# Calcium isotopes reveal the trophic position of extant and fossil elasmobranchs

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## ABSTRACT

Recent calcium isotope studies recovered a trophic level effect in marine ecosystems. However, elasmobranchs are virtually absent from such studies despite their important ecological role, their diversity and their extensive fossil record. Enameloid of extant elasmobranchs representing differing known ecologies were measured for $\delta^{44/42}$Ca. The results reveal that their calcium isotope values have a distribution (from $-0.27$ to $-0.78$‰) that follows a stepwise decrease ($\Delta^{44/42}$Ca) of about $-0.14$‰ across recognized trophic levels: zooplanktivores, primary, secondary, and tertiary consumers. Although the recovered calcium isotope distributions partly match the ecological divisions of extant elasmobranchs, data for marine mammals are more variable and cannot be explained by trophic segregation alone. Nevertheless, our results reopen perspectives for the study of ancient marine assemblages using calcium isotopes. As a case study, the calcium isotope values of a Pliocene fish assemblage parallel the results obtained from extant elasmobranchs and allow inferring that the giant shark *Megaselachus megalodon* fed at a slightly higher trophic level than the contemporaneous Great White shark.

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## 1. Introduction

The isotope reconstruction of dietary ecology and foraging preferences in living marine animals is mostly restricted to carbon and nitrogen isotopes in soft tissues (Minagawa and Wada, 1984). The elemental strontium/calcium (Sr/Ca) and barium/calcium (Ba/Ca) ratios offer an alternative for the fossilizable mineral phase of bone and teeth (Peek and Clementz, 2012). However, diagenesis (Balter et al., 2011) and seawater temperature (Balter and Lécuyer, 2010) are two independent parameters that can overprint the primary dietary Sr/Ca and Ba/Ca signatures. To date, there is little work on alternate isotope markers such as calcium, which is particularly bioavailable in the ocean (average concentration of ~ 420 ppm e.g. Elderfield and Schultz, 1996) and incorporates the shell and bioapatite skeleton of both invertebrate and vertebrate organisms. Moreover, the isotope composition of seawater is identical at the global scale due to the relatively long residence time of calcium in the ocean (~1 Ma e.g. Zhu and Macdougall, 1998) and seawater calcium composition has not significantly changed in the last 5 Ma (De La Rocha and De Paolo, 2000; Fantle and DePaolo, 2005; Griffith et al., 2008) relative to the biological variation measured in previous work (Clementz et al., 2003). Early work on calcium isotopes showed a significant decrease in calcium isotope ratios with increasing

trophic level in both terrestrial and marine ecosystems (Skulan et al., 1997; Skulan and DePaolo, 1999). Subsequently, Clementz et al. (2003) analyzed a number of extant and fossil marine mammals and showed that their calcium isotope values also matched the first noted trend in decreasing values with increasing trophic level.

Because of their high diversity and abundance worldwide, elasmobranchs play an important role in marine ecosystems and previous studies have shown that large sharks have the potential to shape marine communities (Ferretti et al., 2010). But elasmobranch calcium isotope data are virtually absent from the literature. Due to their breadth of feeding ecology (from zooplanktivores to tertiary consumers), the various species of elasmobranchs provide a testable mean to assess the utility of calcium isotopes in dietary reconstruction. If there is a trophic effect on calcium isotopes for marine invertebrates and mammals, then this should also be reflected in extant elasmobranchs, which have high modern diversity, and are also abundant in the fossil record.

Here, we examine the calcium isotope composition of tooth apatite from 18 extant species of elasmobranchs of known ecologies (Table 1) using Multi-Collection Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS). We show that significant differences exist between elasmobranch groups with different feeding ecologies. We discuss the calcium isotope variability of elasmobranchs in the context of marine food webs and finally assess the utility of this geochemical tool in paleoecology with a case study from two Pliocene (*ca.* 5 Ma) fish assemblages.

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**Table 1**

Ca stable isotope values of elasmobranch teeth, fish teeth and marine mammal bone and teeth analyzed in this study together with taxonomic and ecological information. [1]: consumer level inference from this study; for body lengths: *: estimate; ** extrapolation from Adnet et al. (2010); ***extrapolation from Gottfried et al. (1996). Abbreviations: n, replicates; TL: total length for sharks; DW: disk width for rays.

