Effect of chemical composition and microstructure on the mechanical properties of fish scales from *Megalops Atlanticus*

ABSTRACT

Fishes use their scales as a protection against external threats like environmental hazards and predators. This work presents an experimental study of the microstructure, composition and mechanical properties of *Megalops Atlanticus* (Atlantic tarpon) scales. Mechanical properties were evaluated in uniaxial tension as a function of position along the length of the fish (head, mid-length and tail). Additional tensile tests were performed in three different orientations (0°, 45°, and 90°) to evaluate the anisotropic behavior of the scales. Examination of fish scale microstructure and compositions were performed by using Scanning Electron Microscopy (SEM) and RAMAN spectroscopy. The results showed that scales from *Megalops Atlantiticus* are anisotropic, with variations of mechanical properties as a function of body position. *Megalops Atlantiticus* scales display a characteristic hierarchical structure composed of fibrous collagen layered structures and hydroxyapatite crystals that provide multifunctional characteristics, showing to have a non-homogenous distribution within the scale.

KEY WORDS

Fish scales, microstructure, biological materials, chemical composition, layered structures, mechanical properties.

1. INTRODUCTION

Nature is being used as a source of inspiration and model for engineers and scientists in a relatively new field of study known as biomimetics or bio-inspiration (Zhu et al., 2012). Among the natural materials that have received special attention are the highly mineralized "hard" materials like bones, teeth and nacre. Materials with lower mineral content like fish scales have received less attention and these materials could also be a source of inspiration (Garranoa et al, 2012).

Fish scales are a skeletal element that covers and protects the skin of the fish (Torres et al, 2008). There are about 28.000 identified living species of fish, of which approximately 1.000 are cartilaginous fishes (cartilaginous skeleton), 108 are jawless fishes and the remaining are bony fishes (bone skeletons) (Gene et al, 2009). Fish scales can be classified in four basic types depending on the fish species. Placoid scales are found in cartilaginous fishes like sharks and manta rays, while in bony fishes there are three different types of scales: cosmoid, ganoid and teleost (Kardong, 2008). Figure 1 shows the four different types of fish scales.

Placoid scales have similarities with teeth in composition and formation method. They are homologous to integumentary structures (Kardong, 2008). The outer part of placoid scales is composed of enamel, a layer of dentin underlaying the enamel and a living inner structure composed of a vascular nucleus that is supplied with blood (Elliott, 2011), (see Figure 1a). Placoid scales do not increase in size with growing of the fish; instead, new scales are added between older scales (Gene et al, 2009).

Cosmoid scales are found in primitive fishes (lungfishes) and are similar to placoid scales. They are composed in the bottom by a double layer of vascular (supplied by blood) and laminar bone, over the double layer of bone, a layer of dentin and enamel on top (Kardong, 2008), (see Figure 1b). Growth of the scale is by addition of new laminar bone beneath (Gene et al, 2009). Ganoid scales are modified cosmoid scales where the dentin layer disappeared.

They are characterized by a thick surface of enamel on top and beneath it there is a vascular and/or a laminar bone (see Figure 1c). As a final type of scales, Teleost scales are found in most of the bony fish. They evolved from ganoid scales and were simplified to only laminar bone partially calcified (Kardong, 2008). There are two types of teleost scales (see Figure 1d), ctenoid, with spines along its posterior margins, and cycloid, composed of concentric rings called circuli. The circuli are often used to determine the age of the fish. As the teleost fish grows, new circuli rings are added, (Kardong, 2008). Teleost scales are mainly composed by hydroxyapatite (mineral component), type I collagen (organic component) and water. The collagen fibers form sheets stacked on top of each other with different orientations from one another, forming plywood like structure (Ikoma et al, 2003).

The microstructure and mechanical properties of different types of fish scales have been reported in previous studies. For instance, Bigi et al (2001) studied the distribution and orientation of collagen fibers of *Leuciscus Cephalus,* concluding that the collagen fibrils are co-aligned in each layer and the alignment rotates by 36° in successive layers. Ikoma et al (2003) studied the effect of dehydration on the scale and showed an increase of the Young´s modulus with dehydration in scales from *Pagrus major*. Bruet et al. (2008) studied *Polypterus Senegalus* scales using nanoindentation reporting that the change of Young´s modulus across the scale has an important role in protecting the animal, based on overlaying layers of different material's properties. Torres et al. (2008) performed the characterization of scales from the *Arapaima Gigas* fish drawing similar conclusions. They also studied the effect of demineralization of the scale in the mechanical properties. Similarly, Lin (2011) studied the mechanical properties of fish scales from *Arapaima Gigas* arguing that the scales are composed of collagen fibres in the inner layer while a higher concentration of hydroxyapatite can be found in the outer layer of the scales. In addition, they also studied the effects of dehydration on the mechanical properties. Using a different approach, Zhu et al (2011) studied the resistance to penetration of *Morone Saxatilis* scales, showing that fish scales have superior penetration resistance than sveral engineering polymers. Furthermore, Garranoa et al, (2012) studied the mechanical behaviour of scales of *Cyprinus Carpio* in three different positions on the body of the fish (Head, Mid-length and tail) as a function of moisture (from hydrated to dehydrated condition). The results of studying the mechanical properties demonstrated that there is an increase in stiffness with increasing dehydration. The scales obtained from the head having the highest stiffness in the hydrated state and scales with the least stiffness were from the middle and tail of the fish, respectively.

Megalops Atlanticus (Atlantic Tarpon) is a warm water fish that inhabits the tropical Atlantic in America and Africa (Wade, 1962). They belong to the Megalopida family (Joseph, 2006). They are also known to live in a wide variety of habitats, including lakes, rivers, and offshore marine waters (Crabtree et al, 1995, Merrick, 1984). The Tarpon is a prized fish by sports fishermen because strikes hard and makes strong runs that result in spectacular airborne displays (Cyr, 1991). There have been reported at lengths of 2.50 *m* (Whitehead, 1978) and at weights of up to 161 *kg*. Tarpon have about 40-48 cycloid scales in the lateral line. The predators of the tarpon in its maturity are mainly humans, sharks and alligators. These types of predators make them interesting from the evolutionary point of view, since the microstructural characteristics, composition and mechanical properties should have evolved to protect the fish against these predators. The Tarpon is of commercial importance as a food source in the Caribbean according to an ecological study by García & Contreras, (2011).

This work aims at studying the composition, microstructure and mechanical properties of scales from *Megalops Atlanticus*. As the composition in different radial positions within the scale is poorly known, a study was performed in the cross section of the scale in different radial position using RAMAN spectroscopy. Mechanical properties were evaluated in uniaxial tension as a function of position along the length of the fish (head, mid-length and tail), while in order to evaluate anisotropy of the scales additional tensile tests were performed in three different orientations (0°, 45°, and 90°).

2. MATERIALS AND METHODS

Five (5) fresh *Megalops Atlanticus* (Atlantic tarpon) fishes were obtained from a fish market, each fish weighted between 4 - 4.5 kilograms. Fish scales were extracted from three different positions along the length of the fish: Scales close to the gill cover were defined as head scales; scales under the dorsal fin and at the end of the anal fin were defined as middle and tail scales, respectively. A total of 18 scales were extracted from each position, 9 from the left and 9 from the right side of the fish. Of the scales extracted from each side, 3 were from the lateral line (equidistant between the dorsal and pelvic fin), 3 from a line over it and 3 from the line beneath it, as shown in Figure 2. To detach the scale from the fish skin, tweezers were used to avoid any twisting that could end up damaging or pre-cracking the scale. After extraction, the scales were maintained immersed in Hanks Balanced Salt Solution (HBSS) in a fridge and evaluated within two weeks to prevent any loose of organic materials that can influence the results.

Composition

Spatial variations in the mineral to organic ratios of the scales were studied using RAMAN Spectroscopy (Horiba Jobinyvon, LabRAM). Figure 3 illustrates the cross section, areas and points used to evaluate composition. Samples were mounted within cold-cure epoxy resin to avoid damage of the organic content of the scales. After complete curing, the samples were polished using series of silicon carbide abrasive papers down to 1200 grit, until it was reached the centre of the scale (see figure 3a). Then, it was finished with diamond suspensions with 6, 3 and 1 μm particle diameters.

Cycloid scales are composed of concentric rings (Kardong, 2008); as a result the scale composition can be expected to be symmetrically identical from the centre of the scale. Consequently, only three areas in half cross sections of the scale were examined as shown in figure 3b. As fish scales have different sizes along the fish's body (Ibañez et al, 2009), a normalization based on measuring the radial length (L) of each scale from its centre to the right edge was used. Based on the length (L), three areas in half cross sections of the scale were examined. The first area evaluated was in the centre of the scale (0L), the second area was the midway point between the centre and the right edge (0.5L) and the last was close to the right edge (0.8L). For each area of evaluation, ten (10) linear measurements of composition were taken equidistant from the bottom to the top of the scale, evaluating the whole cross section as shown in figure 3c, for a total of 30 measurements per scale. The Raman spectrum dispersion, baseline and areas under each peak were analyzed using commercial software (Origin®). In order to compare the composition in different radial position of the scale, the areas under the peak PO_4 (962 cm^{-1}) and Amide I (1652 cm^{-1}) were used to estimate the mineral to collagen ratios (PO_4/ Amide I). A similar procedure was followed by Ryou et al (2011) to analyze the chemical composition of dentin.

Macro and Microstructure

Macro and Microstructure were studied using Scanning Electron Microscopy (SEM, FEI Phenom G2 PRO). Samples were coated with gold palladium for SEM analysis. The macrostructure study was aimed to observe common features that could be identify by a naked eye. Whereas, microstructure was evaluated in samples used in Raman spectroscopy aiming at studying the cross section of the scale.

Mechanical response

Tensile tests were performed in hydrated conditions on small tensile dog bone specimens extracted from the centre of the scales, as shown in figure 4. The specimens were extracted using a punching process. The resulting sample having a gage length and width of 6 and 5 *mm*, respectively, as shown in figure 4a. Considering that scales exhibit strain rate dependence (Lin, 2011), all specimens were loaded until complete failure at a rate 0.3 *mm/min*, taking over 30 seconds to occur the failure after starting the test. Tensile testing of the specimens was performed under displacement control loading at room temperature using an Instron 3366 universal testing machine. The commercial system has a full-scale load range of 500N and a load precision of 0.03 %.

