Microstructural characterization of the bony plated armor in the prehensile tail of seahorses

Michael M Porter[1], Ana Bertha Castro Ceseña[1], Ekaterina Novitskaya[1], Marc A Meyers[1,2], Joanna McKittrick[1,2]

[1]Materials Science and Engineering Program;
[2]Department of Mechanical and Aerospace Engineering;
University of California, San Diego, USA.

Background

At the University of California San Diego, we look at natural biological materials for insight to design and fabricate new bio-inspired materials and devices. Our group has investigated many biological materials designed for structural support and protection, including: bovine bone, elk antler, sheep and ram horn, armadillo carapace, porcupine quill, bird beaks, turtle shell, crab exoskeleton, abalone shell, and fish scales [1-4]. To add to our collection of characterized biological materials, we studied the microstructure of the bony plated armor in the prehensile tail of the seahorse (*Hippocampus kuda*).

Seahorses, commonly known for their equine profile and vertical swimming posture, are amazingly complex fish with a variety of unique characteristics. Seahorses have a head like a horse, eyes that move independently like a chameleon, a brood pouch like a kangaroo, camouflage skin like a gecko, and a flexible prehensile (i.e., grasping and holding) tail like a monkey [5-7]. From a mechanics perspective, the bony plated armor in the seahorse tail is a fantastic example of a multifunctional material that provides the seahorse structural support, protection, and even the ability to bend in a strict spiral and grasp objects. In this work, the bony plated armor in seahorses was characterized with 3D optical microscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and micro-hardness testing.

Hierarchical structure of bony plates

Seahorses have a prehensile tail composed of approximately 140 bony plates arranged in rings of four overlapping corner plates per tail segment. Fig 1 shows the tail cross-section (skin removed) and the hierarchical structure of the bony plated armor. The bony plates are inorganic/organic composites composed of approximately 45 wt% inorganic, 38 wt% organic, and 17 wt% water. For clarity, the tail segments are numbered, as seen in Fig 1, according to Bruner et al. [5].