| Taxon | Vernacular name | Diet | Provenance | Coll number | Habitat | Consumer level | Total Length or Disc Width (cm) | δ44/42Ca (‰ ICP Ca Lyon) | 2SD | replicate analysis | δ44/42Ca (‰ IAPSO SW) | 2SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Extant* | | | | | | | | | | | | |
| *Cetorhinus maximus* | basking shark | plankton | North Sea | REC0946M | pelagic | zooplanktivore | 383 TL | −0.48 | 0.08 | 5 | −0.89 | 0.10 |
| *Manta birostris* | manta ray (male) | plankton | Baja California | NS83-003 | pelagic | zooplanktivore | 500* DW | −0.27 | 0.07 | 3 | −0.68 | 0.09 |
| *Scyliorhinus stellaris* | spotted dogfish/nursehound | opportunistic | Mediterranean Sea | REC0185M | benthic | primary | 90 TL | −0.51 | 0.03 | 3 | −0.92 | 0.07 |
| *Mustelus punctalatus* | houndshark | crustaceans | Mediterranean Sea | REC0321M | demersal | primary | 121 TL | −0.38 | 0.10 | 6 | −0.79 | 0.12 |
| *Myliobatis aquila* | common eagle ray | mainly malacophagous | Mediterranean Sea | REC1119M | benthic | primary | max. 100* DW | −0.50 | 0.05 | 4 | −0.90 | 0.08 |
| *Oxynotus centrina* | angular roughshark | invertebrates | Mediterranean Sea | REC0251M | demersal | primary | 72 TL | −0.45 | 0.05 | 3 | −0.86 | 0.08 |
| *Dasyatis centroura* | roughtail stingray | invertebrates | Mediterranean Sea | REC0627M | benthic | primary | 85 TL | −0.47 | 0.06 | 3 | −0.88 | 0.09 |
| *Odontaspis ferox* | small-toothed sandtiger shark | fish, crustaceans | Pacific | - | demersal | secondary | max 300* TL | −0.57 | 0.03 | 2 | −0.98 | 0.07 |
| *Galeorhinus galeus* | school shark | piscivorous/opportunistic | Mediterranean Sea | REC0347M | demersal | secondary | 146 TL | −0.57 | 0.07 | 3 | −0.98 | 0.09 |
| *Carcharhinus brevipinna* | spinner shark | piscivorous | Mediterranean Sea | REC0270M | pelagic | secondary | 95 TL | −0.54 | 0.06 | 3 | −0.95 | 0.09 |
| *Prionace glauca* | blue shark | piscivorous/opportunistic | Mediterranean Sea | - | pelagic | secondary | max 300* TL | −0.59 | 0.09 | 4 | −1.00 | 0.11 |
| *Lamna nasus* | porbeagle shark | piscivorous | Mediterranean Sea | REC0136M | pelagic | secondary | 140 TL | −0.58 | 0.19 | 4 | −0.99 | 0.20 |
| *Squalus acanthias* | spiny dogfish | piscivorous/opportunistic | Mediterranean Sea | REC0223M | demersal | secondary | 72 TL | −0.58 | 0.12 | 3 | −0.99 | 0.13 |
| *Alopias vulpinus* | common thresher shark | piscivorous | Mediterranean Sea | REC0159M | pelagic | secondary | 404 TL | −0.63 | 0.15 | 3 | −1.04 | 0.16 |
| *Dalatias licha* | kitefin shark | opportunistic (mainly fish) | Mediterranean Sea | REC0254M | demersal | secondary | 87 TL | −0.46 | 0.08 | 5 | −0.87 | 0.10 |
| *Carcharodon carcharias* | white shark | fish, turtles, birds, mammals | unknown | - | pelagic | tertiary | 300* TL | −0.74 | 0.07 | 3 | −1.15 | 0.09 |
| *Hexanchus griseus* | sixgill shark | opportunistic (inc. dead mammals) | Mediterranean Sea | REC0201M | demersal | tertiary | 58 TL | −0.66 | 0.06 | 3 | −1.07 | 0.08 |
| *Hexanchus griseus* | sixgill shark | opportunistic (inc. dead mammals) | Mediterranean Sea | REC0204M | demersal | tertiary | 200 TL | −0.68 | 0.13 | 3 | −1.09 | 0.14 |
| *Carcharodon carcharias* | white shark | fish, turtles, birds, mammals | Mediterranean Sea | REC0610M | pelagic | tertiary | max 400* TL | −0.78 | 0.12 | 4 | −1.19 | 0.13 |
| Mysticete indet. | whale (vertebra) | plankton | unknown | ISEM-no num | pelagic | zooplanktivore | - | −0.12 | 0.10 | 5 | −0.53 | 0.12 |
| *Dugong dugon* | dugong (tooth) | seagrass | unknown | N170 | coastal | herbivore | - | −0.67 | 0.10 | 3 | −1.08 | 0.12 |
| *Dugong dugon* | dugong (bone) | seagrass | unknown | N170 | coastal | herbivore | - | −0.83 | 0.05 | 4 | −1.24 | 0.08 |
| *Odobenus rosmarus* | walrus (enamel) | invertebrates, fish | unknown | N374 | coastal | omnivore | - | −0.86 | 0.09 | 4 | −1.27 | 0.11 |
| *Odobenus rosmarus* | walrus (bone) | invertebrates, fish | unknown | N374 | coastal | omnivore | - | −1.07 | 0.16 | 4 | −1.48 | 0.17 |
| *Tursiops truncatus* | dolphin (tooth) | mainly fish | unknown | ISEM-no num | pelagic | piscivore | - | −1.33 | 0.07 | 5 | −1.74 | 0.09 |
| *Tursiops truncatus* | dolphin (bone) | mainly fish | unknown | ISEM-no num | pelagic | piscivore | - | −1.64 | 0.11 | 4 | −2.05 | 0.13 |
| *Fossil* | | | | | | | | | | | | |
| Sphyraenidae indet | barracuda | - | Early Pliocene, Libya | ALA 041 | - | primary[1] | max. 100* TL | −0.61 | 0.13 | 5 | −1.02 | 0.14 |
| Sparidae indet. | seabream | - | Early Pliocene, Libya | ALA 042 | - | primary[1] | max. 200* TL | −0.44 | 0.08 | 3 | −0.85 | 0.10 |
| *Myliobatis* sp. | shell-crushing ray | - | Early Pliocene, Libya | ALA 040 | - | primary[1] | max. 70* DW | −0.45 | 0.05 | 2 | −0.85 | 0.08 |
| *Carcharodon carcharias* | white shark | - | Early Pliocene, Libya | ALA 005 | - | tertiary[1] | 450** TL | −0.72 | 0.09 | 5 | −1.13 | 0.11 |
| *Megaselachus megalodon* | megalodon | - | Early Pliocene, Libya | ALA 001 | - | tertiary[1] | 700*** TL | −0.87 | 0.13 | 5 | −1.28 | 0.14 |
| *Megaselachus megalodon* | megalodon | - | Pliocene, New Caledonia | - | - | tertiary[1] | 900*** TL | −0.87 | 0.08 | 3 | −1.28 | 0.10 |

## 2. Methods

### 2.1. Sample collection

Cubes of enameloid of extant elasmobranch teeth were sampled from complete teeth of 19 individuals representing different species curated in the collections of the Institut des Sciences de l'Evolution de Montpellier. In addition, two fossil fish assemblages from Pliocene nearshore environments of Libya (Pawellek et al., 2012) and New Caledonia (Seret, 1987) were also sampled. Finally, four marine mammals were also selected and include a whale bone fragment, as well as a dugong, a walrus and a dolphin sampled for bone and teeth. Small fragments weighting about half a milligram were obtained using a sharp blade under a binocular microscope. Detailed information about the samples is provided in Table 1. Tooth crowns were systematically selected and for each sample, enameloid was sampled.

