USING OXYGEN ISOTOPES TO DETERMINE GROWTH PATTERNS IN THE INVASIVE GASTROPOD *POMACEA INSULARUM*

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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To the Mollusk community

ACKNOWLEDGMENTS

I would like to thank my committee, the geology department at the University of Florida, and my family and friends. My fellow geology graduate students lent me immeasurable assistance. An undertaking such as this is not an individual effort.

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USING OXYGEN ISOTOPES TO DETERMINE GROWTH PATTERNS IN THE INVASIVE GASTROPOD *POMACEA INSULARUM*

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Chair: Mark Brenner
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I measured stable oxygen isotope values ($\delta^{18}O_{aragonite}$) along the shell growth axis of the non-native gastropod, *Pomacea insularum*, to determine the life span and seasonal growth pattern of the exotic snail in Florida lakes. Twenty-two live and deceased specimens were collected from three Florida Lakes: Tulane and Verona in south-central Florida, and Newnans in north-central Florida. Shell oxygen isotope profiles were generated by mass spectrometry using the carbonate (aragonite) drilled at 2-5 mm intervals along the shell whorl. Measured values were compared to expected $\delta^{18}O$ values, the latter calculated from temperatures and isotope values of lake water ($\delta^{18}O_{water}$), collected throughout the year. The age, season of growth, and rate of growth of *P. insularum* were determined from seasonal temperature fluctuations, inferred from the $\delta^{18}O_{aragonite}$ values. In addition to yielding information about the life history of *P. insularum* in Florida, data gathered in this study of modern snails shed light on the feasibility of using gastropods for paleoclimate studies of past seasonality.

Eight of the 22 *P. insularum* lived <1 year, ten lived slightly longer than 1 year, and one lived into its third year. Three snails yielded ambiguous results and age could not

be determined. Snails from Lakes Tulane and Verona deposited the majority of their carbonate during late fall/early winter. Seven of the ten animals collected from these lakes deposited only 0-5% of their carbonate during summer. Snail shell growth in Newnans Lake was divided more evenly between summer and winter. For longer-lived animals, carbonate accretion slowed considerably after the first year of growth. Among specimens taken alive, and for which time of death was known, there were major offsets along the shell margin with respect to equilibrium $\delta^{18}O$ values, i.e. between measured $\delta^{18}O_{aragonite}$ and expected values calculated from water temperature and $\delta^{18}O_{water}$ at the time of death. Within individuals, the $\delta^{18}O_{aragonite}$ values varied across the shell growth margin. Furthermore, $\delta^{18}O_{aragonite}$ values from the shell growth margin were different among individuals captured on the same date. If such variation is typical of species in the genus *Pomacea*, it would preclude their use in archaeological or paleoecological studies that seek to determine season of death.

CHAPTER 1
INTRODUCTION

Background

Research on the introduced invasive freshwater gastropod *Pomacea insularum* shows pronounced ecological effects on aquatic ecosystems (Karatayev et al., 2009). *P. insularum* was introduced into North America from its native South America by the aquarium trade (Karatayev et al., 2009 and Howells et al., 2006). Its spread throughout Southeast Asia is well documented (Naylor, 1996 and Carlsson et al., 2004). There, it was introduced as a potential food source, but the snails dispersed rapidly via irrigation systems and devastated the rice industry. Naylor (1996) estimated the total damage at more than one billion dollars.

Phylogenetic analysis of mtDNA indicates there are populations of *P. insularum* throughout Texas, Florida, and Georgia. Its presence in Florida was first confirmed genetically in 2002. In Florida, the exotic apple snail has had a negative impact on snail kite populations, with serious implications for the endangered bird's survival (Cattau, 2008). Competitive interaction with the native *P. paludosa* threatens the survival of the latter species (Rawlings et al., 2007 and Rhymer and Simberloff, 1996). The high fecundity of *P. insularum* (mean clutch = 2064 eggs, 80% hatch rate), its rapid growth (Cowie, 2002), and its cold tolerance (average monthly minimum of 4-6°C in its native range), will only hasten its spread through the water bodies of the southeastern United States (Barnes et al., 2008 and Rawlings et al., 2007).

Currently, there is limited information on the life history of *P. insularum* in Florida. Specifically, there has been a lack of research into the longevity and seasonal growth rates of the species. Such information is essential if effective species management strategies are to be developed.

P. insularum resembles another *Pomacea* species, *P. canaliculata*, which makes identification difficult. Following Rawlings et al. (2007), *P. insularum* was distinguished from *P. canaliculata* in this study by egg clutch morphology. In the three lakes where I collected snails, only *P. insularum* eggs were identified. Furthermore, Rawlings et al. (2007) only found *P. canaliculata* in California and Arizona.

I measured stable oxygen isotope ($\delta^{18}O$) values along the growth axis of *P. insularum* shells to determine their life span and explore their growth pattern in Florida lakes. I accomplished this by drilling carbonate from shells at close intervals and running the samples on an isotope ratio mass spectrometer. The $\delta^{18}O$ value in the shell of a carbonate-secreting organism is controlled by the $\delta^{18}O$ of the host water in which the animal is growing and the water temperature. Because the $\delta^{18}O$ of water in Florida lakes displays relatively small variations throughout the year, and even from year to year, seasonal temperature variations were thought to be the major control on shell $\delta^{18}O$ variations in *P. insularum.* I set out to answer two primary questions: 1) how long do *P. insularum* live in Florida lakes?, and 2) do growth rates of the snail vary seasonally? Answers to these questions may be helpful for developing strategies to manage these exotic organisms.

A secondary objective was to assess whether seasonal growth patterns in *P. insularum* could be applied to paleoclimate studies on seasonality. If this species ceases to accumulate significant amounts of carbonate during a season, it would be difficult or impossible to use subfossil organisms to compare seasonality of past climates to seasonality today (Andreasson and Schmitz 2000; Steuber, 1996). This research emphasizes the importance of first studying extant organisms, i.e. a modern analog, before attempting to reconstruct past annual temperature ranges from subfossil shells.

Oxygen Isotopes

The basis for the $^{18}O/^{16}O$ thermometer was established by Urey in 1947 and confirmed in laboratory experiments with inorganically precipitated carbonate (McCrea, 1950). These experiments demonstrated that fractionation of oxygen isotopes during incorporation of carbonate into an organism's shell, is temperature-dependent. When water temperatures are colder, the ratio of $^{18}O/^{16}O$ in the shell is higher. As the temperature increases, less ^{18}O is incorporated into the shell. Such temperature dependence of oxygen isotope fractionation has been observed in many studies (Boehm et al., 2000; Zhou and Zheng, 2003; White et al., 1999).

Grossman and Ku (1986) derived an empirical equation that describes the fractionation between biologically precipitated aragonite and the host water. The equation demonstrates that for every 4.34°C increase in temperature, there is a 1‰ decrease in the $\delta^{18}O$ value of the mollusk aragonite. Values are expressed in delta (δ) notation, which is the $^{18}O/^{16}O$ ratio of the sample normalized to an international standard permil (‰). Water temperatures in Florida lakes display a seasonal range of approximately 12-15°C annually (Beaver et al. 1981), whereas lake water $\delta^{18}O$ shows little variation throughout the year in lakes with long water residence times, on the order of 0.60-1.04‰(J. Escobar, personal communication). Assuming temperature is the primary variable that governs $\delta^{18}O$ fluctuations in snail carbonate, oxygen isotope measures on closely spaced samples from a gastropod shell should reflect seasonal water temperature fluctuations.

This isotopic approach has been useful for assessing growth patterns of mollusks (Cespugilo et al., 1999, and Schone et al., 2003) and for reconstructing past climate conditions (Zachos et al., 2001). For instance, Cespugilo et al. (1999) found that *Nassa mutabilis* accreted carbonate year round, but showed relatively greater summer growth. The six gastropods they sampled varied between 11 and 29 months in age. Verdegaal et al. (2005) measured the $\delta^{18}O$ signal across unionid mussel shells and solved for water temperature using the equation developed by Grossman and Ku (1986). The overall pattern of the $\delta^{18}O$ profiles revealed that shell growth ceased below 10-12°C, meaning that only spring, summer and fall values were recorded, but winter values were absent.

This study compares the $\delta^{18}O$ values in *P. insularum*, an aragonitic gastropod collected from three lakes, Newnan's, Verona, and Tulane (Fig. 1-1), with the expected temperature-dependent $\delta^{18}O$ values derived from Grossman and Ku's (1986) aragonite-water fractionation equation. Expected shell aragonite values were calculated from the recorded water temperature and measured $\delta^{18}O_{water}$ composition. The oxygen isotope composition of a water body is controlled by: 1) the $\delta^{18}O$ of source water entering the lake (rainfall, runoff, groundwater), and 2) the relative amount of precipitation to evaporation. Evaporation preferentially removes the lighter isotope (^{16}O) from the liquid phase, leaving the lake water relatively enriched in the heavier (^{18}O) isotope (Gat, 1996). High amounts of rain dilute the ^{18}O in the lake water by replenishing the water body with ^{18}O-depleted precipitation. The more "rainout" from a given air mass, the lower the $\delta^{18}O$ value of the precipitation (Kendall and Coplen, 2001). The $\delta^{18}O$ of a water body will generally be relatively lower in months with abundant rainfall compared to months with less precipitation. Furthermore, as air masses move away from their point of formation, precipitation becomes progressively lighter (Scott et al., 2004; Bradley, 1999). Rayleigh distillation continually removes the heavier isotope in precipitation. The oxygen isotope composition of *P. insularum* shells in Florida lakes is therefore a consequence of several factors, with temperature ultimately exerting the greatest influence on seasonal isotopic variation.

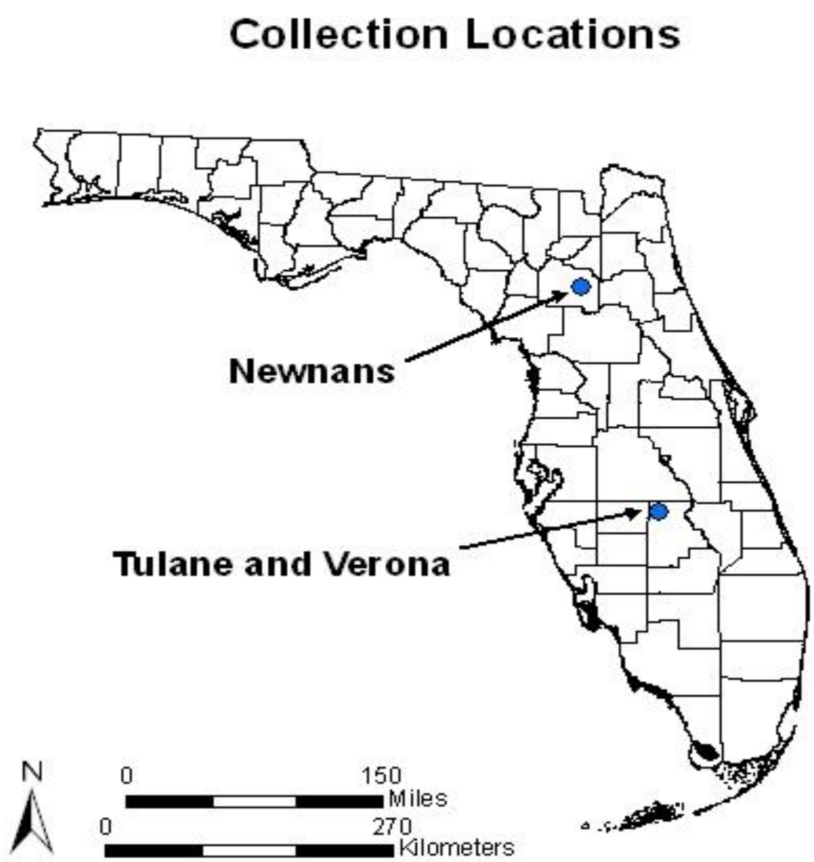


Figure 1-1. *P. insularum* collection sites. Newnans Lake is in Alachua County, Tulane and Verona are in Highlands County, Florida, USA.

CHAPTER 2
MATERIALS AND METHODS

Field Methods

From June 18, 2009 to June 8, 2010, water samples and adult *P. insularum* were collected approximately twice monthly from the mouth of a shallow-water boat launch canal in Newnans Lake, Alachua County, Florida. A Hobo® temperature data logger was emplaced at the collection location (0.5 m depth) and recorded temperature hourly throughout the study. Reported temperatures are averages of water temperatures recorded every hour, three days before and three days after each water collection date. Water samples were collected in CO_2 impermeable vials from three separate locations, spanning the length of the canal. Immediately after collection, the water was treated with 1.13 M copper sulfate ($CuSO_4$) to halt photosynthesis, and refrigerated.

