Thomas Hillard, Samantha Hopkins, Dana Reuter, Scott Blumenthal, Holley Flora

Stable Oxygen Isotope Signature of Extant and Extinct Aplodontiids and Evidence for Water Use.

Abstract:

Aplodontia rufa has a unique kidney anatomy that is inefficient at concentrating urine, and as a result the water intake for the species is high and its range is limited to the high precipitation regions of the Pacific coast. This anatomy is often considered to be a primitive condition for rodents, however we hypothesize it may be a derived character because of the range of extinct members in the clade. Stable oxygen isotope measurements from tooth enamel were used to investigate the water usage in the fossil species *Liodontia alexandrae* alongside the modern *Aplodontia rufa*. Previous studies have shown that animals that are dependent on drinking water and spend more time around water have a lower stable oxygen isotope ratio in their tooth enamel. So, we compared the oxygen isotope ratios of the aplodontines to measurements taken from lagomophs from the same localities that are better at conserving water, including the modern *Sylvilagus bachmani* and the extinct *Oreolagus wallacei*. Our data shows a significant difference between *Aplodontia rufa* and *Sylvilagus bachmani*, but no difference between *Liodontia alexandrae* and *Oreolagus wallacei*. This suggests that *Liodontia* does not have the same renal anatomy as *A. rufa*, and is evidence that the renal anatomy of *A. rufa* is a derived trait in the aplodontiine group.

Introduction:

The mountain beaver, *Aplodontia rufa*, is the only extant member of the broader group Aplodontoidea that includes a great diversity of now extinct rodents including the Mylagaulidae (figrue 1), with the group reaching a peak diversity in the Oligocene and the Miocene, spreading throughout North America and Eurasia with at least 90 species discovered through the evolutionary history of the group (Hopkins, 2008). The modern *Aplodontia rufa* now only exists in a subsection of the pacific northwest, ranging from British Columbia to parts of the Northern California coast and the Sierra Nevada mountain range. *Aplodontia rufa* is restricted to relatively wet habitats and often lives at high elevation (figure 3). The habitat of modern *Aplodontia rufa* is very contrasted from the wide diversity of extinct Aplodontioids as they existed in the much drier habitats of eastern North America and an extended range beyond their modern counterparts (Hopkins 2007). The decline of the group began with the loss of small, more basal aplodontiids in the early Miocene followed by the loss of mylagaulids and most aplodontiines by the end of the Miocene, and the group then disappears from the fossil record during the Pliocene and Pleistocene until the modern day where there is only *Aplodontia rufa* (Hopkins, 2007; figure 1). This decline seems to be correlated with the expansion of C4 grasslands (Hopkins, 2007) that occurred at the end of the Miocene, coupled with the expanded aridity that likely drove this shift (Hynek et. al., 2012).

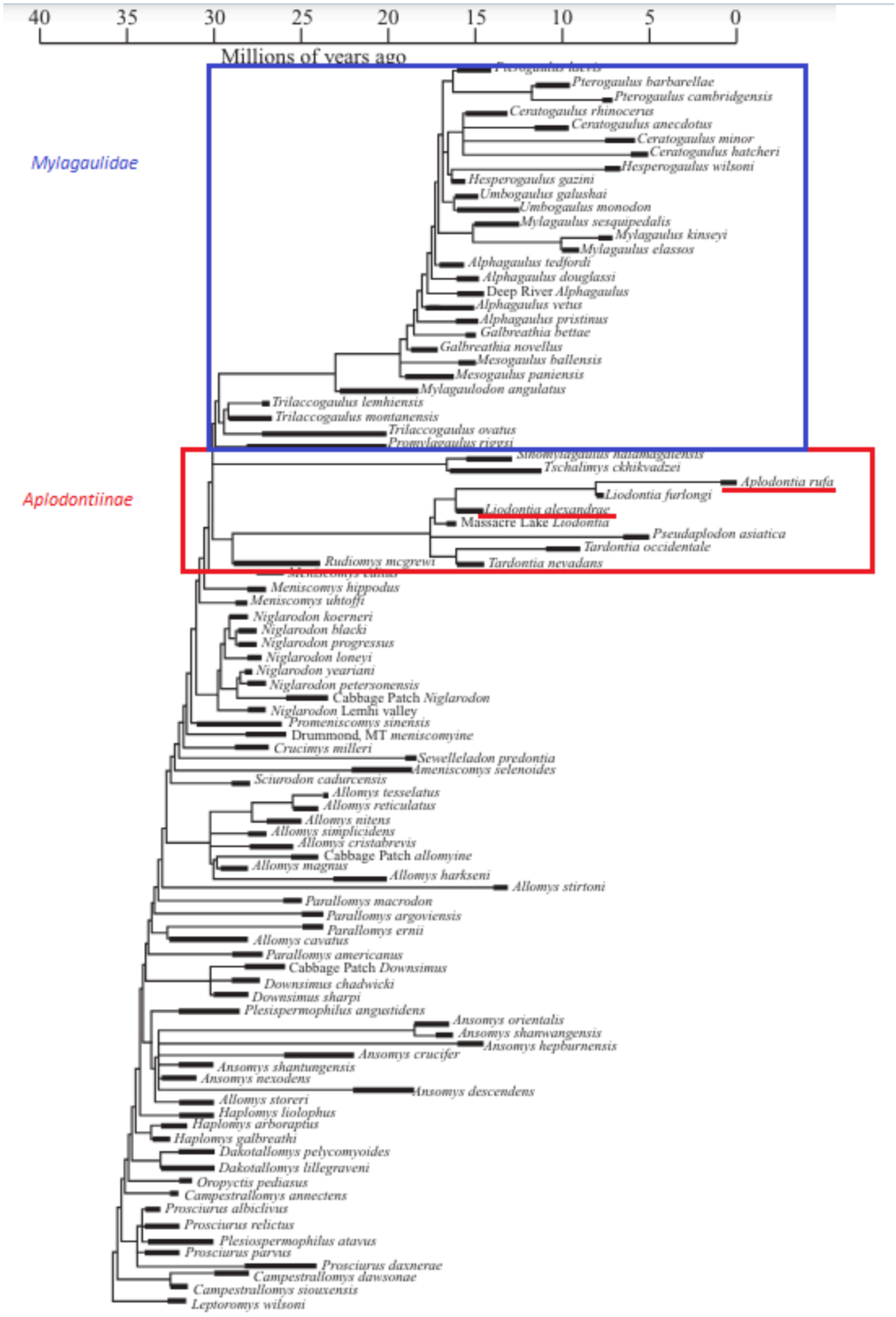
40
35
30
25
20
15
10
5
0
Millions of years ago
Mylagaulidae
Aplodontiinae
Pterogaulus barbarellae
Pterogaulus cambridgensis
Ceratogaulus rhinocerus
Ceratogaulus anecdotus
Ceratogaulus minor
Ceratogaulus hatcheri
Hesperogaulus wilsoni
Hesperogaulus gazini
Umbogaulus galushai
Umbogaulus monodon
Mylagaulus sesquipedalis
Mylagaulus kinseyi
Mylagaulus elassos
Alphagaulus tedfordi
Alphagaulus douglassi
Deep River Alphagaulus
Alphagaulus vetus
Alphagaulus pristinus
Galbreathia bettae
Galbreathia novellus
Mesogaulus ballensis
Mesogaulus paniensis
Mylagaulodon angulatus
Trilaccogaulus lemhiensis
Trilaccogaulus montanensis
Trilaccogaulus ovatus
Tschalimys ckhikvadzei
Aplodontia rufa
Liodontia furlongi
Liodontia alexandrae
Massacre Lake Liodontia
Pseudaplodon asiatica
Tardontia occidentale
Rudiomys mcgrewi
Tardontia nevadans
Meniscomys hippodus
Meniscomys uhtoffi
Niglarodon koerneri
Niglarodon blacki
Niglarodon progressus
Niglarodon loneyi
Niglarodon yeariani
Niglarodon petersonensis
Cabbage Patch Niglarodon
Niglarodon Lemhi valley
Promeniscomys sinensis
Drummond, MT meniscomyine
Crucimys milleri
Sewelleladon predontia
Ameniscomys selenoides
Sciurodon cadurcensis
Allomys tesselatus
Allomys reticulatus
Allomys nitens
Allomys simplicidens
Allomys cristabrevis
Cabbage Patch allomyine
Allomys magnus
Allomys harkseni
Allomys stirtoni
Parallomys macrodon
Parallomys argoviensis
Parallomys ernii
Allomys cavatus
Parallomys americanus
Cabbage Patch Downsimus
Downsimus chadwicki
Downsimus sharpi
Plesispermophilus angustidens
Ansomys orientalis
Ansomys shanwangensis
Ansomys hepburnensis
Ansomys crucifer
Ansomys shantungensis
Ansomys nexodens
Ansomys descendens
Allomys storeri
Haplomys liolophus
Haplomys arboraptias
Haplomys galbreathi
Dakotallomys pelycomyoides
Dakotallomys lillegraveni
Oropyctis pediasus
Campestrallomys annectens
Prosciurus albiclivus
Prosciurus relictus
Plesiospermophilus atavus
Prosciurus parvus
Prosciurus daxnerae
Campestrallomys dawsonae
Campestrallomys siouxensis
Leptoromys wilsoni