### 2.2. Analytical techniques

Chips of tooth apatite were completely dissolved in 1 ml ultrapure concentrated $HNO_3$ overnight, then evaporated and re-dissolved in 0.5 ml 2.0 M $HNO_3$. At this stage, an aliquot of the solutions was taken for concentration analysis of calcium and other elements. This was done on an ICP-AES (iCAP 6000 Series Thermo Electron Corporation).

Calcium was purified as a two-step process from the remaining solution using Eichrom Sr-specific resin (Sr-spec Eichrom®) then cation-exchange resin (AG-50WX-12, 200–400 mesh) with ultrapure solutions of nitric and hydrochloric acids as the elution agents (see detailed method in Tacail et al., 2014). Over 90% of the calcium was collected in order to avoid fractionation processes during column separation. Typical procedural blanks were 0.1 μg while samples were 200–400 μg of calcium, which represents 2000 to 4000 times the size of the procedural blank, therefore being negligible.

The purified fraction of calcium was measured for Ca isotopes on a Thermo Neptune Plus MC-ICP-MS using an Aridus for sample introduction (see Tacail et al., 2014 for details). All samples were diluted in 0.05 M $HNO_3$ before uptake to a concentration of 3 ppm. Measurements were conducted at medium resolution, with each analysis consisting of 40 measurements of 4.2 s integrations on $^{42}Ca^+$, $^{43}Ca^+$ and $^{44}Ca^+$ ions in static mode. 43.5 $m/z$ signals, corresponding to $^{87}Sr^{2+}$, were measured for correction of Sr double charge interferences on Ca isotopes ($^{88}Sr^{2+}$ on $^{44}Ca^+$, $^{86}Sr^{2+}$ on $^{43}Ca^+$, and $^{84}Sr^{2+}$ on $^{42}Ca^+$). Each analysis was preceded by a washout pumping in 0.5 M $HNO_3$ using the Aridus quickwash module. A second washout pumping was carried out in 0.05 M $HNO_3$ and measured as blank, this background being subtracted online before calculation of isotope ratios. Delta values were obtained using the standard bracketing method with the Lyon ICP Ca standard, referred to as ICP Ca Lyon (Tacail et al., 2014) and issued from a Specpure calcium plasma standard solution (Alfa Aesar). SRM915b and SRM1486 standards were used as secondary standards. For each analytical session, samples, SRM1486 and SRM915b were measured in turn. Such a sequence was repeated at least twice to check so that a given sample was replicated. Uncertainties are reported in Table 1 and represent 2 standard deviations of these analyses.

$\delta^{44/42}Ca$ values presented in Table 1 are defined as:

$$\delta^{44/42}Ca = \left( \left( \left(^{44}Ca/^{42}Ca_{sample}\right) / \left(^{44}Ca/^{42}Ca_{ICP\ Ca\ Lyon}\right) \right) - 1 \right) * 1000 \quad (1)$$

where $\delta^{44/42}Ca$ is the normalized difference in per mil (‰) between a sample and our in-house ICP Ca Lyon standard. All measurements in this work are expressed in $\delta^{44/42}Ca$. All $\delta^{44/40}Ca$ data from literature were converted to $\delta^{44/42}Ca$ values by dividing by a 1.9996 factor, calculated using the power fractionation law.

We report a mean $\delta^{44/42}Ca$ value of $-0.15 \pm 0.11$‰ (2SD, n = 13) for the SRM915b standard and a value of $-1.04 \pm 0.11$‰ (2SD, n =

25) for SRM1486, which are close to values published in Tacail et al. (2014) i.e. $-0.12 \pm 0.07$‰ (2SD, n = 11) and $-0.96 \pm 0.14$‰ (2SD, n = 17) respectively. The measured SRM1486 isotope composition is also close to the other published values, which yield a mean value of $-1.00 \pm 0.07$‰, as detailed in Table 2. All measured samples plotted as $\delta^{43/42}Ca$ against $\delta^{44/42}Ca$ fall on a line with a slope of 0.519 ± 0.020, 2SE, which is in general agreement with the 0.5067 slope predicted by the linear approximation of exponential mass-dependent fractionation (Fig. 1).

### 2.3. Intercalibration of Ca isotope compositions

According to the IAPSO seawater values obtained with TIMS and MC-ICP-MS, compiled in Fantle and Tipper (2014) and available in Table 2 (n studies = 16), the average $\delta^{44/42}Ca_{IAPSO-915a} = 0.92 \pm 0.07$‰ (2SD). Considering that $\delta^{44/42}Ca_{915a-915b} = -0.37 \pm 0.08$‰ (2SD) (Heuser and Eisenhauer, 2008; Reynard et al., 2010; Hindshaw et al., 2011; Colla et al., 2013), and that $\delta^{44/42}Ca_{ICP-915a} = 0.15 \pm 0.11$ (2SD, n = 13), the value of $\delta^{44/42}Ca_{IAPSO-ICP\ Ca\ Lyon} = 0.41 \pm 0.07$‰ (2SD). This value is identical to the value measured by Tacail et al. (2014) on the same instruments in Lyon for seawater (0.41 ± 0.06, 2SD, n = 2) as well as seawater measured during the sessions of this present dataset (0.41 ± 0.12, 2SD, n = 5; Table 2).

