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Spines of the porcupine fish: Structure, composition, and mechanical properties

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ABSTRACT

This paper explores the structure, composition, and mechanical properties of porcupine fish spines for the first time. The spine was found to be composed of nanocrystalline hydroxyapatite, protein (collagen), and water using X-ray diffraction, energy-dispersive X-ray spectroscopy, and thermogravimetric analysis. Microstructures have mineralized fibrillar sheets in the longitudinal direction and in a radial orientation in the transverse direction that were observed using light and electron microscopy. Based on the images, the hierarchical structure of the spine shows both concentric and radial reinforcement. Mechanical properties were obtained using cantilever beam and nanoindentation tests. A tapered cantilever beam model was developed and compared to that of a uniform cantilever beam. The tapered beam model showed that while the stresses experienced were similar to those of the uniform beam, the location of the maximum stress was near the distal region of the beam rather than at the base, which allows the porcupine fish to conserve energy and resources if the spine is fractured.

1. Introduction and background

Spines are stiff, tapered structures that protrude from an organism. They are found in mammals (e.g. porcupine, echidna, and hedgehog), plants (e.g. cacti and rose), insects, reptiles, birds, echinoderms, and fish. While spines can be used offensively, for instance in bees and wasps, many organisms use spine structures as a form of defense. Organisms that use spines for defense include porcupines, hedgehogs, cacti, and sea urchins. Spines are used to deter predators by piercing and irritating.

Spine structures can be made of a variety of biological materials. Sea urchin (Fig. 1a) spines are composed of magnesium calcite (Berman et al., 1990; Moureaux et al., 2010); lionfish (Fig. 1b) dorsal spines (Bassett, 1917; Bowes and Murray, 1935; Halstead et al., 1955); and stingray (Fig. 1c) stings are composed of mineralized collagen (Halstead and Modglin, 1950; Ocampo et al., 1953); spines and quills found in hedgehogs, porcupines (Fig. 1d), and echidnas (Fig. 1e) are made of keratin (Martin et al., 2015; Vincent and Owers, 1986); cactus (Fig. 1f) spines are made almost equal parts of crystalline cellulose and amorphous hemicellulose, both of which are polysaccharides (Gindl-Altmutter and Keckes, 2012; Malainine et al., 2003). Meanwhile, scorpion (Fig. 1g), bee (Fig. 1h), and wasp stingers are made of the polysaccharide chitosan (Zhao et al., 2016; Zhao et al., 2015).

Many spine structures, including the previously mentioned scorpion stinger, are known to be functionally graded materials, which have gradients in composition or structural characteristics. The squid beak (Miserez et al., 2008), ancient fish armor (Bruet et al., 2008), and spider fang (Bar-On et al., 2014) all exhibit compositional gradients resulting in a gradual transition in Young's modulus. Functionally graded materials have been found to reduce deformation and damage at material surfaces and dissipate stress by transitioning to a more compliant material (Bechtle et al., 2010; Liu et al., 2016; Pompe et al., 2003).

It is also important to note the multifunctionality that is intrinsic to biological materials. Spines can be useful to an organism for a variety of reasons. For example, in addition to protection, the cactus uses its spines, to prevent water loss in its native desert habitat. Hedgehogs use their quills not only to deter predators, but also to absorb energy when

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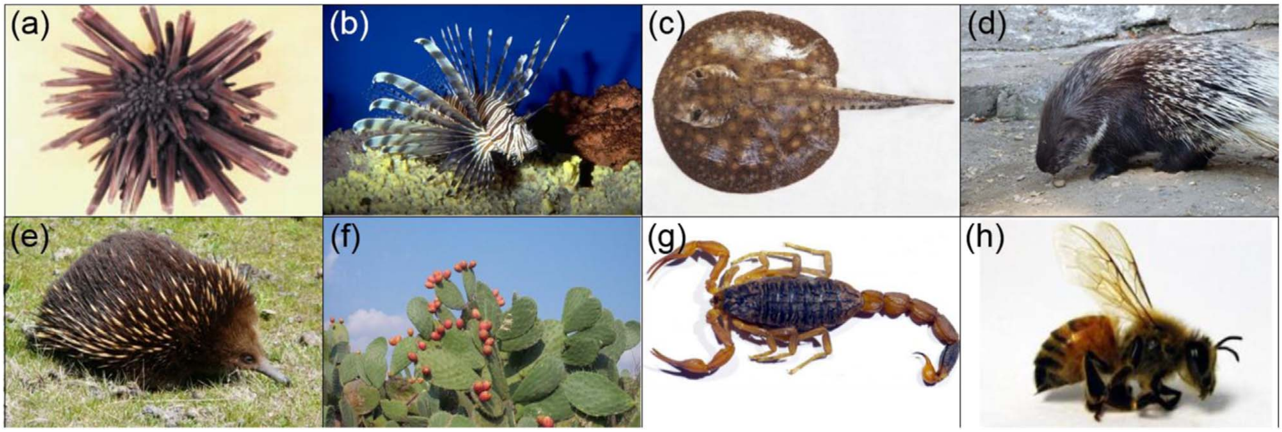

Fig. 1. Various organisms with spine structures. (a) Sea urchin (Su et al., 2000), (b) lionfish (Corsi and Corsi, 2001), (c) stingray (Pedroso et al., 2007), (d) porcupine (Tonge, 2014), (e) echidna (Tonge, 2013), (f) cactus (Rignanese, 2005), (g) scorpion (Zhao et al., 2016), and (h) honey bee (Zhao et al., 2015). Figures are adapted from cited sources.

they fall from high places (Vincent and Owers, 1986). The lionfish, stingray, bee, wasp, and scorpion all use venom to supplement their stings.

