A Representative Volume Element Based Micromechanical Analysis of a Bi-Layered Ganoid Fish Scale

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ABSTRACT

The Mississippi Alligator gar (*Atractosteus spatula*) possesses a flexible exoskeleton armor consisting of overlapping ganoid scales used for predatory protection. Each scale is a two-phase biomineralized composite containing bio-modified hydroxyapatite (hard) minerals and collagen (soft) fibers. The protective layer consists of a stiff outer ganoine layer, a characteristic “sawtooth” pattern at the interface with the compliant bone inner layer. The garfish scale exhibits a decreasing elastic modulus from the external to the internal layers. Scanning electron microscopy (SEM) images of the cross-section revealed a two-layered structure. Elastic moduli, measured from nanoindentation experiments, were correlated to structural changes across each layer. The “material” symmetry of this materially and geometrically nonlinear biomineralized composite is unknown. Therefore, to be able to determine the stiffness tensor requires the use of finite element analysis (FEA). The gar fish scale was computationally modeled using the representative volume element (RVE) based approach. As a result, the unknown symmetry induced by the architecture and material layering require the use of complex FEA boundary conditions. The simulation was conducted in the pure uniaxial strain regimes of tension and shear, which necessitated the mathematical determination so appropriate surface loading conditions could be applied. This paper provides the results from a highly-resolved mesoscale RVE model based on iso-strain boundary conditions (ISBC) to determine the elastic stiffness tensor for the composite system. By assuming isotropic behavior in individual elements, the results for the RVE reveal the fish scale has an “*orthotropic symmetry*” with slight local strain variations occurring at the sawtooth interface.

Keywords: *Layered Structures; Biomaterials; Statistical Properties; Nanoindentation; Finite element analysis (FEA); Representative Volume Element (RVE)*

1. INTRODUCTION

1.1 Biological Materials for Bio-Inspired Design

During the last few decades, research into biological materials such as abalone shell, fish armor, turtle shell, and human bone revealed these biological composites (biocomposites) possess a carefully arranged multilayered structure (Yang et al., 2014) with unique subscale feature achieving mechanical properties which can be superior many man-made materials (Hodo, 2015). The investigation of such optimized systems provides novel insight into the future of advanced engineering materials. Over the last two decades, both design engineers and researchers have turned to nature with the hopes of understanding how biocomposites are efficiently created to have the often mutually exclusive properties of high-strength, high-toughness while maintaining a lightweight configuration (Allison et al., 2013; Song, 2011; Song et al., 2011; Yang et al., 2012; Chandler et al., 2014; Nelms, 2014).

Scientists discovered biocomposites that exhibit such desirable properties when operating under extreme conditions tend to be mineralized tissues (Chen et al., 2012; Meyers et al., 2006; Yang et al., 2012, 2013a, 2013b, 2013c, 2014). Typically these biocomposite consists of primarily two phases: a hard (inorganic minerals) matrix phase integrated with a soft polymer-like reinforcement phase (organic fiber) (Browning et al., 2013; Bruet et al., 2008; Han et al., 2011; Ikoma et al., 2003a, 2003b; Ji & Gao, 2010; Schönborn, 1979; Vernerey et al., 2010; Yang et al., 2014; Youn & Shin, 2009; Zhu et al., 2012), such as the superior performing examples of bone, teeth, shells and fish scales (Hodo, 2015). Designers have recently shown interest in studying fish scales for potential use in a variety of engineering applications. Fish scales were particularly attractive as an in-depth case study because of relative simplicity and involve few discrete components, yet exhibit a superior mechanical response. Since experimental

measurements to determine the mesoscopic response of Mississippi alligator gar's ganoid fish scale are complex, a numerical modeling approach will be appropriate to obtain elastic properties in the present study. Therefore, the experimentally characterized biocomposite was modeled through an iso-strain boundary condition (ISBC) based finite element analysis for determining the effective stiffness tensor.

1.2 BACKGROUND: *Characteristics of Mississippi Alligator gar (Atractosteus spatula)*

The Mississippi alligator gar (*Atractosteus spatula*) has ganoid type scales that are a multilayered biocomposite with graded elastic modulus and hardness properties. The outermost layer having high strength that progressively decreases towards the innermost surface and showing an inverse relationship with toughness. The gar fish scales have a characteristic bi-layers construction at the mesoscale, an outer ganoine layer and inner lamellar bone layer (Allison et al., 2013; Chen et al., 2012, Hodo, 2015; Yang et al., 2013a, 2013b, 2013c). In this protective material system, the primary attribute is to understand how evolution has chemically and geometrically optimized the scales using a two component mixture, which is integrated from the nano-to-millimetric scale, to form its complex hierarchal structure. At this length scale, the bioapatite crystals are mixed with collagen fibers; varying by volume fractions, spatial proximity, and directional orientation to produce a lightweight composite exhibiting high strength and toughness (Hodo, 2015).

