Earth and Planetary Science Letters 298 (2010) 135–142

Contents lists available at ScienceDirect

Earth and Planetary Science Letters

journal homepage: www.elsevier.com/locate/epsl

Revised phosphate–water fractionation equation reassessing paleotemperatures derived from biogenic apatite

E. Pucéat [a,*], M.M. Joachimski [b], A. Bouilloux [c], F. Monna [d], A. Bonin [a], S. Motreuil [a], P. Morinière [e], S. Hénard [f], J. Mourin [g], G. Dera [a], D. Quesne [a]

[a] Université de Bourgogne, UMR CNRS 5561 Biogéosciences, 6 bd Gabriel, 21000 Dijon, France
[b] North Bavarian Center of Earth Sciences, University of Erlangen-Nürnberg, Schloßgarten 5, 91054 Erlangen, Germany
[c] Institut de Physique du Globe de Paris, UMR CNRS 7154, 4 place Jussieu 75252 Paris cedex 05, France
[d] Université de Bourgogne, UMR CNRS 5594 Artehis, 6 bd Gabriel, 21000 Dijon, France
[e] Aquarium La Rochelle, Quai Louis Prunier, 17000 La Rochelle, France
[f] Nausicaä, Centre National de la Mer, Bd Sainte Beuve, 62200 Boulogne sur Mer, France
[g] Aquarium Grand Lyon, Place du Général Leclerc, 69350 La Mulatière, France

ARTICLE INFO

Article history:
Received 21 May 2010
Received in revised form 15 July 2010
Accepted 22 July 2010
Available online 21 August 2010

Editor: M.L. Delaney

Keywords:
apatite
oxygen isotopes
paleotemperature
fractionation

ABSTRACT

Oxygen isotopes of biogenic apatite have been widely used to reassess anomalous temperatures inferred from oxygen isotope ratios of ancient biogenic calcite, more prone to diagenetic alteration. However, recent studies have highlighted that oxygen isotope ratios of biogenic apatite differ dependent on used analytical techniques. This questions the applicability of the phosphate–water fractionation equations established over 25 years ago using earlier analytical techniques to more recently acquired data. In this work we present a new phosphate–water oxygen isotope fractionation equation based on oxygen isotopes determined on fish raised in aquariums at controlled temperature and with monitored water oxygen isotope composition. The new equation reveals a similar slope, but an offset of about +2‰ to the earlier published equations. This work has major implications for paleoclimatic reconstructions using oxygen isotopes of biogenic apatite since calculated temperatures have been underestimated by about 4 to 8 °C depending on applied techniques and standardization of the analyses.

1. Introduction

Since the pioneer work of Longinelli (1966) and Longinelli and Nuti (1973a, 1973b), later refined by Kolodny et al. (1983), oxygen isotopes of biogenic phosphate ($\delta^{18}O_p$) have been used to reconstruct the temperature of ancient oceans (Joachimski et al., 2006; Dera et al., 2009; Trotter et al., 2008). Biogenic phosphates reveal many advantages as (i) apatite is less prone to post-mortem alteration in comparison to biogenic carbonate, (ii) fossil apatite like fish tooth or conodont apatite is widely distributed both stratigraphically and spatially, and (iii) non-equilibrium oxygen isotope fractionation has not been observed during precipitation of biogenic apatite. Due to the high preservation potential of biogenic apatite, isotope studies using conodont or fish tooth $\delta^{18}O_p$ have been used to reconstruct palaeotemperatures and to reassess anomalous temperatures inferred from $\delta^{18}O$ of biogenic carbonate (Pucéat et al., 2007; Trotter et al., 2008; Joachimski et al., 2009).

However, analytical techniques have evolved since the work of Longinelli (1966) and Kolodny et al. (1983), and recent papers have shown that the various analytical techniques currently used can result in significantly different $\delta^{18}O_p$ (up to several per mil; O'Neil et al., 1994; Vennemann et al., 2002; Chenery et al., 2010). These differences in $\delta^{18}O_p$ are based (i) on different chemical protocols used to isolate the phosphate group from biogenic apatite (Crowson et al., 1991; O'Neil et al., 1994), and (ii) on different methods to analyse oxygen isotopes of phosphate-bound oxygen (Vennemann et al., 2002; Chenery et al., 2010). Since oxygen is present in three sites in biogenic apatite, the PO_4^{3-} group has to be isolated prior to isotope analysis. Initially the phosphate group was precipitated as $BiPO_4$ (Longinelli, 1966; Kolodny et al., 1983). More recently, trisilverphosphate is used since Ag_3PO_4 is not hygroscopic and easier to prepare than $BiPO_4$ (Crowson et al., 1991; O'Neil et al., 1994). The $\delta^{18}O$ of $BiPO_4$ and Ag_3PO_4 has been determined either by conventional fluorination (Longinelli, 1966; Crowson et al., 1991), by heating Ag_3PO_4 with graphite in silica tubes, releasing CO_2 (O'Neil et al., 1994), or by online high-temperature reduction in a glassy carbon reactor, releasing CO (Kornexl et al., 1999; Vennemann et al., 2002). Since fluorination, heating in silica tubes, and high-temperature reduction of Ag_3PO_4 samples result in different $\delta^{18}O$ values, phosphate and Ag_3PO_4 standards are used to standardize oxygen isotope analyses

* Corresponding author. Tel.: +33 3 80 38 63 81.
E-mail address: emmanuelle.puceat@u-bourgogne.fr (E. Pucéat).

0012-821X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.epsl.2010.07.034

Table 1
Published $\delta^{18}O$ values for NBS120b and NBS120c analysed using fluorination of Ag_3PO_4 or $BiPO_4$.

Author	Analyte	$\delta^{18}Op$ (‰SMOW)		Analytical reproductibility (1σ)
		NBS120b	NBS120c	
Shemesh et al. (1988)	$BiPO_4$, heated to 130 °C	20.1		±0.3
Shemesh et al. (1988)	$BiPO_4$, heated to 420 °C	20.5		±0.1
Wright and Hoering (1989)	Ag_3PO_4	19.81	19.94	±0.8 for NBS120b, ±0.6 for NBS120c
Crowson et al. (1991)	Ag_3PO_4		21.33	±0.1
Lécuyer et al. (1993)	Ag_3PO_4		21.7	±0.16
Bryant et al. (1994)	$BiPO_4$	19.91		±0.39
Lécuyer et al. (1996)	Ag_3PO_4		21.7	±0.14
Bryant et al. (1996)	Ag_3PO_4		21.36	±0.18
Vennemann et al. (2001)	Ag_3PO_4		22.1	±0.1
Vennemann et al. (2002)	Ag_3PO_4		22.58	±0.09
Chenery et al. (2010)	Ag_3PO_4		21.7	±0.15

$\delta^{18}Op$ is the oxygen isotope composition of the phosphate group of the analyte.

obtained by the different analytical techniques (Vennemann et al., 2002; Chenery et al., 2010). However, a disagreement exists on the oxygen isotope value of the most widely used standard NBS120c (Florida phosphate rock) which is not a certified oxygen isotope standard (Lécuyer et al., 1996; Vennemann et al., 2002; Table 1). Oxygen isotope analyses of phosphate are currently standardized using either a value of NBS120c of 21.7‰ (Lécuyer et al., 2003; Trotter et al., 2008), or of 22.6‰ (Vennemann et al., 2002; Joachimski et al., 2009). The reason for the difference in $\delta^{18}O$ is unclear. Lécuyer et al. (1993) as well as Vennemann et al. (2002) analysed NBS120c by conventional fluorination of Ag_3PO_4 with BrF_5 calibrating the fluorination lines using quartz standard NBS-28. Vennemann et al. (2002) suggested that the 0.9‰ offset in $\delta^{18}O$ of NBS120c may be related to different chemistry used for precipitating Ag_3PO_4.

