**Osedax (Siboglinidae: Annelida) utilizes shark teeth for nutrition**

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**Abstract**

We deployed jaws of the common thresher shark (*Alopias vulpinus*) on the seafloor at ~1000 m depth off Monterey California for 8 months. The jaws disintegrated, with all the hyaline cartilage disappearing, leaving some fragments of tessellated cartilage and the teeth. Two different *O. packardorum* and *O. talkovici* were found to have bored into the roots of some of the teeth, and were using the dentin pulp, which is rich in collagen, as a food source. The enamelled crowns of the shark teeth and the tessellated cartilage showed no signs of *Ose xax* activity. This is the first demonstration of *Ose xax* exploiting a source of food that is not bone. This raises questions as to the original food source of *Ose xax* ‘bone worms’. Examination for the presence of *Ose xax* in the skeletons and teeth of Mesozoic and possibly even Palaeozoic fossil sharks, bony fish and reptiles is warranted.

**Introduction**

Members of *Ose xax* are well known for relying, with the aid of symbiotic bacteria, on sunken vertebrates for their nutrition (Rouse et al., 2004; Goffredi et al., 2005). Initially found living on whale bones (Rouse et al., 2004; Glover et al., 2005; Fujikura et al., 2006), it has subsequently been shown that *Ose xax* can live off the exposed bones of a range of vertebrates, including teleost fish (Jones et al., 2008; Rouse et al., 2011, 2018; McClain et al., 2019). While one of the early descriptions, by Fujikura et al. (2006), reported that *Ose xax japonicus* was found living ‘on the surfaces of tainted spermaceti’ no details were provided. There have been no subsequent reports of extant *Ose xax* living on any other substrate apart from bone, though the unusual *Ose xax jabba* is known to live off buried fragmented pieces of bone buried in the sediment (Rouse et al., 2018). With reference to fossil evidence for *Ose xax* activity that is not bone-related, Kiel et al. (2013) showed bore holes and cavities within the teeth of an Oligocene toothed mysticete whale. Amalfitano et al. (2019) also suggested that circular bore-holes in the fossil vertebrae of the shark *Cetororhynchus montellii*, dating to the Upper Cretaceous, could represent the action of *Ose xax*. However, evidence to support this via internal microCT scans as performed on other fossils to support the presence of *Ose xax* borings (Kiel et al., 2010; Danise & Higgs, 2015), were lacking.

*Ose xax* obtain nutrition via the symbiotic heterotrophic bacteria that live in their roots, which are in contact with the bone matrix. The inorganic component of the bone (calcium hydroxyapatite) is dissolved by acid secreted by the roots (Tresguerres et al., 2013) allowing access to the organic components. The food source was initially hypothesized to be the major organic components of bone such as proteins (e.g. collagen) and hydrocarbons (e.g. cholesterol) (Goffredi et al., 2005). Subsequent studies narrowed this down to the food source likely being collagen (Goffredi et al., 2007, 2014; Miyamoto et al., 2017).

Given the apparent reliance on *Ose xax* and their symbionts on collagen it seems reasonable to examine non-bone marine food sources that they might exploit. Collagen is a primary component of the cartilage of non-bony fishes such as elasmobranchs (Merly & Smith, 2013). Parts of shark cartilaginous skeletons, such as the jaws, may be mineralized as tessellated cartilage (Balaban et al., 2015), allowing resistance to rapid degradation by scavengers. It was hypothesized that this would provide time for colonization and growth of *Ose xax*, which can occur in less than 2 months (Rouse et al., 2008). Also, the teeth of sharks contain various forms of dentin (or dentine), a bone-like matrix with an organic content that is largely collagen (Enax et al., 2012). The dentin of the tooth is surrounded by an extremely hard enamloid ‘crown’ that does not extend over the tooth root (Salomon, 1969; Enax et al., 2012; Jambura et al., 2020). Osteodentine is porous, cellular dentin, harder than bone and is found in the root (base) of all shark teeth (Whitenack et al., 2010). The teeth of most sharks and rays have hollow pulp cavities surrounded by another form of dentin, orthodontine, beneath the enamloid. However, in the clade Lamniformes (and some rays) the pulp cavity is missing, and space beneath the enamloid is filled with osteodentine. These latter teeth are referred to as osteodont or pseudoosteodont (Whitenack et al., 2010; Schnetz et al., 2016; Jambura et al., 2020).

Rouse et al. (2011) reported the deployment of the jaw and several vertebrae of a juvenile mako shark (*Isurus oxyrinchus*, Lamniformes) for 5 months at ~1000 m depth, but the vertebrae and jaws had disintegrated except for some calcareous elements. The teeth were intact and showed no signs of *Ose xax*. Here we report on a deployment of jaws of the common thresher shark (*Alopias*
vulpinus, Lamniformes) for 8 months, and document the occurrence of two known Osedax species using shark teeth as a food source.

