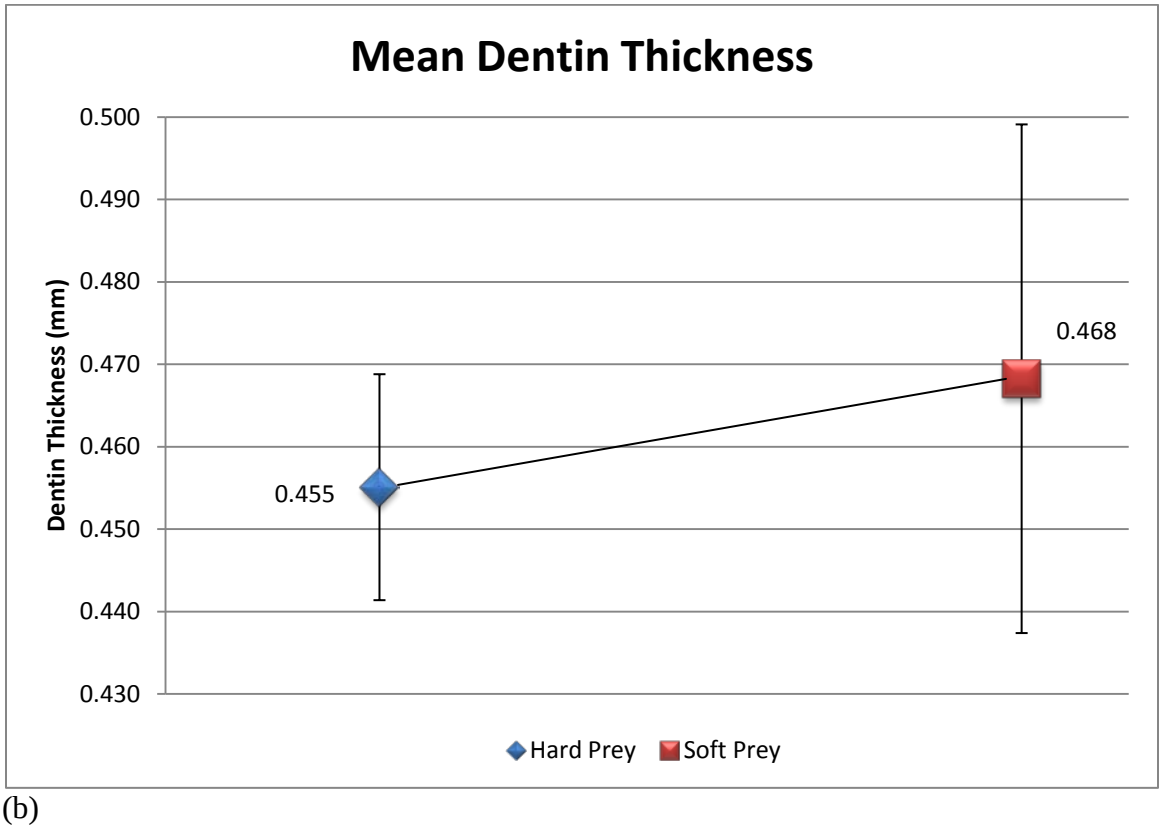


Figure 11. The relationship between the mean tooth height and mean dentin layer in sheepshead after 365 days of hard and soft prey treatment.

There was no significant difference found between the mean enamel and dentin thickness in the hard prey or soft prey treatments after 365 days (Figure 12; $F_{1,15}=0.384$, $P=0.545$; $F_{1,15}=0.205$, $P=0.657$, respectively). The mean enamel layer did demonstrate a general trend toward being thicker in the hard prey treatment, though not significantly so, 0.181 mm and 0.171 mm, respectively. The opposite was found in the mean dentin layer, which demonstrated a general trend toward being thicker in the soft prey treatment, 0.468 mm and 0.455 mm, respectively.



(a)



(b)

Figure 12. (a) The mean enamel and (b) dentin layer (mm) found in the hard and soft prey treatments (+/- SE).

A significant relationship between the standard length of hard prey and soft prey fish and mean tooth height was also found (Figure 13; $F_{1, 15}$=24.683, P<0.001). Standard length data is presented in Table 2. As the standard length of the fish increased, the mean tooth height in both treatments also increased.

Table 2. Standard length (SL) of the hard and soft prey fish after 365 days of treatment (Polohan-Maliao, 2010).

365 DAYS			
Soft-diet		Hard-diet	
Fish #	SL (mm)	Fish #	SL (mm)
1	113	1	91
8	90	4	112
14	103	5	115
15	105	6	111
17	135	7	98
25	115	19	102
		14	102
		15	117
		16	103
		23	125
		27	112

