FRESHWATER MOLLUSCAN FAUNA FROM THE FLORISSANT FORMATION, COLORADO: RECONSTRUCTION OF A LATEST EOCENE, HIGH-ALTITUDE LAKE

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Abstract

The Florissant Formation (Colorado) represents deposition in a late Eocene lacustrine and fluvial depositional environment with associated shales, mudstones, pumice and ash conglomerates (34.07 ± 0.10 Ma). These sediments originated from the reworking of a nearby volcanic field, in which a highly diverse, excellent and uniquely preserved flora and fauna are present. Taxonomic revisions, taphonomy and carbon and oxygen isotope analysis were all used to further reconstruct the past ecology of Lake Florissant, through the use of underutilized molluscan fauna from the lake sediments. This study provides a unique insight into the conditions that supported and preserved this invertebrate fauna. The freshwater molluscan taxa were revaluated, updating work that is more than a century out of date, bringing terminology and identification in line with modern genetic and biologic standards. Taphonomic comparison of spatially and stratigraphically disparate collection sites were preformed to confirm taxonomic identifications and to elucidate the quality and diversity of preservation within this Eocene lake. Stable isotope analyses were additionally performed on unaltered molluscan biogenic carbonates and bedding parallel, post-depositional fibrous calcite to further understand signatures of primary and diagenetic water sources and their potential influences on preservation of carbonates within the Florissant Formation.

The taxonomic revision of the molluscan fauna identified two gastropod genera, *Gyraulus florissantensis* (Planorbidae) and *Lymnaea (Stagnicola) scudderi* (Lymnaeidae) ; and three genera from the bivalve family Sphaeriidae, (*Sphaerium florissantense, Sphaerium* species 1 and *Sphaerium (Musculium)* species 1). Taphonomic analyses found no differences in population dynamics or preservation quality for the *Gyraulus florissantensis* across geographically disparate sites in the middle shale unit, though all specimens did show diagenetic alteration. Overall preservation within the overlying caprock conglomerate unit shows unaltered material. $\delta^{13}C$ and $\delta^{18}O$ data of biogenic carbonates, (i.e. specimens of the Sphaeriidae and Lymnaeidae families), and associated fibrous calcite show two different signatures: (1) the primary water source, and (2) diagenetic pore fluids. The spread observed within the isotope data from the molluscan fauna suggests either seasonal variability and/or taxonomically- specific fractionation. When compared to other ancient lake systems, the relationship between the O and C isotope data reveals that ancient Lake Florissant is within the expected range for a closed (evaporation > precipitation) system. Lake Florissant is interpreted as supporting a diverse population of mollusks that thrived within the lake system and were either diagenetically altered or not at all within the different lithologic units of the deposit. Through taxonomic revisions and

evaluating the degree of preservation of the molluscan fauna within the Florissant lake system, we were able to reconstruct ancient environmental parameters of this late Eocene lacustrine and fluvial depositional.

Introduction

The late Eocene Florissant Formation, Colorado (Fig. 1), consists of lacustrine, stream and floodplain deposits associated with an ancient drainage system that was episodically impounded by volcanic debris flows. The formation's paper shales, mudstones and interbedded ash and pumice conglomerates contain a diverse flora and fauna that was often exceptionally well-preserved. While previous work has focused on the paleo-flora and terrestrial fauna preserved within diatomaceous and smectitic paper-shale couplets and related mudstones, this study aims to analyze a portion of the formation's under-studied molluscan freshwater assemblage.

We investigated gastropods and bivalves from multiple collection localities (stratigraphically and geographically disparate) to investigate paleoecology and past geochemical drivers of preservation within the Florissant Formation. This assemblage is present within almost all of the lithologies and informal stratigraphic units of the formation (Fig. 2), and is therefore well suited, along with diatoms, to analyze taphonomic, geochemical and ecological processes that took place *within* the lake system throughout its duration. Our primary analyses of the molluscan fauna included revising their taxonomy, quantifying and describing physical and chemical preservation, conducting basic community analysis, and measuring stable isotope geochemistry of unaltered biogenic calcites.

Background – prior studies

Lake deposits of the Florissant Formation provide insight into a late Eocene, high-altitude lacustrine environment within the Rocky Mountain region. The formation overall comprises volcaniclastic sediments that cover a small geographic area, occupy a short temporal range, and contain abundant plant, invertebrate and vertebrate fossils. A mean of single-crystal $^{40}Ar/^{39}Ar$ dates on 66 sanidine crystals from four horizons in the upper Florissant Formation yielded a date of 34.07 + 0.10 Ma (Evanoff et al., 2001[1]), placing it geochronologically just before the Eocene/Oligocene (E/O) boundary, which is at 33.9 Ma (Gradstein et al., 2012). At this time there was a rapid shift in global temperatures, a transition from 'greenhouse' to 'ice-house' environmental conditions (Eldrett et al., 2009). Studying the flora and fauna present within the Florissant Formation contributes important understanding of a terrestrial ecosystem that existed around the time of this major climate change.

At the Eocene-Oligocene boundary, based on records from extensive deep-sea data sets, there was an average global temperature drop of ~7^{o}C over 10^5-10^7 years (Zachos et al., 2001). This drop resulted in rapid growth of continental ice-sheets across Antarctica, with an estimated mass of ~50% of present day ice-sheets (Hambrey et al., 1991). Evidence of a drop in atmospheric CO_2, from >1000 ppmv down to ~560 ppmv, indicates that global carbon cycling may ultimately be the driving force for the cooling, although the cause of this CO_2 drop is unknown (DeConto and Pollard, 2003; Eldrett et al., 2009; and Pearson et al., 2009). Opening of

[1] Calibration technique used an age of 27.84 Ma for the Fish Canyon Tuff, an age that has recently suggested to be revised to 28.201 ± 0.04 (Kuiper et al., 2008).

the Drake Passage allowed for the creation of the Antarctic Circumpolar Current, thermally isolating the Antarctic continent (Kennett, 1977; DeConto and Pollard, 2003), thereby influencing glaciation of that continent and also largely contributing to this climatic shift.

Floristic analyses from the extensive fossil flora have been used both to reconstruct the terrestrial Florissant setting and to estimate both paleotemperature and paleoelevation (e.g. Wolfe et al., 1998; Leopold and Clay-Poole, 2001; Meyer, 2001; Smith, 2008). These and other analyses were done on the fruits, seeds, leaves, flowers, wood, pollen and spores preserved within lacustrine shales and mudstones. Through nearest-living relative (NLR), climate leaf analysis multivariate program (CLAMP) and multiple regression techniques, paleobotanists have estimated a range of Mean Annual Temperatures (MATs) for the Florissant lake system, which range from 10.7°C to ≥18°C (Table 1). Paleoelevation estimates, which range from about 305 m to 4133 m (Table 1), have been calculated through lapse rate and paleoenthalpy techniques, (Meyer, 2001). Most estimates range within ±500 m of the current elevation of about 2500 m. This would mean that the Sevier/Laramide uplift in this region of the Rocky Mountains was essentially finished by the time that Lake Florissant existed.

In addition to the macroflora and their pollen, a diverse and unique diatom assemblage has been described by Benson et al. (2012), including 33 genera of which 14 are newly documented in the geologic record. O'Brien et al. (2002 and 2008) in SEM analyses of paper-shales collected from the middle and lower shale units (Fig. 2) discovered that each paper-shale couplet consisted of a few millimeters in thickness of smectitic clay and diatomaceous algal mats, with preservation of fossil material being mainly associated with the algal mats. The authors reasoned that volcanic glass and its weathered equivalent smectite would have dramatically influenced the lake's water chemistry, increasing the amount of dissolved silica within the water, which being a major component of their biology, would promote diatom growth. As these algal (diatom) mats formed, they secreted mucilaginous biofilms, leaves and insects would fall onto them, be trapped, and then sink to the lake bottom encased within the mats after the bloom died off.

Geologic Setting

Stratigraphy

The Florissant Formation was deposited in a paleovalley incised into basement rock of the Precambrian Pikes Peak Granite, situated on top of the Colorado Front Range (Fig. 1; Wobus and Epis, 1978; Evanoff et al., 2001; Meyer and Smith, 2008). Sediments were deposited in lacustrine and fluvial environments affected by episodic volcanic debris flows that dammed the paleo-river valley. The formation has been separated into six informal geologic units (Evanoff et al., 2001), which represent a composite thickness of 74 m (Fig. 2). From bottom to top, the units are: lower shale, lower mudstone, middle shale, caprock conglomerate, upper shale, and upper pumice conglomerate. The units are composed predominantly of volcaniclastic debris flows, ash falls and redeposited volcaniclastic sediments consisting of shale, tuffaceous mudstone and

siltstone, tuff, and arkosic and volcaniclastic sandstone and conglomerate. Most of the sediments originated from a nearby stratovolcano, the Guffey volcanic center, within the Thirtynine Mile volcanic field, with additional volcanic sediments from the Grizzly Peak and Mount Aetna calderas (Evanoff et al., 2001). There is also a granitic component to the sediments from the underlying and surrounding Pike's Peak Granite.

Of the units (Fig. 2), the lower, middle and upper shale are predominantly lacustrine in origin, of which the middle shale is the primary (fossil) quarry interval for the Florissant Fossil Beds National Monument. The middle and upper shale units in all likelihood represent a continuous period of existence for the Florissant lake, into which flowed a major lahar, represented by the caprock conglomerate unit, which is more variable in thickness at different localities than the shale units (Evanoff et al., 2001). The lower mudstone and upper pumice conglomerate units include fluvial channel and floodplain deposits, most prominently represented by the petrified forest near the top of the lower mudstone (Fig. 2). This paper focuses on molluscan fauna recovered from the middle shale and caprock conglomerate units.

The middle shale unit (5-9 m thick) comprises thin layers of ash gray and light brownish gray shale, mudstone, siltstone, silty sandstone and fine pumice conglomerate (Evanoff et al., 2001). These beds were deposited primarily by airfall, by pelagic settling, and by gravity-driven currents. The following observations are based on fossil-bearing specimens examined in our study. Pumiceous grains are common, as is plant debris on bedding planes. Molluscan fossils are common both in thinly laminated, ash-gray "paper shale" and also in more thickly laminated, brownish-gray mudstone and siltstone. There are common, isolated laminae of white ash (very fine silt to granule grain sizes) in the light brownish-gray beds. Sedimentary structures in the thicker laminae are typically planar lamination and normal grading.

The caprock conglomerate unit is a crudely graded, mixed-lithology bed, rich in volcanic clasts, which varies in thickness from 0 to 7.3 m, thinner toward the north and thickest in the axis of the paleo-valley (Evanoff et al., 2001). The caprock conglomerate is interpreted to be the deposit of a lahar that flowed into the Florissant Lake of the time; it is capped by the upper shale unit, which is very similar to the middle shale (Evanoff et al., 2001 and this study), suggesting that the lahar was deposited under water, and that the lake persisted following emplacement of the lahar deposit. The base of the conglomerate is erosive, overlain by a poorly sorted unit rich in clasts of volcanics, granite, and ripped-up mudstone in a muddy matrix. The upper part of the bed is predominantly crudely bedded, coarse-grained sandstone in a muddy matrix. The shells sampled from this unit for this study occur as clasts within this upper portion.