To assess the mechanical response as a function of fish body position (Head, mid-length and tail), five scales were used from each position of each fish, amounting to 75 specimens (3 positions (Head, middle, tail) X (5 specimens) X (5 fishes) = 75). The mechanical responses were analyzed by using ANOVA and Tukey HSD tests. $P < 0.05$ defined significance. The procedure and analysis were similar to Garranoa et al (2012). The Young´s modulus (E), maximum strength (S), Strain to failure (e_f) and modulus of toughness (as a function of e_f) were determined from the stress-strain responses, using the engineering definition. The strain was calculated using the crosshead displacement of the testing machine and the strength was estimated with the sectional area of each specimen.

Tensile tests were performed in three different orientations (0°,45°, 90°). The longitudinal direction on the scale was defined as orientation 0°, the orientations 45° and 90° were taken anti-clock wise as shown in Figure 4b. The maximum strength (S) was studied as a function of orientation. The samples were extracted from the centre of the scale and a total N= 225 specimens were evaluated. Comparisons of the mechanical response were made using ANOVA and Tukey HSD test with defined significance of $P < 0.05$.

3. RESULTS AND DISCUSSION

Composition

The Raman spectrum obtained at the top-right edge of the scale extracted from the head of the fish is shown in figure 5. Well-defined peaks are observed along the cross section of the scale. Characteristic peaks of the spectrogram showing the presence of hydroxyapatite (430, 593, 962, 1073 and 1445 cm^{-1}) (Depaula et al, 2010; Withnall et al, 2003) and type I collagen (1298, 1652, 2933 and 3330 cm^{-1}) (Corinne et al, 2011) were found. Hence, the scales of

Megalops Atlanticus are composed of hydroxyapatite (mineral component) and collagen Type I (organic component). There have been reports on different fish species that indicated the same materials present on fish scales (Ikoma et al, 2003; Zhu et al, 2011; Torres et al, 2008; and Garrano et al, 2012). Further, results obtained from Raman analysis at ten (10) equidistant locations across the right edge of the scale are shown in figure 6. At the bottom of the scale the characteristic peaks of collagen type I show the highest intensity. In contrast, at the top of the scale the peaks showing highest intensity are the characteristic peaks of hydroxyapatite. Changes in crosswise composition for different radial positions of the scale can be seen in figure 7. Figures 7a, 7b and 7c show variations in mineral to collagen ratios, which are denoted by using different gray tones. For instance, the bottom collagen layers which have a mineral to collagen ratio (PO_4/Amide I) lower than 0.5 (low mineral content) are shown in dark grey. It is worth noting that when comparing layers with low mineral content at different radial positions within the scale, at the centre (0L) they cover about 60 % of the scale thickness (figure 7a), while at the midway point between centre and right edge (0.5L) these layers cover about 40 % (figure 7b), and close to the right edge (0.8L) are reduced to only 20 % (figures 7c). As a consequence, low mineral content layers decrease in relation to thickness towards the edge of the fish scale. Moreover, towards the top of the scale there is a gradual change in composition. The mineral to collagen ratio just beneath the top of the scale increased to values between 1 and 2.3 (medium mineral content). Thus, there is more presence of mineral within collagen than for the bottom collagen layers. It is important to underline that medium mineral content layers illustrated in grey in figures 7a, 7b and 7c cover about 30 % of the scale thickness in the centre (0L) (figure 7a) while at the midway point between the centre and right edges (0.5L) as well as in the right edge (0.8L) they are about 50 % mineral content (figure 7b and 7c, respectively). Finally, at the top of the scale there is an external layer, which is highly mineralized, with mineral to collagen ratios higher than 5 (illustrated using light grey in figures 7a, 7b and 7c). The highly mineralized top layer (light grey) goes from 10% thick in (0L) and (0.5L) up to 30 % in (0.8L). Thus, layers with medium and high mineral content increase in relation to the thickness toward the edge of the scale.

Aiming at observing the areas evaluated with RAMAN spectroscopy, Scanning Electron Microscopy (SEM) images were taken in different radial positions of the scale. An assembly of SEM images is shown in figure 7d. Different contrasts can be observed which provide information relating to composition. These changes in contrast are gradual and show the same pattern that the mineral to collagen ratios obtained by using RAMAN spectroscopy. This response was consistent within all the specimens evaluated. Hence, these results indicate that crosswise composition in different radial positions of the scale is non-balanced. Towards the edge of the scale there is a higher mineralization in relation to the thickness than at the centre. Furthermore, changes in composition show that the bottom collagen layers have low mineral content, and the mineralization increases gradually from low mineral content interior to the highly mineralized top of the scale. Hydroxyapatite at the top of the scale is randomly arranged (Schönbörner et al., 1979; Onozato & Watabe, 1979; Oslon & Watabe, 1980), while inside of the scale the crystals are co-aligned in each ply with the collagen fibers (Big et al, 2001; Ikoma et al, 2003). Schönbörner et al., 1979, reported similar findings in composition of teleost scales. Ikoma et al, (2003) reported a mineral content of 46%, which is just about the content of bone (50 %) (Jager & Fratzl, 2000).

Changes in distribution of crosswise composition at different radial positions can be explained by the scale growth. The mineralization process in the scales occurs through the life of the fish, increasing surface area at the periphery and thickness (Schönbörner et al., 1979). The top layer is the first to be formed and the first to be mineralized (Zylberberg et all, 1988) and then new inner layers of collagen are developed (Onozato & Watabe, 1979; Zylberberg & Nicolas, 1982). The circuli indicates that the scale grows from its centre towards the extremities (Bigi et al 2001). As a result, at the extremities of the scale, there is more presence of the highly mineralized top layer, first formed, than from the inner-layers that are growing from the center of the scale. Studies related to changes in composition of bio-materials have concluded that Young´s modulus depends on mineral content, with increasing stiffness with increasing mineral content (Currey & Brear, 1990). This increases the bending stiffness, reducing the deflection of the scale as well as providing resistance to penetration by overlaying materials with different hardness. It has been reported that the change in composition and their order has an important role in protecting the fish based on overlaying layers with different material properties (Bruet et al, 2008; Zhu et al, 2011; Lin, 2011).

Macro and Microstructure

When a single scale of *Megalops Atlanticus* is observed, two regions can be distinguished, exposed and unexposed areas, as shown in figure 8a. The exposed region is the part of the fish scale that is in contact (exposed) to water; while the unexposed region indicates the part of the scale that is covered by others scales. Scales overlap on each other so most of the scale is covered by another scale, allowing a higher protection of the dermis by increasing the density of scales on top of it. Overlapping gives greater flexibility, compared to cosmoid and ganoid scales (Gene et al, 2009).

The top of the scales exhibits a rouged pattern, forming circular ridges around the center, as shown in figure 8b. These ridged patterns have been associated with improved hydrodynamic advantages (Sudo et al, 2002). In addition, the top of the scale shows to have several discontinuities (figures 8a and 8c), which in most of the cases form three lines, but can go from cero up to four discontinuities. These discontinuities are mainly located on scales requiring more flexibility, working as a hinge, allowing increased flexibility of the scale despite of the highly mineralized (stiff) top layer.

The scales show changes in thickness within a single scale. The centre of the scale is thicker than the edges in any direction due to the annular scale growth, which produces variations in thickness within the scale. This change has also been reported in other fish species, e. g. *Leuciscus cephalus* (Bigi et al, 2001) and *Arapaima gigas* (Lin, 2011). When comparing the thickness measured in the centre of the scale as a function of body position, there are statistical differences ($P<0.05$) as shown in figure 9a. In the evaluated areas the thicknesses were between 0.5 – 1 mm. Scales from the mid-length and tail had an average thickness of 0.92 and 0.95 millimetres, respectively; while scales from the head were significantly different from mid-length and tail, with an average thickness of 0.65 mm. Within the fish body, tail and middle scales are mainly used for locomotion. According to Hebrank, (1982), Hebrank & Hebrank, (1986) and Vernerey & Barthelat, (2010) the scales act as an external tendon storing energy. Thus, these differences in thickness may contribute to increasing stiffness in different body positions, where necessary, facilitating storage of elastic energy to make a more efficient swimming.

Figure 9b compares the differences in size of the scales on the three positions evaluated. Comparing the scale's superficial area as a function of body position, there are statistical differences between head, mid-length and tail ($P<0.5$). Scales from *Megalops Atlanticus* had a superficial area of 549, 1197 and 927 mm^2, respectively. Ibañez et al, (2009) and Vernerey & Barthelat, (2010) concluded that depending on the fish needs, the shape, properties and density of scales may emphasize certain functions such as curvature of the fish aiding locomotion, swimming mode and/or protection. A summary of the macroscopic characteristics and statistical comparisons are shown in Table 1.

A Scanning Electron Micrograph (SEM) of the cross section of one scale (figure 10a) shows that the bottom of the scale is unlike the top part of the scale, having a smooth surface. Further, the cross section of the scale displays that *Megalops atlanticus* scales consist of many layers of collagen fibers. A top view of an unidirectional collagen layer is shown in figure 10b. Within each layer the orientation of collagen fibers is the same, whereas between layers there are different orientations. For *Megalops Atlanticus* some collagen layers seem to be oriented about 30 ° from one another as shown in figure 10c. Bigi et al, (2001) reported differences in orientation between collagen layers of 36° for *Leuciscus cephalus* scales. Nonetheless, different orientations have been reported between layers; for instance, Torres et al, (2008), Zylberberg & Bereiter, (1991) and Ikoma et al, (2003) reported orthogonal plywood like structure for different fish species, but this type of microstructural arrangement was not found in *Megalops Atlanticus*. These results are in agreement to the results of Lin, (2011), Zylberberg et all, (1988), Zylberberg & Bereiter, (1991) and Ikoma et al, (2003). Figure 10d shows the rouged pattern (top of the scale highly mineralized), below it, mineralized collagen layers and at the bottom low mineral content layers.

Mechanical response

Figure 11 shows selected stress-strain tensile tests response of scales in three different body positions: head, middle and tail. The stress-strain curves exhibit nonlinearity with similar failure modes. The curves show an initial linear region and then a series of load drops (indicated by arrows), changing the slope of the curve; which are associated with the failure mode of the scale before failure. First appear the fracture of the highly mineralized surface followed by the processes of delamination, tearing and failure of the collagen layers until reaching its maximum strength. In figure 11 there is not a clear shift between linear and nonlinear behavior. Zhu et al (2011) attributed this load drop to the partial failure of the material.

