Oxygen isotope composition (δ¹⁸O) in Sandbar Shark teeth as a proxy to environment: developmental and morphofunctional variability

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Oxygen isotope composition ($\delta^{18}O$) in Sandbar Shark teeth as a proxy to environment: developmental and morphofunctional variability

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Abstract: Oxygen isotope composition is a well-known environmental proxy, studied in both fossil and living organisms. In this research project, the ratio of $\delta^{18}O$ was studied in the biomineral of enameloid tissue of Sandbar Shark teeth. All the sharks were kept in the tropical ocean tank of the Blackpool Sea Life Center (UK). The $\delta^{18}O$ in 6 lateral teeth from different individuals have been measured by SIMS and had average ratio of about 21‰. 20 teeth of different morphotypes and developmental ages from one individual have been measured by TC-EA MS and showed average $\delta^{18}O$ values of 22‰, with no significant difference between the teeth of different morphotypes and developmental ages.

Keywords: Oxygen isotopes, Sandbar Shark, enameloid, shark teeth

Introduction

It was 1966 when Longinelli first analyzed the ratio of stable isotopes of oxygen-18 and oxygen-16 in fossil and living organisms, trying to understand their connection to the living environment [1]. This project work concerns the $\delta^{18}O$ ratio in biomineral of Sandbar Shark teeth. The shark teeth are usually divided into three parts: crown, neck and root. The outermost layer of the crown part of the tooth is enameloid. Enameloid is a very hard and compact tissue. Dentine is the inner layer of the crown. Sandbar Shark ($Carcharhinus plumbeus$) has similar tooth as the Great White Shark ($Carcharodon carcharias$). Some of the species has orthodentine among inner layers of the crown, and a large pulp cavity in the center, like $Galeocerdo cuvier$. While $Carcharhinidae$ like Great White Shark and Sandbar Shark only have pallialdentine and osteodentine with no pronounced pulp cavity inside. The root of the tooth usually contains osteodentine only (Fig. 1) [2].

The shape of Sandbar Shark teeth are pointed lower teeth and triangular upper teeth. The combination of these two types of teeth are useful in biting big prey, as Sandbar Shark usually eats large mammals or fish. The serrated edges can cut the large pieces of the prey into smaller ones which are easier to swallow. It is easy to find that the lower teeth are narrower, and the edge of the lower teeth are more finely serrated comparing to the upper ones (Fig. 2). There are differences between the teeth in the same line. Only the front teeth are erect and symmetrical. Besides, the front teeth are extremely oblique when they move towards the corner of the jaw, as a result, they are also the smallest teeth [3].
Shark teeth are counted as follows: rows of teeth are counted along the line of the jaw, while series of teeth are counted from the front of the jaw inwards. One tooth row contains one or more functional teeth at the front of the jaw, and several substitutes behind. Sharks like Bull Shark can have 50 rows of teeth in 7 series. Usually, only the outermost teeth are functional. The small teeth at the joint where left and right halves of the jaws meet, are usually counted separately. They don’t belong to either side. As we all know, shark teeth are replaced very fast, in average losing at least one tooth per week. Because of sharks have so many rows and series of teeth, the shedded tooth can be replaced within only one day [4].

There are 4 main morphotypes of shark teeth: Symphyseal (S), Anterior (A), Lateral (L) and Posterior (P) teeth. S teeth are located at the joint of the jaw and A teeth locate beside them which are quite big. Sandbar Sharks usually have 3 A teeth on one side of each jaw. L teeth go next to the A teeth and are also big and more numerous than A teeth. At the end of the jaw are P teeth which are smaller than A and L teeth. Figure 3 is a picture of the developmental model of dentition in alternate file order in Grey Reef sharks which also belongs to Carcharhinidae family, same as Sandbar Shark. Single cusp teeth are first initiated along the jaw, formed as mineralized tissue in embryos with one tooth row (stage 1), then two rows (stage 2) and, later in development, nine tooth rows (stage 9). Jaw positions (distal to proximal) numbered 1 – 12 from the symphyseal tooth (S), first as even number positions, then odd in the second
row. Smallest teeth (black, stage 1) then larger alternate teeth with pointed shape (grey, stage 2); later, larger teeth with lateral cusps form row 3. Sequential tooth initiation in a clonal set (arrows, direction of timing for teeth 1 – 9) shows the alternate timing of tooth initiation order in adjacent tooth files 6 and 7 (SAT unit tf 6+7), with the next putative tooth germ (pg) to form in odd number row position. An example, as if it was a single file, a sequential addition model is superimposed on this alternate model at file position 2 [5].

Fig. 3 The development stages of Grey Reef Shark teeth [5].

Fig. 4 The teeth from the upper jaw (left) and the lower jaw (right) of the Sandbar Shark [6].

