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# Effects of Fish Scale Microstructure on Mechanical Performance of the Scales: A Review

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**Abstract**

Fish scales are a skeletal element covering the skin of fish whose main purpose is to protect the body of the fish from external predation. Due to unique properties such as flexibility and specific strength, fish scales are currently an inspiration for the design of novel engineering bio-materials. The microstructure of many fish species mainly comprises of two layers. Each layer comprise of organic type I collagen component reinforced with hydroxyapatite mineral component. Literature review shows that there exist variations in mineral content from limiting layer to external elasmodine layer of fish scale. This explains the reduction in tensile and in macro, micro and nano indentation properties from outer to inner surface of fish scales. On the other hand, the scanning electron microscope (SEM) micrographs showed that fibrils in adjacent lamellae are rotated at an angle of approximately $60^0$. Thus, the scale is a collagen composite laminate. In contrast, other studies have concluded that the laminate structure of fish scale cannot be treated as a homogeneous, uniform structure as past studies had reported. However, not much research is available to support this conclusion as current review literature indicates that the laminate fish scale structure is uniform and homogeneous. The purpose of this study is to review the microstructure of fish scales and its effects on mechanical performance of fish scales.

**Keywords:** Fish scales, mechanical performance, microstructure & layers

## 1. INTRODUCTION

Fish scales have drawn considerable attention due to their laminated composite structure, their low resistance to bending i.e. flexibility and their specific strength and toughness [1]. For instance, the scales of some fish species have been found to be overlapping in order. The overlapping arrangement not only provides for flexibility in movement but also protection barrier against predators. Besides flexibility, fish scales have unique properties such as being ultra-thin and lightweight thus enabling the fish to swim in water with sufficient rigidity for protection from predators. These properties are currently an inspiration for the design of novel engineering bio-materials [2,3]. A study by [4] concluded that scales in the exposed area of the fish body overlap each other on the surface of the fish such that each scale is covered by other scale[s]. Depending on fish species, scales are broadly classified into four major classes: placoid, ganoid, elasmoid and cosmoid scales Similarly, research on teleost fish scales showed that the structure of the scale displayed alternate rows of overlapping scales forming a quasi-periodic pattern [5]. This overlapping significantly increase the thickness of the scale on top of fish skin hence enhanced protection against predators and increased flexibility. This study provides a review on the microstructure of fish scales and its effects on mechanical performance of the scales. Therefore, a review of fish scale microstructure and its effects on mechanical performance of scales are presented in this work.

## 2. MICROSTRUCTURE OF FISH SCALES

Research by [6] showed that the microstructure of *A. gigas* scales has layers composed of collagen fibrils of approximately 100 nm diameter. These fibrils form fiber bundles with diameters of about 1–5 µm. Fibre bundles are organized into lamellae with an average thickness of 50 µm. The scanning electron microscope micrographs showed that fibrils in adjacent lamellae are rotated at an angle of approximately, $60^0$. Thus, the study concluded the scale is a collagen composite laminate. This structure is similar to structures observed in other fish scales with slight variations in dimensions. For instance, the lamella of *P. reticlata* and *C. auratus* fish scales are approximately 1 µm and 5 µm, respectively. Also, within the scale structure, the angle between layers varies from $36^0$ for teleost scales [7] to $90^0$ for *P. major* [8] and less than $90^0$ for *H. bimaculatus* scales [9].

Studies by [7,10] on teleost scales showed that each scale comprised of many axially aligned plies or layers arranged parallel to the scale surface. Further, the studies revealed that individual layers were symmetrically arranged forming a plywood pattern across the scale thickness. This structure is analogous to laminated structures found in other mineralized collagen structures such as teeth, bones and mineralized tendons [11,12]. A study by [8] on *P major* scales concluded that each layer mainly comprises of closely packed type I collagen fibrils of about 70-80 nm. These fibrils are reinforced by a thin calcium deficient hydroxyl apatite (Hap) coating. The study further observed a plywood-like structure as a result of $90^0$ alternate alignment between neighboring lamellae in the scale structure. Similar conclusions were deduced by [13] for *A. gigas* scales.