Data compiled in Blättler et al. (2012) presenting invertebrate values relative to IAPSO were converted to ICP Ca Lyon using the compiled IAPSO values (Table 2). In the present study, our bracketing in-house standard is a Specpure calcium plasma standard solution (Alfa Aesar) referred to as ICP Ca Lyon below (see also Tacail et al., 2014). Normalizing standards used in previous studies are summarized in Table 2. Skulan et al. (1997) then Skulan and DePaolo (1999) used an ultrapure calcium carbonate as their in-house normalization standard. In order to permit comparisons of our results with those of the literature, variations in the calcium isotope ratios need to be expressed relative to a single normalization standard. However, there is no international normalization standard but several in-house normalization standards. Skulan et al. (1997) and Skulan and DePaolo (1999) report seawater $\delta^{44/40}Ca$ values of 0.92 ± 0.18‰ and 0.86 ± 0.04‰ (2SD), respectively, which corresponds to $\delta^{44/42}Ca$ values of 0.46 ± 0.09‰ and 0.43 ± 0.02‰ (2SD). This corresponds to a mean value of 0.45 ± 0.04‰ (2SD), which is 0.04‰ higher than our measured seawater value at 0.41‰. Then, we chose to correct the values reported in Skulan et al. (1997) and Skulan and DePaolo (1999) datasets by subtracting 0.04‰. Thanks to this slight correction, these data are comparable with our dataset. On the other hand, the data published by Clementz et al. (2003) are ambiguous to compare with our dataset. This is because Clementz et al. (2003) did not use a seawater standard for interlaboratory comparison. Despite this, Clementz et al. (2003) directly compared their data with those of Skulan et al. (1997) and Skulan and DePaolo (1999) because they did replicate two taxa of marine mammals previously analyzed by these authors. As purely indicative, we added their data in our Fig. 2. We also added a few marine mammal data in order to be able to compare marine mammal values with those of elasmobranchs.

## 3. Results

All measured data (both extinct and modern) as well as previously published data are reported in Table 1, Figs. 1 and 2 and in the supplementary material. All measured elasmobranch values for $\delta^{44/42}Ca$ in this study have a range of 0.5‰, from $-0.27$ to $-0.78$‰. Values for marine mammals analyzed in this study range from $-0.12$ to $-1.64$‰. Including previously published data, the total range of values for marine organisms, normalized to ICP Ca Lyon, is 1.71‰, with a $\delta^{44/42}Ca$ value for seawater being the most positive (0.41‰) and a bone of a dolphin being the most negative ($-1.64$‰).

The 18 extant elasmobranch values have an intermediate position between invertebrates, fishes and mammals representing higher-level

**Table 2**

Ca isotope compositions of several standards, relative to ICP Ca Lyon. Conversions of literature values to ICP Ca Lyon standard were carried out using a SRM915a $\delta^{44/42}$Ca value of $-0.37‰$ relative to SRM915b (Heuser and Eisenhauer, 2008; Reynard et al., 2010; Hindshaw et al., 2011; Colla et al., 2013) and the measured SRM915b $\delta^{44/42}$Ca value of $-0.15‰$ relative to ICP Ca Lyon. For each standard with literature values, average $\delta^{44/42}$Ca of all published values is given together with detailed isotope compositions from each publication. TIMS or MC-ICP-MS measurement technique is also presented as well as the number $n$ of measurements for each value. The * corresponds to propagated error.

| Standard | $\delta^{44/42}$Ca (‰, rel to ICP Ca Lyon) | | | | Reference | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SRM915b | −0.15 | ± | 0.11 | (2SD, $n = 13$) | This study | | | | |
| | −0.12 | ± | 0.07 | (2SD, $n = 11$) | Tacail et al. (2014) | | | | |
| SRM1486 | −1.04 | ± | 0.11 | (2SD, $n = 25$) | This study | | | | |
| | −0.96 | ± | 0.14 | (2SD, $n = 17$) | Tacail et al. (2014) | | | | |
| | −1.01 | ± | 0.05 | (2SD) | Heuser and Eisenhauer (2008) (TIMS) | −1.02 | ± | 0.14 | (2SD*, $n = 142$) |
| | | | | | Heuser et al. (2011) (TIMS in Mainz, Germ.) | −1.03 | ± | 0.14 | (2SD*) |
| | | | | | Heuser et al. (2011) (TIMS in Münster, Germ.) | −0.98 | ± | 0.14 | (2SD*) |
| Seawater | 0.41 | ± | 0.12 | (2SD, $n = 5$, Belize) | This study | | | | |
| | 0.41 | ± | 0.06 | (2SD, $n = 2$, Belize) | Tacail et al. (2014) | | | | |
| | 0.41 | ± | 0.07 | (2SD, IAPSO seawater) | Wieser et al. (2004) (MC-ICPMS) | 0.36 | ± | 0.18 | (2SD*) |
| | | | | | Chang et al. (2004) (MC-ICPMS) | 0.40 | ± | 0.19 | (2SD*) |
| | | | | | Steuber and Buhl (2006) (MC-ICPMS) | 0.38 | ± | 0.17 | (2SD*) |
| | | | | | Hippler et al. (2013) (MC-ICPMS) | 0.37 | ± | 0.17 | (2SD*) |
| | | | | | Farkaš et al. (2007a,b) EPSL (MC-ICPMS) | 0.36 | ± | 0.18 | (2SD*) |
| | | | | | Amini et al. (2008) (TIMS) | 0.41 | ± | 0.31 | (2SD*) |
| | | | | | Amini et al. (2009) (TIMS) | 0.37 | ± | 0.21 | (2SD*) |
| | | | | | Böhm et al. (2006) (TIMS) | 0.40 | ± | 0.56 | (2SD*) |
| | | | | | Farkaš et al. (2007a,b) EPSL (TIMS) | 0.41 | ± | 0.24 | (2SD*) |
| | | | | | Farkaš et al. (2011) (TIMS) | 0.43 | ± | 0.26 | (2SD*) |
| | | | | | Farkaš et al. (2007a,b) GCA (TIMS) | 0.41 | ± | 0.20 | (2SD*) |
| | | | | | Hippler et al. (2003) (TIMS) | 0.49 | ± | | n.a. |
| | | | | | Huang et al. (2010) (TIMS) | 0.43 | ± | 0.18 | (2SD*) |
| | | | | | Huang et al. (2012) (TIMS) | 0.43 | ± | 0.19 | (2SD*) |
| | | | | | Müller et al. (2011) (TIMS) | 0.46 | ± | 0.29 | (2SD*) |
| | | | | | Teichert et al. (2009) (TIMS) | 0.37 | ± | 0.17 | (2SD*) |
| SRM915a | −0.52 | ± | 0.08 | (2SD) | Colla et al. (2013) (MC-ICP-MS) | −0.52 | ± | 0.11 | (2SD*, $n = 2$) |
| | | | | | Heuser and Eisenhauer (2008) (TIMS) | −0.49 | ± | 0.19 | (2SD*, $n = 56$) |
| | | | | | Hindshaw et al. (2011) (TIMS) | −0.50 | ± | 0.17 | (2SD*, $n = 46$) |
| | | | | | Reynard et al. (2010) (MC-ICP-MS) | −0.58 | ± | 0.21 | (2SD*, $n = 38$) |
| BSE | −0.02 | ± | 0.02 | (2SE) | Concept as defined in Nielsen et al. (2011) | | | | |

consumers (Fig. 2). Their main feeding habits have been synthetized from the literature (Cortes, 1999; Wertherbee and Cortés, 2004; Frisk, 2010).

