Contributions of the layer topology and mineral content to the elastic modulus and strength of fish scales

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A B S T R A C T

Fish scales are an interesting natural structural material and their functionality requires both flexibility and toughness. Our previous studies identified that there are spatial variations in the elastic properties of fish scales corresponding to the anatomical regions, and that they appear to be attributed to changes in the microstructure. In the present study, a model is proposed that describes the elastic behavior of elasmoid fish scales in terms of the relative contributions of the limiting layer and both the internal and external elasmodine. The mechanical properties of scales from the *Megalops atlanticus* (i.e. tarpon) were characterized in tension and compared with predictions from the model. The average error between the predicted and the experimental properties was 7%. It was found that the gradient in mineral content and aspect ratio of the apatite crystals in the limiting layer played the most important roles on the elastic modulus of the scales. Furthermore, misalignment of plies in the external elasmodine from the longitudinal direction was shown to reduce the elastic modulus significantly. This is one approach for modulating the fish scale flexibility for a high mineral content that is required to increase the resistance to puncture.

1. Introduction

Over the past decade, natural dermal armors have served as a source of inspiration for the design of new engineering materials (Li et al., 2012; Chintapalli et al., 2014; Funk et al., 2015; Martini and Barthelat, 2016). Among the wide range of dermal armors being explored (Yang et al., 2013), several studies have focused on elasmoid fish scales (Lin et al., 2011; Garrano et al., 2012; Zhu et al., 2012; Yang et al., 2013; Gil-Duran et al., 2016), which are unique from the more rigid cosmoid, ganoid and placoid scales (Sherman et al., 2016). Elasmoid scales are much thinner and stacked or over-layered with respect to one another. They are characteristic of fish with greater speed, and could be considered multi-functional as their flexibility is arguably just as important as their resistance to puncture (Bruet et al., 2008; Jandt, 2008). Flexibility in these dermal armors is achieved by their construction, and apparently from the stacking sequence of the principal layers.

The overall structure of elasmoid scales consists of three distinct layers across its thickness (Fig. 1A). The Limiting layer (LL) is the most highly mineralized region of the scale. It serves as the outermost coating of the scales and as such is regarded as the first barrier against penetration (Zhu et al., 2012). The elasmodine is a laminate consisting

of discrete plies of unidirectionally aligned collagen fibers with a diameter of between 100 and 160 nm (Zylberberg and Nicolas, 1982; Garrano et al., 2012). The individual plies of the elasmodine are rotated at specific angles with respect to one another and exhibit a unique thickness, which depends on the fish (Murcia et al., 2016a). Based on earlier reports (Zylberberg and Nicolas, 1982; Zylerberg, 1985; Sire and Huysseune, 2003), the mineralization front extends from the LL into the underlying collagen matrix of the elasmodine. According to the distribution in mineral content, the elasmodine is further divided into external (EE) and internal (IE) regions, with the EE possessing the larger mineral content of the two.

Previous studies on the mechanical behavior of fish scales have revealed that the strength and elastic modulus of the scales are largely dependent on the number of mineralized plies of the EE, and the ratio of the elasmodine thickness to the total scale thickness (i.e. elasmodine ratio) (Murcia et al., 2015, 2016b). The rule of mixtures is commonly adopted as a first approach to describe the elastic behavior of composite materials based on the contributions of the constituents. Although a model for the elastic properties of scales has been presented (Zhu et al., 2012), it was not based on an explicit description of the contributions from the LL, EE and IE of the scales. Owing to the differences in mineral

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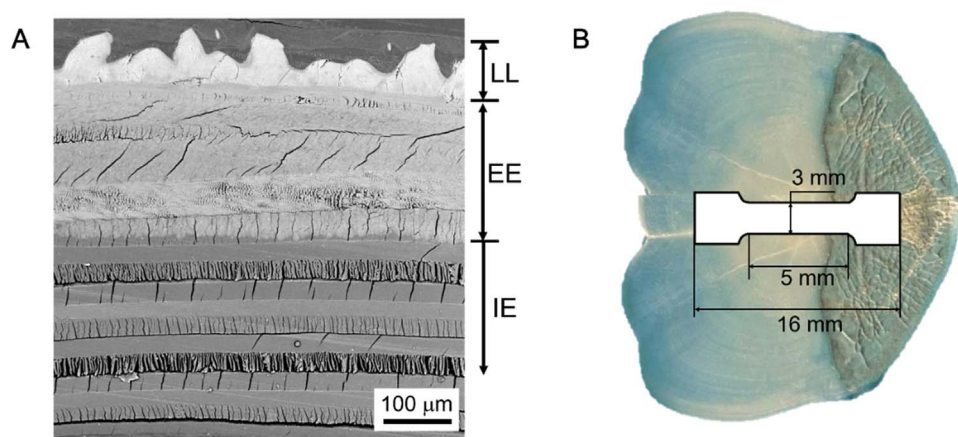E-mail address: darola@uw.edu (D. Arola).