Collection localities and sampling methods

Mollusk specimens for this study were collected by interns and staff at the Florissant Fossil Beds National Monument from numerous localities within the Florissant Formation (FLFO); locality numbers are from the internal National Monument FLFO system. Two localities, P-9 and P-39, were sampled from the middle shale unit, and one locality, P-16, was sampled from the caprock conglomerate unit (Fig. 2). Locality P-9, previously described in

Henning et al., (2012) consists of siltstone and mudstone with a few interbedded paper shales. P-9 was sampled sub-meter (measured down from the caprock conglomerate) by Henning et al., 2012, for taphonomic analysis of insects within the Florissant Formation. Our study utilizes the molluscan assemblage obtained during their study. Locality P-39 consists predominantly of paper shale, with some mudstone and siltstone and is characterized by abundant pumice deposits. Original P-39 samples were collected en masse, from the lower to middle part of the middle shale unit, from material that was salvaged from a road cut during flood mitigation. Fossils from this P-39 material, were separated out during summer field seasons from 2008 through 2014. Locality P-16 is situated stratigraphically above the middle shale unit and located geographically near P-9. Author Buskirk with three others sampled fresh, weathered and float specimens at P-16, which at the sampling horizon is a medium-grained, sandy volcaniclastic conglomerate (overlying a coarser basal unit). For our study, only newly exposed, 'fresh', samples were utilized.

Taxonomy of Molluscan Fauna

Introduction

The Florissant molluscan assemblage from the caprock conglomerate and middle shale units includes three sphaeriid bivalve and two pulmonate gastropod species. Preservation within the paper shales has resulted in shell compression that has eliminated or covered essential characteristics, notably the hinge line of the bivalves. The original freshwater Florissant molluscan descriptions were published by Cockerell in 1906, and the type specimens are housed in the American Museum of Natural History and the University of Colorado Museum. Taxonomic identifications were re-asserted by Hartman (1998), although no formal revisions have been published. These fossil taxa were placed within extant genera, and more recent descriptions of the living species include both shell and soft-body characteristics. Molecular phylogenetic studies of freshwater mollusks, most notably for pulmonate gastropods (e.g. Remigio, 2002; Albrecht et al., 2007), indicate that shell morphologies are not reliable characters for systematic placement. Cryptic species and the paucity of shell morphologies in the thin-shelled lotic and lentic taxa have resulted in major re-arrangements of numerous higher taxonomic units based on genetic character sets (Lee and Ó Foighill, 2003; Lee, 2004). Shell characteristics of these late Eocene freshwater mollusks are insufficient to erect new genera.

Gastropods from the Florissant Formation are placed into two genera; *Gyraulus* (family Planorbidae) and *Lymnaea* (family Lymnaeidae). Planorbid and lymnaeid families were previously included in suborder Basammatophora; however Bouchet and Rocroi (2005) reassigned these families into the unranked higher clade Hygrophila to replace the polyphyletic suborder. Bivalves from the Florissant Formation are placed into the genus *Sphaerium* (family Sphaeriidae).

Materials and Methods

Specimens from the Florissant Formation used in this study were collected from two localities, P-9 and P-39, in the middle shale unit and one locality, P-16, from the caprock conglomerate unit (Figs. 1 and 2). Shell measurements (Fig. 3) included a) for Planorbidae: body width and umbilicus width (Figs. 3 and 4); b) for Lymnaiedae: axial height and width of largest body whorl; and c) for Sphaeriidae: height and length. Shells were measured with digital calipers and photographed using a light microscope.

Systematic Paleontology

Class GASTROPODA Cuvier, 1795
Unranked clade HYGROPHILA Férussac, 1822
Superfamily PLANORBOIDEA Rafinesque, 1815
Family PLANORBIDAE Rafinesque, 1815
Genus GYRAULUS Charpentier, 1837

Caillaudia Bourguignat, 1883
Glyptanisus Iredale, 1943

Type diagnosis. – Shell planispiral or rarely (in some ancient lakes) pseudodextral with elevated spire; 2-10 mm in diameter, with 3 to 5 whorls, which may be rounded or angular, with or without a keel. Penial morphology, particularly a dagger-like stylet (emended by Brown, 2001, p. 19).

Brown & Van Eeden (1969) added the following description to the genus: shell discoid, never septate, of less than 5 whorls, penis with chitinous stylet.

Gyraulus florissantensis (Cockerell, 1906)
Figure 5a

Planorbis florissantensis Cockerell, 1906

Type diagnosis. – The usual diameter is about 4 ½ mm, but a few have been found as large as nearly 7 mm. The periphery is rounded (not sharply keeled), especially in the young, but the shell is evidently very flat, and probably somewhat flexible, as it could endure pressure without much breaking. The oblique striae on the last whorl are quite strong. One specimen was dark brown, which is probably the original color.

Material examined. – 111 specimens from the middle shale unit.

Description. – Shells planispiral, deeply umbilicate, with 3-4 whorls. Shell width 1.3 mm to 6.2 mm. Shell with closely spaced striae parallel to the aperture lip, present on all teleconch whorls. Protoconch smooth. Shell periphery rounded to slightly subangular.

Remarks. – Two end members of the range in apertural face dimensions suggested two morphologies, one flared and the other non-flared. In an attempt to illustrate this morphological variability, body width and umbilicus width (Fig. 3 and 4) were measured on 111 specimens. Graphical comparison of these two measurements showed a wide range, but no differentiation into recognizable clusters (Fig. 4). Additional morphometric measurements of the shell may show discernible sub-populations. Hartman (1998) reported that the additional planorbid genus *Promenetus* was present at Florissant, although our study did not observe any carinated specimens.

Superfamily LYMNAEOIDEA Rafinesque, 1815
Family LYMNAEIDAE Rafinesque, 1815
Genus LYMNAEA Lamarck, 1799

Limnophysa Fitzinger, 1833
Lymnaea (Stagnicola) Lamarck, 1799

Type Diagnosis. – Lymnaea Lamarck. Shell oblong, sometimes turreted; spire prominent. Aperture entire, longitudinal. Lip sharp, its inferior part rising upon the columella, forming a very oblique fold as it enters the aperture. No operculum (translated by Gould, 1833).

Subgenus STAGNICOLA Jeffreys, 1830

Type Diagnosis. –Stagnicola communis Jeffreys, 1830, p. 376

Animal lutescenti-fuscum. *Tentacula* subconica, acuminata. *Testa* oblonga, acuminata, glabra, interdùm spiraliter rugosa, et sub lente striis transversis seriatim dispositis ornata, fusco-cornea. *Anfractus* 6—7. *Apertura* ovata: *labro* intùs sæpè violaceo, subincrassato.
Long. 0.875.—Diam. 0.325.
Limneus palustris. *Drap. Hist. des Moll. p.* 52. *t.* 2. *f.* 40, 41. & *t.* 3. *f.* 1, 2.
Helix limosa. *Linn. Syst. Nat.* 1. *p.* 1249?
—— palustris. *Gmel. Syst. Nat.* 1. *p.* 3658.
Buccinum palustre. *Müll. Verm.* 2. *p.* 131.
Stagnicola communis. *Leach MSS.*

Lymnaea (Stagnicola) scudderi Cockerell, 1906
Figure 5b(1)

Lymnaea scudderi Cockerell, 1906, p. 461, Fig. 4

Type diagnosis. – Length about 6 mm, breadth about 4 mm, the spire short, about 1½ mm, smooth and shining, without any strong sculpture; apex obtuse.

Material examined. – 38 specimens from the middle shale unit, 7 from the caprock conglomerate unit, and 2 from the lower mudstone unit.

Description. – Shells are dextral, small to medium in size, range in height 1.5 - 10 mm (average 5.6 mm), except for a singular lower mudstone specimen, 25 mm; elongately conical and imperforate with shouldered whorls. Striae present on well preserved specimens and on all whorls, parallel to aperture lip.

Remarks. –Lip and columella morphologies from middle shale specimens are not visible. Incomplete three-dimensional specimens from the caprock conglomerate unit bear a lip that is straight and reflected. Most specimens from this unit are decollate (missing their spires).

Additional specimens observed from the lower mudstone unit (e.g. CU Boulder specimens: UCM 36097) were higher spired and more elongate than the typical *L. scudderi*. Some of these may belong in the species *Lymnaea sieverti,* another species from the Florissant Formation, described by Cockerell in 1906.

In 1908, Cockerell wrote a taxonomic diagnostic key to the Florissant lymnaeids which noted that *L. sieverti* had a "spire quite long, acute, the apex slender." No specimens of this species were noted in our collection from the middle shale and caprock conglomerate units. In 1908, Cockerell also introduced the new species *Lymnaea florissantica*, distinguished from *L. scudderi* only by size (*florissantica* > 20 mm, *scudderi* < 6 mm). While the one specimen (25 mm high) observed from the lower mudstone unit (e.g. CU Boulder specimen: UCM 4625) may be *L. florissantica,* all other specimens are ≤10 mm high, and were placed in the *L. scudderi taxonomic grouping.*

Lymnaea sieverti Cockerell, 1906, p. 461, Fig. 3 – Long. 8 mm, lat. 4 ¼ mm, with about five rounded whorls; length of aperture about 5 mm; sutures impressed; sculpture weak; aperture contracted.

Lymnaea florissantica Cockerell, 1908a, p. 69. – Length 21 mm, diameter about 10½; spire short, scarcely over 5 mm long, the whorls moderately convex; body-whorl not very convex, with coarse, shallow, vertical grooves. (Fig. 5(2))

Anderson (2008) placed *Stagnicola* as a sub-genus of *Lymnaea* based on DNA studies.

Class BIVALVIA Linnaeus, 1758
Subclass HETERODONTA Neumayr, 1884
Order VENEROIDA H. Adams and A. Adams 1856
Superfamily SPHAERIODEA Deshayes, 1855 (1820)
Family SPHAERIIDAE Deshayes, 1855 (1820)
Genus SPHAERIUM Scopoli, 1777

Cyclas Lamarck, 1799

Type diagnosis. – Shell small, rather thin; moderately convex, broadly ovoid and more or less equilateral in outline. Beak low, located at or slightly in front of mid-length. Hinge plate narrow;

cardinal teeth two, or occasionally three, on each valve, usually nearly parallel to hinge line; anterior and posterior lateral teeth two on right valve, one on left valve. Surface with concentric growth lines, one or more of which may accentuated (emended by Russell, 1975).