A comparison of the mechanical properties as a function of body position (head, mid-length and tail) is shown in Figure 12. Among the regions evaluated there were statistical differences in Young´s modulus as a function of body position ($P<0.5$) (Figure 12a). Scales from the head were statistically different from scales from the middle and tail. There were no statistical differences between scales from the middle and tail. A summary of the mechanical properties and statistical comparison are shown in Table 2. Studies on the mechanical behaviour of scales from *Cyprus Carpio* showed statistical differences between Young´s modulus as a function of body position head 0.39 GPa, middle 0.30GPa and tail 0.18 GPa. Garrano et al, (2012) attributed these changes in Young's modulus to variations in the degree of the mineralization of scales in three different body positions. Weiner, (1998) also reported that the mineral phase works as a reinforcement of the collagen, providing stiffness and playing an important role in the strength of bone.

The Young´s modulus reported by Garrano et al, (2012) were slightly higher than the ones found here for *Megalops Atlanticus* (Head=0.30 GPa, middle=0.21 GPa and tail=0.19 GPa). Both studies suggest a decrease of Young's modulus from head to tail. However, on *Megalops Atlanticus* scales there were no statistical differences between the middle and tail regions, opposite to the results found by Garrano et al, (2012). Studies of fishes' swimming modes have observed that among the different types of propulsive patterns fishes display, those fishes that use the body and/or caudal fin tend to perform oscillatory and/or undulatory movements (Lindsey, 1978; Breder, 1926). Fish like *Megalops atlanticus* that lives mostly in open waters, tend to make ondulatory movements in order to generate swimming power (Moyle & Cech, 2004). Ondulatory movements generally use about half of the animal body. In contrast, animals that swim in slow moving waters like *Cyprus Carpio*, tend to move in a oscillatory manner, using only parts of the body around the tail. Therefore, the mechanical properties of fish scales within the fish seem to be swimming mode dependent, satisfying specific species needs. It is also interesting to note that scales from *Megalops Atlanticus* from the head were the smallest in size and thickness but having the highest Young´s modulus. The scale through mineralization may compensate the lack of thickness, increasing protection as well as reducing weight.

Figure 12 shows statistical comparisons of mechanical properties as a function of body position. The results show that among the three regions of evaluation there were no statistical differences observed ($p > 0.05$). Neither of the response variables showed differences as a function of body position.

Fish scales can be considered a bio-composite where nanofibres of collagen are reinforced with hydroxyapatite crystals. Collagen plays an essential role as it homogenizes the stresses, allowing the scale to be tough (Ji & Gao, 2004). Collagen also exhibits significant viscoelasticity (Sasaki et al, 1993). According to Ji & Gao, (2004) the viscoelasticity of protein may help dissipate fracture energy under dynamic loads due to large deformations before fracture. This mineral content has also been attributed by Ji & Gao (2004) to work well under dynamic loads and large deformations. These types of characteristics are found in natural materials that undergo multi-axial stresses (Zhu et al, 2011) like bone (Wagner & Weiner,1992), teeth (Elliott, 1994; Cate, 2003) and elk antler (Launeya et al, 2010) among others.

A comparison of the results of the mechanical behaviour of the scales at different orientations (0°, 45°, and 90°) is shown in Figure 13. When comparing the maximum strength, there are statistical differences ($p < 0.05$) between the orientations evaluated (0°, 45°, and 90°). Specimens tested in orientation (0°) had the highest strength (23.6 MPa) followed by orientation (45°) and (90°), with values of 20.2 and 16.7 MPa, respectively. Differences in mechanical properties can be attributed to the orientation of collagen layers and hydroxyapatite crystals. As fish scales are composed of many layers of collagen fibers, their orientation will directly affect the mechanical properties of fish scales. Figure 14 shows the preferential orientation of mineralized collagen layers within the scale. The layers show three main orientations 0°, -30°and +30°, as indicated with numbers in figure 14a. This sequence seems to be repeated for the remaining layers of the scale. This result indicates that many of the collagen layers in *Megalops Atlanticus* are oriented mainly longitudinally to the fish (orientations 0 °) as shown in figure 14b, while in the transverse direction (orientation 90°) there are not fibers reinforcing, explaining the reduction of strength of samples tested at 90°

(figure 13). It is important to mention that the discontinuities that the scales show at the top layers may contribute to the standard variation of the results. These findings agree with the fact that the scale needs more bending stiffness in the longitudinal direction (0°) than in the transverse direction (90°), helping in the swimming process.

4. CONCLUSIONS

Compositional analysis of the scales showed to have changes crosswise. In general, towards the extremes, the scale has higher mineralization in relation to the thickness of the scale. The changes are explained by the natural growth of the scale. The mineral content of the scale plays an essential role by helping to compensate for the low mechanical strength of proteins, maintaining in this way the stiffness of the scale where needed. At the top of the scale there is a highly mineralized layer with several collagen fibers layers, yielding resistance to penetration while keeping some toughness. As a result, the combination and distribution of materials within the scales impart multifunctional characteristics such as resistance to penetration, flexibility and hydrodynamic advantages.

A comparison of the mechanical properties as a function of body position (head, mid-length and tail) showed differences in Young´s modulus. Scales from the head were statistically different from scales from the middle and tail. There were no statistical differences between scales from the middle and tail. These changes are different from previous works done on *Cyprus Carpio,* which is a fish that swims in an oscillatory way rather than ondulatory as *Megalops Atlanticus*. Therefore, the mechanical properties of fish scales appear to be swimming mode dependent and natural evolution has produced a fish exoskeleton that satisfies specific-species needs.

A comparison of the strength of scales on different orientations showed that *Megalops Atlanticus* scales are basically anisotropic. Specimens tested at 0° of orientation (longitudinal direction of the fish) had the highest strength, followed by 45° and 90°, respectively. These findings agree with the fact that the scale needs more bending stiffness in the longitudinal direction (0°) than in the transverse direction (90°) helping on the swimming process.

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REFERENCES

Bigi, A. Burghammer, M. Falconi R, Koch MH, Panzavolta S, Riekel C. (2001). Twisted Plywood Pattern of Collagen Fibrils in Teleost Scales: An X-ray Diffraction Investigation. *Journal of Structural Biology.* 136. 137–143

Breder CM. (1926). The locomotion of fishes. *Zoologica* 4. 159–297.

Bruet, B. J. F, Song J, Boyce M. C, Ortiz C. (2008). Materials design principles of ancient fish armour. *Nature Materials.* 7. 748 – 756

Cate A. R. T., (2003) Oral histology: development, structure, and function. 7th ed. St. Louis: Mosby.

Corinne G. Leanne L. Kevin H. Laurent K. (2011).Surface-Sensitive Raman Spectroscopy of Collagen I Fibrils. *Biophysical Journal*. 100. 1837–1845

Crabtree, R.E. Cyr, E.C. Dean, J.M. (1995). Age and growth of tarpon, *Megalops Atlanticus*, from South Florida waters. *Fishery. Bulletin*. 93:619-628.

Currey, J.D. Brear, K.. (1990). Hardness, Young_s modulus and yield stress in mammalian mineralized tissues. *The Journal of Materials Science: Materials in Medicine 1*, 14–20

Cyr, E.C. (1991). Aspects of the life history of the tarpon, *Megalops atlanticus*, from South Florida. Ph.D. Thesis. University of South Carolina, Columbia

DePaula S.M., Huila M.F.G. , Araki K. , Toma H.E. (2010). Confocal Raman and electronic microscopy studies on the topotactic conversion of calcium carbonate from *Pomacea lineate* shells into hydroxyapatite bioceramic materials in phosphate media. *Micron*. 41. 983–98.

Elliott D.G., (2011). In Encyclopedia of Fish Physiology, edited by Anthony P. Farrell. Academic Press. San Diego. Pages 476-488,

Elliott, J.C., 1994. Structure and Chemistry of the Apatites and Other Calcium Orthophosphates. *Elsevier Press*, Amsterdam,Chapter 3.

García, C. B. & Contreras, C. C. (2011). Trophic levels of fish species of commercial importance in the Colombian Caribbean. *Revista de Biología Tropical*. 59. 1195-1203.

Garrano, A. M. G., La Rosa, G., Zhang, D., Niu, L. N., Tay, F.R., Majd, H., Arola, D. (2012). On the mechanical behavior of scales from Cyprinus carpio. *Journal of the Mechanical Behavior of Biomedical Materials*. 7. 17 – 29

Gene H, Bruce B. Collette, Douglas E. Facey, Brian W. B. (2009). *The Diversity of Fishes: Biology, Evolution, and Ecology, 2nd Edition*. Wiley-Blackwell. ISBN: 978-1-4051-2494-2

Hasegawa K., Turne C.H. Burr D.B. (1994). Contribution of collagen and mineral to the elastic anisotropy of bone. *Calcified Tissue International.* 55. 381–386.

Hebrank, M.R., 1982. Mechanical-properties of fish backbones in lateral bendingand in tension. Journal of Biomechanics. 15. 85–89.

Hebrank, M.R., Hebrank, J.H., 1986. The mechanics of fish skin – lack of an external tendon role in 2 teleosts. *Biological Bulletin.* 171. 236–247.

Ibañez a. L., Cowx , i. G. Higgins P. O. (2009). Variation in elasmoid fish scale patterns is informative with regard to taxon and swimming mode. *Zoological Journal of the Linnean Society*, 155, 834–844.

Ikoma, T., Kobayashi H., Tanaka, J., Walsh D., and Mann S. (2003). Microstructure, mechanical, and biomimetic properties of fish scales from Pagrus major. *Journal of structural Biology*. 142. 327–333

Jager, I., Fratzl, P., (2000). Mineralized collagen Mbrils: a mechanical model with staggered arrangement of mineral particles. *Biophysical Journal*. 79, 1737–1746.

Ji B, Gao H, (2004) Mechanical properties of nanostructure of biological materials, *Journal of the Mechanics and Physics of Solids*. 52. 1963-1990.

