PLOS | ONE

RESEARCH ARTICLE

Seasonality and Paleoecology of the Late Cretaceous Multi-Taxa Vertebrate Assemblage of "Lo Hueco" (Central Eastern Spain)

Laura Domingo[1,2,3]*, Fernando Barroso-Barcenilla[2,4], Oscar Cambra-Moo[5]

1 Departamento de Geología Sedimentaria y Cambio Medioambiental, Instituto de Geociencias IGEO-CSIC-UCM, Madrid, Spain, **2** Departamento de Paleontología, Universidad Complutense de Madrid, Madrid, Spain, **3** Earth and Planetary Sciences Department, University of California Santa Cruz, Santa Cruz, California, United States of America, **4** Grupo de Investigación IberCreta, Departamento de Geología y Geografía, Universidad de Alcalá de Henares, Alcalá de Henares, Spain, **5** Laboratorio de Poblaciones del Pasado, Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid, Spain

* laudomingo@gmail.com

OPEN ACCESS

Citation: Domingo L, Barroso-Barcenilla F, Cambra-Moo O (2015) Seasonality and Paleoecology of the Late Cretaceous Multi-Taxa Vertebrate Assemblage of "Lo Hueco" (Central Eastern Spain). PLoS ONE 10(3): e0119968. doi:10.1371/journal.pone.0119968

Academic Editor: Matthew C. Mihlbachler, NYIT College of Osteopathic Medicine, UNITED STATES

Received: February 27, 2014

Accepted: January 28, 2015

Published: March 25, 2015

Funding: This study was supported by research projects CGL2009-09000 and CGL2009-10766 (Spanish Ministry of Economy and Competitivity). LD acknowledges postdoctoral fellowships of the University of California Santa Cruz, PICATA program (UCM-UPM Moncloa Campus of International Excellence, Spain), and Juan de la Cierva program (Spanish Ministry of Economy and Competitivity). Part of this research was carried out within projects CGL2011-25894 and CGL2012-35199 (Spanish Ministry of Economy and Competitivity). The funders had no role in study design, data collection and

Abstract

Isotopic studies of multi-taxa terrestrial vertebrate assemblages allow determination of paleoclimatic and paleoecological aspects on account of the different information supplied by each taxon. The late Campanian-early Maastrichtian "Lo Hueco" Fossil-Lagerstätte (central eastern Spain), located at a subtropical paleolatitude of ~31°N, constitutes an ideal setting to carry out this task due to its abundant and diverse vertebrate assemblage. Local $\delta^{18}O_{PO4}$ values estimated from $\delta^{18}O_{PO4}$ values of theropods, sauropods, crocodyliforms, and turtles are close to $\delta^{18}O_{H2O}$ values observed at modern subtropical latitudes. Theropod $\delta^{18}O_{H2O}$ values are lower than those shown by crocodyliforms and turtles, indicating that terrestrial endothermic taxa record $\delta^{18}O_{H2O}$ values throughout the year, whereas semiaquatic ectothermic taxa $\delta^{18}O_{H2O}$ values represent local meteoric waters over a shorter time period when conditions are favorable for bioapatite synthesis (warm season). Temperatures calculated by combining theropod, crocodyliform, and turtle $\delta^{18}O_{H2O}$ values and gar $\delta^{18}O_{PO4}$ have enabled us to estimate seasonal variability as the difference between mean annual temperature (MAT, yielded by theropods) and temperature of the warmest months (TWMs, provided by crocodyliforms and turtles). ΔTWMs-MAT value does not point to a significantly different seasonal thermal variability when compared to modern coastal subtropical meteorological stations and Late Cretaceous rudists from eastern Tethys. Bioapatite and bulk organic matter $\delta^{13}C$ values point to a C_3 environment in the "Lo Hueco" area. The estimated fractionation between sauropod enamel and diet is ~15‰. While waiting for paleoecological information yielded by the ongoing morphological study of the "Lo Hueco" crocodyliforms, $\delta^{13}C$ and $\delta^{18}O_{CO3}$ results point to incorporation of food items with brackish influence, but preferential ingestion of freshwater. "Lo Hueco" turtles showed the lowest $\delta^{13}C$ and

analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

$\delta^{18}O_{CO3}$ values of the vertebrate assemblage, likely indicating a diet based on a mixture of aquatic and terrestrial C_3 vegetation and/or invertebrates and ingestion of freshwater.

Introduction

The Mid-Cretaceous thermal maximum, which peaked in Turonian times and constituted the warmest climate warming of the last 144 Ma [1–2] was followed by a long-term cooling trend beginning at the early Campanian and detected in both the terrestrial and marine realms [3–8]. This trend was characterized by alternating cooling and warming episodes across the Campanian and Maastrichtian [9–11]. In spite of this cooling pattern, overall warmer conditions than today persisted until the end of the Cretaceous, as suggested by high atmospheric CO_2 concentrations and a lack of permanent ice at the poles [11–14]. During this time, enhanced ocean heat transport along with the maintainance of a low albedo at high latitudes, due to the presence of forests, contributed to a reduced latitudinal thermal gradient [15].

Although stable isotope studies on Cretaceous terrestrial vertebrate taxa can help to better understand continental climatic and environmental conditions, they are not as common as those performed on Cenozoic vertebrate taxa due to the scarcity of adequate sample sizes of fossil material, the destructive nature of the stable isotope technique, as well as pervasive diagenetic effects observed in fossils of this age. Some stable isotope studies on Cretaceous vertebrate assemblages have focused on the analysis of $\delta^{18}O$ on the bioapatite with the aim of characterizing paleoclimate [16–18], faunal behavior and/or paleohydrology [18–20], and metabolism with special interest on dinosaurian body temperature [21–23]. $\delta^{13}C$ values have been used to make inferences on paleoecology and to study the fractionation between diet (vegetation) and dinosaur enamel [24–26]. Domingo *et al.* [27] carried out a preliminary stable isotope study on the "Lo Hueco" vertebrate paleofauna (central eastern Spain) as a first approach to evaluate the potential, validity and degree of preservation of the bioapatite from this locality and as a first approach to characterize paleoclimatic ($\delta^{18}O_{H2O}$, temperature) and paleoecological-/paleoenvironmental (diet, type of vegetation) variables existing during the late Campanian-early Maastrichtian in the southeastern area of the Iberian region. Albeit the Late Cretaceous Iberian geological record shows a good representation of vertebrate localities and outcrops, there are very scarce stable isotope studies on this area and time period [27–28]. Therefore, the isotopic investigation of the "Lo Hueco" vertebrate assemblage provide valuable data, which may shed light on climatic and environmental conditions that occurred in this subtropical setting located in the Tethys realm.

The main objectives of our study are three-fold: 1) to determine seasonal thermal variability in the Iberian Late Cretaceous, 2) to characterize the isotopic offset between $\delta^{13}C$ values of dinosaur tooth enamel and their diet ($\Delta^{13}C_{enamel\text{-}diet}$), and 3) to unveil dietary and habitat affinities for "Lo Hueco" crocodyliforms and turtles. The richness and diversity of the Late Cretaceous "Lo Hueco" Fossil-Lagerstätte [29–30] have allowed us to approach the aforementioned questions by performing multi-taxa stable isotope analyses ($\delta^{18}O_{PO4}$, $\delta^{18}O_{CO3}$ and $\delta^{13}C$) on dinosaurs, crocodyliforms, turtles, gars and bulk organic matter. Most isotopic studies dealing with Mesozoic vertebrates focused on one or two groups [16–17, 19, 25–26, 31–32], so the advantage of considering co-existing taxa relies on the fact that they can shed light on differences in habitat and resource utilization, informing about climatic, environmental and ecological variables such as hydrology of the area, seasonality, type of vegetation, resource/habitat overlap and/or partitioning.

While different paleontological, geochemical and modelling studies offered a good characterization of long-term climatic variability for the Middle and Late Cretaceous (e.g., [1–3, 6, 16, 33], among others), the evaluation of seasonal thermal variability has proven challenging on account of the lack of proxies capable of recording intra-annual information. Controversy arises when discerning whether Middle and Late Cretaceous climate was more seasonally thermally equable (low mean annual range of temperatures) [34–39], although, there seems to be an agreement indicating a higher seasonal thermal variability (i.e., lower equability) during the colder Late Cretaceous in comparison to the warmer Middle Cretaceous [38].

$\delta^{18}O$ values in the phosphate and carbonate fractions of vertebrate bioapatite record the $\delta^{18}O$ value of their body water ($\delta^{18}O_{bw}$). In the case of terrestrial endothermic species, $\delta^{18}O_{bw}$ is a reflection of oxygen uptake (inspired O_2 and water vapor, drinking water, dietary water, oxygen in food dry matter) and loss (excreted water and solids, expired CO_2, and water vapor). Dinosaur metabolism is a controversial issue that remains under debate [21, 40–45]. Fricke and Rogers [22] performed $\delta^{18}O_{PO4}$ analyses on Late Cretaceous theropods and crocodilians from fossil sites located at different latitudes and found that the reconstructed theropod slope of the $\delta^{18}O_{PO4}$-latitude relationship was steeper than the one observed for crocodilians (ectotherms) and similar to that estimated for present-day endotherms such as mammals and birds. Amiot *et al.* [23] used a similar approach to that of Fricke and Rogers [22] on a wider suite of Cretaceous dinosaurs and obtained body temperatures of 36–38°C for dinosaurs inhabiting high and low latitudes. Other authors such as O'Connor and Dodson [46] and Gillooly *et al.* [47] argued that dinosaur body temperature depended on body mass (inertial homeothermy), ranging from 25°C at 12 kg to 41°C at 13,000 kg [47]. However, Fricke and Rogers [22] observed similar metabolisms for Late Cretaceous theropods showing different body mass (e.g., *Albertosaurus*: 1.3–1.7 tons; *Majungasaurus*: 1.5 tons; *Saurornitholestes*: 10 kg). Amiot *et al.* [23] observed a widespread endothermy in different groups of Late Cretaceous dinosaurs (theropods, sauropods, ornithopods, and ceratopsians). Eagle *et al.* [48] performed clumped isotope analyses on sauropods and estimated body temperatures between 4 and 7°C lower (36 to 38°C) than those proposed by Gillooly *et al.* [47], arguing that sauropods were able to regulate their body temperatures, preventing overheating. Ectothermic semi-aquatic taxa, such as crocodiles and turtles, form their bioapatite within a narrow thermal window, and their bioapatite $\delta^{18}O$ is a reflection of the $\delta^{18}O_{H2O}$ value of warm months when conditions are favorable for apatite synthesis [20, 49–50]. In the case of ectothermic aquatic taxa (gars), bioapatite forms in isotopic equilibrium with ambient water and since they do not thermoregulate, their bioapatite $\delta^{18}O$ values record both $\delta^{18}O_{H2O}$ and ambient temperature independently of body temperature [51]. Therefore, it is possible to estimate temperature values from gar $\delta^{18}O$ values if the $\delta^{18}O_{H2O}$ value is independently estimated [20, 51–52].

Studies dealing with dinosaur $\delta^{13}C$ values have mainly focused on herbivore taxa (e.g., [24–26, 53]). These authors obtained relatively high $\delta^{13}C$ values for hadrosaurian and ceratopsian dinosaurs for a typical C_3 environment (C_4 plant expansion took place in the late Miocene–Pliocene). Plants following the C_3 or Calvin-Benson photosynthetic pathway (trees, shrubs, forbs, and cool-season grasses) strongly discriminate against ^{13}C during fixation of CO_2, yielding tissues with $\delta^{13}C$ values averaging $\sim$ -27.5‰ (VPDB) (ranging from -36‰ to -22‰). The most negative $\delta^{13}C$ values of this range (-36‰ to -30‰) reflect closed canopy conditions due to recycling of ^{13}C-depleted CO_2 and low irradiance. The highest values (-25‰ to -22‰) correspond to C_3 plants from high insolated, arid, or water stressed environments [54–56]. When considering fossil taxa, it is necessary to account for shifts in the $\delta^{13}C$ value of atmospheric CO_2 ($\delta^{13}C_{atmCO2}$), the source of plant carbon, including anthropogenic modification due to fossil fuel burning, which has decreased the $\delta^{13}C$ value of atmospheric CO_2 from a value of −6.0‰ in the Late Cretaceous [57] to a modern value of −8.0‰ [58–59]. Accounting for these

shifts in baseline and assuming the modern fractionation of ~ -19.5‰ between $\delta^{13}C_{atmCO2}$ and C_3 vegetation $\delta^{13}C$ values [60], Late Cretaceous mean $\delta^{13}C$ value for C_3 plants would be ~ -25.5‰ (ranging from -34‰ to -20‰), with the most negative $\delta^{13}C$ values (-34‰ to -28‰) reflecting closed canopy conditions and the highest values (-23‰ to -20‰) reflecting high insolated, arid, or water stressed environments. Studies of $\delta^{13}C$ offsets between terrestrial vertebrate enamel and diet ($\Delta^{13}C_{enamel\text{-}diet}$) have been mainly carried out on modern mammalian taxa, which allows for extrapolation on paleontological studies dealing with mammals, so that a consistent $\Delta^{13}C_{enamel\text{-}diet}$ of +12 to +14‰ has been observed between herbivore mammalian tooth enamel and vegetation $\delta^{13}C$ values [61–63], while the difference between mammalian carnivore and herbivore tooth enamel $\delta^{13}C$ values ($\Delta^{13}C_{carnivore\text{-}herbivore}$) has been proposed to be ~ -1.3‰ [64]. Johnson *et al.* [65] and Angst *et al.* [66] also investigated this tissue-diet fractionation on modern birds by analyzing ostrich eggshells and diet, obtaining a $\Delta^{13}C_{eggshell\text{-}diet}$ value of +16‰ and +13.4‰, respectively. The lack of modern counterparts in the case of dinosaurs makes it difficult to assess this offset and we can just rely on the fossil record geochemical imprint to determine tissue-diet fractionation values. In this vein, analyzing herbivore dinosaur bioapatite and bulk organic matter $\delta^{13}C$ values, Fricke and Pearson [25] and Fricke *et al.* [26] argued that $\Delta\delta^{13}C_{enamel\text{-}diet}$ for ornithischian dinosaurs was ~ +18‰, whereas Tütken [32] estimated a $\Delta\delta^{13}C_{enamel\text{-}diet}$ value of ~ +16‰ for sauropods.