Remark. Cox et al. (1969) gave the following emended diagnosis: Ovate, quadrate, bluntly triangular in some, shell moderately solid, concentrically striate; beaks near median; animal with two siphons. Carter et al. (2011) designated an alternative classification at the order level.

Sphaerium florissantense Cockerell, 1906
Figure 5c

Musculium florissantense (Cockerell) Sterki, 1916

Material examined. – 15 specimens from the caprock conglomerate unit.

Type diagnosis. – Length 8¼ mm, breadth 10 mm, with rounded outline like that of the European *S. corneum*; beaks not protruding or prominent; sculpture consisting of fine but distinct concentric striae, 4 to 6 in about 150μ.

Description. – Shells are moderately convex, sub-triangular to oval, with a centrally positioned umbo, small to medium in size (width: 5.0 - 7.5 mm, height: 4.0 - 5.75 mm). Hinge with a single posterior and anterior lateral tooth. Outer surface striated.

Sphaerium species 1
Figure 5d

Material examined. – 4 specimens from the caprock conglomerate unit.

Description. – Shell oval to inequilateral, moderately convex, with umbo deflected from the midline, with the beak pointed toward the anterior end, medium in size (width: 5.0-8.5 mm, height: 3.7 - 6.3 mm). Anterior tooth reduced. Outer surface striated.

Subgenus MUSCULIUM Link, 1807

Musculium Müller, 1921
Calcyculina Clessin 1872
Carneola Westerland 1873
Primella Cooper 1890

Type diagnosis. – Hinge plate weak, embryonal shell prominent, commonly set off by a groove (emended by Cox et al., 1969, p. N670).

Sphaerium (Musculium) species 1

Figure 5e

Material examined. – 11 specimens from the caprock conglomerate unit.

Description. – Inflated shells that are subtriangular to triangular; a centrally positioned umbo with a raised umbonal cap. Small to medium in size (width: 3.4 - 5.9 mm, height: 3.0 - 4.8 mm). Outer surface striated.

Remarks. – This species differs from *S. florissantense* by the presence of prominent calyculi. Fossil shells show a well-developed hinge, which is more prominent than on either species of *Sphaerium* from Florissant. Hornbach et al. (1980, p. 1703) translated Link's original description as: Presence of calyculi or caps on the shell.

While *Musculium* was an accepted genus both on shell morphologies and life histories (Heard, 1977; Hornbach et al., 1980) modern studies on ribosomal and mitochondrial phylogenetics indicate that it is a subgenus of *Sphaerium* (e.g. Lee and Ó Foighil, 2003).

Life Histories

Mollusk Community Structure

The total number of individuals from each taxon was recorded for isotopic analyses and taphonomic studies. Molluscan taxa from the Florissant Formation include the planorbid and lymnaeid gastropods *Gyraulus florissantensis* and *Lymnaea (Stagnicola) scudderi*, and three sphaeriid bivalves *Sphaerium florissantense*, *Sphaerium* species 1, and *Sphaerium (Musculium*) species 1. All these taxa were collected from all three fossil localities, P-9, P-16 and P-39.

From the samples of caprock conglomerate (collection locality P-16), 48 individual specimens were sampled for isotopic analyses (carbonate material was completely removed from mollusks): 40 *Sphaerium*, seven *Stagnicola* and one *Gyraulus*. In the upper 130 cm of the middle shale (locality P-9), of the specimens completely counted, a total of 70 individuals were taxonomically identifiable to a generic level (> 66% complete), of which 75 were *Gyraulus*, 29 *Lymnaea (Stagnicola)*, and 7 *Sphaerium*. Additional specimens were collected and counted from both the P-9 and P-39 localities (Table S1), but are not reported here due to inequality in collection type (stratigraphically controlled vs. remediation of county road work). Fossilized excrement, in the form of either coprolite or vomit is preserved in the middle shale locality P-9. This material comprises mollusk shells (sphaeriid and lymnaeid) contained within an organic matrix (Fig. 5f), but individual specimen resolution in each is inadequate for detailed description. Full analyses of these excretions could contribute to an overall community analysis. Both pulmonates and sphaeriids are common prey for a wide variety of predators, commonly fish and birds, and also small mammals.

Mollusk Lifestyles

Bivalves described from the Florissant Formation are species from the Family Sphaeriidae (fingernail clams), which comprise the non-unionoid freshwater bivalves in North America (Sturm et al., 2006). Inhabiting a variety of niches including large rivers and lakes, springs, peat bogs and temporary pools, their highest diversity is observed within lakes, ponds and small rivers (Sturm et al., 2006). Living sphaeriids are burrowing clams that predominantly live within soft sediment substrates (fine sand, muddy sand and mud), rarely inhabiting rocks and gravels (Sturm et al., 2006). They are mostly interstitial suspension- or deposit-feeders, feeding on bacteria, small organic detritus (Kořínková, 2011a; Dillon, 2000) and phytoplankton (Sturm et al., 2006).

Sphaeriidae clams are hermaphroditic and viviparous, retaining their eggs and then hatched juveniles within brood pouches internal to their demibranchs. Larvae are incubated for one or up to several months within the parental brood pouch, during which primary shell growth starts. Juveniles are released generally twice per year, summer and fall seasons, although some can be released as early as late spring and as late as early winter (Kořínková, 2011b; Mackie, 1979; Strum et al., 2006). In modern *Sphaerium* clams, full growth is obtained within a few months, sometimes as fast as 50 to 70 days. Sphaeriids generally only live 6 to 15 months, up to 24 in rare cases, with (the subgenus *Musculium* living shorter lives than other *Sphaerium* species (Heard, 1977; Kořínková, 2011b; Mackie, 1979; Strum et al., 2006). A few species of the subgenus *Musculium*, can go into hibernation or aestivation to wait for more favorable environmental conditions. This allows for overwintering populations that will produce the next summer's generation. Semelparity (one reproduction phase per lifespan or season) is the most common mode of reproduction in sphaeriid clams, though iteroparity (more than one reproduction phase) has also been observed (Kořínková, 2011b; Mackie, 1979). In general, the parental generation dies after reproduction, though some might subsist into the next season to produce additional litters.

In some species, several brood pouches can be in development at once within a parent, though growth of the larvae stops when the parent obtains full growth, retarding growth of the incubating larvae (Mackie, 1979; Strum et al., 2006). If one brood pouch is not released before death, parental shells can be found with incubated larvae still within their gills. This creates a situation in which upon collection of sphaeriid shells, modern or fossil, multiple generations could potentially be obtained: dead or dying adults, reproductive adults, newborns and larvae. Generation overlaps are most common in continuously flooded habitats, whereas complete die out of the parental generation after giving birth is observed in temporary waters (Kořínková, 2011b).

Of the gastropods, the genus *Gyraulus* is classified into the Family Planorbidae, and the genus *Lymnaea* within the Family Lymnaeidae, both within the informal taxon unit Pulmonata. Pulmonates are freshwater and land-living gastropods that breathe by pulling air over the inner surface of their mantle, the pallial lungs (Dillon, 2000; Sturm et al., 2006). Planorbids (planispiral shells) and lymnaeids (dextrally coiled, slender shells) bear no operculum and are typically thin shelled. They live on a variety of surfaces, and can free float, depending on feeding

strategies; they can carry an air bubble within their mantle to control buoyancy and to help them inhabit waters that have low dissolved oxygen content, e.g., calm, warm and even stagnant waters (Dillon, 2000; Sturm et al., 2006). Both lymnaeids and planorbids live within the epilimnion, close to the surface, but deep enough in the water column where wave, wind and possibly predators are less likely to be a disturbance. Planorbids preferentially live closer to shore, whereas lymnaieds are reported to travel out farther within a lake (Dillon, 2006).

Planorbids and lymnaeids are grazers, using a radula--a jaw-like appendage with teeth, to grab food off of a substrate. Planorbids prefer firmer, muddier sediments, rich in decaying matter and slow currents (Dillon, 2000); they feed preferentially off of algae, macrophytes, organic detritus and diatoms. Lymnaeids live and feed off of practically any surface, rocky or soft sediment, within a lotic or lentic environment, grazing on algae, macrophytes, organic detritus, small animal fragments, vascular water plants and diatoms (Dillon, 2000; Sturm et al., 2006).

While reproductively hermaphroditic and therefore capable of self-fertilization, pulmonates more commonly alternate between the female or male role and will find a partner to reproduce (Dillon, 2000). Young are hatched from eggs, reaching maturity within a few weeks, although this timing is entirely dependent on environmental conditions and availability of food. Mature adults typically reproduce semelparously (once per year), in either spring or late summer, with the parents generally dying after reproduction. Some adults may subsist into the next season to reproduce once or twice more. Thus there can be multiple generations existing within an environment at a given time (Dillon, 2000). Most pulmonates live for less than 12 to 24 months (Dillon, 2000); shells are continuously grown throughout life, much more slowly after reproduction.

Community discussion

Differences in population make-up from both the P-16 and P-9 localities are understood to be due to environmental, facies and taphonomic differences between the sites. The caprock conglomerate sampled at P-16 is understood to be the deposit of a geographically constrained lahar that flowed into the Florissant lake and churned up the lake shore, whereby it picked up predominantly the infaunal, shallow-water bivalves, disarticulating them, and then sealing them internally in the unit when it was emplaced on the lake bottom. A preservation factor to consider is that pulmonates may be underrepresented within the caprock conglomerate purely due to the thin nature of their shell, in that if and when they were picked up by the lahar, they preferentially were destroyed and not preserved.

The middle shale facies represented in P-9 and P-39 is more indicative of offshore lacustrine sediment deposition. Planorbids and lymnaeids, able to free-float and move around within the epilimnion of a lake, are more common in this facies than sphaeriids, who, living in nearshore, benthic environments, would have to be transported after death to reach offshore settings. Planorbids and lymnaeids, upon death, could have either been transported from near shore environments to the lower anoxic portions of the lake, or they could have simply fallen out of the overlying water column.

Taphonomy

Introduction

The Florissant Formation has long been recognized for its exceptional preservation of fossils (Meyer, 2003; Meyer and Smith, 2008), in particular, of detailed compression and impression fossils of insects and plants. Exceptional preservation is commonly associated with anoxic bottom waters, for example in lake beds. Fossil preservation in the Florissant lake beds has been attributed also to rapid burial by volcanic ash (e.g., Cockerell, 1908b). Recent studies have documented that preservation of insect and plant fossils are associated with biofilms of extracellular polymeric substances (EPS), which suppress the action of bacteria and grazers (e.g., O'Brien et al., 2008). Fine grain size can also contribute to fine preservation, but in a taphonomic study examining insect preservation quality by grain size, Henning et al. (2012) found no significant difference amongst those grain sizes from shale to siltstone.