Joseph S. N. (2006). *Fishes of the World, 4th Edition*. Wiley-Blackwell ISBN: 978-0-471-25031-9

Kardong K. V. (2008). *Vertebrates: Comparative Anatomy, Function,Evolution.* New York: McGraw-Hill. ISBN-13: 978-0073524238

Launeya M.E, Chenb P. Y, McKittrick J, Ritchiea R.O. (2010). Mechanistic aspects of the fracture toughness of elk antler bone. Acta Biomaterialia. 6. 1505–1514.

Lin, Y.S. (2011). Mechanical properties and the laminate structure of Arapaima gigas scales. *Journal of the Mechanical Behavior of Biomedical Materials*. 7. 1145-1156.

Lindsey CC. (1978). Form, function and locomotory habits infish. In: Hoar D, Randall DJ, eds. Fish physiology, Vol. VII. New York: Academic Press, 1–100.

Merrick JR, G. S. (1984). Australian freshwater fishes -biology and management. . *Griffin Press*.

Moyle P. B, Cech J.J Jr. (2004). Fishes, an introduction toichthyology, 5th edn. Upper Saddle River, NJ: Prentice Hall

Onozato, H., Watabe, N., (1979). Studies on fish scale formation and resorption. *Cell and Tissue Research*. 201, 409–422.

Oslon, O.P., Watabe, N., 1980. Studies on formation and resorption of fish scales. *Cell ad Tissue Research*. 211, 303–316.

Ryou H. Amin N. Ross A. Eidelman N. Wang D. H. Romberg E. Arola D. (2011) Contributions of microstructure and chemical compositionto the mechanical properties of dentin. *The Journal of Materials Science.* 22. 1127–1135.

Sasaki, N., Yoshikawa, M., Enyo, A., (1993). Stress relaxation function of bone and bone collagen. *Journal of Biomechanics.* 26. 1369–1376.

Schönbörner, A. A., Boivin, G., and Baud, C. A. (1979) The mineralization process in teleost fish scales. *Cell Tissue Research*. 202, 203–212.

Sudo S., K. Tsuyuki, Y. Ito, T. Ikohagi (2002). A Study on the Surface Shape of Fish Scales. *JSME International Journal*, 45, 1100-1105.

Torres, F.G., Troncoso, O.P., Nakamatsu, J., Grande, C.J., Gómez, C.M. (2008). Characterization of the nanocomposite laminate structure ocurring in fish scales from Arapaima Gigas. *Materials Science and Engineering*. 28. 1276-1283.

Vernerey, F.J., Barthelat, F., (2010). On the mechanics of fishscale structures. *International Journal of Solids and Structures.* 17. 2268–2275.

Wade, R. A. (1962). The Biology of the Tarpon, *Megalops Atlanticus*, and the Ox-Eye, Megalops Cyprinoides, with Emphasis on Larval Development. *Bulletin of Marine Science,* 12. 545-622.

Wagner H.D., Weiner S.(1992). On the relationship between the microstructure of bone and its mechanical stiffness, Journal of Biomechanics. 25. 1311-1320.

Weiner, H. W. (1998). THE MATERIAL BONE: Structure-Mechanical Function Relations. *Annual Review of Materials Research* . 28. 271–298.

Whitehead, P. a. (1978). FAO species identification sheets for fishery purposes. Field guide to the commercial marine resources of the Gulf of Guinea. Rome: In W. Fischer.

Witherington D. (2014). Atlantic tarpon, *Taken with permission from the autor from:* http://www.drawnbydawn.com/biological-illustration/illustration-fish

Withnall R., Babur Z. Chowdhry, Jack Silver, Howell G.M. Edwards, Luiz F.C. de Oliveira. (2003). Raman spectra of carotenoids in natural products. *Spectrochimica Acta Part A.* 59. 2207- 2212.

Zhu, D., Fuentes, C.; Motamedi, R. (2012). "Structure and Mechanical Performance of a ''Modern'' fish scale". *Advanced Engineering Materials*, 14. B184 - B195.

Zylberberg, L. Bereiter-Hahn, J. Sire, J. (1988). Cytoskeletal organization and collagen orientation in the fish scales. *Cell Tissue Research.* 253 , 597–607.

Zylberberg, L., Bereiter-Hahn, J., 1991. The distribution of Tyr- and Glu-microtubules during fish scale regeneration. *European Journal of Cell Biology* 54, 132–139.

Zylberberg, L., Nicolas, G., (1982). Ultrastructure of scales in a teleost (Carassius auratus L.) after use of rapid freeze–fixation and freeze–substitution. *Cell Tissue Resarch*. 223, 349–367.

TABLES

Body Position	Thickness (mm)	Area (mm2)
Head	0.955 a	1197 a
Middle	0.926 a	927.1 b
Tail	0.650 b	549.8 c
P-value	***1,04 e-7***	***1,67 e-5***

Table 1. Macroscopic characteristics and statistical analysis of the scales studied. Values are averaged. Rows with different letters indicate significant differences.

Body Position	Young´s Modulus (MPa)	Fracture Strain (mm/mm)	Modulus of Toughness (MPa)	Maximun Strength (MPa)
Head	301,0 a	0.262 a	4,39 a	20.69 a
Middle	218.1 b	0.240 a	4,35 a	20.54 a
Tail	194.1 b	0.238 a	3.62 a	19.27 a
P-value	***0,003***	***0,08***	***0,22***	***0,58***

Table 2. Mechanical properties and statistical analysis of the scales studied. Values are averaged. Rows with different letters indicate significant differences.

FIGURES

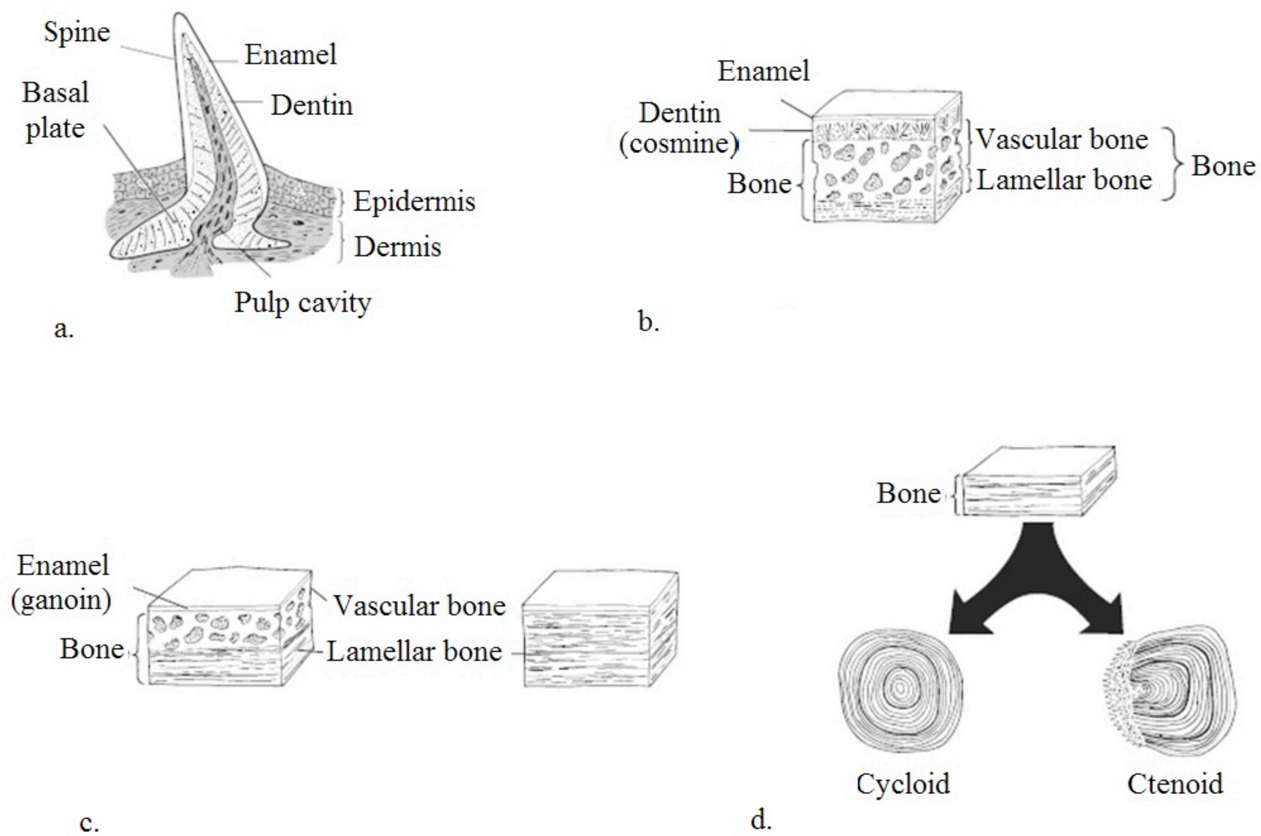


Figure 1. Types of fish scales (Kardong, 2008). a. Placoid; b. Cosmoid; c. Ganoid ; and d. Teleost.

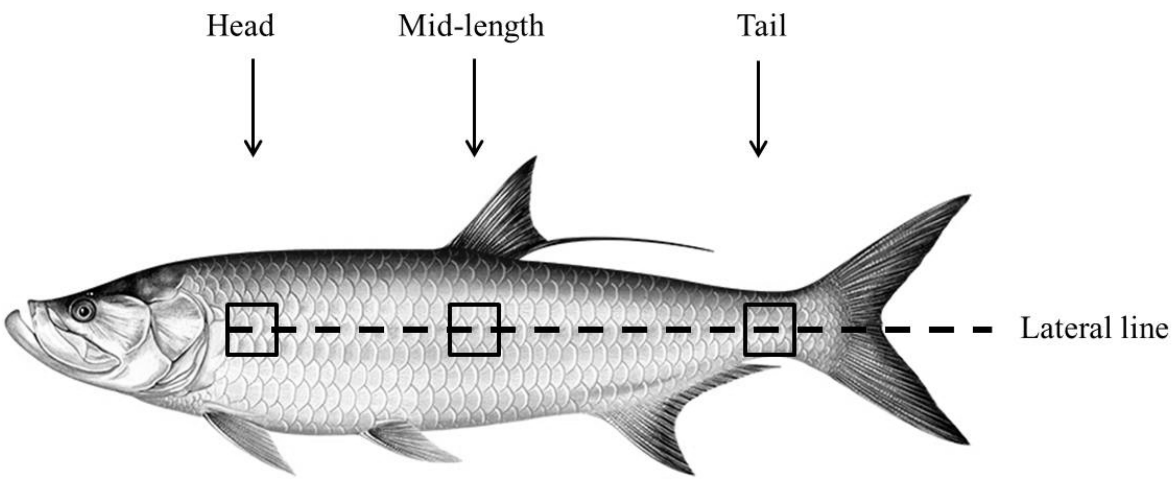


Figure 2. *Megalops Atlanticus* (Atlantic tarpon) fish. Sample extraction positions are shown. Figure courtesy of D. Witherington (2014).