Geographic and geological setting

The Late Cretaceous "Lo Hueco" Fossil-Lagerstätte is located in the province of Cuenca (central eastern Spain: 2° 02'50"W, 39° 57'15"N) (Fig. 1). It was fortuitously discovered in 2007, while constructing the Madrid-Levante high-speed railway. More than 10,000 macrofossils of different taxonomic groups of flora and fauna were unearthed. The stratigraphic position and associated fauna support a late Campanian–early Maastrichtian age [29, 67–68]. The "Lo Hueco" site outcrops in Garumn facies, which is the informal term that designates marl, clay, and gypsum, mainly of red color, deposited in shallow marine, coastal, and/or continental environments of southwestern Europe during the latest Cretaceous and the earliest Paleogene [69]. It corresponds to the upper part of the Villalba de la Sierra Formation (Fig. 2A), a lithological unit interpreted as a coastal marsh with distributary channels and sporadic establishment of sabkhas [29]. Six stratigraphic levels were defined at the "Lo Hueco" outcrop named V, G1, R1, G2, R2, and M from bottom to top [29] (Fig. 2B). This succession appears slightly modified laterally by a lower sulphate interval (S1) that cuts the V level in the eastern area of the outcrop, by a sandy channel structure (C) that interrupts V, G1, and R1 levels in the southern area, and by an upper sulphate interval (S2) that cuts part of G2 in the northeastern area (Fig. 2B). Four bonebeds have yielded the majority of fossils: the C structure, the G1 and G2 levels and the lower part of the R2 level (Fig. 2B) [29, 68]. The G1 level is interpreted as a proximal muddy floodplain (close to the distributary channels) and vertebrate fossils usually appear complete and associated. More than 14 partially articulated sauropod skeletons were recovered from this level [28, 68]. Exceptionally well-preserved plant remains have been also described from this lithosome [30]. The G2 level corresponds to the distal part of a poorly drained muddy floodplain (distal from the distributary channels) and points to a relatively calm aquatic environment (i.e., marsh/swamp) exposed to partial or total desiccation, although there is a low degree of articulation of preserved skeletons [29, 68]. Recent fluid inclusion and geochemical analyses on the sulphates from S1 and S2 have pointed to a near coast playa-lake environment with mainly brackish and freshwater influence in the area, while not totally ruling out some extent of marine influence [70]. During the late Campanian-early Maastrichtian, the "Lo Hueco" locality was placed at a subtropical paleolatitude of ~ 31°N.

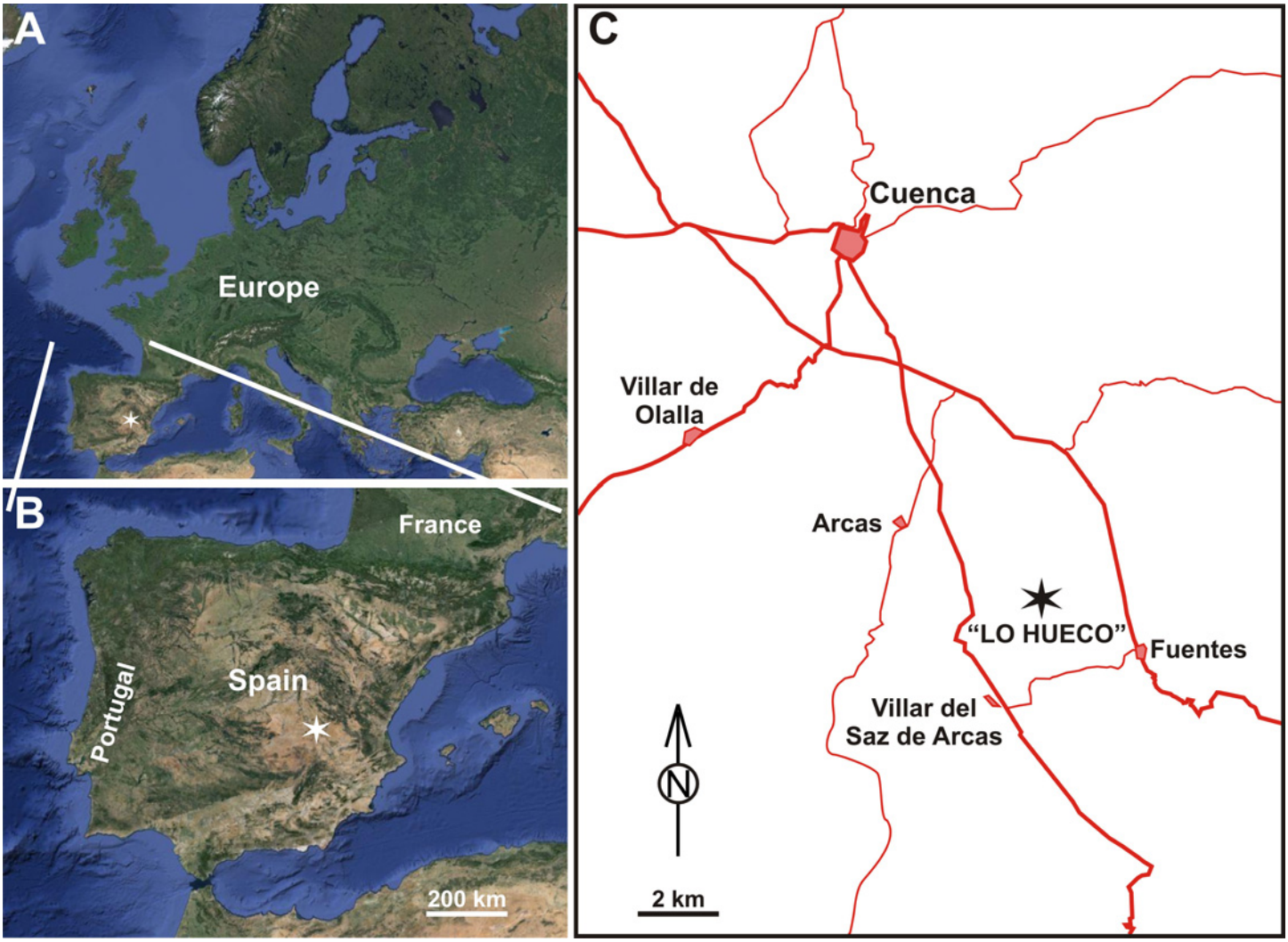

Fig 1. Geographic setting of the "Lo Hueco" fossil site. Satellite images of A) Europe, and B) Iberian Peninsula; the star shows the situation of the "Lo Hueco" fossil site. C) Close-up view of "Lo Hueco" near the town of Fuentes showing other towns, and the main and secondary roads. Europe and Iberian Peninsula satellite images from USGS EROS (Earth Resources Observatory and Science (EROS) Center) http://eros.usgs.gov/#.

doi:10.1371/journal.pone.0119968.g001

Materials and Methods

The rich and diverse vertebrate assemblage of the "Lo Hueco" fossil site allowed us to collect multi-taxa samples including dinosaur (theropods: n = 11, sauropods: n = 4) and crocodyliform (n = 36) tooth enamel, turtle shell (n = 9) and gar ganoine (n = 19) from levels G1 and G2. Paleontological prospection and excavation undertaken at the "Lo Hueco" fossil site were authorized by the Dirección General de Patrimonio y Museos of the Junta de Comunidades de Castilla-La Mancha (Spain) (permit number: 04–0392-P11). "Lo Hueco" vertebrate remains are housed at Museo de las Ciencias de Castilla-La Mancha (Cuenca, Spain). Complete repository information including specimen number, stratigraphic situation and geographic location are given in S1 Dataset. Dinosaurs, crocodyliforms and turtles were analyzed for their $\delta^{18}O_{PO4}$, $\delta^{18}O_{CO3}$ and $\delta^{13}C$ values, whereas gars were analyzed for their $\delta^{18}O_{PO4}$ values. Dinosaur dentine was also analyzed in order to check for diagenetic effects. Although three major groups of dinosaurs (theropods, sauropods and ornithopods) are represented in "Lo Hueco", in this

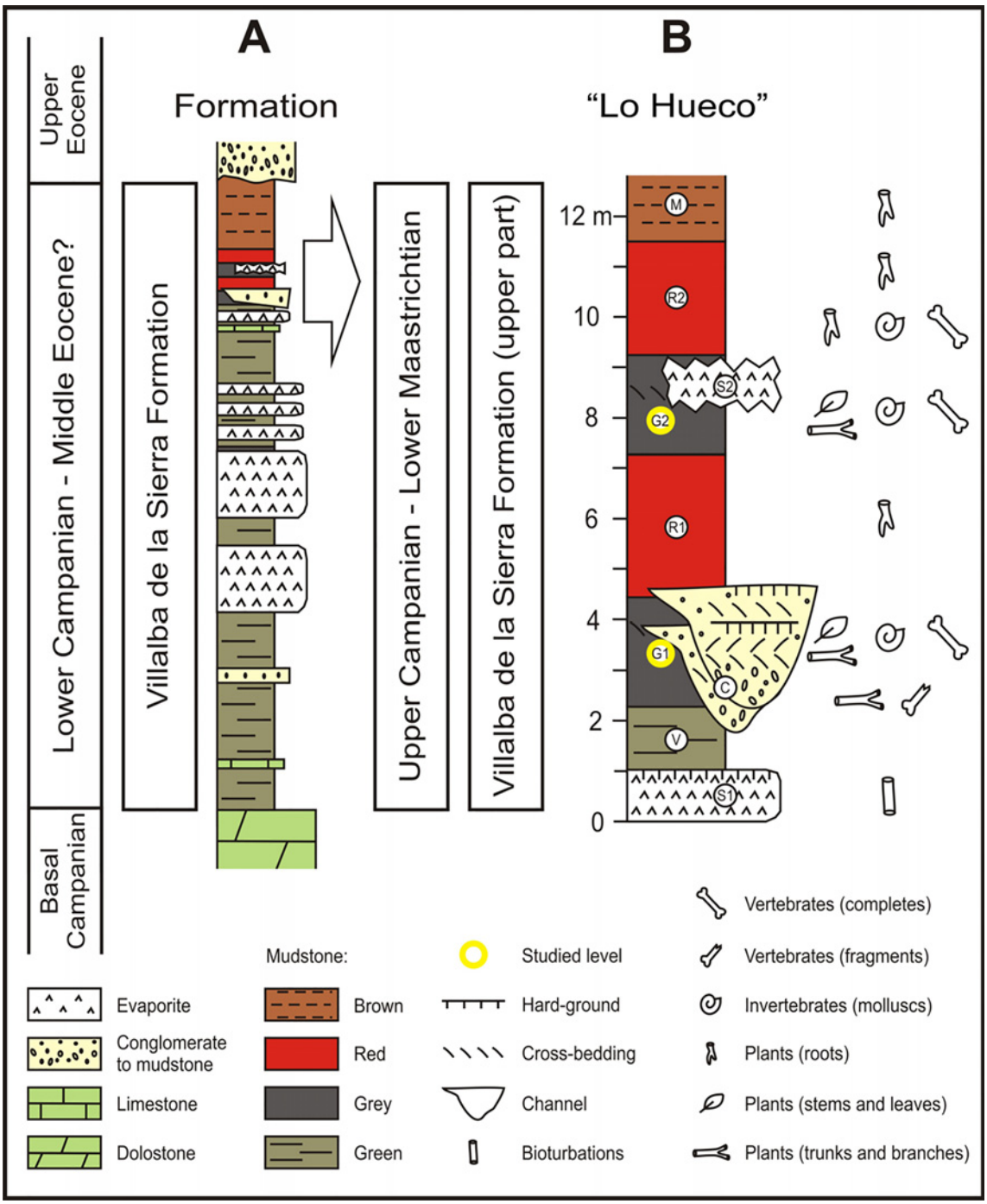

Fig 2. Stratigraphic context of the "Lo Hueco" fossil site. A) The Villalba de la Sierra Formation. B) Detailed stratigraphic section of "Lo Hueco" showing the studied levels G1 and G2.

doi:10.1371/journal.pone.0119968.g002

study we had access to theropods belonging to Dromaeosaurinae and Velociraptorinae Eumaniraptora and to sauropods, which are presumably representing two titanosaurian species, one of them close to the genus *Ampelosaurus* [71–73]. Eusuchian crocodile remains are very abundant at this locality, although the record is monotonous and seems to be represented by two

genera close to *Allodaposuchus* [74]. Most of the turtle material probably belongs to two forms of Pleurodira, specifically to members of Bothremydidae [29, 75–76]. Finally, actinopterygians from "Lo Hueco" are mainly represented by lepisosteids, commonly known as gars [29]. We also performed $\delta^{13}C$ analyses on bulk organic matter from the "Lo Hueco" G1 (n = 10) and G2 (n = 8) levels with the aim of characterizing the type of vegetation that was present in the area.

The carbon and oxygen isotope results are reported in the δ-notation. $\delta^{H}X_{sample} = [(R_{sample}—R_{standard})/R_{standard}] \times 1000$, where X is the element, H is the mass of the rare, heavy isotope, and R = $^{13}C/^{12}C$ or $^{18}O/^{16}O$. Vienna Pee Dee Belemnite (VPDB) is the standard for $\delta^{13}C$ values, whereas $\delta^{18}O$ values for both bioapatite carbonate and phosphate are reported relative to Vienna Standard Mean Ocean Water (VSMOW).