Figure 1. Phylogeny of aplodontid species through time with bolded lines representing known stratigraphic range of the taxa and thin lines showing inferred ranges. Taxa used for this study are underlined in red (*Aplodontia rufa* and *Liodontia alexandrae*). The aplodontiinae clade is delineated in the red box and the mylagaulidae clade is delineated in the blue box. From Hopkins, 2008.

This restriction in the range of modern *Aplodontia* to wet climate regimes in the pacific northwest is attributed to the reduced kidney structure of *Aplodontia* that limits the ability for them to concentrate their urine (Nungesser & Pfeiffer, 1965). Their kidneys contain a very reduced inner section with a small amount of medullary substance and they have simple vasa recta with no loops (Carraway & Verts, 1993; Pfeiffer et. al., 1960) (Figure 2). Furthermore, the majority of nephrons present in the kidney's cortex are cortical nephrons with very short loops of Henle that do not enter the medulla, and the loops of Henle have no thin section that assists with ion transport to concentrate urine. The majority of the other nephrons have short loops that only barely enter the medulla with a reduced thin section (Pefiffer et. al., 1960). The loops of Henle are important in the process of concentrating the urine and reabsorbing water back into the bloodstream, especially when they enter the medullary region. Having a longer loop of Henle with a thin section is associated with greater water reabsorption. Thus, it is believed that animals living in arid climates tend to have a longer loop of Henle (Beuchat, 1996) because it increases the efficiency of reabsorbing water back into the bloodstream, conserving the limited amount of water available to them. It has also been found that mammals living in arid climates also tend to have thicker medullary sections in their kidneys (Al-Kahtani et. al., 2004) which also pertains to greater water reabsorption from the urine as the long loops of Henle pass through the medullary section to reabsorb water.