Through SEM imaging, a study by [14] on structure and mechanical properties of *A. Spatula* scales showed that the outer layer of fractured surface of fish scale comprised of arranged nanorods fixed in matrix analogous to the morphology of tooth enamel. Similarly, the inner layer consisted of fibre-like nanostructure resembling that of ganoine layer reported by [15]. Optical imaging of the microstructure of gar *A. spatula* scales revealed that the scale is made of two layers, a bony layer and a ganoine layer. Further analysis of fish microstructure through SEM showed that the structure of the inner layer and outer bony layer comprise of collagen fibrils oriented parallel to the scale surface [15,16].

A study carried by [8] concluded that *P. major* scales have two layers, an upper layer and a lower layer. Other studies, [17] for *C. carpio*, [13] for *A. gigas*, [18] for *Pristipoides sieboldii, Cyprinus carpio, Carassius auratus and M. cephalus* scales and [19] for *Grass carp* have concluded that scales comprise of two layers. That is, an inner soft collagen layer and an outer hard bony layer. A study by [8] showed that the 2-3 µm thick upper layer comprises of randomly packed collagen fibres while the second layer consisted of closely stacked co-aligned collagen fibres. The X-ray diffraction (XRD) broad peaks observed by the authors revealed the apatite structure with absence of other carbonates and phosphates of calcium. Similarly, XRD broad peaks of *A. Gigas* scales corresponded to the apatite structure with low crystallinity levels [13]. The observed low crystallinity is attributed to small hydroxyapatite crystals ranging from 1.5 to 4 µm. These findings are consistent with HA proportions reported in other mineralized collagen structures such as bones, teeth, etc. [20,21].

Besides textural variations, the two layers showed variations in terms of thickness and mineralization levels. The research by [13] concluded that the inner layer is 9 times wider than the external layer. On mineralization levels, the study showed the inner layer is formed by partially mineralized tissues whose collagen fibrils are organized in a plywood pattern. On the other hand, the outer layer showed highly mineralized tissues without clear collagen fibril organization. This difference in mineralization levels between inner and outer layer influences the mechanical properties such as tensile strength, micro-hardness, elastic modulus, fracture toughness, among other properties of the scales.

Further investigation of the elasmodine layer showed that it consists of two distinct layers, internal elasmodine (IE) and external elasmodine (EE) [17]. Although collagen fibrils are arranged in such a way that they form plywood structure in both IE and EE, the two layers of elasmodine are differentiated by relative mineral content. Research by [22] concluded that the mineral content in EE is higher than IE. However, the mineral content in EE is far less than that of the limiting layer. Based on these findings, the elasmoid scale has three distinct layers and not two as reported by the study. A research by [23] showed circuli pattern on top of limiting layer with both layers having several plies of unidirectional collagen fibrils. The study concluded that the structure of *megalops atlanticus* scales has three layers, LL, EE and IE. In addition, recent studies by [24] for *Megalops atlanticus, C. carpio* and *A. gigas* and [25] for *Megalops atlanticus* have reported that the cross-sections of these scales comprise of three layers, LL, EE and IE. The three layers exhibit differences in degree of mineralization whereby the outer limiting layer with higher mineralization level tends to be thicker and darker than the inner layer [6]. Research by [24] reported progressive reduction in mineral content from outer layer to inner layer for *Megalops atlanticus, C. carpio* and *A. gigas* scales. Similarly, studies by [22,23] reported progressive decrease in mineral content from the limiting layer to internal elasmodine layer for carp and *Megalops atlanticus* scales, respectively. Research by [24] on structure and properties of limiting layers of fish scale from different fish types concluded that apatite that is, mineral component volume percentage reduced from 60% for the outer layer to 40% for the inner layer for arapaima scales. On the other hand, apatite reduced from 70% for the outer layer to 20% for the inner layer for carp and tarpon fish scales. Variations in apatite volume percentage within, say, outer layer can be associated with differences in growth rate and environmental conditions among fish species.