Sample chemical treatments followed those described in Bassett *et al.* [77] for phosphate in bioapatite, Koch *et al.* [78] for carbonate in bioapatite and Domingo *et al.* [79] for bulk organic matter. All samples were analyzed at the Stable Isotope Laboratory of the University of California Santa Cruz (USA). Bioapatite $\delta^{18}O_{PO4}$ values were measured using a Thermo Finnigan Delta plus XP isotope ratio mass spectrometer coupled via continuous flow to a high temperature conversion elemental analyzer (TCEA). Bioapatite $\delta^{13}C$ and $\delta^{18}O_{CO3}$ analyses were conducted on a Thermo MAT253 dual-inlet isotope-ratio mass spectrometer coupled to a Kiel IV carbonate device. Bulk organic matter $\delta^{13}C$ analyses were performed using a Carlo Erba 1108 elemental analyzer interfaced to a ThermoFinningan Delta Plus XP isotope ratio mass spectrometer. The standards used in the case of phosphates were Fisher Standard ($\delta^{18}O$ = 8.4‰), UCSC Low standard ($\delta^{18}O$ = 11.3‰), UCSC High standard ($\delta^{18}O$ = 19.0‰) and Kodak standard ($\delta^{18}O$ = 18.1‰) (all of them are silver phosphate). Standard deviations for repeated measurements of Fisher (n = 52), UCSC Low (n = 13), UCSC High (n = 16), and Kodak (n = 7) standards were 0.51‰, 0.55‰, 0.21‰, and 0.36‰ for $\delta^{18}O_{PO4}$. As suggested by Suarez *et al.* [20], we used NIST 120c as a quality control standard and not as a calibration standard since its value is highly dependent on both pretreatment and analysis methods. NIST 120c value (n = 8) was 21.5±0.4. Replicate $\delta^{18}O_{PO4}$ analyses were carried out in ∼80% of the samples. The average absolute difference for $\delta^{18}O_{PO4}$ was 0.02‰ and the standard deviation of this average difference was 0.20‰. The standards used in the case of carbonates were Carrara Marble (CM, $\delta^{13}C$ = 2.05‰ and $\delta^{18}O$ = -1.91‰, both VPDB), NBS-18 ($\delta^{13}C$ = -5.03‰ and $\delta^{18}O$ = -23.01‰, VPDB) and NBS-19 ($\delta^{13}C$ = 1.95‰ and $\delta^{18}O$ = -2.20‰, VPDB). The standard deviations for repeated measurements of CM (n = 16), NBS-18 (n = 19), and NBS-19 (n = 10) were 0.03‰, 0.33‰, and 0.04‰ for $\delta^{13}C$, respectively, and 0.08‰, 0.07‰, and 0.09‰ for $\delta^{18}O$, respectively. Duplicate analyses were performed for ∼50% of the samples. The average absolute difference for $\delta^{13}C$ and $\delta^{18}O_{CO3}$ was 0.03‰ and 0.14‰, respectively, and the standard deviations of these average differences were 0.02‰ and 0.05‰ for $\delta^{13}C$ and $\delta^{18}O_{CO3}$, respectively. The standards used in the case of bulk organic matter analyses were Pugel ($\delta^{13}C$ = -12.60‰) and Acetanilide ($\delta^{13}C$ = -29.53‰). The standard deviations for repeated measurements of Pugel (n = 11) and Acetanilide (n = 3) were 0.15‰ and 0.02‰, respectively. Statistical tests were performed using the program SPSS PASW Statistics 18.0 software.

We used the following equations to calculate $\delta^{18}O_{H2O}$ values from "Lo Hueco" vertebrates:

Dinosaurs: $\delta^{18}O_{H2O} = 1.11\delta^{18}O_{PO4}–26.44$ [16] (1)

Crocodyliforms: $\delta^{18}O_{H2O} = 0.82\delta^{18}O_{PO4}–19.13$ [80] (2)

Turtles: $\delta^{18}O_{H2O} = 1.06\delta^{18}O_{PO4}–21.6$ [81] (3)

Equation (1) was calculated by Amiot *et al.* [16] constructing a database with modern mammalian $\delta^{18}O_{PO4}$ values and the $\delta^{18}O$ value of meteoric water from IAEA stations. Since most of dinosaur samples from "Lo Hueco" belong to theropods, we considered them as endotherms following most authors [22–23, 45], and therefore we applied equation (1) as Amiot *et al.* [16] suggested in their study. Equation (2) was experimentally determined by Amiot *et al.* [80]

analyzing modern crocodilian $\delta^{18}O_{PO4}$ values and the $\delta^{18}O$ value of the water in which they live. Equation (3) was determined by Coulson *et al.* [81] for freshwater and marine extant turtle datasets combining experimental and literature results. This equation was adjusted to correct for the difference in NIST 120c value obtained by Coulson *et al.* [81] (22.6‰) and in our study (21.5±0.4).

Once dinosaur, crocodyliform and turtle $\delta^{18}O_{H2O}$ values were estimated, we used gar ganoine $\delta^{18}O_{PO4}$ values as an independent proxy to calculate temperature values and to eventually determine temperature offsets related to seasonal patterns (see Discussion). Following Domingo *et al.* [82], we selected lepisosteid middle flank scales since they record the greatest number of layers of ganoine per unit of time and grow all year round. In the case of dinosaurs, we have only used theropod $\delta^{18}O_{PO4}$ values on account of 1) the low number of sauropod samples, and 2) the uncertainty about whether sauropods obtained water mainly from drinking (i.e., obligate drinkers) or from vegetation (i.e., non-obligate drinkers).

Different phosphate-water oxygen isotope fractionation equations have been proposed as paleothermometers based mainly on studies carried out on extinct and extant fish and invertebrate phosphates. The utilization of a given equation may yield significant differences in calculated temperature values when compared to other equations, which can be as large as 8–9°C [83–84]. Here, we applied three different equations in order to check whether they can be used to calculate temperature offsets related to seasonal patterns independently of the absolute temperature values yielded by each of them (see Discussion). We considered the following equations:

$T(°C) = 119.3\ (\pm 12.9) - 4.38\ (\pm 0.54)\ (\delta^{18}O_{PO4} - \delta^{18}O_{H2O})$ [51] (4)

This equation was rescaled for a NBS 120b value of 21.4‰ (see [84])

$T(°C) = 118.7\ (\pm 4.9) - 4.22\ (\pm 0.20)\ (\delta^{18}O_{PO4} - \delta^{18}O_{H2O})$ [85] (5)

$T(°C) = 117.4\ (\pm 9.5) - 4.50\ (\pm 0.43)\ (\delta^{18}O_{PO4} - \delta^{18}O_{H2O})$ [84] (6)

Results

Table 1 shows dinosaur, crocodyliform, turtle and gar mean $\delta^{18}O_{PO4}$, $\delta^{13}C$, $\delta^{18}O_{CO3}$, and $\delta^{18}O_{H2O}$ values. Individual values per sample and statistical tests are given in S1 and S2 Datasets, respectively.

$\delta^{18}O_{PO4}$ results

Fig. 3 shows mean $\delta^{18}O_{PO4}$ values for theropods, sauropods, crocodyliforms, turtles and gars. Mean $\delta^{18}O_{PO4}$ value for theropods is 20.8±0.9‰, with a non-significant increase of ~0.5‰ between G1 and G2 ($t = -1.146$, $p = 0.276$). Mean $\delta^{18}O_{PO4}$ value for sauropods is 20.9 ± 0.4‰ and there is also an increase of ~0.5‰ in $\delta^{18}O_{PO4}$ between G1 and G2, although in this case no statistical tests were done since only one sauropod per level could be sampled. Mean $\delta^{18}O_{PO4}$ value for crocodyliforms is 19.4 ± 0.9‰ with a significant increase of ~1.0‰ between G1 and G2 ($t = -3.491$, $p = 0.002$). Turtles show a mean $\delta^{18}O_{PO4}$ value of 18.3‰ ± 0.7‰, with a non-significant increase of ~0.8‰ between G1 and G2 ($t = -2.208$, $p = 0.063$). Crocodyliforms and turtles show statistically consistent lower $\delta^{18}O_{PO4}$ values than dinosaurs for the total dataset (Table 1 and S2 Dataset). Mean $\delta^{18}O_{PO4}$ value for gars is 19.8 ± 1.0‰, with a non-significant decrease of ~0.2‰ between G1 and G2 ($t = 0.475$, $p = 0.641$). ANOVA tests show significant differences for $\delta^{18}O_{PO4}$ values when comparing all taxa for the total dataset and for G1 and G2 independently (S2 Dataset).

Table 1. Mean and standard deviation (SD) $\delta^{18}O_{PO4}$ (‰ VSMOW), $\delta^{18}O_{H2O}$ (‰ VSMOW), $\delta^{13}C$ (‰ VPDB), and $\delta^{18}O_{CO3}$ (‰ VSMOW) values of "Lo Hueco" theropods, sauropods, crocodyliforms, turtles and gars for the total dataset (TOTAL) and independently for G1 and G2 levels.

	$\delta^{18}O_{PO4}$ (‰ VSMOW)		$\delta^{18}O_{H2O}$ (‰ VSMOW)		$\delta^{13}C$ (‰ VPDB)		$\delta^{18}O_{CO3}$ (‰ VSMOW)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
TOTAL								
Theropods	20,8	0,9	-3,5	1,0	-10,7	0,8	30,0	1,1
Sauropods	20,9	0,4	-	-	-10,5	0,8	29,0	0,1
Crocodyliforms	19,4	0,9	-3,2	0,7	-10,4	1,9	28,2	1,0
Turtles	18,3	0,7	-2,2	0,7	-11,8	0,6	27,1	0,3
Gars	19,8	1,0						
G1								
Theropods	20,2	1,2	-4,1	1,4	-10,7	1,2	31,2	0,2
Sauropods	20,6	-	-	-	-10,6	1,0	29,0	0,02
Crocodyliforms	19,0	0,7	-3,6	0,6	-10,3	2,1	28,1	1,1
Turtles	17,9	0,6	-2,7	0,6	-12,5	0,1	27,0	0,3
Gars	19,1	1,5						
G2								
Theropods	20,7	0,5	-3,5	0,5	-10,9	0,6	29,7	1,1
Sauropods	21,1	-	-	-	-10,3	0,9	29,0	0,1
Crocodyliforms	20,0	0,7	-2,7	0,6	-10,6	1,8	28,2	1,1
Turtles	18,7	0,5	-1,8	0,5	-11,3	0,2	27,2	0,2
Gars	19,7	0,9	-	-	-	-	-	-

doi:10.1371/journal.pone.0119968.t001

$\delta^{18}O_{H2O}$ results

Mean calculated $\delta^{18}O_{H2O}$ value for theropods is -3.5 ± 1.0‰, with a non-significant increase of ~0.6‰ between G1 and G2 (t = -0.670, p = 0.566). Mean $\delta^{18}O_{H2O}$ value for crocodyliforms is -3.2 ± 0.7‰, with a significant increase of ~0.9‰ between G1 and G2 (t = -2.541, p = 0.019).

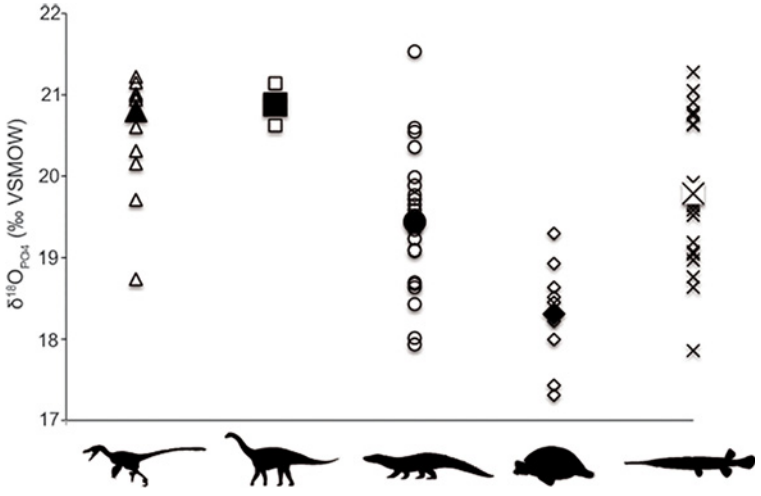

Fig 3. $\delta^{18}O_{PO4}$ (‰ VSMOW) values of "Lo Hueco" vertebrates. Theropods (triangles), sauropods (squares), crocodyliforms (circles), turtles (diamonds), and gars (crosses). Large black symbols represent mean $\delta^{18}O_{PO4}$ values.

doi:10.1371/journal.pone.0119968.g003

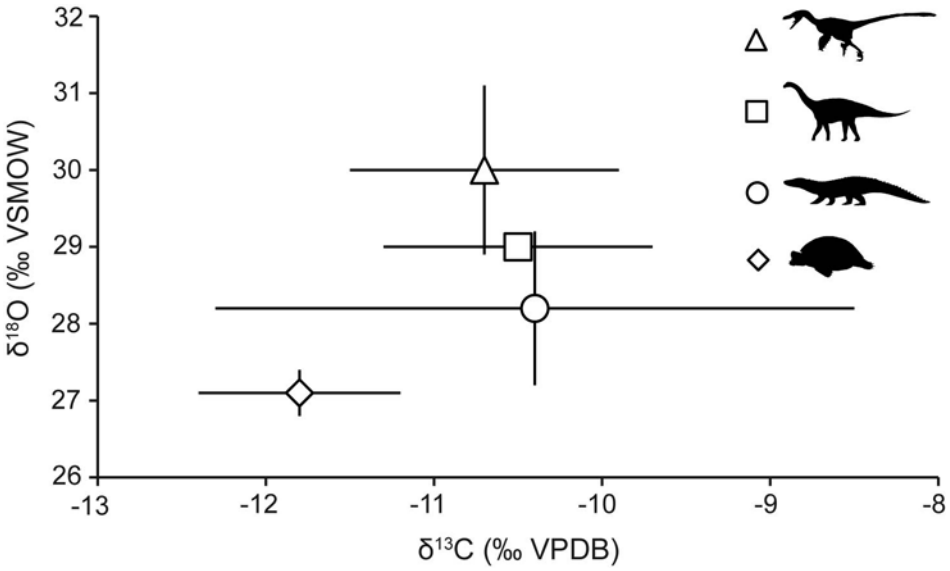

Fig 4. Mean ± 1 SD $\delta^{13}C$ (‰ VPDB) and $\delta^{18}O_{CO3}$ (‰ VSMOW) values of the "Lo Hueco" vertebrates. Theropods (triangles), sauropods (squares), crocodyliforms (circles), and turtles (diamonds).

doi:10.1371/journal.pone.0119968.g004

Turtle mean $\delta^{18}O_{H2O}$ value is -2.2 ± 0.7‰, with a non-significant increase of ~0.9‰ between G1 and G2 (t = -2.215, p = 0.062). ANOVA test perfomed for the total dataset and for levels G1 and G2 showed significant differences in $\delta^{18}O_{H2O}$ value among theropods, crocodyliforms and turtles (S2 Dataset).