These differences have major implications for the reconstruction of paleotemperatures. To calculate temperatures from $\delta^{18}O_p$ of fish teeth, conodonts, or phosphatic marine invertebrates, the phosphate–water fractionation equations of Longinelli and Nuti (1973a, 1973b) or Kolodny et al. (1983) have been used, independently of the analytical technique and the value of NBS120c used to standardize the data. Yet in contrast to recent studies, the phosphate–water oxygen isotope fractionation equations have been established by analysing $BiPO_4$ by conventional fluorination, without using NBS120c for standardization as this standard was not available at that time. As a result, (i) these fractionation equations may not be applicable to data acquired using the most recent techniques, and (ii) large differences in reconstructed paleotemperature (4 °C using Kolodny et al., 1983) arise between data sets analysed assuming a $\delta^{18}O$ value of 21.7 or 22.6‰ for standard NBS120c.

In this work, we raised seabreams (*Sparus aurata*) in aquariums at a controlled temperature and monitored water oxygen isotope composition ($\delta^{18}O_w$). The oxygen isotope composition of the phosphate group of fish teeth was analysed using the most recent techniques for which several apatite standards have been made available. With these data we established a new phosphate–water fractionation equation that allows us to discuss the applicability of earlier published equations on recently acquired data.

2. Experimental

Seabreams (*S. aurata*) were placed in eleven aquariums at the Aquarium of La Rochelle (La Rochelle, France), Nausicaä (Boulogne sur Mer, France) and the Aquarium du Grand Lyon (Lyon, France) where they lived for 4 to 5 months, depending on the availability of the aquariums at the different sites. Waters in the aquariums were maintained at constant temperatures (±0.5 °C) ranging from 8 to 28 °C.

Aquariums of 200 to 600 l were used for the experiments. The aquariums were filled with water with different $\delta^{18}O$ values, depending on the facilities available at each site. For the site of Nausicaä, seawater is permanently pumped from the nearby Channel and stored in a tank of 100 m^3 before being redistributed in every aquarium of the site, including those of our experiment. Water is then constantly renewed in every aquarium (open system). For the site of Lyon, seawater from the Mediterranean Sea was imported and diluted with osmosis water to obtain a salinity of 32. For the site of La Rochelle, artificial seawater with a salinity of 35 was produced using osmosis water and the commercial salt Instant Ocean. In the aquariums of La Rochelle and Lyon, the water was recycled in a closed system. Water with an identical oxygen isotopic composition as the initial water was regularly added to compensate for evaporation and to maintain aquarium water salinity and $\delta^{18}O$ as constant as possible. Water $\delta^{18}O_w$ was measured once per month. Experimental setups are summarized in Table 2.

In order to study the short-term variability of water $\delta^{18}O$ for the aquariums of Nausicaä, in which the water was constantly renewed, the 12 °C warm water of the aquarium was sampled every day during one week of the experiment (Table 3). In order to study the impact of regular addition of waters in aquariums with a closed system (La Rochelle and Lyon), the aquariums at 16, 18, 20, and 22 °C were sampled before and after the addition of new water during the month of April 2008 (Table 3).

Every fish was injected in the peritoneal area with 2% calceine (40 mg of calceine per kg of fish; Trébaol et al., 1991) when introduced into the aquarium in order to identify the parts of the teeth that formed while the fish were raised. Calceine remains less than one week in fish internal fluids and marks precipitating apatite with a yellow–brown colour under natural light and with a bright green fluorescence under UV light, allowing the selection of apatite formed during the experiment (Fig. 1).

3. Material and methods

At the end of the experiment, yellow–brown teeth in the functional position can be seen very clearly on seabream jaws (Fig. 1). In order to select with certainty apatite that mineralized when the fishes were living in the aquariums at controlled temperature, only teeth

Table 2
Experimental setups.

Site	Location	Number of aquariums	Temperature of the aquariums	Origin of water	Water renewal
Nausicaä	Boulogne sur Mer (France)	4	8, 10, 12, 14 °C	Seawater from the Channel, stored in a tank before redistribution	Open system (water constantly renewed)
Aquarium de La Rochelle	La Rochelle (France)	4	16, 18, 20, 22 °C	Artificial seawater with a salinity of 35, produced from osmosis water and the commercial salt Instant Ocean	Closed system (water recycled within the aquariums, with regular addition to compensate for evaporation)
Aquarium du Grand Lyon	Lyon (France)	3	24, 26, 28 °C	Seawater from the Mediterranean Sea diluted with osmosis water to a salinity of 32	Closed system (water recycled within the aquariums, with regular addition to compensate for evaporation)

Table 3
Oxygen isotope composition of water samples.