This study, to our knowledge, is the first taphonomic analysis conducted on the Florissant freshwater molluscan fauna. The purpose of this study was to understand possible geochemical (diagenetic) alteration that would affect stable-isotope analyses and to qualify types of preservation seen within the formation. We examined specimens from shaley to silty beds from the middle shale unit localities P-9 and P-39) and from the caprock conglomerate unit (locality P-16), which locally is a muddy, very coarse sandstone. The most significant finding is that of all specimens studied, only fossil shells from the caprock conglomerate unit preserve aragonite; shelly material in the middle shale unit has been removed or apparently replaced by silica.

Physical taphonomy – methods, results, discussion

Methods. Specimens from the middle shale unit from two different localities (P-9, P-39) were assessed on a diversity of criteria to explore modes of and potential influences on preservation (Table S1a). Observations per individual shell or impression, preserved on a fissile surface, were made under a binocular microscope. Observations included sediment surface and sample lithology (grain size, color, lamination, etc.), preservation type (presence of shell material), color of specimen, proportion of completeness, amount of shell breakage, and amount of preserved detail (e.g. growth lines). Each complete or near-complete individual was also measured with a digital caliper for basic morphological properties (Fig. 3). In gastropod specimens, additional properties were noted, specifically coiling direction, relative height of spire, and aperture shape. Bivalves were analyzed for articulation characteristics and for shell orientation (convex up or down) within the lithologic sample itself (Table S1b). All additional notes taken during taphonomic observations are recorded in Table S1c.

Results. Because the primary factors for physical taphonomy are basic physical characteristics such as shell shape, and because taphonomic observations were made on many specimens not formally identified taxonomically, we report results here at the family level: planorbids, lymnaeids, and sphaeriids. In examining modes and quality of preservation of

molluscan fossils in the middle shale unit via binocular microscope, we found very few to no significant differences between middle shale collection localities (P-9, P-39) or among sample grain size. (Table S1). However, there are some differences based on (family-level) shell type (Fig. 6). In terms of shell completeness, almost all (97%) planorbids and most (88%) lymnaeids were more than 2/3 complete; a lower proportion (67%) of sphaeriids were complete shells, with more incidences of fragmentary sphaeriid shells. In terms of shell cracking, lymnaeids were observed to be significantly more cracked than planorbids and sphaeriids (Fig. 6), with 83% of lymnaeids observed to have "throughgoing" cracks, compared to 26% and 33% for the others. In the category of preserved detail of growth lines and shell striation, there was not a significant difference among shell types (Figure 6), with about two thirds of shells retaining "some" to "fine" detail.

Discussion. The range of grain sizes and other lithologic parameters (bed thickness, composition, organic content, etc.) in the middle shale unit is apparently not sufficient to affect the preservation quality of shelly fossils. This study of physically preserved characteristics remains incomplete until a similar study is conducted on the caprock conglomerate, or other stratigraphic horizons to understand the mode and type of preservation throughout the entirety of the Florissant Formation and the Florissant lake itself. The higher level of completeness of gastropods is attributed to their likely settling from the water column, while the bivalves were more likely physically transported from nearer to shore. The higher level of cracking in the lymnaeids we attribute to their being more three-dimensional, with a prominent outer whorl susceptible to cracking during burial and compaction.

Chemical taphonomy via X-ray diffraction – methods, results, discussion

Methods. The mineralogy of the specimens' shell material was analyzed by X-ray diffraction of samples from both the middle shale and the caprock conglomerate (Table S2). Specimens from all three recognized taxonomic families were analyzed. Analyses were made with a Bruker F8 Focus Powder X-Ray Diffractometer at the Material Sciences facility, University of Washington. Crystal phases were compared to the International Center Diffraction Data (ICDD) database. Samples were picked and cleaned with a metal dental pick or a pin-vice, and then shell material was crushed with a metal pestle within a glass sample vial. All samples were analyzed with 0.6mm and Ni diffraction slits, and 40kv and 40ma diffractometer parameters. Angle measurement, 2θ, varied from sample to sample (Table S2), but all were in the 10-90 2θ range, specifically to analyze for aragonite mineralogy (ICDD). Step size was 0.020 to 0.030, depending on sample. Samples were mounted to analysis slides in one of two ways, either by pressing powdered material into a silicon grease, or by pressing the powdered sample down onto double-sided sticking tape with a microspatula. In one sample (P16-Bulk-XRD1) a version of both methods was used, with the sample mounted onto a slide cover with silicon grease, which was then held onto the analyses slide with double stick tape. Thin sections (<60μm in thickness) were also made of sphaeriid and lymnaeid specimens, from the caprock conglomerate unit, which were then analyzed using a petrographic microscope with cathodoluminescence

attachment. This was done to investigate possible diagenesis of the meta-stable aragonite shell material when compared with the more stable calcite crystal of calcium carbonate, but the results were ambiguous and are not included within this paper.

Results. The X-Ray diffraction analyses of skeletal shell material from specimens of the Florissant Formation show that the caprock conglomerate specimens retain their original[2] aragonite mineralogy, while the middle shale unit specimens have been replaced entirely with silica (Fig. 7). Positions of the peaks, and their relative intensities, seen within the diffraction profile of the caprock conglomerate specimens agrees well with the ICCD accepted spectra for aragonite crystal forms (Kontoyannis and Vagenas, 2000). Any shift in the data or broadening of the peaks can be attributed to noise within the machine, non-uniformity in the powder size of the sample, or relative height of the sample to the detector slide, in addition to amorphous crystal forms (Connolly, 2005). The diffraction profile for specimens from the middle shale unit (Fig. 7) do not fit well with the expected aragonite diffraction profile, or even with calcite, an expected diagenetic alteration of the original aragonite shell. In fact, the middle shale specimens show a wide, high intensity peak at ~2θ, which is indicative of amorphous silica within the sample analyzed (Morris et al., 1981).

Discussion. Original aragonite shell is preserved only in specimens from the caprock conglomerate unit but not in specimens from the middle shale unit. This result has a counterintuitive component because we might have expected pore fluids to have moved more easily through the coarser-grained bed, promoting dissolution. There are at least two explanations for the difference in preservation, which are not mutually exclusive. 1) Rapid deposition of the caprock conglomerate removed the included shelly material from interaction with pore waters; subsequent diagenetic processes did not affect the specimens in this unit. 2) Biologically mediated pore-water chemistry in the diatom/biofilm-rich layers of the middle shale produced conditions conducive to $CaCO_3$ dissolution and SiO_2 replacement. The importance of these findings relates to the goal of stable isotope analyses of these shells: only original, unaltered shell material will provide reliable data on the original environmental parameters of the lake.

Stable Isotopes

[2] Freshwater invertebrates currently and in the past are understood to precipitate aragonite shells (many references). Carter et al. (1998) suggest reasons why: because the amount of dissolved Mg within terrestrial environments is enough to 'poison' other types of calcium carbonate precipitation and because there is an evolutionary taxonomic preference on what type is precipitated, e.g. aragonite precipitation evolved first and has been retained evolutionarily in freshwater mollusks, amongst others.

Stable isotopes have been shown to be effective in elucidating the paleotemperatures and paleoenvironments of past ecosystems. Many marine and freshwater mollusks are good indicators of paleoenvironments because they precipitate their $CaCO_3$ shells in isotopic equilibrium with their habitat and have essentially no vital effects (Fritz and Poplawski, 1974; Kaplan and Selleck, 2008). Thus the $\delta^{18}O$ and $\delta^{13}C$ signature of the ambient water will be preserved in the shell of a specimen, if that shell is unaltered. Our goals were to determine lake-water chemistry, to investigate possible differences between taxonomic groups, and to test for potential seasonal signatures in individual specimens.

Of the mollusks sampled in this study, the specimens from the caprock conglomerate were shown to consist of aragonite, which is probably original biogenic carbonate, a possibility we can interrogate with the isotopic data. We refer to this shell material as "biogenic carbonate." We analyzed oxygen and carbon stable isotopes in samples of shell material from *Sphaerium florissantense, Sphaerium* sp. 1, *Sphaerium (Musculium)* sp. 1, *Lymnaea (Stagnicola)scudderi* and *Gyraulus* sp. Additionally, we sampled and analyzed bedding-parallel veins of fibrous calcite with crystal growth perpendicular to bedding, also from the caprock conglomerate unit, to determine potential diagenetic fluid interactions with the biogenic carbonate.

Methods

All samples are from the caprock conglomerate unit, site P-16; each numbered sample is from a single valve (e.g., P16-1). A total of 48 biological specimens were analyzed: 26 Sphaeriidae identified only at the family level, 11 *Sphaerium florissantense,* 3 *Sphaerium (Musculium)* sp. 1, 7 *Lymnaea (Stagnicola) scudderi* and one *Gyraulus* sp. The number of samples from each taxon indicates their relative abundance in processed material. Four powdered samples of the fibrous calcite were also analyzed. Two individual specimens (P16-46, P16-47) of sphaeriids were subsampled "across" the shell, from umbo to ventral lip, to analyze for variation within the original water source during a single organism's shell growth; however, the umbo was not retained in sample P16-47. These subsamples were then prepared according to Method 2 (see below) and run twice each.

Samples were picked and cleaned with a metal dental pick or pin-vice. Then we used two different methods of crushing shell material within a glass vial. In Method 1, the sample was partially crushed and powdered (with a metal pestle), then subsampled with a micro-spatula. In Method 2, the sample was fully powdered and then subsampled. For most samples where Method 1 was used, a total (from both methods) of at least three subsamples were run. Because Method 2 better ensured that the analysis represented the entire sample and that the subsample was fully digested (as confirmed by uniformity of results), two runs were considered sufficient. With a few exceptions, both methods generated consistent isotope data among subsamples from one individual (Supplement Table S3). Analyses were replicated to ensure correct methodologies and to obtain averaged data sets. Analyses were only dropped when data were flagged by the reduction script due to low CO_2 content, to high standard deviations in $\delta^{18}O$ and $\delta^{13}C$, or to memory effects present within the analyses (5 runs total were dropped).

Samples for oxygen and carbon stable isotope analyses were run on a Finnigan Delta Plus mass spectrometer with attached Kiel III Carbonate Device in the University of Washington's Isolab. Values are reported in standard delta notation:

$$\delta^{18}O = \left(\frac{\left(\frac{^{18}O}{^{16}O}\right)_{sample}}{\left(\frac{^{18}O}{^{16}O}\right)_{standard}} - 1 \right) * 1000\ ^{o}/_{oo}$$

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) * 1000\ ^{o}/_{oo}$$

Where $\delta^{18}O$ and $\delta^{13}C$ are a measurements of the ratios of the amount of the heavier isotope to lighter isotope of an element, in a sample, against that of a standard, the Vienna - Pee Dee Belemnite (V-PDB). Errors are 0.1 ‰ for $\delta^{18}O$, and 0.05 ‰ for $\delta^{13}C$. Data were reduced using Matlab scripts available through the Isolab.