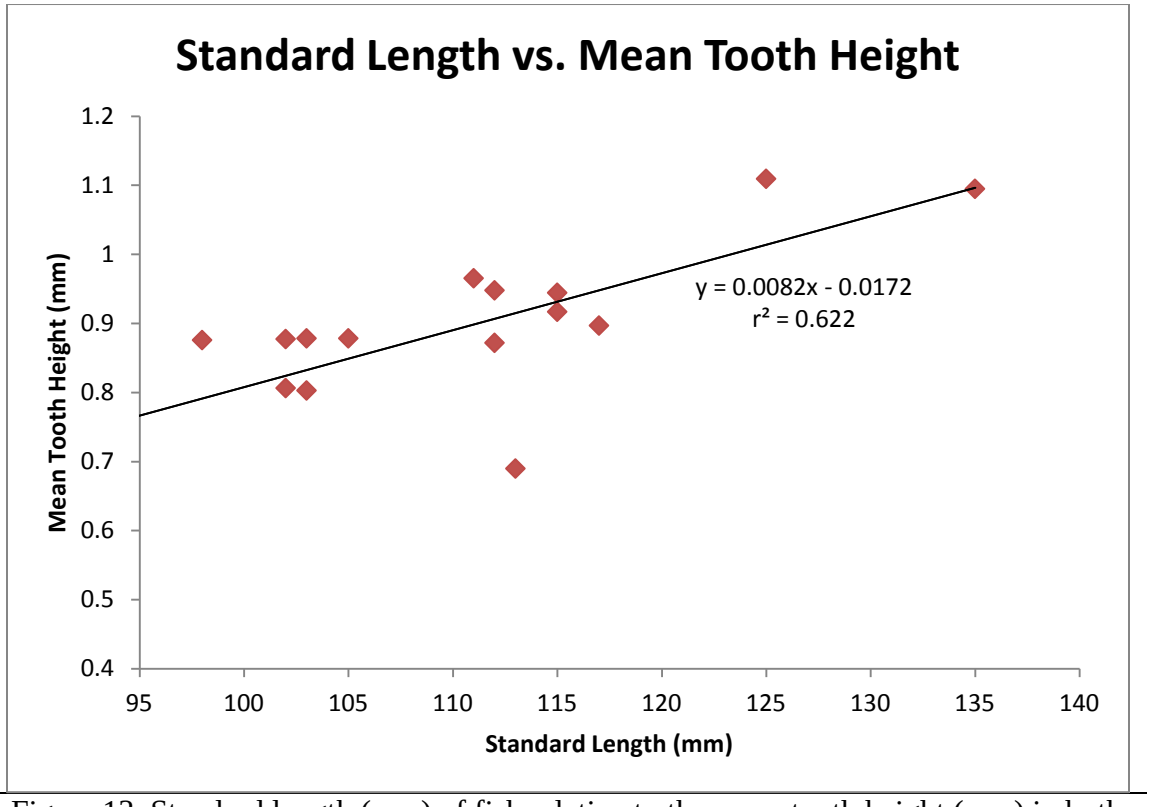


Figure 13. Standard length (mm) of fish relative to the mean tooth height (mm) in both treatments.

The calcium content of the *A. probatocephalus* did not demonstrate a significant difference in the enamel or dentin layers between the two treatments ($F_{1, 15}$=3.136, P=0.097; $F_{1, 15}$=1.494, P=0.241, respectively: Table 3). Calcium content did demonstrate a general trend toward being greater in hard prey fish, though not significantly greater. (Figure 14).

Table 3. ANOVA results testing the effect of diet on calcium content of enamel and dentin layers in *Archosargus probatocephalus* teeth.

Variable (wt. %)	df	F	P	Hard, Mean (±S.E.)	Soft, Mean (±S.E.)
Enamel Layer Calcium	1,15	3.136	0.097	39.325(±0.347)	37.447(±1.312)
Dentin Layer Calcium	1,15	1.494	0.241	37.081(±0.469)	35.690(±1.301)

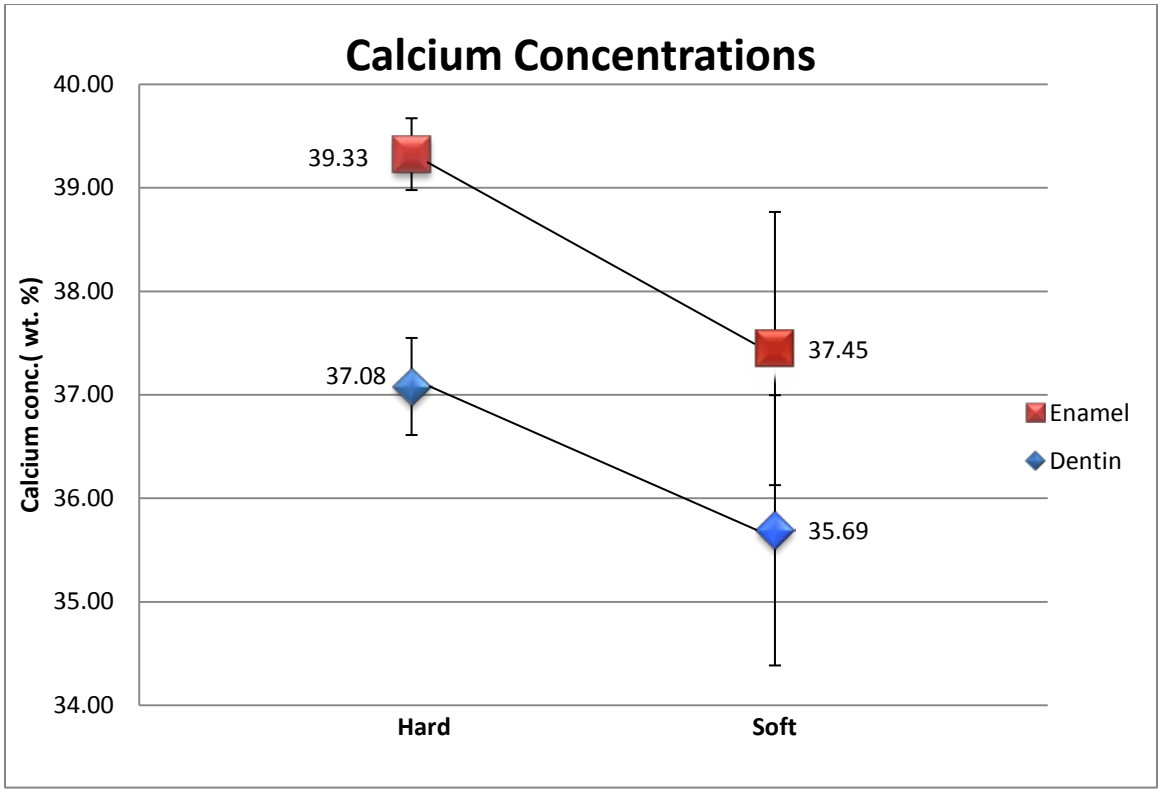


Figure 14. Calcium concentrations (wt. %) in enamel and dentin layers (+/- S.E.) of sheepshead molariform teeth after being fed hard prey and soft prey for 365 days.