At Lakes Tulane and Verona, Highlands County, Florida, snails were collected three times, in spring, summer, and winter, from March 2009-January 2010. Only live snails were collected from Lake Verona, whereas both living and deceased specimens were collected from Lake Tulane. A total of 22 individuals were collected throughout the year. Monthly water-column $\delta^{18}O$ values for Lakes Tulane and Verona were provided by Jaime Escobar (personal communication) and represent values obtained from June 2006 to June 2007. Proxy water temperature data for Lakes Tulane and Verona came from nearby (~40 km) Lake Annie, Highlands County, Florida, and were provided by the Archbold Biological Station. Reported temperatures are for the time span during which snails were collected in Lakes Tulane and Verona. I compared temperature data collected from Lakes Tulane, Verona, and Annie for the period February-September 2007 to test the validity of using the Lake Annie values (Fig. 2-1). This time period captures the range of intra-annual temperature variability fairly well. Average annual surface temperatures of Lakes Verona, Tulane, and Annie were 26.5°C, 25.6°C, and 25.8°C, respectively. The most significant monthly temperature deviation, 2.6°C, was recorded in April, when temperature in Lake Annie was 22.3°C and temperatures in Lakes Tulane and Verona were 24.3°C and 24.9°C, respectively. This difference may reflect the fact that data were collected on different dates within the same month. With the exception of the April data, the average departure in temperature in all months between Lakes Annie and Lakes Tulane/Verona was only 0.8°C.

Laboratory Methods

Water Samples

Two ml Wheaton glass septa top vials were filled with 200 µl of lake water. Open vials were placed in a glove bag that was filled with CO_2 to purge ambient air. Next, the vials were sealed with caps containing a rubber septum and removed from the glove bag. Sample vials were placed into an aluminum block and heated to 45°C for 9 hours to allow oxygen in the water to equilibrate with CO_2 in the vial headspace. Following equilibration, a hollow needle was used to pierce the septum and headspace gas was drawn out of the multiprep vial and into a water trap, before entering a VG Isogas Prism II mass spectrometer. The oxygen isotopic signature of the water samples was expressed in delta notation with respect to Standard Mean Ocean Water (VSMOW).

Snail shells

Once returned to the laboratory, snails were frozen to prevent further growth. After five days, individuals were thawed at room temperature and tissues were removed from the shells. The organic periostracum and residual tissue were dissolved overnight in 6% sodium hypochlorite (NaClO) (Fastovsky et al., 1993).

The most recent growth band in all shells was identified microscopically and sampled using a 0.025-cm drill bit attached to a SHERLINE Model 5100 vertical mill. From that point, the growth axis of each shell was spot-sampled with the mill at 2-5 mm intervals, to a depth of 100 µm, yielding 36-113 samples per snail. In one shell from Newnans Lake, 10 samples were drilled along a single growth band ~2 cm from the shell margin. Two to four samples were also drilled along the growth margin of all shells from Newnans Lake. Portions of one shell from Lake Verona were analyzed for mineralogy with a Rigaku Ultima IV multipurpose x-ray diffractometer (XRD) and a Zeiss Evo-10 scanning electron microscope (SEM).Both the outer and inner prismatic layers were composed entirely of aragonite.

A *P. insularum* egg clutch was also collected from Newnan's Lake and juveniles were hatched and raised in a tank under constant temperature (24°C) using local well water. Four well water samples were analyzed over a four month period. All returned a δ^{18}O value of -3.85 +/-0.2‰. After six months of growth, shells of four individuals were drilled along the growth axis at 5-mm intervals, from a section near the center of the whorl. I removed three to five samples per shell and used the average of these samples to represent mean "control" isotope values and tested for species-specific offsets (vital effects) in the $\delta^{18}O_{aragonite}$.

After drilling, 30-60 µg of shell powder from each sample was loaded into a glass vial. The vials were placed into a Kiel III preparation device and reacted with phosphoric acid to liberate CO_2 from the carbonate. Gas then entered a Finnigan-MAT 252 isotope ratio mass spectrometer for oxygen isotope analyses. Analytical precision for δ^{18}O analyses was ± 0.06‰ for carbonates and _ for water. All results are presented in standard delta notation relative to the Vienna Peedee Belemnite (VPDB) standard as follows:

$$\delta^{18}O(‰) = \left(\frac{\frac{^{18}O}{^{16}O}sample}{\frac{^{18}O}{^{16}O}PDB\ standard} - 1\right) \times 1000 \tag{2-1}$$

The $\delta^{18}O$ values measured in each snail shell were compared to “expected” values calculated from lake water temperature and $\delta^{18}O$ values, using the equation of Grossman and Ku (1986), as modified by Gat, 1987:

$$\delta^{18}O_{aragonite} = \frac{Temperature\ (°C) - 20.6}{4.34} + (\delta^{18}O_{water} - 0.27) \tag{2-2}$$

This was done to explore whether the measured shell $\delta^{18}O$ values do indeed record seasonal conditions in the lake waters.

The maximum $\delta^{18}O$ value measured in each shell presumably represents carbonate precipitated under coolest conditions, while the minimum value is thought to represent carbonate precipitated from warmest waters. To establish the age of each individual, $\delta^{18}O$ values were plotted from the apex of the shell to the aperture, i.e. from the oldest to most recent accreted carbonate. Starting at the apex, I identified the first major high or low $\delta^{18}O$ value in the growth sequence and counted subsequent $\delta^{18}O$ peaks or valleys, assuming that each high or low represented aragonite deposited during a seasonal temperature extreme. Thus, age determination for each animal was estimated using the number of isotopic maxima and minima in each shell, with a full cycle (minimum to minimum or maximum to maximum) representing one year. If a shell’s isotopic range did not approximate the expected range, it was assumed that the individual did not deposit carbonate during both seasonal temperature extremes.

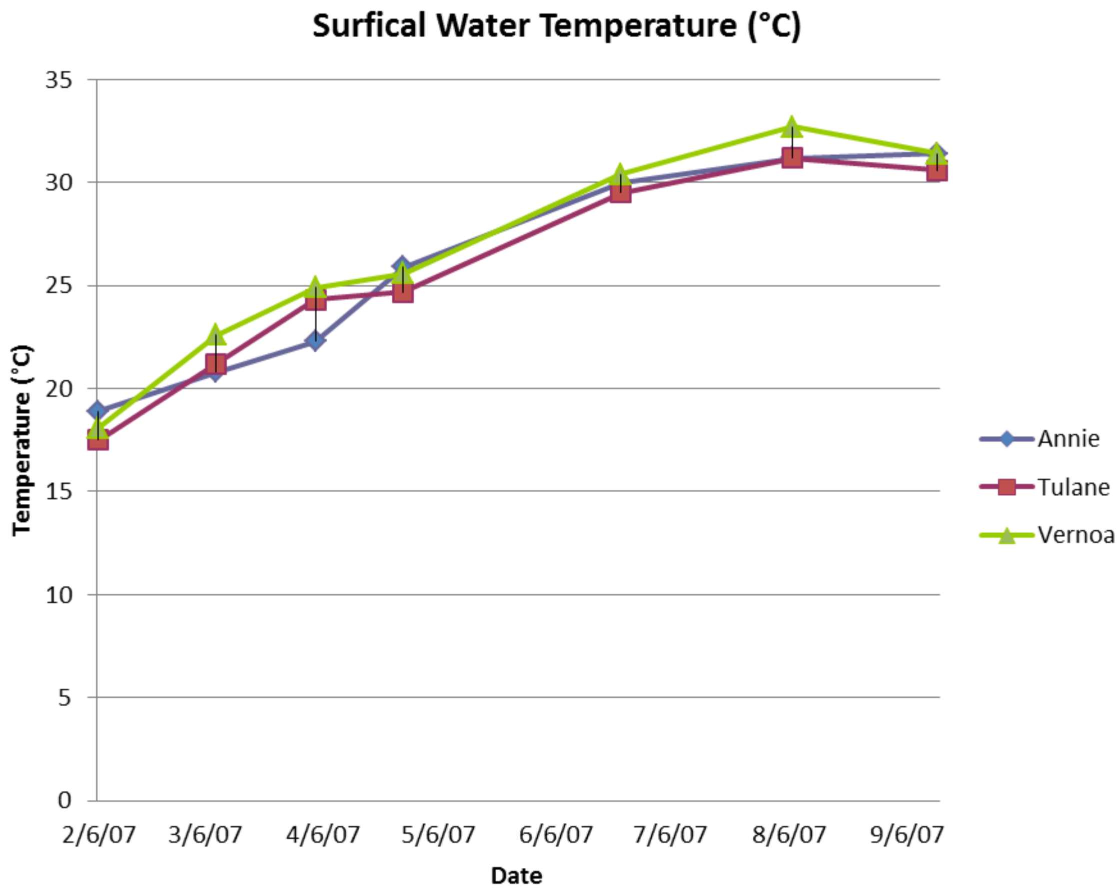


Figure 2-1. Water temperature comparisons of Lake's Tulane, Verona, and Annie over an eight month period during 2007.

CHAPTER 3
RESULTS

Lake Water Temperature and Isotope Values

Lakes Tulane and Verona

Monthly temperature in Lake Annie varied from 18.9 to 32.1°C, with a range of 13.2°C (Fig. 2-1). The average annual $\delta^{18}O_{water}$ value for Lake Tulane in 2006/2007 was 0.32‰, with a range of only 0.60‰. In Lake Verona, the average annual $\delta^{18}O_{water}$ value for the same period was -0.01‰, with a range of only 1.04‰. For both lakes, the highest $\delta^{18}O$ values were recorded from June-August, and the most depleted $\delta^{18}O$ values were measured in December for Tulane and November for Verona (Figs. 3-1 and 3-2) (J. Escobar, personal communication). Summer ^{18}O enrichment is likely explained by relatively greater evaporation, with preferential loss of the lighter ^{16}O in the late spring and summer months. Conversely, lower evaporation rates in winter leave the lake water relatively less enriched in ^{18}O (Sharp, 2007).

Newnans Lake

From June 2009 to June 2010, the average water temperature at the collection site was 22.87°C. The highest average temperature, 31.7°C, was recorded on 9 June 2010, and the lowest average temperature, 11.02°C, was recorded on 17 February 2010. The range of average water temperatures throughout the year at a depth of 0.5 m was 20.67°C (Fig. 3-3).

The $\delta^{18}O$ value of the Newnans Lake water (0.5 m depth) fluctuated more than that of the water in the south-central Florida lakes. The average annual $\delta^{18}O_{water}$ value was 0.54‰, and the annual range was 2.88‰ (Fig. 3-4). The boat launch canal where samples were collected had a pronounced $\delta^{18}O$ minimum during winter and two less obvious maxima during late fall and early summer. From June 2009 to January 2010, the $\delta^{18}O$ variability was only ~38% of that from January to June 2010. From late January to mid-February 2010, the $\delta^{18}O$ dropped from 0.56‰ to -1.33‰ and remained negative until late April.

Snail Shells

Tank-Raised Snails

The snails raised in a controlled setting were all enriched in ^{18}O relative to the expected value (Fig. 3-5). Additionally, despite constant temperature and $\delta^{18}O$ water, there were small intra-shell and inter-shell fluctuations in $\delta^{18}O_{aragonite}$. Table 3-1 compares the oxygen isotopic variability within and among the four control snails. The average standard deviation of $\delta^{18}O_{aragonite}$ within each shell was 0.15‰. The maximum departure from the mean value in each shell ranged from 0.21 to 0.39‰. The $\delta^{18}O_{aragonite}$ values from the four shells were enriched by an average of 1.35‰ compared to the expected values.

Shell Margin and Growth Line Samples

Maximum $\delta^{18}O_{aragonite}$ variability along the lip of Newnans Lake shells was 3.03‰, with an average range of $\delta^{18}O_{aragonite}$ of 1.03‰ (Table 3-2). The $\delta^{18}O_{aragonite}$ values measured along the single growth line, ~2 cm from the margin, showed very low variability (<0.23‰), i.e. within the precision of the method (Fig. 3-6).

Lake Verona Snails

The expected annual $\delta^{18}O$ range for Lake Verona shells was calculated using the Grossman and Ku (1986) aragonite-water fractionation equation (Figure 3-7). Over the course of a year, the expected range of $\delta^{18}O_{aragonite}$ values was 3.10‰. The maximum expected value was 0.04‰, and the minimum expected value was -3.07‰. Shells from Lake Verona displayed an average range of $\delta^{18}O_{aragonite}$ of 2.75‰. The average maximum value was 0.48‰ and the average minimum value was -2.27‰ (Table 3).