Elasmobranch isotope values are distributed along three groups, which encompass the four ecological groups established in the literature (zooplanktivores, primary, secondary and tertiary consumers), with some overlap between their lower and upper ends. The group



**Fig. 1.** Mass fractionation line for all standards and samples measured in this study. Black diamonds: elasmobranch and teleost samples; white circles: marine mammals; white squares: SRM1486 and SRM915b; reversed triangle: seawater.

with the most positive values (average value of $-0.44‰ \pm 0.08$ 1SD; n = 7, 7 sp.) includes two ecological groups: elasmobranchs that feed on plankton (*i.e.* zooplanktivores) and primary consumers that feed on benthic invertebrates (*i.e.* rays and small sharks). The second group comprises elasmobranchs that feed on pelagic cephalopods and fishes (*i.e.* secondary consumers) (average value of $-0.57‰ \pm 0.05$ 1SD; n = 8, 8 sp.) and differs significantly from the group feeding on benthic invertebrates (Wilcoxon–Mann–Whitney, $p^{**} = 0.0064$). The third group has the most negative $\delta^{44/42}$Ca values and includes relatively large elasmobranchs that feed on fishes, turtles and mammals (*i.e.* tertiary consumers) (average value of $-0.72‰ \pm 0.05$ 1SD; n = 4, 2 sp.), which significantly differs from elasmobranchs that feed on pelagic cephalopods and fishes (Wilcoxon–Mann–Whitney, $p^{**} = 0.0082$).

To the exception of a whale bone ($-0.12‰$), the three other marine mammals (dugong, walrus and dolphin) show the most negative values of the dataset from $-0.67$ to $-1.64‰$. For each of these three individuals, bone is systematically lower than tooth (enamel + dentine) by $0.23‰$ on average.

## 4. Discussion

### 4.1. Calcium isotopes and elasmobranch ecology

Before conducting our calcium isotope analysis, we selected elasmobranch teeth that are representative of the diversity of feeding ecologies reported in the literature (Cortes, 1999; Wertherbee and Cortés, 2004; Frisk, 2010). This allowed us to measure the calcium isotope composition of zooplanktivores (n = 2), primary consumers (n = 5), secondary consumers (n = 8) and tertiary consumers (n = 4) and discuss their isotope distribution with their known ecology.