Sample name	Month of sampling	Date of sampling (day/month/year)	Days from the beginning of the experiment[a]	Aquarium Temperature (°C)	Site	δ^{18}Ow (‰SMOW)	Mean δ^{18}Ow (‰SMOW) per month	Mean δ^{18}Ow (‰SMOW) per aquarium	1σ
N1-8	May	15/05/2008	105	8	Aquarium of Nausicaa	−1.51	−1.51		
N2-8	June	19/06/2008	140	8	Aquarium of Nausicaa	−1.36	−1.36	−1.30	0.18
N3-8	July	29/07/2008	180	8	Aquarium of Nausicaa	−1.21	−1.21		
N4-8	August	27/08/2008	209	8	Aquarium of Nausicaa	−1.11	−1.11		
N1-10	May	15/05/2008	105	10	Aquarium of Nausicaa	−1.49	−1.49		
N2-10	June	19/06/2008	140	10	Aquarium of Nausicaa	−1.36	−1.36	−1.26	0.20
N3-10	July	29/07/2008	180	10	Aquarium of Nausicaa	−1.11	−1.11		
N4-10	August	27/08/2008	209	10	Aquarium of Nausicaa	−1.08	−1.08		
N1-12	May	15/05/2008	105	12	Aquarium of Nausicaa	−1.49	−1.49		
N2-12a	June	15/06/2008	140	12	Aquarium of Nausicaa	−1.31			
N2-12b		16/06/2008	140	12	Aquarium of Nausicaa	−1.39			
N2-12c		17/06/2008	140	12	Aquarium of Nausicaa	−1.32	−1.35	−1.28	0.17
N2-12d		18/06/2008	140	12	Aquarium of Nausicaa	−1.37			
N2-12e		19/06/2008	140	12	Aquarium of Nausicaa	−1.34			
N3-12	July	29/07/2008	180	12	Aquarium of Nausicaa	−1.16	−1.16		
N4-12	August	27/08/2008	209	12	Aquarium of Nausicaa	−1.14	−1.14		
N1-14	May	15/05/2008	105	14	Aquarium of Nausicaa	−1.44	−1.44		
N2-14	June	19/06/2008	104	14	Aquarium of Nausicaa	−1.38	−1.38	−1.31	0.16
N3-14	July	29/07/2008	180	14	Aquarium of Nausicaa	−1.13	−1.13		
R1-16	February	21/02/2008	21	16	Aquarium of La Rochelle	−4.67	−4.67		
R2-16	March	16/03/2008	45	16	Aquarium of La Rochelle	−4.16	−4.16		
R3-16a[b]	April	24/04/2008	84	16	Aquarium of La Rochelle	−3.84	−3.83	−4.14	0.57
R3-16b[c]		24/04/2008	84	16	Aquarium of La Rochelle	−3.81			
LR-16	May	19/05/2008	109	16	Aquarium of La Rochelle	−3.35	−3.35		
R216	June	25/06/2008	146	16	Aquarium of La Rochelle	−4.68	−4.68		
R1-18	February	21/02/2008	21	18	Aquarium of La Rochelle	−3.92	−3.92		
R2-18	March	16/03/2008	45	18	Aquarium of La Rochelle	−3.34	−3.34		
R3-18a[b]	April	24/04/2008	84	18	Aquarium of La Rochelle	−2.51	−2.57	−2.87	0.75
R3-18b[c]		24/04/2008	84	18	Aquarium of La Rochelle	−2.63			
LR-18	May	19/05/2008	109	18	Aquarium of La Rochelle	−2.50	−2.50		
R500	June	25/06/2008	146	18	Aquarium of La Rochelle	−2.04	−2.04		
R1-20	February	21/02/2008	21	20	Aquarium of La Rochelle	−4.07	−4.07		
R2-20	March	16/03/2008	45	20	Aquarium of La Rochelle	−3.45	−3.45		
R3-20a[b]	April	24/04/2008	84	20	Aquarium of La Rochelle	−2.79	−2.88	−3.45	−3.45
R3-20b[c]		24/04/2008	84	20	Aquarium of La Rochelle	−2.96			
LR-20	May	19/05/2008	109	20	Aquarium of La Rochelle	−4.02	−4.02		
R501	June	25/06/2008	146	20	Aquarium of La Rochelle	−2.84	−2.84		
R1-22	February	21/02/2008	21	22	Aquarium of La Rochelle	−4.62	−4.62		
R2-22	March	16/03/2008	45	22	Aquarium of La Rochelle	−3.94	−3.94		
R3-22a[b]	April	24/04/2008	84	22	Aquarium of La Rochelle	−3.28	−3.24	−4.15	0.64
R3-22b[c]		24/04/2008	84	22	Aquarium of La Rochelle	−3.20			
LR-22	May	19/05/2008	109	22	Aquarium of La Rochelle	−4.88	−4.88		
R502	June	25/06/2008	146	22	Aquarium of La Rochelle	−4.08	−4.08		
L1-24	February	19/02/2008	19	24	Aquarium Grand Lyon/Aqualand	−1.20	−1.20		
L2-24	March	24/03/2008	53	24	Aquarium Grand Lyon/Aqualand	−0.05	−0.05	−0.20	0.58
L3-24	April	21/04/2008	81	24	Aquarium Grand Lyon/Aqualand	−0.05	−0.05		
L4-24	May	20/05/2008	110	24	Aquarium Grand Lyon/Aqualand	0.03	0.03		
L5-24	July	03/07/2008	154	24	Aquarium Grand Lyon/Aqualand	0.28	0.28		
L1-26	February	19/02/2008	19	26	Aquarium Grand Lyon/Aqualand	−1.20	−1.20		
L2-26	March	24/03/2008	53	26	Aquarium Grand Lyon/Aqualand	−0.32	−0.32		
L3-26	April	21/04/2008	81	26	Aquarium Grand Lyon/Aqualand	−0.87	−0.87	−0.80	0.32
L4-26	May	20/05/2008	110	26	Aquarium Grand Lyon/Aqualand	−0.73	−0.73		
L5-26	July	03/07/2008	154	26	Aquarium Grand Lyon/Aqualand	−0.86	−0.86		
L1-28	February	19/02/2008	19	28	Aquarium Grand Lyon/Aqualand	−1.73	−1.73		
L2-28	March	24/03/2008	53	28	Aquarium Grand Lyon/Aqualand	−0.21	−0.21		
L3-28	April	21/04/2008	81	28	Aquarium Grand Lyon/Aqualand	−0.53	−0.53	−0.64	0.62
L4-28	May	20/05/2008	110	28	Aquarium Grand Lyon/Aqualand	−0.37	−0.37		
L5-28	July	03/07/2008	154	28	Aquarium Grand Lyon/Aqualand	−0.38	−0.38		

[a] Because of aquarium availability on the different sites, the experiment begun January the 31th 2008 in La Rochelle, February the 8th 2008 in Lyon, and May the 7th in Nausicaa. The days from the beginning of the experiments represent the number of day from the beginning in La Rochelle.
[b] Water sampled before addition of new water (see Section 2).
[c] Water sampled after addition of new water (see Section 2).

still located inside the bone on the distal side of the jaws were sampled. These teeth, that were located behind functional yellow–brown teeth and mineralized after them (Rosecchi, 1985), were completely white under natural light and did not present any bright green fluorescence under UV (Fig. 1). They therefore mineralized after calceine had dissipated from the internal fluid of the fish. The seabreams in the aquarium at 14 °C died after 2 months of experiment and did not remain in the aquarium long enough to be able to identify with certainty teeth that were entirely mineralized in the aquarium on the basis of calceine marking. These teeth were not used for oxygen isotope analysis.