The shell $\delta^{18}O$ values indicate that none of the Lake Verona specimens lived much longer than one year. Samples LVL 001 and LVL 011 had two defined $\delta^{18}O$ minima and one maximum, indicating they lived >1 year (Figs. 3-8 and 3-9). Individuals LVL 002, LVL 012, and LVL 022 had only one $\delta^{18}O$ minimum and one maximum (Figs. 3-10, 3-11, and 3-12), indicating that they lived <1 year. All of the samples, except LVL 001, exceeded the expected $\delta^{18}O$ maximum value, by an average of 0.61‰. Only sample LVL 022 reached the expect $\delta^{18}O$ minimum value of -2.99‰. Nevertheless, general agreement between the pattern of measured and expected $\delta^{18}O$ values suggests the majority of shell growth in Lake Verona snails occurred between October and February, when all the organisms deposited >87% of their carbonate (Table 3-3). Only two of the five snails deposited measurable amounts of carbonate between June and September (Table 3-3).

Lake Tulane Snails

The expected annual $\delta^{18}O$ range for Lake Tulane shells was calculated using the Grossman and Ku (1986) aragonite-water fractionation equation (Figure 3-7). The expected $\delta^{18}O_{aragonite}$ range, maximum, and minimum calculated for Lake Tulane were 2.91‰, 0.27‰, and -2.63‰, respectively (Fig. 3-13). The average $\delta^{18}O_{aragonite}$ range, maximum, and minimum for the five snails in the lake were 2.11‰, 0.49‰, and -1.62‰ (Table 3-4).

Tulane sample LTL 001 was the longest-lived specimen collected from the southern lakes. The $\delta^{18}O_{aragonite}$ data showed three minima (Fig. 3-14) and indicate it was collected as it began its third year of growth. The $\delta^{18}O_{aragonite}$ showed less detail after the first year of growth. This was expected, as the animals accumulate carbonate rapidly during the early stages of growth to escape predation (Gittenberger et al., 1998; Verdegaal et al., 2005). Samples LTD 001 and LTD 005 contained two isotope minima, indicating they lived slightly longer than a year (Figs. 3-15 and 3-16). LTD 002 and LTD 012 were difficult to interpret because they lacked a clear seasonal isotope signal (Figs. 3-17 and 3-18). It is possible that LTD 002 deposited all of its carbonate during a single season. Similar to the shells from Verona, all but one sample collected from Lake Tulane exceeded the expected winter $\delta^{18}O$ maximum, while no shells reached the expected summer $\delta^{18}O$ minimum. All of the snails in Lake Tulane deposited the majority of their carbonate outside of the summer months (June-September) (Table 3-4). In the summer months (June-September), snails LTD 001, 002, 005, and LTL 001 deposited <5% of their total carbonate (Table 3-4).

Newnans Lake Snails

Newnans Lake snails were collected in a boat launch canal, where the water temperature and $\delta^{18}O_{water}$ fluctuated considerably. Temperature even varied substantially on short timescales, with a maximum daily temperature fluctuation of 8.3°C. Expected $\delta^{18}O_{aragonite}$ values were calculated using the temperature and $\delta^{18}O_{water}$ data (Fig. 3-19). The expected minimum (-2.42‰) is similar to values calculated for the other lakes, but the maximum (2.68‰), and therefore range (5.11‰), are higher. Measured $\delta^{18}O_{aragonite}$ values did not capture the expected extremes, and yielded a mean maximum of 1.23‰, a mean minimum of -2.18‰, and mean range of 3.42‰ (Table 3-5). Failure to capture the extremes was not unexpected. Even spot sampling of the shell involves some “smoothing” of the record, and it is probable that the true minimum and maximum values lie between sampled intervals in the shell, i.e. were not drilled. Furthermore, snails themselves may achieve some “smoothing” by preferential deposition of aragonite at optimum temperatures.

A seasonal trend was nevertheless apparent in most of the Newnans Lake samples. NLL 011, 012, 023, 031, 052, and 131 all lived at least a year (Figs. 3-20, 3-21, 3-22, 3-23, 3-24, and 3-25), and samples NLL 024, 041, 061, 091, and 101 all lived less than a year (Figs 3-26, 3-27, 3-28, 3-29, and 3-30). The isotope profile for NLL 063 was ambiguous, and age could not be determined (Fig. 3-31). Four of the samples reached the minimum expected $\delta^{18}O$ value, while none reached the maximum expected $\delta^{18}O$ value. Carbonate deposition was split fairly evenly between warm and cool periods (Table 3-5).

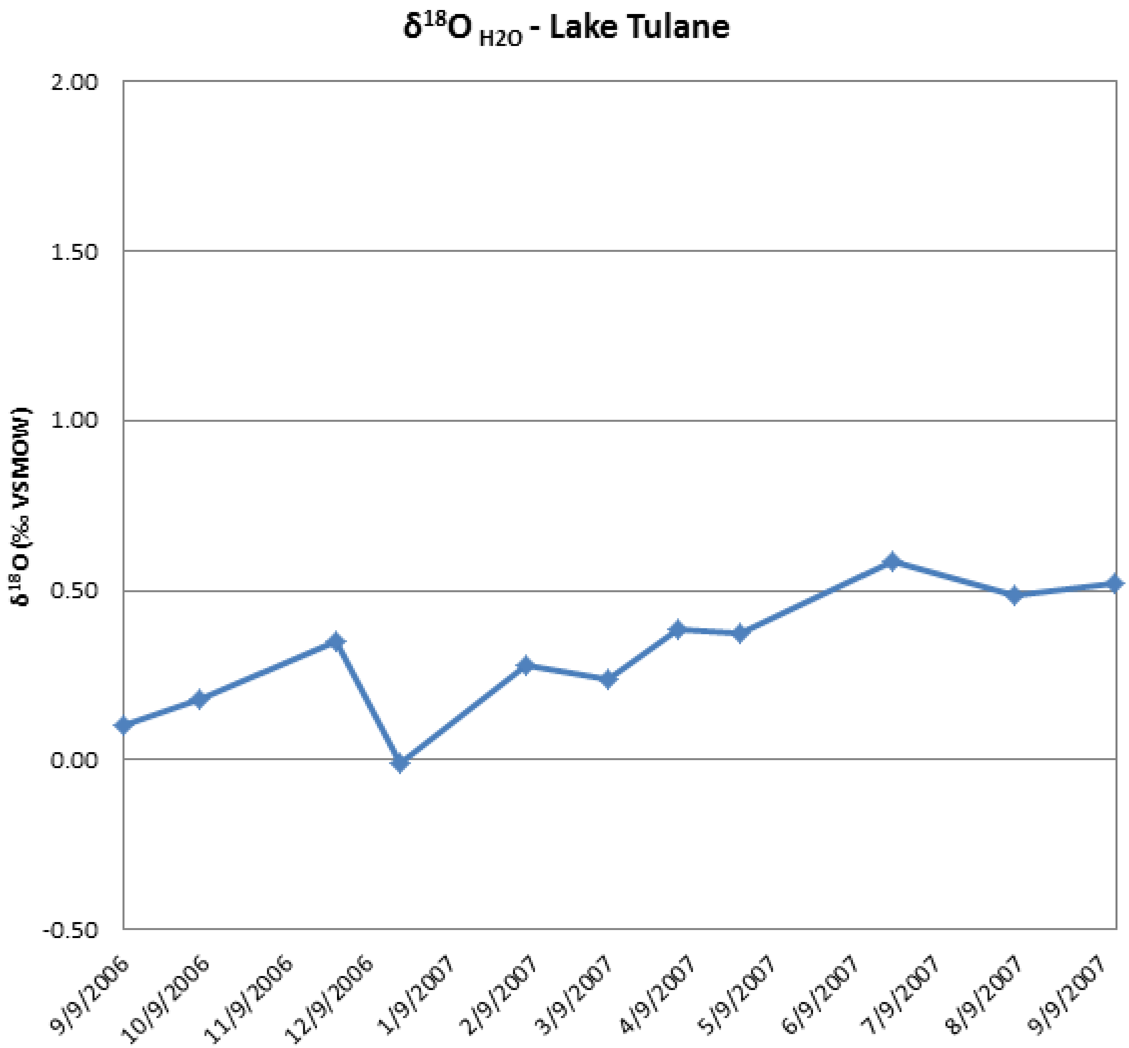


Figure 3-1. Annual range of oxygen isotopes in the surface waters of Lake Tulane.

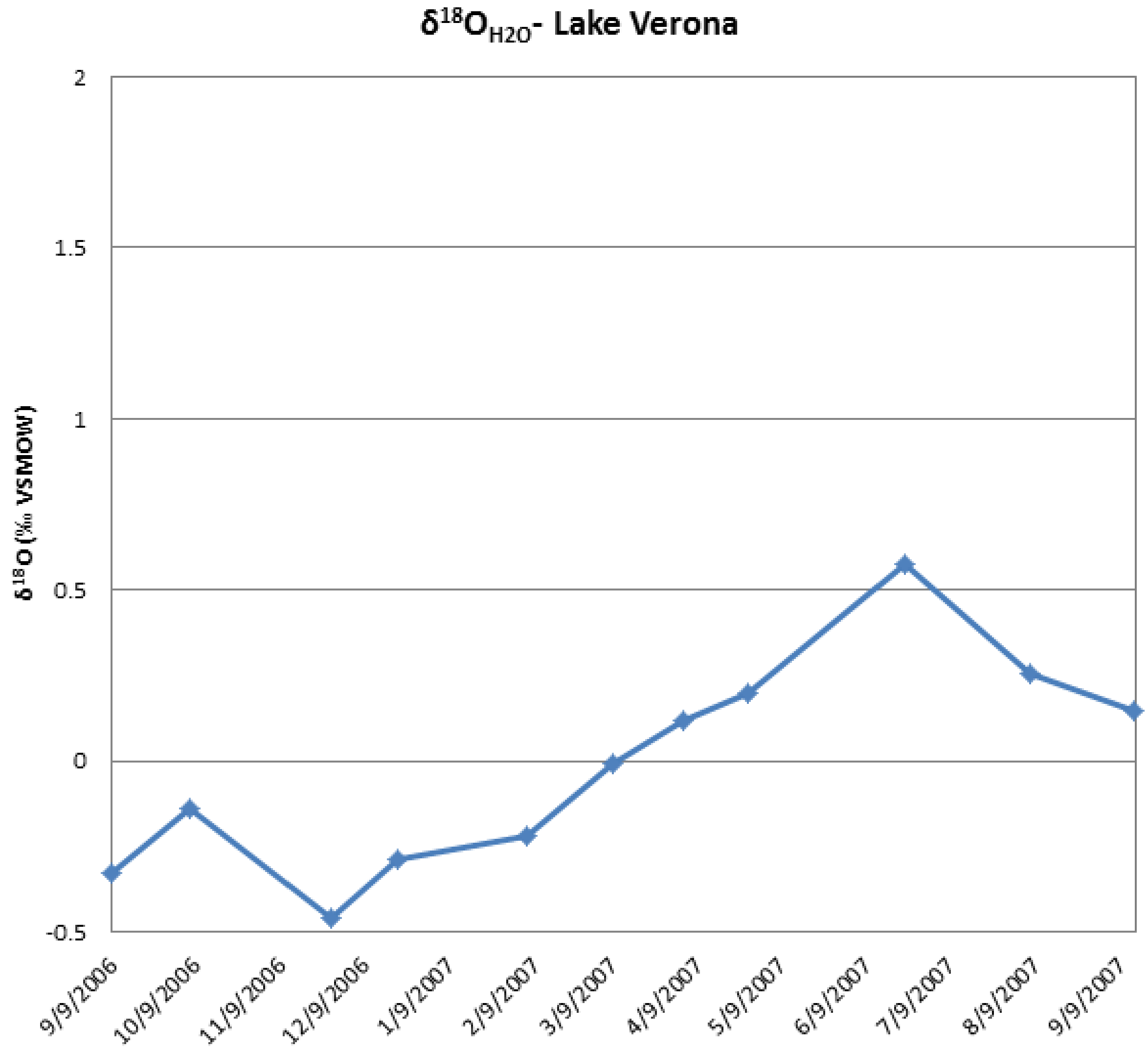


Figure 3-2. Annual range of oxygen isotopes in the surface waters of Lake Verona.