$\delta^{13}C$ and $\delta^{18}O_{CO3}$ results

Fig. 4 shows a biplot $\delta^{13}C$- $\delta^{18}O_{CO3}$ graph for the "Lo Hueco" vertebrate assemblage. Theropod mean $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values are -10.7 ± 0.8‰ and 30.0 ± 1.1‰, respectively, with a decrease in both values between G1 and G2, although only significant in the case of $\delta^{18}O_{CO3}$ (t = 2.603, p = 0.022). Sauropod mean $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values are -10.5 ± 0.8‰ and 29.0 ± 0.1‰, respectively, with a non-significant increase in $\delta^{13}C$ between G1 and G2 (t = -0.334, p = 0.770) and with the same $\delta^{18}O_{CO3}$ value in both levels. Crocodyliform mean $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values are -10.4 ± 1.9‰ and 28.2 ± 1.0‰, respectively, with a non-significant decrease in $\delta^{13}C$ between G1 and G2 (t = 0.214, p = 0.834) and a non-significant increase in $\delta^{18}O_{CO3}$ between G1 and G2 (t = -0.204, p = 0.842). Finally, turtle mean $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values are -11.8 ± 0.6‰ and 27.1 ± 0.3‰, respectively, with increases in both values between G1 and G2, although only significant in the case of $\delta^{13}C$ (t = -14.300, $p < 0.001$). $\delta^{13}C$ values do not show statistically significant differences among taxa for the total dataset, G1 and G2, whereas $\delta^{18}O_{CO3}$ values do show significant differences in all cases (S2 Dataset).

Bulk organic matter mean $\delta^{13}C$ value is -25.1±1.4‰, with a non-significant increase of ~0.2‰ between G1 and G2 (t = -0.302, p = 0.767).

Diagenesis

Several lines of evidence point to a low degree of diagenetic alteration for the "Lo Hueco" fossil vertebrates:

1) Tooth enamel was the selected tissue for dinosaurs, crocodyliforms, whereas ganoine was analyzed in the case of gars. Relative to dentine, bone and isopedine, tooth enamel and ganoine

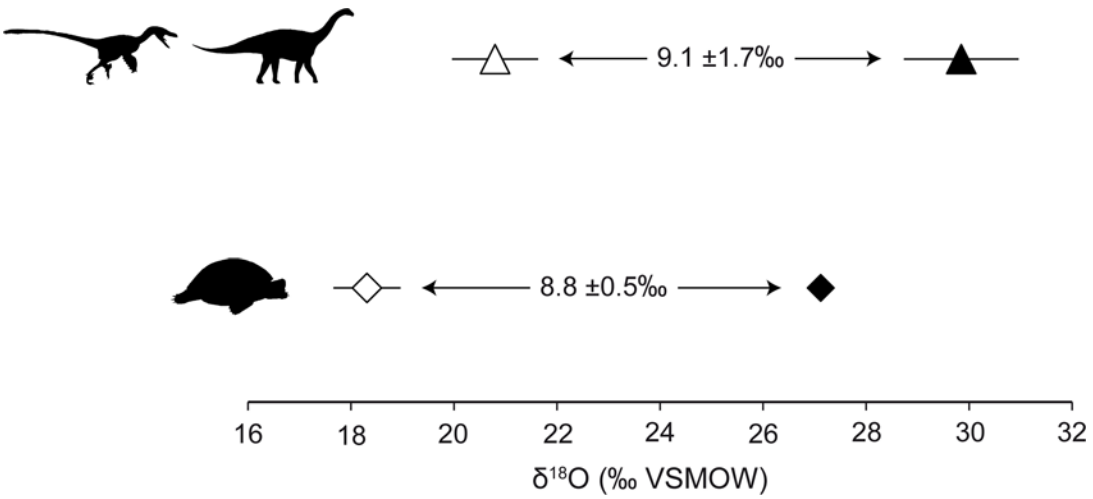

Fig 5. $\Delta^{18}O_{CO3\text{-}PO4}$ values for dinosaurs (theropods and sauropods) and turtles. Both values are within the range of the expected equilibrium difference for modern bioapatites (~8.6–9.1‰) [87–88]. Open symbols represent $\delta^{18}O_{PO4}$ values and solid symbols correspond to $\delta^{18}O_{CO3}$.

doi:10.1371/journal.pone.0119968.g005

have larger apatite crystals, a lower content of organic matter, and a low porosity, making them the least prone tissues to undergo diagenetic alteration [86].

2) Several studies of living organisms point to a difference between bioapatite $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ values ($\Delta^{18}O_{CO3\text{-}PO4}$) of ~8.6–9.1‰ [87–88]. Obtaining a $\Delta\delta^{18}O_{CO3\text{-}PO4}$ value near this range in fossil bioapatite has been viewed as an indication that both phases retain pristine isotopic values [87, 89–91]. The mean $\Delta^{18}O_{CO3\text{-}PO4}$ value for "Lo Hueco" dinosaurs is 9.1 ± 1.7‰ and thus, it is in the range of the expected equilibrium difference supporting a low degree of diagenetic alteration (Fig. 5). Although this relationship was established for endotherms, "Lo Hueco" turtle $\Delta^{18}O_{CO3\text{-}PO4}$ value is also in that range (8.8 ± 0.5‰) (Fig. 5), probably reflecting sustained and constant body temperature during mineral growth (preferentially during summer months: [49–50]). The fact that we only sampled compact bone from the outermost part of turtle shells reduces the possibility of diagenetic alteration. $\Delta^{18}O_{CO3\text{-}PO4}$ value could not be calculated for crocodyliforms since $\delta^{18}O_{CO3}$ and $\delta^{18}O_{PO4}$ values did not come from the same samples.

3) Dinosaur enamel and dentine $\delta^{18}O_{PO4}$ analyses were performed in order to evaluate the response of these tissues against postburial alteration. Several authors have observed higher isotopic values in dinosaur dentine compared to enamel (e.g., [24–26]). Fricke and Pearson [25] argued that this difference was due to a variable exposure of dentine to diagenetic fluids, as well as more isotopic exchange and/or secondary mineral formation in dentine compared to enamel. In the case of the "Lo Hueco" dinosaurs, we also observed higher isotopic values in dentine with theropods showing $\Delta^{18}O_{PO4dentine\text{-}enamel}$ = 1.04, and sauropods showing $\Delta^{18}O_{PO4dentine\text{-}enamel}$ = 1.34. Significant differences have been detected between both tissues when considering the total dataset (t = -2.983, p = 0.007).

4) An additional line of evidence to assess the preservation of original $\delta^{18}O_{PO4}$ values was outlined by Fricke and Rogers [22] when trying to determine the type of metabolism of dinosaurs. According to these authors, differences in body temperatures between ectotherms and endotherms give rise to differences in $\delta^{18}O_{PO4}$ values as a function of latitude in such a way that at high latitudes, ectotherms have higher $\delta^{18}O_{PO4}$ values than endotherms (due to lower body temperature for ectotherms). Conversely, at low latitudes, endotherms show higher $\delta^{18}O_{PO4}$ values than ectotherms. Amiot *et al.* [16] stated that diagenetic processes would bring

about the homogenization of isotopic values and therefore, the aforementioned differences would not be observable. The "Lo Hueco" fossil site was situated at a low paleolatitude (~31°N) during the Late Cretaceous [92]. Theropod dinosaurs, which have been proposed to be endotherms [22–23, 93], show consistently higher $\delta^{18}O_{PO4}$ values in comparison to crocodyliforms and turtles (ectotherms) at the "Lo Hueco" fossil site when considering the total dataset as well as G1 and G2 levels separately (Table 1), in agreement with the results observed by Fricke and Rogers [22] and Amiot *et al.* [16]. In addition to the difference observed in $\delta^{18}O_{PO4}$ values between endotherms and ectotherms, lower $\delta^{18}O_{PO4}$ values for aquatic gars and semi-aquatic crocodyliforms and turtles, and higher $\delta^{18}O_{PO4}$ values for terrestrial theropods constitute another line of evidence pointing to the preservation of the original isotopic signal, as previously stated by other authors [19–20].

Discussion

Seasonal thermal variability in the Iberian Late Cretaceous

Controversy arises when discerning whether Middle and Late Cretaceous climate witnessed a lower mean annual range of temperatures (i.e., seasonal thermal equability) than today [34–39], although it seems that during the colder Late Cretaceous there was a higher seasonal thermal variability (ie., lower equability) in comparison to the warmer Middle Cretaceous [38]. Multi-taxa studies as the one carried out here allow us to investigate this issue on account of differences in the timing of bioapatite growth, thus tracking different moments throughout the year. While in general, $\delta^{18}O_{H2O}$ values calculated for theropods, crocodyliforms and turtles from "Lo Hueco" correspond to typical precipitation waters in subtropical locations [94], theropods recorded consistently lower $\delta^{18}O_{H2O}$ and temperature values than crocodyliforms and turtles in G1 and G2 levels as well as in the total dataset (Table 1). Lower $\delta^{18}O_{H2O}$ values of terrestrial endothermic taxa such as theropods would correspond to ingested water probably consumed during the whole year and hence, recording all seasons. In contrast, $\delta^{18}O_{H2O}$ data of semiaquatic ectothermic taxa such as crocodyliforms and turtles would represent local meteoric waters over a shorter time scale when the conditions are favorable for apatite synthesis (i.e., growth during the warm season and higher $\delta^{18}O_{H2O}$ values) [21, 49, 80]. Temperature values calculated from theropod $\delta^{18}O_{H2O}$ values would track mean annual temperature (MAT), whereas temperature values estimated from crocodyliform and turtle $\delta^{18}O_{H2O}$ values would record temperature of the warmest months (TWMs), when apatite synthesis is more likely to occur. Albeit the seasonal thermal amplitude is usually calculated as the difference between temperature of the warmest months (TWMs) *minus* temperature of the coldest months (TCMs), in our approach it is not feasible to work out this latter value with the available proxies. We opted to infer the semi-seasonal thermal variability characterized as the difference between TWMs and MAT (ΔTWMs-MAT). Since the "Lo Hueco" site was located in a coastal subtropical setting, we created a database with modern meteorological information from coastal stations situated within the subtropics in both hemispheres (25°–35°) compiling MAT and TWMs (i.e., July, August, September) data and calculating ΔTWMs-MAT (S3 Dataset). We also considered seasonal data provided by Tethyan Cretaceous rudists [36, 38]. These authors argued that during the colder Early Cretaceous (late Barremian-middle Albian), seasonal thermal variability was more intense than during warmer Cretaceous episodes (late Albian-early Campanian). Fig. 6 shows ΔTWMs-MAT *vs* subtropical latitude range (25°–35°) for data calculated from vertebrate taxa from "Lo Hueco", modern subtropical stations, and Santonian-Campanian eastern Tethyan rudists (Greece and Turkey: [36, 38]). $\Delta TWMs_{\text{crocodyliforms-}}MAT_{\text{theropods}}$ values from "Lo Hueco" are 2.2±0.1°C, 3.5±0.1°C, and 2.7±0.1°C for G1, G2, and the total dataset, respectively (S4 Dataset), whereas $\Delta TWMs_{\text{turtles-}}MAT_{\text{theropods}}$ yielded higher values of 6.1±0.2°C, 7.4

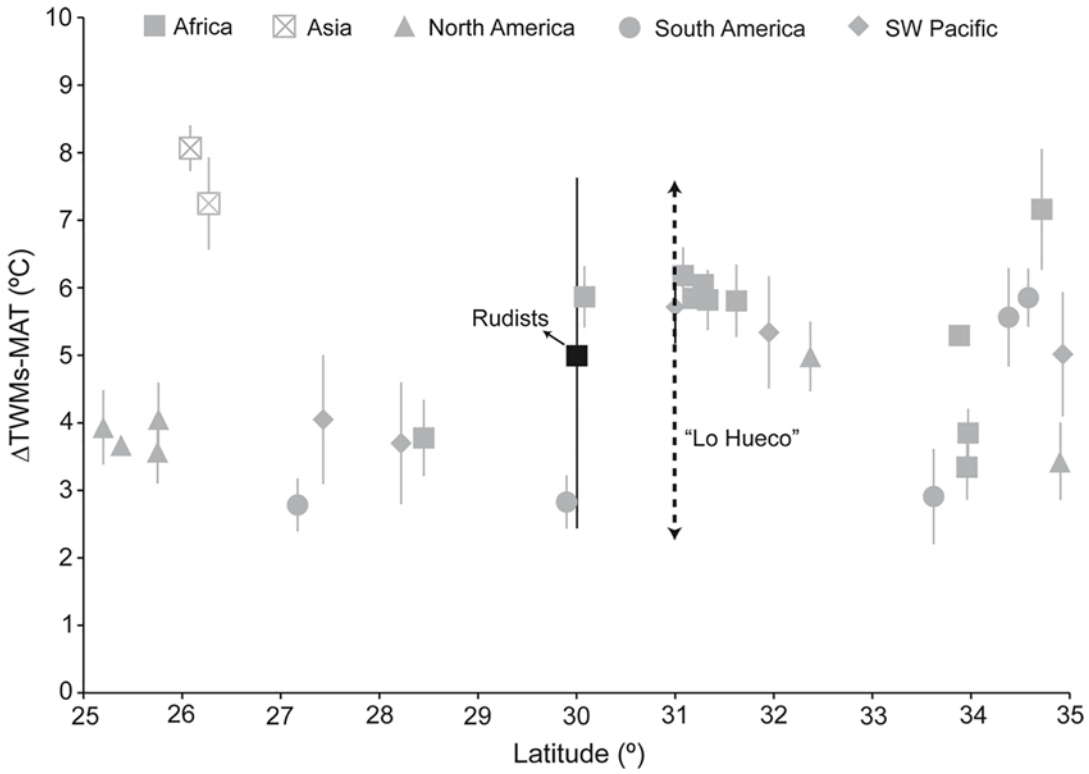

Fig 6. ΔTWMs-MAT (°C) *vs* latitude (°) for "Lo Hueco" (black dotted arrow), Late Cretaceous eastern Tethyan rudists from Steuber [36] and Steuber *et al*. [38] (black square), and modern meteorological stations located at latitudes 25° to 35° in both hemispheres from Africa (grey square), Asia (crossed square), North America (grey triangle), South America (grey circle), and Southwest Pacific (grey diamond). Absolute latitude is shown including North and South hemispheres.

doi:10.1371/journal.pone.0119968.g006

±0.20°C, and 6.7±0.2°C for G1, G2, and the total dataset, respectively (S4 Dataset). Similar $\Delta TWMs_{\text{crocodyliforms-}}MAT_{\text{theropods}}$ and $\Delta TWMs_{\text{turtles-}}MAT_{\text{theropods}}$ values were obtained when using equations (4), (5) and (6) (S4 Dataset). ΔTWMs-MAT values from modern data vary between minimum values of ~2.8±0.4°C (Easter Island, Chile, 27.17°S) and maximum values of ~8.1±0.3°C (Fuzhou, China, 26.08°N). Finally, the mean sea-surface ΔTWMs-MAT value from Late Cretaceous Tethyan rudists is ~5.0±2.6°C (Greece and Turkey, ~30°N). No significant statistical differences have been found between the "Lo Hueco" ΔTWMs-MAT values and those shown by modern meteorological stations and Cretaceous rudists (S5 Dataset) indicating that the climatic conditions in the subtropical western area of the Tethys during the late Campanian-early Maastrichtian were not more significantly equable than those observed today. Previous studies have suggested that during mid-Cretaceous greenhouse conditions the latitudinal thermal gradient was weaker than the one observed today, whereas during the cooler late-Cretaceous, the latitudinal thermal gradient has been proposed to be either weaker or similar to that observed today [6, 95–96]. Cool greenhouse periods, as Huber *et al.* [6] refer to mid-Campanian-Maastrichtian, showed warm temperatures in high latitudes and cool temperatures in the subtropics and tropics (e.g., [95]). Cooler conditions have been traditionally associated to a lower thermal equability and *vice versa* [37, 97] and in this vein, Steuber *et al.* [38], studying seasonal thermal variability on Tethyan rudists, observed a lower thermal seasonality during the warmer Cretaceous episodes, whereas cooler Cretaceous episodes show a higher thermal seasonality. "Lo Hueco" temperature data do not show a significant different seasonal thermal variability when compared to modern data pointing to a similar seasonal thermal

amplitude between central eastern Iberia during the late Campanian-early Maastrichtian and today (S3 and S5 Datasets). The determination of past seasonality remains a difficult issue to determine due to the limitation of the seasonal information yielded by paleoproxies and the shortage of these indicators in terrestrial settings. Isotopic studies of multi-taxa vertebrate assemblages as the one from the "Lo Hueco" locality help to characterize past seasonality. Future clumped isotope analyses on soil carbonate and invertebrate carbonate from the "Lo Hueco" locality may allow us to double-check TWMs values and compare them with those provided by crocodyliforms and turtles.