Results

Averaged measurements (and non-averaged for the single samples P16-20, P16-32) of $\delta^{13}C$ and $\delta^{18}O$ of molluscan biogenic carbonates and non-biogenic (fibrous) calcite are reported by taxa and sample type (Fig. 8, Table 2). Individual, non-averaged, subsample values are reported in Supplementary Table S3. Because there are no major differences in the data among specimens from the Sphaeriidae family, and because taxonomic identification was poor, they are reported at a family level in Figure 8. Most of the molluscan biogenic carbonate values plot within an elongated cluster between +2 ‰ and -5 ‰ $\delta^{18}O$, and 0 ‰ and -5.5 ‰ $\delta^{13}C$, with one major outlier [a *Lymnaea (Stagnicola) scudderi*] at -9.76 ‰ $\delta^{18}O$, -11.93 ‰ $\delta^{13}C$. Also, some of the averaged data for the lymnaeid samples plot slightly off the general cluster.

Single-shell variation results are shown in more detail in Fig. 9. Variation is observed for both specimens, a total difference of 0.72 ‰ in $\delta^{18}O$ and 1.66 ‰ in $\delta^{13}C$ in P16-46, and 0.44 ‰ in $\delta^{18}O$ and 0.57 ‰ in $\delta^{13}C$ in P16-47. The four analyzed fibrous calcite values are characterized by substantially more depleted $\delta^{18}O$ and enriched $\delta^{13}C$ results (averages: $\delta^{18}O$: -14.45 ‰ and $\delta^{13}C$: +4.68 ‰), plotting distinctly away from the rest of the measured stable isotope values (Fig. 8).

Discussion

The consistent grouping of the molluscan stable isotope values may be explained by one of two hypotheses; 1) all the data were collected from organisms that precipitated their shells in or near to equilibrium with their water source, or 2) all the samples were diagenetically altered by the same process. We reject the diagenetic argument for the following reasons. According to the x-ray diffraction data presented herein (Fig. 7, Supplement Fig. XRD) sphaeriid specimens

retain (interpreted original) aragonite material, and other taxa plot in the same cluster. The observed slight variance within the *Lymnaea* (*Stagnicola*) *scudderi* data might be due to a vital effect, but data are generally consistent with the rest of the molluscan specimens sampled. The isotope values overall are consistent with other ancient lacustrine carbonates (*cf.* Talbot, 1990) (Fig. 10). Furthermore, the biogenic carbonate data do not trend toward the fibrous calcite isotope data, which are far removed from the former. Accordingly we tentatively accept the hypothesis that the mollusks grew in isotopic equilibrium with their ambient lake waters while the fibrous calcite was precipitated later (see below).

The sphaeriid specimens sampled for single-shell variation show more variation in $\delta^{13}C$ than in $\delta^{18}O$ (Fig. 9), which we attribute to a signal in the $\delta^{13}C$ of seasonal variations in productivity. Though these values could also be attributed to changes in productivity over multi-season precipitation of shell material or continuous shell growth throughout the mollusk's life (e.g. what is observed in lymnaeids and planorbids), both of these would be counter to the single growth-season known for most sphaeriids (Kořínková, 2011b; reported above). Lymnaeids and planorbids were not sub-sampled for seasonal variation, but because both groups continuously precipitate their shells for their whole life (generally less than one year, rarely two for the genera included in this study), their isotopic values, homogeneously sampled, would reflect average sub-annual to annual variations of carbon within their environment.

Covariance of $\delta^{18}O$ and $\delta^{13}C$ from stable isotope measurements from authigenic and biologic freshwater specimens is generally associated with whether the lake is a closed system (long water residence time, no effective surface outflow) or an open system (short residence time, inflow and outflow). Strong co-variance ($r \geq 0.7$) between $\delta^{13}C$ and $\delta^{18}O$ values has been shown to be indicative of closed lake systems with relatively long residence times (Leng et al., 2005; Li and Ku, 1997; Talbot, 1990). The C and O stable isotope values obtained from this study also show a strong covariance (all molluscan data: $r = 0.66$; sphaeriids: $r = 0.76$) in the $\delta^{13}C$ and $\delta^{18}O$ values, suggesting that, for at least a short period of time, Lake Florissant was a closed system. This is supported by the geologic history, in that the lake was only formed after the damming of a paleo-river valley (Evanoff et al., 2001). As the basin filled with water, deposition and geochemical values would reflect a closed lake system, until either the dam was broken, or until water levels over-filled basin constraints and the lake would switch to an open system. While closed, evaporation would have the largest affect on the $\delta^{18}O$ values (Talbot, 1990; Leng et al., 2005), substantially depleting the lake water in ^{16}O, creating more positive values in $\delta^{18}O$.

Carbon. Carbon isotopes are affected by changes in productivity -- the amount of biomass living and decomposing within an environment (Leng et al., 2005). Photosynthetic plants and algae such as diatoms preferentially utilize ^{12}C to make organic matter, depleting the total dissolved organic carbon (TDIC) in an environment of ^{12}C, and producing more positive $\delta^{13}C$ values. O'Brien et al. (2002; 2008) identified diatom fossils within the lacustrine shales, which they interpreted to be part of bacterial and diatom seasonal blooms during warmer spring-early summer months, when increased run-off from seasonal rainfall augmented silica input into

the lake, which in turn supported a greater population of silica-secreting phytoplankton. Phytoplankton on average will deplete the TDIC $\delta^{13}C$ values of an environment by 20 ‰, typical values ranging -47 to -26 ‰ (Leng et al., 2005) (Fig. 11). C3 plants, present in the surrounding area, would also have an impact on the TDIC within a freshwater environment, either through removal of ^{12}C or enrichment through decomposition of organic material. C3 plants present at Florissant would include macrophytes (aquatic plants), $\delta^{13}C$ values -50 to -11 ‰, and terrestrial flora, $\delta^{13}C$ values -32 to -20 ‰ (Fig. 11). During colder months and non-phytoplankton blooming seasons, the expected values for freshwater $\delta^{13}C$ would be more enriched in ^{12}C, creating more negative delta values.

The enriched $\delta^{13}C$ values from the Florissant mollusk shells, 0 ‰ to -5.5 ‰ compared to potential sources of TDIC for the lake (Fig. 11), likely indicates that the shells were precipitated during times of high productivity in the lake system. This idea agrees well with our understanding of modern Sphaeriidae lifestyles, where shells of sphaeriids are predominantly precipitated during the warmer late spring-through-summer-early fall seasons. Single season precipitation of the Sphaeriidae biogenic carbonates accounts for the range observed within the sphaeriid intra-shell analyses, and potentially for the general range within the $\delta^{13}C$ values at any given value of $\delta^{18}O$. Especially if there was a diatom bloom during a sphaeriids season of growth, for example, this would shift the $\delta^{13}C$ value within a short span of time, where the shell may record $\delta^{13}C$ values reflective of the lake water before, during and after these phytoplankton bloom events.

The slightly offset, lower $\delta^{13}C$ values observed in the lymnaeids can be accounted for by understanding that they filled a different ecological niche than the sphaeriids or planorbids, and consumed food, e.g., organic detritus, algae, diatoms, macrophytes etc., more enriched in ^{12}C. This may also explain the one isotopic outlier we have in our data, from a single specimen that was identified as a *Lymnaea (Stagnicola) scudderi* (Fig. 8). For example(s), either the lymnaeid could have been captured from another water body by the lahar that created the caprock conglomerate unit as it moved over the landscape. Or in the case misidentification, the specimen could be a terrestrial snail that was caught up similarly. In either instance the shell stable isotopes would reflect a different primary signature. Terrestrial snails have previously been described from Florissant by Cockerell, 1906.

The $\delta^{13}C$ values from the biogenic samples are toward the heavy end of the spectrum for lacustrine carbonates (Sharp, 2007), consistent with a highly productive lake with relatively low organic decomposition. The excellent preservation of non-lacustrine fossil fauna and flora, in particular of organic insects and leaves, is indicative of a stratified lake with anoxic bottom waters, also consistent with high productivity.

Oxygen. Primary oxygen isotope signatures within a terrestrial water source can be influenced by a broad range of variables, including latitude of the continental mass, altitude, and 'continentality' (how "interior" a catchment basin is within a continent, relative to its coastline) (Albarède, 2011). Evaporation can shift the signal towards more positive values up to about +10 ‰, preferentially removing ^{16}O from soils and terrestrial bodies of water making the signal more

enriched in ^{18}O (Chamberlain et al., 2012). While the processes that affect the $\delta^{18}O$ signal of a water source are thermally regulated, temperature is often considered to be a secondary effect because a large temperature shift is needed to change $\delta^{18}O$ values by more than just a few permil (Chamberlain et al., 2012). Latitude probably also had only a small effect on the signal seen in this study, because it only decreases the $\delta^{18}O$ value of precipitated waters by 0.002 ‰ per km northward progression of water vapor (Craig, 1961). With the Florissant Fossil Beds <10° latitudinally away from its assumed water vapor sources--the Pacific Ocean or Gulf of Mexico, the influence on the $\delta^{18}O$ values by latitude would be insignificant.

Because of the large effect that evaporation can have on a freshwater, closed water system, up to about +10 ‰ (Chamberlain et al., 2012),it is likely one of the primary influences on our oxygen isotopic signature. Evaporation over a season could easily account for the range observed in our $\delta^{18}O$ values, +2 ‰ to -5 ‰. If our values don't represent evaporation, and are not from a closed system, an additional hypothesis is that they could reflect input into the system of a heavier oxygen isotopic component. This is a possibility due to the relative proximity, ~25 km, of the 39-Mile Volcanic Complex whence most of the lithological components within the lake system originated. Interaction of hydrothermal fluids and atmospheric CO_2 (Fig. 11) sourced from this volcanic complex, near the watershed of the Florissant Lake, could affect both the oxygen and carbon stable isotope inputs into the system, though more work would be needed to confirm this hypothesis.

The stable isotope values for the fibrous calcite data, (Fig. 8) are considered to represent the signal of later pore fluids, which precipitated calcium carbonate. These pore waters had a different signature than those recorded by contemporary precipitation in lake Florissant. Possible fluid sources include: 1) over-pressurized fluids from overburdened sediments (as in Cobbold et al., 2013) or 2) hydrothermal fluids sourced from the nearby volcanic field (as in Fig. 11). These O and C isotopes would have been affected by processes dissimilar to those found within a normal lake, e.g., higher temperatures, interaction with buried carbon sources, and higher pressure of the source.

Conclusions

Overall, the late Eocene Florissant Lake had a healthy molluscan fauna living nearshore, within the epilimnion and associated benthic sediments. The lymnaeid and planorbid organisms would have grazed upon bacteria, algae and diatoms present within the lake; organic detritus and macrophytes; and in the case of lymnaeids, small animal fragments and vascular water plants. The sphaeriids, interstitial-filter feeders of benthic sediments and associated waters, would have subsisted on interstitial organic debris and bacteria. The high-molluscan shell concentration in irregular masses of organic detritus, thought to be preserved feces (coprolites) or boluses (regurgatilites) of predators, is an indicator of higher level of the trophic structure within the lake. A few fish and shorebirds have been described from the Florissant Formation and are thought to be the most likely sources of this organic material.

