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Structure, property, and function of sheepshead (*Archosargus probatocephalus*) teeth

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ABSTRACT

Objectives: This paper studies *A. probatocephalus* teeth and investigates the mechanical properties and chemical composition of the enameloid and dentin.

Design: Nanoindentation tests with a max load of 1000 μN and X-ray Energy Dispersive Spectroscopy (EDS) were performed along the diameter of the polished sample. Microstructural analysis of the dentin tubules was performed from SEM images.

Results: From nanoindentation testing, the dentin of the sheepshead teeth has a nanoindentation hardness of 0.89 ± 0.21 (mean ± S.D.) GPa and a reduced Young's modulus of 23.29 ± 5.30 GPa. The enameloid of *A. probatocephalus* has a hardness of 4.36 ± 0.44 GPa and a mean reduced Young's modulus of 98.14 ± 6.91 GPa. Additionally, nanoindentation tests showed that the enameloid's hardness and modulus increased closer to the surface of the tooth. X-ray Energy Dispersive Spectroscopy (EDS) data further suggests that the gradient may be a result of the wt% fluoride within the enameloid, where an increase in fluoride results in an increase in reduced Young's modulus and hardness.

Conclusion: The microstructural characterization of the number density and area of the dentin tubules were used to address the porosity effect in the dentin to achieve the experimentally validated microhardness. The mechanical properties of the sheepshead teeth were also compared with previous nanoindentation tests from other aquatic species. The sheepshead teeth exhibit a greater reduced Young's modulus and hardness compared to shark and piranha teeth.

1. Introduction

Archosargus probatocephalus, commonly referred to as the sheepshead fish, live along the coasts of the Atlantic and the Gulf of Mexico. They can grow to a length of about 75 cm and to a weight of 10 kg and are known for its bizarre, almost human-like in appearance, teeth (Bigelow & Shroeder, 1953). Previous studies have focused on the bones, scales, diet, and oral jaw strength of *A. probatocephalus* (Fernandez & Motta, 1997; Ogawa et al., 2004); however, the biomechanics and the material properties of the teeth have not been examined. In general, teeth can be subjected to large loads during feeding, producing a stress and strain that may result in the failure of teeth, which is even more of a danger for the sheepshead with its dietary regimen ranging from small invertebrate species (copepods, amphipods, and mysids) to hard-shelled animals such as barnacles, crabs, oysters, and clams (Bigelow & Shroeder, 1953; Overstreet & Heard, 1982; Sedberry, 1987).

Teeth are considered one of the hardest substances found in animals (He & Swain, 2008). They exhibit exceptional mechanical properties that allow them to bear various imposed loads while retaining their shape and are ideal for biting and chewing (Braly, Darnell, Mann, Teaford, & Weihs, 2007). Teeth are composed of highly mineralized enamel and dentin. For example, human enamel is 85 vol% mineralized and is the hardest tissue in the human body (Cate, 1994). The function of enamel is to provide a hard surface for crushing and slicing food and, for some species, wounding its prey (Currey, 2002). Conversely, human dentin is 50 vol% mineralized, 30 vol% organic components, and 20 vol% fluids with a microstructure characterized by distinct tubules (Habelitz, Marshall, Balooch, & Marshall, 2002). Fish teeth are made of enameloid and dentin. Enameloid is analogous to mammalian enamel, even though the developmental process is different. The individual properties of both enameloid and dentin were studied to understand the function and strength of the teeth of the sheepshead.

The chemical composition of teeth can provide insight into its

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mechanical strength, particularly the wt% of calcium and phosphorus (Cuy, Mann, Livi, Teaford, & Weihs, 2002; Jeng, Lin, Hsu, Chang, & Shieh, 2011). Calcium and phosphorus are present in hydroxyapatite, $Ca_5(PO_4)_3(OH)$, the mineral strengthening human teeth; however, unlike human teeth, some fish teeth are strengthened by fluorapatite, $Ca_3(PO_4)F$ (Enax, Prymak, Raabe, & Epple, 2012). Compared to hydroxyapatite, fluorapatite has a higher bulk modulus, stiffness constants, and elastic modulus (Brunet et al., 1999; Gardner, Elliott, Sklar, & Briggs, 1992). In this paper, the chemical composition of the sheepshead enameloid and dentin are investigated through X-ray Energy Dispersive Spectroscopy (EDS). The micromechanical properties of the enameloid and dentin were captured using nanoindentation, which is a common technique used to determine the mechanical properties of hard biological tissues (Kinney, Balooch, Marshall, Marshall, & Weihs, 1996; Rho, Tsui, & Pharr, 1997). Previous research on the mechanical properties of fish teeth have focused on sharp teeth whose primary function is to tear through the flesh of its prey. In contrast, the function of *A. probatocephalus* teeth is to crush the shells of its prey. Experimental results from this study will be compared to previous nanoindentation tests done on the bonnethead shark, sand tiger shark, great white shark, and piranha (Chen et al., 2012; Whitenack, Simkins, Motta, Hirai, & Kumar, 2010). The goal of this paper is to investigate the microstructure, chemical composition, and material properties of *A. probatocephalus* teeth to gain insight into the structure's integrity.