"Lo Hueco" vertebrate isotopic paleoecology

Carbon isotope data have not been widely investigated in Cretaceous multi-taxa assemblages and studies have mainly focused on herbivore terrestrial taxa (e.g., [24–26]). We have analyzed $\delta^{13}C$ values of sauropods, theropods, crocodyliforms and turtles as well as $\delta^{13}C$ values of the bulk organic matter from the "Lo Hueco" sediments. Sauropod mean $\delta^{13}C$ value is -10.5±0.8‰ and bulk organic matter mean $\delta^{13}C$ value is -25.1±1.4‰ (Fig. 7). While we are aware that the low number of "Lo Hueco" sauropod tooth enamel samples may prevent us from attaining conclusive results, we still believe that it is worth discussing them in the context of other isotopic studies focused on herbivore dinosaurs. The "Lo Hueco" bulk organic matter mean $\delta^{13}C$ value is almost identical to the estimated Late Cretaceous C_3 vegetation mean $\delta^{13}C$ value (-25.5‰), assuming a $\delta^{13}C_{atmCO2}$ value of ∼-6‰ for the late Campanian-early Maastrichtian [57] and the modern fractionation value of ∼-19.5‰ between $\delta^{13}C_{atmCO2}$ and C_3 vegetation $\delta^{13}C$ values [60]. This suggests a pure C_3 environment for the "Lo Hueco" locality for that time period. Assuming that the "Lo Hueco" bulk organic matter mean $\delta^{13}C$ value is representative of the $\delta^{13}C$ value of the vegetation present in the area, then the fractionation between the "Lo Hueco" sauropod enamel and diet ($\Delta^{13}C_{enamel\text{-}diet}$) would be ∼15‰. This offset lies between the one estimated for mammals and ostriches (12 to 14‰: [61–63, 66]) and the one estimated for sauropod dinosaurs and ostriches (∼16‰: [32, 65], respectively), and it is narrower when compared to the one estimated for ornithischian dinosaurs (i.e., 18‰: [25–26]). Stanton-Thomas and Carlson [24], Fricke and Pearson [25] and Fricke *et al.* [26] obtained higher $\delta^{13}C$ values for hadrosaur and ceratopsian tooth enamel (-4‰ to -5.9‰) from different Late Cretaceous North American localities located in the vicinity of the Western Interior Seaway when compared to "Lo Hueco" sauropod $\delta^{13}C$ values. Since bioapatite $\delta^{13}C$ values of terrestrial vertebrates are ultimately controlled by $\delta^{13}C$ values of diet giving rise to consistent $\Delta^{13}C_{enamel\text{-}diet}$ values [63, 86], the difference between the fractionation values obtained in our study and those from Stanton-Thomas and Carlson [24], Fricke and Pearson [25] and Fricke *et al.* [26] might be likely associated to metabolic differences among taxonomic groups (sauropods *vs*. ceratopsians and hadrosaurs) (Fig. 8) [22–23, 46–48]. Bulk organic matter mean $\delta^{13}C$ values showed by Fricke and Pearson [25] are ∼-24.0‰, just 1‰ higher than our bulk organic matter mean $\delta^{13}C$ values, supporting differences in dinosaur metabolism in a scenario with similar isotopic values of vegetation (Fig. 7). Fricke and Pearson [25] pointed out that differences in the methane production in herbivore dinosaur stomachs during digestion along with different utilization of plant organic compounds (e.g., carbohydrates, proteins, lipids) and/or plant parts (e.g., leaves, seeds, wood) may also explain the differences observed in $\Delta^{13}C_{enamel\text{-}diet}$. The larger ceratopsian and hadrosaur $\Delta^{13}C_{enamel\text{-}diet}$ may be due to their ability to incorporate low quality, fibrous vegetation [25], whereas the smaller sauropod $\Delta^{13}C_{enamel\text{-}diet}$ value may be indicative of consumption of more digestible food items (e.g., soft leaves), fact also supported by the dental morphology of "Lo Hueco" titanosaur sauropods (i.e., chisel-like teeth) more adapted to leave behind the least digestible tissues [98–99]. The 3‰ difference observed in $\Delta^{13}C_{enamel\text{-}diet}$ values

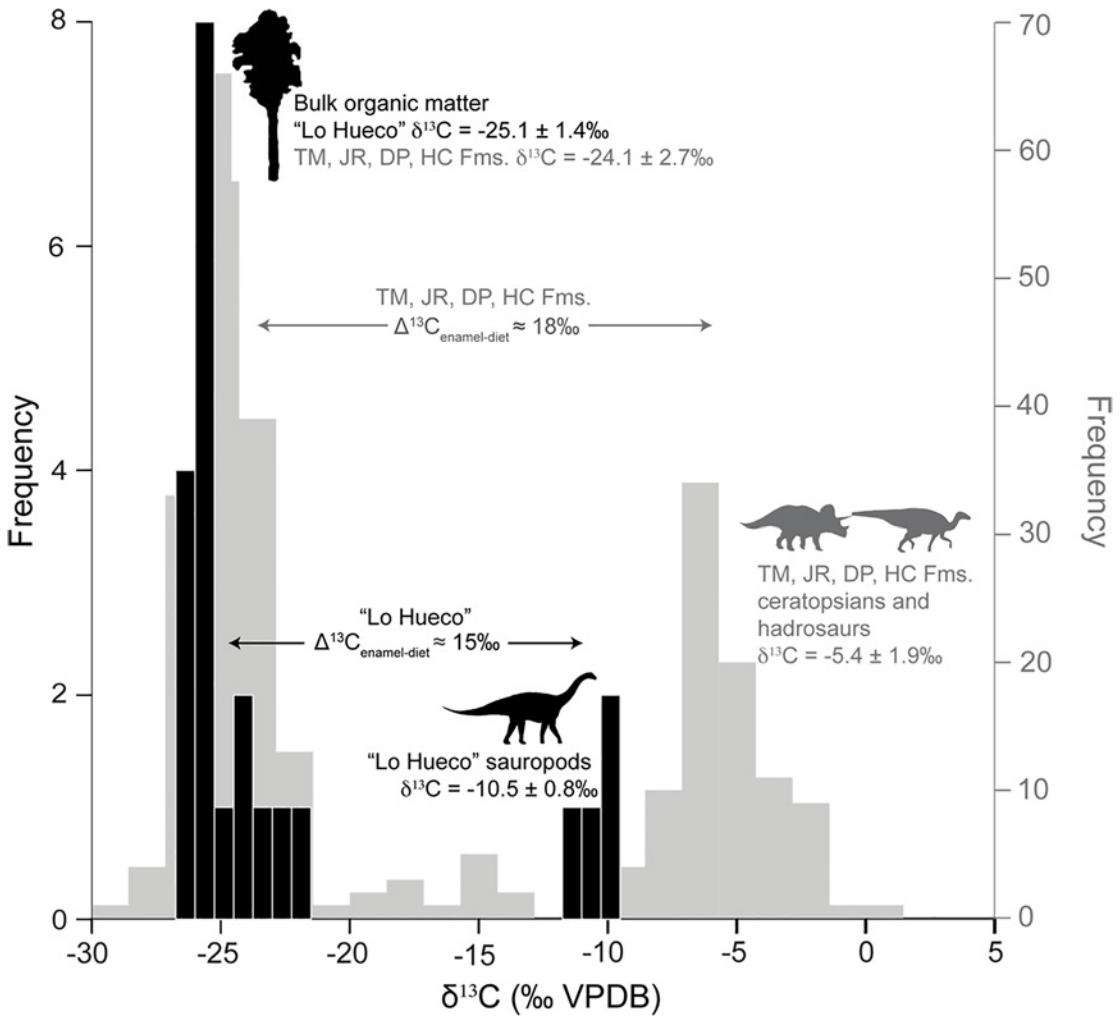

Fig 7. Fractionation between herbivore enamel and herbivore diet ($\Delta^{13}C_{enamel\text{-}diet}$). "Lo Hueco" bulk organic matter and sauropod mean ± 1 SD $\delta^{13}C$ (‰ VPDB) (black). Two Medicine (TM), Judith River (JR), Dinosaur Park (DP), and Hell Creek (HC) Formations bulk organic matter and ceratopsian and hadrosaur mean ± 1 SD $\delta^{13}C$ (‰ VPDB) (grey) [25–26]. "Lo Hueco" $\Delta^{13}C_{enamel\text{-}diet}$ is 15‰, when analysing sauropods, whereas Fricke and Pearson [25] and Fricke *et al*. [26] obtained a value of 18‰ when analysing ceratopsians and hadrosaurs. Note different frequency scale for "Lo Hueco" (black) and Fricke and Pearson [25] and Fricke *et al*. [26]´s data (grey).

doi:10.1371/journal.pone.0119968.g007

for ornithischians from Fricke and Pearson [25] and Fricke *et al.* [26] and sauropods from our study is statiscally significant (t = -4.481, p = 0.003), and therefore, even considering the variability yielded by dinosaur and bulk organic matter $\delta^{13}C$ values, these two sets of data do not significantly overlap. Stanton-Thomas and Carlson [24] stated that higher $\delta^{13}C$ values observed in hadrosaurs might be related to ingestion of vegetation subject to high salinity levels and/or consumption of gymnosperms (which show an enrichment of ~1.1 to 2‰ compared to mean C_3 $\delta^{13}C$ values). "Lo Hueco" sauropods may have relied heavily on angiosperms as revealed by the palynological content of the "Lo Hueco" sediments showing pollen assemblages dominated by angiosperms (93%: [100]). Specifically, the palynological assemblage from "Lo Hueco" is dominated by freshwater palynomorphs, spores and pollen grains related to swamp or local wetland vegetation [100]. The preferential incorporation of angiosperms by "Lo Hueco" sauropods may also lie behind the difference observed between our $\Delta^{13}C_{enamel\text{-}diet}$ value and the one estimated by Tütken [32]. Interestingly, in the compilation of sauropod $\delta^{13}C$

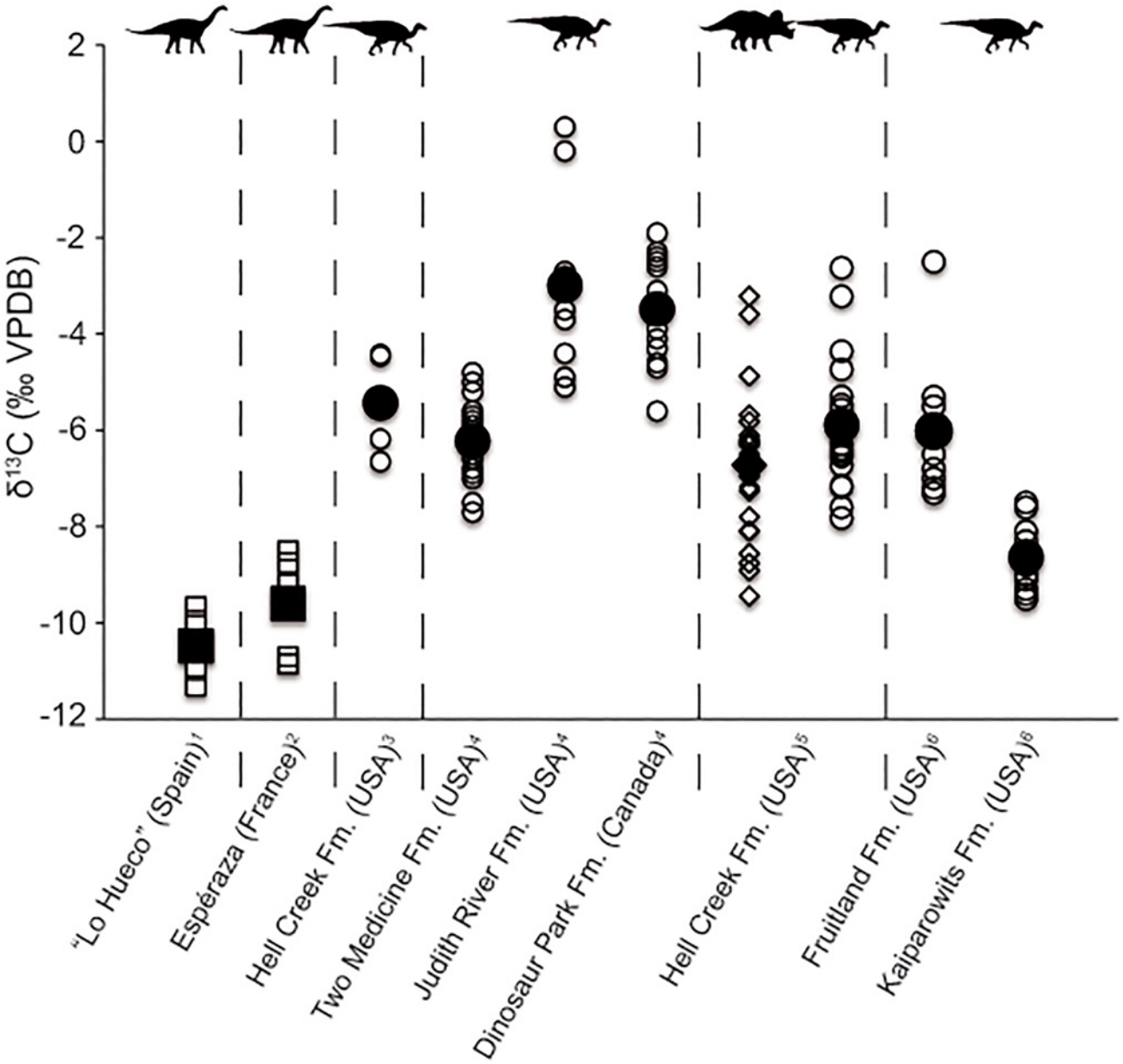

Fig 8. $\delta^{13}C$ (‰ VPDB) values for Campanian-Maastrichtian herbivore dinosaurs: titanosaur sauropods (squares), hadrosaurs (circles), and ceratopsians (diamonds). Large black symbols represent mean $\delta^{13}C$ values. [1]This study; [2]Tütken [32]; [3]Stanton-Thomas and Carlson [24]; [4]Fricke *et al*. [26]; [5]Fricke and Pearson [25]; [6]Fricke *et al*. [53].

doi:10.1371/journal.pone.0119968.g008

values carried out by this author, *Ampelosaurus* showed the lowest $\delta^{13}C$ values, and according to Knoll *et al*. [73], one of the "Lo Hueco" sauropod taxa remains close to this genus. These uncertainties regarding herbivore dinosaur isotopic paleoecology open up new lines of investigation dealing with the question of the type of dietary behavior and physiology of different genera giving rise to different $\Delta^{13}C_{enamel\text{-}diet}$ offsets.