The δ¹⁸O in shark tooth mainly comes from the phosphate and carbonate oxides of the fluorapatite[2]. The ratio of δ¹⁸O is depends on the environment, or the δ¹⁸O ratio of the ambient water. As we all know, H₂δ¹⁸O molecule is heavier than normal water molecule which means H₂δ¹⁸O is more difficult (need higher temperature) to evaporate into the atmosphere. As a result, tropical sea water has lower δ¹⁸O ratio compared to the cold sea water. By analyzing the ratio of ¹⁸O and ¹⁶O in shark teeth, we can easily know the temperature of the sea water where the shark lives, or at least the temperature where the shark stays when this tooth developed. From the difference in δ¹⁸O between the teeth of the same or different individuals we can we can better understand their lifestyle. Measuring the ratio of δ¹⁸O in fossil
shark teeth, we can reveal their ecology and environment. The environment of ancient earth, including isotopic composition of ocean water, could be quite different from today, and $\delta^{18}O$ from shark teeth is one of the tools to help us to understand the ancient world. Oxygen isotopes have been used in measuring dinosaur body temperature \[^7\]. Other isotopes, such as $\delta^{14}C$, are also widely used in archeology and paleontology \[^8\].

**Materials and Methods**

26 teeth of the Sandbar Shark (*Carcharhinus plumbeus*) have been analyzed. Sharks were kept in the tropical ocean tank of the Blackpool Sea Life Center (UK), and their teeth were collected from the tank substrate after being shedded naturally. All the teeth analyzed were grown in the same monitored constant water temperature (~24.5°C) and salinity, seawater being recharged every 2 days directly from the Irish Sea.

6 teeth for SIMS analyses, inside the tooth/enameloid variability – collected from aquarium substrate (could be from several individuals), 20 of teeth using TC-EAMS mass spectrometry, one values per tooth (enameloid) – from one individual.

**SIMS**

Six teeth of the Sandbar Shark (*Carcharhinus plumbeus*) have been analyzed. Sharks were kept in the tropical ocean tank of the Blackpool Sea Life Center (UK), and their teeth were collected from the tank substrate after being shedded naturally. All the teeth analyzed were grown in the same monitored constant water temperature (~24.5°C) and salinity, seawater being recharged every 2 days directly from the Irish Sea.

Preparation of samples was performed in the Laboratory of Isotope Geology at the Natural History Museum of Stockholm (Sweden). Cleaned and sectioned teeth were mounted in epoxy resin to obtain best histological exposure, polished and gold-coated. $^{18}O/^{16}O$ isotope ratio analyses were performed in-situ by secondary ionization mass spectrometry (SIMS) by a high precision and high spatial-resolution CAMECA IMS 1280 ion microprobe following methods similar to those described by Whitehouse & Nemchin \[^9\], utilizing a critically focused Cs$^+$ primary beam with a spot size of ca. 10 μm, a low energy, normal incidence electron flooding device for charge compensation and simultaneous detection of $^{16}O$ and $^{18}O$ in two Faraday detectors. Durango apatite mounted together with the teeth was used as the primary isotope standard, assuming a $\delta^{18}O$ VSMOW value of 9.40 ‰ (where V-SMOW refers to Vienna Standard Mean Ocean Water) independently determined by gas source isotope ratio mass spectrometry at the Stable Isotope Laboratory of the University of Erlangen-Nuremberg, Germany. All the $\delta^{18}O$ values below are given in VSMOW. Two pairs of Durango analyses bracketed each batch of 6 - 8 unknowns, with a mean standard deviation of its replicate analyses over the analytical sessions of 0.34 ‰, ±0.01% (1SD), which was propagated together with the withen run uncertainty. Minor linear drift corrections of between 0.015‰ to 0.03‰ per run were applied over each session to minimize this external uncertainty. All the analyses, including sample preparation, were performed at the Swedish Natural History Museum, Stockholm.
TC-EA MS

20 of S, A, L and P teeth were analyzed at the University of Erlangen, Germany. Tissue-selectively scratched mineral powder samples of enameloid (each sample 0.8 to 1 mg) have been dissolved in nitric acid and have been chemically converted into Ag₃PO₄, using the method described by Joachimski et al. [10]. Oxygen isotopes have been measured on CO generated by reducing tri-silver phosphate using a high-temperature conversion-elemental analyzer (TC-EA). The analyzer has been connected online to a Thermo Finnigan Delta Plus mass-spectrometer. From each of the samples three measurements have been made. Accuracy and reproducibility of measurements have been monitored by multiple analyses of laboratory prepared tri-silver phosphate and of the NBS120c tri-silver phosphate standards. The average oxygen isotope composition of the laboratory standard has been 17.2‰ of Vienna Standard Mean Ocean Water (V-SMOW), and the mean δO18 value of NBS120c has been 22.4‰ V-SMOW, which is relatively close to the value of 22.58‰ V-SMOW determined by Vennemann et al. [11]. The overall reproducibility has been determined by replicate analyses of tri-silver phosphate standards, and replicate sample analysis has been better than ±0.2‰ (1σ).