2. Materials and methods

2.1. Sample preparation

Specimens of *A. probatocephalus* were acquired from the Mississippi Gulf Coast. Sheepshead teeth were kept in an ambient dry condition before testing, and all samples were extracted from one fish. Fig. 1 shows the extracted teeth used for this study. Molar teeth were removed, cold mounted in epoxy resin, and polished transversely approximately halfway down to create the surface for analysis. Samples were polished with a Struers TegraPol-11 (Struers Inc., Cleveland, OH) using interchangeable silicon carbide grinding discs of decreasing grit size (e.g. 320, 500, 1200, 4000). Diamond and silica suspensions with grit size 1 μm and 0.04 μm, respectively, were used for the final polishing.

2.2. Microstructural analysis

Optical micrographs of polished samples were taken using a ZEISS Axiovert 200 m. The microstructure of the dentin and enameloid were characterized by Scanning Electron Microscopy (SEM) at 20 kV using a ZEISS SUPRA 40 FESEM (Carl Zeiss Inc., Thornwood, NY). Fig. 2 diagrams the surface area used for nanoindentation, optical microscope, and SEM. The dentin tubule number density, dentin tubule diameter size, and nearest neighbor distances of the dentin tubules were quantified using ImageJ analysis software (Schneider, Rasband, & Eliceiri, 2012). The results are shown in Fig. 3.

2.3. Nanoindentation

Specimens were tested using a Triboindenter TI-900 (Hysitron Inc., Minneapolis, MN) with a diamond Berkovich tip of radius of 150 nm. A total number of 75 indentations with a max load of 1000 μN was performed on both the enameloid and dentin sections. Specimens were indented at a load rate and unload rate of 200 μN/s. The max load was held for 5 s before unloading. Load versus displacement was continuously recorded throughout the testing. Additionally, 100 indentations spaced 26 μm apart with a max load of 1000 μN were performed along the entire diameter of the polished sample to observe the changes in material properties based on the test location of the tooth. The data was analyzed using standard routines (Oliver & Pharr, 1992) to determine the hardness, *H*. The hardness for each test is defined by Eq. (1):

$$H = \frac{P_{\max}}{A} \quad (1)$$

where $P_{\max}$ is the peak load, and *A* is the projected indentation area indenter. The reduced Young's modulus is calculated during the unloading phase and is described by Eq. (2):

$$E_r = \frac{1}{2}\sqrt{\frac{\pi}{A}}\frac{dP}{dh} \quad (2)$$

where dP/dh is the slope of the unloading curve. For the sake of this convenience, the reduced Young's modulus will be referred to as modulus for the remainder of this paper.

2.4. Chemical analysis

After nanoindentation, the chemical composition of both the enameloid and dentin was explored by X-ray Energy Dispersive Spectroscopy (EDS). Testing was performed using a ZEISS SUPRA 40 FESEM equipped with an EDAX PV7715/89 ME analyzer (EDAX, Mahwah, NJ). The local variations in chemical composition were

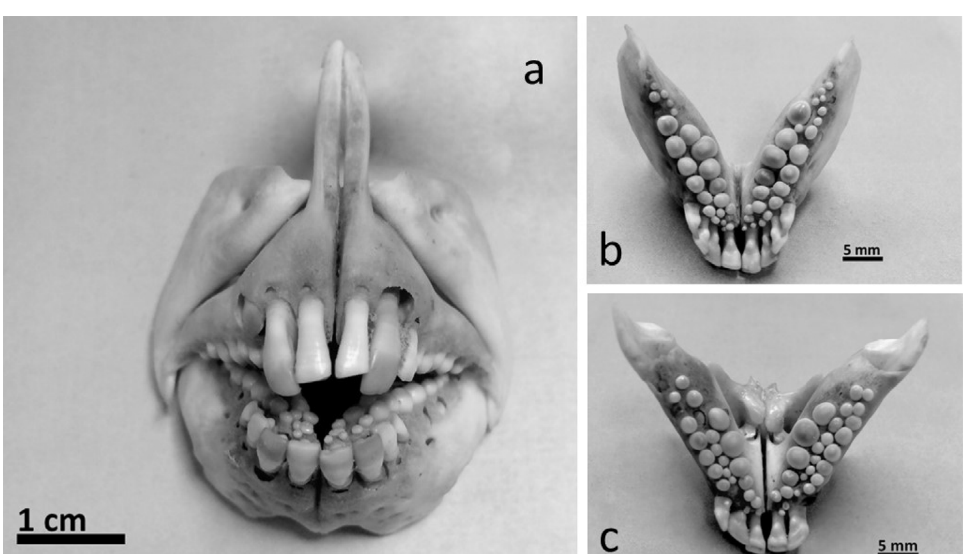

Fig. 1. (a) *A. probatocephalus* teeth. (b) Upper section. (c) Lower section.