"Lo Hueco" theropod mean $\delta^{13}C$ value is -10.7±0.8‰, statistically indistinguishable from the sauropod mean $\delta^{13}C$ value (-10.5±0.8‰) (Fig. 4, S2 Dataset). To the best of our knowledge, there are no isotopic studies dealing with herbivore and carnivore dinosaur feeding

paleoecology from the same locality. Isotopic studies on ancient vertebrate predator-prey systems have mainly focused on Quaternary mammals [64, 101–103] and to a lesser extent on Miocene mammals [104]. Clementz *et al.* [64] observed a $\delta^{13}C$ offset between predators (wolves) and their prey (moose and elks) ($\Delta^{13}C_{carnivore\text{-}herbivore}$) of $\sim$ -1.3‰. These authors argued that lower tooth enamel $\delta^{13}C$ values for carnivores might be due to differences in their digestive physiology in comparison to herbivores. The lack of significant differences between "Lo Hueco" carnivore and herbivore dinosaur tooth enamel $\delta^{13}C$ values ($\Delta^{13}C_{carnivore\text{-}herbivore}$ = -0.2‰, S2 Dataset) may be due to differences in metabolic and/or physiological mechanisms between dinosaurs and mammals, however a more plausible explanation is that titanosaur sauropods were not likely prey for theropods belonging to Dromaeosaurinae and Velociraptorinae subfamilies on account of body mass differences. Interestingly, the lack of significant differences in $\delta^{13}C$ values between both theropod subfamilies ($t = 0.516$, $p = 0.618$), along with the similarity in their body masses and likely hunting strategies point to some degree of resource overlap. Also, the lack of significant differences in variance of $\delta^{13}C$ values between these subfamilies of theropods (Levene test; $F = 1.582$, $p = 0.240$) suggests that none was more versatile than the other in resource utilization.

In the case of aquatic ectotherms (crocodyliforms and turtles), $\delta^{13}C$ values are a reflection of food oxidation (respiration) and ambient water (e.g., disolved inorganic carbon or DIC can constitute an additional source of carbon) [105–106]. "Lo Hueco" crocodyliforms belong to forms close to the genus *Allodaposuchus* [74]. Since it is not possible to determine physiological tolerance to salinity from the morphological standpoint in the basal Eusuchia from "Lo Hueco", (J.L. Sanz and F. Ortega, personal communication), the information supplied by stable isotopes may shed light about salt tolerance, dietary behavior and habitat occupancy. We have compared the isotopic values of "Lo Hueco" crocodyliforms to those provided by Wheatley *et al.* [107] for Louisiana and Florida modern crocodylians. We are aware that extrapolation between extinct and extant organisms is not straightforward due to unknown physiological mechanisms in ancient taxa as well as different environmental conditions. However, we still believe that a comparison can be made since 1) the marine-freshwater isotopic threshold values are well established not only in modern studies, but also in the past, 2) the marine $\delta^{18}O$ value has remained fairly constant throughout the geological time and 3) the Wheatley *et al.* [107]´s study considered modern crocodylians from Louisiana and Florida with a similar latitude to the one suggested for "Lo Hueco" (25–30°N for Louisiana and Florida *vs* 31°N for "Lo Hueco"). "Lo Hueco" crocodyliforms show a mean $\delta^{13}C$ value, which is statistically indistinguishable from the value observed by Wheatley *et al.* [107] for Louisiana and Florida modern coastal *Alligator mississippiensis* ($p = 0.999$) and Florida modern coastal *Crocodylus acutus* ($p = 0.868$), while it is significantly different from Florida modern inland *A. mississippiensis* ($p < 0.001$), whereas "Lo Hueco" crocodyliforms show a significantly lower mean $\delta^{18}O_{CO3}$ value, when compared to coastal and inland *A. mississippiensis* ($p = 0.019$ and $p = 0.001$, respectively) and coastal *C. acutus* ($p < 0.001$) (Fig. 9A). These results pose interesting issues concerning the salinity discrimination and osmoregulation capacity of the "Lo Hueco" eusuchian crocodyliforms. Jackson *et al.* [108] in a study of salinity tolerance and osmoregulation mechanisms by modern crocodilians argued that reptiles inhabiting marine and estuarine waters keep a constant plasma osmolality by behavioral modifications (avoiding drinking seawater) and/or morphological adaptation (salt-secreting glands and reduced integumental permeability). Crocodyliforms from "Lo Hueco" are under study and with the current information we cannot determine their physiological tolerance to salinity from a morphological standpoint (e.g., presence or absence of salt-secreting glands) (JL Sanz and F Ortega, personal communication, 2014). While waiting for these morphological studies, isotopic results suggest that the "Lo Hueco" crocodyliforms may have incorporated food items from brackish waters as

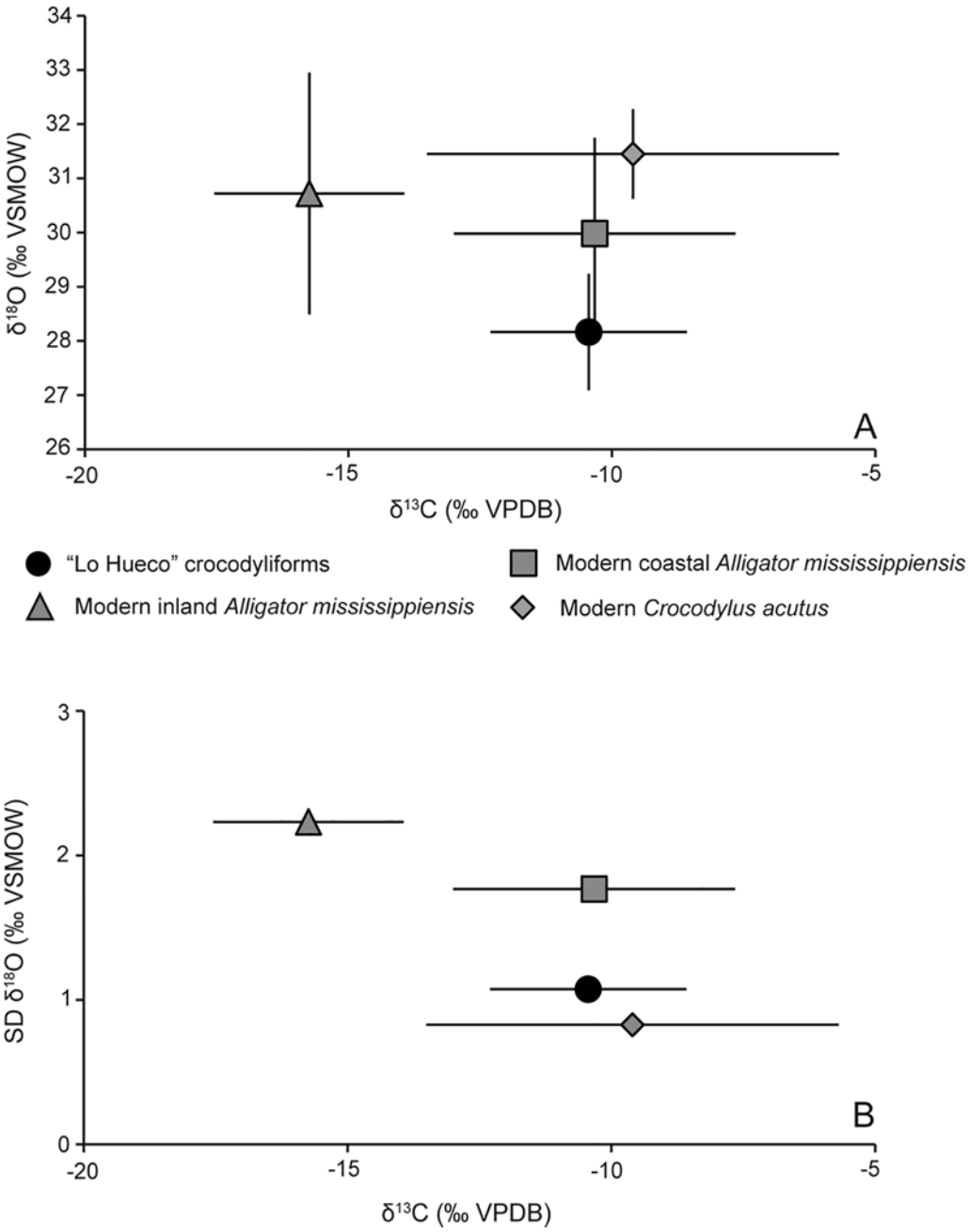

Fig 9. Comparison between isotopic values of "Lo Hueco" crocodyliforms and modern crocodilians. A) $\delta^{18}O_{CO3}$ (‰ VSMOW) *vs* $\delta^{13}C$ (‰ VPDB) mean ± 1 standard deviation (SD) values of the "Lo Hueco" crocodyliforms (black circle), and modern inland *Alligator mississippiensis* (grey triangle), coastal *Alligator mississippiensis* (grey square) and *Crocodylus acutus* (grey diamond) [107]. B) SD $\delta^{18}O_{CO3}$ (‰ VSMOW) *vs* $\delta^{13}C$ (‰ VPDB) values of the "Lo Hueco" crocodyliforms and modern crocodilians (symbols as in A).

doi:10.1371/journal.pone.0119968.g009

shown by their $\delta^{13}C$ values, whereas they avoided ingesting this water and consumed preferentially freshwater, as suggested by their $\delta^{18}O_{CO3}$ values. Clementz and Koch [109] and Wheatley *et al.* [107] argued that animals incorporating marine food items and drinking seawater show a lower variability in their $\delta^{18}O_{CO3}$ values. Even though "Lo Hueco" crocodyliforms likely drank freshwater, its $\delta^{18}O_{CO3}$ variability (1.0‰) is not as high as that shown by either inland (2.2‰)

and coastal (1.8‰) *A. mississippiensis*, and it is more similar to the one shown by saltwater tolerant *C. acutus* (0.8‰) (Fig. 9B). This might indicate that $\delta^{18}O$ values of the water ingested by the "Lo Hueco" crocodyliforms may have remained homogeneous during the time window in which bioapatite mineralized (i.e., warmest months of the year).

"Lo Hueco" turtle mean $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values are the lowest when compared to dinosaurs and crocodyliforms (Fig. 4) (although significant differences are only observed between turtles and dinosaur $\delta^{18}O_{CO3}$ values, S2 Dataset). "Lo Hueco" turtles belong to two forms of Pleurodira, specifically to members of Bothremydidae [29, 75–76]. They are omnivore freshwater turtles, so low $\delta^{13}C$ values may be indicative of a diet based on a mixture of aquatic and terrestrial C_3 vegetation and/or invertebrates, whereas low $\delta^{18}O$ values may indicate ingestion of water with an inland source. Unlike crocodyliforms, there are not isotopic studies dealing with bone carbonate of freshwater turtles from the subtropics and they focused on marine turtles [105–106]. As observed in the case of "Lo Hueco" crocodyliforms, "Lo Hueco" turtles show a low variability in their mean $\delta^{18}O_{CO3}$ value, in spite of inhabiting freshwater environments, when compared to modern marine loggerhead turtles reported by Wheatley *et al.* [107] (0.3‰ *vs*. 0.6‰, for "Lo Hueco" and marine loggerhead turtles, respectively) pointing to a low variability of $\delta^{18}O_{CO3}$ values and presumably of the $\delta^{18}O$ values of the ingested water for the time of bioapatite mineralization (i.e., warmest months of the year).

Conclusions

Isotopic analyses on the multi-taxa terrestrial vertebrate assemblage of the "Lo Hueco" locality (central eastern Spain) provides valuable information about climatic and environmental conditions existing in southeastern Iberia during the late Campanian-early Maastrichtian (Late Cretaceous). Seasonal thermal varibility has been inferred as the difference between temperature of the warmest months (TWMs), supplied by crocodyliforms and turtles (whose bioapatite mineralizes during the warm season) and mean annual temperature (MAT), provided by theropods (that record ingested water throughout the year). "Lo Hueco" ΔTWMs-MAT results do not point to a significant different seasonal thermal variability to that observed today. From the paleoecological standpoint, $\delta^{13}C$ values of the "Lo Hueco" taxa point to consumption of pure C_3 vegetation, fact that agrees well with bulk organic matter $\delta^{13}C$ values from the "Lo Hueco" sediments. The estimated fractionation between sauropod enamel and diet ($\Delta^{13}C_{enamel\text{-}diet}$) is $\sim$15‰, lower than other fractionation values calculated for sauropods ($\sim$16‰) and ornithischians ($\sim$18‰), and likely indicating differences in metabolic and/or physiological processes or different utilization of plant organic compounds and/or plant parts. Since "Lo Hueco" titanosaur sauropods may have not been likely prey for theropods belonging to Dromaeosaurinae and Velociraptorinae subfamilies on account of differences in their body mass, no conclusive information concerning dinosaurian predator-prey $\delta^{13}C$ offset could be attained. Although "Lo Hueco" crocodyliform material remains under study and no paleoecological conclusions have been drawn from a morphological standpoint, isotopic results indicate that they may have incorporated food items from brackish waters as shown by their $\delta^{13}C$ values, whereas they avoided ingesting saline water and consumed preferentially freshwater, as suggested by their $\delta^{18}O_{CO3}$ values, when compared with isotopic values of modern crocodilians inhabiting subtropical regions. "Lo Hueco" turtles show the lowest $\delta^{13}C$ and $\delta^{18}O_{CO3}$ values of the vertebrate assemblage likely indicating a diet based on a mixture of aquatic and terrestrial C_3 vegetation and/or invertebrates and ingestion of water with an inland source, a fact that agrees well with their taxonomic designation.