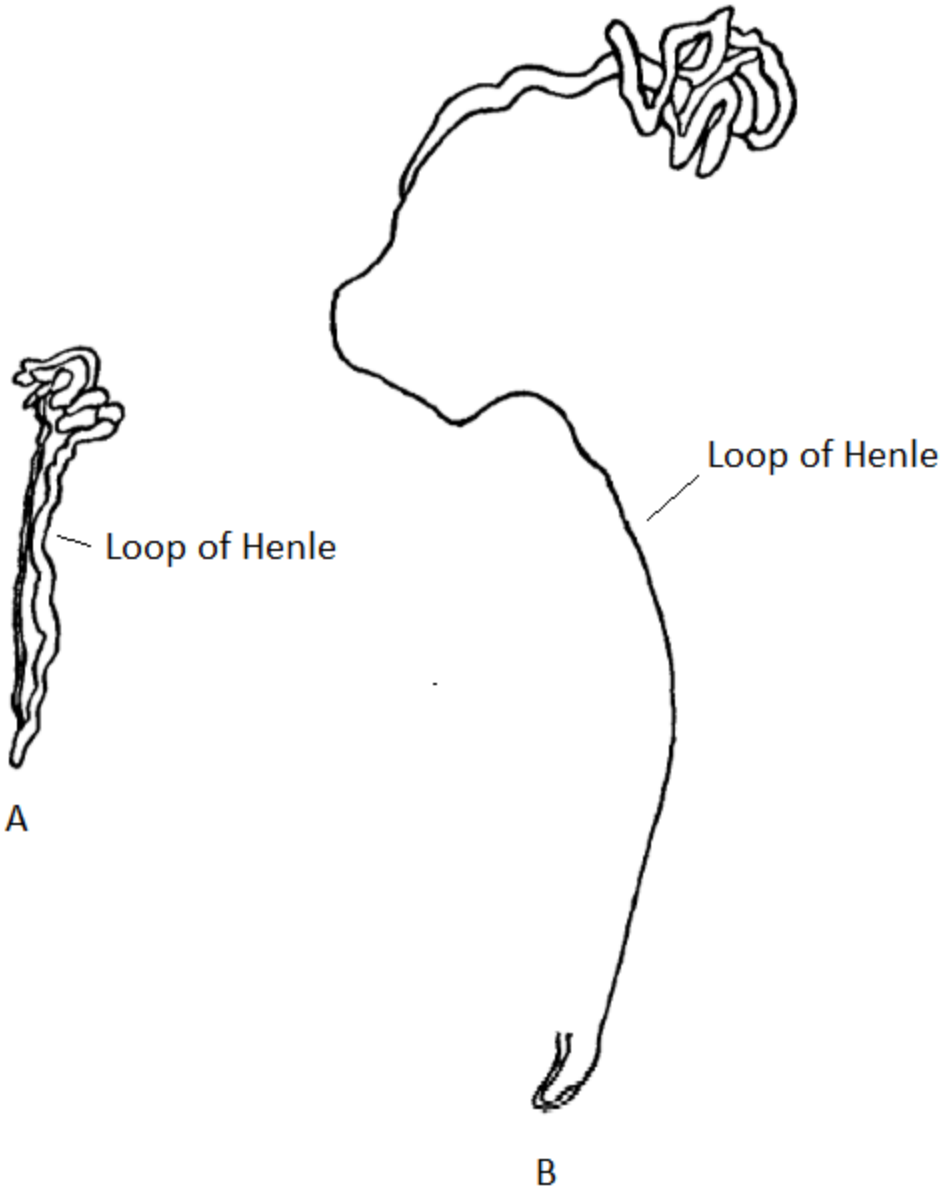

Figure 2. Example of anatomy of nephron of *Aplodontia rufa*. A represents a cortical nephron with a very short loop of henle, which represents the majority of the nephrons in *A. rufa*. B represents a nephron with a long loop of henle for comparison. These are camera lucida drawings, taken from Pfeiffer et. al., 1960.

Aplodontia lacks these anatomical adaptations in their kidneys and instead have short loops of Henle with little medullary substance and few thin sections (Carraway & Verts, 1993), likely causing an inability to produce hypertonic, or concentrated, urine

(Pefiffer et. al., 1960) and are thus less efficient with water and need to consume more of it. The inefficient water concentration in *A. rufa* has been demonstrated experimentally (Dicker & Eggleton, 1964; Schmidt-Nielson & Pfeiffer, 1970) showing that the concentration of urine is low and that the water drinking requirements are high. This has been attributed to the high water requirements of *Aplodontia* (Nungesser & Pfeiffer, 1965), evidenced by how often they drink in captivity (Crocker et. al., 2007). The inefficient kidney anatomy of *Aplodontia* could explain why its range occurs in the wet climates found in parts of the pacific northwest and California (Carraway & Verts, 1993).

As a consequence of how reduced the kidneys are, many consider the renal anatomy of *Aplodontia* to be a primitive trait of rodents (Nungesser & Pfeiffer, 1965). Modern phylogenetic analyses place the Aplodontioidea within the most basal rodent clade alongside squirrels (Fabre et. al., 2012), and many consider *Aplodontia* to be a primitive rodent because it lacks the modified jaw muscles that other rodents have, as it has reduced forms of many muscles in the head and neck, specifically within the zygomasseter muscle system that is more developed in other rodents (Carraway & Verts, 1993). However, the fossil record of aplodontioid rodents suggests that the renal anatomy may not be primitive in rodents. The range of extinct aplodontioids stretches much farther east than the range of modern *Aplodontia*, in climates that have been reconstructed as much more dry than the Pacific Northwest is today. It is instead more likely that the inefficient kidney of *Aplodontia* may be a derived trait, potentially to deal with a diet of mostly ferns that are toxic to other herbivores (Carraway and Verts, 1993), and that fossil members of the Aplodontioidea had kidneys that more resembled those

of other rodents (Hopkins, 2008). While it is impossible to actually examine the renal anatomy of extinct aplodontioids, it is possible to infer some aspects of the renal anatomy from stable oxygen isotopes of the tooth enamel of fossil Aplodontioids.

Stable oxygen isotopes have an abundance of applications throughout paleobiology, especially within the field of paleoecology. Evaporation, humidity, and amount of rainfall affect the oxygen isotopic composition of surface water across the landscape (Kohn et. al., 1996). Stable oxygen isotope measurements from soil carbonates and clays can give information on climate and precipitation changes (Kukla et. al., 2022) as well as changes in topography and biome regime (Kukla et. al., 2021). Stable oxygen isotope values from mammal tooth enamel can give information on what environment that specific animal was living in consequently. The oxygen isotope signature measured from tooth enamel is determined by the net oxygen flux through the body, and the factors that fractionate the oxygen isotopes. This includes oxygen in food and water taken into the body as well as the metabolic water and CO_2 produced from respiration (Luz et. al., 1984). The enamel of teeth is made of bioapatite which is highly resistant to wear and diagenesis and is thus in abundance in the fossil record (Longinelli, 1984). Many studies have shown that the stable isotopic composition of enamel can be used to infer the oxygen isotopic composition of the meteoric water that animal was drinking, which allows for paleoclimatic and environmental reconstructions (Drewicz & Kohn, 2013; Cerling et. al., 1997; Wang et. al., 2008). While ungulates are more commonly used, rodents can also be used to measure stable oxygen isotope ratios and have a faster lifespan and a smaller range that provides more local measurements of isotope ratios (Kimura et. al., 2013).

The enamel isotope values for oxygen should reflect the drinking water or food water consumed, with the relative amount of drinking water to food consumed causing variation between the measurements of different animals (Royer et. al., 2013; Hynek et.al., 2012). Previous studies have found that animals living in arid environments that get more water from food show a heavier oxygen isotope signature than animals that drink more water, because of the evaporative enrichment happening in plants (Jeffrey et. al., 2015). Hence, animals that rely strongly on drinking water to meet their water needs will show a relatively lower oxygen isotope value similar to that of the meteoric water. This has been shown to be true for large mammals that spend a lot of time in water like the hippopotamus (Clementz et. al., 2008). This difference between water-dependent and water-independent taxa can be traced in the enamel isotope values of many different kinds of herbivorous taxa, and the distinction between the two has been used as a proxy to measure aridity of an environment showing that it is a detectable difference (Levin et. al., 2006; Blumenthal et. al., 2017).