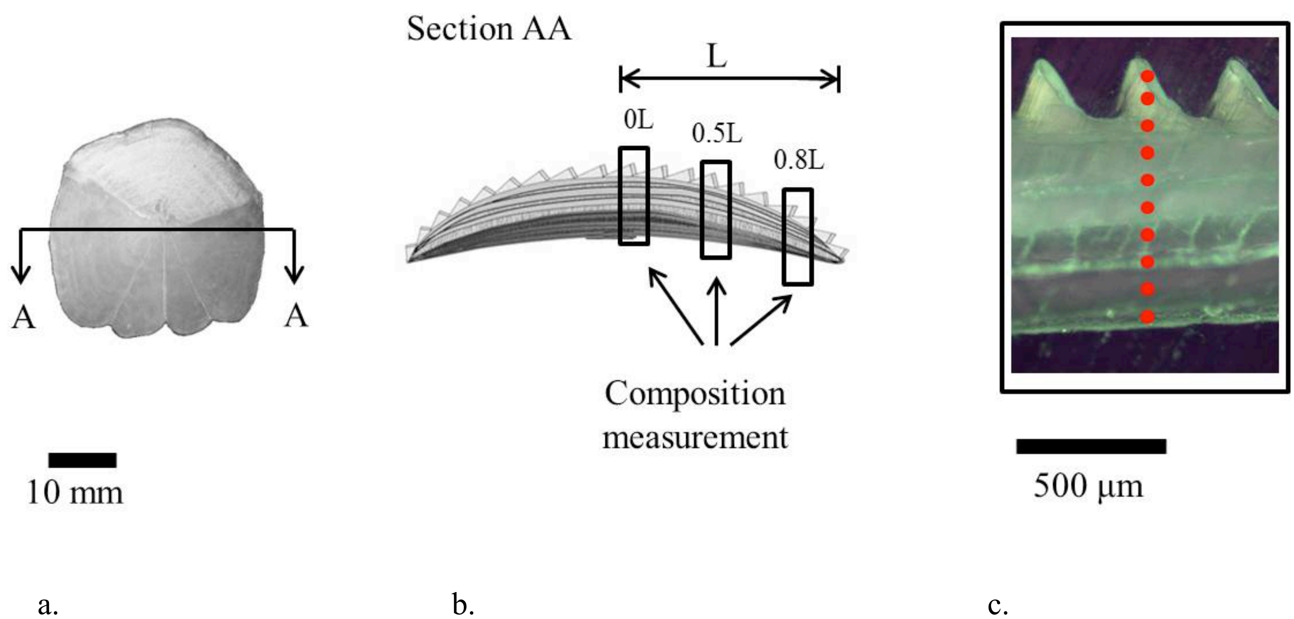
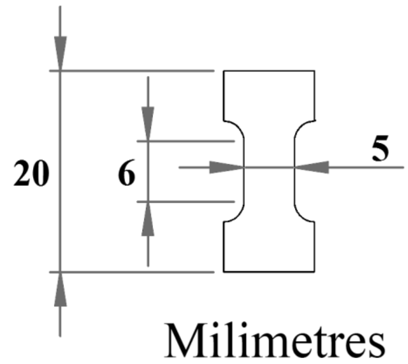
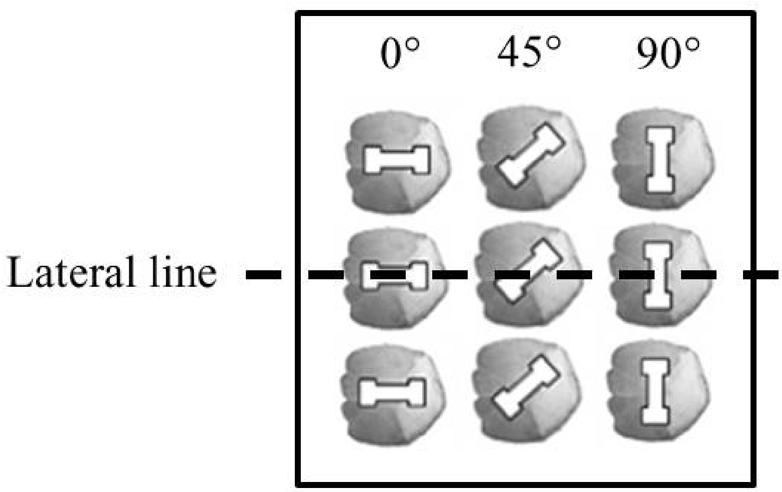


Figure 3. Fish scale cross section, areas and points of composition evaluation. **a.** Cross section, where the composition was analyzed in a single scale. **b.** Radial positions on the cross section where the composition was measured. **c.** Ten (10) equidistant points where composition was measured.



a.



b.

Figure 4. Specimens for tensile tests. **a.** The specimen geometry **b.** Orientations studied.

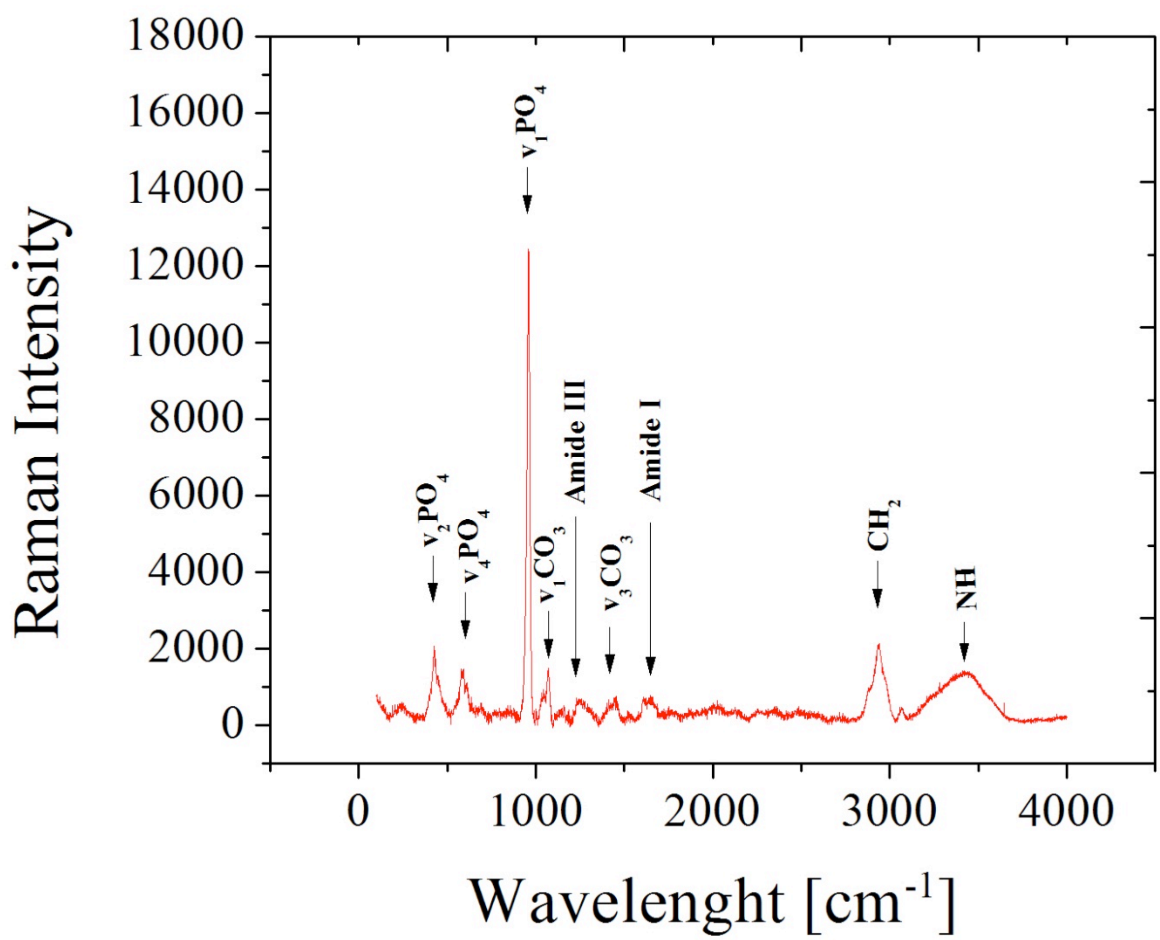


Figure 5. Raman spectrum of a point at the top-right edge cross section of a selected scale.