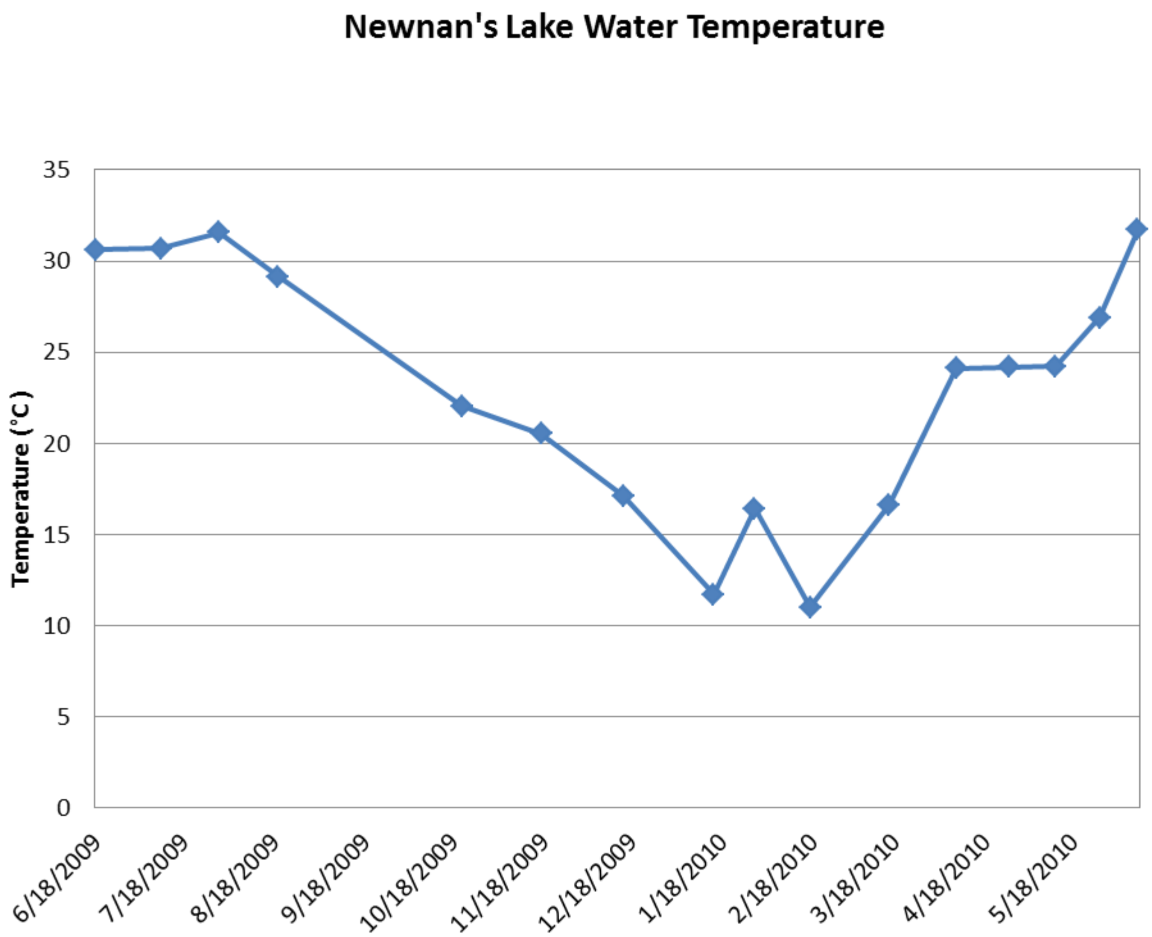


Figure 3-3. Annual water temperature variation taken at 0.5 meter depth. The temperature range in Newnans Lake is greater than Tulane or Verona. This is attributed to temperature fluctuations at the mouth of the shallow canal in Newnans Lake where samples were taken.

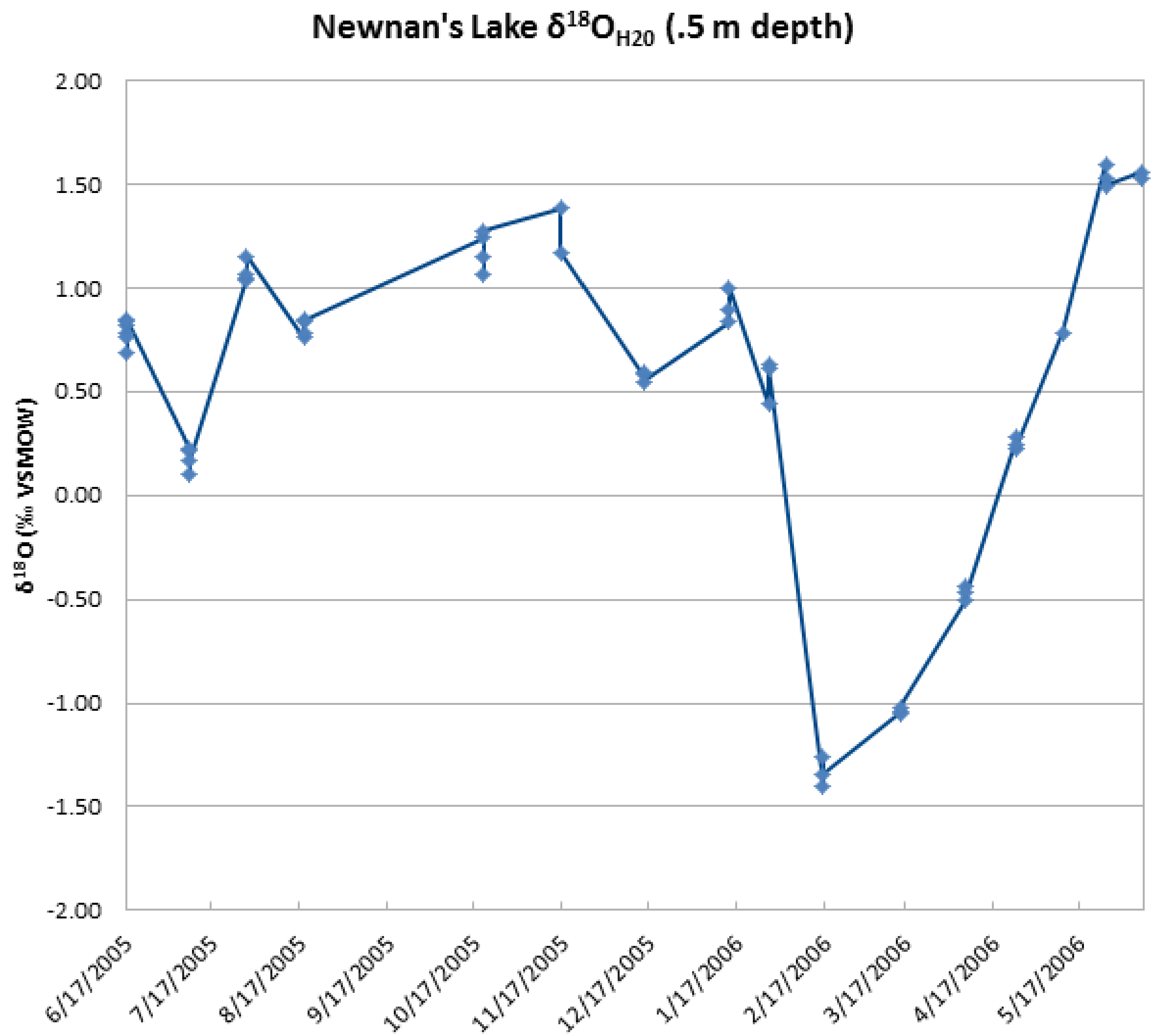


Figure 3-4. Annual range of oxygen isotopes taken monthly from three separate locations along the canal of Newnans Lake.

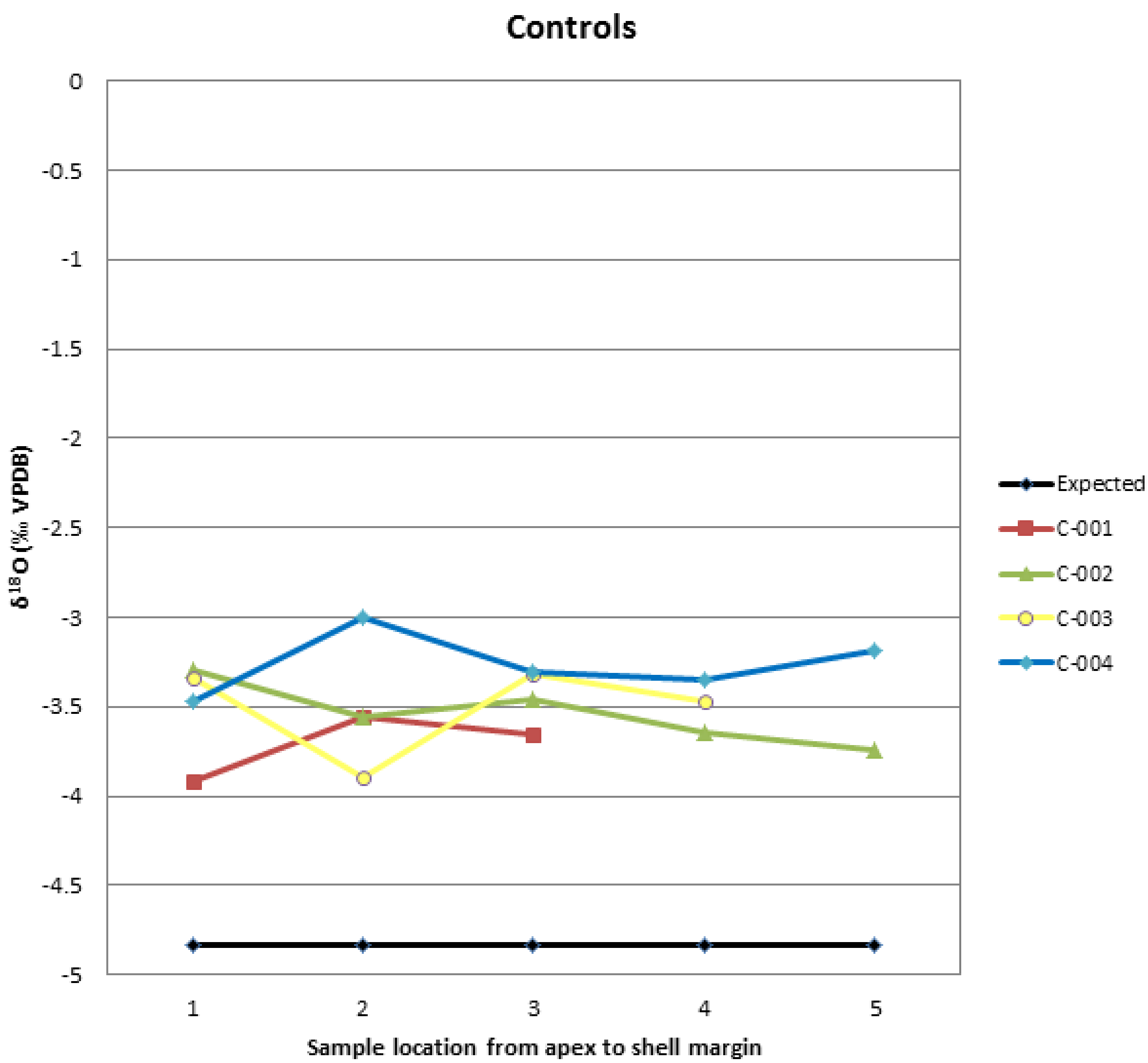


Figure 3-5. Oxygen isotopic profiles generated from drilling along the growth axis of four *P. insularum* raised in tanks with the temperature kept at 25° +/- 1°C. The black line represents the expected isotopic profile.