As a result of the hierarchal ordering, the gar fish scale exhibits strength and toughness comparable to those of most metal alloys. Uniquely, the bulk mechanical properties are several orders of magnitude higher than those found for the discrete ganoine and bone layers. The engineering properties of the individual layers have shown to be analogous to dental enamel (hard) for the ganoine and cancellous bone for the inner layer (soft). The hard enamel is

lightweight, high-strength, but brittle with low fracture toughness. Whereas the sponge-like cancellous bone is also lightweight, but it is soft, low-strength and has high fracture toughness. However, when the two materials are combined, they make a lightweight composite that has high-strength with good fracture toughness.

A particular feature of interest is the distinct geometric interaction at the interface between stiff ganoine and compliant bone in gar fish scale. The interlocked interface has a distinct periodic sawtooth structure, or rasp, with the ganoine layer intruding into the interfacial region (Allison et al., 2013). In addition, the discrete layers have a modulus mismatch which researchers believe has a role in dissipating stress/energy across the interface (Ji & Gao, 2010). Until recently, research has overlooked the role of the sawtooth in efficiently transferring load across the ganoine-bone interface without debonding/delaminating (Chandler et al. 2013; Nelms, 2014).

Borrowing from the engineered composites community, the experimental characterization was used to determine an idealized volume for computational analysis (Shan & Gokhale 2002). In order to analyze the effects of various material and structural features on elastic response, the first step is to determine the material symmetry of the fish scale through the use of high-resolution mesoscale finite element modeling (FEM) with the representative volume element (RVE) based approach. The averaging field theorems have shown to provide an adequate prediction of the effective mechanical properties for functionally graded composite plates (Cheng & Batra, 1999; Reiter et al., 1997) similar to that of gar fish scale.

The main focus of this paper is to (1) determine the material symmetry for the fish scale by applying appropriate boundary conditions to a 740 μ m cubic RVE with 7.4 μ m resolution, and (2) understand the effect of geometric interlocking at the ganoine-bone interface with respect to

the local strain state under global iso-strain conditions. Using optical microscopy techniques, Wilson in 1848 sketched the architecture of the two layer-lamina of the Needlenose gar (*Lepisosteus osseus*) and determined that continuous ridges of ganoine intrude into the bone layer, characteristically described as a sawtooth-shaped rasp. Similarly, with the advent of advanced 3-D μ CT capabilities, Hodo (2015) obtained images of continuous sawtooth-like features in the Mississippi Alligator gar fish scales. The geometry of fish scale necessitates a three-dimensional (3-D) finite element analysis (FEA) to model the interface geometry and determine if the interlocking may contribute to elastic anisotropy in the gar fish scales. The local strain variations at interfaces, especially around the sawtooth features can be captured using the 3-D analysis.

In the FEA, iso-strain boundary conditions (ISBC), in terms of surface displacements, were applied to the RVE to generate pure uniaxial stress and pure shear conditions (Kelkar et al. 1992). The concept of ISBC and application are discussed in detail in Section 3. Because the fish scale exhibits unknown material symmetry, kinematic and periodic boundary conditions alone will not lead to pure uniaxial stress states. By applying proper surface loading, it is then possible to accurately determine the material symmetry of the fish scale. Knowledge of symmetry will help both scientist and engineers to better understand the mechanisms nature uses to strengthen biocomposites. Such knowledge can guide future researchers to better understand the engineering behavior of flexible, yet protective fish scale and other natural occurring biomineralized tissues.

To understand the fish scale's protective attributes, this study combined microscopy, mechanical experiments, and high fidelity computational simulations to discern what are the advantageous design principles regarding the structure and mechanical properties that appear in

the protective gar fish scale. The following sections provide a) details of the preparation of fish scales for further characterization, Section 2, b) the theoretical background and application of iso-strain boundary conditions, Section 3, c) results from high-resolution scanning electron microscopy (SEM) imaging for interface geometry and assembly, Section 4.1, d) nanoindentation mechanical measurements for determining the variation of young's modulus along the layers, Section 4.2, e) details of the computational model generation, Section 4.3, and f) the RVE determined stiffness tensor for the fish scale as well as the resulting internal strain field, Section 5.

2. MATERIALS AND LABORATORY CHARACTERIZATION METHODS

The Alligator gar fish specimens were live caught from a swamp adjacent to the Mississippi River located near Vicksburg, MS, by the biologist team at Environmental Laboratory of the US Army Engineer Research and Development Center (ERDC). The fish was euthanized, and scales were removed according to the ERDC animal care guidelines (#EL-6009-2011-5), which encompass the *NIH Guide for Care and Use of Laboratory Animals*, Fig. 1.

Figure 1. (Single column figure)

2.1 Scanning Electron Microscopy

High fidelity imaging was performed at the *U.S. Army ERDC Geotechnical and Structures Laboratory (Vicksburg, MS)*, using Scanning Electron Microscopy (SEM). An FEI Nova NanoSEM 630 SEM with a low Voltage high Contrast Detector (VCD) was used to determine the interfacial geometric structure between the ganoine and bone layers.

2.2 Nanoindentation

Nanoindentation was performed to determine the elastic moduli's spatial variations by expanding on previous research from a single line scan to a uniform, high-resolution grid thus

providing improved analysis on the variance present in highly heterogeneous gar fish scale. The experiments were performed with a iNanoTM nanoindenter provided by Nanomechanics, Inc. using a “Dynamic Indentation Constant Strain Rate” testing protocol. The experimental setup parameters are defined in Table 1. As seen in Figure 3a, a Berkovich grid indented array, totaling 1680 indents with a 10 μ m spacing, was used to map the fish scale’s cross-section.