Further review of literature by [25] on contributions of the layer topology and mineral content to the elastic modulus and strength of fish scales reported drastic reduction of mineral content from LL to IE. The study reported maximum mineral content of 70% at the surface of the LL. The mineral content reduced linearly from LL to EE to attain 17% mineral content at the LL/EE interface. Within the EE, the mineral content remained slightly constant before decreasing drastically to negligible content within the IE. Due to negligible mineral content within the IE, the study concluded that IE layer comprises of plies of unidirectional type I collagen lacking mineral reinforcement. On the contrary, type I collagen in LL and EE is reinforced by minerals mainly apatite crystals. Similar conclusion of reduction in mineral content from outermost region to region closest to the body of the fish has been deduced [26]. This observation explains the decrease of mechanical performance of fish scale from the harder limiting layer to a more flexible internal elasmodine layer.

Fish scales just like other mineralized structures such as teeth, bones and tendons comprise of collagen organic component, hydroxyapatite mineral component and water [27].

The study by [8] concluded that the mineral phase of *pagrus major* scales comprises of calcium-deficient hydroxyapatite with small proportions of magnesium, carbonate and sodium ions. However, differences in proportions and spatial organization of these components account for differences in mechanical properties exhibited amongst these bone-like materials. A research by [13] reported weight ratios of 16%, 39% and 45% while [8] reported 13%, 46% and 41% for water, inorganic and organic component, respectively. On the other hand, research by [28] reported 13%, 40% and 47% mass ratios for water, inorganic and organic components, respectively for teleost scales. In addition, the study established that organic components are completely destroyed and absent at $700^0$C. These variations in mineral phase content reported in literature could be attributed to differences in fish scale types and structure as well as environmental conditions.

Studies have reported different proximate compositions for different fish species. A study by [29] on dried lizard fish scales reported high protein and ash content of 38.9% and 43.2%, respectively. However, the study showed that the scale had very few lipids of 0.067% with fairly moderate moisture content of 12.1%. The proximate composition of lizardfish scales reported by the study is slightly lower than those reported in literature. A research by [30] on deep-sea red fish scales reported 39.4% ash content and 56.9% protein whereas a study by [31] on sea beam scales reported 51.2% protein, 47.3% inorganic matter and 0.1% fat.

Comparative morphological studies of fish scales extracted from anatomical locations of head, mid-length and near tail have shown microstructural variations from head to tail. A research by [26] reported the number of plies for the tail, mid-length and head as 20, 30 and 37, respectively, showing increase in the number of plies from the tail to the head. Similarly, the thickness of the scales increased from the tail to the head by 50% from 20 to 30 µm, respectively. The increase in the number of plies or layers and scale thickness serves to increase protection against predation from tail to head. On the contrary, other research studies [23,32] reported maximum scale thickness at the mid-length, which reduced progressively towards the edges of the fish as a result of annular growth. Similar distribution of thickness from thickest centre/middle to the thinnest edges of the fish has been reported for different fish species such as *Leuciscus cephalus* [7] and *Arapaima gigas* [27]. Besides variations in thickness in the three anatomical regions, a study by [32] reported differences in scale sizes over the entire body of the fish. The scales extracted from near the head and near the tail were smallest in size while those extracted at a third distance of fish length from the head were twice as large. Research by [33] concluded that the size of individual scale is dependent on the age of the fish.

## 3. MECHANICAL PROPERTIES OF FISH SCALES

A review of the tensile and nano-indentation properties of fish scales as well as the effect of testing direction on these mechanical properties is presented in this section.

### 3.1 Tensile properties

A study to characterize *Arapaima gigas* fish scales reported tensile properties under dry and humid conditions. Dry scales showed higher Young's modulus of 1.38 GPa compared to 0.83 GPa for humid scales represent 39.9% variation. Also, dry scales reported higher tensile strength of 53.86 MPa against 22.26 MPa for humid scales [13]. This represents 59% decrease in tensile strength from dry to humid scales. Similarly, a research by [27] on investigation of mechanical properties of *Arapaima gigas* reported higher Young's modulus of 1.2 GPa for dry scales while hydrated scales reported Young's modulus of 0.1 GPa representing 92% margin. Furthermore, the study reported 46% decrease in ultimate tensile strength from 46.7 MPa for dry scales to 25.2 MPa for hydrated scales.