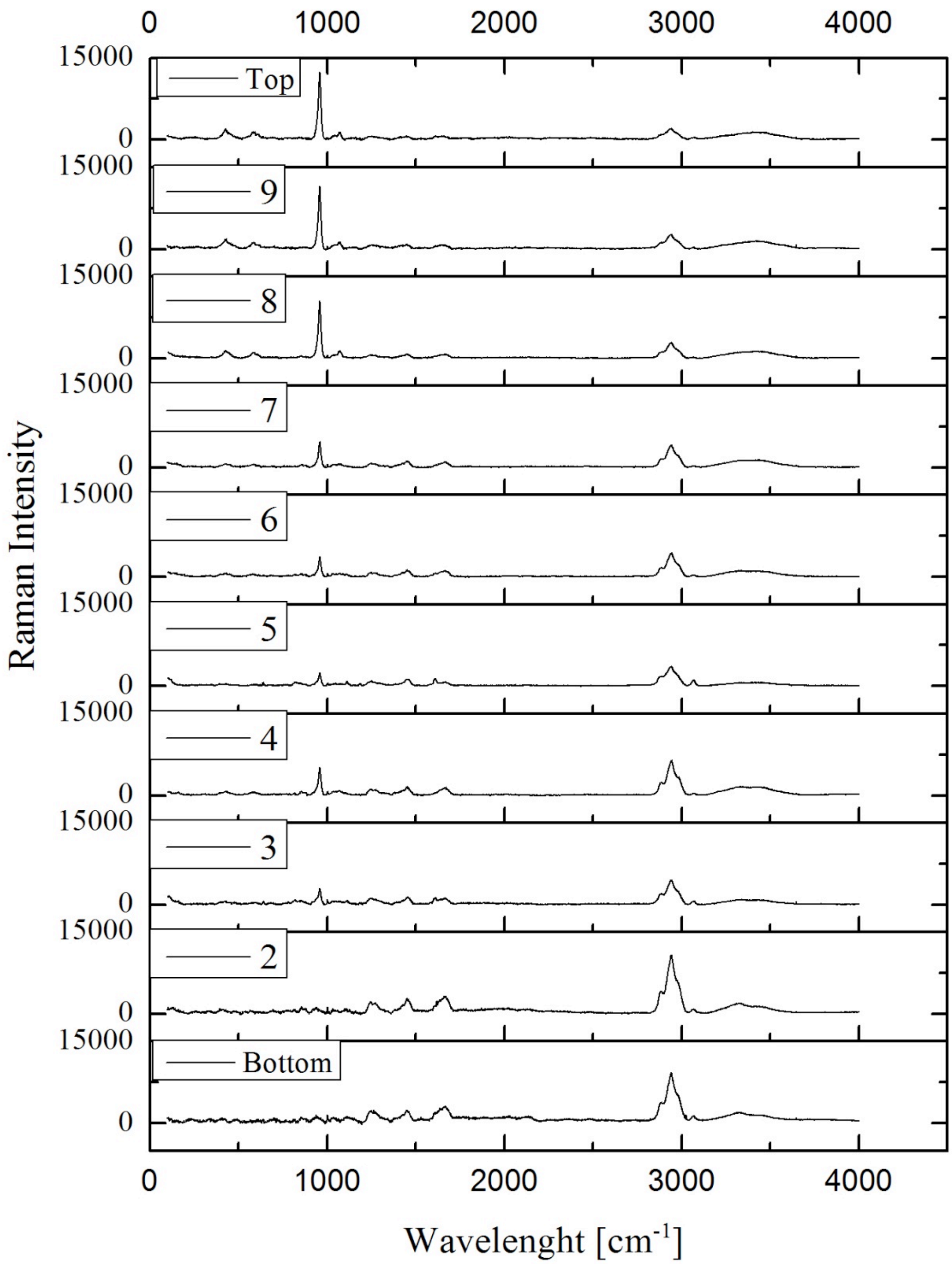


Figure 6. Raman intensity spectrums obtained across the right edge of the scale. 10 equidistant measurements are shown.

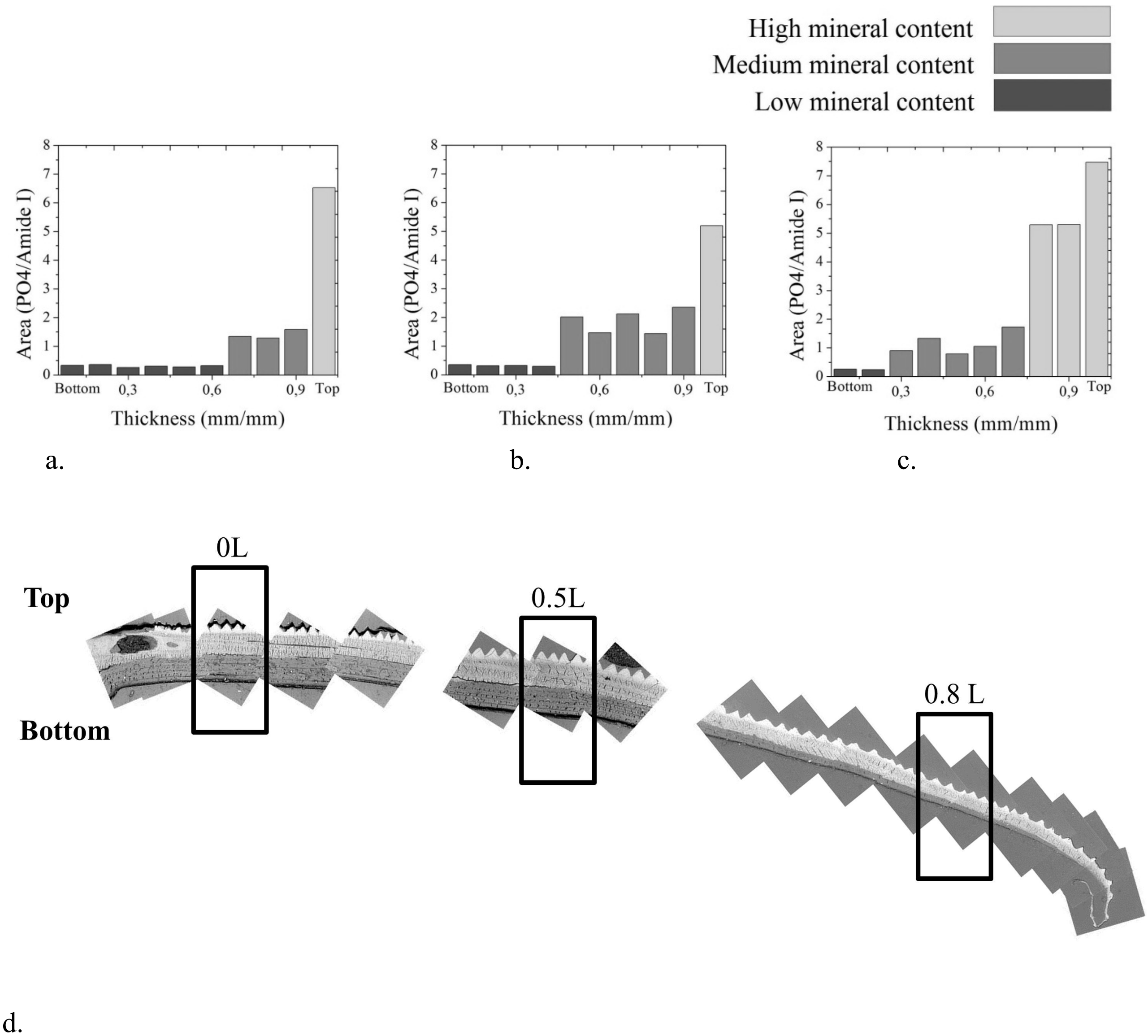


Figure 7. Crosswise composition at different radial positions. **a.** Mineral to collagen ratios of the centre (0L). **b.** Mineral to collagen ratios of the midway point between the centre and the right edge (0.5L). **c.** Mineral to collagen ratios of the right edge (0.8L). **d.** Scanning Electron Micrograph (SEM) assembly of images from half cross section of the scale.

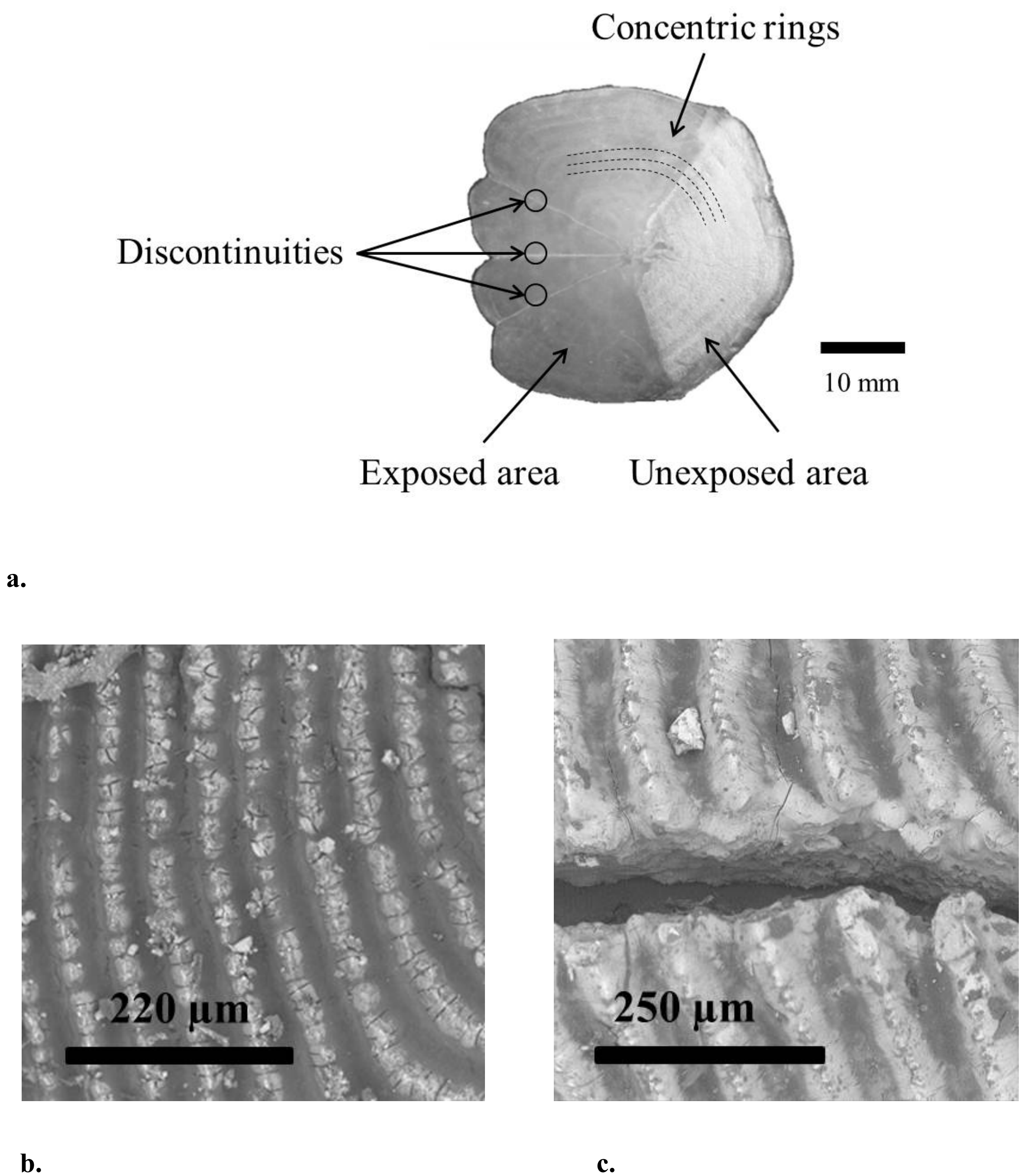
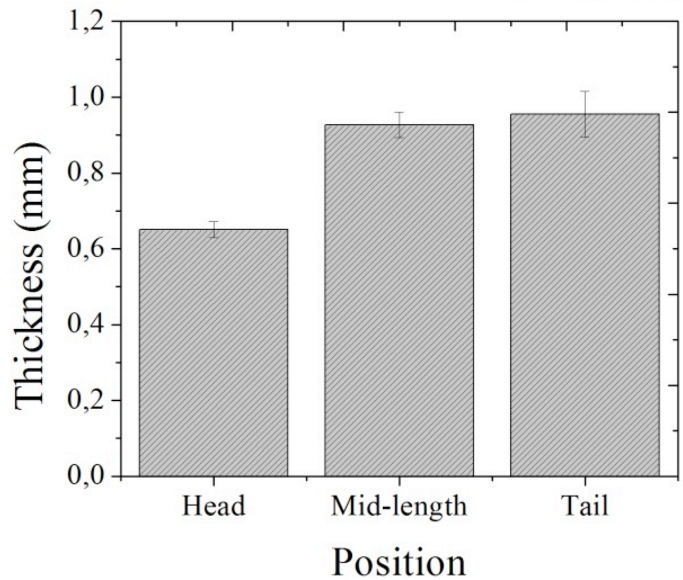
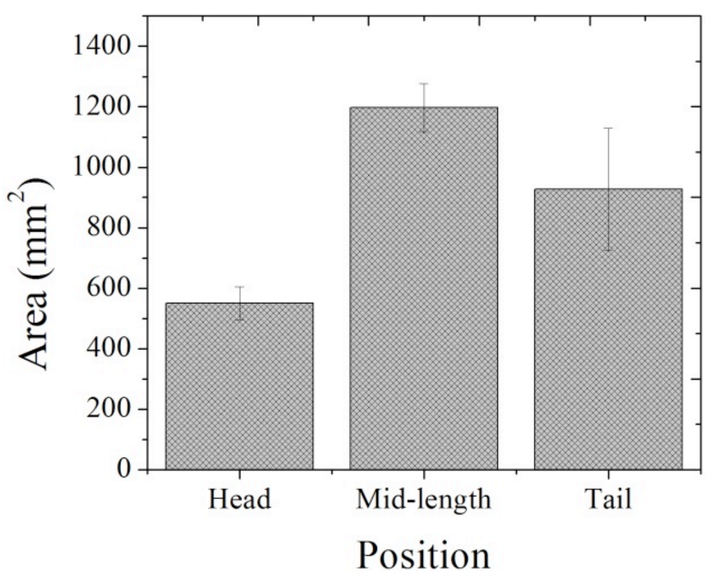