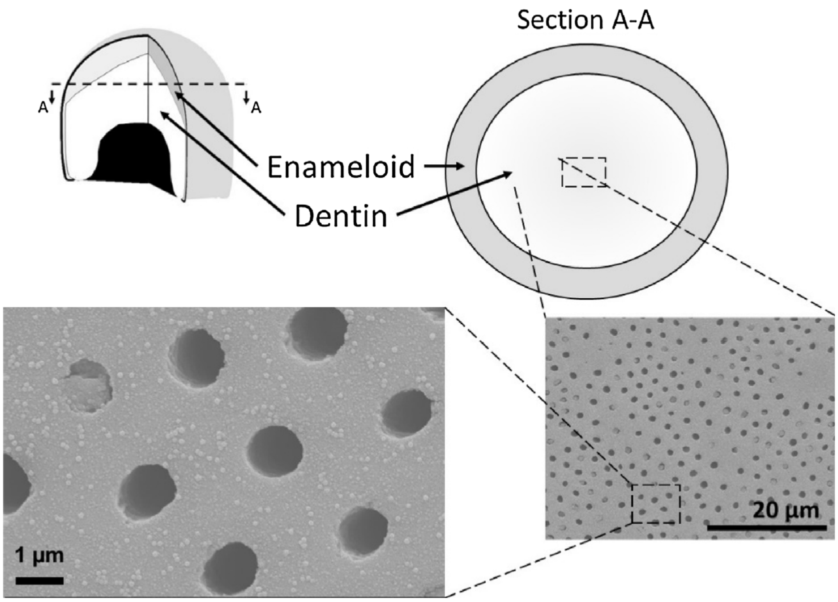

Fig. 2. *A. probatocephalus* tooth (top left), surface area for structural and chemical analysis (Section A-A), and SEM image of dentin tubules (bottom).

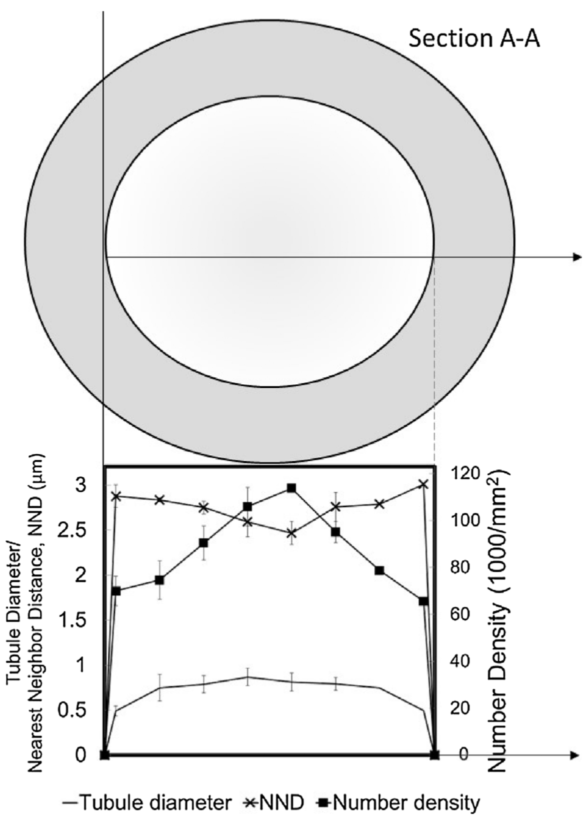

Fig. 3. Dentin tubule quantification as a function of spatial location.

recorded to characterize and identify the changes in chemistry throughout the diameter of the cross section of the tooth. The weight percent of the main and trace elements were acquired through the EDAX GENESIS EDS software. Of particular interest is the weight percent of calcium, phosphorus, and fluoride.

3. Results

3.1. Microstructural analysis

Fig. 3 displays the ImageJ analysis of the dentin tubules, quantifying the changes in number density, diameter size, and nearest neighbor distance. The number density increases from the dentin-enameloid junction towards the center of the dentin region, increasing from 65,000 per mm^2 to 115,000 per mm^2. Likewise, the tubule diameter is larger in the center of the dentin region, increasing from 0.50 μm to 0.87 μm. The nearest neighbor distance of the dentin tubules decreases towards the center of the dentin, from 3.01 μm to 2.47 μm.

3.2. Nanoindentation

From the nanoindentation testing, the dentin of the sheepshead teeth has a hardness of 0.89 ± 0.21 (mean ± S.D.) GPa and a modulus of 23.29 ± 5.30 GPa. The enameloid of *A. probatocephalus* has a hardness of 4.36 ± 0.44 GPa and a modulus of 98.14 ± 6.91 GPa.