Similarly, a study by [8] on mechanical properties of *P. major* fish scales reported an elastic modulus of 2.2 GPa and tensile strength of 93 MPa for dry scales. For dry scales, the elastic modulus and tensile strength reported for *P. major* are slightly higher than similar values for *A. gigas* scales. These variations in tensile properties can be attributed to differences in mineral contents between the two fish species. For instance, demineralization of *P. major* scales reduced elastic modulus by 76% from 2.2 to 0.53 GPa while tensile strength reduced by 61.3% from 93 to 36 MPa. This clearly shows that the mechanical properties of scales are mineral content dependent. Thus, the study concluded that interactions between organic collagen fibres and mineral hydroxyapatite crystals are of fundamental significance in determining the mechanical properties of scales. Scale failure under tensile loading occurred due to pulling out and fracture of collagen fibres as well as sliding of collagen lamellae. On the other hand, [15] concluded that stretching, sliding and fracturing of the collagen fiber are the dominant failure mechanisms during tensile loading of *P. senegalus* scales.

Besides, studies by [26,34,35] have concluded that tensile strength and elastic modulus of scales increase with reduction in water content. Similarly, a study by [36] for *A. gigas* scales reported higher impact results for dry samples compared with humid samples for both longitudinal and transverse directions. Also, a study by [37] to characterize the tensile and compressive properties of *A. spatula* concluded that the scales exhibit inelastic behavior when hydrated hence lower tensile properties.

Thus, the differences in mechanical behavior between wet and dry scales reported in literature show that water content significantly influence the mechanical properties of scales. According to [13], soaking fish scales in water increases the water content to approximately 30% of its dry weight. As the water content increases, the volume fraction of hydroxyapatite mineral crystals decreases leading to decline in mechanical properties due to direct relationship between mechanical properties and mineral content. However, the research by [8] concluded that in hydrated or humid scales, collagen fibre and water molecules interact with each other causing gradual pulling out as well as slippage of collagen lamellas. This results to plastic deformation of collagen fibres hence reduced mechanical properties. Additionally, collagen fibres interact with each other in dehydrated or dry scales thus increasing the mechanical properties. Nevertheless, a study by [38] showed that water molecules affect the bonding between fibrils. This study concluded that hydration significantly reduced the density of collagen by 11.2% from 1.34 to 1.19 g/cm$^3$ accompanied by 81.6% decrease in elastic modulus to 0.6 GPa down from 3.26 GPa.

In addition to water content, the mechanical properties of fish scales are mineral content dependent. For instance, the linear initial portion of tensile stress-strain curve of *P. major* fish scales showed Young's modulus of 2.2 GPa. Compared with other mineralized tissues, fish scales have the lowest mineral content at 46% compared to 50% and 80% mineral content for red deer and axis deer with a corresponding Young's modulus of 6.1 GPa and 31.6 GPa, respectively [8]. Within the structure of fish scale, tensile properties vary with distance from internal to external surface of the scales. For instance, a study by [39] reported higher Young's Modulus ranging from

30 to 40 GPa on the exterior surface compared to 15 GPa on the interior surface of the scale. Similarly, the research by [27] showed 64.3% variation in Young's modulus from 46.8 GPa to 16.7 GPa for mineral and collagen layers, respectively. These findings are consistent with a study by [24] which reported decrease in modulus as distance increased from exterior to interior surface of all fish scales investigated.

In contrast, the collagen layer obtained after removal of external mineralized layer of *A. gigas* scales reported higher tensile strength than the entire scale in both longitudinal and transverse directions. For instance, the collagen layer reported 36.9 MPa while the entire scale reported 23.6 MPa in longitudinal direction [34]. According to the study, the highly mineralized external layer is designed for compressive strength thus protecting fish body against predation. Hence, scales tend to be brittle and weak in tension. However, these findings are counterintuitive to previous findings that have reported higher tensile properties for mineral layer compared to collagen layer. Similarly, the increase in cross-section of fractured surface of entire scale through predator attack relative to collagen layer accounts for increased strength of collagen layer.