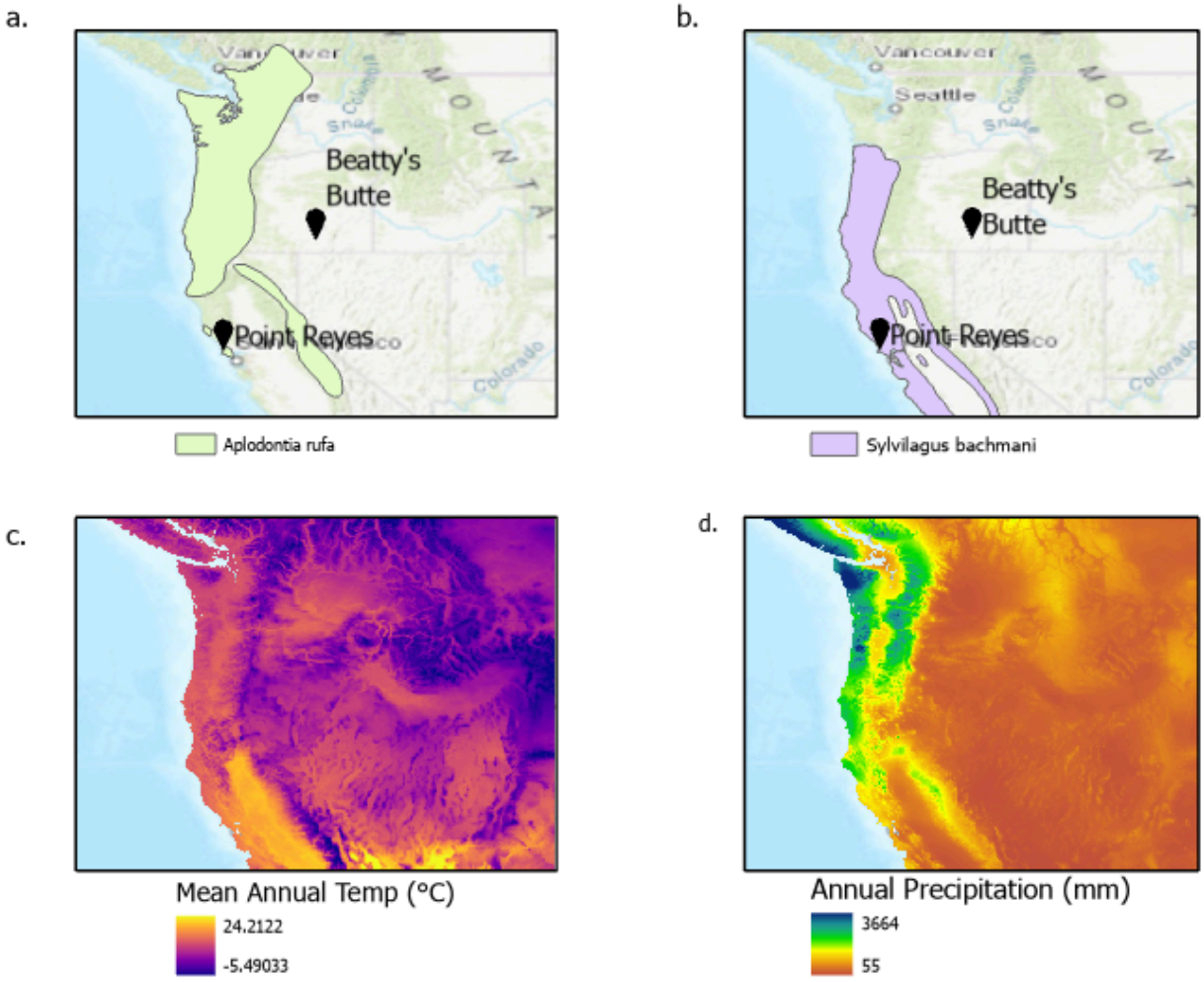

Figure 3. Geographic map of western North America showing the modern range for *Aplodontia rufa* (yellow, a.) and *Sylvilagus bachmani* (purple, b.). The locations where modern samples were collected in Point Reyes and the fossil samples in Beatty's Butte are also shown. The bottom maps show the mean annual temperature in degrees celsius (c.) and the annual precipitation in mm (d.) for this same region. Climate data was obtained from worldclim.org. Range distributions were obtained from the IUCN. ArcGIS pro was used to make the map.

In this study we will be comparing the isotope oxygen values from the enamel of modern *Aplodontia rufa* to the values of modern *Sylvilagus bachmani*, the brush rabbit. The fact that *Aplodontia* has high drinking needs because of the unique kidney structure suggests that the oxygen isotope signature may be lower than the comparison species. *Sylvilagus*, unlike *Aplodontia*, is able to concentrate its urine more, so it can get water it

needs from food; as a consequence, it drinks less water and has a broader geographic range (Chapman, 1974; Heisinger & Breitenbach, 1969) (figure 3). We hypothesize that because of the kidney structure of *Aplodontia* that causes them to drink more water, the oxygen isotope values for *Aplodontia* will be lower than *Sylvilagus*. This is a result of the isotopic difference in fractionation between drinking water and water obtained from plants, with *Sylvilagus* getting more water from plants. We can then apply this logic to fossil aplodontiines and compare them to fossil lagomorphs from a drier environment in eastern Oregon (figure 3), and this way we can see if the kidneys of fossil aplodontiines were similar to the modern *Aplodontia*. If the extinct aplodontiine is also offset from the lagomorph then we can assume it also has a similar kidney structure to modern *Aplodontia* because it needs to drink more water. Having a greater understanding of the past kidney structures of aplodontiines will expand our knowledge of rodent evolution and the evolutionary history of this rodent lineage.

Methods:

Location and Samples:

Fossil samples were collected from Beatty's Butte in southeast Oregon (figure 3). The butte itself is a volcanic cone and the fossils are found within various tuff and volcanic ash deposits around the flanks of the butte (Wallace, 1946). The assemblage contains animals from the Barstovian North American Land Mammal Age of the middle Miocene and includes both browsing and grazing ungulates, as well as a species of *Mylagaulus* (Wallace, 1946), which seems to suggest a mixed open and closed habitat. Specimens of *Liodontia alexandrae*, the only aplodontiine species from this site, were

used for stable isotope analysis to test their kidney function. *Oreolagus wallacei* is a lagomorph that was also sampled from the site to compare the values of the aplodontiine to an animal that has normal kidneys. Several tooth fragments from each species were taken from the University of California Museum of Paleontology to be measured for stable isotope analysis.

Modern samples of *A. rufa* and *S. bachmani* were both collected from the same locality in Point Reyes peninsula (figure 3) on the coast of California. Samples were collected between February and December with most collected between May and June. This is a wet area that receives a lot of rainfall due to its proximity to the ocean, making it an ideal habitat for mountain beavers (Evens, 2008). This area is home to a population of *Aplodontia rufa* known as the Point Reyes Mountain Beaver, *Aplodontia rufa phaea* (Evens, 2008). Specimens of this species were used for stable oxygen isotope analysis to look at the isotope signature of a known, unique kidney structure. *Sylvilagus bachmani* samples were used as a comparative lagomorph to compare to the *Aplodontia* with a signature from a normal kidney structure. These tooth fragment samples from both species were obtained from the Museum of Vertebrate Zoology for stable isotope analysis.

Stable Oxygen Isotopes:

Stable oxygen isotopes are used in paleoecology to understand the climate and precipitation patterns of ancient environments. This reflects the differential amount of evaporation and precipitation of water containing the stable oxygen isotopes, with water molecules containing heavier 18O isotopes being preferentially precipitated and the

water molecules containing lighter 16O isotopes being preferentially evaporated from the environment (Craig, 1961). One way in which this can be analyzed is through the tooth enamel of herbivores. Tooth enamel is mineralized in bioapatite and more resistant to erosion and alteration compared to other parts of an animal, leaving an abundance in the fossil record that can be analyzed (Kohn et. al., 1996). The isotopic signature from the enamel reflects environmental conditions because the isotopes recorded in enamel are obtained from the plants that the herbivores consume, and the plants get this oxygen from the environment. At each step the oxygen isotopes undergo fractionation between the environment and the plants and the herbivore. But these fractionations are relatively constant, so it is possible to extrapolate the environmental isotope values from the tooth enamel. Some studies have suggested that the fractionation could be dependent on other environmental variables like the temperature affecting the fractionation of oxygen within a plant (Xia & Yu, 2020).