Additionally, an uncertainty analysis was performed on the nanoindentation data. Eq. (3) shows the confidence interval associated with a normal probability distribution (Fisher, 1915).

$$\bar{x} - t_{\nu,\alpha}\frac{S_x}{\sqrt{N}} \le \mu \le \bar{x} + t_{\nu,\alpha}\frac{S_x}{\sqrt{N}} \tag{3}$$

where $\bar{x}$ is the average of the data points, S_x is the standard deviation of N measurements, and t represents a constant based on the confidence level, α, and degrees of freedom, ν. The 95% confidence interval for the hardness of the dentin and enameloid are 0.89 ± 0.05 GPa and 4.36 ± 0.14 GPa, respectively. The 95% confidence interval for the modulus of the dentin and enameloid are 23.29 ± 1.22 GPa and 98.14 ± 2.21 GPa, respectively.

Nanoindentation was also performed along the entire diameter of the polished sample to determine the changes in the modulus and hardness. Fig. 4 shows the changes in modulus and hardness along the diameter of the tooth. Within the dentin, the modulus and hardness are constant. However, in the enameloid region, a gradient of material properties is present, with the harder material being closer to the surface of the tooth.

3.3. Chemical analysis

The chemical composition for the enameloid and dentin regions are shown in Table 1. Additionally, the changes in chemical composition along the enameloid region for calcium, phosphorus, and fluoride are shown in Fig. 5.

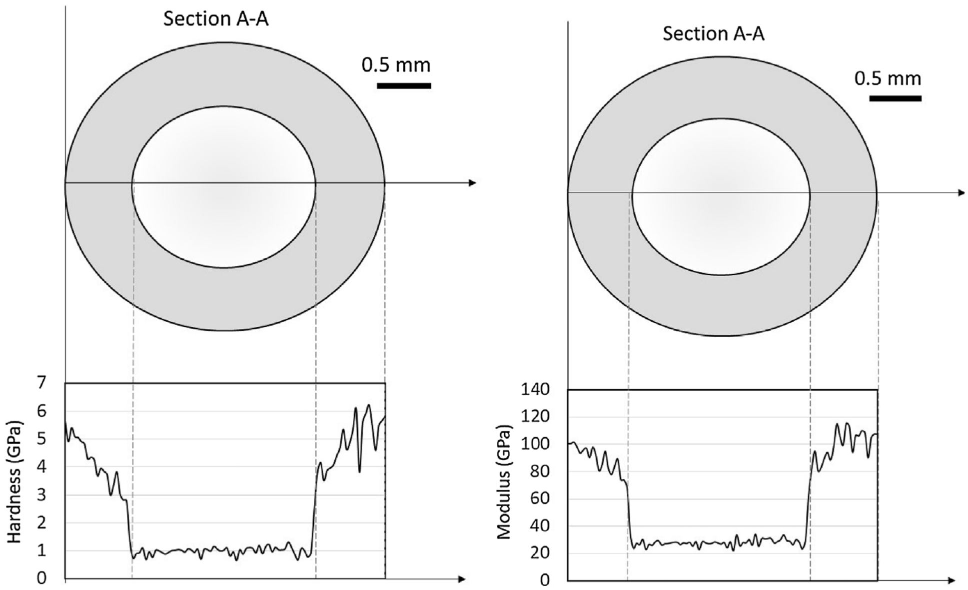

Fig. 4. Hardness (left) and modulus (right) along the cross section of the *A. probatocephalus* tooth.

4. Discussion

The purpose of this study was to investigate the microstructure, chemical composition, and material properties of *A. probatocephalus* (sheepshead) fish teeth. The results are the first reported for the sheepshead and provide material properties for a fish that has a diet consisting of hard-shelled organisms that include barnacles, crabs, oysters, and clams.

The presence of fluoride in the sheepshead teeth have also been noted in other fish by Suga, Taki, and Wada (1983) who investigated the presence of fluoride in various other fishes. The fluoride concentration observed in this paper coincides with the findings in Suga et al. (1983) that showed a range of 1.5 wt%–5.5 wt% fluoride in the enameloid of different fishes. Though marine fishes, such as the sheepshead, live in an environment that naturally contains fluoride, Suga et al. (1983) observed that the fluoride concentrations in the enameloid of fishes were not dependent on its environment but rather the phylogeny of the fish.

From Fig. 4, there is a gradual increase in modulus and hardness within the enameloid region traversing to the tooth outer layer, which may be due to the chemical composition gradient within the enameloid. Previous research by Cuy et al. (2002) and Jeng et al. (2011) showed that the changes in material properties of the human enamel correlate with the wt.% of calcium and phosphorus; whereby, an increase in calcium and phosphorus led to an increase in hardness; however, from Fig. 5 the wt.% of calcium and phosphorus for the sheepshead tooth does not change within enameloid region. Instead, a change in the wt.% of fluoride is present. Fig. 6 compares the similar trends of the modulus and wt.% fluoride, suggesting a direct correlation between the two.