Outcomes from the C and O stable isotope analyses of the molluscan biogenic calcites from the caprock conglomerate unit of the Florissant Formation have been useful in providing a snapshot of quantitative information about primary hydrological sources of a freshwater system. The strong covariance in the $\delta^{18}O$ and $\delta^{13}C$ values is consistent with a closed lake system, with a long-residence time and with no effective outflow, at least during the seasons of growth for the mollusks sampled. Evaporation would have been the primary source of change for the $\delta^{18}O$ values, explaining the values and the range in the data observed. $\delta^{13}C$ values observed within Sphaeriidae, would also be controlled by seasonal effects, primarily by bacterial and diatom blooms during the warm summer months, when the sphaeriids would have been preferentially precipitating their shells. Where the values observed within the lymnaeid and planorbid specimens are more indicative of the variation of carbon within the environment over a sub-annual to annual time span influenced by both high and low productivity within the system.

The geologically instantaneous nature of the caprock conglomerate unit, and the short growth season of the sampled mollusks, gives us a snapshot of the ecology and geochemistry of a freshwater lake system that existed just before the Eocene/Oligocene boundary. With the additional taxonomic and taphonomic analyses completed on other molluscan specimens from two middle shale unit collection localities, we know that active populations of mollusks were present throughout the duration of the lake, with *Gyraulus florissantensis* comprising a large part of the population. By analyzing the stable isotopes, taxonomy and taphonomy of freshwater mollusks, a better understanding of this unique freshwater system has been reached, and has filled a gap in our knowledge of the Eocene Florissant Lake.

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TABLE 1. Climatic and paleoelevation estimates based on paleoflora of the Florissant Formation (modified and updated from Leopold and Clay-Poole, 2001; Meyer, 2001)

Source reference	*Mean Annual Temperature (MAT °C)*	*MAT Technique**	*Paleoelevation (m) based on lapse rates or paleoenthalpy (Wolfe et al., 1998; Meyer, 2001)*
MacGinitie (1953)	≥18	NLR	305-915
Gregory and Chase (1992)	10.7 ± 1.5 to 11.6 ± 1.5	MR	2300 ± 400, 3200 ± 800
Wolfe (1993)	12 to 12.5	CLAMP	--
Wolfe (1994)	10.8	CLAMP	4133 and 2255
Gregory (1994a)	10.7 ± 1.5	MR	2300 ± 370, 3300 ± 750
Gregory and McIntosh (1996)	12.8	MR	3100 ± 800, 1900 ± 500, 2900 ± 700
Wolfe et al. (1998)	11.8	CLAMP	3800
Leopold and Clay-Poole (2001)	17.5	NLR	--
Boyle et al. (2008)	15.6 ± 2.5, 14.7 ± 2.2	higher taxa, modern forests	--

*NLR = Nearest Living Relative; Asia Clim = Asiatic climate nomogram of Wolfe (1979); CLAMP = Climate-Leaf Analysis Multivariate Program of Wolfe (1993); MR = Multiple Regression technique of Gregory and Chase (1992)

Table 2. Summary of stable-isotope analyses (see Table S3)

		d13C vs VPDB (per mil)		d18O* vs VPDB (per mil)	
Sample #	Taxonomy	Sample Average	S.D.	Sample Average	S.D.
P16-1	sphaeriid	-2.357	0.6354	-1.3392	1.0337
P16-2	sphaeriid	-1.7385	1.4973	-1.3624	0.6822
P16-3	*Sphaerium florissantense*	-1.1026	0.0904	0.3962	0.0711
P16-4	sphaeriid	-4.0061	0.2282	-2.4825	0.2658
P16-5	sphaeriid	-1.9999	0.7944	-1.7443	1.1187
P16-6	sphaeriid	-3.693	0.3872	-2.7034	0.3294
P16-7	sphaeriid	-3.1174	0.2853	-3.2362	0.0658
P16-8	sphaeriid	-1.7657	0.5219	-3.0176	0.51
P16-9	sphaeriid	-3.3986	0.2277	-3.3969	1.0877
P16-10	sphaeriid	-2.3309	0.1066	-4.7923	3.1084
P16-11	sphaeriid	-0.1474	0.1969	1.0546	0.2881
P16-12	sphaeriid	-1.9278	0.0902	-2.8783	0.7351
P16-13	*Sphaerium (Musculium)* sp. 1	-0.059	0.3547	0.9028	0.7057
P16-14	*Sphaerium florissantense*	-0.9472	0.1295	-0.2946	0.415
P16-15	sphaeriid	-0.8871	0.0449	-0.454	0.7966
P16-16	*Sphaerium florissantense*	-0.4662	0.3623	1.6808	0.2029
P16-17	sphaeriid	-1.5692	0.0906	-1.503	0.3293
P16-18	sphaeriid	-2.8758	0.5728	-1.6193	0.46
P16-19	*Sphaerium florissantense*	-1.3509	0.113	0.4649	0.0826
P16-20	*Sphaerium* sp.1	-2.4563	not averaged	-1.7558	not averaged
P16-21	sphaeriid	-2.5323	1.0632	-2.6055	0.8825
P16-22	sphaeriid	-1.884	0.5669	-0.9989	0.4116
P16-23	*Sphaerium (Musculium)* sp. 1	-2.6456	0.5945	-2.3012	0.5683
P16-24	*Sphaerium florissantense*	-3.6559	0.3259	-3.2097	0.5122
P16-25	sphaeriid	-2.5665	0.301	-2.5834	0.6175
P16-26	*Sphaerium florissantense*	-3.1645	0.2876	-2.4767	0.5904
P16-27	sphaeriid	-2.7846	0.2059	-0.5893	0.0784
P16-28	*Sphaerium florissantense*	-2.3687	0.1185	-1.5777	0.2264
P16-29	sphaeriid	-1.6157	0.1959	-0.8163	0.1571
P16-30	sphaeriid	-5.3658	1.2246	-3.7153	1.341
P16-31	sphaeriid	-3.5591	0.1169	-3.4028	0.2204
P16-32	*Sphaerium florissantense*	-1.8458	not averaged	-0.2488	not averaged
P16-33	sphaeriid	-1.4032	0.1213	-0.4146	0.1633
P16-34	*Lymnaea (Stagnicola) scudderi*	-11.9311	0.0395	-9.7647	0.4808
P16-35	*Sphaerium florissantense*	-2.251	0.1259	-0.5495	0.253
P16-36	*Sphaerium florissantense*	-1.3592	0.0924	-1.6086	0.0405
P16-37	*Lymnaea (Stagnicola) scudderi*	-3.8286	0.1031	-1.4654	0.3497
P16-38	sphaeriid	-2.2408	0.1792	-0.3338	0.2522
P16-39	*Sphaerium florissantense*	-2.2198	0.0232	-1.1292	0.1423
P16-40	*Sphaerium (Musculium)* sp. 1	-1.0923	0.0699	-0.6755	0.3377
P16-41	*Lymnaea (Stagnicola) scudderi*	-3.6179	0.1484	-0.0998	0.2058
P16-42	*Lymnaea (Stagnicola) scudderi*	-3.8383	0.0199	-0.7802	0.0977
P16-43	*Lymnaea (Stagnicola) scudderi*	-2.8896	0.01	-2.5101	0.0265
P16-44	*Lymnaea (Stagnicola) scudderi*	-4.243	0.2228	-2.6382	0.0961
P16-45	*Lymnaea (Stagnicola) scudderi*	-2.4549	0.0426	-1.1868	0.0893
P16-46a	*Sphaerium (Musculium)* sp. 1	-3.2824	0.0228	-1.8622	0.0132
P16-46b	*Sphaerium (Musculium)* sp. 1	-2.3158	0.13	-1.1349	0.0873
P16-46c	*Sphaerium (Musculium)* sp. 1	-1.6177	0.0448	-1.6648	0.0153
P16-47b	sphaeriid	-0.4709	0.0448	0.6431	0.0028
P16-47c	sphaeriid	-1.0448	0.0064	1.087	0.025
P16-48	*Gyraulus* sp.	-1.6035	0.0767	-0.9094	0.1688
P16-FC1_ Bulk	Fibrous Calcite	5.1637	not averaged	-14.5048	not averaged
P16-FC2	Fibrous Calcite	4.1144	not averaged	-14.1583	not averaged
P16-FC3	Fibrous Calcite	4.8823	not averaged	-14.6289	not averaged
P16-FC4	Fibrous Calcite	4.5486	not averaged	-14.5009	not averaged
	Ave Fibrous Calcite	4.6773	0.4517	-14.4482	0.2022

*with respect to sample mineralogy

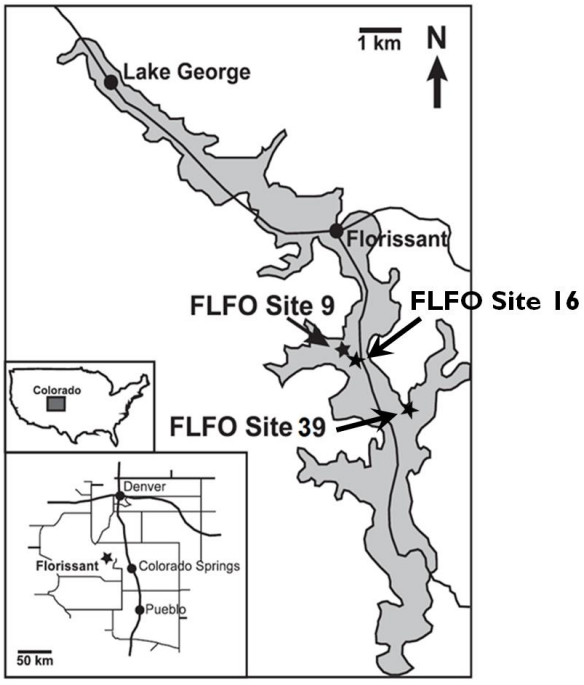


Figure 1. Collection site locations and geographic extent of the Florissant Formation, modified from Henning et al., 2012.