Porcupine fish belong to a family within the order Tetraodontiformes called Diodontidae (Santini et al., 2013). While the order Tetradontiformes is an extremely diverse group that includes the boxfish and triggerfishes, the porcupine fish is most closely related to the families Tetraodontidae and Molidae, which include pufferfish and ocean sunfish, respectively (Santini et al., 2013). Porcupine fish are preyed upon by pelagic predators including tuna, dolphinfish, and wahoo (Aquarium of the Pacific, 2015). Once porcupine fish reach the adult stage and become too large to swallow, the number of predators decreases significantly. The main predator of the adult porcupine fish is the tiger shark.

To protect themselves, porcupine fish, like their pufferfish relatives, inflate their bodies up to three times their original volume (Brainerd, 1994). During inflation, water or air is pumped into the stomach. In addition to inflation, porcupine fish also have long spines across their bodies that are erectile when the fish inflates (Leis, 2006). These spines are actually modified scales and serve to both irritate the predator and increase the effective volume of the porcupine fish, making it harder for predators to swallow.

Apart from general observations, little to no work has been done on the composition and microstructure of the porcupine fish spine. However, some work has been done to understand the spine structure in the family Tetraodontidae. Hertwig et al. (1992) observed that the dermal spines in *Tetraodon steindachneri* have bilateral symmetry and a laminated longitudinal cross-section. The spines of the *T. steindachneri* and *Takifugu obscurus* can be stained using alizarin red-S, a histological dye that indicates the presence of calcium (Byeon et al., 2011; Hertwig et al., 1992). Of note, these spines have a dense outer layer of collagen around a mineralized core (Hertwig et al., 1992), as well as numerous concentric circles in the transverse cross-section of the spines (Byeon et al., 2011).

The composition of porcupine fish spines is largely unknown, since alizarin red only signifies the presence of calcium, but does not specify whether the spines contain calcium carbonate, hydroxyapatite, or both minerals. As modified scales, the spines can be expected to be compositionally similar to that of other fish scales, comprising highly aligned type I collagen, calcium phosphate (e.g. hydroxyapatite and tricalcium phosphate), and in some cases, calcium carbonate (Ehrlich, 2015; Lin et al., 2011; Zylberberg et al., 1992). Sire et al. (2009) noted that the dermal plates in tetraodontiforms are composed of only bone. However, the order Tetraodontiformes is so diverse in morphology that it is unlikely that every modified scale across the order is compositionally identical.

In the porcupine fish, Brainerd (1994) identified three different regions of the spine: the spinous process, the lateral processes, and the axial process. Leis (1978) referred to these regions as the spine shaft, the lateral arms of the base, and shaft extension, respectively. Spines generally have bilateral symmetry with two lateral processes, one spinous process, and one axial process.

This work aims to characterize the composition, structure and mechanical properties of the spines from two porcupine fish, *Diodon holocanthus* (Long-spine) and *Diodon hystrix* (Spot-fin). The spines must be able to withstand the force of a predator's jaw to maintain structural integrity. There are two main hypotheses we have explored: (1) the composition of the spines will be similar to that of other scales, meaning that the spines will likely contain collagen and calcium phosphate mineral, and (2) the morphology and consequent mechanical properties of the spines help prevent spine fracture.

2. Materials and methods

One of each *D. holocanthus* (Museum ID: SIO 65–679) and *D. hystrix* (Museum ID: SIO H52-415) (Fig. 2) were received from the Scripps Institution of Oceanography. Samples had been fixed with 10% formalin and post-fixed in 50% isopropyl alcohol and deionized water. Spines were extracted from the right lateral side of the fish using a

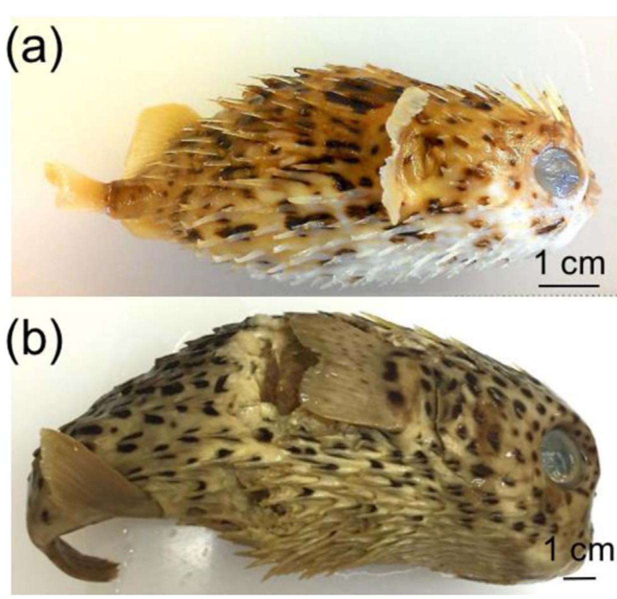

Fig. 2. Photographs of the specimens used in this study, (a) *Diodon holocanthus* (slender-bodied long-spine porcupine fish) and (b) *Diodon hystrix* (round-bodied spot-fin porcupine fish) samples received from the Scripps Institution of Oceanography.