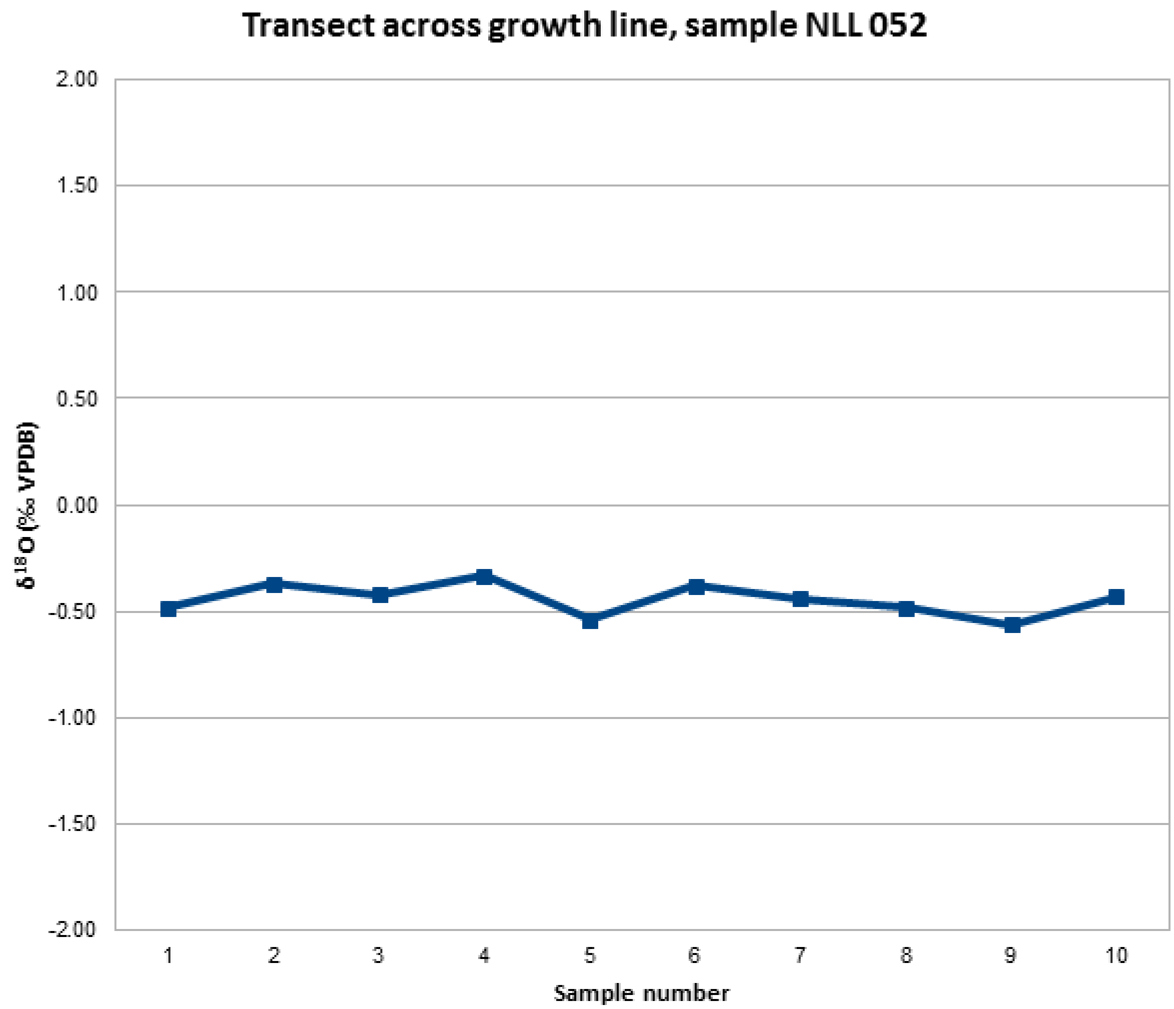


Figure 3-6. Ten samples taken along a single growth line, ~2 cm from the shell margin, in Newnans Lake shell 052.

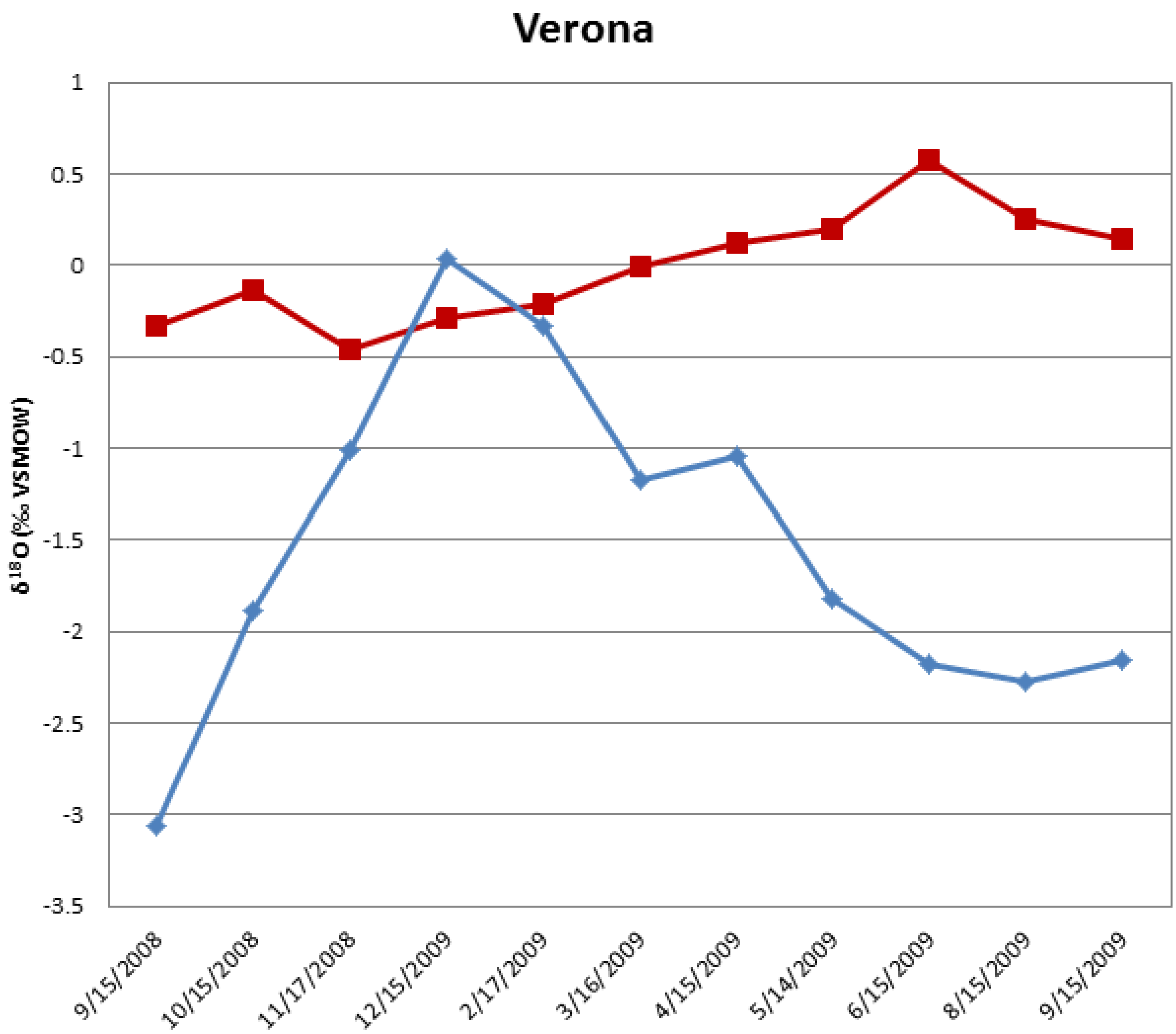


Figure 3-7. Comparison between the expected shell $\delta^{18}O$ profile generated by Grossman and Ku's equation (blue), and the surface water isotopes from Lake Verona (red), showing the strong effect of temperature on shell isotope variation.

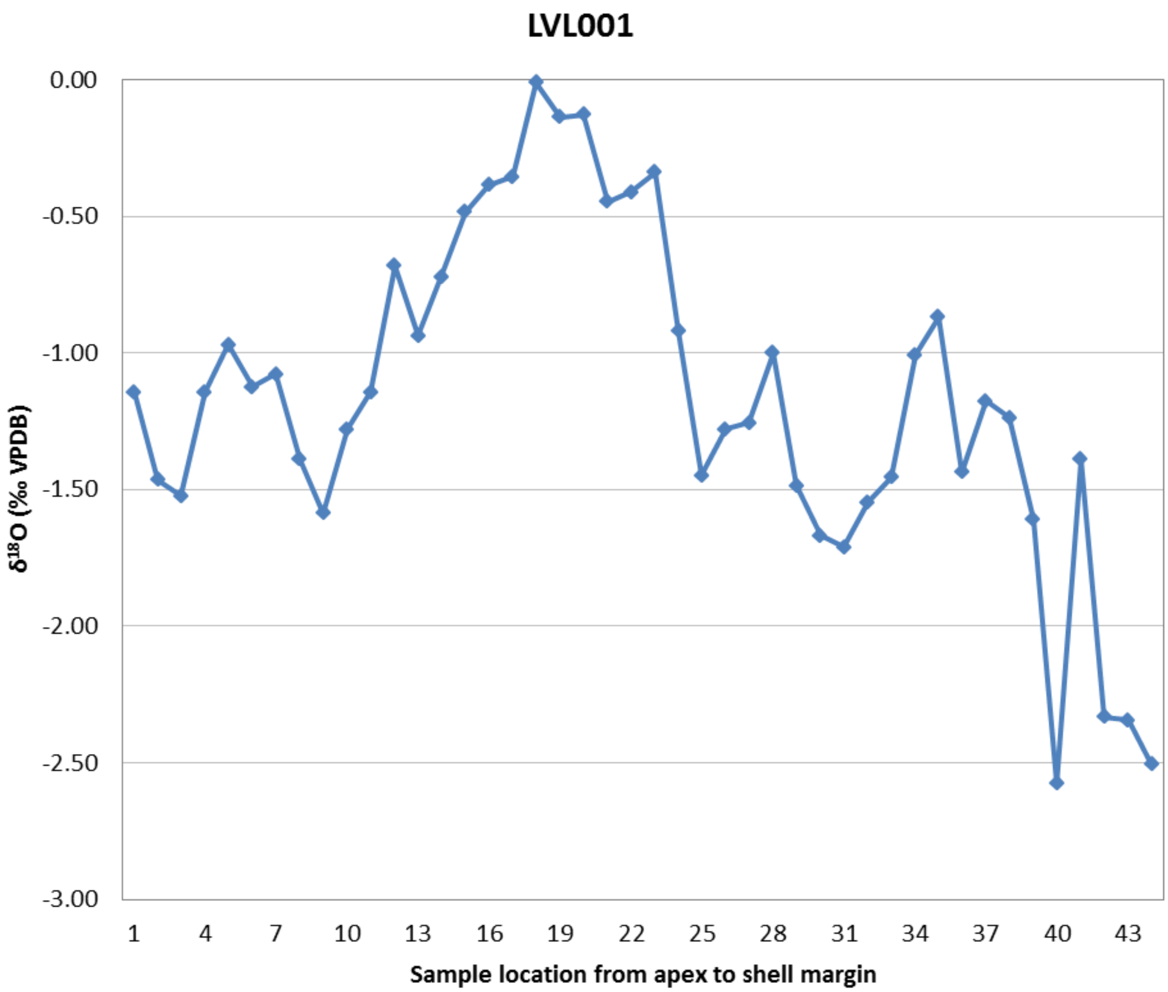


Figure 3-8. Shell profile generated by drilling along the growth axis of Lake Verona shell LVL 001. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