Different information can also be determined from stable oxygen isotopes based on the species being sampled. Traditionally, larger ungulate herbivores have been used as their large size makes sampling easier and relatively less destructive. However, smaller herbivores like rodents can also be used, and these can reflect different information as rodents do not migrate as much and have a much more limited home range, allowing for analysis of a more local isotopic signature (Kimura et. al., 2013; Hynek et. al., 2012). Additionally, rodent teeth grow much faster than ungulate teeth, so a rodent tooth records a much more seasonal and short-term signature than an ungulate tooth (Royer et. al., 2013).

For the purposes of this study, we are comparing aplodontiid teeth to lagomorph teeth that are sampled in the same time and place, so the differences in the oxygen isotope signature between the aplodontiid and lagomorph would be due to different sources of water. Consuming plants confers an additional fractionation on the oxygen isotope than drinking water (Clementz et. al., 2008), so it would be expected that the isotopic signature of the oxygen would be different between these two. The inefficient kidney structure of *Aplodontia rufa* requires the animal to drink water (Nungesser & Pfeiffer, 1965), so the stable oxygen isotopic composition of *Aplodontia* tooth enamel will not be the same as that of *Sylvilagus bachmani* which gets more of its water from evaporatively enriched plants..

Sample Measurements:

Modern samples were run at the University of California Davis using a Micromass Optima isotope ratio mass spectrometer (IRMS). These samples were also drilled to obtain enamel powder of tooth using a rotary tool. The powder was treated with 30% hydrogen peroxide and then soaked in 0.1 N acetic acid to remove contaminants, and finally rinsed with 100% ethyl alcohol. These were than heated and mixed with phosphoric acid to get gas, that was then measured using the mass spectrometer to measure the stable oxygen isotope ratio. Stable oxygen isotope ratio measurements were given based on the VPDB standard.

Fossil taxa samples were run at the University of Oregon stable isotope lab in the Termo-Finnigan MAT 253 mass spectrometer. To measure the tooth enamel, samples were drilled to obtain enamel powder using a rotary tool. Samples were loaded with

CaCO3 into a heating block at 70 degrees celsius and mixed with phosphoric acid. Samples were measured in the mass spectrometer for d18O on the gas bench. Stable oxygen isotope measurements were given based on the VSMOW standard and were then converted to the VPDB standard to match the modern measurements using equation 1.

Equation 1. Conversion from stable oxygen isotope measurements under the VSMOW standard to the VPDB standard in per mil. From Brand et. al., 2014.

$$\delta^{18}O_{VPDB} = 0.97001 * \delta^{18}O_{VSMOW} - 29.99\ ‰ \qquad (1)$$

Results:

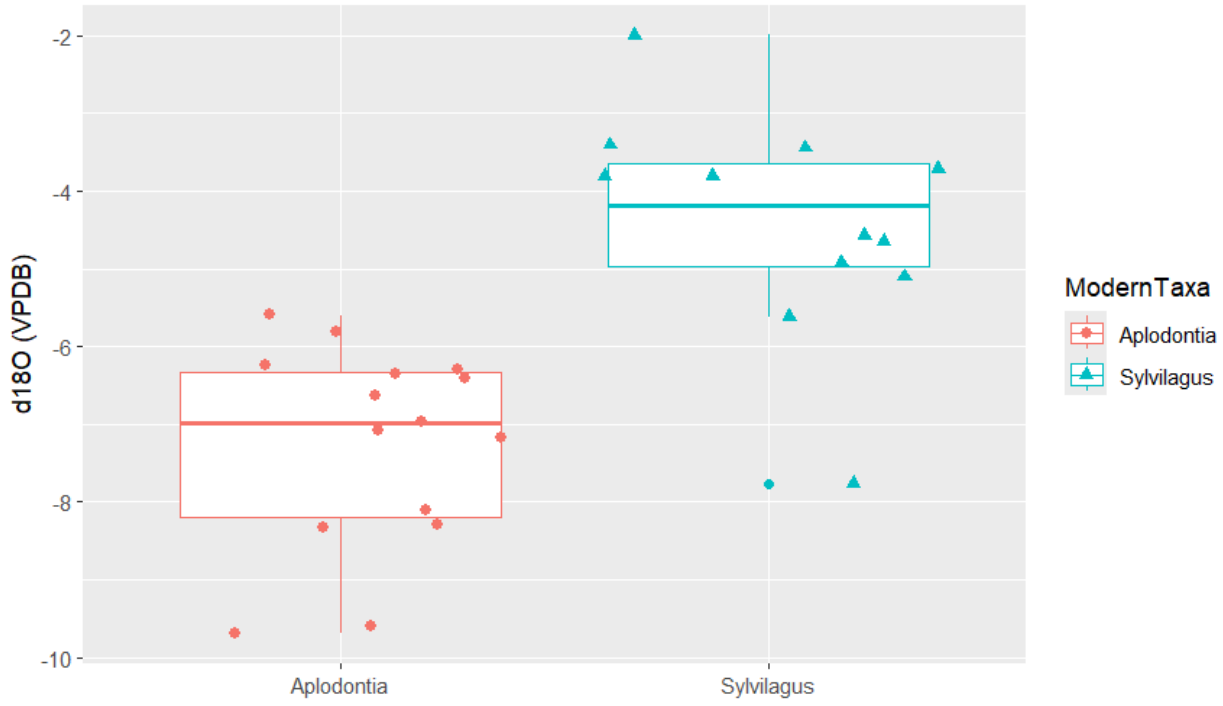

Figure 4. Box plot distribution of stable oxygen isotopes for modern taxa from Point Reyes, *Aplodontia rufa* and *Sylvilagus bachmani*. Measured in d18O values. *Aplodontia* is represented in red circles and *Sylvilagus* is represented in blue triangles.