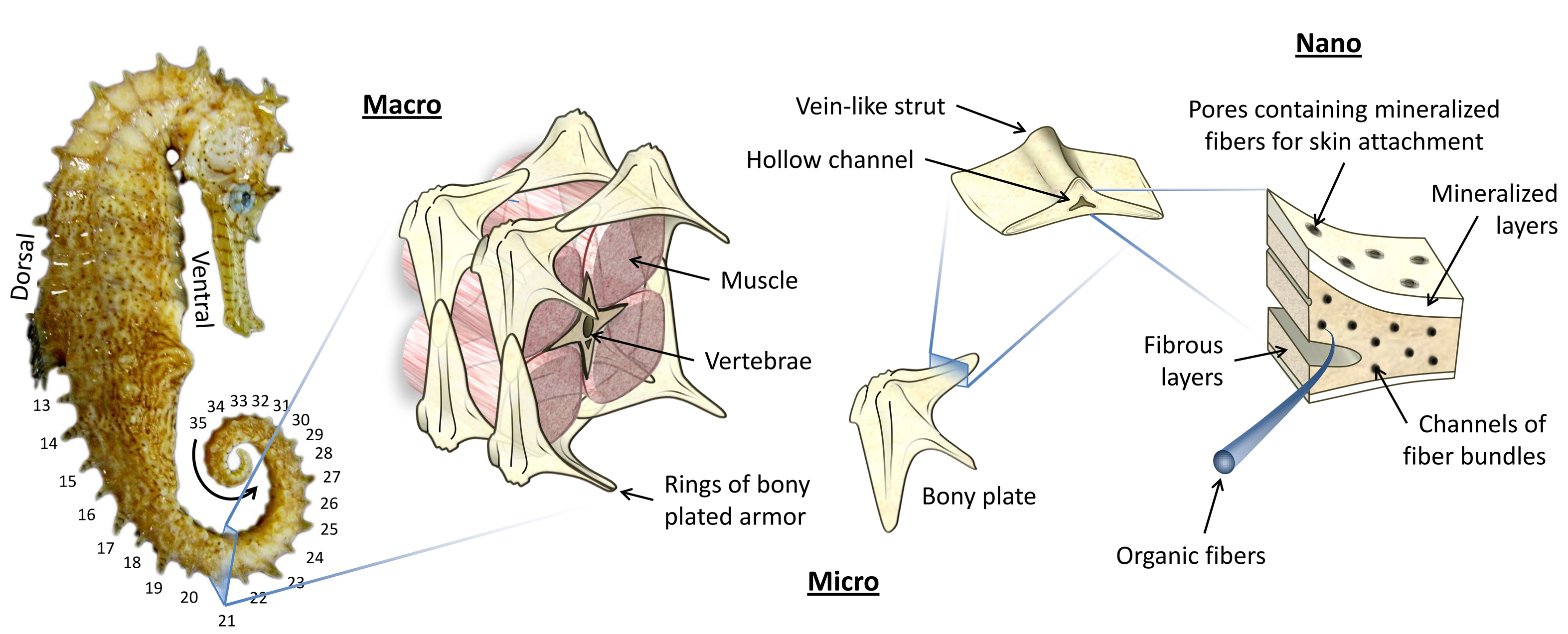


Fig 1. Right side view of a seahorse (unspecified species) showing the dorsal and ventral sides, the bone number of each tail segment, the tail cross-section, and the hierarchical structure of a bony plate from the macro to nano scales.

Overlap and sequence of bony plates

The bony plates overlap longitudinally and laterally giving seahorses the necessary flexibility for axial and lateral bending, grasping and holding [6]. To visualize the succession of overlapping plates, several seahorse tails were partially deproteinated with 12.5% NaClO for 0-60 mins (Fig 2). The longitudinal overlap between two adjacent plates is much like a joint, where a male shaft fits neatly into its female counterpart (Fig 3a). On the right and left sides of the tail, the ventral plates always overlap the dorsal plates (Fig 3b). The right-left (left-right) overlap on the dorsal and ventral sides of the tail, however, are randomly sequenced from segment to segment, and may be distinct to each individual seahorse, similar to a human fingerprint (Fig 3c). Longitudinal overlapping allows seahorses to bend their tails ventrally in a strict spiral. Slight lateral bending may occur concurrently with ventral bending [6]; although, the ventral-dorsal overlap seems to prevent significant lateral movement.

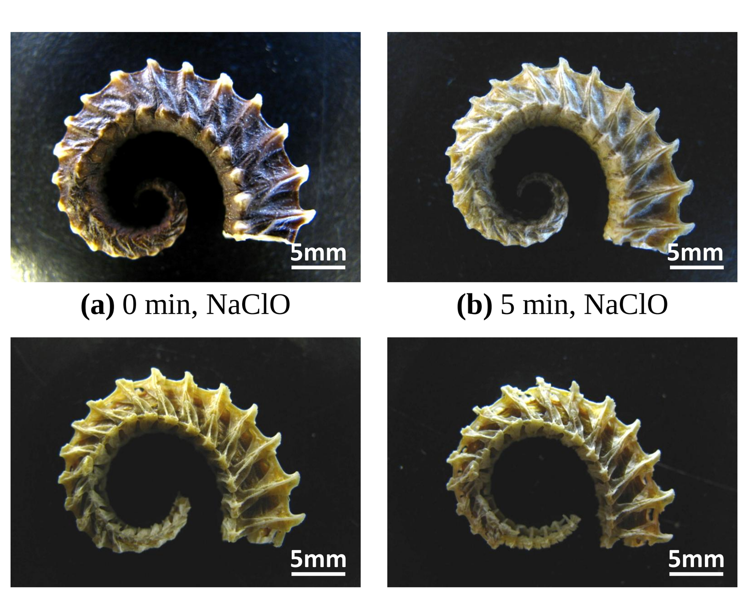


Fig 2. Time course of seahorse tail subjected to 12.5% NaClO in ambient conditions. Treatment used to determine the size, placement, overlap, and sequence of bony plates.