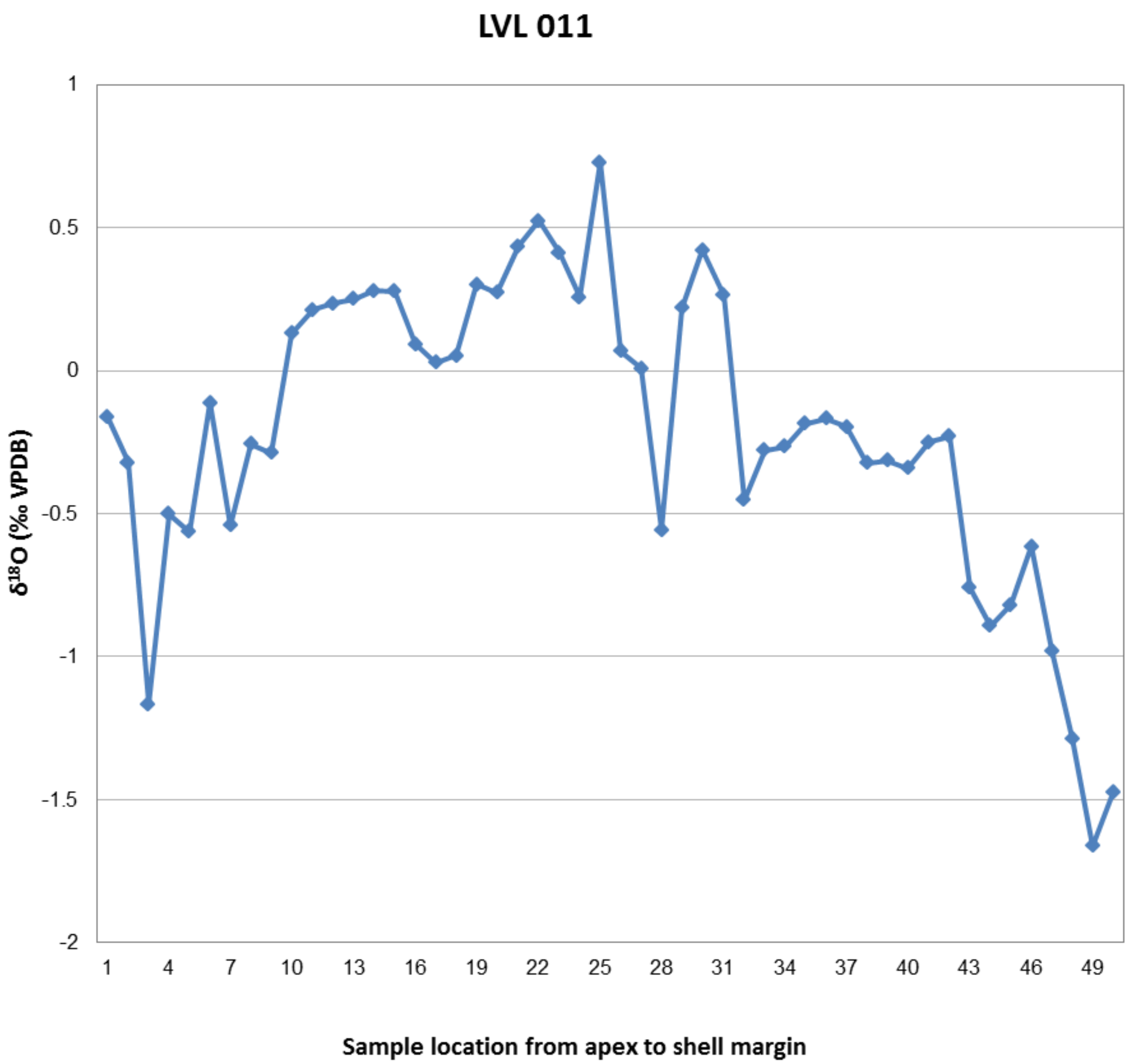


Figure 3-9. Shell profile generated by drilling along the growth axis of the Lake Verona shell LVL 011. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

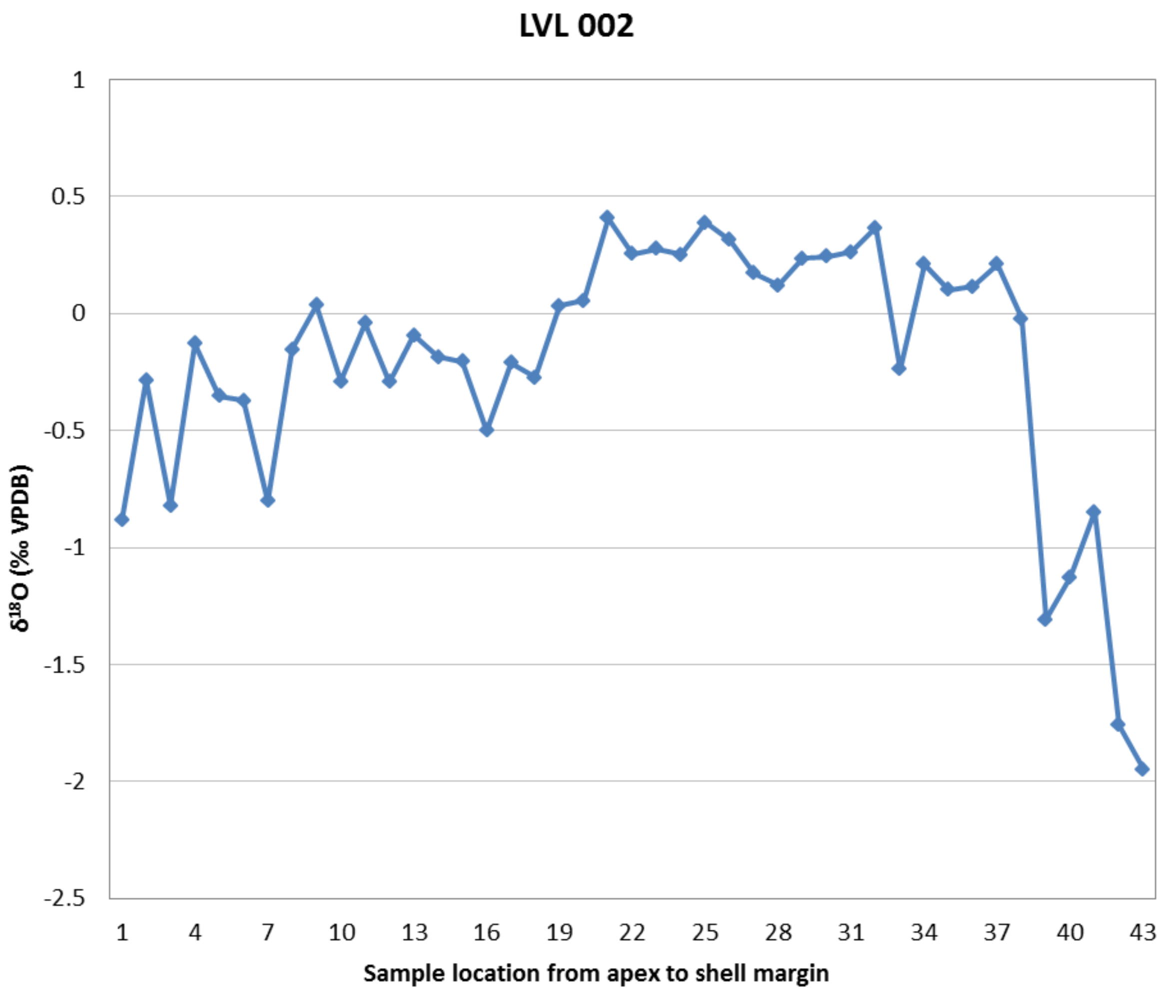


Figure 3-10. Shell profile generated by drilling along the growth axis of the Lake Verona shell LVL 002. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lacks multiple maxima or minima, indicating that it lived less than one year.

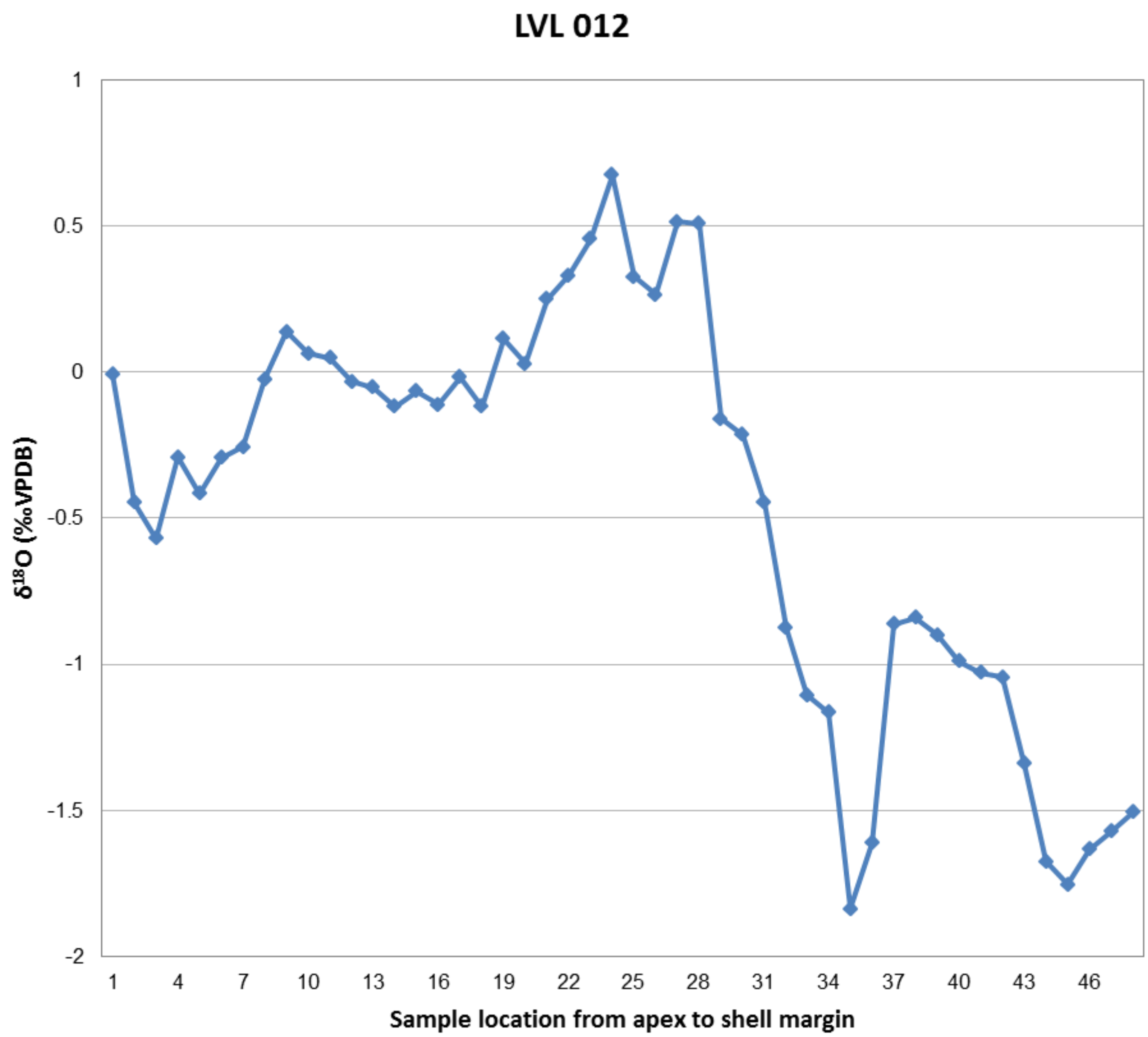


Figure 3-11. Shell profile generated by drilling along the growth axis of the Lake Verona shell LVL 012. More positive values (enriched in ^{18}O) correspond to winter months, while the more negative values (depleted in ^{18}O) correspond to summer months. An annual cycle spans one isotopic minimum or maximum to the next. This snail lacks multiple maxima or minima, indicating that it lived less than one year.

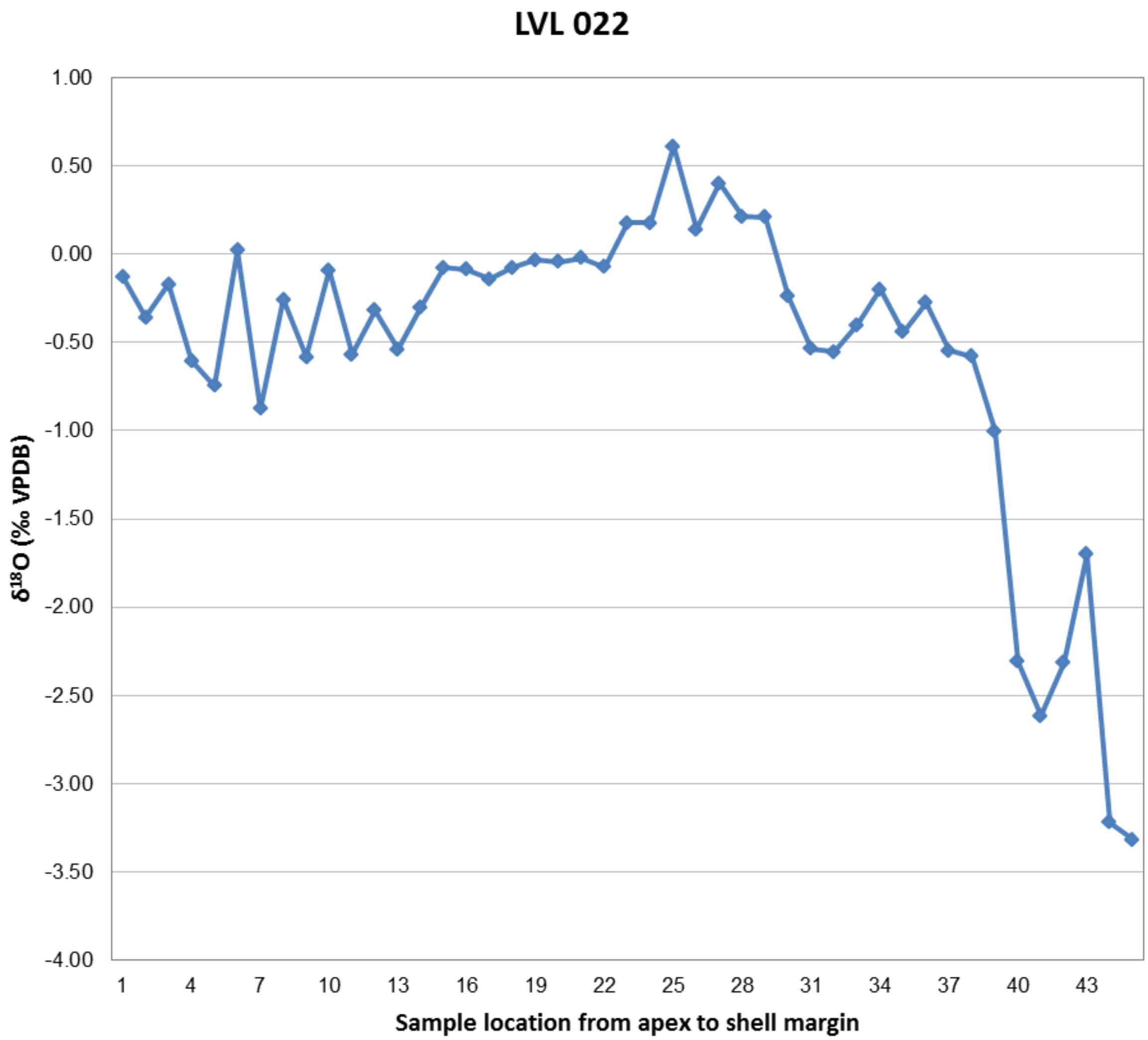


Figure 3-12. Shell profile generated by drilling along the growth axis of the Lake Verona shell LVL 022. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lacks multiple maxima or minima, indicating that it lived less than one year.