There was a significant difference found in the percentage of functional jaw surface that was covered by teeth between the two treatments (Figure 15; $F_{1, 15}$=4.771, P=0.045; Table 4). The percentage of teeth covering the functional jaw surface of the hard prey fish was greater than the soft prey group, 57.20 % vs. 50.31%.

The mean functional jaw surface area appeared to be greater in the soft prey than the hard prey, 37.03 mm^2 and 31.22 mm^2, respectively. However, there was no significance found between the mean functional jaw surface area in the hard prey and soft prey treatments (Figure 16; $F_{1, 15}$=3.027, P=0.102; Table 4).

There was no significant difference found between the mean total tooth surface area of the hard prey, 18.13 mm^2, and the soft prey, 18.88 mm^2 (Figure 17; $F_{1,15}$=0.077, P=0.784; Table 4). Interestingly, the mean total surface area was statistically the same in

the two treatments, but the percent of functional jaw surface covered by teeth was significantly greater in the hard prey group.

Table 4. ANOVA results testing the effect of diet on total surface area of teeth, functional jaw surface area, and percent coverage of the jaw by teeth.

Variable	df	F	P	Hard, Mean (± S.E.)	Soft, Mean (± S.E.)
Functional Jaw Surface Area	1,15	3.027	0.102	31.221(±1.809)	37.031(±3.11)
Total Tooth Surface Area	1,15	0.077	0.784	18.133(±1.603)	18.882(±2.149)
Percent Coverage by Teeth	1,15	4.772	**0.045**	57.202(±1.011)	50.308(±3.382)

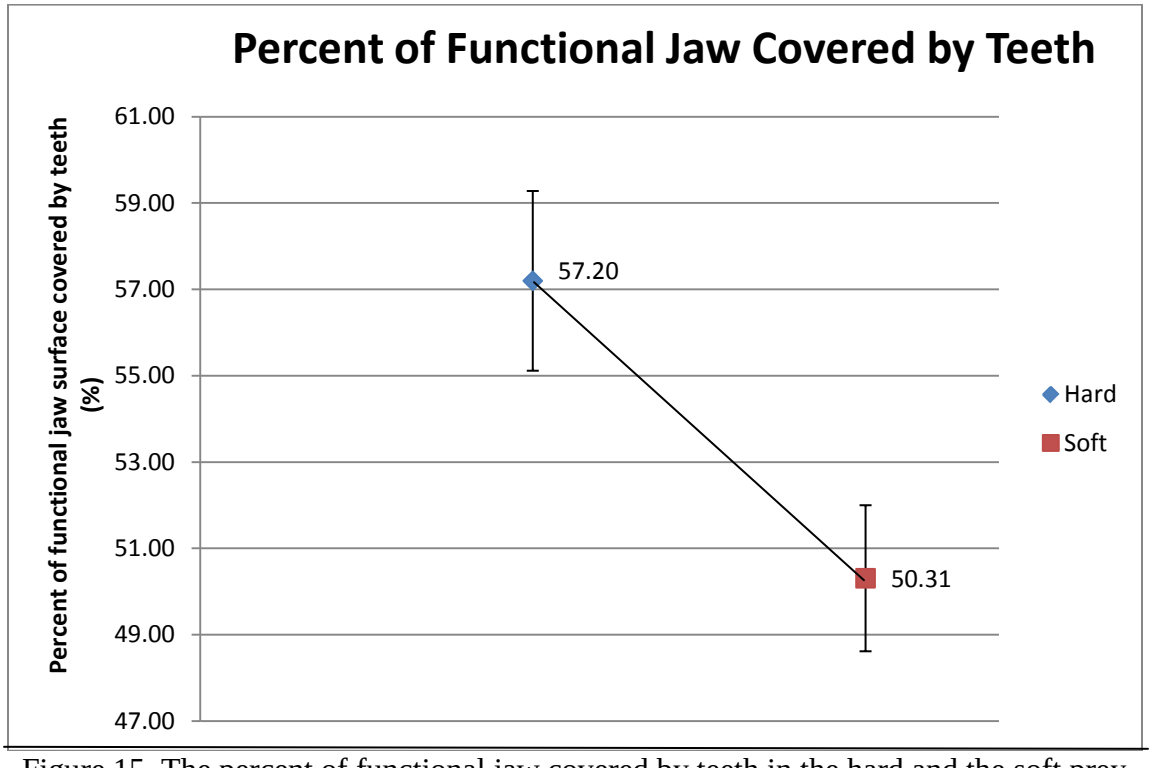


Figure 15. The percent of functional jaw covered by teeth in the hard and the soft prey treatments (+/- S.E.).