The distribution of $\delta^{44/42}$Ca values spreads among three isotope groups, which partly match the ecological divisions defined above: zooplanktivores and primary consumers, with the highest values,
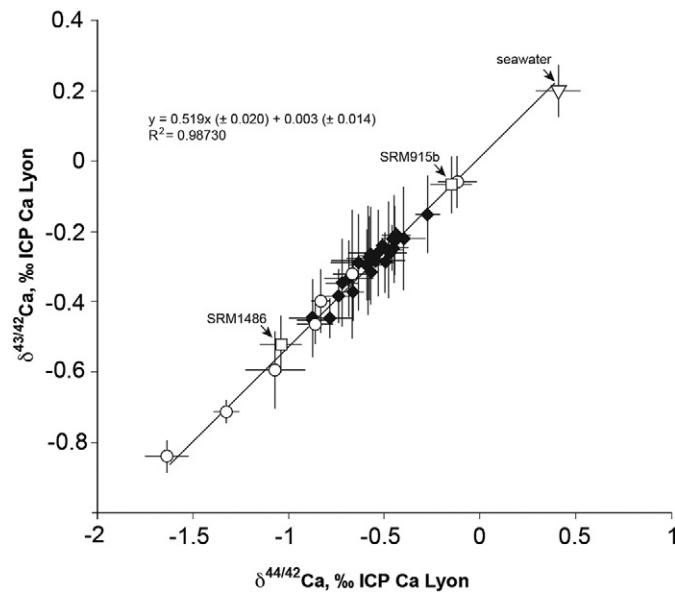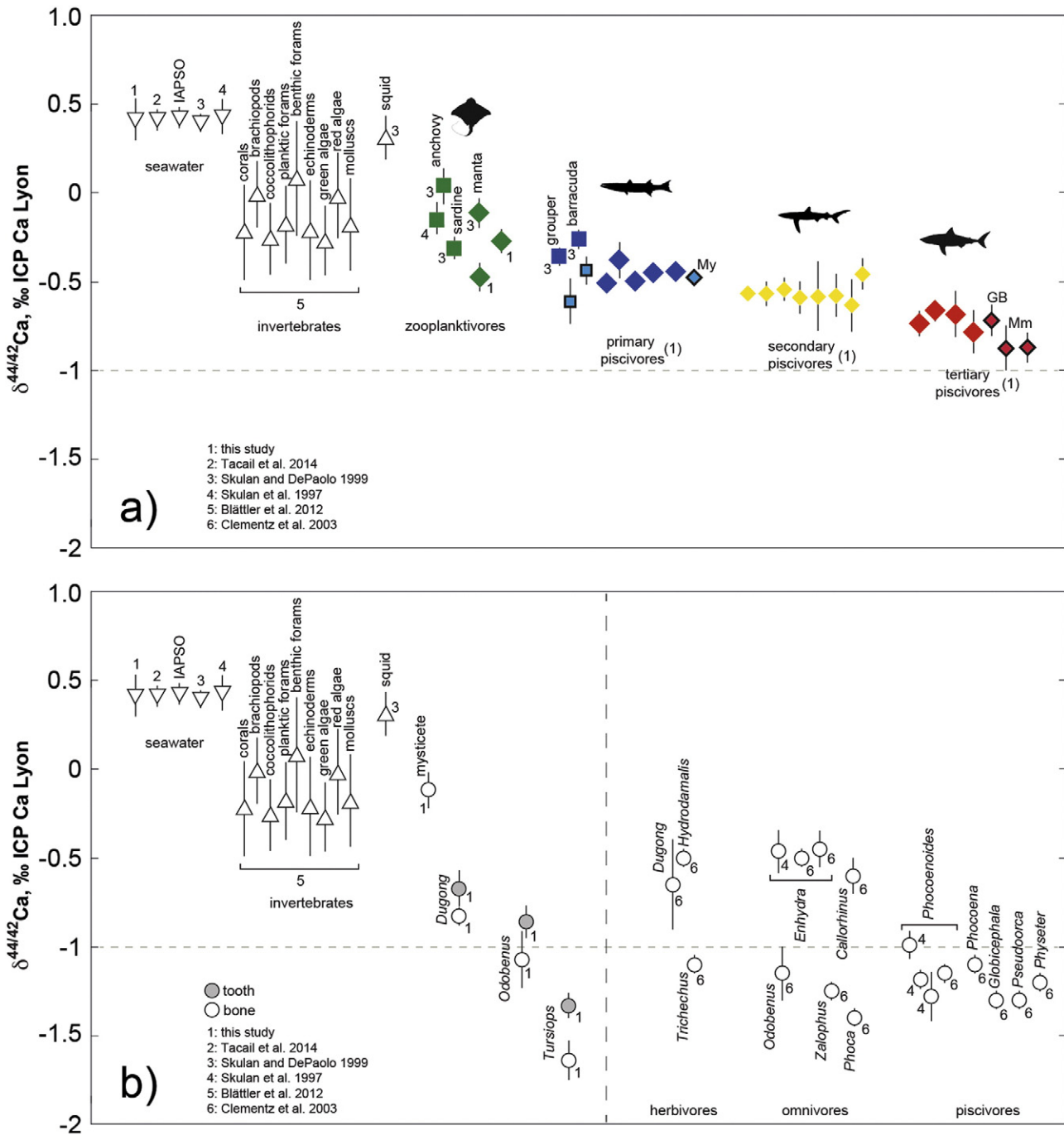
**Fig. 2. a)** Elasmobranch $\delta^{44/42}$Ca (in ‰) variability replaced in the broader context of marine ecosystems. Note the general stepwise decrease in isotope ratio with increasing trophic level (from left to right). Top elasmobranch predators have calcium isotope values higher than their potential mammalian preys (b). This inconsistent distribution is possibly due to a buffering effect in fish caused by the ingestion of seawater, which possesses an isotopically heavy calcium value. Extant elasmobranchs and teleosts are indicated with filled diamonds and squares, respectively. Pliocene elasmobranchs and teleosts are indicated with blackout lined diamonds and squares, respectively: GB, *Carcharodon carcharias*; Mm, *Megaselachus megalodon*; My, *Myliobatis* sp. Data from previous publications are indicated with a number. Inverted triangles correspond to seawater values according to this study; Tacail et al., 2014; IAPSO values (Table 2); Skulan et al., 1997; Skulan and DePaolo, 1999; triangles correspond to mean invertebrate values as compiled from Blättler et al., 2012. **b)** marine mammal values replaced in the context of marine ecosystems. Data from this study and data to the right of the stippled line compiled from Skulan et al., 1997 and Clementz et al., 2003.

secondary consumers, with the intermediate values and tertiary consumers, with the lowest values. We also describe a stepwise decrease expressed as $\Delta^{44/42}$Ca between (zooplanktivores + primary consumers) and secondary consumers of $-0.13‰$ and another $\Delta^{44/42}$Ca between the secondary consumers and the tertiary consumers of $-0.15‰$.

Zooplanktivores cannot be discerned isotopically from primary consumers. These elasmobranchs have the highest $\delta^{44/42}$Ca values (average $= -0.44‰$; n $= 7$) and almost exclusively feed on

invertebrates, *i.e.* plankton or mollusks. The highest value in this group is the manta ray ($-0.27‰$), which is comparable to the value measured by Skulan and DePaolo (1999). With the exception of the large planktivorous species (the basking shark and the manta ray), the maximal total body length (TBL) for species in this group does not surpass 120 cm and are therefore the smallest species in our dataset. This group contains small sharks and rays and their diet relies on invertebrates or can sometimes be opportunistic by including fishes. The diet of the hound shark almost exclusively includes crustaceans

and this species shows one of the highest $\delta^{44/42}$Ca values analyzed here ($-0.38 \pm 0.10‰$). The common eagle ray is principally malacophagous and its $\delta^{44/42}$Ca value is comparable to the opportunistic nursehound shark ($-0.50 \pm 0.05‰$ in both cases).

The second group includes elasmobranchs from the secondary consumers group, feeding on pelagic cephalopods and fishes. Elasmobranchs in this group have intermediate TBL between 100 and 300 cm and an average $\delta^{44/42}$Ca value of $-0.58‰$ (n = 8). The diet of the small-toothed sand tiger includes demersal sharks, teleosts and prawns (Fergusson et al., 2007). The isotope value of the kitefin shark is surprisingly high ($-0.46 \pm 0.08‰$) for a species reported to include demersal fishes and small sharks in its diet (Navarro et al., 2014). However, this is a relatively poorly studied species that lives at depth and we cannot exclude the diet of this particular individual was unusual or resembles the diet of the closely related species, the cookiecutter shark, which plugs out flesh from large fish or marine mammals (Heithaus, 2004). Species with the most negative values in this group are the common thresher shark, which feeds on a variety of school fishes and pelagic invertebrates ($-0.63 \pm 0.15‰$) as well as the most wide-ranging shark species, the blue shark ($-0.59 \pm 0.09‰$).