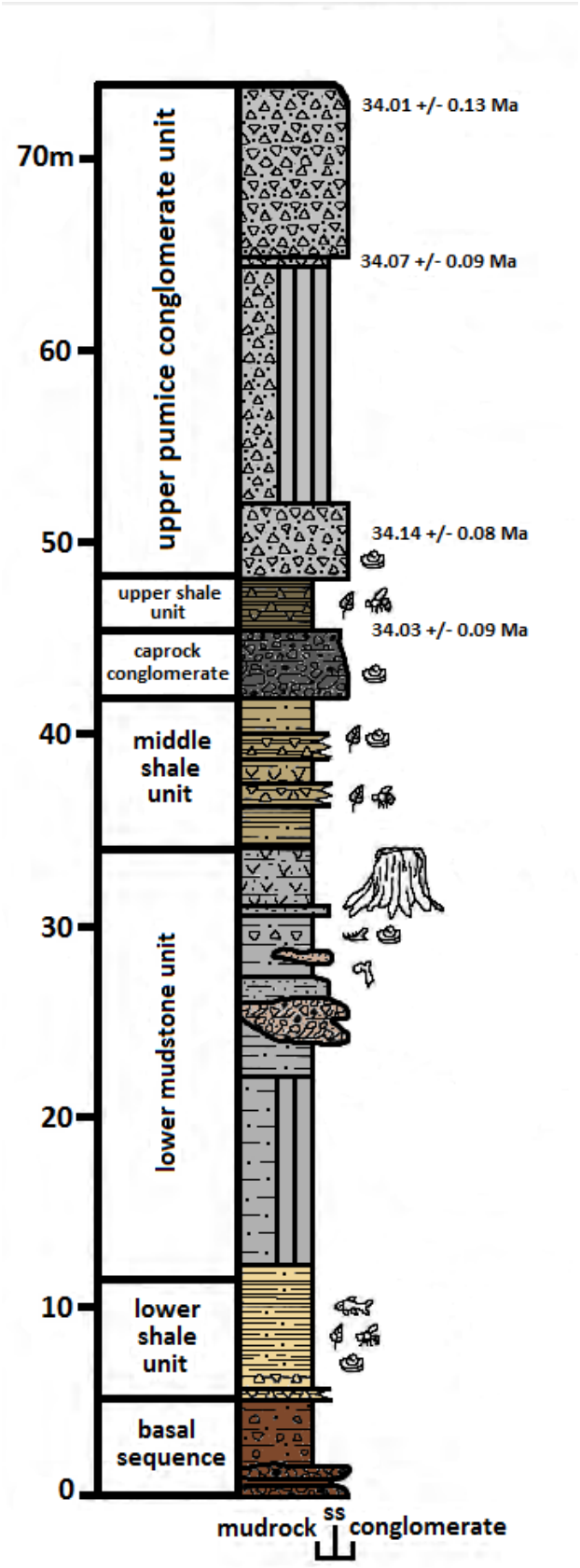
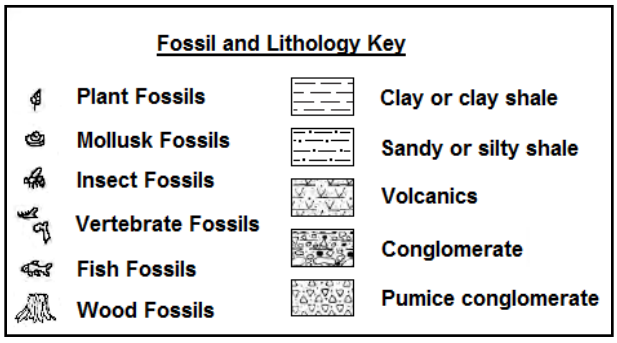


Figure 2: Detailed stratigraphic column of the Florissant Formation; related fossils and single-crystal sanidine $^{40}Ar/^{39}Ar$ dates noted; adapted from Evanoff et al. (2001). Collection sites denoted at right.



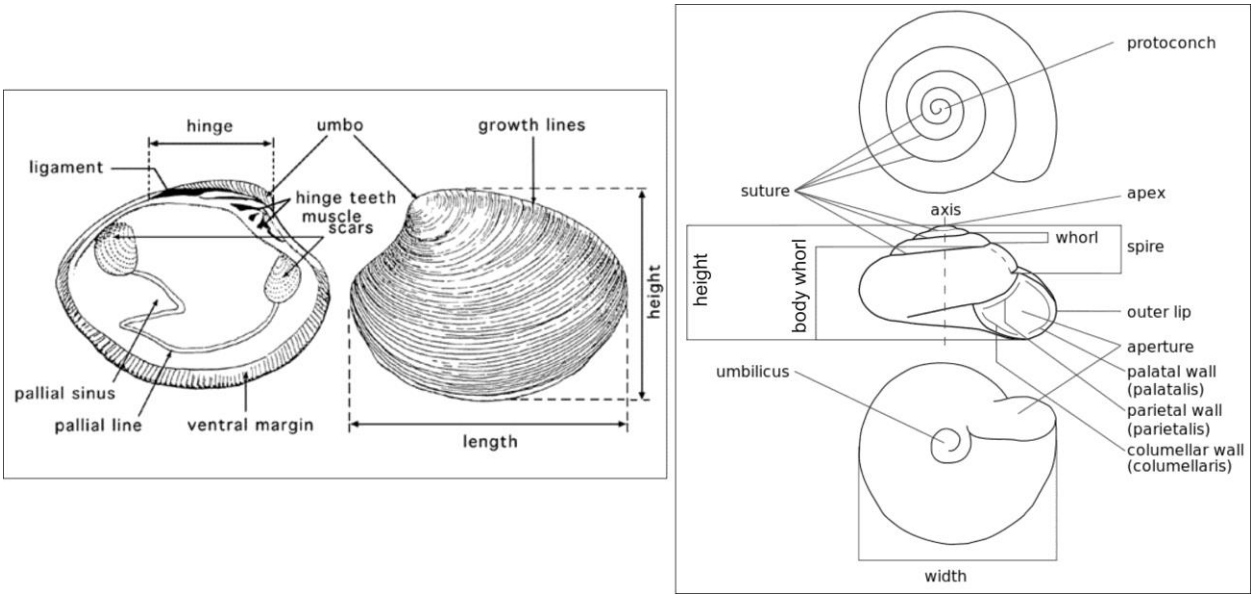


Figure 3: Morphological terminology for bivalves (left) and gastropods (right).

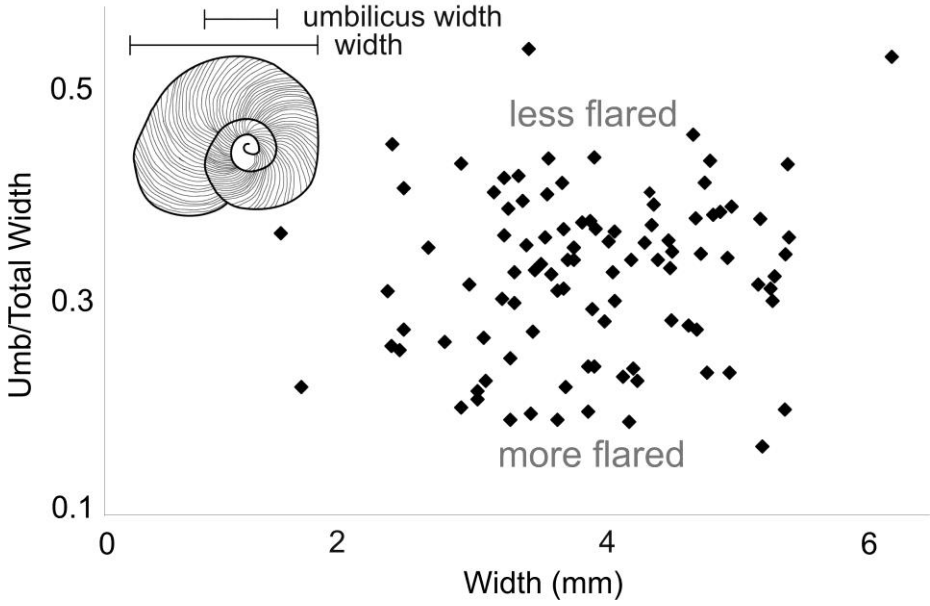


Figure 4: Ratio of umbilicus width/total width (measures degree of flaring) plotted against shell width (measures size) for 111 *Gyraulus sp.* individuals. Lower ratios (small umbilicus relative to total width) are more flared; higher ratios are less flared. The data show a continuous distribution both in ratios and in size.

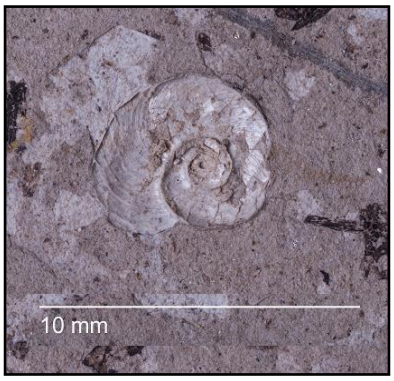


Figure 5a: *Gyraulus florissantensis* (Cockerell, 1906) UCM-35173, photo courtesy of UCM.

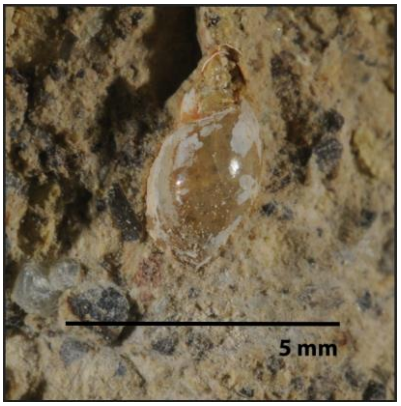


Figure 5b: 1)*Lymnaea (Stagnicola) scudderi* Cockerell, 1906 2)UCM-4625 counterpart (*Lymnaea florissantica* syntype, Cockerell, 1908a), photo courtesy of UCM.

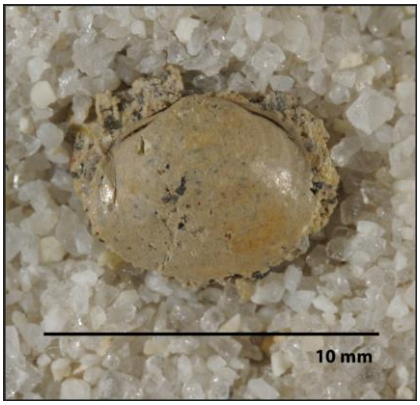


Figure 5c: *Sphaerium florissantense* Cockerell, 1906.

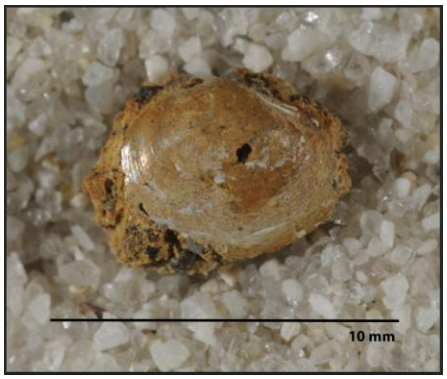


Figure 5d: *Sphaerium* species 1.

Figure 5e: *Sphaerium (Musculium)* species 1.