The work by [23] on effect of chemical composition on mechanical behavior of *megalops atlanticus* fish scales reported higher elastic modulus of 0.3 GPa for scales extracted from the head region compared to 0.22 GPa and 0.195 GPa for scales from mid-length and tail, respectively. This represents 35% decrease in elastic modulus from head to tail and 11.4% decrease from mid-length to tail. Hence, according to the study, there was no significant difference in elastic modulus of scales from mid-length to tail. Further review literature by [26] on the mechanical behavior of *cyprinus carpio* scales concluded that the elastic modulus of the scales extracted from three regions [head, mid-length and tail] was significantly different. The scales from head region reported the highest elastic modulus of 0.39 GPa against 0.30 GPa and 0.18 GPa for scales extracted from the middle and tail regions, respectively representing 53.8% decrease from head to tail. Dehydrating the scales for 2 to 24 hours showed similar trend of decreasing elastic modulus from head to tail by 23.7%.

A study by [18] has reported similar findings of gradient decrease of mechanical properties of fish scales from head to tail. The study compared the morphology, structures and mechanical properties of four different kinds of fish scales: *Cyprinus carpio, Carassius auratus, Mugil cephalus, Pristipomoides sieboldii*. The scales were extracted from three anatomical locations: head, mid-length and tail. The tensile strength, elastic modulus and toughness of *Carassius auratus* scales showed a gradient decline from the head to tail. This observation can be explained by previous research findings that have reported increase in the number of plies or layers as well as thickness of scales from tail to head [26]. Also, the scale showed similar ductility under tensile loading as the three anatomical regions had negligible ultimate strain values. The study further observed that on tensile strength testing, all the fish scales except *Cyprinus carpio* showed decrease in strength from head to tail by 11.6 to 16.7%.

On the contrary, *Cyprinus carpio* showed increase in tensile strength from head to tail. A study by [17] on *cyprinus carpio* scales reported superior tear resistance for head scales due to larger external elasmodine layer of scales from head region. Review literature shows a consistent trend of decreasing mechanical properties from head to tail. Nevertheless, there is no scientific rationale available in literature to support the increase in tensile strength and tear resistance from head to tail for *Cyprinus carpio* scales reported [18]. The study further revealed that the elastic modulus and tensile strength of *Cyprinus carpio* was lowest by 51% and 40.4%, respectively of the four fish scales investigated. The lower composition of bone layer in this scale type was responsible for lower modulus and strength. Consequently, *Cyprinus carpio* reported the highest ultimate strain due to inverse relationship between tensile strength and ultimate strain.

### 3.2 Nano-indentation Properties

In addition to tensile tests, macro, micro and nano indentation tests have also been used to assess the damage and deformation mechanism of the scales under penetration and impact loading [14]. A study on structure-property relationship for *A. gigas* using nanoindentation tests reported higher reduced modulus values of between 5-30 GPa under 15% humidity condition [40] compared to elastic modulus of 1.38 GPa under dry condition [13]. Although differences in dry and humid testing conditions can be attributed to these variations, reduced modulus measured by indentation technique is usually over 50% higher than elastic modulus determined by tensile testing. According to study by [40], the specific micromechanical properties of the scale are captured in more detail through nanoindentation testing technique. In contrast, only average properties are captured through tensile testing technique.

A research by [27] on mechanical properties and the laminate structure of *Arapaima gigas* scales showed higher micro-indentation hardness of 550 MPa for external layer compared to 200 MPa for internal layer. Similarly, nanoindentation hardness and nanoindentation modulus decreased from exterior to interior surface of the scale. Nanoindentation hardness reduced from 2.0 to 0.6 GPa for external surface and internal surface, respectively representing 70% decrease. On the other hand, nanoindentation modulus reduced by 64.3% from 46.8 to 16.7 GPa as distance increased from external to internal surface. Variations in hardness and modulus from outer to inner layer can be attributed to higher degree of mineralization on the external layer than internal layer.

Similarly, a study by [15] for *P. senegalus* scales reported 88% decrease in scale nano-indentation hardness from 4.5 GPa for external layer to 0.54 GPa for internal layer. Similarly, nanoindentation modulus decreased by 72.6% from 62 to 17 GPa for exterior surface and interior surface, respectively. The nanoindentation hardness reported by this study is 50% higher than that of enamel. The higher nano-indentation reported is due to the presence of ganoine layer covering the external surface of the scale. Besides, the study revealed that the scale had unique protection mechanism due to distinct reinforcing layers of different hardness comprised of highly mineralized rod-like crystallites of apatite. Microscopic imaging of hardness indents revealed that the surface of the scale showed circumferential fracture behavior at 2.0 N load.