SEM images in Fig. 7 capture the tubule structures existent within the dentin layer of the tooth. As aforementioned, tubule number density differences exist between the tubules located along the dentin-enameloid junction and those located within the center of the dentin region (Fig. 3). Differences in tubule density and diameter have been

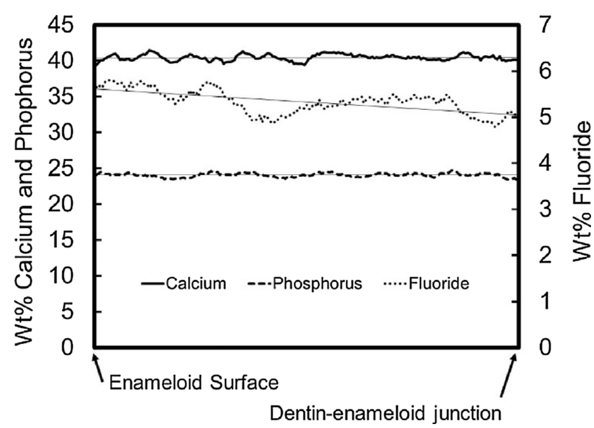

Fig. 5. Energy Dispersive Spectroscopy (EDS) results showing the wt% of calcium, phosphorus, and fluoride within the enameloid region of the sheepshead tooth.

previously been reported by Komabayashi, Nonomura, Watanabe, Marshall, and Marshall (2008) who observed a factor of two increase in the dentin tubule density of human mandibular canines from the outer to the inner surface. Similarly, a sheepshead's tooth exhibits an increase in tubule density by a factor of 1.75 from the outer to inner surface. Additionally, human teeth studied by Jakiel, Szyszkowska, Malicka, Rahnama, and Dawidowicz (2017) noted the increase of dentin tubule diameter towards the root of the tooth. Because of the nanoindentation methodology used within our paper, the results observed in Fig. 4 were not able to show the effects of dentin tubules on the hardness and modulus properties of the dentin. However, previous research using a microhardness technique has noted an inverse relationship between the tubular density and the hardness (Pashley, Atsuko, & Parham, 1985). For the current study, the average modulus of 23.29 GPa observed in the dentin in Fig. 4 is more of a representation of the intertubular

Table 1
Energy Dispersive Spectroscopy (EDS) results for the enameloid and dentin regions of *A. probatocephalus*.

Element Weight Percent					
	Calcium (Ca)	Phosphorus (P)	Oxygen (O)	Fluoride (F)	Magnesium (Mg)
Enameloid	40.3	24	21.9	5.3	2.2
Dentin	40.2	22.4	23.8	0.4	0.5

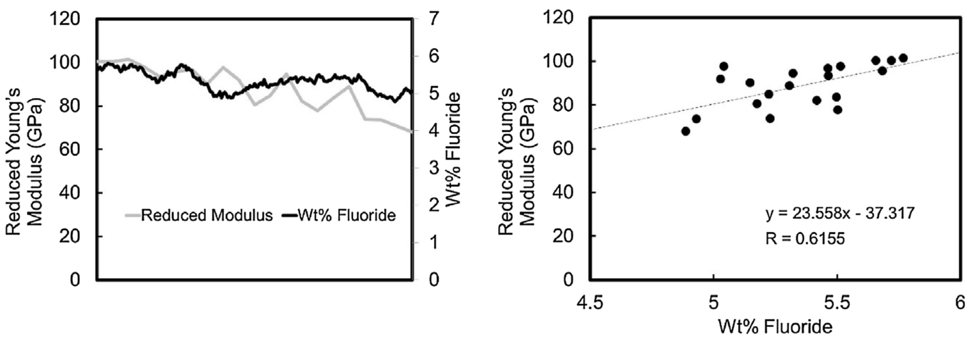

Fig. 6. Modulus and wt.% Fluoride throughout the enameloid region (left). Modulus vs wt.% Fluoride (right).

dentin (or the dentin between the tubules). Additionally, the dentin hardness does not show a spatial dependence, which is observed in previous studies conducted on the intertubular dentin in human teeth (Kinney et al., 1996). The existence of peritubular dentin would also effect the dentin strength (Kinney et al., 1996); however, Fig. 2 shows the absence of peritubular dentin, which has also been noted by previous microstructural research on the dentin of other fishes (Isokawa, Van Huysen, & Kosakai, 1964; Kubota et al., 1971).

Currently, a relationship describing the effect of tubular density and tubule diameter on the microhardness of the dentin does not exist. To estimate the effect of the tubule density and diameter within the sheepshead dentin, an approach similar to that done by Voigt (1889) and Kachanov (1958) is applied:

$$h = h^{*}(1 - \varphi) \quad (4)$$

where h is the microhardness of the dentin, h^{*} is the hardness of the intertubular dentin, and φ is the area fraction of the pores caused by the dentin tubules. Eq. (4) can be rewritten as:

$$h = h^{*}(1 - \eta\nu) \quad (5)$$

where η and ν are the number density and cross-sectional area of the dentin tubules, respectively (c.f. Horstemeyer, Lathrop, Gokhale, & Dighe, 2000). Using this approach to determine the microhardness properties of human dentin produces results that are comparable to previous microhardness results. Data taken from Sumikawa, Marshall, Gee, and Marshall (1999) and Marshall, Balooch, Gallagher, Gansky, and Marshall (2001) were used to estimate the microhardness of the outer region of the human dentin. These calculations compared well with microhardness results from Pashley et al. (1985). Data used for this comparison is compiled in Table 2.