The last group includes the largest elasmobranchs of the dataset, and is only composed of sharks (average $\delta^{44/42}$Ca value of $-0.72‰$, n = 4). The Great White shark is an epipelagic swimmer but the sixgill shark lives in demersal habitats of continental shelves. These two species have a diet consisting of pelagic fishes and marine reptiles and mammals. The Great White shark has a varied diet including molluscs, crustaceans, fishes, cephalopods, turtles, birds and marine mammals (Fergusson et al., 2000; Stevens, 2010). Two individuals have been measured here and we estimate the TBL of the most fractionated one ($-0.78 \pm 0.12‰$) to approximate 400 cm, which is not significantly more fractionated than the other individual ($-0.74 \pm 0.07‰$) for which a total body length is roughly estimated to 300 cm. Great White sharks reach a maximum TBL of 600 cm and this species will eventually hunt marine mammals on a regular basis when it reaches a certain size, which could correspond to the isotope values recorded here being the lowest of the extant dataset. One of the largest predators included in our dataset, the bluntnose sixgill shark *Hexanchus griseus* has a varied diet that includes crabs (Barans and Ulrich, 1994), elasmobranchs and swordfish (Ebert, 1994), odontocetes (*i.e.* toothed whales such as dolphins) (Heithaus, 2001) and scavenges on mysticetes (Ebert, 2003). The smallest individual, which is a newly hatched individual (TBL = 58 cm for $-0.66 \pm 0.06‰$) has a $\delta^{44/42}$Ca value similar to the adult individual (TBL = 200 cm for $-0.68 \pm 0.13‰$) even if its diet is clearly different from adults (Adnet and Martin, 2007), which is likely indicating that the calcium used for building its first set of teeth has a maternal origin (see also Olin et al., 2011).

Proposing a fractionation mechanism for the observed trophic partitions appears premature because calcium isotope cycling at the scale of the elasmobranch body is unknown. Recent studies on terrestrial mammals including humans found that the calcium isotope composition of urine was heavier than diet (Heuser and Eisenhauer, 2010; Morgan et al., 2012; Tacail et al., 2014). Considering a mass balance between dietary input and excretion, the whole body should be depleted in heavy calcium isotopes. This could constitute the dietary baseline for the next trophic level even for marine mammals for which excretion fluxes are difficult to sample. Elasmobranchs and mammals are physiologically not comparable and such a process remains speculative pending continued research at the scale of organs and cells.

### 4.2. Elasmobranch calcium isotope values in the broader context of marine food webs

Prior to the present study, evidence for a trophic level effect in the marine environment based on calcium isotopes was documented for seawater, planktonic invertebrates, fish/snails and marine mammals with respectively decreasing isotope ratios (see review in DePaolo,

2004). Then for elasmobranchs, only a single value from a manta ray tooth (Skulan and DePaolo, 1999) was published. Elasmobranchs today are an important part of marine habitats. They are extremely diverse and dominate different predatory niches in a variety of climatic zones from polar to tropical environments. The observed range in extant elasmobranch isotope values presented here ($-0.27$ to $-0.78‰$) reflects their varied ecological positions but is also framed, albeit with some overlap, between teleost fishes and mammals (Fig. 2). As explained in the Methods, all data from marine invertebrates and vertebrates published previously have been expressed relative to the same reference standard as our data in order to allow comparisons. Marine mammal values from the works of Skulan et al. (1997), Skulan and DePaolo (1999) and Clementz et al. (2003) mostly consist of bone and a direct comparison with our elasmobranch dataset may not be exactly appropriate. Because of its crystalline arrangement, the structure of elasmobranch enameloid better compares with mammalian enamel or dentine than with bone. Marine mammal bone values should therefore be adjusted. According to Heuser et al. (2011), dentine, which is approximated to bone in their work, has heavier Ca isotope values than enamel by about 0.3‰ (expressed as $\delta^{44/40}$Ca). However, the dataset of Heuser et al. (2011) consists exclusively of archosaurian (crocodiles, birds, dinosaurs) dentine and enamel values and no tooth-bone pairs in a single individual were measured. Extrapolating the dentine-enamel offset of Heuser et al. (2011) to the marine mammal bone values of Skulan et al. (1997), Skulan and DePaolo (1999) and Clementz et al. (2003) does not seem appropriate. On the other hand, our small dataset of marine mammals shows that 1) bone values for the walrus and the dugong are consistent with the values obtained for these two taxa by Clementz et al. (2003); and 2) in the three marine mammals analyzed here, measurements of pairs of bone-tooth on each individual show that bone has systematically lower values than tooth (contra Heuser et al., 2011) by 0.23‰ on average.

Our elasmobranch dataset overlaps with the invertebrate dataset of Blättler et al. (2012), the lowest half of the fish group and the highest third of the marine mammal group (Fig. 2). Considering an approximate $-0.65‰$ difference between consumer's bone and diet (the $\Delta_{diet-bone}$ offset of Skulan and DePaolo, 1999), our elasmobranch dataset should correspond with a dietary source ranging from $-0.10‰$ to $+0.40‰$. This is partly consistent in the comparative dataset with invertebrate preys such as mussels ($0.07 \pm 0.09‰$), some fishes such as anchovy ($0.04$ to $-0.15‰$) and the mysticete, which shows the highest value of the mammalian dataset (Table 1, Fig. 2). However, the $\Delta_{diet-enameloid}$ offset is unknown, elasmobranchs have a cartilaginous skeleton, and their physiology is not comparable to that of mammals.

As discussed in the previous paragraph, the Great White and the sixgill sharks (all third level consumers) all include a considerable amount of marine mammal preys in their diet, but these taxa have comparable or higher delta values than most marine mammals. Data available for several otarids, the walrus and all odontocetes show $\delta^{44/42}$Ca values well below the lowest value of the elasmobranch dataset. These apex shark predators are known to scavenge on carcasses, hunt actively porpoises and dolphins and hunt in colonies of pinnipeds (see review in Heithaus, 2001). Also, a significant amount of marine mammals have been reported in stomach contents of Great White sharks of the Mediterranean (Fergusson et al., 2000), which is part of our dataset. However, our $\delta^{44/42}$Ca values for this third group of elasmobranchs (*i.e.* the third-level consumers) are inconsistent with the range of inferred dietary sources, even if a hypothetical $\pm 0.2‰$ enamel-bone offset is taken into account for marine mammal bone (Heuser et al., 2011 suggest $-0.2‰$; Tacail et al., 2014 suggest $-0.3‰$; this study suggests $+0.2‰$), but other dietary sources such as soft tissue are also non-negligible. This is the case for reported intakes of several kg of whale blubber (Carey et al., 1982), which implies comparatively lower intake of skeletal calcium in the diet. But isotope variations between soft tissue reservoirs of vertebrates are poorly constrained. Finally, calcium isotope variability in marine mammals will have to consider specific

physiological factors involving Ca fractionation such as pregnancy or milking for both mother and juvenile, which remain poorly understood yet.