The dynamic indentation technique was used to acquire continuous elastic modulus as function of depth. The elastic modulus with respect to the penetration depth showed excellent convergence in the ganoine and bone regions by 300 nm of surface penetration for the fish scale. The measured modulus for each indent was analyzed over the penetration depth range from (500 – 800) nm.

Table 1. Nanoindentation setup parameters for determining minimum penetration depth

Input Parameter	Value
Tip Geometry (Angle/Curvature)	Berkovich (142.3°/~150 nm)
Tip Material Type	Diamond
Indentation Strain Rate	0.2 s ⁻¹
Maximum Indention Depth, (D _{Max})	1- μ m
Spacing Between Indents, 10x(D _{Max})	10- μ m
Poisson’s Ratio, (ν)	0.35
Penetration Range for Averaging Results	(500-800)- μ m

3. Theory and Calculations

3.1 Iso-strain Based Finite Element Boundary Conditions

In this study, the mechanical response, in terms of the effective stiffness tensor, was determined by using iso-strain boundary conditions (ISBC) on a representative domain. In layered composite systems, the length scale reflects the size of the fiber or fiber bundles. However, in a biological system, the RVE length scale is influenced by the salient features, such

as the sawtooth interface and layered geometry. The fish scale architecture necessitates that the entire thickness establishes the appropriate domain size. The mechanical response of a “bio-inspired” macroscopic system consisting of layers of fish scales would require the effective properties, as determined from the RVE, for each element in a FEM model for the region highlighted in Figure 1.

The ISBC enables one to obtain the elastic moduli and Poisson’s ratio, shown in Sections 3.2.1 and 3.2.2, under uniaxial stress state without any shear due to material anisotropy, i.e. cross-sectional planes remain planar. The equations are based on the relation between surface displacements (a traction boundary value problem) and resulting strain field required by the compatibility conditions (Truesdell & Toupin, 1960). Due to geometric nonlinearity and modulus variations, it is non-trivial to achieve a “pure” uniaxial stress distribution based on conventionally employed kinematic and periodic boundary conditions thus necessitating the ISBC. Furthermore, the heterogeneity and anisotropic nature of the fish scale, a simple stretching in tension would introduce significant shear in the RVE, which will not truly yield the correct elastic response. Therefore, the boundary conditions originally developed for woven composite lamina (Kelkar et al., 1992; Chaphalkar & Kelkar, 2001) were applied to simulate “pure” tension or “pure shear” condition in the fish scale RVE. The purpose of the ISBC is to create a uniform deformation state where all internal faces remain planar and have a zero net reaction force on faces transverse to the applied displacement (i.e. only one face with a net reaction force).

3.2 Application of Is-Strain Boundary Conditions (ISBC)

The results from the various numerical simulations were combined into a set of linear equations to compute the RVE's effective properties. Surface displacements were independently applied to determine the material symmetry, such as orthotropic stiffness, resulting from

geometric configuration and property variations. In the following sections, the application of the ISBC as well as the analytical equations required to determine effective elastic properties followed by the stiffness tensor are discussed.

3.2.1 Principal Moduli and Poisson's Ratio Determination

To obtain the RVE's effective principal moduli and Poisson's ratio, the ISBC's were applied as principal direction displacements over the entire surface of the model and given by the displacement field, $u_i = E_{ij}x_j$, required to produce a uniform macroscopic strain, E_{ij} , as defined the average theorem, $E_{ij} = \frac{1}{V} \int \varepsilon_{ij} dV$. To determine the principal moduli for the RVE the prescribed displacements were applied as defined by Cases I-III:

Case I: $u(0)=0; u(l)=u_o; v(0)=v(l)=0; w(0)=w(z_o)=0$

Case II: $u(0)= u(l)=0; (b) v(0)=0; v(l)= v_o; (c) w(0)=w(z_o)=0$

Case III: $u(0)= u(l)=0; (b) v(0)= v(l)=0; w(0)=0; w(z_o)= w_o$

In the above cases, u_o , v_o , and w_o are the displacements prescribed on the x , y and z surfaces, respectively, as referenced by the coordinate system in Fig. 2. The displacements in the three principal directions are applied independently and superposed to an equivalent combined loading case through the summation of the resulting reaction forces, eqs. 1-3. The reaction forces were combined with Hooke's law to predict the elastic modulus and Poisson's ratio for the functionally graded RVE followed by the corresponding stiffness tensor. The superscript designates the applied case and the subscript the spatial direction. For the ISBC, the reaction forces at the restricted surfaces for each case were summed to determine the effective stiffness. Equations 1, 2 and 3 are set of linear equations with scalar constants a and b which relate the elastic properties to the simulation results for Cases I, II, and III (superscripts).