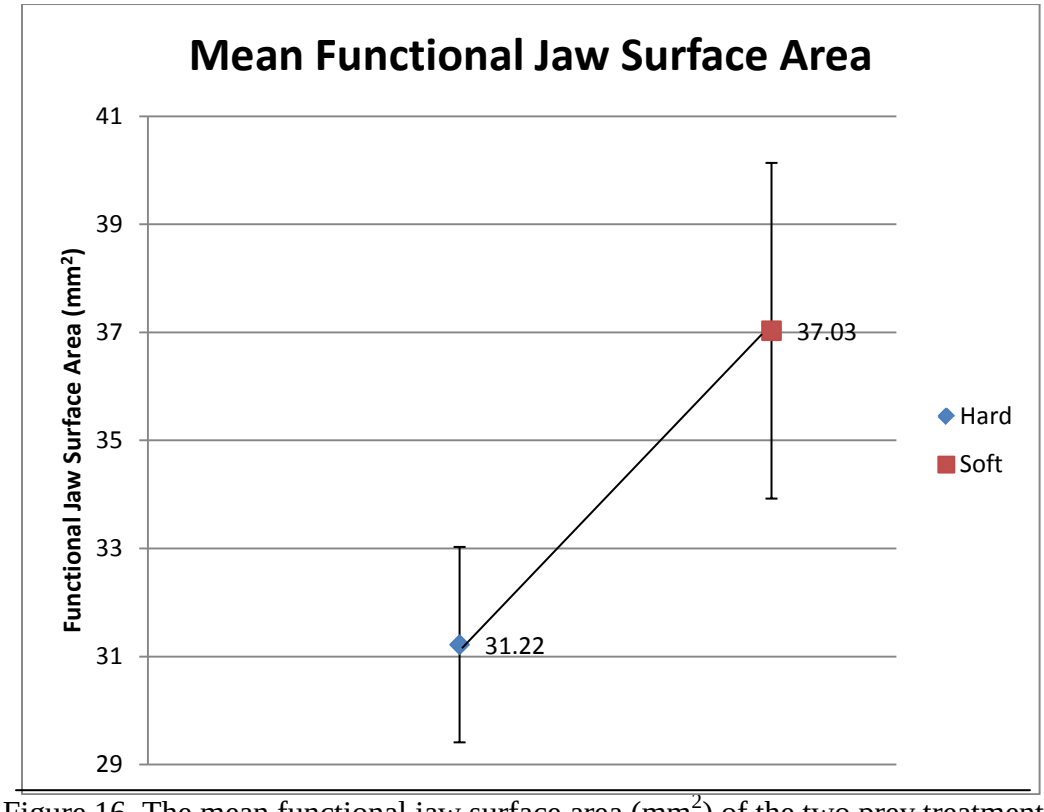


Figure 16. The mean functional jaw surface area (mm^2) of the two prey treatments (± S.E.).

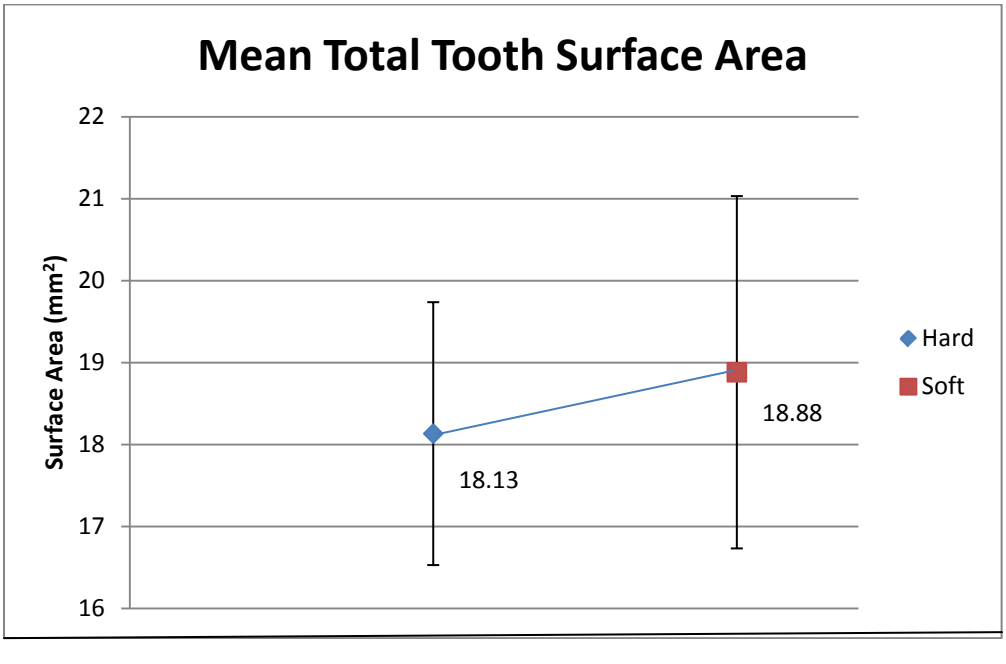


Figure 17. Mean total tooth surface area (mm^2) of the hard and soft prey treatment groups (± S.E.).

Finally, the mean number of teeth on each jaw was compared between the hard prey, 36.59 teeth, and soft prey, 38.92 teeth, and no significant difference was found (Figure 18; $F_{1, 15}$=0.691, P=0.419). There was a very high standard error for the soft prey fish, note the large error bars. This could have been due to the low representative sample of soft prey fish received.