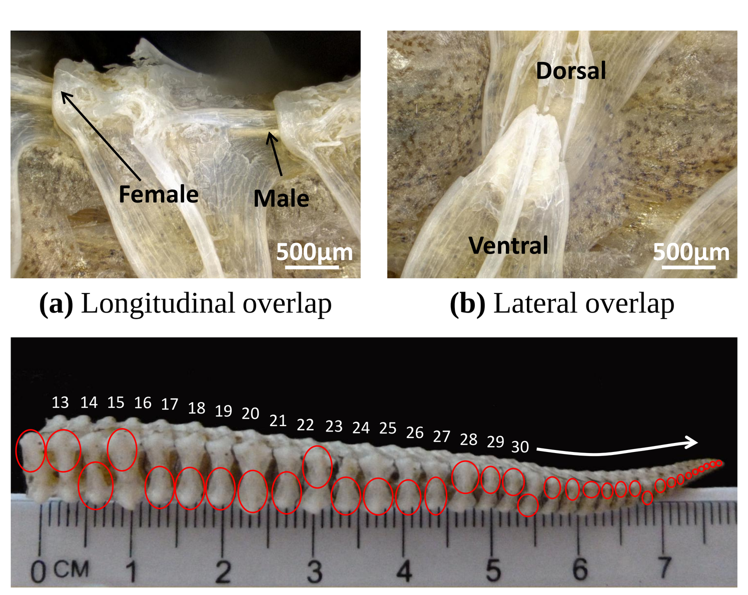


Fig 3. Images of overlapping bony plates after bleaching: **(a)** longitudinal overlap, **(b)** lateral overlap, and **(c)** random overlap sequence of dorsal plates over the tail length.

Microstructural features and surface roughness of bony plates

The bony plated armor in the prehensile tail of a seahorse is a complex inorganic/organic composite with unique microstructural features (Fig 4). Each plate is a thin, V-shaped bone with a narrow shaft (male joint) and rounded inset (female joint) positioned at the front and back of the V-junction, respectively. The overall shape of the bony plates resembles a 3-dimensional coordinate system axis, where the two plate wings (right/left and ventral/dorsal) are positioned along the x and y axes and the male shaft projects along the z axis. The outer surfaces of the plate wings have a central, vein-like strut and small tendrils running the length of the wings for added structural support. At the junction of the plate wings and the male joint, a distinct ridge of rounded nodules and pores makeup the four pointed corners of each tail segment. The inner surface of the entire bony plate is relatively smooth, with directionally oriented mineral and protein fibers.

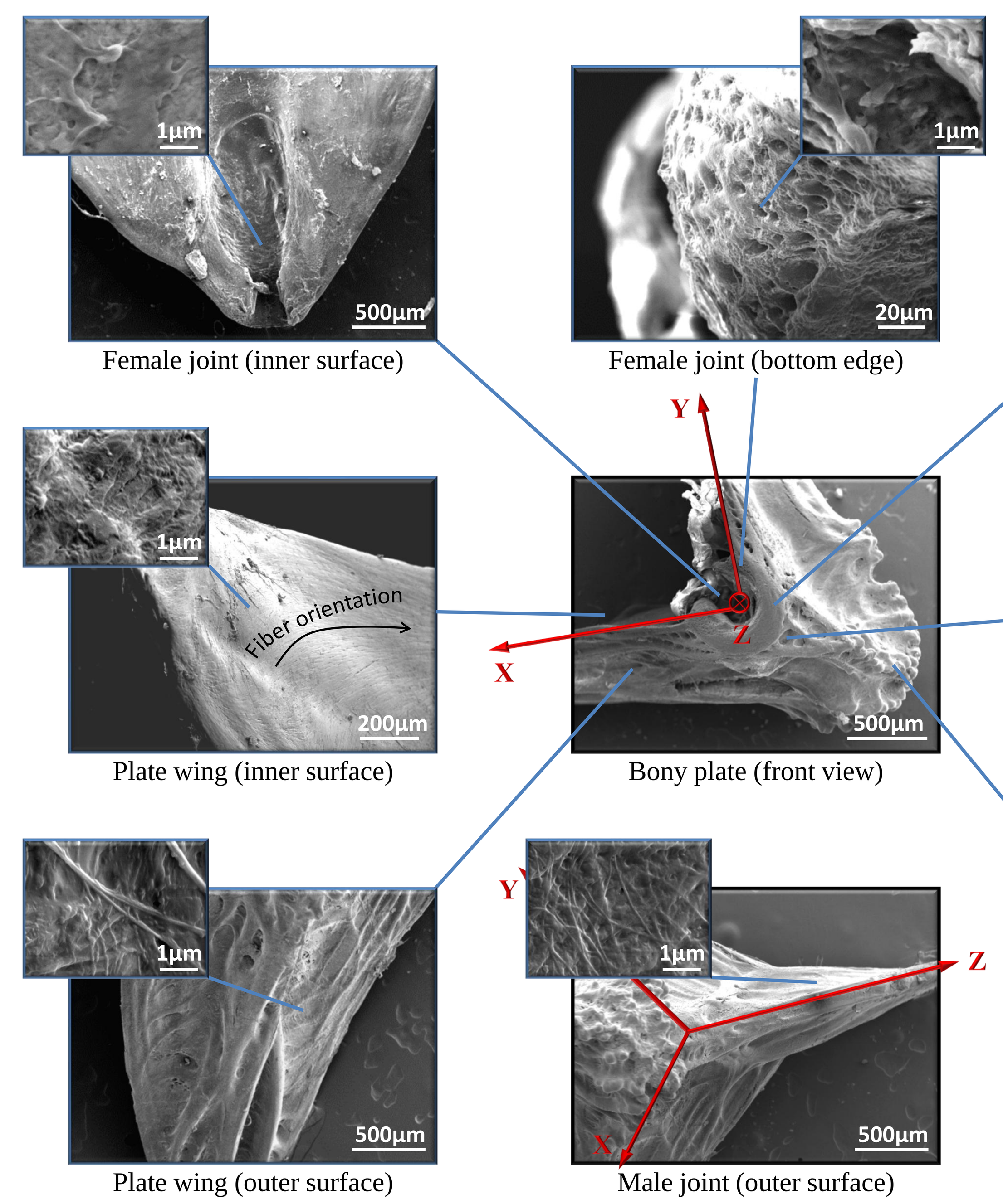
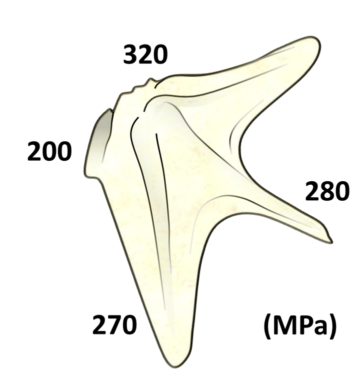