$$F_x^T = F_x^I + aF_x^{II} + bF_x^{III} \quad (1)$$

$$F_y^T = F_y^I + aF_y^{II} + bF_y^{III} \quad (2)$$

$$F_z^T = F_z^I + aF_z^{II} + bF_z^{III} \quad (3)$$

Constants a and b can be determined by setting reactions forces in the transverse directions equal to zero. By setting the two reaction force equations equal to zero, a and b can be found by simultaneously solving the set of linear equations. In equations 5-18, a and b are combined with the applied displacements and RVE's edge length, l , to relate them to the various elastic properties, for both principal and shear conditions. For E_x , eqs. 4-7 were used to determine the cubic RVE's elastic properties are as follows:

$$F_x^T \neq 0, F_y^T = 0, F_z^T = 0 \quad (4)$$

$$E_x = \frac{1}{u_o l} (F_x^I + aF_x^{II} + bF_x^{III}) \quad (5)$$

$$\nu_{xy} = \frac{-av_o}{u_o} \quad (6)$$

$$\nu_{xz} = \frac{-bw_o}{u_o} \quad (7)$$

To calculate E_y , the x and z reaction forces were set equal to zero. Equations 8-11 were then used to determine effective elastic properties with respect to the y -axis.

$$F_y^T \neq 0, F_x^T = 0, F_z^T = 0 \quad (8)$$

$$E_y = \frac{1}{av_o l} (F_y^I + aF_y^{II} + bF_y^{III}) \quad (9)$$

$$\nu_{yx} = \frac{-u_o}{av_o} \quad (10)$$

$$\nu_{yz} = \frac{-bv_o}{au_o} \quad (11)$$

To determine E_z , F_x and F_y in eqs. 1 and 3, were set equal to zero to determine the elastic properties with respect to the z -axis using eqs. 12-15.

$$F_z^T \neq 0, F_x^T = 0, F_y^T = 0 \quad (12)$$

$$E_z = \frac{1}{bw_0l} (F_z^I + aF_z^{II} + bF_z^{III}) \quad (13)$$

$$v_{zy} = \frac{-av_0}{bw_0} \quad (14)$$

$$v_{zx} = \frac{-u_0}{bw_0} \quad (15)$$

Additionally, the shear moduli can be determined by using the same approach of applied displacements to produce an effective RVE strain then superposed to determine the effective shear stiffness. Section 3.2.2 describes how the shear response can be determined using the ISBC.

3.2.2 Shear Moduli Determination

Three displacement conditions were applied to determine the effective shear moduli of the gar fish RVE. Again, the resulting reaction forces were acquired and used to compute the remaining elastic properties. The displacement boundary conditions were applied to the surface of the model to create a small average shear strain. Similar to the principal moduli, three shear displacement cases were applied and combined with Hooke's law to calculate the effective response of the gar fish RVE. The resulting reaction forces were obtained to compute the remaining elastic properties from the following displacement boundary conditions:

$$\text{Case } \mathbf{G}_{xy}: u(l) = u_o*(y/l); u(v(l)) = u_o; v(0) = v(l) = 0$$

$$\text{Case } \mathbf{G}_{xz}: u(l) = u_o*(z/l); u(w(l)) = u_o; w(0) = w(l) = 0$$

$$\text{Case } \mathbf{G}_{yz}: v(l) = v_o*(z/l); v(w(l)) = v_o; w(0) = w(l) = 0$$

The respective shear moduli were determined by summing the nodal reaction forces at the restrained faces. Equations 16 – 18 were used to calculate the effective shear moduli.

$$G_{xy} = \frac{1}{2v_o} \left(\frac{F_x^T}{l} + \frac{F_y^T}{l} \right) \quad (16)$$

$$G_{xz} = \frac{1}{2w_o} \left(\frac{F_x^T}{l} + \frac{F_z^T}{l} \right) \quad (17)$$

$$G_{yz} = \frac{1}{2w_o} \left(\frac{F_y^T}{l} + \frac{F_z^T}{l} \right) \quad (18)$$

Using the implied orthotropic symmetry of the ISBC, it is possible to determine the stiffness tensor by first computing the compliance tensor, $\mathbf{S}$, eq. 19.

$$\mathbf{S} = \begin{bmatrix} \frac{1}{E_x} & \frac{-\nu_{yx}}{E_y} & \frac{-\nu_{zx}}{E_z} & 0 & 0 & 0 \\ \frac{-\nu_{xy}}{E_x} & \frac{1}{E_y} & \frac{-\nu_{zy}}{E_z} & 0 & 0 & 0 \\ \frac{-\nu_{xz}}{E_x} & \frac{-\nu_{yz}}{E_y} & \frac{1}{E_z} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{G_{yz}} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{G_{zx}} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{G_{xy}} \end{bmatrix} \quad (19)$$

Results can then be inverted to determine the constants of the orthotropic stiffness tensor, $\mathbf{C}$ in eq. 20, and analyze the resulting material symmetry.

$$\mathbf{S}^{-1} = \mathbf{C} = \begin{bmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{21} & C_{22} & C_{23} & 0 & 0 & 0 \\ C_{31} & C_{32} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{55} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{bmatrix} \quad (20)$$

4. Experimental Results and Discussion

4.1 Scanning Electron Microscopy

The SEM images exhibited an interlocked interface with the inner bone layer, Fig. 2, and displayed the characteristic sawtooth pattern occurring at the ganoine-bone transition with ganoine protruding into the bone. Scale bars are 500 and 100 μm for the images in fig. 2a and fig. 2b, respectively.