Stable isotope analyses were performed at the GeoZentrum Nordbayern of the University of Erlangen-Nuremberg (Germany). The teeth were soaked for 12 h in 2.5% NaOCl to remove soluble

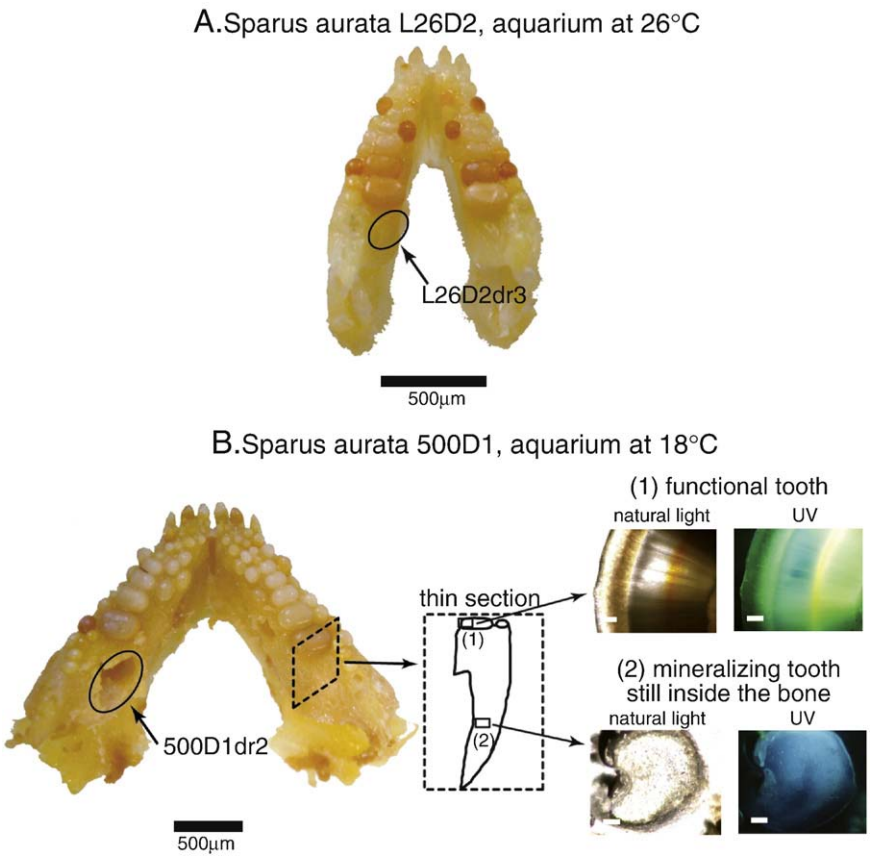

Fig. 1. Pictures of (A) the lower jaw of specimen L26D2, raised at 26 °C and (B) the lower jaw of specimen 500D1, raised at 18 °C. White scale on the four detailed pictures in (B) represents 50 µm. The plain black ellipses indicate the location of tooth L26D2dr3 and 500D1dr2 that were not yet erupted. (A) The brown-yellow teeth mineralizing after injection of calceine at the beginning of the experiments are clearly visible. (B) The functional tooth on the detailed picture of the thin section (1) shows brown parts under natural light, that display a bright green luminescence under UV due to the presence of calceine in the apatite. By contrast, the tooth inside the bone located under this functional tooth (2) is white under natural light and does not show any bright green luminescence under UV but a slight deep blue luminescence that occurs naturally in fish tooth apatite.

organic matter, washed several times in distilled water and soaked for 48 h in 0.125 M NaOH to remove humic acids (Stephan, 2000). After several rinse cycles with distilled water, 36 apatite samples (0.5 to 1 mg) were dissolved in nitric acid and chemically converted to Ag_3PO_4 using the method described by Joachimski et al. (2009). Oxygen isotope ratios were measured on CO using a High Temperature Conversion Elemental Analyzer (TC-EA) connected online to a ThermoFinnigan Delta plus mass spectrometer. All $\delta^{18}O$ values are reported in per mil relative to V-SMOW (Vienna Standard Mean Ocean Water). Accuracy and reproducibility ($<\pm 0.2$‰, 1σ) were monitored by multiple analyses of Ag_3PO_4 from NBS120c and several Ag_3PO_4 standards (TUI-1, TUI-2, YR-2; $n=10$). The average oxygen isotope compositions of TUI-1, TUI-2 and YR-2 standards were 21.3, 5.5, and 13.2‰ V-SMOW, respectively. The mean $\delta^{18}O$ value of NBS120c was 22.6‰ V-SMOW, comparable to the value of 22.6‰ V-SMOW determined by Vennemann et al. (2002) by conventional fluorination.

Water oxygen isotope composition was analysed from 0.5 ml water subsamples at the Leibniz Laboratory for Radiometric Dating and Stable Isotope Research in Kiel with a Finnigan Gasbench II connected to a Finnigan DeltaPlusXL mass spectrometer applying the CO_2-water isotope equilibration techniques. The $\delta^{18}O$ data are expressed versus V-SMOW. Accuracy and reproducibility was monitored by analysing two laboratory standards (Kiel ground water: −7.7‰ V-SMOW, $n=10$; Mediterranean Sea water: 1.05‰ V-SMOW, $n=2$) that have been calibrated using the international standards V-SMOW, SLAP, and GISP. Analytical precision was ±0.04‰ (1σ).

4. Results

4.1. Variation of the water oxygen isotope composition during the experiment

In Nausicaä, the oxygen isotope composition of the water was very similar in each of the 4 aquariums and remained very stable both on short (weekly) and longer (monthly) scale, with values from about −1.50‰ at the beginning to about −1.10‰ at the end of the experiment (Table 3, Fig. 2). The low $\delta^{18}O_w$ values in the aquariums of La Rochelle range from −3.9 to −4.7‰ at the beginning of the experiment, reflecting values of the local osmosis water that has been used to produce artificial seawater. By contrast, the higher $\delta^{18}O_w$ values in the aquariums of Lyon are between −1.73 and −1.20‰ at the beginning of the experiment and reflect the oxygen isotope composition of imported Mediterranean Sea water diluted with osmosis water. Water oxygen isotope ratios generally tends to increase in all aquariums of Lyon and La Rochelle, with some variation observed throughout the experiment. Isotope values varied by 1.33‰ to 1.88‰ for aquariums at La Rochelle, and by 0.88‰ to 1.85‰ at Lyon (Table 3, Fig. 2).

4.2. Variations of phosphate–water oxygen isotope fractionation with temperature

Due to the observed fluctuations in $\delta^{18}O_w$ in the aquariums and because it is not possible to constrain the exact time of tooth apatite

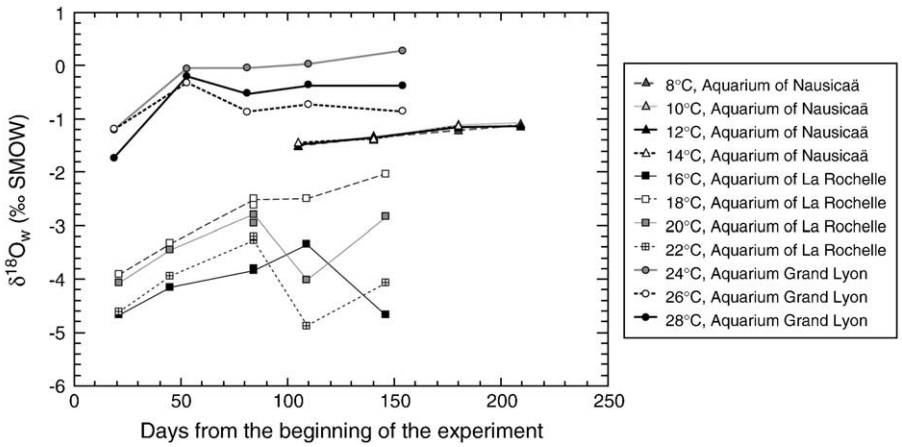

Fig. 2. Variations of the oxygen isotope composition of aquarium waters during the experiment.

precipitation during the experiment, we calculated the mean $\delta^{18}O_w$ value for each aquarium and used the mean values to calculate $\delta^{18}O_p$–$\delta^{18}O_w$ values (Table 3, Table 4). Values of $\delta^{18}O_p$–$\delta^{18}O_w$ vary from 25.2‰ on average at 8 °C to 21.7‰ at 28 °C.