**Materials and methods**

Heads of Alopias vulpinus had been donated to the NOAA Southwest Fisheries Science Center, La Jolla, California by anglers as part of the Oceanside Anglers’ Club thresher shark sport fishing tournament in May 2018. Frozen heads were then provided for this project. Three jaws were dissected, two lower and one upper (Figure 1A) and placed in mesh bags and then into a half of a minnow trap that was weighted (Figure 1B), in order to prevent the teeth from being buried in the sediment, as in a previous deployment of mako shark jaws. The trap was deployed near a whale fall ‘Francisco’ at 1018 m depth in Monterey Submarine Canyon (36.77°N 122.08°W) by the ROV ‘Doc Ricketts’ from the R/V ‘Western Flyer’ on 13 December 2018, dive 1105, and recovered on 8 August 2019, dive 1172. Upon collection the remains of the jaws and teeth were removed from the mesh bags and examined for Osedax traces with a Leica S8APO stereomicroscope with a Pentax WG-III handheld camera. Some teeth were dissected open at this point and then fixed and preserved in 95% ethanol. All remains of the jaws and all other teeth including some with Osedax traces were also fixed and preserved in 95% ethanol. These samples were examined further with a Leica MZ9.5 stereomicroscope and photographed with a Canon Rebel T6s camera. A dwarf male found in the tube of one Osedax specimen was photographed with a Leica DMR compound microscope. Osedax specimens were subsampled for DNA analysis and the remaining tissue accessioned into the Benthic Invertebrate Collection at Scripps Oceanography. Mitochondrial cytochrome c subunit I (COI) sequences were obtained following methods outlined previously (Vrijenhoek et al. 2009).

**Results**

There was no trace of any hyaline cartilage after the 8 months on the seafloor. There were some fragments of the outer layer of

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**Figure 1.** (A) Jaws of thresher shark Alopias vulpinus prepared for deployment. (B) Jaws in mesh bags in a weighted basket (black arrow) deployed near a whale fall at 1018 m depth in Monterey Submarine Canyon. (C) Tessellated cartilage fragment from mesh bags recovered after 8 months on the sea floor. The hyaline cartilage had disappeared. There were no obvious signs of Osedax activity on these fragments. (D) Most of the ~40 recovered teeth were intact. (E) Two teeth photographed on the initial recovery before fixation. Each has an obvious Osedax inside the tooth root and an emergent tube. (F) A tooth root dissected on the initial recovery showing a live Osedax, probably O. talkovici. Most of the dentin pulp has been digested, though the enameloid crown of the tooth was untouched.
tessellated cartilage that showed no traces of *Osedax* (Figure 1C). Of the ~40 teeth that were recovered most showed no obvious traces of *Osedax* (Figure 1D). These intact teeth, which had a width of up to 8 mm across the root section, showed a distinct nutritive pore on one side (Figure 1D). Ten teeth were found to have obvious *Osedax* occupying the root area of the tooth and we obtained COI sequences for six of these (GenBank OQ814198-OQ814203), identified as either *Osedax packardorum* or *O. talkovici*. Several teeth that were observed and photographed on the initial recovery (Figure 1E) showed the typical gelatinous tubes of *Osedax* attached to the root portion. The *Osedax* specimens had all retracted, with colours visible inside the tooth root suggestive of *Osedax* roots (green) and crown (red). The outer surfaces of the tooth roots were relatively intact (Figure 1E) but on dissection of one specimen there was no little to no dentin visible inside the root, or the crown of the tooth (Figure 1F).

In one case there was a cluster of four teeth bound together (Figure 2A) and all four showed evidence of *Osedax* (Figure 2B) in their respective roots. One of these specimens was sequenced for COI (GenBank OQ814199) and was found to be *Osedax packardorum* Rouse et al., 2018, with a 98.6% similarity to the sequence of the holotype of *Osedax talkovici* (MG262313). Four other *Osedax talkovici* were also found in other teeth (GenBank OQ814200-OQ814203). The tube of the female specimen in Figure 3A, B was examined, and a single dwarf male was present (Figure 3C, D). The enameloid crowns of the teeth were unaffected with no trace of *Osedax* borings.