Figure 2. (1.5 column figure)

The baseline dimensions of sawtooth structures were determined from direct measurement of the SEM imagery. High fidelity images were employed to measure the sawtooth tip projection distance as well as the geometric spacing scaled by the 100 μm scale bar in Fig. 2b. The spacing and height results were rounded to the nearest voxel dimensions as determined by the FE mesh resolution. The spacing was approximately 148 μm with a height of 40 μm . This data provided the baseline values for the idealized volume representation presented in section 4.3. Since the volume fraction of sawtooth features is relatively low as compared to the total volume of the fish scale, minor deviations from ideal sawtooth shape and size should not cause any significant statistical deviations in the average moduli values. The SEM analysis combined with the following nanoindentation results (Section 4.2) are used to investigate the effect of material/geometric variations on the bulk elastic response through FEM in Section 5.

4.2 Nanoindentation results

Nanoindentation experiments revealed material property grading within each layer. The gradation showed decreasing elastic modulus from the outer towards the internal layers and corroborates previous results as determined by Chen et al. (2012) and Allison et al. (2013) as well as expands from line scans to a full 2-D grid. The results showed two distinct layers, a high

modulus ganoine and low modulus bone layer, connected by a mechanical transition zone near the internal layer boundary. The material property grading is most drastic in the interface zone followed by a progressive decrease to the ultimate minimum at the medial surface of the scale. Shown in Fig. 3a is the dynamically determined elastic modulus for the nanoindentation grid. The data was reduced to find the median value as an input into the computational model. Medians are shown by the red lines on the Tukey's boxplot in Fig. 3b. The median probability values were chosen to idealize the local variations within the layers. Significant local variance can be seen in the grid, with a distinctive moduli gradation from the ganoine to the bone over approximately a 40 μ m layer.

Figure 3. (Single column)

Additionally, the SEM images and nanoindentation experiments qualitatively showed significant heterogeneity of the underlying microstructure, so statistical analysis was conducted to show, in quantitative terms, the role structural (material/geometric) variance has on mechanical response. The modulus data was analyzed using Tukey's boxplot with outlier bounds set a 1.5 times the inner quartile and box defined by 25th and 75th quartiles as seen in Fig. 3b. Table 2 summarizes the measured nanoindentation modulus results. Poisson's ratio was determined using open literature for ganoine and bone (Han et al., 2011; Rupin, 2008; Jager & Fratzl, 2000).

Table 2. Ganoine, interface and bone modulus variations for the ganoine, interface and bone layers present in the Mississippi Alligator gar scale

Layer	Thickness (μ m)	Elastic Modulus (GPa)	Poisson's Ratio
Ganoine	80	62-48	0.28
Interface	40	48-20	0.3
Bone	620	20-13	0.35

As previously stated, the laboratory experiments were performed to guide the computational analysis. The reduced data was used to develop an experimentally driven representative model which includes the functionally graded modulus and sawtooth interface geometry. The layer properties were combined with the imaging for finite element analysis described in the following section and computationally characterized for the representative elastic properties.

4.3 Generation of Representative Volume Element (RVE) Model

The RVE computational modeling of the Alligator gar scale allows insight into functional aspects of the internal fish scale structure and property variations that cannot be determined through direct experimental measurements. A high-resolution computational approach guided the finite element model development using isotropic elastic material behavior for all voxels. The RVE model, made use of preprocessor scripts written in-house and developed at the University of Mississippi, to include structural and mechanical property variations observed during the laboratory experiments. Simulations were performed using commercially available ABAQUS[®] finite element (FE) software.

The determination of a suitable length scale for the RVE model has always been a challenge. The rationale used in the present study was based on the typical overall size of a single scale of the gar fish so all structural features, such as the sawtooth rasps, are captured in the RVE. Therefore, the size and length scale for the gar fish RVE was chosen as the typical scale thickness of 740-microns in the ganoine covered region visible in fig. 2. The RVE was discretized into one million elements using 100x100x100 cubic voxels. The SEM images and nanoindentation measurements provided the dimensions of the idealized RVE, number of different layers, and graded distribution for the corresponding elastic modulus within the ganoine

and bone layers. The nanoindentation grid was normalized with respect to the distance from the interface to account for the slight curvature present in the physical scale. Since the goal is to determine only the material symmetry and not the damage processes at the interface, the surface roughness present at the ganoine-bone boundary has been neglected, consistent with existing RVE approaches to model the bulk elastic properties of woven or unidirectional fiber-composite laminates.