Fig 4. SEM micrographs of the untreated bony plates of a seahorse (*Hippocampus kuda*), illustrating the various surface features and roughness at several different locations, the 3-dimensional shape axes and the fiber orientation of the inner plate surface.

Micro-hardness of bony plates at different locations

For protection, the bony plates must be sufficiently hard, yet tough enough to withstand stresses induced by bending and grasping. The female joint is nearly 10% softer than the male, enabling it to absorb stresses caused by joint movement (see right). The top ridge is the hardest region of the bone, which may serve as a hard protective shield against high impact (see right). Compared to bovine femur bone (~67 wt% mineral, ~500 MPa), the bony plates in seahorses have a much lower mineral content (45 wt%) and hardness (270 MPa). The superior toughness and overlapping nature of the bony plates may enhance tail flexibility, resulting in greater resistance to crushing and brittle fracture.



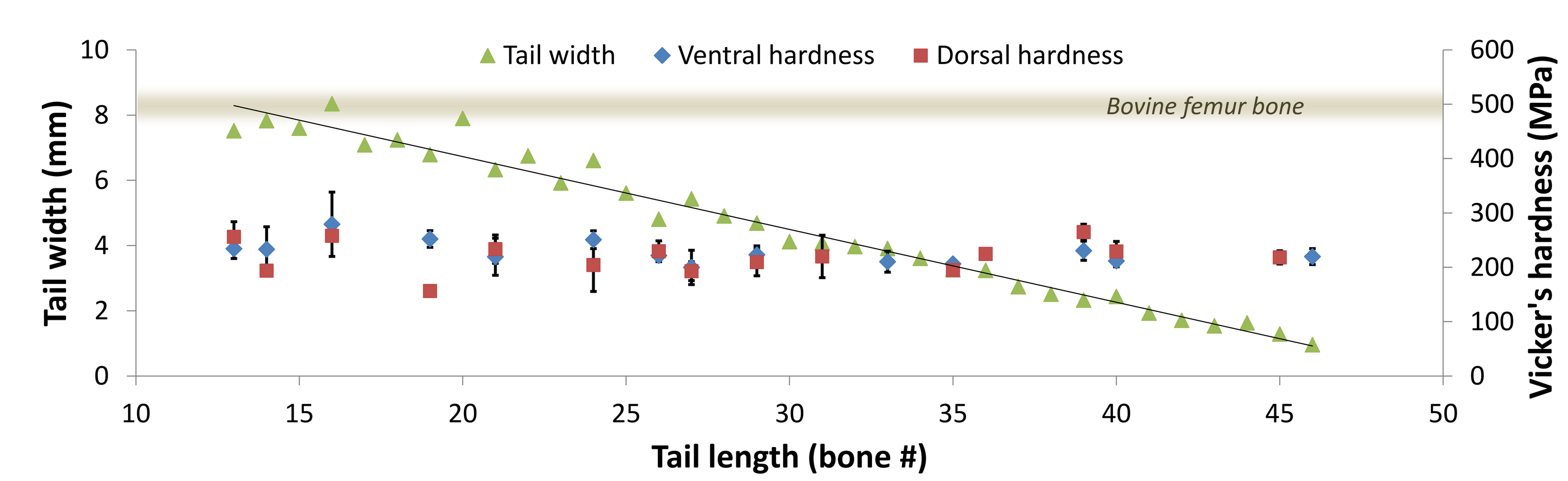


Fig 5. (Top image) Representative illustration of a bony plate and the measured micro-hardness (Vicker's hardness) at various locations (data from dorsal plates 13-19 – see Fig 1). **(Plot)** Tail width and micro-hardness of the bony plated armor (unspecified surface locations) versus seahorse tail length by bone # [5] (see Fig 1). Micro-hardness of bovine femur bone is shown for comparison.

Untreated (UT), deproteinated (DP), and demineralized (DM) bony plates

The bony plates in seahorses are composed of 45 wt% calcium phosphate, 38 wt% protein, and 17 wt% water. To analyze the mineral and protein constituents individually, untreated (UT) bony plates were compared to those treated with 12.5% NaClO for 24 hrs (deproteinated - DP) and 0.6 N HCl for 24 hrs (demineralized - DM), respectively. Elemental analysis of the bony plates with energy dispersive X-ray spectroscopy (EDX) confirmed the compositions (Fig 6). Fracture surfaces of the bony plates illustrate their hierarchical complexity, showing multiple inorganic/organic layers of directionally aligned fibers at the submicron scale (Fig 6a-c). The deproteinated bony plates exhibited a brittle fracture behavior, while the untreated and demineralized plates were much tougher and more elastic. The hardness of the untreated bony plates (270 MPa) is higher than would be expected from a simple mixture of its mineral (336 MPa) and protein (168 MPa) constituents: $H_{mix} = \varphi_m H_m + \varphi_p H_p$ where φ is the wt% and H is the hardness of the mineral (m) and protein (p) constituents, respectively. The higher than expected hardness of the untreated bony plates is likely due to the complex structural hierarchy.