**Discussion**

This study provides the first direct evidence for *Osedax* to exploit a substrate other than bone for nutrition. The worms appear to have used the inner parts of the shark teeth, composed of osteodentine and orthodentine (Goto, 1991; Whitenack et al., 2010). The root surfaces were largely intact with erosion only near the *Osedax* tubes. This appears to be a general strategy of *Osedax*, where surface regions of bone are left alone while the roots ramify inside (Rouse & Goffredi, pers. obs.). It was notable that one tooth at least was penetrated by *Osedax* via the nutritive pore (Figure 1E), though based on the position of the tubes, other *Osedax* accessed the interior via other parts of the tooth root, such as the lateral region (Figures 1E, 2B & 3A). Dentin has a similar hardness to bone and its organic matrix is also largely composed of collagen (MacDougall & Javed, 2010). The dentin of shark teeth is about 20% organic matrix (Enax et al., 2012), comparable to that seen in sperm whales (Brault et al., 2014).
and humans (LeGeros, 1981). This makes dentin an obvious food source for Osedax if accessible. The teeth of Alopias vulpinus studied here had roots that obviously could be penetrated by Osedax. In a previous experiment using the jaws of a juvenile mako shark (Isurus oxyrinchus), deployed at a similar depth, no Osedax occurrences were found after 5 months of deployment (Rouse et al., 2011). Both these sharks belong in Lamniformes and so have similar osteodont teeth with the crown full of dentin and no pulp cavity (Jambura et al., 2020). It may be that Osedax larvae simply did not recruit to the mako shark teeth during the deployment period, since they fell to the bottom of the deployment trap and may have been buried, while the teleost vertebrae in the experiment were secured above the sediment (see Figure 1A in Rouse et al., 2011). For the present experiment the thresher shark jaws were placed in mesh bags (Figure 1A), which stopped the teeth being buried when the jaws disintegrated.

There was no evidence of Osedax boring through the outer tooth crown, which is made up of enameloid, a much harder substrate than dentin or bone, and with a much lower proportion of organic matrix (Enax et al., 2012). Also, shark enameloid incorporates significant amounts (2.5% or more) of fluoride (LeGeros, 1981; Miake et al., 1991) and this may resist the acid deployed by Osedax (Tresguerres et al., 2013). These factors could preclude Osedax larvae from surviving long enough to bore through the enameloid crown to access the organic-rich dentin beneath. Kiel et al. (2013) showed evidence of boreholes in the teeth of Oligocene whales, and these also appeared to be restricted to the root with little evidence of boring into enamel crown. Tessellated cartilage, with its collagen matrix (Maisey et al., 2021), is a possible food source for Osedax, however, there was no evidence of Osedax utilizing this calcified sheath that surrounded the hyaline cartilage of the jaw. Further study on the possible utility of the various forms of cartilage found in skeletons of sharks and rays is warranted.

Osedax was discovered living on a whale skeleton and two species were initially described (Rouse et al., 2004). Subsequently Osedax species were shown to be able to utilize other mammal bones, initially with the deployment of cow bones (Jones et al., 2008). This was dismissed as not being ecologically relevant and that Osedax was most likely a whale fall specialist (Glover et al., 2013).
Further deployment experiments revealed that Osedax could colonize the bones of marine teleosts (Rouse et al., 2011), seals (Rouse et al., 2015), marine turtles birds (Rouse et al., 2018) and alligators (McClain et al., 2019), rendering the whale fall specialist argument untenable. Also Osedax appears to have evolved well before the appearance of whales (Vrijenhoek et al., 2009; Danise & Higgs, 2015) and so its original nutritional choice remains unknown. The two species of Osedax found to exploit dentin in this study, O. packardorum and O. talkovi, are phylogenetically quite separated in the context of the overall diversity of the clade, belonging to subclades IV and I, respectively (Rouse et al., 2018). The ability of disparate Osedax lineages to exploit bones as well as dentin suggests calcified collagenous tissues may be an ancestral preference, rather than bone as previously proposed. Rouse et al. (2018) summarized the various bone substrates that had been specifically colonized by different Osedax up to that time. Osedax talkovi was notable for having been found on the widest range of bones deployed at 600–1000 m depth (cow, whale, elephant seal, teleost, turkey, turtle) and to this can now to be added shark teeth. Osedax packardorum lives across a similar depth range as O. talkovi (600–1000 m depth) but had only been found on whale and cow bones until now.

The discovery that Osedax can exploit the dentin of teeth raises the obvious question of the evolutionary origin of the group and its original nutritional source. Modern sharks (crow group Elasmobranchia) have a fossil record extending back into the Devonian (Frey et al., 2019), with bony fishes (Osteichthyes) even older. Thus, bones and teeth have been available as food sources in the sea for over 400 million years. Sharks notably show substantial turnover of teeth in their lifetimes (Botella et al., 2009) with shed teeth falling to the seafloor and potentially being available for Osedax. Osedax is nested within Siboglinidae, which contains taxa such as Frenulata, Sclerolinum and Vestimentifera, taxa that also utilize symbiotic bacteria for their nutrition, though their energy source is hydrogen sulphide or methane from seeps and/or vents (Rouse et al., 2022). Estimates of the origin of Siboglinidae based on molecular clocks have yet to be fully explored, though such estimates for the origin of crown group Osedax and Vestimentifera both fall in the late Cretaceous (Vrijenhoek et al., 2009; Vrijenhoek, 2013; Taborda et al., 2015). The presence of fossils attributed to Osedax and Vestimentifera date to the mid-Cretaceous and Jurassic, respectively (Danise & Higgs, 2015; Georgieva et al., 2019), suggest an earlier origin of Siboglinidae as a whole. Close examination for Osedax traces in fossilized marine bones and teeth from earlier in the Mesozoic and possibly back into the Paleozoic may well be warranted.

Data. All relevant data are within the manuscript or on GenBank.

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References


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