For each aquarium water temperature, $\delta^{18}O_p$–$\delta^{18}O_w$ values scatter by up to 2‰ (Fig. 3). Despite this scattering, a clear relation between $\delta^{18}O_p$–$\delta^{18}O_w$ and temperature is observed, with the fractionation between water and phosphate increasing with decreasing temperature.

Table 4
Oxygen isotope composition of fish teeth.

Sample Name	Number of teeth analysed altogether	Aquarium temperature (°C)	δ^{18}Op (‰SMOW)	Mean δ^{18}Ow (‰SMOW)	δ^{18}Op–δ^{18}Ow (‰SMOW)
N8D1dr1	1	8	24.0	−1.3	25.3
N8D1dr3dr8	2	8	23.8	−1.3	25.1
N10D1dr4dr5	2	10	23.6	−1.26	24.8
N10D1dr1dr2	2	10	23.0	−1.26	24.3
N10D1dr5	1	10	23.7	−1.26	25.0
N12D1dr5	1	12	24.0	−1.28	25.3
216D1dr1	1	16	18.8	−4.14	22.9
216D1dr1[a]	1	16	19.2	−4.14	23.3
216D2dr2	1	16	19.8	−4.14	24.0
500D1dr2	1	18	20.7	−2.87	23.5
500D1dr2[a]	1	18	20.0	−2.87	22.8
500D2dr1	1	18	21.3	−2.87	24.2
501D1dr1	1	20	19.9	−3.45	23.4
501D1dr2	1	20	19.6	−3.45	23.0
501D1dr5	1	20	20.2	−3.45	23.7
501 D1dr5[a]	1	20	19.7	−3.45	23.1
501D1dr6	1	20	20.2	−3.45	23.7
501D1dr7	1	20	19.7	−3.45	23.1
501D2dr3-4	3	20	20.0	−3.45	23.5
502D1dr1	1	22	18.2	−4.15	22.3
502D1dr1[a]	1	22	18.3	−4.15	22.5
502D2dr1	1	22	20.0	−4.15	24.2
L24D2dr1	1	24	22.8	−0.2	23.0
L24D2dr2dr4	2	24	21.2	−0.2	21.4
L24D2dr5	1	24	22.4	−0.2	22.6
L26D1dr4	1	26	20.9	−0.8	21.7
L26D1dr3	1	26	21.2	−0.8	22.0
L26D2dr3	1	26	20.9	−0.8	21.9
L26D2dr1-2-4	3	26	21.1	−0.8	21.9
L28D1dr1	1	28	20.6	−0.64	21.3
L28D1dr1[a]	1	28	20.7	−0.64	21.4
L28D3dr1-2-3	3	28	21.9	−0.64	22.6

δ^{18}Op is the oxygen isotope composition of apatite phosphate group.
δ^{18}Ow is the mean oxygen isotope composition of water in every aquarium (see Table 1).
[a] Duplicates from different fragments of the same tooth.

5. Discussion

5.1. Comparison with earlier published fractionation equations

The observed scattering in $\delta^{18}O_p$–$\delta^{18}O_w$ of up to 2‰ for each aquarium water temperature (Fig. 3) arises from the combination of (i) analytical errors on both $\delta^{18}O_p$ (±0.2‰) and $\delta^{18}O_w$ (±0.04‰) analyses, and (ii) variable evolution of $\delta^{18}O_w$ in the different aquariums during the experiment (Fig. 2). In Nausicaä, the relative stability of $\delta^{18}O_w$ is likely related to water storage in the large tank before redistribution in the aquariums resulting in a buffering of possible $\delta^{18}O$ fluctuations of the pumped nearby seawater. By contrast, the aquariums in both La Rochelle and Lyon, working in a closed system, encountered larger fluctuations in $\delta^{18}O_w$. These variations likely result from uncontrolled evaporation processes that were not entirely compensated by the regular addition of water during the experiment.

In order to establish the relationship between temperature and $\delta^{18}O_p$–$\delta^{18}O_w$, we applied a linear regression model to our data set. Because temperature was held constant at ±0.5 °C in each aquarium, the error/range ratio of this parameter is low by comparison to the one of $\delta^{18}O_p$–$\delta^{18}O_w$. Instead of a classical approach (Davis, 2002), we directly searched for the $T = f(\delta^{18}O_p–\delta^{18}O_w)$ equation as already applied in previous studies (Longinelli and Nuti, 1973b; Erez and Luz, 1983; Kolodny et al., 1983). Although this procedure is supposed to provide a biased model, several authors concluded that predictions derived from this procedure, called inverse calibration, are more reliable than those operated on the basis of the seemingly more appropriate classical approach (Centner et al., 1998; Grientschnig, 2000; Tellinghuisen, 2000). The linear regression calculated from our data set provides the following equation:

$$T(°C) = 124.6(\pm 9.5) - 4.52(\pm 0.41)\,(\delta^{18}O_p - \delta^{18}O_w), \quad (1)$$

$$r = 0.8848, p < 0.001.$$

The errors on coefficients are given at 1σ. Both parameters correlate significantly and should be regarded as varying jointly. This property is depicted by the ellipse in Fig. 4 which represents the 95% confidence region of both estimates (slope and intercept), so that any attempt in considering their errors independently to each other, in other terms as possibly varying within a rectangle region, would be wrong (Draper and Smith, 1998). From Fig. 4, it becomes clear that the fractionation equations previously reported by Longinelli and Nuti (1973b) and Kolodny et al. (1983) are significantly different from Eq. 1. The slope of the new equation (4.52) is not significantly different from the slopes of 4.38 and 4.30 calculated by Kolodny et al. (1983) and Longinelli and Nuti (1973b). By contrast, our data present