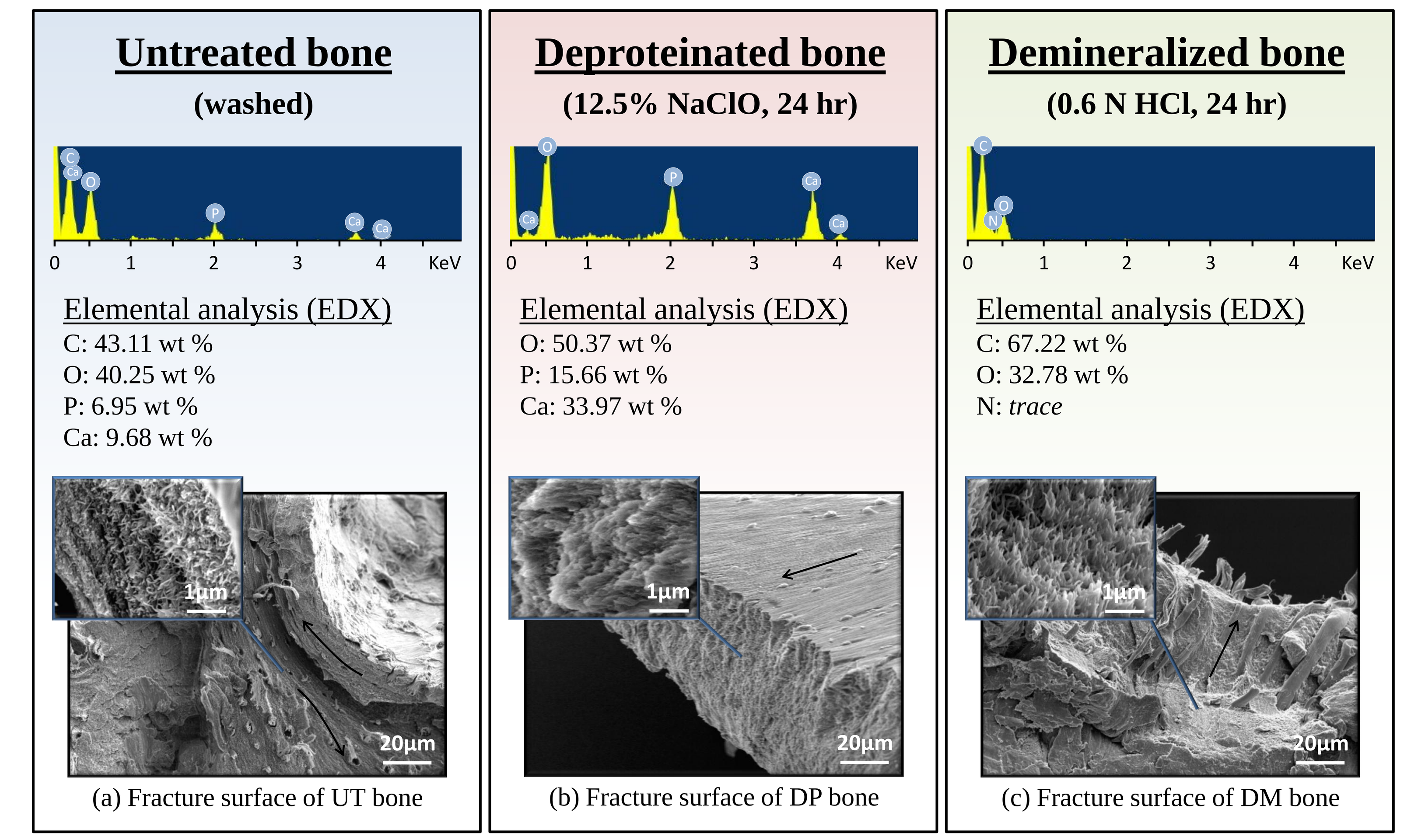


Fig 6. (Top) EDX elemental analysis and **(Bottom)** representative SEM micrographs showing fracture surfaces and fiber orientations of the plate wing of untreated (left), deproteinated (middle), and demineralized (right) bony plated armor.

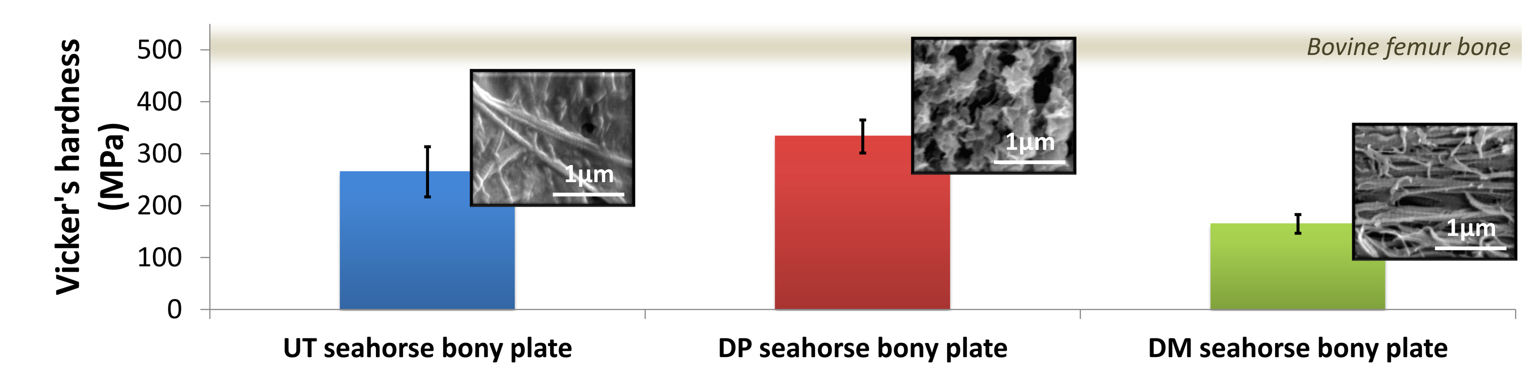


Fig 7. Micro-hardness and SEM insets of untreated (UT), deproteinated (DP), and demineralized (DM) seahorse bony plates compared to bovine femur bone. (All data from dorsal plates 13-19 at unspecified locations – see Fig 1).

Conclusions

The bony plated armor in the prehensile tail of the seahorse is a multifunctional material that provides structural support, protection, and mobility. The bony plates are arranged in rings of four overlapping plates per tail segment, with approximately 35 segments spanning the length of the tail. When compared to bovine femur bone, the bony plates in the seahorse tail have a much lower mineral content and average hardness. The distribution of hardness across a single bony plate, however, seems to be tailored to specific tasks - harder on the outer surface for protection, and softer at the overlapping joints for mobility. The structural hierarchy of the mineral and protein constituents, along with the distinct hardness distribution and overlapping nature of the bony plates, gives the prehensile tail its exceptional toughness and flexibility required to perform specific functions.

Acknowledgements

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