In contrast, a research by [14] reported circumferential cracking pattern at 5 N and 10 N loads for *A. spatula* scales. Unlike radial fracture, circumferential fracture is a clear indication that the deformation and fracture under loading are locally confined at the indentation area as opposed to propagating throughout the material for radial fracture. The formation of circumferential cracking behavior witnessed for *P. senegalus* and *A. spatula* scales suggests the presence of multilayered structure in the scales. Circumferential cracking is beneficial material property that inhibits damage propagation from damaged to undamaged surface. Further review literature on protective role of *Arapaima gigas* shows that there is deflection, stretching and necking of the scale prior failure. This characteristic behavior serves to localize the damage to the vicinity of the indenter thus inhibiting crack initiation and propagation [34].

Also, a research by [3] showed that cross-like fracture patterns in the outer layer of the multilayered structure of elasmoid fish scale localized penetration inhibiting crack propagation. A study by [37] showed that the fracture toughness of *cyprinus carpio* scales ranged between 2.5 to 6.0 $MPa.m^{1/2}$. Similarly, [41] concluded that teleost fish scales from striped bass are among toughest material in nature with fracture toughness of 15-18 $kJ/m^2$. These values for fracture toughness are comparable with those of other mineralized tissues such as bone and dentin [42].

Review literature by [6] on battle in the Amazon between *Arapaima gigas* and Piranha predator reported higher hardness on the outer layer due to higher degree of mineralization of the outer layer. The outer layer reported micro-hardness of 600 MPa compared to 200 MPa for the inner layer. This represents 67% decrease from outer to inner layer indicating higher mineralization level on the outer layer. On nano-indentation hardness testing, the study reported consistent nano-hardness results of 0.6 GPa and 2.0 GPa for internal region and external region, respectively representing 233.3% increase from inner to outer layer. Further review literature shows that the reduced modulus of *Arapaima gigas* scales gradually decreased by 53.4% from 33.7 GPa for exterior surface to 15.7 GPa for interior surface. A study by [39] reported a similar trend of decreasing hardness by 61.5% from 1.3 to 0.5 GPa for external region and internal region, respectively. This shows that scales exhibit an increasing bio-mineralization gradient from internal to external layer of the scales. Thus, the external surface of the scale is highly mineralized and resistant to predator teeth penetration. However, nano-indentation hardness reported by the study was slightly lower than the values reported by [15]. The variations in nanoindentation hardness were attributed to the presence of ganoine on external surface of *P. senegalus* responsible for outstanding hardness.

A study by [39] reported variations in nanoindentation results between the ganoine and bony layers. The external ganoine layer reported superior nanoindentation modulus and nanoindentation hardness of 70.8 GPa and 3.6 GPa, respectively. In contrast, the internal bony layer reported lower nanoindentation modulus and nanoindentation hardness of 20.5 GPa and 0.7 GPa, respectively. Moreover, SEM images showed a clear interface between the two layers with the bony layer showing mineralized collagen fiber. In addition, [14] showed differences in nano-mechanical properties between the outer layer and inner layer. The stiffer and thinner outer layer reported higher indentation modulus and indentation hardness of 69 GPa and 3.3 GPa, respectively. On the other hand, the thicker inner layer reported indentation modulus of 14.3 GPa and indentation hardness of 0.5 GPa.

These results are consistent with other studies [15,16,40] which reported reduction of both nanoindentation modulus and hardness when moving from exterior to interior layer. According to these studies, the decline in nano-indentation hardness and nano-indentation modulus from exterior to interior surface of the fish scale can be spatially correlated to the decrease in mineralization from outer to inner layer. Furthermore, studies by [14,39] reported abrupt change in nanoindentation modulus and hardness at the interface region between inner and outer layers. In contrast, [15] reported gradual change in modulus and hardness at the interface due to the presence of dentin layer in the interface region for *P. senagalus* scales.

