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See-through teeth, clearly

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Summary
The teeth of the deep-sea dragonfish are sharp, hard, stiff, and transparent. Here we compare them to other teeth and their structure, which may determine both scattering and mechanical behavior of teeth in diverse animals.

If you want to catch fish in deep sea, where no sunlight penetrates you need to develop tools highly specialized for the task. The dragonfish is a master of such tricks. It evolved to be dark like the abyss surroundings; it grew a barbel protruding from its chin and holding a bioluminescent lantern to attract its prey, it adapted its jaw so that its mouth can open much larger than comparable size fish so it can prey on fish that are 50% its body mass; and it evolved a set of teeth that are the latest discovery in biomaterial science: they are not only razor-sharp, hard, and stiff, but they are completely transparent when wet, that is, their index of refraction must exactly match that of seawater, and they do not scatter light at all. Why bother? Because if they scattered light, when illuminated by bioluminescent light either from the dragonfish itself, or by prey bioluminescence the prey would discover the ferocious teeth and quickly swim away to save themselves. Thus, besides their structure and mechanical properties, the dragonfish teeth must have sophisticated optical behavior, as cleverly discovered and shown by Velasco-Hogan *et al.* (1).

Transparent teeth are not *per se* unique, other animals have them too, e.g. the radula teeth of the red abalone (Figure 1A), but in abalone teeth transparency is accidental, not functional, since the mouth radula is located under the shell and under the animal's soft body, where nobody can see it during the animal's life. In dragonfish teeth, instead, the function of transparent teeth is clear. Pun intended.

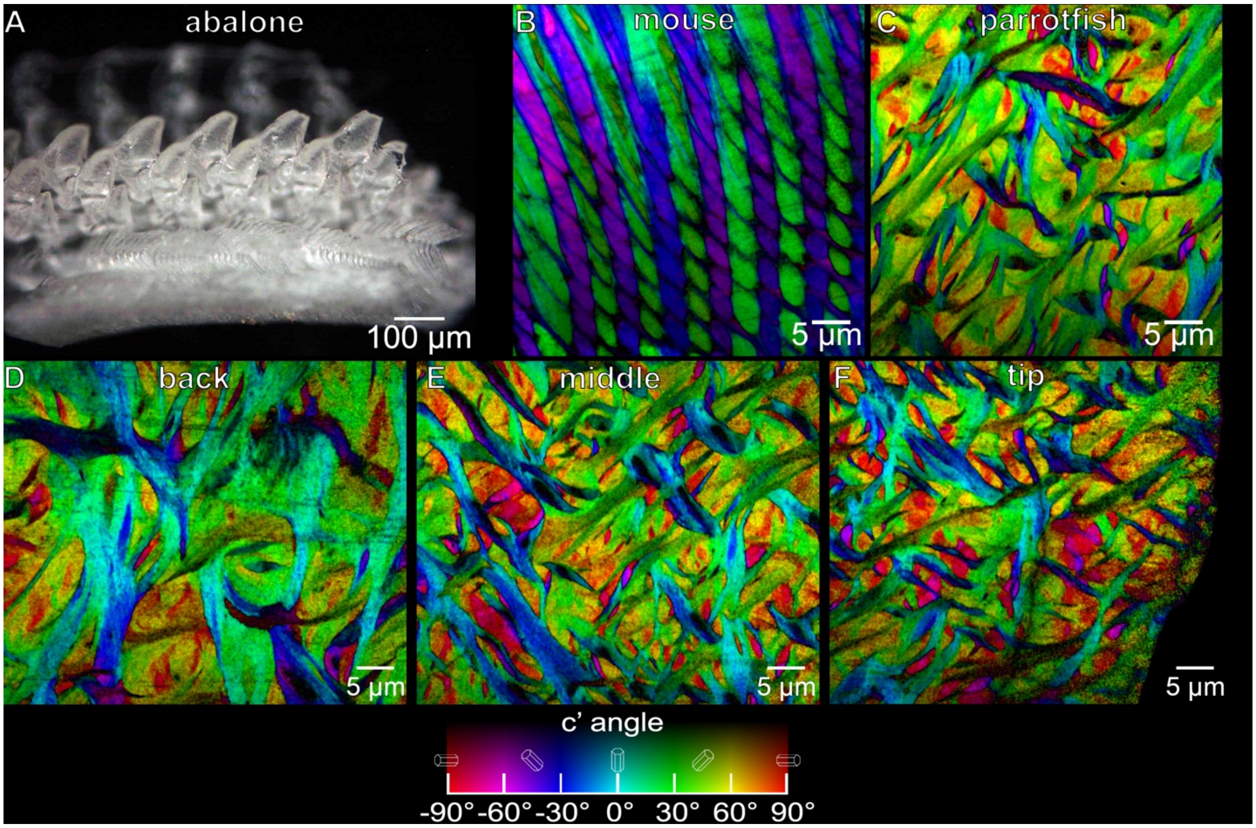
So, dragonfish teeth are transparent, and usefully so. But how is transparency achieved? The first-discovery paper by Velasco-Hogan *et al.* (1), addresses this point only in part, by presenting transmittance and reflectance data, and scattering calculations based on simplified assumptions, which hint at the possible role of nanocrystal size. Extremely informative density and refractive index measurements, however, remain to be done. So does the full characterization of the teeth surface: the enamel-like layer. One of the open questions is: How does a mineralized material match the density and refractive index of water? Granted, the latter is deep seawater, which is denser than surface water (with density 1.033 g/cm^3 at 1000 m depth, compared to 1.028 g/cm^3 at the surface, but the density varies a lot from place to place, depending on depth, temperature, and salinity.)