The layer properties as defined in Table 2, provided the material constant (elastic modulus and Poisson's ratio) for the gar fish scale model. It should be noted the RVE model closely followed the actual specimen used for nanoindentation. The layer thicknesses were held constant while the interface properties were varied to determine the effect any geometric perturbations in the sawtooth rasps, i.e. waviness, would have on the elastic properties and resulting stiffness tensor. To establish the effects of material property variations and structured interface on the homogenized elastic properties, three interface conditions were considered: (a) explicitly modeled sawtooth rasps as a constant 2-D projection in the z-direction, (b) removing all of the ganoine, i.e. interface consisting of all bone, to provide a lower bound on the stiffness tensor, and (c) making the interface consist entirely of ganoine thereby proving an upper bound. A comparative analysis of the resulting properties and stiffness tensors can provide insight into the effects the interface makeup has on the effective elastic response. Shown in Fig. 4 is the linear modulus gradation for the piecewise function. Region (a) is the modulus variation present in the sawtooth, while lines (b) and (c) define the lower and upper bounds of the interface moduli.

Figure 4. (Single column)

The RVE model described by region (a) included periodic rasp intrusions, each having a spacing of 148 μm through the entire interfacial thickness of the scale. Illustrated in Fig. 5, is the 3-D RVE configuration and modulus variations including the embedded ganoine. The most significant variations occur, in the x-directions, from the outer to inner layers and along with sawtooth configuration from the imaging provided by SEM. As a consequence, the elastic properties and structure were not varied laterally in the z-direction. Note the reference axis orientation for later discussion purposes. Figure 5a represents the combined interface condition, region (a) in fig. 4, which includes the explicitly modeled sawtooth rasps and fig. 5b the white section indicates the region where the properties were varied according to lines (b), all bone, and (c), all ganoine, defined in fig. 4 as well.

Figure 5. (Single column)

5. Computational Results and Discussion

The numerical simulations were performed according the iso-strain methodology presented in Section 3 and the experimentally determined RVE discussed in Section 4.3. The computational analysis provided the reaction forces for each of the three principal case and three shear cases independently. High-resolution FE modeling with micron size elements provided insight into strain de-localization as well as the strain field progressing into the soft bone layer.

5.1 Determination of Elastic Properties

The ISBC provided a uniform global strain distribution required for determining the homogenized elastic properties. The surface nodal forces were summed to determine the total reactions in each principal direction to calculate the effective elastic moduli and Poisson's ratio using equations 1-18. The resulting effective properties determined using the ISBC (section 3) are given in Tables 3a-c. Tables 3a, 3b, and 3c, correspond to the interface conditions as defined

by region (a), line (b) and line (c) in fig. 4 for consistency. The simulations determined the elastic properties for the combined interface (Table 3a) lie between the two extreme interface conditions, all compliant bone (Table 3b) or all stiff ganoine (Table 3c).

Tables 3a-c. Homogenized principal elastic moduli and Poisson's ratio of the Mississippi alligator gar fish scale as determined by the Iso-Strain Boundary Conditions

Effective Elastic Properties	Gar Fish Modulus (GPa)
(a) Interface: ganoine/bone material (sawtooth)	
E_x, ν_{xy}, ν_{xz}	19.09, 0.310, 0.310
E_y, ν_{yx}, ν_{yz}	21.37, 0.348, 0.327
E_z, ν_{zy}, ν_{zx}	21.41, 0.327, 0.348
G_{xy}	8.70
G_{xz}	8.70
G_{yz}	9.19
(b) Interface: bone material (planar)	
E_x, ν_{xy}, ν_{xz}	18.95, 0.313, 0.313
E_y, ν_{yx}, ν_{yz}	21.10, 0.348, 0.328
E_z, ν_{zy}, ν_{zx}	21.10, 0.328, 0.348
G_{xy}	8.60
G_{xz}	8.60
G_{yz}	9.06
(c) Interface: ganoine material (planar)	
E_x, ν_{xy}, ν_{xz}	19.46, 0.307, 0.307
E_y, ν_{yx}, ν_{yz}	22.12, 0.348, 0.317
E_z, ν_{zy}, ν_{zx}	22.12, 0.317, 0.348
G_{xy}	8.94
G_{xz}	8.94
G_{yz}	9.51

The principal moduli show a negligible reduction of 0.5% when the sawtooth is removed (Table 3b) and an increase of 2% when the interface is completely replaced with ganoine (Table 3c). The effect is more pronounced on the shear moduli showing a decrease of 1.1% for the bone interface condition, case (b), and an increase of 5% for ganoine interface, case (c), due to the

inclusion of additional high modulus material. The Poisson's ratio showed variations for the three interface conditions with a maximum difference of 1.2% relating the yz response. Additionally, given the slight difference in elastic stiffness due to changes in interface configuration, the material symmetry determined for the RVE model without the geometric perturbations (i.e., waviness or surface roughness) present in the gar fish scale can be considered as reasonable approximation of the elastic response.

When investigating the effects of structured interface on material symmetry, the volume percentage of the sawtooth domain compared to total volume is not significant enough to show any major difference between E_y and E_z (Table 3a), yet does show the effects of the property variations by a significantly softer response for E_x . This variation can be attributed the orientation of the material variations. The material “layup” in the x -direction most closely aligns with the Reuss bound assumptions while the y/z directions are more consistent with the Voigt approximation to predict the effective stiffness for composite materials. Consequently, the x -direction is expectedly softer than the y and z elastic response.