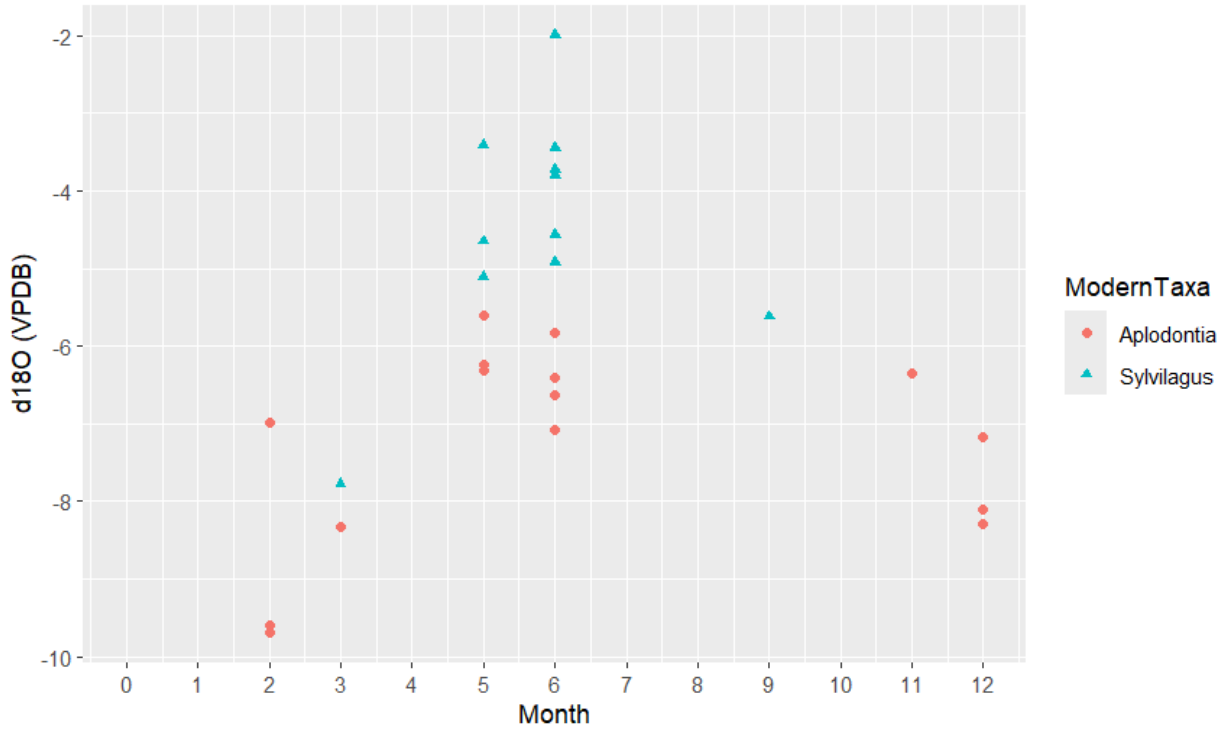

Figure 5. Scatter plot of the stable oxygen isotope values for the modern sample measurements from Point Reyes by collection month. Measured in d18O ratio values. *Aplodontia rufa* is represented in red circles and *Sylvilagus bachmani* is represented in blue triangles.

For the modern taxa, the average δ18O value for *Aplodontia* is -7.24 +/- 1.29‰ and it ranges from -9.69‰ to -5.60‰. *Sylvilagus* on the other hand has an average δ18O value of -4.40 +/- 1.43‰ and it ranges from -7.77‰ to -1.99‰ (figure 4). Based on the distribution of values in the boxplot and a T test from the results (t(22.4) = -5.35, p = 0.0000211), it appears that the mean stable oxygen isotope values is significantly different between *Sylvilagus* and *Aplodontia* as the boxplot quartiles do not overlap with each other (figure 4). This especially true when you consider the minimum value for *Sylvilagus* is an outlier and the rest of the values for *Sylvilagus* lie outside of the range of values for *Aplodontia* (figure 4). Samples were collected from throughout the year with most samples being collected in May and June. However, δ18O values for

Sylvilagus are consistently higher than those for *Aplodontia* throughout the year (figure 5).

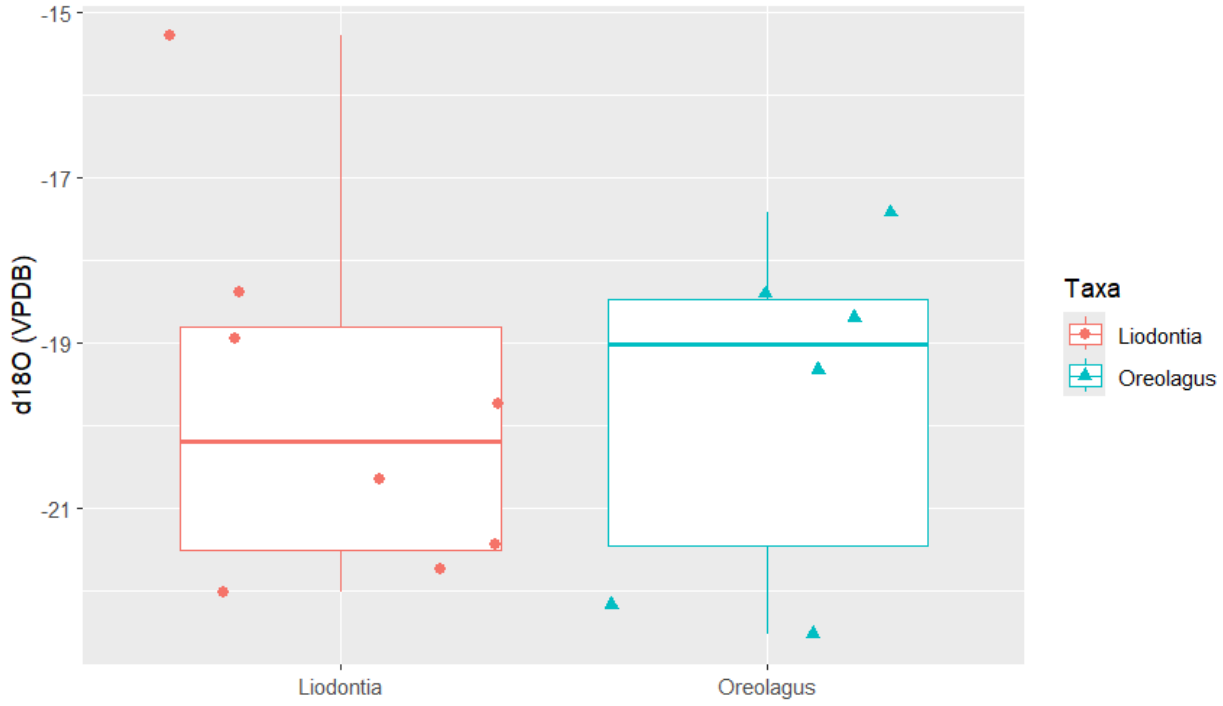

Figure 6. Box plot distribution of stable oxygen isotopes for fossil taxa from Beatty's Butte, *Liodontia alexandrae* and *Oreolagus wallacei*. Measured in d18O values. *Liodontia* is represented in red circles and *Oreolagus* is represented in blue triangles.

For the fossil taxa, the average δ18O for *Liodontia* is -19.77 +/- 2.24‰ and the values range from -22.02‰ to -15.28‰. For *Oreolagus*, the average δ18O is -19.75 +/- 2.10‰ and the values range from -22.52‰ to -17.42‰ (figure 6). Unlike the modern taxa, the distribution of values for the fossil taxa seems to overlap strongly between the two species. This alongside the T test of this data (t(11.3) = -0.017, p = 0.986) suggests that they are not statistically different from each other and have similar isotopic

signatures (figure 6). One of the values for *Liodontia* is unusually high and likely is a strong outlier, however it does not affect how the distributions of values overlap with each other. The fossil taxa overall also seem to be more variable than the modern taxa with a bigger overall spread in the data and a bigger range in the quartile values (figure 6).