Figure 8. Top view of a scale. **a.** Single scale showing concentric rings and the exposed and unexposed areas of the scale. **b.** Scanning Electron Micrograph (SEM) of the circular ridges at the top of the scale. **c**. SEM image of discontinuities in the scale.



a.



b.

Figure 9 Comparison of the macroscopic characteristics of scales from *Megalops atlanticus.* The scales were taken from the lateral line. **a.** Differences in thickness between three positions compared. **b.** Differences in size of the scale between the three positions compared. The error bars indicate standard deviation.

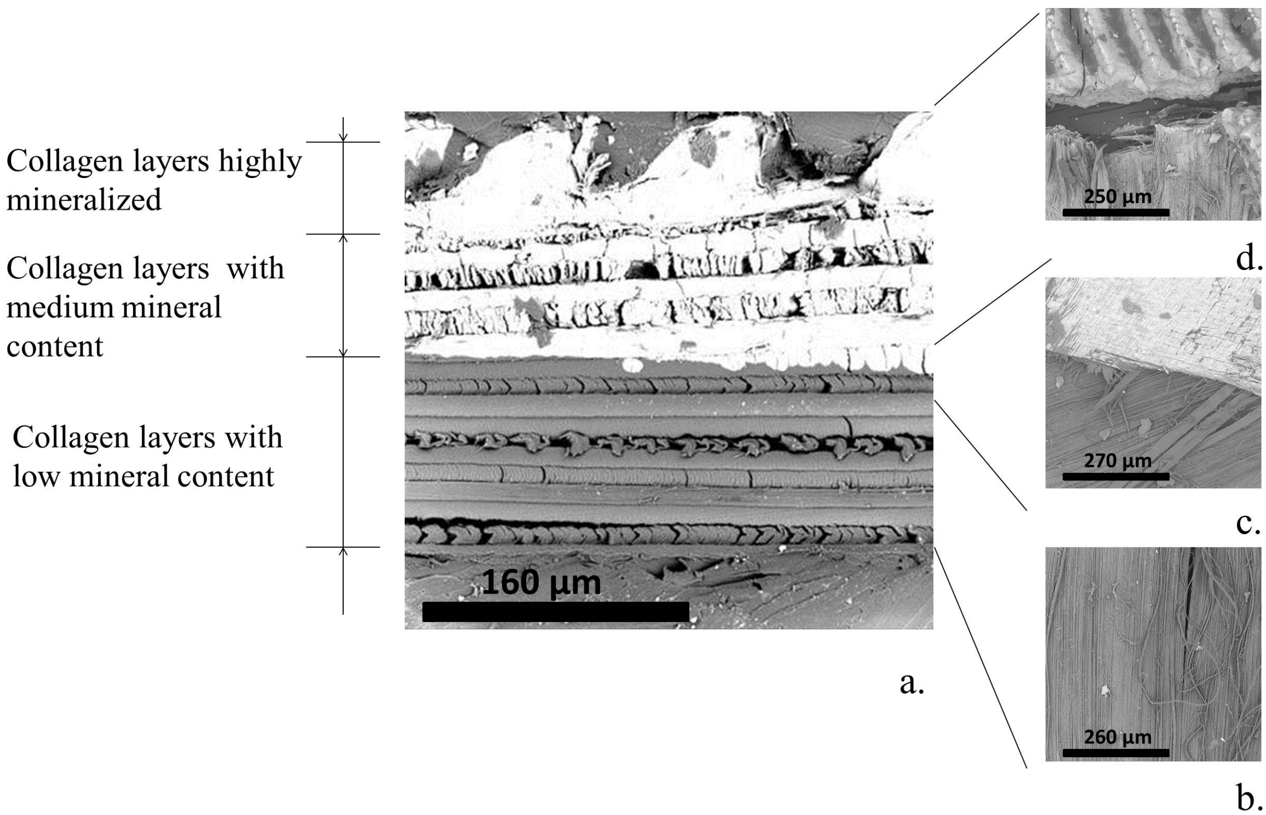


Figure 10. Microstructure of the scale. **a.** Scanning Electron Micrograph (SEM) of the cross section of an individual scale showing different layers. **b.** SEM image of a unidirectional collagen ply. **c.** SEM image of the transition between a low mineral content layer and a medium mineral content layer. **d.** SEM image of the rouged pattern, mineralized collagen layers and collagen ply.

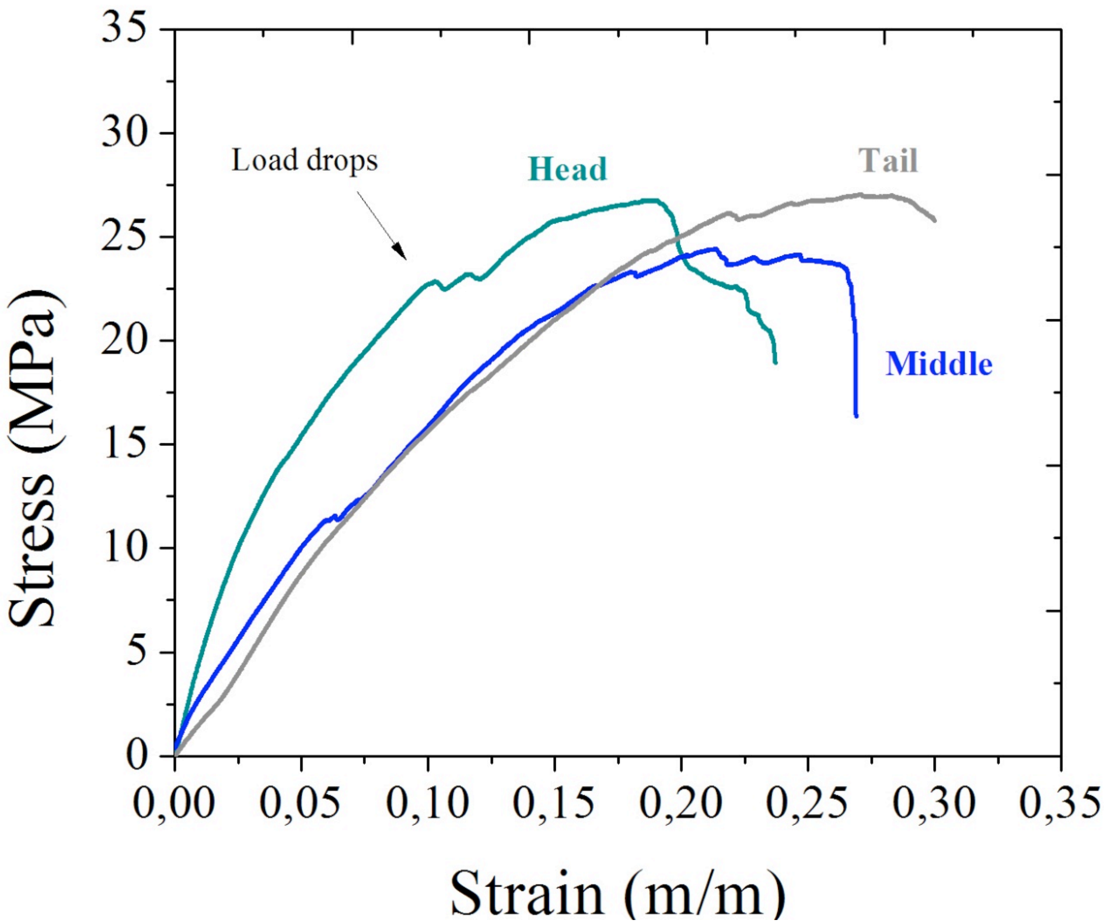
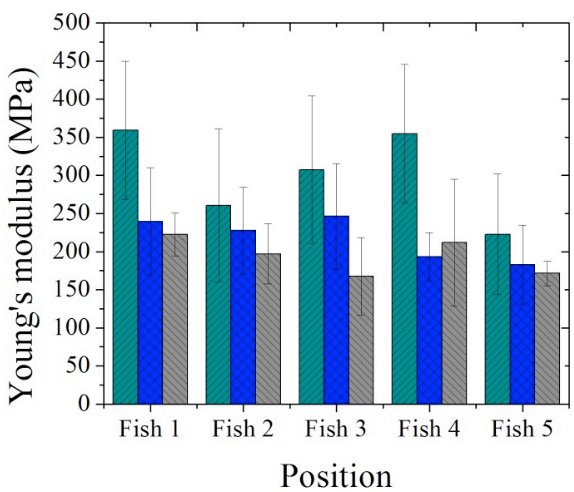
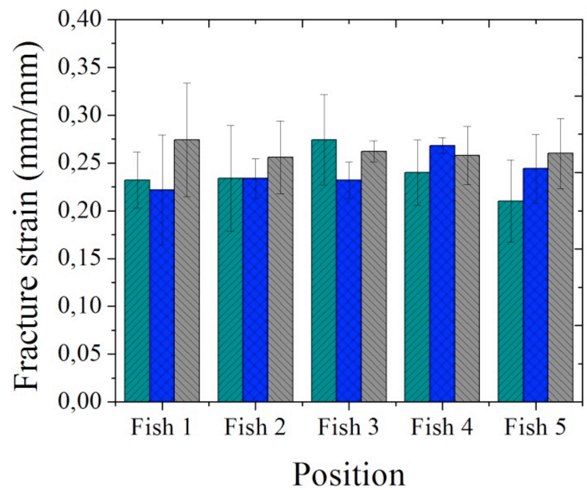