Based on the computed elastic tensor, the fish scale material exhibits an orthotropic symmetry with small variations in y and z directions. The results show a 6.3% increase in shear modulus with the largest variation (12%) observed in the principal moduli. The major and minor Poisson's ratio were determined to be 0.348 to 0.310 between the the xy/xz and yx/zx response. Lower moduli and increased Poisson's ratio for properties related to the x -direction point toward the bone dominance of the material properties. Conversely, the increase in the E_y and E_z moduli as well as the reduced Poisson's ratio point to ganoine dominance of the mechanical response and potentially indicate a preferential material layup. Given the typical predatory event, i.e. penetration, would occur along the x -axis where the increased stiffness in the y and z directions

would provide transverse stiffness similar to a confinement stress applied on an isotropic material.

Next, the effective elastic properties in Tables 3a-c were used to estimate the orthotropic elastic stiffness tensor, $\mathbf{C}$ seen in Fig. 6a-c. First, the compliance tensor, $\mathbf{S}$, as defined by eq. 19, was computed directly from the elastic constants. The results were then inverted, eq. 20, to provide the compliance tensors for each of the three cases, (a), (b), and (c). Due to the equivalence of the C_{22} and C_{33} as well as the C_{12} and C_{13} , the response can be further refined as transversely isotropic symmetry for the three interface conditions.

Figure 6. (Single column)

The interfacial region accounts for 5% total volume, and the sawtooth rasps 1.7%. Therefore the results do not indicate any geometric stiffening due to the structural interactions at interface. This can be attributed to relatively low number of rasps leading to a diffuse strain field between the features (section 5.2). It appears the symmetry is dominated by the through-thickness property variations, i.e. functionally graded elastic modulus. Since structured interfaces provide potential for local load redistribution, the following section investigates the strain response due to the sawtooth rasps as well as validates the effective strain is equivalent to the average of the internal strain field.

5.2 Representative Strain Response

To develop a better understanding of the effects of graded properties and the presence of sawtooth structures on the strain response of the fish scale as modeled through the RVE, the spatial and statistical strain distribution were plotted in Figures 7a-d and show results for “pure” tension loading conditions (Section 3.2.1 Case I). As can be seen from the contour plots, the normal strains ϵ_{11} (X-axis) and ϵ_{22} (Y-axis), Figures 7a and 7c respectively, as well as the shear strain ϵ .

12, Figure 7b, are uniformly distributed except for the local deviation around the sawtooth regions. While there is a significant strain gradient present around the sawtooth rasp, the strain fields do not interact enough, i.e. diffuse, to cause any variance in the bulk response. Though the RVE is under overall tension, the local sawtooth region at ganoine interface layer exhibits a combined shear and compressive strains, about 24% and 5% of the maximum axial strain, respectively. Whereas the out of plane shear strains: ϵ_{13} and ϵ_{23} were found to be negligible ($\sim 10^{-7}$) which indicates the sawtooth structure distributed along the 3rd (or Z) direction does not induce shearing motion while the same structure produces significant shear in the X-Y plane. Also, the anchoring of mineralized high-stiffness ganoine layer into the soft low-stiffness bone layer induced a complex distribution of strain (shear, tensile, and compressive). We speculate that one of the efficient ways to dissipate energy is the use of damage nucleation and growth in the local sawtooth tip. To fully understand and evaluate the influence of sawtooth structural features would require modeling of the nonlinear response of the fish scale using a much finer RVE.

Figure 7. (2 column)

Since the fish scale is a highly heterogeneous system, to understand how the strains are distributed within a slice of the RVE, a histogram plot was considered. Each slice contains 10,000 elements. A histogram of the data allows for a quantitative look at the number of elements experiencing a given strain state as well as the distribution and variance within the slice. Shown in Fig. 7b is the maximum strain of 0.14%, a minimum of 0.04%, and 0.1% average strain consistent the applied boundary conditions. In general, the strain distribution is spatially correlated with a highly localized response occurring at the interface. The sawtooth intrusions produce a tensile strain in the y-direction, shown in Fig. 7c, while the transverse strain in the z-directions is near zero for the majority of the RVE. The shear response, Fig. 7d, shows a similar

concentration occurring at the sawtooth tip with the majority of elements and average being at zero strain. The strain and strain energy, shown in Fig. 8, concentrate at the soft inner surface of the bone; allowing the protective ganoine layer to stay at reduced strain energy levels, an important characteristic for brittle materials.

Figure 8. (2 column)

The strain energy density contour in Fig. 8a provides insight into the potential localization effects related to material and geometry variations as well as hot spots for elastic energy-related phenomena such as linear elastic fracture. Highlighting the importance of understanding the sawtooth's effect on the interfacial integrity. Fig. 8b shows the mechanical response of the RVE for u_{11} displacement (or predatory loading) condition is dominated by the bone layer and reliant on its soft nature to lower the energy state in the protective ganoine.