Discussion:

The data for the modern species seems to suggest a significant difference between the stable oxygen isotopes of *Aplodontia rufa* and *Sylvilagus bachmani* with the *Aplodontia* measurements being isotopically lighter than the *Sylvilagus* (figure 4). This lines up with our hypothesis that *Aplodontia* will have a different isotopic signature because of the inefficient water concentration ability in its kidneys and the need to drink more water. As previous studies have suggested, animals that live in more water stressed environments have a different isotopic signatures because of the added fractionation involved in obtaining water from foods like plants (Blumenthal et. al., 2017; Levin et. al., 2006). In these studies, animals that spent more time in the water have a lower stable oxygen isotope ratio (Clementz et. al., 2008; Clementz & Koch, 2001). So, it makes sense that *Aplodontia* has a lower isotopic ratio because of its dependence on drinking water.

The fossil aplodontiine, *Liodontia alexandrae*, does not seem to have significantly different stable oxygen isotope values from the lagomorph *Oreolagus* that was used as a comparison taxon (figure 6). This seems to suggest that *Liodontia* does not have the same kind of unique and inefficient kidney structure that *Aplodontia rufa* has because it

does not seem to need to drink as much water and is able to obtain the water that it needs from plants like lagomorphs. So the stable oxygen isotope values between *Liodontia* and *Oreolagus* are mostly the same likely because *Liodontia* does not have the same kidney structure as *Aplodontia*, so the water requirements for the animal are lower.

An additional point of evidence for *Liodontia* not having the same kidney structure as *Aplodontia* comes from the habitat of Oregon at the time of its existence. Similar to the modern day climate, eastern Oregon during the middle Miocene was much drier than western Oregon is today, as evidenced by the stable oxygen isotope values from the region from this time (Chamberlain et. al., 2012). A drier and more arid climate limits access to water, which would make it hard for an animal like *Aplodontia rufa* to live in with its inefficient kidney structure. This is why the range of *Aplodontia rufa* is limited to the western side of the cascade mountain range in the modern day where there is no rain shadow and higher precipitation (figure 3), which can support the high water demand of *Aplodontia* (Nungesser & Pfeiffer, 1965). So the presence of *Liodontia* in a dry habitat implies that the animal had better water reabsorption capabilities in its kidneys than *A. rufa*.

If *Liodontia* had a normal kidney structure, the phylogeny of aplodontioids thus implies that the renal anatomy of *Aplodontia rufa* is a derived trait and not a primitive characteristic of rodents (figure 1). If this was an ancestral characteristic, then we would expect to see the trait in older species of aplodontiids within the same clade as *A. rufa*. But because *Liodontia* is a close relative of *Aplodontia rufa* and within the same clade of aplodontiinae (figure 1), then the absence of a unique kidney structure in *Liodontia*

alexandrae strongly suggests that this was a trait that was uniquely developed in *Aplodontia rufa*.

Another compounding piece of evidence to support that the kidney structure is not an ancestral trait, is the range extent of the mylagaulidae, the sister group to the aplodontiines. While the range of aplodontiines is somewhat limited to the western United States, mylagaulids have been found as far east as the great plains region and the open and arid habitats of the central United States (Hopkins, 2008; Carraway & Verts, 1993). This heavily implies that mylagaulids did not have the same renal anatomy of *Aplodontia* because if they were inefficiently concentrating their urine in the same way, their range would not be able to extend to the much more arid great plains because of the high water demand they would have. So, the fact that mylagaulids and *Liodontia* do not have the unique renal anatomy of *Aplodontia rufa* shows that this is likely a derived character in that species.

Future iterations of this study should look more broadly at different members of the aplodontioid clade and collect more stable oxygen isotope data for different species to try and pinpoint where the kidney anatomy of *Aplodontia* would have evolved in the phylogenetic tree. Although the habitat range of mylagaulids strongly suggests that they are able to concentrate their urine efficiently, it would be beneficial to run samples on members of this clade in order to fully understand their renal anatomy and confirm that this is a derived trait. Future studies should investigate samples of the smaller, more basal members of the aplodontioid clade like *Meniscomys* or *Allomys* (figure 1). Finally, we should investigate more samples within the aplodontiine clade to figure out which species of aplodontiines had this anatomy. Within this clade, it is difficult to pinpoint

exactly when this anatomy was evolved because of the lack of diversity within aplodontiinae and the lack of specimens within the Pliocene and Pleistocene (Hopkins, 2008). However, running samples of *Liodontia furlongi* or even fossil samples of *Aplodontia rufa* could provide more information on the evolution of this character within the aplodontioid clade.

Conclusion:

This study found that the stable oxygen isotope ratio for *Aplodontia rufa* was significantly lower than the ratio for *Sylvilagus bachmani*, however there was no difference between stable oxygen isotope values of *Liodontia alexandrae* and *Oreolagus wallacei*. This disparity suggests that *Liodontia* does not share the renal anatomy of *Aplodontia rufa* as the isotope ratio is more comparable to the lagomorph. The range of *Liodontia* and other extinct aplodontioids is also evidence that they lack this unique anatomy as their ranges extend into more arid regions without access to water. This is evidence that the renal anatomy of *A. rufa* is a derived trait and is not a retained primitive characteristic of rodents as some authors claim. Future studies should look at other fossil species of aplodontioids to determine when this trait would have evolved and what other taxa may have it in the fossil record.

References:

Al-Kahtani, M. A., Zuleta, C., Caviedes-Vidal, E., & Garland, Jr, T. (2004). Kidney mass and relative medullary thickness of rodents in relation to habitat, body size, and phylogeny. *Physiological and Biochemical Zoology*, *77*(3), 346-365.

Beuchat, C. A. (1996). Structure and concentrating ability of the mammalian kidney: correlations with habitat. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *271*(1), R157-R179.

Blumenthal, S. A., Levin, N. E., Brown, F. H., Brugal, J. P., Chritz, K. L., Harris, J. M., ... & Cerling, T. E. (2017). Aridity and hominin environments. *Proceedings of the National Academy of Sciences*, *114*(28), 7331-7336.

Brand, W. A., Coplen, T. B., Vogl, J., Rosner, M., & Prohaska, T. (2014). Assessment of international reference materials for isotope-ratio analysis (IUPAC Technical Report). *Pure and Applied Chemistry*, *86*(3), 425-467.

Carraway, L. N., & Verts, B. J. (1993). Aplodontia rufa. *Mammalian Species*, (431), 1-10.

Cerling, T. E., Harris, J. M., Ambrose, S. H., Leakey, M. G., & Solounias, N. (1997). Dietary and environmental reconstruction with stable isotope analyses of herbivore tooth enamel from the Miocene locality of Fort Ternan, Kenya. *Journal of Human Evolution*, *33*(6), 635-650.