If scattering, or lack thereof, is really the key to dragonfish teeth transparency, one needs to first measure and fully characterize the structure, including composition, size, shape, orientation, and arrangement of crystals at the enamel-like surface, and then use these structural parameters to calculate scattering efficiency. Clearly, the business end of a visible light scatterer is its surface, not its bulk, which is the primary focus of this first-discovery paper by Velasco-Hogan *et al.* (1).

In other animals, enamel, or enamel-like, or enameloid, as enamel is called in parrotfish, sharks, and a few other fish, is made of hydroxyapatite (HAP) or fluorapatite (FAP) nanocrystals 20-100 nm in diameter, many microns long thus resembling nano-spaghetti, all grouped into bundles of parallel nano-spaghetti that, like most other biominerals are space-filling, thus leave no open spaces at all between one nano-spaghetti and the adjacent ones, even at the atomic scale (2). How the bundles of nano-spaghetti elongate, straight or curved, and intertwine with one another is extremely different from one animal to another. The bundles (termed rods, prisms, or simply bundles) are straight in mouse enamel, curved in human and fish enamels, well organized into alternating directions in both mouse and human enamel, but not in parrotfish, where the pattern of interwoven bundles is more intricate than in any other animal thus far observed. Parrotfish enameloid is the stiffest, and one of the hardest biomaterials ever measured, with a mean of 124 GPa and 7.3 GPa, respectively. Presumably, the complex structure of bundles has something to do with these outstanding mechanical performances.

The arrangement and orientations of HAP or FAP crystals varies wildly. Compare, for example, the simplicity of mouse enamel with the intricacy of parrotfish enameloid. In mouse enamel each bundle contains perfectly co-oriented nano-spaghetti crystals (1 color, either green, or blue for most bundles in Figure 1B), and all bundles are cylindrical, with a circular or elliptical cross-section depending on their orientation with respect to the polished surface, with all rods parallel to one another in each layer. In adjacent layers, bundles change direction, by a precise angle, and then repeat, so the layers of bundles alternate between two angles as presented in Figure 1B. In parrotfish enameloid FAP nano-spaghetti also align into space-filling bundles, but the bundles are not co-oriented, they exhibit crystal lattice tilting (3), and they are not straight. They curve in all directions in three dimensions, generating a much more intricate pattern, as presented in the polished cross-section of Figure 1C.

Furthermore, in parrotfish the gradual decrease in crystal bundle sizes from the back of the enameloid layer to the tip of the tooth elegantly correlates with a gradient in hardness and stiffness, as shown in Figure 1D-F.



A. The transparent radula teeth of the red abalone *Haliotis rufescens*. Micrograph obtained on a Zeiss visible light microscope, with crossed-polarizers, at 50x magnification, from the middle of the extracted radula, in air. (Photo credit: Pupa Gilbert)
B,C. Comparison of mouse enamel with parrotfish enameloid. In these polarization-dependent imaging contrast (PIC)(4) maps the HAP or FAP crystal *c*-axis orientation in each 60-nm pixel is measured at a synchrotron with minimal radiation damage (5), and then displayed in colors, according to the color legend at the bottom. B. Mouse *Mus musculus* HAP enamel in the middle of the enamel layer, between the dentin-enamel junction (DEJ) and the surface of the mouse incisor. In each vertical layer bundles are all parallel to one another, thus they all appear elliptical in cross-section, and with the same color. Layers of green and blue elliptical bundles alternate as ABABAB in the horizontal direction. C. Parrotfish *Chlorurus microrhinos* FAP enameloid, as it appears in the middle of the enameloid layer, between the DEJ and the biting tip. Elongated crystal bundles exhibit frequent crystal lattice tilting, which appears as gradually changing colors. Bundles are curved, intertwined, and do not form a periodic pattern. Reproduced with permission from (6) and (7). D,E,F. PIC maps of parrotfish FAP enameloid. The bundle sizes change gradually from the back, to the middle, to the tip of the tooth, from 5-10 µm to 1-5 µm in bundle width. The hardness and stiffness increase from the back of the tooth to the tip. The mean hardness H increases from 4 GPa to 7.3 GPa, and the Young's modulus E from 80 GPa to an astonishing 124 GPa. Reproduced with permission from (7).

The dragonfish teeth exhibit a gradient in density from the inside to the outside, as observed by x-ray tomography (1). It is possible, therefore, that further investigations will reveal a structure-properties correlation, but this is an open question.

Another intriguing finding by Velasco-Hogan *et al.* (1) is the extremely sharp tip of the dragonfish teeth: a radius of curvature of only 5 µm or less, compared to shark or piranha teeth, which have 14 µm, and sea urchins, which have 24 µm. How do they stay so sharp with repeating use? Do they self-sharpen like the sea urchin teeth? And if so, do they use a self-sharpening mechanism similar to that found in sea urchin teeth (8), that is, breaking at pre-determined locations like perforated paper, but along a sharp profile? Or do they have their own, creative solution to this problem?

Now that the teeth of the dragonfish have been discovered by material scientists, some light must be shed on the many remaining dark, deep sea mysteries.

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