Fig. 1. Details of the evaluation involving tensile testing of the scales. A) cross-section of a representative tarpon scale denoting the three distinct regions including the limiting layer (LL), external elasmodine (EE) and internal elasmodine (IE). B) Location and geometry of a stamped tensile specimen from a representative tarpon scale.

content of these three layers and their distribution, these factors should be considered in descriptions of the elastic behavior. Furthermore, the lamina orientations in the elasmodine is not the same for all fish (Murcia et al., 2016a) and is potentially an important contributor to the elastic properties as well.

Earlier studies concerning the contribution of the individual layers of scales to the mechanical properties have evaluated the strength and elastic modulus of the IE and mineralized layers separately. A summary of the tensile properties from these investigations is listed in Table 1. The elastic modulus of the mineralized layers is significantly larger than that of the whole scale, which apparently results from the large mineral gradient. Whereas properties of the IE are largely controlled by the stretching of unidirectional collagen fibrils, the properties of the EE and LL have not been reported individually due to the difficulty in achieving separation. For the *M. saxatilis,* the properties of the mineralized layers were estimated using the rule of mixtures assuming the volume fraction of the IE is equal to the sum of the volume fractions of the EE and LL. However, it is not clear that this ratio is constant over the length of the fish.

Understanding the relative contributions of the mineralized layers and IE to the mechanical behavior of fish scales is necessary to distinguish the principles that guide the scale development and its ability to achieve its multifunctional performance. According to the results of previous studies, the tensile properties and Mode III tear resistance of elasmoid scales are highly correlated with the EE ratio (Murcia et al., 2015, 2016b). However, a detailed analysis of the importance of spatial variations in the scale microstructure on mechanical behavior was not conducted. To the authors' knowledge, no study has developed a microstructure-based model that quantitatively details contributions of the microstructural characteristics to the tensile properties of scales. Therefore, the objective of this study is to develop new knowledge regarding the contributions of the layer topology and the layer composition to the elastic modulus and strength of elasmoid fish scales.

2. Materials and methods

2.1. Experimental approach

Scales from a wild captured *Megalops atlanticus* (i.e. tarpon) were extracted from the body of a single tarpon within three characteristic regions including adjacent to the head, mid-length and near the tail, following established procedures (Murcia et al., 2016a). After extraction the scales were stored in Hanks Balanced Salt Solution (HBSS) at 4 °C and evaluated within a week of harvesting the fish. Conventional dog-bone shaped tensile specimens were sectioned from the scales using a punch and stamping process (Zhang et al., 2007). In recognition of the variation in thickness of the scales (Zhu et al., 2013), a single sample was stamped from the center of each scale where the thickness is most uniform. The specimens possessed a gage section length and width of approximately 5 mm and 3 mm, respectively (Fig. 1). The thickness was measured over the length of the gage section to assess the variation, and in general varied by less than 50 μm over the gage section length. The lowest value was used in calculating stress. In recognition that the scales may exhibit anisotropy due to the different alignment of the collagen fibers (Zhu et al., 2012; Yang et al., 2014; Murcia et al., 2015, 2016a), all of the specimens were obtained with alignment parallel to the fish length.

After sectioning, the specimens were maintained in an HBSS bath at room temperature. Subsequently, the remaining area of the scales outside of the stamped tensile specimen was used for microstructural analysis. A fixation process was adopted that started with submersion in 2% glutaraldehyde buffered with 0.1 M sodium cacodylate with pH = 7.2 for 4 h. They were then rinsed in 0.1 M sodium cacodylate buffer followed by post-fixation in 1% osmium tetroxide buffered with 0.1 M sodium cacodylate for 2 h. After rinsing, the scales were dehydrated through an ascending ethanol series from 50% to 95%, followed by 100% acetone and instant dehydration in 2,2- dimethoxypropane (DMP) for 5 h, and then followed by infiltration of the scales in Mollenhauer resin. The microstructure and fiber orientation of individual lamina were analyzed following established procedures

Table 1
Elastic modulus (E) and strength (S) of scales reported for the *Arapaima gigas* (arapaima)*, Megalops atlanticus* (tarpon) *and Morone saxatilis* (striped bass) in the longitudinal direction. The properties are defined in terms of the whole scale thickness, as well as the contribution of the individual layers where available. For the striped bass, the mineralized layer properties were estimated using the rule of mixtures assuming the volume fraction of the IE is equal to the sum of the volume fractions of the EE and LL. All quantities are listed in MPa.

	Entire Scale		IE		EE + LL	
Fish	**E**	**S**	**E**	**S**	**E**	**S**
Arapaima gigas (Yang et al., 2014)	860 ± 320	23.6 ± 7.2	470 ± 250	36.9 ± 7.4	–	–
Megalops atlanticus (Gil et al., 2016)	300 ± 59	24.4 ± 7.1	170	40 ± 8	–	–
Morone saxatilis (Zhu et al., 2012)	∼ 860 ± 150	∼ 30 ± 10	∼ 450 ± 150	∼ 65 ± 15	∼ 1250	∼ 45