Using Eq. (5), the microhardness of the sheepshead dentin can be calculated from the image analysis results from this paper. Fig. 8 shows the spatially dependent microhardness of the dentin. This approach can be used to analyze the dentin region of different animals.

Additionally, nanoindentation tests have been performed on the teeth of the sand tiger shark (Whitenack et al., 2010), bonnethead shark (Whitenack et al., 2010), piranha (Chen et al., 2012), and Great white shark (Chen et al., 2012). Table 3 and Fig. 9 compare the results from different fish species with the results obtained from the sheepshead. From Fig. 9, the sheepshead teeth have a higher average modulus and hardness when compared to the results from other fishes. The material properties of the sheepshead enameloid (4.5 GPa hardness and 100 GPa modulus) are also comparable to human enamel, which have exhibited a maximum hardness of 6 GPa and modulus of 110 GPa at the tooth's surface (Cuy et al., 2002).

The differences in mechanical properties observed in Fig. 9 may be a result of dietary habits and functions of the teeth. Previous biomechanical studies have examined the form and function of shark teeth (Frazzetta, 1988; Ramsay & Wilga, 2007). The primary predatory strategy of the great white shark is to severely injure its prey by taking a huge bite of its flesh, bring it close to death, and then feed (Diamond, 1986). Similarly, piranhas are known to use their sharp teeth to take

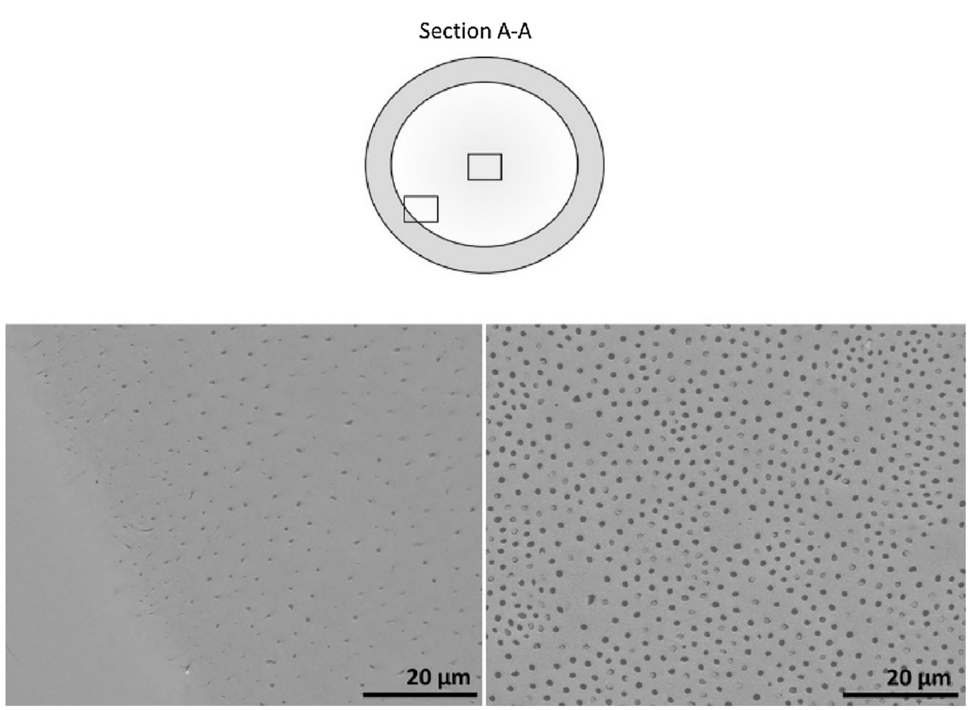

Fig. 7. Scanning Electron Microscope (SEM) images of a polished tooth surface comparing the dentin tubule density near the dentin-enameloid junction (left) and in the center of the tooth (right).

Table 2
Microhardness calculated from Eq. (5): $h = h^*(1 - \eta\nu)$ compared with microhardness experiments done by Pashley et al. (1985). Tubule number density and cross sectional data were taken from Sumikawa et al. (1999); Intertubular data was taken from Marshall et al. (2001).

Tooth	Region	Tubule Density, η (#/mm^2)	Tubule Cross Sectional Area, ν (mm^2)	Intertubular Hardness, h^* (GPa)	Calculated Microhardness: $h = h^*(1 - \eta\nu)$	Micro-hardness, h (GPa)	Percent difference
Lateral Incisor							
	Distal Area	29,400	1.52E-06	0.74	0.707	0.686	2.90%
	Central Region	38,200	1.52E-06	0.74	0.697	0.686	1.53%
Primary Canine							
	Distal Area	24,100	1.96E-06	0.74	0.705	0.686	2.64%
	Central Region	18,300	1.96E-06	0.74	0.713	0.686	3.79%