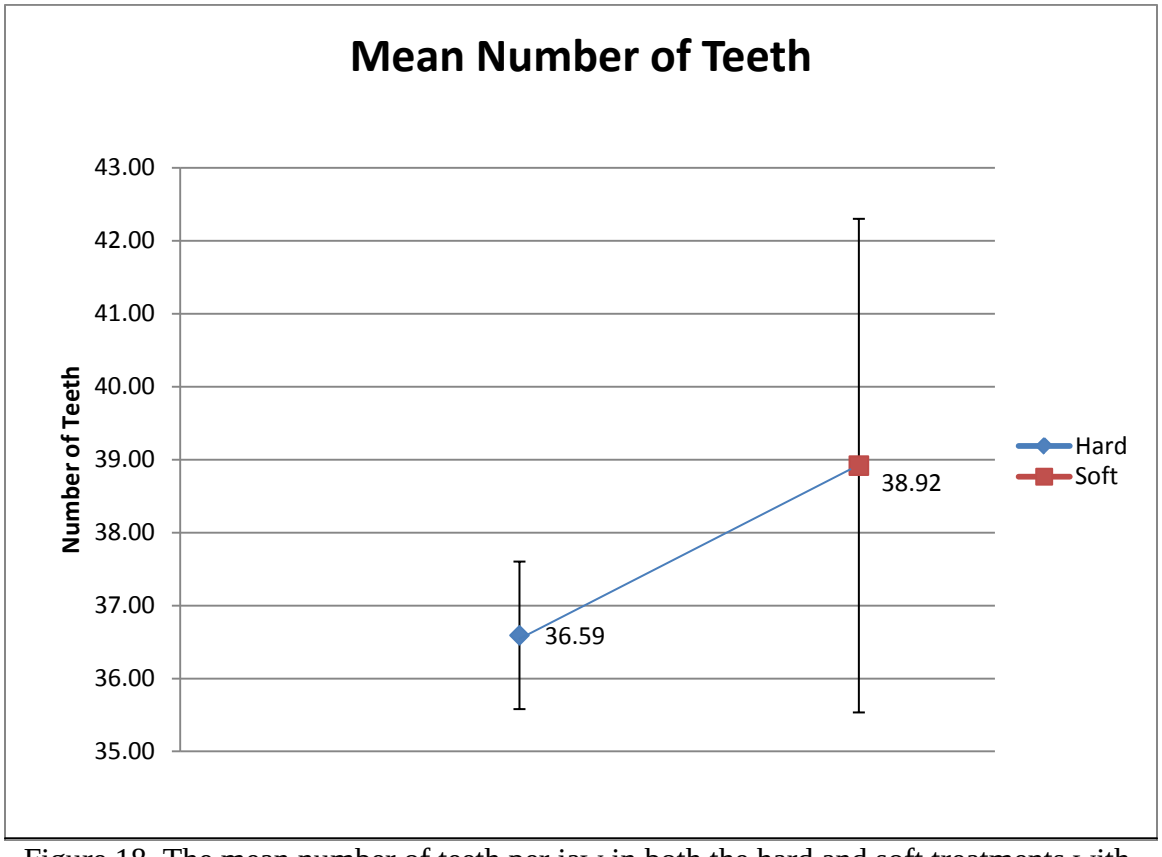


Figure 18. The mean number of teeth per jaw in both the hard and soft treatments with (± S.E.).

DISCUSSION

After 365 days of rearing, there was a positive correlation found between the standard length of the sheepshead and the mean tooth height. It is common knowledge that larger fish have larger teeth; but it is important to note that the individual fish with the larger mean tooth height in the hard prey treatment group, also demonstrated a significant increase in the mean enamel layer thickness. The correlation is distinct enough that, by examining the SEM photo of a tooth section's enamel layer, the prey treatment could be determined, especially in longer specimens. The opposite was found in the soft prey group, in which a significant positive correlation was found between an increase in the dentin layer and the mean tooth height. As the mean height of the tooth increased, the dentin layer of the hard prey teeth did not increase significantly.

The length of the sheepshead teeth does not demonstrate a wide degree of variation and therefore seemed to be genetically influenced based on the length of the fish. However, the proportions of the teeth layers, enamel and dentin, seems to be influenced by food resources, or a prey-induced reaction norm (Stearns, 1989; Via, et al., 1995). If the proportions of enamel and dentin in the teeth were based on fixed genetic factors, one might expect that the dentin and enamel layer would increase at the same rate proportionate to the increase in standard length, for both treatments. This was not found during this study to be true. The hard prey fish had an increased rate of enamel layer thickness associated with tooth size. The soft prey fish had an increased rate of dentin layer thickness associated with tooth size.

Fish eating hard prey benefit from changing their morphological feeding mechanism, having a thicker enamel layer, to aid in crushing the shell of the *Mercenaria* sp (Hulsey, et al., 2008; Polohan-Maliao, 2010). Fougerolle (2000) found that when *A.*

probatocephalus was fed hard prey items, saw bone thickness increased “for more resistance to stress for biting and crushing hard prey.” Like bone, when the enamel layer of teeth is thicker this might also make the tooth more resistant to stress while crushing the shell of hard prey items. Also, since the enamel layer of fish teeth is 4-5 times harder than the dentin layer, increasing the enamel to dentin ratio would increase the overall hardness of the teeth and make crushing hard prey less stressful on the fish (Chen, et al., 2012).

Although there was the previously stated correlation, there was no clear visible difference between the enamel and dentin layer heights when examined directly. This is surprising and could be due to the low representative sample of soft prey jaws received, which can provide a conservative estimate of treatment differences. If the sample size was larger the correlations might be more evident for phenotypic plasticity. The experiment was set up with 15 jaws in each treatment for the 365 day rearing stage, but there was high mortality among subjects while at Florida Institute of Technology.