Supporting Information

S1 Dataset. Signature, level, sample, δ18OPO4 (‰ VSMOW), δ13C (‰ VPDB), δ18OCO3 (‰ VSMOW), and δ18OH2O (‰ VSMOW) values.
(XLS)

S2 Dataset. ANOVA and post-hoc tests for δ18OPO4, δ18OH2O, δ13C and δ18OCO3 values for the total dataset (TOTAL), and G1 and G2 levels.
(XLS)

S3 Dataset. Continent, country, station, latitude, longitude, altitude, year of record, mean annual temperature (MAT), temperature of the warmest months (July, August, September) (TWMs) and difference between TWMs and MAT (ΔTWMs-MAT).
(XLS)

S4 Dataset. A) ΔTWMs crocodyliforms-MATtheropods and ΔTWMs turtles-MATtheropods calculated using equations (4), (5) and (6) for the total dataset (TOTAL) and independently for levels G1.
(XLS)

S5 Dataset. Post-hoc Tukey p-values comparing pairs of ΔTWMs-MAT values for "Lo Hueco" locality, Late Cretaceous eastern Tethyan rudists [36, 38] and modern meterological stations from different continents (http://www-naweb.iaea.org/napc/ih/IHS_resources_isohis.html).
(XLS)

Acknowledgments

We thank JL Sanz (Universidad Autónoma de Madrid), F Ortega (Universidad Nacional de Educación a Distancia), A Pérez-García (Universidad Complutense de Madrid) and A Torices (University of Alberta) for the access to the analyzed material and suggestions that helped to improve the manuscript. We are indebted to PL Koch (University of California Santa Cruz) for valuable comments and access to his laboratory, and to D Andreasen (University of California Santa Cruz) for his help with stable isotope analyses. O Sanisidro is acknowledged for illustrations. We thank editor P Dodson and reviewers C Suarez and R Amiot for their valuable comments.

Author Contributions

Conceived and designed the experiments: LD FBB OCM. Performed the experiments: LD. Analyzed the data: LD FBB OCM. Contributed reagents/materials/analysis tools: LD FBB OCM. Wrote the paper: LD FBB OCM.

References

1. Poulsen CJ, Gendaszek AS, Jacob RL. Did the rifting of the Atlantic Ocean cause the Cretaceous thermal maximum? Geology. 2003; 31: 115–118.
2. Forster A, Schouten S, Baas M, Sinninghe Damsté JS. Mid-Cretaceous (Albian–Santonian) sea surface temperature record of the tropical Atlantic Ocean. Geology. 2007; 35: 919–922.
3. Parrish JT, Spicer RA. Late Cretaceous terrestrial vegetation: A near polar temperature curve. Geology. 1988; 16: 22–25.
4. Jenkins HC, Gale AS, Corfield RM. Carbon- and oxygen-isotope stratigraphy of the English chalk in Italian Scaglia and its paleoclimatic significance. Geol Mag. 1994; 131: 1–34.

5. Huber BT, Hodell DA, Hamilton CP. Middle–Late Cretaceous climate of the high southern latitudes: Stable isotope evidence for minimal equator to pole thermal gradients. Geol Soc Am Bull. 1995; 107: 1164–1191.
6. Huber BT, Norris RD, MacLeod KG. Deep-sea paleotemperature record of extreme warmth during the Cretaceous. Geology. 2002; 30: 123–126.
7. Friedrich O, Herrle JO, Wilson PA, Cooper MJ, Erbacher J, Hemleben C. Early Maastrichtian carbon cycle perturbation and cooling event: Implications from the South Atlantic Ocean. Paleoceanography. 2009; 24: PA2211.
8. Jenkyns HC, Forster A, Schouten S, Sinninghe Damsté JS (2004) High temperatures in the Late Cretaceous Arctic Ocean. Nature 432: 888–892. PMID: 15602558
9. Nordt L, Atchley S, Dworkin S. Terrestrial evidence for two greenhouse events in the latest Cretaceous. GSA Today. 2003; 13: 4–9. PMID: 14655765
10. MacLeod KG, Huber BT, Isaza-Londoño C. North Atlantic warming during global cooling at the end of the Cretaceous. Geology. 2005; 33: 437–440.
11. Royer DL. CO_2-forced climate thresholds during the Phanerozoic. Geochim Cosmochim Acta. 2006; 70: 5665–5675.
12. Frakes LA, Francis JE, Syktus JI. Climate Modes of the Phanerozoic. Cambridge: Cambridge University Press; 1992.
13. Markwick PJ, Rowley DB. The geological evidence for Triassic to Pleistocene glaciations: Implications for eustasy. In: Pindell JL, Drake CL, editors. Paleogeographic Evolution and Non-Glacial Eustasy, Northern South America: Society for Sedimentary Geology (SEPM) Special Publication, Tulsa, Oklahoma. 1998; 58: 17–43.
14. DeConto RM, Brady EC, Berggren J, Hay WW. Late Cretaceous climate, vegetation, and ocean interactions. In: Huber BT, MacLeod KG, Wing SL, editors. Warm Climates in Earth History. Cambridge: Cambridge University Press; 2000. pp. 275–296.
15. DeConto RM, Hay WW, Thompson SL, Bergengren JC. Late Cretaceous climate and vegetation interactions: Cold continental paradox. In: Barrera E, Johnson C, editors. The evolution of Cretaceous ocean/climate systems. Geological Society of America Special Paper. 1999; 332: 391–432.
16. Amiot R, Lécuyer C, Buffetaut E, Fluteau F, Legendre S, Martineau F. Latitudinal temperature gradient during the Cretaceous upper Campanian–middle Maastrichtian: $\delta^{18}O$ record of continental vertebrates. Earth Planet Sci Lett. 2004; 226: 255–272.
17. Amiot R, Wang X, Zhou Z, Wang X, Buffetaut E, et al. Oxygen isotopes of East Asian dinosaurs reveal exceptionally cold Early Cretaceous climates. Proc Natl Acad Sci USA. 2011; 108: 5179–5183. doi: 10.1073/pnas.1011369108 PMID: 21393569
18. Straight WH, Barrick RE, Eberth DA. Reflections of surface water, seasonality and climate in stable oxygen isotopes from tyrannosaurid tooth enamel. Palaeogeogr Palaeoclimatol Palaeoecol. 2004; 206: 239–256.
19. Amiot A, Buffetaut E, Lécuyer C, Wang X, Boudad L, et al. Oxygen isotope evidence for semi-aquatic habits among spinosaurid theropods. Geology. 2010; 38: 139–142.
20. Suarez CA, Gonzalez LA, Ludvigson GA, Cifelli RL, Tremain E. Water utilization of the Cretaceous Mussentuchit Member local vertebrate fauna, Cedar Mountain Formation, Utah, USA: Using oxygen isotopic composition of phosphate. Palaeogeogr Palaeoclimatol Palaeoecol. 2012; 313–314: 78–92.
21. Barrick RE, Showers WJ, Fisher AG. Comparison of thermoregulation of four ornithischian dinosaurs and a varanid lizard from the Cretaceous Two Medicine Formation: Evidence from oxygen isotopes. Palaios. 1996; 11: 295–305.
22. Fricke HC, Rogers RR. Multiple taxon-multiple locality approach to providing oxygen isotope evidence for warm-blooded theropod dinosaurs. Geology. 2000; 28: 799–802.
23. Amiot R, Lécuyer C, Buffetaut E, Escarguel G, Fluteau F, Martineau F. Oxygen isotopes from biogenic apatites suggest widespread endothermy in Cretaceous dinosaurs. Earth Planet Sci Lett. 2006; 246: 41–54.
24. Stanton-Thomas KJ, Carlson SJ. Microscale $\delta^{18}O$ and $\delta^{13}C$ isotopic analysis of an ontogenetic series of the hadrosaurid dinosaur *Edmontosaurus*: Implications for physiology and ecology. Palaeogeogr Palaeoclimatol Palaeoecol. 2004; 206: 257–287.
25. Fricke HC, Pearson DA. Stable isotope evidence for changes in dietary niche partitioning among hadrosaurian and ceratopsian dinosaurs of the Hell Creek Formation, North Dakota. Paleobiology. 2008; 34: 534–552.
26. Fricke HC, Rogers RR, Backlund R, Dwyer CN, Echt S. Preservation of primary stable isotope signals in dinosaur remains, and environmental gradients of the Late Cretaceous of Montana and Alberta. Palaeogeogr Palaeoclimatol Palaeoecol. 2008; 266: 13–27.

27. Domingo L, Barroso-Barcenilla F, Cambra-Moo O. Paleoenvironmental reconstruction of the "Lo Hueco" fossil site (Upper Cretaceous, Cuenca, Spain): Preliminary stable isotope analyses on crocodilians and dinosaurs. Palaios. 2013; 28: 195–202.

28. Lécuyer C, Bogey C, Garcia JP, Grandjean P, Barrat JA, et al. Stable isotope composition and rare earth element content of vertebrate remains from the Late Cretaceous of Northern Spain (Laño): Did the environmental record survive? Palaeogeogr Palaeoclimatol Palaeoecol. 2003; 193: 457–471.

29. Barroso-Barcenilla F, Cambra-Moo O, Escaso F, Ortega F, Pascual A, et al. New and exceptional discovery in the Upper Cretaceous of the Iberian Peninsula: The palaeontological site of "Lo Hueco", Cuenca, Spain. Cretaceous Res. 2009; 30: 1268–1278.

30. Cambra-Moo O, Barroso-Barcenilla F, Coruña F, Postigo-Mijarra JM. Exceptionally well-preserved vegetal remains from the Upper Cretaceous "Lo Hueco" site, Cuenca, Spain. Lethaia. 2013; 46: 127–140.

31. Tütken T, Pfretzschner HU, Vennemann T, Sun G, Wand YD. Paleobiology and skeletochronology of Jurassic dinosaurs: implications from the histology and oxygen isotope compositions of bones. Palaeogeogr Palaeoclimatol Palaeoecol. 2004; 206: 217–238.

32. Tütken T. The diet of sauropod dinosaurs: Implications from carbon isotope analysis of teeth, bones, and plants. In: Klein N, Remes K, Sander M, editors. Biology of the Sauropod Dinosaurs: Understanding the Life of Giants. Bloomington, IN: Indiana University Press; 2011. pp. 57–79.

33. Markwick PJ. Fossil crocodilians as indicators of Late Cretaceous and Cenozoic climates: implications for using palaeontological data in reconstructing palaeoclimate. Palaeogeogr Palaeoclimatol Palaeoecol. 1998; 137: 205–271.

34. Sloan LC, Barron E. "Equable" climates during Earth history? Geology. 1990; 18: 489–492.

35. Bush ABG, Philander SGH. The late Cretaceous: Simulation with a coupled atmosphere-ocean general circulation model. Paleoceanography. 1997; 12: 495–516.

36. Steuber T. Isotopic and chemical intra-shell variations in low-Mg calcite of rudist bivalves (Mollusca: Hippuritacea)—disequilibrium fractionations and Late Cretaceous seasonality. Int J Earth Sci. 1999; 88: 551–570.

37. Golovneva LB. The Maastrichtian (Late Cretaceous) climate in the Northern Hemisphere. In: Hart MB, editor. Climates: Past and Present. Geological Society, London, Special Publications. 2000; 181: 43–54.

38. Steuber T, Rauch M, Masse JP, Graaf J, Malkoc M. Low-latitude seasonality of Cretaceous temperatures in warm and cold episodes. Nature. 2005; 437: 1341–1344. PMID: 16251961

39. Hunter SJ, Valdes PJ, Haywood AM, Markwick PJ. Modelling Maastrichtian climate: investigating the role of geography, atmospheric CO_2 and vegetation. Clim Past. 2008; 4: 981–1019.

40. Weaver JC. The improbable endotherm: the energetics of the sauropod *Brachiosaurus*. Paleobiology. 1983; 9: 173–182.

41. Dunham AE, Overall KL, Porter WP, Forster CA. Implications of ecological energetics and biophysical and developmental constraints for life-history variation in dinosaurs. In: Farlow JO, editor. Paleobiology of the dinosaurs. Geological Society of America, Special Paper. 1989; 238: 1–19.

42. Seebacher F. Dinosaur body temperatures: the occurrence of endothermy and ectothermy. Paleobiology. 2003; 29: 105–122.

43. McNab BK. Resources and energetics determined dinosaur maximal size. Proc Natl Acad Sci USA. 2009; 106: 12184–12188. doi: 10.1073/pnas.0904000106 PMID: 19581600

44. Sander PM, Christian A, Clauss M, Fechner R, Gee CT, et al. Biology of sauropod dinosaurs: the evolution of gigantism. Biol Rev. 2011; 86: 117–155. doi: 10.1111/j.1469-185X.2010.00137.x PMID: 21251189

45. Clarke A. Dinosaur Energetics: Setting the bounds on feasible physiologies and ecologies. Am Nat. 2013; 182: 283–297. doi: 10.1086/671259 PMID: 23933721

46. O'Connor MP, Dodson P. Biophysical constraints on the thermal ecology of dinosaurs. Paleobiology. 1999; 25: 341–368.

47. Gillooly JF, Allen AP, Charnov EL. Dinosaur fossils predict body temperatures: PLOS BIOL. 2006; 4: e248. PMID: 16817695

48. Eagle RA, Tütken T, Martin TS, Tripati AK, Fricke HC, et al. Dinosaur body temperatures determined from isotopic (^{13}C-^{18}O) ordering in fossil biominerals. Science. 2011; 333: 443–445. doi: 10.1126/science.1206196 PMID: 21700837

49. Barrick RE, Fisher AG, Showers WJ. Oxygen isotopes from turtle bone: applications for terrestrial paleoclimates? Palaios. 1999; 14: 186–191.