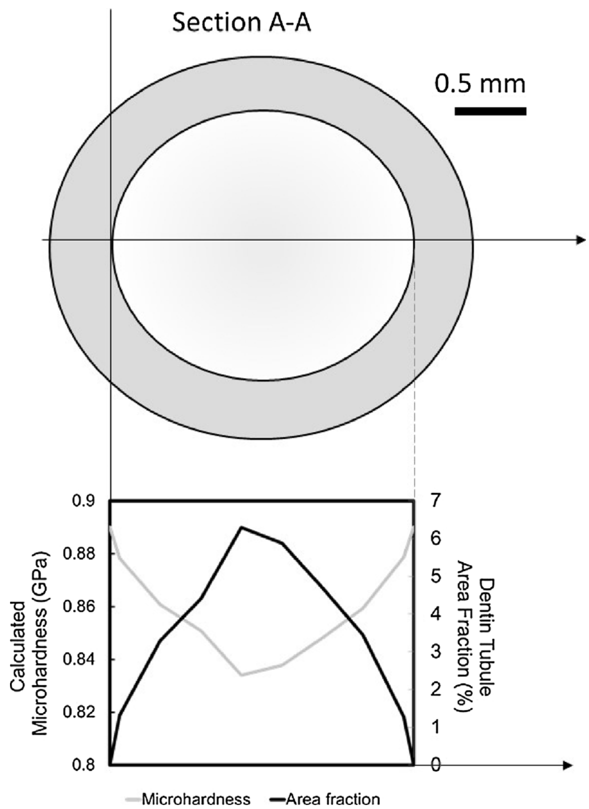

Fig. 8. Spatially dependent microhardness calculated from Eq. (5) ($h = h^*(1 - \eta\nu)$) and area fraction of the dentin tubules within the sheepshead dentin based on image analysis quantification.

bits of flesh from fishes and other vertebrates (Nico & Taphorn, 1988). Additionally, the structure of the shark and piranha tooth contain serrations, which are known to enhance cutting efficiency (Atkins, 2009). As such, the shape and structure of the piranha and shark tooth are ideal for the fishes' strategy of attacking the flesh of its prey. Alternately, because many of the hard-shelled prey of sheepshead fish are much slower, and even stationary, the need to impair its prey is unnecessary. Instead, the typical behavior for feeding on hard shelled animals consists of positioning their prey over the teeth, forcefully biting, and ejecting any loose shell debris (Fernandez & Motta, 1997).

Where the great white shark and piranha primarily attack the flesh of its prey, the sheepshead often have to break through the shells of its diet. The function of the sheepshead teeth requires stronger mechanical properties, which allow the fish to fracture crabs, oysters, barnacles, and clams. Fig. 10 compares the hardness and elastic moduli obtained from previous studies of the prey of the sheepshead to the elastic modulus and hardness of the teeth of the sheepshead (Lian & Wang, 2014; Lee, Kim, & Choi, 2008; Raman & Kumar, 2011). The elastic modulus exhibited by the some of the sheepshead prey is much greater than recorded values observed for fish bones encountered in the prey of sharks and piranhas. The mean modulus values of carp and tilapia rib bones were found to be 7.5 GPa and 8.6 GPa, respectively (Cohen et al., 2012).

The nanoindentation data was performed using dehydrated samples. Nanoindentation done by Habelitz et al. (2002) and research done by Lewis and Nyman (2008) show the effects of specimen storage and dehydration on the nanoindentation results of teeth. Ethanol, which is used to clean the sample and remove the polishing debris, has also been found to affect the mechanical properties of teeth (He & Swain, 2008). Further tests should be performed to capture the hydration effects on the nanoindentation of sheepshead teeth. Additionally, the microstructure of the enameloid should be investigated to investigate its effects on the material properties.

5. Conclusions

In this study of *A. probatocephalus* (sheepshead fish) teeth the mechanical properties and chemical composition of the enameloid and dentin were studied. Nanoindentation tests showed that the modulus

Table 3
Hardness and modulus properties of teeth from different fish species.

Species	Material	Common Name	H (GPa)	E_r (GPa)	Reference
Carcharias taurus	Dentin	Sand tiger shark	1.21 ± 0.16	28.44 ± 2.21	Whitenack et al. (2010)
Sphyrna tiburo	Dentin	Bonnethead shark	0.97 ± 0.07	22.49 ± 1.72	Whitenack et al. (2010)
Serrasalmus manueli	Dentin	Piranha	0.8 ± 0.3	23.0 ± 6.0	Chen et al. (2012)
Carcharodon carcharias	Dentin	Great white shark	0.7 ± 0.2	20.4 ± 5.6	Chen et al. (2012)
Archosargus probatocephalu	Dentin	Sheepshead	0.89 ± 0.21	23.29 ± 5.30	current study
Carcharias taurus	Enameloid	Sand tiger shark	3.20 ± 0.20	72.61 ± 4.73	Whitenack et al. (2010)
Sphyrna tiburo	Enameloid	Bonnethead shark	3.53 ± 0.30	68.88 ± 1.50	Whitenack et al. (2010)
Serrasalmus manueli	Enameloid	Piranha	4.1 ± 0.9	86.5 ± 15.9	Chen et al. (2012)
Carcharodon carcharias	Enameloid	Great white shark	4.1 ± 1.1	84.4 ± 19.9	Chen et al. (2012)
Archosargus probatocephalu	Enameloid	Sheepshead	4.36 ± 0.44	98.14 ± 6.91	current study