A lack of food resource recognition (i.e. naivety) could have contributed to the high mortality rates, 60%, in the soft prey treatment. The reason for this difference in mortality was not clear but it was apparent that the hard prey fish did eat more clams than the soft prey treatment, and that the soft prey treatment seemed to ignore the clam prey more by comparison (Polohan-Maliao, 2010). The soft prey fish may not have recognized the clam prey as a food source because the unshelled clams are not found in nature and is a novel prey item. When exposed to novel prey stimuli in laboratory conditions, prey-capture success of individual fish depends on chronological age and prior feeding experience (Godin, 1978; Brown, Davidson, & Laland, 2003). The fish in this experiment

were captured very young and had no previous experience with this type of prey to establish it as a food source.

Novel prey introduction could account for the 26.7% mortality rate in the hard prey treatment during the rearing phase of this experiment. This rate could have also been due to an inability of the fish to remodel their enamel and dentin thickness to consume the newly introduced hard prey. The inability of some fish to adjust might suggest that phenotypic plasticity itself might be an adaptive trait not exhibited by every fish in a population (Frost, et al., 2007).

The calcium results were also unexpected. A fish that is eating hard prey items—such as the hard shells in this experiment—was expected to have both a thicker and more calcium-laden enamel layer in order to withstand the structural pressures of consuming such a hard prey. As stated earlier, the teeth with increased calcium levels in their elemental composition of enamel have greater microhardness of enamel (Davidson, Hoekstra, & Arends, 1974). Thus, calcium level is a good indicator of enamel hardness; nonetheless, there was no significant difference found in the calcium data between the two treatments.

The ability to increase the amount of calcium in response to stimulus may have been out of the phenotypic response of this species, but the treatment group did significantly increase the proportion of enamel versus the proportion of dentin in the teeth. This response to the hard prey stimulus suggests that there are two ways to strengthen the enamel layer of teeth: increase calcium content or thicken the enamel layer. It would appear that *Archosargus probatocephalus* does the latter.

When consuming a hard prey item like *Mercenaria* an increase in the percent of teeth in the jaw would increase the points of contact on the shell and would cause more

surface area to apply pressure and therefore cause the shell to be crushed more easily. This would be an advantage in consuming hard prey items and a phenotypic response to food stimulus. Fish morphology is the underlining variation in feeding ability and a key role in shaping diet (Wainwright & Barton, 1995). The jaw morphology would have to change if the hard prey group was going to consume the intact shells.

This experiment revealed a significant difference in the percentage of functional jaw surface that was covered by teeth between the two prey treatments (Figure 15). The hard prey treatment had significantly more percent of functional jaw surface covered by teeth than the soft prey treatments. This increase in the percentage of teeth that can come in contact with hard food resources is necessary to utilize this resource.

There are trade-offs for individuals that change or enhancement phenotypes to combat environmental stressors. For example, a study was performed on pumpkinseed sunfish, *Lepomis gibbosus*, to examine the effect of prey resources, hard and soft, on pharyngeal jaw structure. The resulting fish from the soft prey group did have a longer standard length, but had much smaller jaws than the hard prey group (Mittlebach, Osenberg, & Wainwright, 1999). While the hard prey group invested mineral and calorie resources into tooth structure, the soft prey group put nutrients and minerals into growth. This trade-off resulted in each group investing materials in different physical structures depending on the food resource utilized to increase their fitness.

Does phenotypic plasticity mean that any animal can change its phenotype to any need in a habitat affected by environmental stressors? No, of course not. Every individual is restricted to the amount of their plasticity based on the evolutionary history of the animal and ancient developmental genes that make up their ridged framework (Stearns, 2009). For example, a fish is not going to look up at land and one day relocate. There is

too much evolutionary history such as gill development versus lungs, etc. that restrict the plasticity of that organism. If the same fish had a change in food resources in an area though, it might have enough phenotypic plasticity to utilize the new food resource in that habitat (Kerfoot, Lorenz, & Turingan, 2011).

Phenotypic plasticity has been thought of as a "non-genetic" response because by definition, it means a phenotypic change that can result without changing the genotype of the organisms confronting environment variation. However phenotypic plasticity is itself a trait, and is subject to natural selection and evolutionary change. Therefore it would be a mistake to think of it as "non-genetic" (West-Eberhard, 1989).

When environmental factors such as food resources stress individuals in a population, the plasticity response by individuals may be different from one another, so that different food resources in that habitat can be consumed. This term is resource polymorphism, and is defined as the occurrence of different morphotypes within a single population using different resources and has been found in many different taxa (Ruehl & DeWitt, 2007; Andersson, et al., 2007). This type of resource response is usually found in species that are cannibalistic and usually during the early stages of development including egg and larval stages found in some species (Andersson, et al., 2007). This may be found in some populations of *Archosargus* but the population would have to be under greater stress conditions for this to occur.

The adaptive role of phenotypic plasticity has been studied in relation to resource exploitation and morphology (Cutwa & Turingan, 2000; Selvaraj, 2010), but never has the different layers of the *Archosargus probatocephalus* tooth been examined using SEM techniques to determine the phenotypic plasticity of fish as in this experiment. This is a new avenue for research of the phenotypic response to environmental stimuli. This

procedure could be used on countless number of fish species and in a relatively short time period. One of the drawbacks with this type of experimentation is that a SEM is required and that is a very expensive purchase or rental. There is also the training that is required to make the pictures correctly using the SEM and the various computer programs associated with this project. Each program and skill takes time to learn and perfect. Additionally, when working with such small pieces of teeth it is also possible to lose a test subject very easily. Another drawback in this type of experimentation is the unpredictable nature of live animal testing. When working with live test subjects there are multiple factors that could negatively affect the results of the experiment, including previously mentioned mortality rate, the test subjects not performing as expected or as needed to collect data, etc.