Nevertheless, this inversion between the $\delta^{44/42}$Ca values of apex elasmobranch predators and their potential prey (marine mammals, especially odontocetes), is best understood when considering the input of heavy calcium through the ingestion of seawater. Seawater is the ultimate source of heavy calcium in the marine environment with an average value of ~0.4‰ relative to ICP Ca Lyon. Fish, including elasmobranchs, constantly filter calcium from seawater through their gills, which represents the main source for building their skeleton (Flik and Verbost, 1993). Another source of calcium for elasmobranch can be incidental ingestion of their teeth, as currently reported in juveniles (Klimey and Ainley, 1998) and which are constantly renewed during their lifetime. In mammals, seawater can be incidentally ingested during surface breathing or food intake, but most of the time seawater is voluntarily discarded (Ortiz, 2001). Mammals could therefore be considered a rather closed system and contrary to fish and elasmobranchs, seawater calcium will not buffer Ca isotope compositions of their tissues toward more positive values. Noteworthy in our dataset is the whale bone, which shows the most enriched $\delta^{44/42}$Ca value of the mammalian dataset ($-0.12 \pm 0.10$‰). This animal gulps considerable amount of krill, which isotope composition should be close to that of seawater. Also consistent with the idea that seawater positively buffers Ca isotope values is the sea otter ($-0.43 \pm 0.11$‰), which ingests more seawater than any other marine mammals (Ortiz, 2001) and shows particularly enriched $\delta^{44/42}$Ca values in the mammalian dataset. Considering the mammalian body as a rather closed system, not influenced by the input of enriched seawater calcium, may explain why even apex shark predators have more positive $\delta^{44/42}$Ca values than odontocetes.

### 4.3. Early Pliocene case study

Calcium amounts to ~40% of biogenic hydroxylapatite ($Ca_{10}(PO_4)_6(OH)_2$) and unless calcium is entirely replaced, modifying the calcium isotope composition of bone or teeth through diagenesis is unrealistic (Heuser et al., 2011). Thus, measuring Ca isotopes in fossil vertebrates most likely reflects the initial conditions under which bone or teeth formed. Although the calcium isotope composition of seawater has changed in the last 20 million years (De La Rocha and De Paolo, 2000; Fantle and DePaolo, 2005; Griffith et al., 2008), its value at 5 million years ago, which corresponds to the age of the analyzed fossil fauna, is slightly lower than present seawater value by about 0.06‰ (Fantle and DePaolo, 2005). To test for a paleoecological signal in fossil elasmobranchs, we analyzed two sets of fish teeth described from the Early Pliocene of Libya (Pawellek et al., 2012)and New Caledonia (Seret, 1987). The Libyan assemblage includes a seabream, a barracuda, a shell-crushing ray and two large sharks: the Great White *Carcharodon carcharias* and the extinct giant shark *Megaselachus megalodon* (Table 1) altogether constituting an assemblage with representatives of diverse ecologies. The Libyan and New Caledonian fossil assemblages represent one of the last stratigraphic co-occurrences of the Great White and the giant shark, before the latter became extinct. The distribution of calcium isotope values in taxa from this fossil assemblage follows the distribution recovered in this study for extant relatives (Fig. 2) with the shell-crushing ray and the seabream and barracuda having the highest values that correspond to the primary consumers. The Great White shark from the Pliocene of Libya has a value very close to the two extant Great White sharks measured in this study (Table 1), which corresponds to the tertiary piscivore group. The giant sharks from the Pliocene of Libya and New Caledonia yield undistinguishable values ($-0.87 \pm 0.13$‰ and $-0.87 \pm 0.08$‰ respectively) and despite relatively large error bars, have calcium isotope values distinctly lower than those of the Great White shark (Table 1). This possibly indicates that the giant shark was feeding at a higher trophic level. Although a dietary overlap is likely, as is often observed between most species of

sharks, the low calcium isotope value of the giant shark implies that it had access to food not available to Great White sharks. Current fossil evidence confirms that giant sharks and Great White sharks were both feeding on large odontocetes and mysticetes (Bianucci et al., 2000; Aguilera et al., 2008). Whether the present isotope data indicate that the Great White shark was mostly feeding on small mammals while the giant shark was also feeding on large whales will have to be clarified. These data suggest that Ca isotopes are a valuable tool for assessing trophic relationships among fossil marine organisms.

### 5. Conclusions

We assessed calcium isotope ratios for a set of extant elasmobranch taxa with varying foraging ecologies in order to complete the picture of the natural variation of calcium isotopes in marine food webs and to test for the validity of calcium isotopes as trophic tracers. To the exception of the Kitefin shark, we found that calcium isotope values corroborate expectations based on the known foraging ecology of elasmobranchs, as reported in the literature, discerning three levels of consumers: species feeding on plankton and invertebrates, species feeding on pelagic invertebrates and fishes, and large predatory species feeding on fishes and marine mammals. In addition, we record a stepwise decrease ($\Delta^{44/42}$Ca) of ~ 0.14‰ between these feeding groups. Elasmobranchs and marine mammals calcium isotope data do not show a congruent distribution. Physiological difference behind calcium cycling in the body of fish and mammals might be responsible for such discrepancy. Further data on marine mammals will permit a refinement of this hypothesis. Nevertheless, application of calcium isotopes to ancient marine food webs, such as our case study from the Pliocene of Libya and New Caledonia, is promising and our data on extant elasmobranchs will serve as a reference to understand the distribution and significance of calcium isotope values in fossil elasmobranchs and associated vertebrate faunas.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.chemgeo.2015.09.011.

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