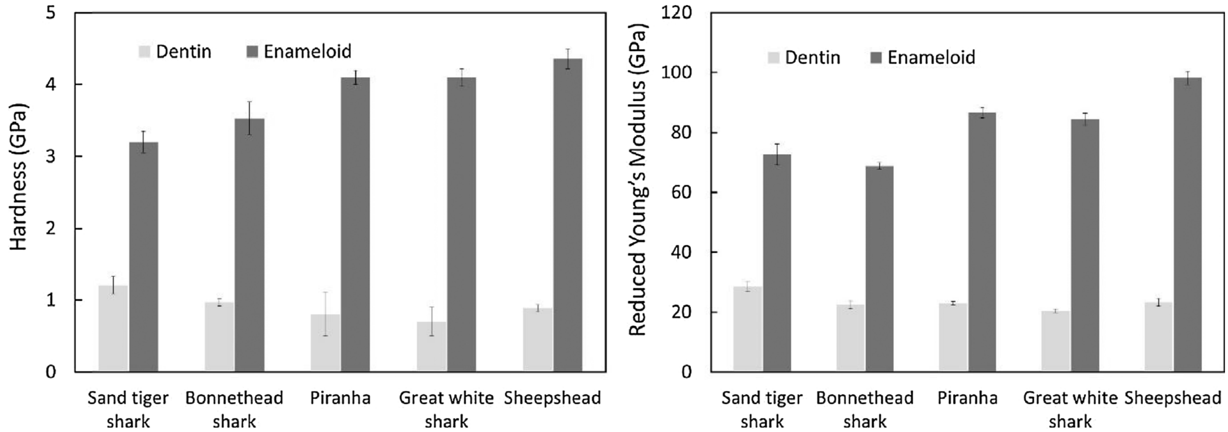

Fig. 9. Comparison of hardness (left) and modulus (right) for various aquatic species (Chen et al., 2012; Whitenack et al., 2010). Error bars represent t_{95}, a 95% confidence interval.

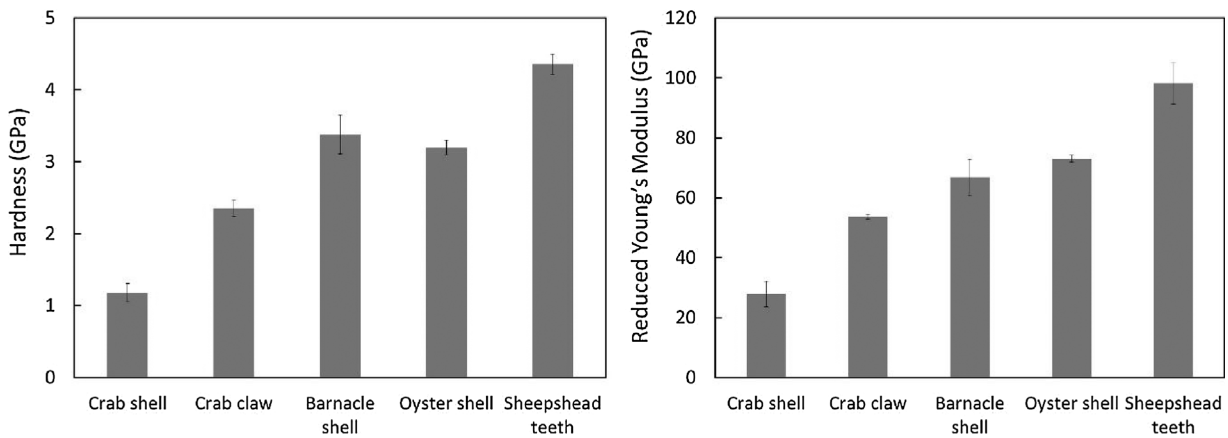

Fig. 10. Hardness (left) and modulus (right) of the sheepshead teeth compared with the shells of common animals in the sheepshead's diet (Lee et al., 2008; Lian & Wang, 2014; Raman & Kumar, 2011). Error bars represent standard deviation.

and hardness of the enameloid increased closer to the surface of the tooth. EDS results further suggest that the modulus and hardness gradient are a result of the weight percent fluoride within the enameloid, where an increase in fluoride results in an increase in modulus and hardness. Also, an approach to determine the effect of tubule diameter and number density on the microhardness was presented in this paper. The mechanical properties of the sheepshead teeth were also compared to other aquatic species, exhibiting a higher modulus and hardness when compared to shark and piranha teeth.

Conflicts of interest

No conflicts of interests.

Sources of funding

Army ERDC laboratory.

Ethical approval

Manuscript was approved by Army ERDC laboratory correspondent.

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