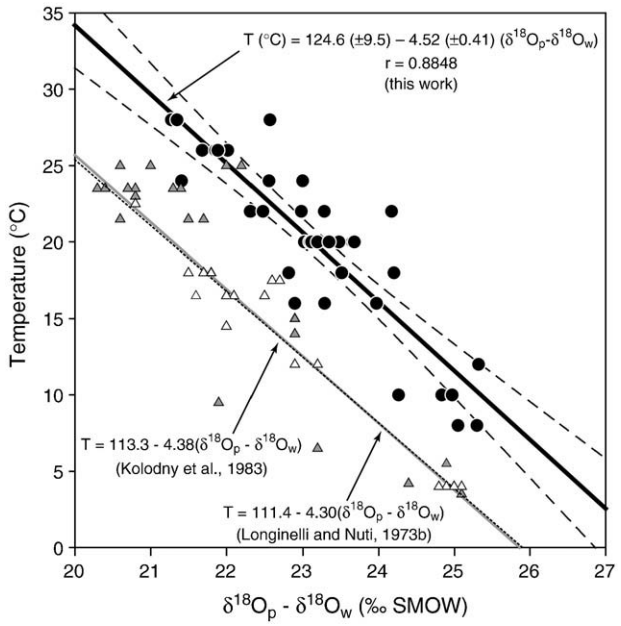

Fig. 3. Temperature versus $\delta^{18}O_p$–$\delta^{18}O_w$ for fish teeth measured in this study (black closed circle). Values published by Longinelli and Nuti (1973b) and Kolodny et al. (1983) are shown as white and grey triangles, respectively. The linear regression (black bold line) has been computed for the present study data as well as its 95% confidence interval (long dashed lines). Fractionation equations provided by Kolodny et al. (1983) and Longinelli and Nuti (1973b) are shown for comparison (grey line and black dotted line, respectively). Regression analysis has been computed using the method implemented in R (http://www.r-project.org).

an offset of +1.7 to +1.9‰ in the 5–35 °C range in comparison to the previously published equations (Fig. 3).

Kolodny et al. (1983) and Longinelli and Nuti (1973b) did not provide a value for NBS120c since this standard was not available at

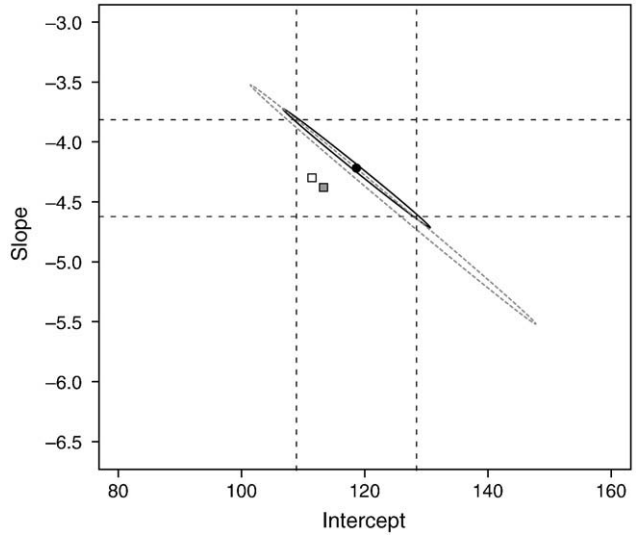

Fig. 4. Joint confidence area for the slope and intercept of the linear regression (95% confidence level) using our data (grey dashed ellipse) and pooled data (black ellipse). Joint confidence region is significantly reduced if our data are pooled with values of Kolodny et al. (1983) and Longinelli and Nuti (1973b), corrected by adding 2.2‰ (see section 5.2). Least squares estimates of both slope and intercept for pooled data and their 95% individual confidence intervals are plotted as black circle and dashed black line, respectively. The slope and intercept of the fractionation equations published in Longinelli and Nuti (1973b) and Kolodny et al. (1983) are given as white and grey square, respectively. Joint confidence regions of estimates were computed with R using the ELLIPSE package, following an adapted version of the procedure described in Cornillon and Matzner-Løber (2007).

that time. However, Shemesh et al. (1988) and Bryant et al. (1994) using the same laboratory and techniques (fluorination of $BiPO_4$) as Kolodny et al. (1983) reported values for NBS120b of 20.1 ± 0.3‰ and 19.91 ± 0.39‰, respectively. NBS120b and NBS120c are different aliquots of a Florida phosphate rock and previous studies reported differences in $\delta^{18}O$ of NBS120c and NBS120b between 0.1 and 1.7‰ dependant on used analytical methods (Wright and Hoering, 1989; Stephan, 2000; Chenery et al., 2010). Analyses of NBS120b using the trisilverphosphate and TC-EA methodology (this study) gave a value of 22.2 ± 0.2‰ (adopting a $\delta^{18}O$ of NBS120c = 22.6‰). In comparison, Chenery et al. (2010) reported a value of 21.4‰ for NBS120b (adopting a $\delta^{18}O$ of NBS120c = 21.7‰). This 1.4‰ offset in $\delta^{18}O$ with earlier analyses of NBS120b using fluorination of $BiPO_4$ (Shemesh et al., 1988; Bryant et al., 1994) would increase by 0.9‰ and total 2.3‰ if all data are normalized using a value of 22.6‰ for NBS120c. A comparable offset has also been reported by O'Neil et al. (1994) with an average offset of +1.2‰ being observed for biogenic apatites analysed as Ag_3PO_4 by sealed tube combustion and as $BiPO_4$ by conventional fluorination. Since O'Neil et al. (1994) reported a value of 21.7‰ for NBS120c, this offset would increase to 2.1‰ if normalized using a value for NBS120c of 22.6‰ (Fig. 5). The average difference of 2.2‰ calculated from the work of Chenery et al. (2010) and O'Neil et al. (1994) is comparable to the offset documented between the temperature equation reported in this study and by Kolodny et al. (1983) and Longinelli and Nuti (1973b).

5.2. A new phosphate–water fractionation equation

In order to better constrain the regression parameters of the new phosphate–water fractionation equation, we corrected the $\delta^{18}O$ data given by Longinelli and Nuti (1973b) and Kolodny et al. (1983) by adding 2.2‰ and pooled these data with our data set (Fig. 4 and Fig. 6).The linear regression calculated from the pooled data is given in Eq. (2). This new equation is not significantly different from Eq. (1), but pooling of the data reduces the errors of estimates considerably (Fig. 4):

$$T(°C) = 118.7(\pm 4.9) - 4.22(\pm 0.20)\left(\delta^{18}O_p - \delta^{18}O_w\right), \quad (2)$$

$$r = 0.9192,\ p < 0.001.$$

Various laboratories either use a $\delta^{18}O$ value for standard NBS120c of 21.7‰ (e.g. Lécuyer et al., 1993) or 22.6‰ (Vennemann et al., 2002). In order to account for this problem, we modified the phosphate–water fractionation equation (Eq. 2) by including a correction term for the used $\delta^{18}O$ value of NBS120c:

$$T(°C) = 118.7 - 4.22\left[\left(\delta^{18}O_p + \left(22.6 - \delta^{18}O_{NBS120c}\right)\right) - \delta^{18}O_w\right]. \quad (3)$$

With this equation, temperatures can be calculated independently of the adopted value of NBS120c used for standardization of the analyses.