Even with these drawbacks, the techniques used in this experiment could be used for further investigation of phenotypic plasticity in different species utilizing a variety of food resources. The duration of the experiment could also be changed to see if a shorter treatment or longer treatment duration would have any effect on the morphological response. Future research could examine if the phenotypic response diminishes when changing between prey types multiple times during the life of a fish or with the age of the fish. *Archosargus probatocephalus* would be an optimal test subject for this type of research because as previously stated it has a life expectancy of 20 years plus.

"The ability of a fish species to inhabit different environments depends upon its propensity to adapt to local conditions, by making use of available prey-resources" (Huskey & Turingan, 2001). Phenotypic plasticity allows organisms to change to the variations in environments and utilize new resources to realize new niches (Wintzer, 2004; Ghalambor, et al., 2007). The change in jaw morphology would allow sheepshead

to move into new environments and utilize the food resources in that new habitat. Sheepshead phenotypic plasticity could account for the large range of this species.

The ability of sheepshead to change jaw morphology during a relatively short time, 365 days, was found during this experiment. The percentage of teeth covering the functional jaw surface of the hard prey fish was greater than the soft prey group, 57.20 % vs. 50.31%, respectively. There was a significant positive correlation between the thickness of the enamel layer in the hard prey group and the mean tooth height, with a slope of 0.2844 and 0.1192, respectively. Finally, there was a significant positive relationship found between the dentin layer of the soft prey group and the mean tooth height, with a slope of 0.4263 and 0.217, respectively.

No significant difference was found in the enamel thickness or the amount of calcium, the mean functional jaw surface area, number of teeth per jaw, or the combined surface area of the teeth between treatments. It is apparent that phenotypic plasticity can increase an individual's ability to survive in a variable food resource environment by changing some aspects of jaw/tooth morphology, but the ability to change in response to stimuli was not found in all areas of tooth structure.

BIBLIOGRAPHY

Andersson, J., Par, B., Claessen, D., Persson, L., & De Roos, A. M. (2007). Stabilization of population fluctuations due to cannibalism promotes resource polymorphism. *American Society of Naturalists*, 169(6), 820-829.

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, 13(1), 115-151.

Brown, C., Davidson, T., & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology*, 63, 187-196.

Brown, T. L. (2003). *Chemistry: the central science, 9th ed.* Upper Saddle River, N.J.: Prentice Hall.

Chen, P.Y., Schirer, J., Simpson, A., Nay, R., Lin, Y.S., Yang, W., Lopez, J.L., Olevsky, E. A., Meyers, M.A.(2012). Predation versus protection: Fish teeth and scales evaluated by nanoidentation. *Journal of Materials Research*, 27(1), 100-112.

Clifton, K. B., & Motta, P. J. (1998). Feeding morphology, diet, and ecomorphological relationships among five Caribbean labrids (Teleostei, Labridae). *Copeia*, 1998(4), 953-966.

Cutwa, M. M., & Turingan, R. G. (2000). Intralocality variation in feeding biomechanics and prey use in *Archosargus probatocephalus* (Teleostei, Sparidae), with implications for the ecomorphology of fishes. *Environmental Biology of Fishes*, 59(2), 191-198.

Davidson, C. L., Hoekstra, I. S., & Arends, J. (1974). Microhardness of sound, decalcified and etched tooth enamel related to the calcium content. *Caries Research*, 8(2), 135-144.

Evans, D. H. (1998). *The Physiology of Fishes.* Boca Raton: CRC Press LLC.

Ferry-Graham, L. A., Wainwright, P. C., & Bellwood, D. R. (2001). Prey capture in long-jawed butterflyfishes (Chaetodontidae): the functional basis of novel feeding habits. *Journal of Experimental Marine Biology and Ecology*, 256, 167-184.

Flegler, S. L., Heckman, J. W., & Klomparens, K. L. (1993). *Scanning and Transmission: Electron Microscopy An Introduction.* New York: Oxford University Press, Inc.

Fougerolle, M. F. (2000). *Prey-induced changes in the feeding biomechanics of the sheepshead, Archosargus probatocephalus.* (Master's thesis). Florida Institute of Technology. Melbourne, FL.

Frost, A. J., Winrow-Giffen, A., Ashley, P. J., & Sneddon, L. U. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? *The Royal Society of Biological Sciences*, 274, 333-339.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology, 21*(3), 394-407.

Godin, J.-G. (1978). Behavior of juvenile pink salmon (*Oncorhynchus gorbuscha* Walbaum) toward novel prey. *Environmental Biology Fisheries*, 3(3), 261-266.

Hernandez, L. P., & Motta, P. J. (1997). Trophic consequences of differential performance: ontogeny of oral jaw-crushing performance in the sheephead, *Archosargus probatocephalus* (Teleostei, Sparidae). *Journal of Zoology London*, 243, 737-756.

Herold, R. C., Graver, H. T., & Christner, P. (1980). Immunohistochemical localization of amelogenins in enameloid of lower vertebrate teeth. *Science (New York, NY), 207*(4437), 1357.

Horn, M. H., Martin, K. L., & Chotkowski, M. A. (1999). *Intertidal Fishes: Life in Two Worlds.* San Diego CA: Academic Press.