Figure 5f: Part and counter-part of a concentration of lymnaeid shells in organic detritus, regurgatilites.

	completeness			
	>95% (n)	95-66% (n)	66-33% (n)	***>66% (%)***
planorbids	72	69	4	***97***
lymnaeids	8	13	3	***88***
sphaeriids	5	3	2	***67***

	cracking			
	much (n)	some (n)	little (n)	***much (%)***
planorbids	38	72	37	***26***
lymnaeids	20	1	3	***83***
sphaeriids	4	4	2	***40***

	detail			
	fine (n)	some (n)	little (n)	**some or fine (%)**
planorbids	22	79	47	**69**
lymnaeids	5	13	6	**75**
sphaeriids	3	5	4	**67**

Figure 6: Simplified summary of taphonomic observations of specimens from the middle shale unit (see Table S1a), generalized to the familial level. Observations are estimates or qualitative; the total number of specimens counted varies due to some disqualified individual cases. In the case of "cracking," "much" was described as throughgoing cracks (across whole shell), "some" as deep (but not throughgoing) cracks, and "little" as edge cracks or no cracks.

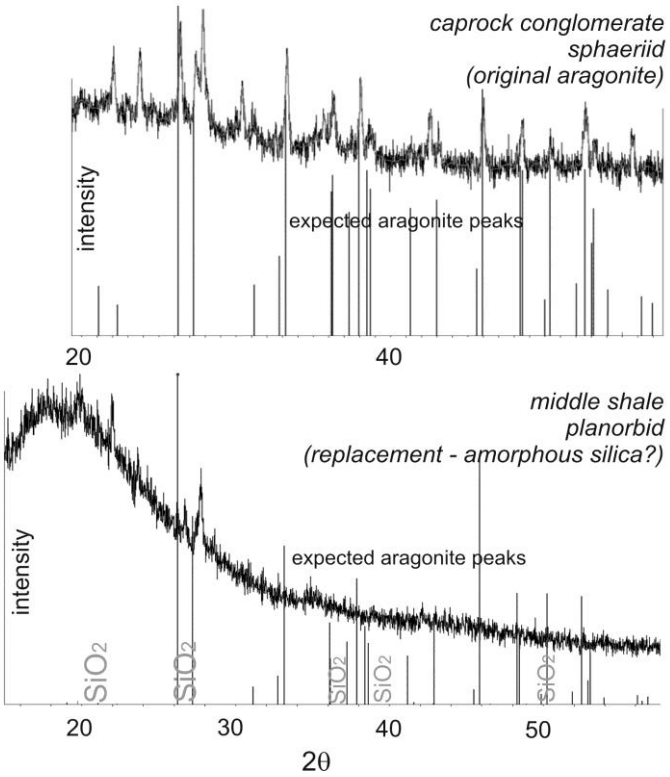


Figure 7: X-ray diffraction spectra for representative specimens from the caprock conglomerate (above, sample P16-XRD3) and middle shale (below, sample P39-XRD2) (see Table S2 and Figure Sx for other x-ray results). Expected location of aragonite peaks are shown on both; on the lower figure, approximate location of major silica peaks are also shown.

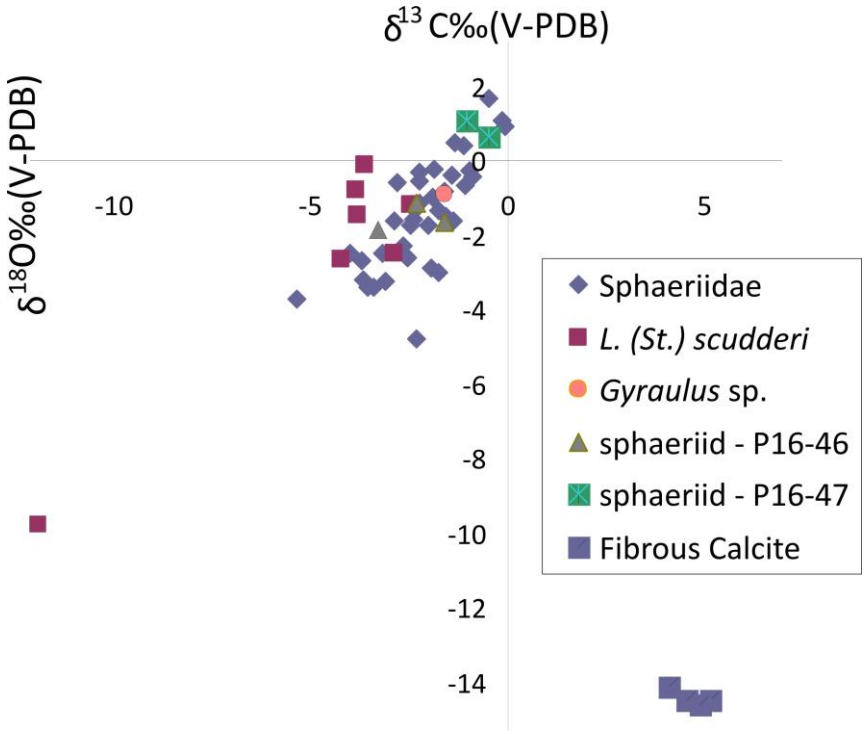


Figure 8: Average carbon and oxygen isotope values (Table 2) for molluscan samples analyzed on a Finnigan Delta Plus mass spectrometer with attached Kiel III Carbonate Device, against **V**ienna - **P**ee **D**ee **B**elemnite (V-PDB) standard. See Figure 9 for individual sphaeriid runs. See Table S3 for full-run data.

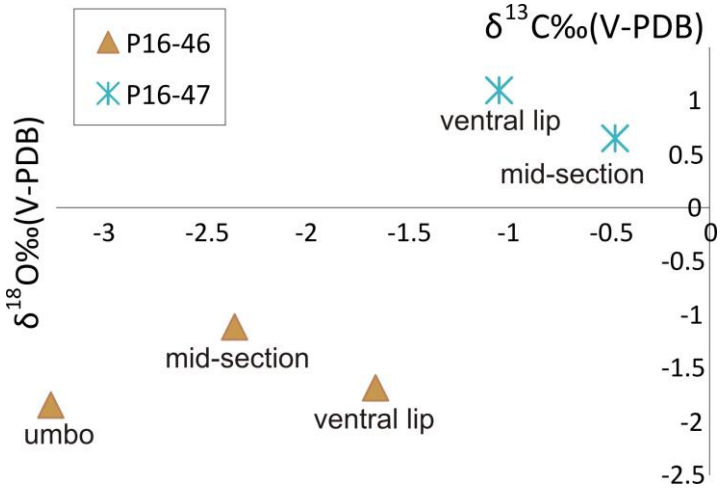


Figure 9: Carbon and oxygen values for sphaeriid samples that were analyzed for single shell variation. Samples were analyzed on a Finnigan Delta Plus mass spectrometer with attached Kiel III Carbonate Device, against **V**ienna - **P**ee **D**ee **B**elemnite (V-PDB) standard.

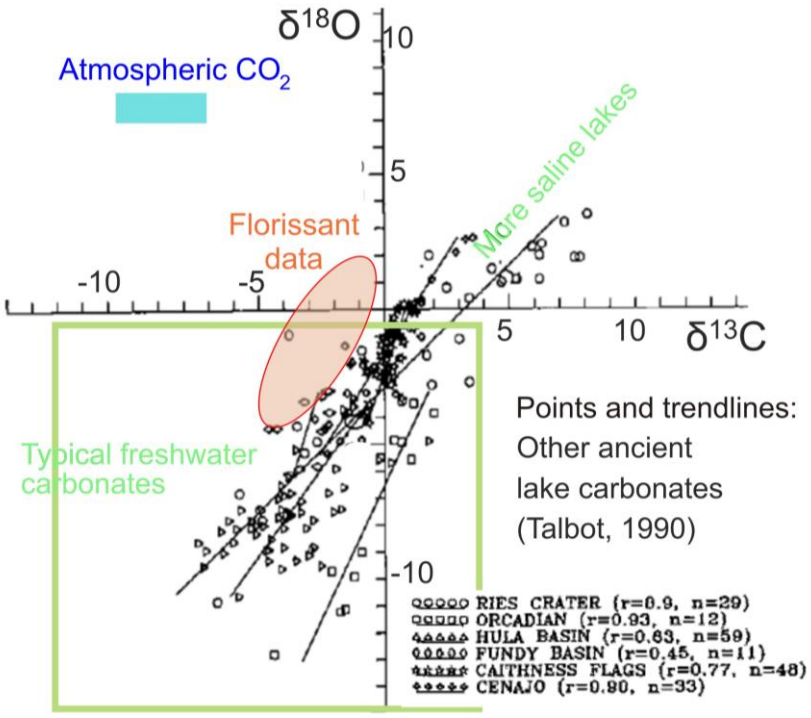


Figure 10: Comparison of Florissant isotope data (from aragonite from the caprock conglomerate; see previous figure) to plots from other ancient lake carbonates from closed and open lakes ($r \geq 0.7$) (Talbot, 1990); the field for typical freshwater carbonates (Sharp, 2007); and a trendline for more saline lakes (Leng et al., 2005).

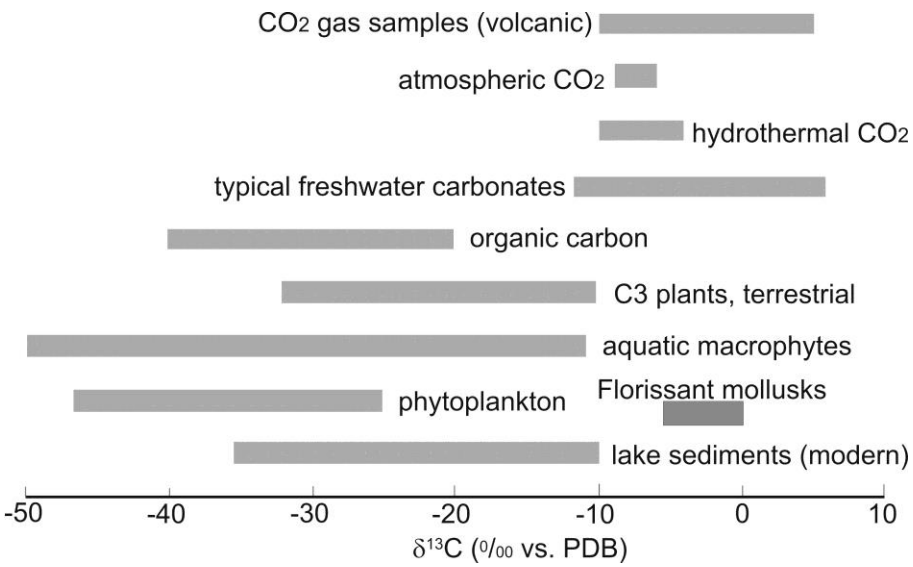


Figure 11: Range of δ13C in sources related to freshwater lakes, compared to the Florissant mollusks. Adapted from data in Leng et al., 2005 (after Leng and Marshall, 2004); Sharp, 2007.