5.3. Implication for previously published paleotemperatures

The new paleotemperature equation has major implications for marine paleotemperatures calculated from oxygen isotopes measured on biogenic phosphate using the most recent techniques. Our results imply that calculated paleotemperatures in all studies using a value for standard NBS120c of 21.7 or 22.6‰, which is the case in most studies since the work of O'Neil et al. (1994), have been underestimated by about 4 or 8 °C, respectively. For example, palaeotemperatures calculated from $\delta^{18}O$ of Palaeozoic conodont apatite (Joachimski et al., 2009, Trotter et al. 2008) will increase substantially. Trotter et al. (2008) reported an increase in $\delta^{18}O$ of conodont apatite from low $\delta^{18}O$ values in the Early to high $\delta^{18}O$ values

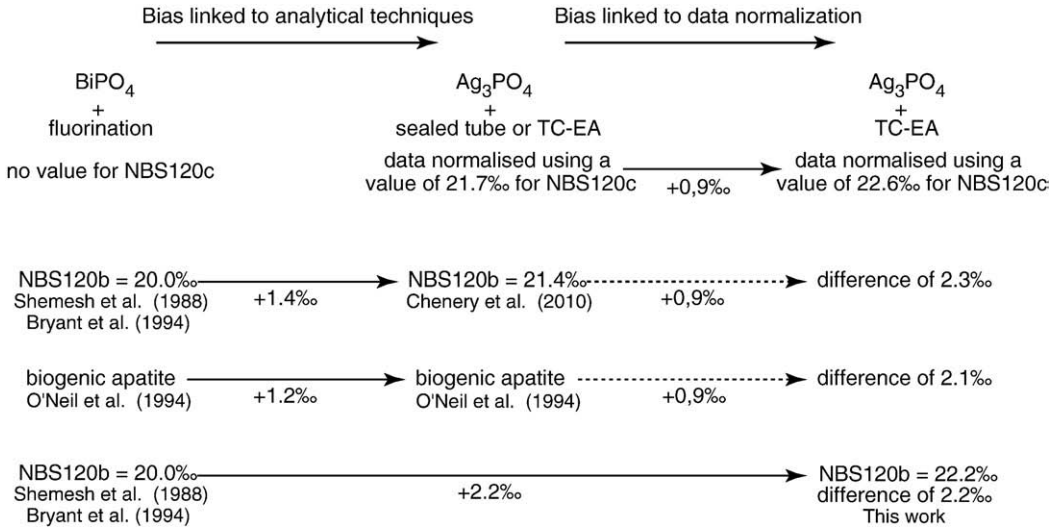

Fig. 5. Analytical and data normalization bias for oxygen isotope ratio of standard NBS120b and biogenic apatite samples based on O'Neil et al. (1994), Chenery et al. (2010), and this study. See section 5.1 for details.

in the Middle/Late Ordovician. This increase in $\delta^{18}O$ was interpreted as evidence for major climatic cooling that gave rise to the Ordovician biodiversification event. Using the revised phosphate–water equation, Early Ordovician palaeotemperatures will increase to 42° to 50 °C. These high sea surface temperatures exceed the lethal temperature limit of modern marine invertebrates and question whether the Early Ordovician $\delta^{18}O$ values mirror a secular decrease in the oxygen isotope composition of the Early Palaeozoic oceans as well as temperature as suggested by Veizer et al. (1999). This question is of importance and has been intensively debated, as such a secular evolution of the $\delta^{18}O$ of seawater would require changes in oceanic hydrothermal processes (Shields et al., 2003; Kasting et al., 2006).

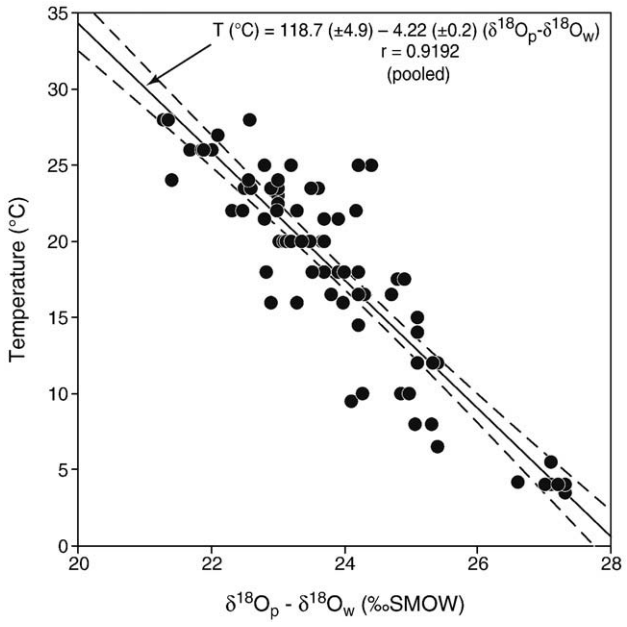

Fig. 6. Temperature versus $\delta^{18}O_p$–$\delta^{18}O_w$ for pooled data (black circles), that include both data from this study, Kolodny et al. (1983) and Longinelli and Nuti (1973b). Data from Kolodny et al. (1983) and Longinelli and Nuti (1973b) were corrected by adding +2.2‰ (see text). The computed linear regression and its 95% confidence interval are represented as black bold and dashed lines, respectively. Regression analysis computed as in Fig. 1.

6. Conclusion

The new phosphate–water fractionation equation obtained from fish raised in aquariums at a controlled temperature and monitored oxygen isotope composition of ambient waters, shows a similar slope but an offset of about 2.2‰ with earlier published fractionation equations. Analyses of standard NBS120b confirm that this offset is the consequence of different techniques used to analyse phosphate $\delta^{18}O$. Our new data imply that most of previously published marine palaeotemperatures have been underestimated by 4 to 8 °C depending on the adopted value of standard NBS120c. The new fractionation equation integrates the value of NBS120c used for data standardization and allows to correct previously published marine palaeotemperatures.

Acknowledgments

We warmly thank A. Langert, Y. Kolodny, and A. Shemesh (Hebrew University of Jerusalem) who provided us with an aliquot of NBS120b. We are grateful to N. Andersen for water analyses. We thank especially E. Rosinski for reviewing English. We are very grateful to J.-M. Maggiorani, F. Cousin and A. Decay who took care of the fish and of the quality of water during the experiments. We thank the Aquarium of La Rochelle, the Aquarium Grand Lyon, and Nausicaä for providing the aquariums and facilities needed for this work. This work was funded by a FABER project from the Région de Bourgogne.

References

Bryant, J.D., Luz, B., Froelich, P.N., 1994. Oxygen isotopic composition of fossil horse tooth phosphate as a record of continental paleoclimate. Palaeogeogr. Palaeoclimatol. Palaeoecol. 107, 303–316.
Bryant, D.J., Froelich, P.N., Showers, W.J., Genna, B.J., 1996. Biologic and climatic signals in the oxygen isotope composition of Eocene–Oligocene equid enamel phosphate. Palaeogeogr. Palaeoclimatol. Palaeoecol. 126, 75–89.
Centner, V., Massart, D.L., de Jong, S., 1998. Inverse calibration predicts better than classical calibration. J. Anal. Chem. 361, 2–9.
Chenery, C., Müldner, G., Evans, J., Eckardt, H., Lewis, M., 2010. Strontium and stable isotope evidence for diet and mobility in Roman Gloucester, UK. J. Arch. Sci. 37, 150–163.
Cornillon, P.-A., Matzner-Løber, E., 2007. Régression — Théorie et Applications. Springer-Verlag France, Paris.
Crowson, R.A., Showers, W.J., Wright, E.K., Hoering, T.C., 1991. Preparation of phosphate samples for oxygen isotope analysis. Anal. Chem. 63, 2397–2400.
Davis, J.C., 2002. Statistics and Data Analysis in Geology. Wiley, New York.
Dera, G., Pucéat, E., Pellenard, P., Neige, P., Delsate, D., Joachimski, M., Reisberg, L., Martinez, M., 2009. Water mass exchange and variations in seawater temperature in the NW Tethys during the Early Jurassic: evidence from neodymium and oxygen isotopes of fish teeth and belemnites. Earth Planet. Sci. Lett. 286, 198–207.