Hulsey, C. D., Roberts, R. J., Lin, A. S., Guldberg, R., & Streelman, J.T. (2008) Convergence in a mechanically complex phenotype: detecting structural adaptations for crushing in cichlid fish. *Evolution,* 62(7), 1587-1599.

Huskey, S., & Turingan, R. (2001). Variation in prey-resource utilization and oral jaw gape between two populations of largemouth bass, *Micropterus salmoides. Environmental Biology of Fishes*, 61, 185-194.

Jong, G. d. (2004). Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytologist*, 166(1), 101-118.

Kerfoot, J. R., Lorenz, J. J., & Turingan, R. G. (2011). Environmental correlates of the abundance and distribution of *Belonesox belizanus* in a novel environment. *Environmental Biology of Fishes*. 92, 125-139.

Liao, H., Haga, S., Jones, C. M. (1991). *Virginia Recreational Development Fund Summary Project.* Center for Quantitative Fisheries Ecology. Old Dominion University. Norfolk, VA: Virginia State Records.

Liem, K. (1980). Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. *Environmental Physiology of Fishes*, 35, 299-334.

Meyer, A. (1987). Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution,* 1357-1359.

Mittlebach, G. G., Osenberg, C. W., & Wainwright, P. C. (1999). Variation in feeding morphology between pumpikseed populations: phenotypic plasticity or evolution. *Evolutionary Ecology Research*, 1, 111-128.

Motta, P.J. (1987). A quantitative analysis of ferric iron in butterfly teeth (Chaetodontidae, Perciformes) and the relationship to feeding ecology. *Canadian Journal of Zoology*. 65. 106-112.

Moyle, P., & Cech, J. J. (2004). *Fishes: An Introduction to Ichthyology.* San Francisco: Pearson Benjamin Cummings.

Nelson, A. E., Hildebrand, N. K., & Major, P. W. (2002). Mature dental enamel [Calcium Hydroxyapatite, Ca10(PO4)6(oh)2] by XPS. *Surface Science Spectra*, 9(1), 250-259.

Norton, S. F. (1995). A functional approach to ecomorphological patterns of feeding in cottid fishes. *Environmental Biology Fishes,* 44(1), 61-78.

Polohan-Maliao, B. (2010). *Feeding mechanism of sheephead, Archosargus probatocephalus.* (Master's thesis). Melbourne, Flordia: Florida Institute of Technology.

Price, T. D., Qvarnstrom, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *The Royal Society*, 270(15230, 1433-1440.

Reytan. (2006). Department of Histology, Jagiellonian University Medical College. Retrieved May 28, 2012, from: http://www.histologia.cm-uj.krakow.pl/index.html

Ray, C., & Robins, C. R. (1999). *A field guide to Atlantic coast fishes: North America* (Vol. 32). Houghton Mifflin Harcourt.

Ruehl, C. B., & DeWitt, T. J. (2007). Trophic plasticity and foraging performance in red drum, *Sciaenops ocellatus* (Linnaeus). *Journal of Experimental Marine Biology and Ecology.* 349. 284-294.

Scheiner, S. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, 24, 35-68.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and environment.* Cambridge: Cambridge University Press.

Schmitt, R. J., & Holbrook, S. J. (1984). Ontogeny of prey selection by black surfperch *Embiotoca jacksoni* (Pisces Embiotocidae): the role of fish morphology, foraging behavior, and patch selection. *Marine Ecology*, 18, 225-239.

Sedberry, G. (1989). Feeding habits of sheephead, *Archosargus probatocephalus*, in inshore reef habitats of the southeastern continental self. *Norteast Gulf Science*, 87(4), 29-37.

Selvaraj, T. (2010). *"Environment-induced phenotypic plasticity in the teeth of hatchery and wild largemouth bass, Micropterus floridanus".* (Master's thesis). Retrieved from TopSCHOLAR: Masters Theses & Specialist Projects. Western Kentucky University. (Paper 200)

Stearns, S. C. (2009, Sept). Principles of evolution, ecology, and behavior. New Haven, CT, USA.

Stearns, S. C. (1989). The evolutionary significance of phenotypic plasticity. *BioScience,* 39(7), 436-445.

Turingan, R. G., & Huskey, S. H. (2000). *Estimated biting strength of fishes and the effects of substratum type and jacket color on the vulnerability of underwater cables to fish bites.* Melbourne, FL: Florida Institute fo Technology.

Underwood, C. J., Mitchell, S. F., & Veltkamp, C. J. (1999). Microborings in mid-Cretaceous fish teeth. *Proceedings of the Yorkshire Geological Society, 52*(3), 269-274.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S., Schlichting, C., & Tienderen, P. (1995). Adaptive phenotypic plasticity: consensus and controversy. *TREE*, 10(5), 212-217.

Wainwright, P. C., & Barton, R. A. (1995). Predicting patterns of prey use from morphology of fishes. *Environmental Biology of Fishes,* 44, 97-113.

Webb, A. L. (2011). *Sound production in two loricariid catfishes*. (Master's thesis) Retrieved from TopSCHOLAR: Masters Theses & Specialist Projects. Western Kentucky University. (Paper 1089)

West-Eberhard, M. J. (1989). Phenotypic plasicity and the origins of diversity. *Annual review of ecological systems,* 20, 249-78.

Wintzer, A. P.(2004). *Morphological and behavioral consequences of rearing Florida largemouth bass with non-elusive prey.* (Master's thesis) Retrieved from Scholar Commons. University of South Florida. Paper 1307.