Result

Fig. 5 shows the ratio of δ¹⁸O in the enameloid of the L-teeth Tooth 5-10. Tooth 10 has the highest average value 23.19‰, 1.47‰ higher than Tooth 8 which has the lowest value with 21.72‰ in average. Fig. 6 shows the ranges of each tooth which we can see that Tooth 8 has the largest range from 20.43‰ to 23.21‰ in span of 2.78‰. The tooth which has the smallest range is Tooth 7, from 22.01‰ to 23.68‰ in span of 1.66‰, and standard deviation of it is also the lowest one among the L-teeth within 0.37‰. What is interesting that although Tooth 8 has a wide range, it has the second lowest standard deviation within only 0.39‰, a little bit higher than Tooth 7(fig. 7). The values of Tooth 5 are very scattered with a standard deviation of 0.79‰. Beside Tooth 10 and Tooth 8, the other four L-teeth’s averages are between 22‰ and 23‰ therefore we can say that the overall ratio of δ¹⁸O in enameloid of L-teeth is around 22‰.
Fig. 5  The values of the teeth Tooth 5-10.

Fig. 6  The averages, standard deviations and data variability of each group.
Fig. 7  The relationship of average and standard deviation.

<table>
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<tr>
<th>Tooth</th>
<th>$\delta^{18}O/%$</th>
<th>Variability/%</th>
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<tr>
<td>L40</td>
<td>21.68</td>
<td>0.31</td>
</tr>
<tr>
<td>S22</td>
<td>21.72</td>
<td>0.11</td>
</tr>
<tr>
<td>A32</td>
<td>20.89</td>
<td>0.11</td>
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<tr>
<td>L52</td>
<td>21.34</td>
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<tr>
<td>L52</td>
<td>21.00</td>
<td>0.33</td>
</tr>
<tr>
<td>P52</td>
<td>21.66</td>
<td>0.35</td>
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<tr>
<td>P52</td>
<td>21.87</td>
<td>0.17</td>
</tr>
<tr>
<td>A25</td>
<td>22.00</td>
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</tr>
<tr>
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</tr>
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<td>L55</td>
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<tr>
<td>P15</td>
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<tr>
<td>A25</td>
<td>21.33</td>
<td>0.31</td>
</tr>
</tbody>
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N2-Peak
Fig. 8 The ratio of $\delta^{18}$O in different morphotypes of teeth. Letters S, A, L and P indicate the morphotypes of shark teeth, which are symphyseal teeth, anterior teeth, lateral teeth and posterior teeth. The first number in the name of the tooth marks the lateral position of the tooth and the second number marks which row the tooth locate, so the second number also reflects the age of the tooth. For example, the tooth A32 means the third anterior tooth count from the middle which locates in the second row. Those teeth which share the same name were located in same position, but on the different sides of the jaw.

Discussion

All the six L-teeth have an average ratio of $\delta^{18}$O at a level of 22‰ indicates that there are no significant differences among L-teeth, even when they come from different individual. As all the teeth are from the Sandbar Sharks which were living in the same environment, therefore any variation could only be due to biological factors or caused by measuring techniques. The data variability of the average values is 1.47‰, even less than the data variability of single tooth, which suggests that there is no significant difference in $\delta^{18}$O among these six L-teeth. The reason of the high variability of each tooth could be various. It is easy to think that the mass-spectrometer has inherent deviation and each value has an error at a level of 0.40‰, takes up less than 2% of the overall amount. However, there is still variability even within the consideration of the mass-spectrometer deviation. Sometimes organic impurity, such as collagen from dentine canals, can influence the result. As in-situ SIMS technique takes such a small mass for individual measurement, this kind organsics-related inaccuracy can be expected. When analyzing all the values, I find that the data follow normal distribution. (Fig. 9) Despite the number of samples in relatively small, I can still figure out that most of the data is located near the average. The further from the average, the less dense data points become. Although this is not enough to claim that the data follow normal
distribution, I can still consider the reason of the deviation is accidental error, which happens randomly in some cases.

Fig. 9 It shows where most of the data are located. I have chosen three teeth with most numerous data points. The data points gather near the average value.

As for different rows and different morphotypes of the teeth, the variability of average $\delta^{18}O$ is 0.27‰ and 0.53‰ respectively, smaller than the variability of the teeth of the same morphotype. This is no doubt due to the different measuring technique: these data were obtained from the bulk enameloid powder samples by TCEA-MS (see Methods section). The difference takes up only 2% of the overall amount. There is also no obvious trend among different rows or morphotypes. This indicates that the ratio of $\delta^{18}O$ in the enameloid of these Sandbar Sharks’ teeth has no relationship with age and morphotype. In-situ measurements of $\delta^{18}O$ (intra-tooth variability) therefore appear less reliable than conventional (TCEA-MS) mass-spectrometry of enameloid powder.

Conclusion

According to the results, we can conclude that the Sandbar Shark kept living in same environment, confirming known initial condition of this experiment. And most importantly, I did not observe any difference in the ratio of $\delta^{18}O$ among the different teeth of different developmental age, or different...
morphotypes. Also, there was no significant difference between the teeth of same morphotype from different individuals. Therefore, I can conclude that there is no biological variability in $\delta^{18}$O ratio in Sandbar Shark.

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References