Draper, N.R., Smith, H., 1998. Applied Regression Analysis. Wiley-Interscience, New York.
Erez, J., Luz, B., 1983. Experimental paleotemperature equation for planktonic foraminifera. Geochim. Cosmochim. Acta 47, 1025–1031.
Grientschnig, D., 2000. Relation between prediction errors of inverse and classical calibration. J. Anal. Chem. 367, 497–498.
Joachimski, M.M., von Bitter, P.H., Buggisch, W., 2006. Constraints on Pennsylvannian glacioeustatic sea-level changes using oxygen isotopes of conodont apatite. Geology 34, 277–280.
Joachimski, M.M., Breisig, S., Buggisch, W., Talent, J.A., Mawson, R., Gereke, M., Morrow, J.M., Day, J., Weddige, K., 2009. Devonian climate and reef evolution: insights from oxygen isotopes in apatite. Earth Planet. Sci. Lett. 284, 596–599.
Kasting, J.F., Howard, M.T., Wallmann, K., Veizer, J., Shields, G., Jeffries, J., 2006. Paleoclimates, ocean depth, and the oxygen isotopic composition of seawater. Earth Planet. Sci. Lett. 252, 82–93.
Kolodny, Y., Luz, B., Navon, O., 1983. Oxygen isotope variations in phosphate of biogenic apatites. I. Fish bone apatite — rechecking the rules of the game. Earth Planet. Sci. Lett. 64, 398–404.
Kornexl, B.E., Gehre, M., Höfling, R., Werner, R.A., 1999. On-line $\delta^{18}O$ measurement of organic and inorganic substances. Rapid Commun. Mass Spectrom. 13, 1685–1693.
Lécuyer, C., Grandjean, P., O'Neil, J.R., Cappetta, H., Martineau, F., 1993. Thermal excursions in the ocean at the Cretaceous–Tertiary boundary (northern Morocco): δ18O record of phosphatic fish debris. Palaeogeogr. Palaeoclimatol. Palaeoecol. 105, 235–243.
Lécuyer, C., Grandjean, P., Emig, C.C., 1996. Determination of oxygen isotope fractionation between water and phosphate from living linguids: potential application to palaeoenvironmental studies. Palaeogeogr. Palaeoclimatol. Palaeoecol. 126, 101–108.
Lécuyer, C., Picard, S., Garcia, J.-P., Sheppard, S., Grandjean, P., Dromart, G., 2003. Thermal evolution of Tethyan surface waters during the Middle–Late Jurassic: evidence from d18O values of marine fish teeth. Paleoceanography 18 (3), 1076.
Longinelli, A., 1966. Ratios of oxygen-18: oxygen-16 in phosphate and carbonate from living and fossil marine organisms. Nature 211, 923–927.
Longinelli, A., Nuti, S., 1973a. Revised phosphate–water isotopic temperature scale. Earth Planet. Sci. Lett. 19, 373–376.
Longinelli, A., Nuti, S., 1973b. Oxygen isotope measurements of phosphate from fish teeth and bones. Earth Planet. Sci. Lett. 20, 337–340.
O'Neil, J.R., Roe, L.J., Reinhard, E., Blake, R.E., 1994. A rapid and precise method of oxygen isotope analysis of biogenic phosphate. Isr. J. Earth Sci. 43, 203.
Pucéat, E., Lécuyer, C., Donnadieu, Y., Naveau, P., Cappetta, H., Ramstein, G., Huber, B.T., Kriwet, J., 2007. Fish tooth $\delta^{18}O$ revising Late Cretaceous meridional upper ocean water temperature gradients. Geology 35, 107–110.
Rosecchi, E., 1985. Ethologie alimentaire des Sparidae Diplodus annularis, D. sargus, D. vulgaris, Pagellus erythrinus et *Sparus aurata* du golfe du Lion et des étangs Palavasiens. Thesis, University of Montpellier.
Shemesh, A., Kolodny, Y., Luz, B., 1988. Isotope geochemistry of oxygen and carbon in phosphate and carbonate of phosphorite francolite. Geochim. Cosmochim. Acta 52, 2565–2572.
Shields, G.A., Carden, G.A.F., Veizer, J., Meidla, T., Rong, J.-Y., Li, R.-Y., 2003. Sr, C, and O isotope geochemistry of Ordovician brachiopods: a major isotopic event around the Middle–Late Ordovician transition. Geochim. Cosmochim. Acta 67, 2005–2025.
Stephan, E., 2000. Oxygen isotope analysis of animal bone phosphate: method refinement, influence of consolidants, and reconstruction of paleotemperatures for Holocene sites. J. Arch. Sci. 27, 523–535.
Tellinghuisen, J., 2000. Inverse vs. classical calibration for small data sets. J. Anal. Chem. 368, 585–588.
Trébaol, L., Francillon-Vieillot, H., Meunier, F.J., 1991. Etude de la croissance des mâchoires pharyngiennes chez Trachinotus teraia (Carangidae, Perciformes) à l'aide de la technique du marquage vital. Cybium 15, 263–270.
Trotter, J.A., Williams, I.S., Barnes, C.R., Lécuyer, C., Nicoll, R.S., 2008. Did cooling oceans trigger Ordovician biodiversification? Evidence from conodont thermometry. Science 321, 550–554.
Veizer, J., Karem Azmy, D.A., Bruckschen, P., Buhl, D., Bruhn, F., Carden, G.A.F., Diener, A., Ebneth, S., Godderis, Y., Jasper, T., Korte, C., Pawellek, F., Podlaha, O.G., Strauss, H., 1999. $^{87}Sr/^{86}Sr$, $\delta^{13}C$, and $\delta^{18}O$ evolution of Phanerozoic seawater. Chem. Geol. 161, 59–88.
Vennemann, T.W., Hegner, E., Cliff, G., Benz, G.W., 2001. Isotopic composition of recent shark teeth as a proxy for environmental conditions. Geochim. Cosmochim. Acta 65, 1583–1599.
Vennemann, T.W., Fricke, H.C., Blake, R.E., O'Neil, J.R., Colman, A., 2002. Oxygen isotope analysis of phosphates: a comparison of techniques for analysis of Ag_3PO_4. Chem. Geol. 185, 321.
Wright, E.K., Hoering, T.C., 1989. Separation and purification of phosphates for oxygen isotope analysis. Annual Report of the Director, Geophysical Laboratory, Carnegie Institute, 2150, pp. 137–141.