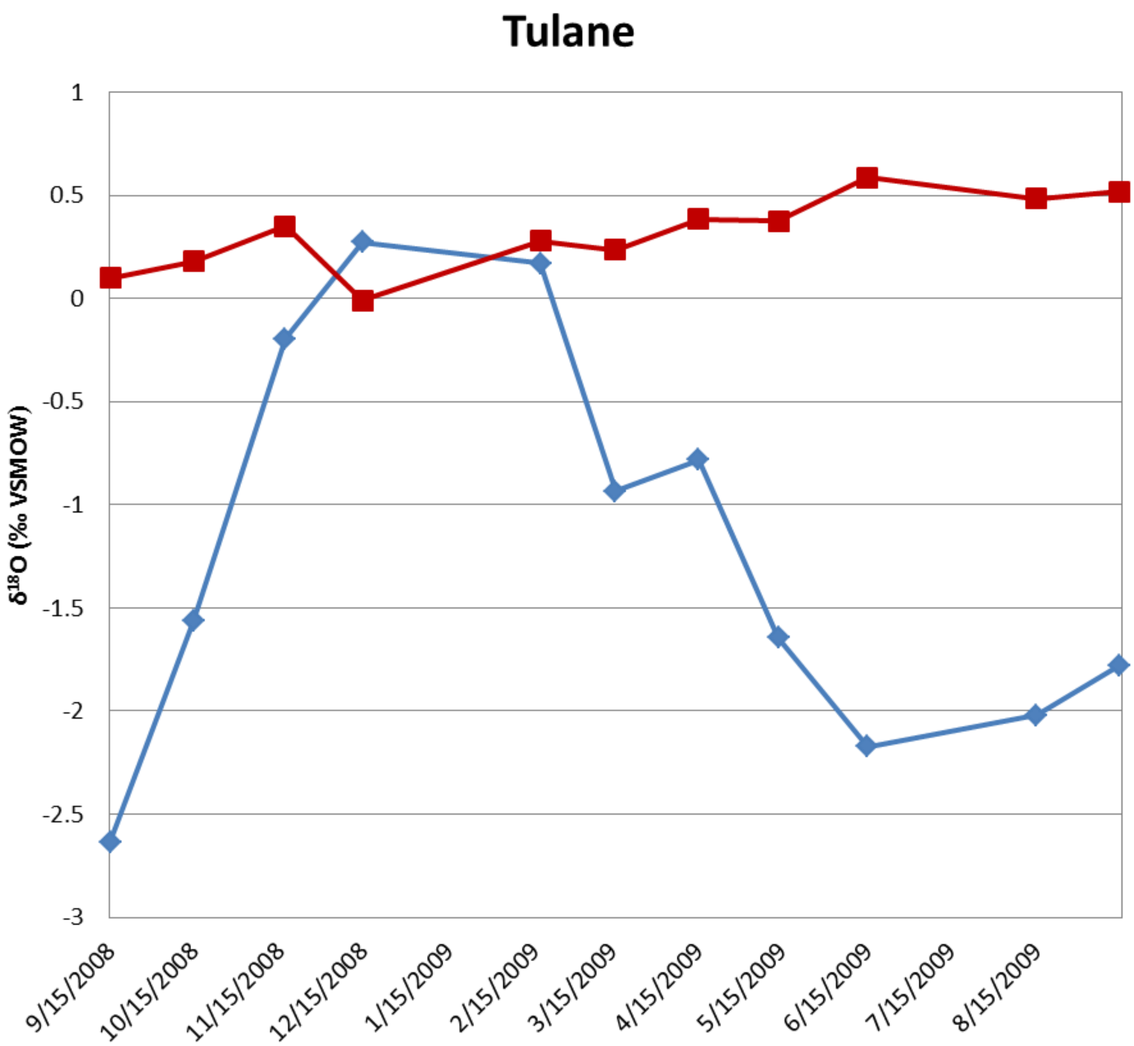


Figure 3-13. Comparison between the expected shell $\delta^{18}O$ profile generated by Grossman and Ku's equation (blue), and the surface water isotopes from Lake Tulane (red), showing the strong effect of temperature on shell isotope variations.

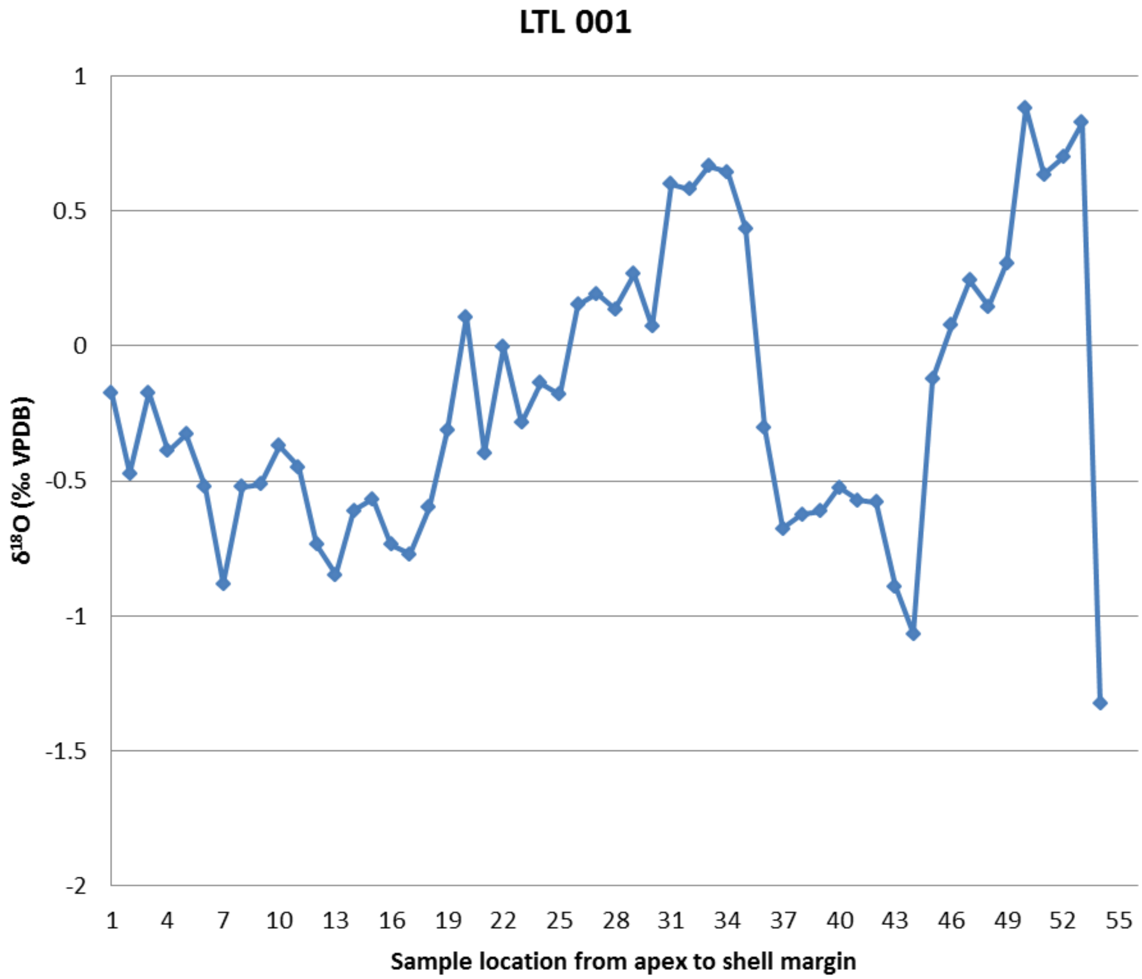


Figure 3-14. Shell profile generated by drilling along the growth axis of the Lake Tulane shell LTL 001. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived slightly longer than two years.

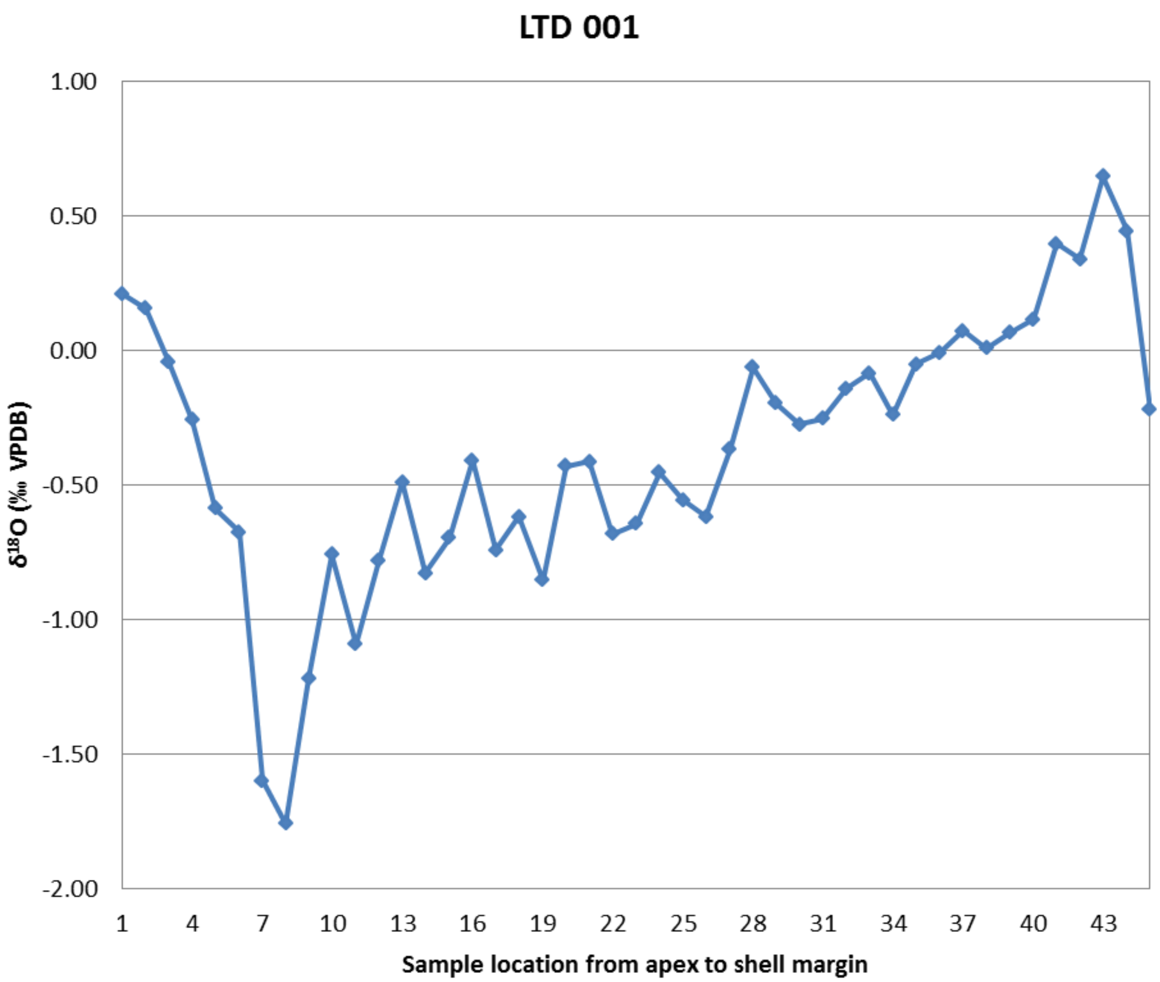


Figure 3-15. Shell profile generated by drilling along the growth axis of the Lake Tulane shell LTD 001. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived just over one year.