### 3.3 Direction of testing

A research by [36] on impact and fracture analysis of *A. gigas* scales in transverse and longitudinal directions confirmed the anisotropic behavior of fish scales. For both testing conditions that is, dry, humid and cryogenic, the impact strength in transverse direction was higher than longitudinal direction. For instance, the energy absorbed by dry scales was 42.49 $kJ/m^2$ and 35.11 $kJ/m^2$ in transverse and longitudinal directions, respectively representing 17.4% variation. Similarly, scales in humid condition absorbed 17.08 $kJ/m^2$ in transverse direction compared to 11.92 $kJ/m^2$ in longitudinal direction, representing 30.2% variation. Just like tensile strength and modulus, dry scales reported higher impact strength than humid scales. Further review literature [34] on structure and mechanical properties of *A. gigas* scales reported 27.2% higher energy dissipation of 1.47 MPa in longitudinal orientation against 1.07 MPa in transverse orientation. Similarly, the scales reported 39.8% higher tensile strength of 23.6 MPa in longitudinal orientation compared to 14.2 MPa in transverse orientation. In contrast, [32] reported higher tensile strength of 60 MPa with an orientation of $45^0$ or $90^0$ to the horizontal axis compared to $0^0$ orientation.

The impact test results reported in literature are at least 2 to 3 times higher compared to other collagen structures such as bones [36]. Although both fish scales and bones are made of hydroxyapatite and collagen as the main building blocks, the structure of fish scale is built in a way similar to that of a laminate fibre reinforced composite material. A research by [13] concluded that both *A. gigas* scales and laminated composite material have similar mechanical behavior. Thus, fish scales have improved ability to dissipate energy during impact tests, hence, higher impact strength compared to other collagen structures such as bones.

The analogy between fish scales and fibre reinforced composites has been further supported by similarities in failure mechanisms. A study by [43] concluded that delamination, fibre breakage and matrix crack propagation are the main failure mechanisms in fibre reinforced composites. These failure mechanisms have also been reported in fish scale. A research by [36] reported delamination failure mode under impact testing. The study further showed that delamination began prior to scale fracture and facilitated reduction in maximum fracture energy followed by crack propagation on the laminate structure. Further, study by [23] reported delamination and tearing failure mechanisms for *Megalops Atlanticus* scales without the mineralized layer. Similar failure mechanism has been reported [3] for striped bass scale while collagen bundles "pull out" has been reported for coelacanth scales after fracture [44].

However, fish scales have been described by several studies as plywood structures composed of collagen lamellae assembled from mineralized collagen fibrils arranged in layers [27]. On the other hand, [34] described fish scale as a Bouligand type twisted plywood structure. A Bouligand type structure ensures that the scale exhibit in-plane isotropy in mechanical response. Further, the structure of coelacanth scales exhibited in-plane isotropy. The scale showed no significant difference between the mechanical properties along transverse and longitudinal directions. The study reported tensile strength of 50 MPa in both directions while Young's modulus reported was 210 MPa and 250 MPa in longitudinal and transverse directions, respectively [44].

## 4. SUMMARY AND DISCUSSIONS

Fish scales are a skeletal element covering the skin of fish whose main purpose is to protect the body of the fish from external predation [13]. Depending on fish species, scales are broadly classified into four major classes: placoid, ganoid, elasmoid and cosmoid scales. Placoid scales are found in cartilaginous fish like sharks while most of the bony fish have elasmoid scales. The elasmoid scales mainly consist of mineral hydroxyapatite component, organic type I collagen component and water [45]. Ganoid scales have a thick surface layer of enamel, also known as ganoine, on top of a bony base. On the other hand, cosmoid scales have a double bone layer consisting of vascular and lamellar bone with the outermost layer considered as a dentin-like cosmine [34].

Microstructural studies on elasmoid scales have shown that scales comprise of two main layers, the outermost layer also known as limiting layer and the elasmodine layer. The limiting layer has a high mineralization level composed of apatite crystals reinforced by thin collagen fibres. On the other hand, the elasmodine layer has plies of unidirectional type I collagen fibrils. Besides, elasmoid scales comprise of hydroxyapatite and type I collagen as the primary building blocks [3,23,26,27]. Studies by [7] and [8] concluded that the co-alignment of type I collagen fibrils in the elasmodine layer is meant to enhance strength along the fibre direction. However, not much research is available on effects of co-alignment of collagen fibrils on strength of scales.