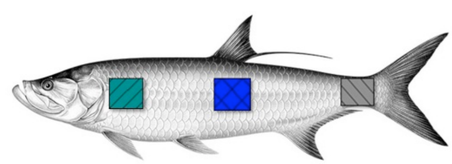
Figure 11. Typical stress-strain response of wet scales from *Megalops Atlanticus*. Specimens obtained from fish 1.

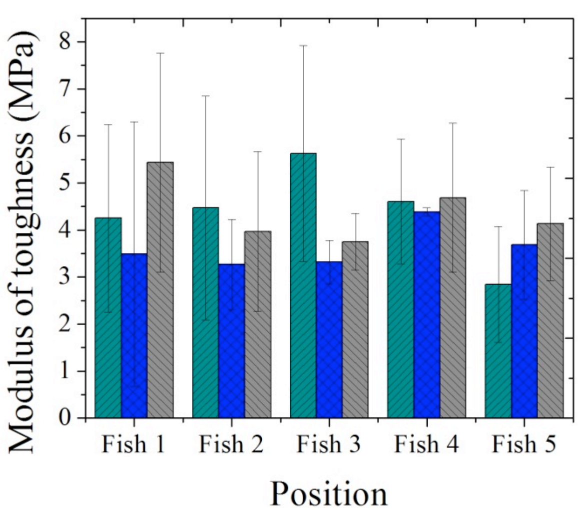


a.

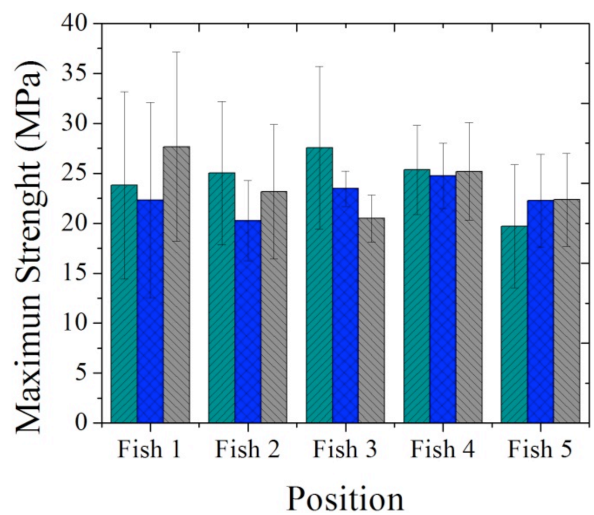


b.





c.



d.

Figure 12. Mechanical properties of scales as a function of body position (head, middle and tail). **a.** Young´s modulus **b.** Fracture strain **c.** Modulus of toughness **d.** Maximum strength. The error bars indicate standard deviation.

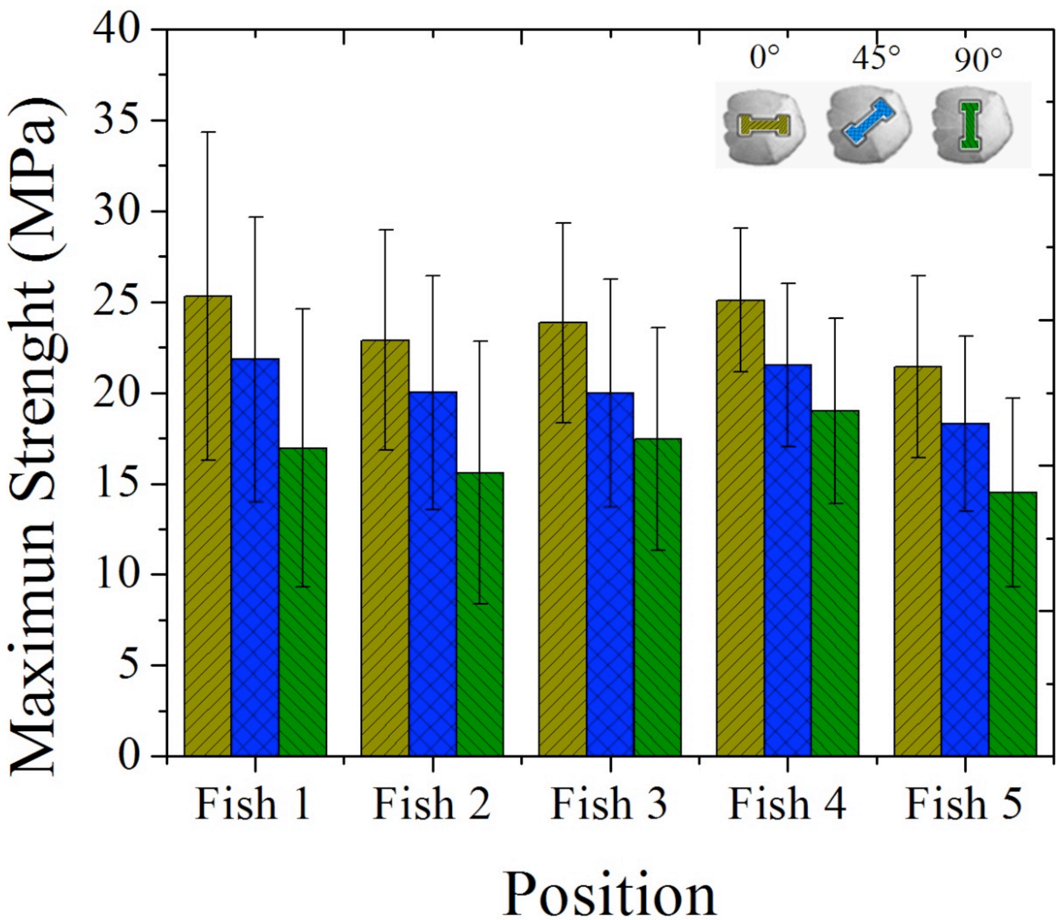
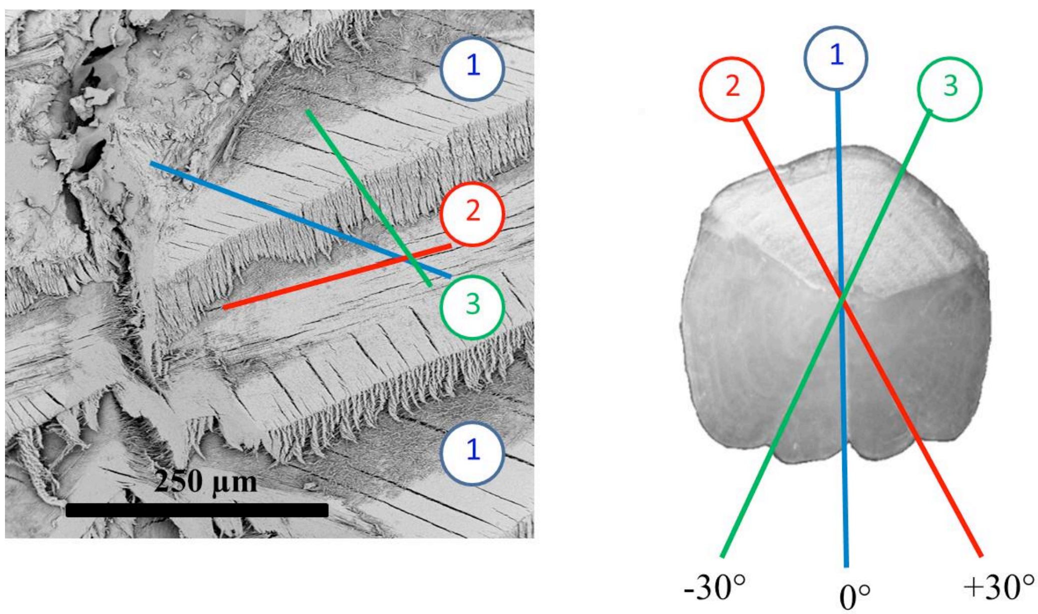


Figure 13. Maximum tensile strength at different orientations (0 °, 45 °, 90 °). The error bars indicate standard deviation.



a. b.

Figure 14. Preferential orientation of collagen layers within the scale. **a.** SEM image of the cross section of the scale. **b.** Orientation of collagen layers on the scale.