Chamberlain, C. P., Mix, H. T., Mulch, A., Hren, M. T., Kent-Corson, M. L., Davis, S. J., ... & Graham, S. A. (2012). The Cenozoic climatic and topographic evolution of the western North American Cordillera.

Chapman, J. A. (1974). Sylvilagus bachmani. *Mammalian Species*, (34), 1-4.

Clementz, M. T., & Koch, P. L. (2001). Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. *Oecologia*, *129*, 461-472.

Clementz, M. T., Holroyd, P. A., & Koch, P. L. (2008). Identifying aquatic habits of herbivorous mammals through stable isotope analysis. *Palaios*, *23*(9), 574-585.

Cooke, S. B., & Crowley, B. E. (2018). Deciphering the isotopic niches of now-extinct Hispaniolan rodents. *Journal of Vertebrate Paleontology*, *38*(5), e1510414.

Craig, H. (1961). Isotopic variations in meteoric waters. *Science*, *133*(3465), 1702-1703.

Crocker, D. E., Kofahl, N., Fellers, G. D., Gates, N. B., & Houser, D. S. (2007). High rates of energy expenditure and water flux in free-ranging Point Reyes mountain beavers Aplodontia rufa phaea. *Physiological and Biochemical Zoology*, *80*(6), 635-642.

Dicker, S. E., & Eggleton, M. G. (1964). Renal function in the primitive mammal Aplodontia rufa, with some observations on squirrels. *The Journal of Physiology*, *170*(1), 186.

Drewicz, A. E., & Kohn, M. J. (2018). Stable isotopes in large herbivore tooth enamel capture a mid-Miocene precipitation spike in the interior Pacific Northwest. *Palaeogeography, palaeoclimatology, palaeoecology*, *495*, 1-12.

Evens, J. (2008). *Natural History of the Point Reyes Peninsula: Fully Revised and Expanded Edition* (No. 94). Univ of California Press.

Fabre, P. H., Hautier, L., Dimitrov, D., & P Douzery, E. J. (2012). A glimpse on the pattern of rodent diversification: a phylogenetic approach. *BMC evolutionary biology*, *12*, 1-19.

Grimes, S. T., Collinson, M. E., Hooker, J. J., Mattey, D. P., Grassineau, N. V., & Lowry, D. (2004). Distinguishing the diets of coexisting fossil theridomyid and glirid rodents using carbon isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *208*(1-2), 103-119.

Heisinger, J. F., & Breitenbach, R. P. (1969). Renal structural characteristics as indexes of renal adaptation for water conservation in the genus Sylvilagus. *Physiological Zoology*, *42*(2), 160-172.

Hopkins, S. S. (2007). Causes of lineage decline in the Aplodontidae: testing for the influence of physical and biological change. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *246*(2-4), 331-353.

Hopkins, S. S. (2008). Phylogeny and evolutionary history of the Aplodontoidea (Mammalia: Rodentia). *Zoological Journal of the Linnean Society*, *153*(4), 769-838.

Hynek, S. A., Passey, B. H., Prado, J. L., Brown, F. H., Cerling, T. E., & Quade, J. (2012). Small mammal carbon isotope ecology across the Miocene–Pliocene boundary, northwestern Argentina. *Earth and Planetary Science Letters*, *321*, 177-188.

Jeffrey, A., Denys, C., Stoetzel, E., & Lee-Thorp, J. A. (2015). Influences on the stable oxygen and carbon isotopes in gerbillid rodent teeth in semi-arid and arid environments: Implications for past climate and environmental reconstruction. *Earth and Planetary Science Letters*, *428*, 84-96.

Kimura, Y., Jacobs, L. L., Cerling, T. E., Uno, K. T., Ferguson, K. M., Flynn, L. J., & Patnaik, R. (2013). Fossil mice and rats show isotopic evidence of niche partitioning and change in dental ecomorphology related to dietary shift in Late Miocene of Pakistan. *PLoS One*, *8*(8), e69308.

Kohn, M. J., Schoeninger, M. J., & Valley, J. W. (1996). Herbivore tooth oxygen isotope compositions: effects of diet and physiology. *Geochimica et Cosmochimica Acta*, *60*(20), 3889-3896.

Kukla, T., Ibarra, D. E., Rugenstein, J. K. C., Gooley, J. T., Mullins, C. E., Kramer, S., ... & Chamberlain, C. P. (2021). High-resolution stable isotope paleotopography of the John Day Region, Oregon, United States. *Frontiers in Earth Science*, *9*, 635181.

Kukla, T., Rugenstein, J. K. C., Ibarra, D. E., Winnick, M. J., Strömberg, C. A., & Chamberlain, C. P. (2022). Drier winters drove Cenozoic open habitat expansion in North America. *AGU Advances*, *3*(2), e2021AV000566.

Levin, N. E., Cerling, T. E., Passey, B. H., Harris, J. M., & Ehleringer, J. R. (2006). A stable isotope aridity index for terrestrial environments. *Proceedings of the National Academy of Sciences*, *103*(30), 11201-11205.

Longinelli, A. (1984). Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research?. *Geochimica et cosmochimica Acta*, *48*(2), 385-390.

Luz, B., Kolodny, Y., & Horowitz, M. (1984). Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. *Geochimica et Cosmochimica Acta*, *48*(8), 1689-1693.

Nungesser, W. C., & Pfeiffer, E. W. (1965). Water balance and maximum concentrating capacity in the primitive rodent, Aplodontia rufa. *Comparative Biochemistry and Physiology*, *14*, 289-297.

Pefiffer, E. W., Nungesser, W. C., Iverson, D. A., & Wallerius, J. F. (1960). The renal anatomy of the primitive rodent, Aplodontia rufa, and a consideration of its functional significance. *The Anatomical Record*, *137*(3), 227-235.

Royer, A., Lécuyer, C., Montuire, S., Amiot, R., Legendre, S., Cuenca-Bescós, G., ... & Martineau, F. (2013). What does the oxygen isotope composition of rodent teeth record?. *Earth and Planetary Science Letters*, *361*, 258-271.

Schmidt-Nielsen, B. O. D. I. L., & Pfeiffer, E. W. (1970). Urea and urinary concentrating ability in the mountain beaver Aplodontia rufa. *American Journal of Physiology-Legacy Content*, *218*(5), 1370-1375

Wallace, R. E. (1946). A Miocene mammalian fauna from Beatty Buttes, Oregon. *Carnegie Inst Wash Contrib Paleo pub*, *551*, 13-134.

Wang, Y., Kromhout, E., Zhang, C., Xu, Y., Parker, W., Deng, T., & Qiu, Z. (2008). Stable isotopic variations in modern herbivore tooth enamel, plants and water on the Tibetan Plateau: Implications for paleoclimate and paleoelevation reconstructions. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *260*(3-4), 359-374.

Xia, Z., & Yu, Z. (2020). Temperature-dependent oxygen isotope fractionation in plant cellulose biosynthesis revealed by a global dataset of peat mosses. *Frontiers in Earth Science*, *8*, 307.