6. CONCLUSIONS

Since the Mississippi Alligator gar provides a unique case-study for bio-inspired protective material systems given its highly protective dermal armor, a finite element based modeling approach was considered in the present work to model the mechanical response of its ganoid fish scale. SEM and nanoindentation experimental methods have enabled the determination of both spatial and mechanical characterization of the bi-layer ganoid fish scale. The interface between ganoine and bone layers is geometrically interlocked with circumferentially oriented sawtooth projections. A parametric study was performed to investigate the influence of geometrical/material nonlinearities on the computed stiffness tensor. Based on the present results the idealized RVE model with sawtooth geometry was determined to be adequate for obtaining the effective elastic tensor for the fish scale. The elastic modulus of the gar fish scale progressively decreases along the thickness. Using the through thickness graded

properties estimated from these characterizations, and explicitly modeling the microstructural features, a 740 μ m cubic RVE was constructed with one-million voxels. By applying iso-strain based loading conditions at the boundaries of RVE, pure tension, and pure shear conditions were simulated for determining the effective stiffness tensor of the heterogeneous and anisotropic fish scale. Interestingly, the RVE analysis predicted the presence of an orthotropic material symmetry in the fish scale due to its architecture and the graded properties in the ganoine-bone bilayer. Furthermore, the global strain was shown to be uniform; the strain field internally showed significant variations as well as strain energy accumulation at the sawtooth tip. As a result of the iso-strain boundary condition implementation, the analysis was useful in quantifying the effect of the structural-mechanical property variations on the macroscopic elastic properties.

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Appendix: Figures and detailed captions

Figure 1. Section dissection and freshly cleaned scales dissected from middle region of the Mississippi Alligator gar fish using the ERDC animal care guidelines (#EL-6009-2011-5) for use in in the nanoindentation and microscopy experimental analysis (Hodo, 2015).

Figure 2. High resolution SEM of the juvenile Mississippi Alligator gar fish scale with (a) 500µm scale bar showing the periodic sawtooth rasp and (b) 100µm scale bar for geometric analysis. The light region shows the ganoine layer and sawtooth rasps while the adjacent gray is the underlying bone.

Figure 3. (a) Surface map of the dynamic nanoindentation modulus average from (500-800 μm) and (b) the corresponding data analysis Tukey's box plot for the Mississippi alligator gar fish scale. The red lines indicate the grid line median, the box provides the bounds of the 25th and 75th percentile. Rod dots indicate outliers using the traditional data bounds of 1.5 times the inner quartile.

Figure 4. Functional grading of elastic modulus variations to idealize the Mississippi Alligator gar fish scale computational model showing the linear piecewise functions that define the modulus variations for the three computational models. (a), (b), and (c) correspond to the three interface conditions analyzed for their effective elastic properties found in Tables 3a-c. Region (a) providing the material variations present in the sawtooth rasps, the line (b) provides the lower bound and (c) the upper bound for the interface elastic properties and corresponding computational models.

Figure 5. ParaView visualization of the computational Representative Volume Element (RVE) model's cubic configuration with (a) explicit inclusion of sawtooth and (b) interface region, seen in white, consisting bone to provide the lower bound and ganoine to provide the upper bound on the elastic stiffness tensor determined using the ISBC.

$$\begin{aligned}
(a) \quad \mathbf{C} &= \begin{bmatrix} 28.16 & 14.58 & 14.58 & 0 & 0 & 0 \\ 14.58 & 31.47 & 15.37 & 0 & 0 & 0 \\ 14.58 & 15.37 & 31.47 & 0 & 0 & 0 \\ 0 & 0 & 0 & 9.19 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8.70 & 0 \\ 0 & 0 & 0 & 0 & 0 & 8.70 \end{bmatrix} \\
(b) \quad \mathbf{C} &= \begin{bmatrix} 28.05 & 14.55 & 14.55 & 0 & 0 & 0 \\ 14.55 & 31.20 & 15.31 & 0 & 0 & 0 \\ 14.55 & 15.31 & 31.20 & 0 & 0 & 0 \\ 0 & 0 & 0 & 9.06 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8.60 & 0 \\ 0 & 0 & 0 & 0 & 0 & 8.60 \end{bmatrix} \\
(c) \quad \mathbf{C} &= \begin{bmatrix} 28.48 & 14.70 & 14.70 & 0 & 0 & 0 \\ 14.70 & 32.32 & 15.62 & 0 & 0 & 0 \\ 14.70 & 15.62 & 32.32 & 0 & 0 & 0 \\ 0 & 0 & 0 & 9.51 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8.94 & 0 \\ 0 & 0 & 0 & 0 & 0 & 8.94 \end{bmatrix}
\end{aligned}$$

Figure 6. Orthotropic elastic stiffness tensor of the Mississippi Alligator gar fish scale calculated using the properties shown in Tables 3a-c for (a) explicitly models sawtooth rasp, (b) bone interface, and (c) ganoine interface. Constants show transversely isotropic response with increased stiffness in transverse direction for all three interface types with minimal variations in stiffness.

Figure 7. To highlight the internal strain distribution contours during for the u_{11} displacement boundary condition (Case I) at a mid-plane slice taken along the z-axis: (a) ϵ_{11} strain, (b) ϵ_{11} strain distribution histogram, (c) ϵ_{22} strain, and (d) ϵ_{12} strain.

Figure 8. (a) The schematic shows the spatial strain energy density for the center cross-sectional cut of the gar fish RVE and (b) shows the strain energy density distribution for case I boundary conditions applied to the gar fish RVE. The red dashed line indicates the average strain energy of the cross section.