Further comparative morphological study [25] on *Megalops atlanticus* scales extracted from head, mid-length and tail concluded that the thickness of the external elasmodine and internal elasmodine layers is significantly higher than that of the limiting layer. The ultra-thin structure of the limiting layer in addition to higher mineralization level enhances mechanical response of the scales against predation. Further review literature [22] shows that for same fish species, the mineral content of the individual layers is similar irrespective of the anatomical location. Thus, differences in mechanical response observed in these anatomical regions could be due to variations in thickness of limiting layers in these regions. In a head to tail direction, a study on leptoid scale from teleost fish revealed that the scales are arranged in a manner resembling the structure of roof tiles [5]. Similarly, [2] reported that fish scales overlap in a manner similar to roof tiles forming an invisible membrane.

In addition, nanoindentation test results [24] showed decreasing nanoindentation modulus with increasing distance from the external surface for all three types of fish: *tarpon*, *carp* and *arapaima* scales. However, there were variations in nanoindentation hardness with *tarpon* scale recording the highest hardness followed by *carp* then *arapaima* scales, respectively. These variations in hardness amongst the three fish species can be attributed to differences in the structure of apatite resulting from differences in environmental conditions and growth rate. On the contrary, indentation results reported [40] for *A. gigas* showed that the hardness followed a cyclic saw-tooth shaped pattern. This study further revealed that the peaks of these cyclic saw-tooth patterns were found in the inner layer which appeared dark. This is because in the inner layer, the indentation plane is perpendicular to laminate fibrils. The study presented some modifications in the organization and chemical structure of fish scale. Further, the study concluded that the laminate structure of fish scale cannot be treated as a homogeneous, uniform structure as past studies had reported. However, not much research is available to support this conclusion as current review literature indicates that the laminate fish scale structure is uniform and homogeneous [46].

Further review literature [19,37,47] on *Grass carp* and *A. spatula* scales have reported anisotropic mechanical behavior. Moreover, *A. gigas* scales exhibited anisotropic mechanical behavior for both the entire scale and collagen layer without external mineralized layer [34]. Similarly, the study by [23] reported anisotropic mechanical behavior for *Megalops Atlanticus* scales without the top mineralized limiting layer and external elasmodine. The $0^0$ orientation was established to give the highest strength followed by $45^0$ and then $90^0$ orientations. In contrast, the research by [3] concluded that the whole scale of *striped bass* fish manifested in-plane anisotropic behavior while the collagen layer displayed an in-plane isotropic behavior in terms of strength and modulus. However, not much research is available on in-plane isotropic behavior of collagen layer.

A research by [46] concluded that fish skin is a strongly anisotropic shell with the bending stiffness capability only experienced for longitudinal bending. A study to compare the tear energy required to fracture *Cyprinus carpio* scales from three regions; head, mid-length and tail in three orientations: $0^0$, $45^0$ and $90^0$ reported moderate anisotropy for scales extracted from head [18]. More tear energy was required to fracture scales obtained from head region in the $0^0$ orientation than the energy required for the $45^0$ and $90^0$ orientations. However, the scales extracted from the middle and head region did not manifest anisotropy [17]. The structural anisotropy manifested by fish scale plays a significant role in avoiding indentation attacks from predators [16].

## 5. CONCLUSION

The limiting layer of fish scale has a high mineralization level composed of apatite crystals reinforced by thin collagen fibres. On the other hand, the elasmodine layer has plies of unidirectional type I collagen fibrils and hydroxyapatite and type I collagen as the primary building blocks. However, not much research is available on effects of co-alignment of collagen fibrils on strength of scales. Furthermore, studies have concluded that the laminate structure of fish scale cannot be treated as a homogeneous, uniform structure as past studies had reported. However, not much research is available to support this conclusion as current review literature indicates that the laminate fish scale structure is uniform and homogeneous. Additionally, there has not been much research done on in-plane isotropic behavior of fish scales in terms of strength and modulus.

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