50. Pough FH, Janis CM, Heiser JB. Vertebrate Life, 6th ed. Upper Saddle River, NJ: Prentice Hall; 2002.

51. Kolodny Y, Luz B, Navon O. Oxygen isotope variations in phosphate of biogenic apatites, I. Fish bone apatite rechecking the rules of the game. Earth Planet Sci Lett. 1983; 64: 398–404

52. Grimes ST, Mattey DP, Hooker JJ, Collinson ME. Paleogene paleoclimate reconstruction using oxygen isotopes from land and freshwater organisms: the use of multiple paleoproxies. Geochim Cosmochim Acta. 2003; 67: 4033–4047.

53. Fricke HC, Rogers RR, Gates TA. Hadrosaurid migration: Inferences based on stable isotope comparisons among Late Cretaceous dinosaur localities. Paleobiology. 2009; 35: 270–288.

54. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotopic discrimination and photosynthesis. Annu Rev Plant Biol. 1989; 40: 503–537.

55. Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW. Climate change and the evolution of C_4 photosynthesis. Trends Ecol Evol. 1991; 6: 95–99. doi: 10.1016/0169-5347(91)90183-X PMID: 21232434

56. Hayes JM. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. Rev Mineral Geochem. 2001; 43: 225–277.

57. Ekart DD, Cerling TE, Montañez IP, Tabor NJ. A 400 million year carbon isotope record of pedogenic carbonate: implications for paleoatmospheric carbon dioxide. Am J Sci. 1999; 299: 805–827.

58. Friedli H, Lotscher H, Oeschger H, Siegenthaler U, Stauver B. Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. Nature. 1986; 324: 237–238.

59. Marino BD, McElroy MB. Isotopic composition of atmospheric CO_2 inferred from carbon in C_4 plant cellulose. Nature. 1991; 349: 127–131.

60. Koch PL. Isotopic reconstruction of past continental environments. Annu Rev Earth Planet Sci. 1998; 26: 573–613.

61. Cerling TE, Harris JM. Carbon isotope fractionation between diet and bioapatite in ungulate mammals and implications for ecological and paleoecological studies. Oecologia. 1999; 120: 347–363.

62. Passey BH, Robinson TF, Ayliffe LK, Cerling TE, Sponheimer M, et al. Carbon isotope fractionation between diet, breath CO_2, and bioapatite in different mammals. J Archaeol Sci. 2005; 32: 1459–1470.

63. Koch PL. Isotopic study of the biology of modern and fossil vertebrates. In: Michener R, Lajtha K, editors. Stable Isotopes in Ecology and Environmental Science, 2nd Edition. Boston: Blackwell Publishing; 2007. pp. 99–154.

64. Clementz MT, Fox-Dobbs K, Wheatley PV, Koch PL, Doak DF. Revisiting old bones: coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. Geol J. 2009; 44: 605–620.

65. Johnson BJ, Fogel M, Miller GH. Stable isotopes in modern ostrich eggshell: A calibration for paleoenvironmental applications in semi-arid regions of southern Africa. Geochim Cosmochim Acta. 1998; 62: 2451–2461.

66. Angst D, Lécuyer C, Amiot R, Buffetaut E, Fourel F, et al. Isotopic and anatomical evidence of an herbivorous diet in the Early Tertiary giant bird *Gastornis*. Implications for the structure of Paleocene terrestrial ecosystems. Naturwissenschaften. 2014; 101: 313–322. doi: 10.1007/s00114-014-1158-2 PMID: 24563098

67. Ortega F, Sanz JL, Barroso-Barcenilla F, Cambra-Moo O, Escaso F, et al. El yacimiento de macrovertebrados fósiles del Cretácico Superior de "Lo Hueco" (Fuentes, Cuenca). Palaeontologica Nova, Publicaciones del Seminario de Paleontología de Zaragoza. 2008; 8: 119–131.

68. Cambra-Moo O, Barroso-Barcenilla F, Berreteaga A, Carenas A, Coruña F, et al. Preliminary taphonomic approach to "Lo Hueco" palaeontological site (Upper Cretaceous, Cuenca, Spain). Geobios. 2012; 45: 157–166. doi: 10.1093/jncimonographs/lgs029 PMID: 23271767

69. Gutiérrez G, Robles F. Consideraciones sobre la utilización del término "Garumniense" en la Cordillera Ibérica: Cuadernos de Geología Ibérica. 1979; 5: 127–214.

70. González-Acebrón L, Barroso-Barcenilla F, Cambra-Moo O, Carenas B, Segura M. Environmental significance of gypsum-bearing layers at the "Lo Hueco" palaeontological site (Upper Cretaceous, Cuenca, Spain): petrography, fluid inclusions and isotopic relations. Facies. 2014; 60: 755–771.

71. Díez Díaz V, Ortega F, Sanz J. Microwear patterns of the titanosaurian teeth from the Late Cretaceous of "Lo Hueco" (Cuenca, Spain). Program and Abstracts of the 73rd Meeting of the SVP. Journal of Vertebrate Paleontology. 2013; 116.

72. Knoll F, Witmer LM, Ridgely RC, Ortega F, Sanz JL. A new titanosaurian sauropod neurocranium from the Late Cretaceous of Spain. Program and Abstracts of the 73rd Meeting of the SVP. Journal of Vertebrate Paleontology. 2013; 155.

73. Knoll F, Ridgely RC, Ortega F, Sanz JL, Witmer LM. Neurocranial osteology and neuroanatomy of a Late Cretaceous titanosaurian sauropod from Spain (*Ampelosaurus* sp.). PLOS ONE. 2013; 8: e54991. doi: 10.1371/journal.pone.0054991 PMID: 23355905

74. Narváez I, Ortega F, Brochu C, Escaso F. A new basal eusuchian crocodile from the Late Cretaceous of "Lo Hueco" (Cuenca, Spain). In: Torcida Fernández-Baldor F, Huerta P, editors. VI International Symposium about Dinosaur Paleontology and their Environment, abstract book; 2013. pp. 96–97.

75. Pérez-García A, Ortega F, Murelaga X. A new genus of Bothremydidae (Chelonii, Pleurodira) in the Cretaceous of southwestern Europe. Geobios. 2012; 45: 219–229.

76. Pérez-García A, Ortega F, Murelaga X. Two synchronic and sympatric Bothremydidae taxa (Chelonii, Pleurodira) in the Late Cretaceous site of "Lo Hueco" (Cuenca, Spain). In: Morphology and Evolution of Turtles. Vertebrate Paleobiology and Paleoanthropology. Brinkman; 2013. pp. 251–259.

77. Bassett D, Macleod KG, Miller JF, Ethington RL. Oxygen isotopic composition of biogenic phosphate and the temperature of Early Ordovician seawater. Palaios. 2007; 22: 98–103.

78. Koch PL, Tuross N, Fogel ML. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. J Archaeol Sci. 1997; 24: 417–429.

79. Domingo L, López-Martínez N, Leng MJ, Grimes ST. The Paleocene–Eocene Thermal Maximum record in the organic matter of the Claret and Tendruy continental sections (South-central Pyrenees, Lleida, Spain). Earth Planet Sci Lett. 2009; 281: 226–237.

80. Amiot R, Lécuyer C, Escarguel G, Billon-Bruyat JP, Buffetaut E, et al. Oxygen isotope fractionation between crocodilian phosphate and water: Palaeogeogr Palaeoclimatol Palaeoecol. 2007; 243: 412–420.

81. Coulson AB, Kohn MJ, Shirley MH, Joyce WG, Barrick RE. Phosphate–oxygen isotopes from marine turtle bones: Ecologic and paleoclimatic applications. Palaeogeogr Palaeoclimatol Palaeoecol. 2008; 264: 78–84.

82. Domingo L, Grimes ST, Soler-Gijón R, López-Martínez N. Analysis of the geochemical variability in lepisosteid scales from the Fontllonga-3 site (early Danian, Tremp Formation, South Central Pyrenees, Spain): implications for palaeoenvironmental studies. Palaeogeogr Palaeoclimatol Palaeoecol. 2009; 274: 204–214.

83. Coulson AB, Kohn MJ, Barrick RE. Isotopic evaluation of ocean circulation in the Late Cretaceous North American seaway. Nat Geosci. 2011; 4: 852–855.

84. Lécuyer C, Amiot R, Touzeau A, Trotter J. Calibration of the phosphate $\delta^{18}O$ thermometer with carbonate–water oxygen isotope fractionation equations. Chem Geol. 2013; 347: 217–226.

85. Pucéat E, Joachimski MM, Bouilloux A, Monna F, Bonin A, et al. Revised phosphate–water fractionation equation reassessing paleotemperatures derived from biogenic apatite. Earth Planet Sci Lett. 2010; 298: 135–142.

86. Kohn MJ, Cerling TE. Stable isotope compositions of biological apatite. Rev Mineral Geochem. 2002; 48: 455–488.

87. Bryant JD, Koch PL, Froelich PN, Showers WJ, Genna BJ. Oxygen isotope partitioning between phosphate and carbonate in mammalian apatite. Geochim Cosmochim Acta. 1996; 60: 5145–5148.

88. Iacumin P, Bocherens H, Mariotti A, Longinelli A. Oxygen isotope analyses of coexisting carbonate and phosphate in biogenic apatite: A way to monitor diagenetic alteration of bone phosphate? Earth Planet Sci Lett. 1996; 142: 1–6.

89. Fricke HC, Clyde WC, O'Neil JR, Gingerich PD. Evidence for rapid climate change in North America during the latest Paleocene thermal maximum: Oxygen isotope compositions of biogenic phosphate from the Bighorn Basin (Wyoming). Earth Planet Sci Lett. 1998; 160: 193–208.

90. Tütken T, Vennemann TW, Janz H, Heizmann EPJ. Palaeoenvironment and palaeoclimate of the middle Miocene lake in the Steinheim basin, SW Germany: A reconstruction from C, O, and Sr isotopes of fossil remains. Palaeogeogr Palaeoclimatol Palaeoecol. 2006; 241: 457–491.

91. Domingo L, Cuevas-González J, Grimes ST, Hernández Fernández M, López-Martínez N. Multiproxy reconstruction of the paleoclimate and paleoenvironment of the middle Miocene Somosaguas site (Madrid, Spain) using herbivore tooth enamel. Palaeogeogr Palaeoclimatol Palaeoecol. 2009; 272: 53–68.

92. Hay WW, DeConto R, Wold CN, Wilson KM, Voigt S, et al. Alternative global Cretaceous paleogeography. In: Barrera E, Johnson C, editors. The Evolution of Cretaceous Ocean/Climate Systems. Geological Society of America Special Paper. 1999; 332: 1–47.

93. Pontzer H, Allen V, Hutchinson JR. Biomechanics of running indicates endothermy in bipedal dinosaurs. PLOS ONE. 2009; 4: e7783. doi: 10.1371/journal.pone.0007783 PMID: 19911059

94. Rozanski K, Araguás-Araguás L, Gonfiantini R. Isotopic patterns in modern global precipitation. In: Swart PK, Lohmann KC, McKenzie J, Savin S, editors. Climate Change in Continental Isotopic Records. Geophysical Monograph Series. 1993; 78: 1–36.

95. Pucéat E, Lécuyer C, Sheppard SMF, Dromart G, Reboulet S, et al. Thermal evolution of Cretaceous Tethyan marine waters inferred from oxygen isotope composition of fish tooth enamels. Paleoceanography. 2003; 18: 1029.

96. Pucéat E, Lécuyer C, Donnadieu Y, Naveau P, Cappetta H, et al. Fish tooth $\delta^{18}O$ revising Late Cretaceous meridional upper ocean water temperature gradients. Geology. 2007; 35: 107–110.

97. Greenwood DR, Wing SL. Eocene continental climates and latitudinal temperature gradients. Geology. 1995; 23: 1044–1048.

98. Hummel J, Clauss M. Sauropod feeding and digestive physiology. In: Klein N, Remes K, Sander M, editors. Biology of the Sauropod Dinosaurs: Understanding the Life of Giants. Bloomington, IN: Indiana University Press; 2011. pp. 11–33.

99. Calvo J. Jaw mechanics in sauropod dinosaurs. Gaia. 1994; 10: 183–193.

100. Peyrot D, Barroso-Barcenilla F, Cambra-Moo O. Paleoecology of the late Campanian/early Maastrichtian Fossil-Lagerstätte of "Lo Hueco" (Cuenca, Spain): Palynological insights. Palaeogeogr Palaeoclimatol Palaeoecol. 2013; 387: 27–39.

101. Fox-Dobbs K, Leonard JA, Koch PL. Pleistocene megafauna from Eastern Beringia: paleoecological and paleoenvironmental interpretations of stable carbon and nitrogen isotope and radiocarbon records. Palaeogeogr Palaeoclimatol Palaeoecol. 2008; 261, 30–46.

102. Yeakel JD, Guimarães PR Jr, Novak M, Fox-Dobbs K, Koch PL. Probabilistic patterns of interaction: the effects of link-strength variability on food web structure. J R Soc Interface. 2012; 9: 3219–3228. doi: 10.1098/rsif.2012.0481 PMID: 22832361

103. Yeakel JD, Guimarães PR Jr, Bocherens H, Koch PL. The impact of climate change on the structure of Pleistocene food webs across the mammoth steppe. Proc R Soc B. 2013; 280: 20130239. doi: 10.1098/rspb.2013.0239 PMID: 23658198

104. Domingo MS, Domingo L, Badgley C, Sanisidro O, Morales J. Resource partitioning among top predators in a Miocene food web. Proc R Soc B. 2013; 20122138.

105. Vennemann TW, Hegner E, Cliff G, Venz GW. Isotopic composition of recent shark teeth as a proxy for environmental conditions. Geochim Cosmochim Acta. 2001; 65: 1583–1599.

106. Biasatti DM. Stable carbon isotopic profiles of sea turtle humeri: implications for ecology and physiology. Palaeogeogr Palaeoclimatol Palaeoecol. 2004; 206: 203–216.

107. Wheatley PV, Peckham H, Newsome SD, Koch PL. Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA. Mar Ecol Prog Ser. 2012; 447: 211–229.

108. Jackson K, Butler DG, Brooks DR. Habitat and phylogeny influence salinity discrimination in crocodilians: implications for osmoregulatory physiology and historical biogeography. Biol J Linn Soc Lond. 1996; 58: 371–383.

109. Clementz MT, Koch PL. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia. 2001; 129: 461–472.