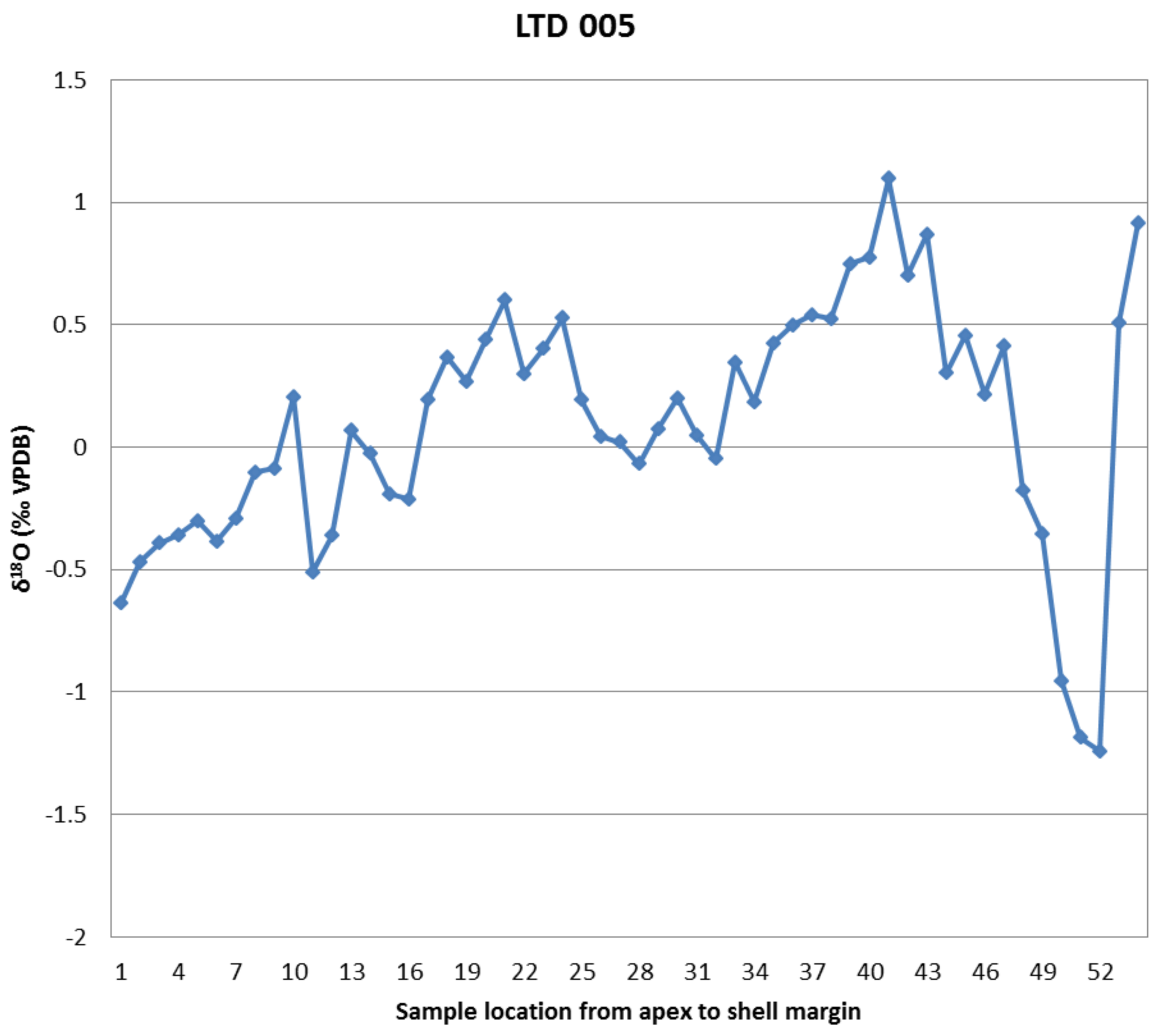


Figure 3-16. Shell profile generated by drilling along the growth axis of the Lake Tulane shell LTD 005. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived slightly longer than one year.

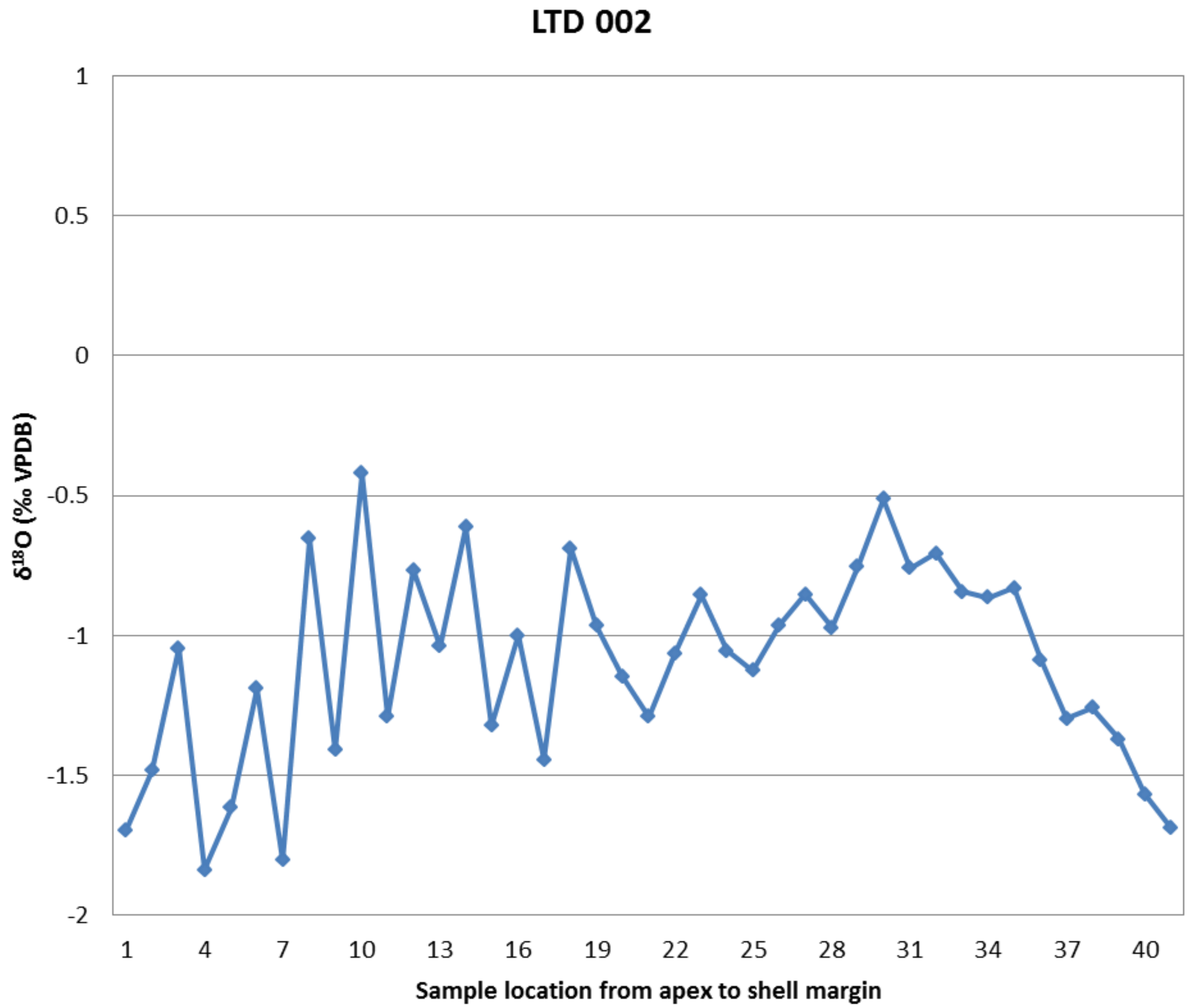


Figure 3-17. Shell profile generated by drilling along the growth axis of the Lake Tulane shell LTD 002. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. Without a clearly defined maximum and minimum this profile is difficult to interpret.

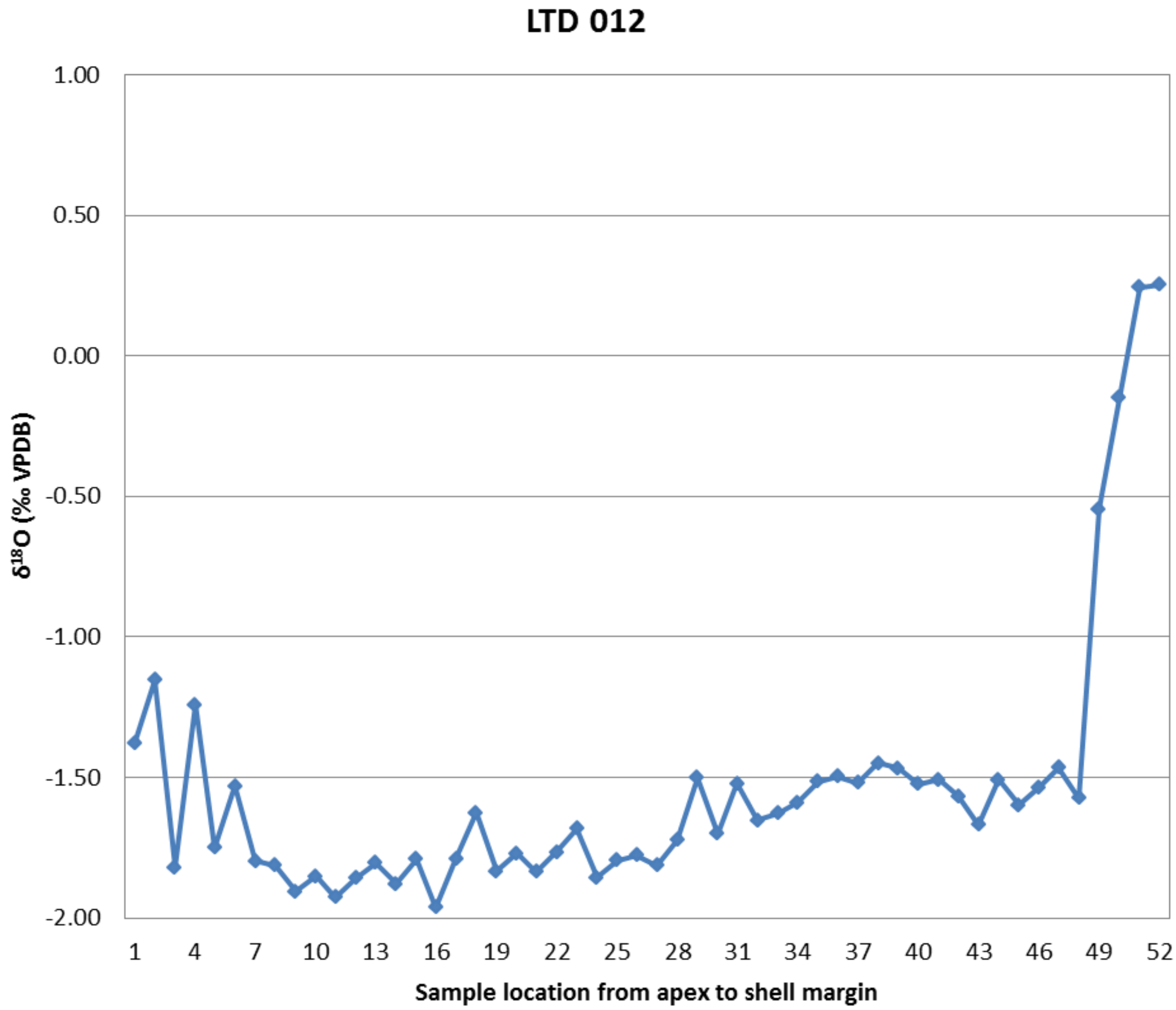


Figure 3-18. Shell profile generated by drilling along the growth axis of the Lake Tulane shell LTD 012. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. Without a clearly defined maximum and minimum this profile is difficult to interpret.

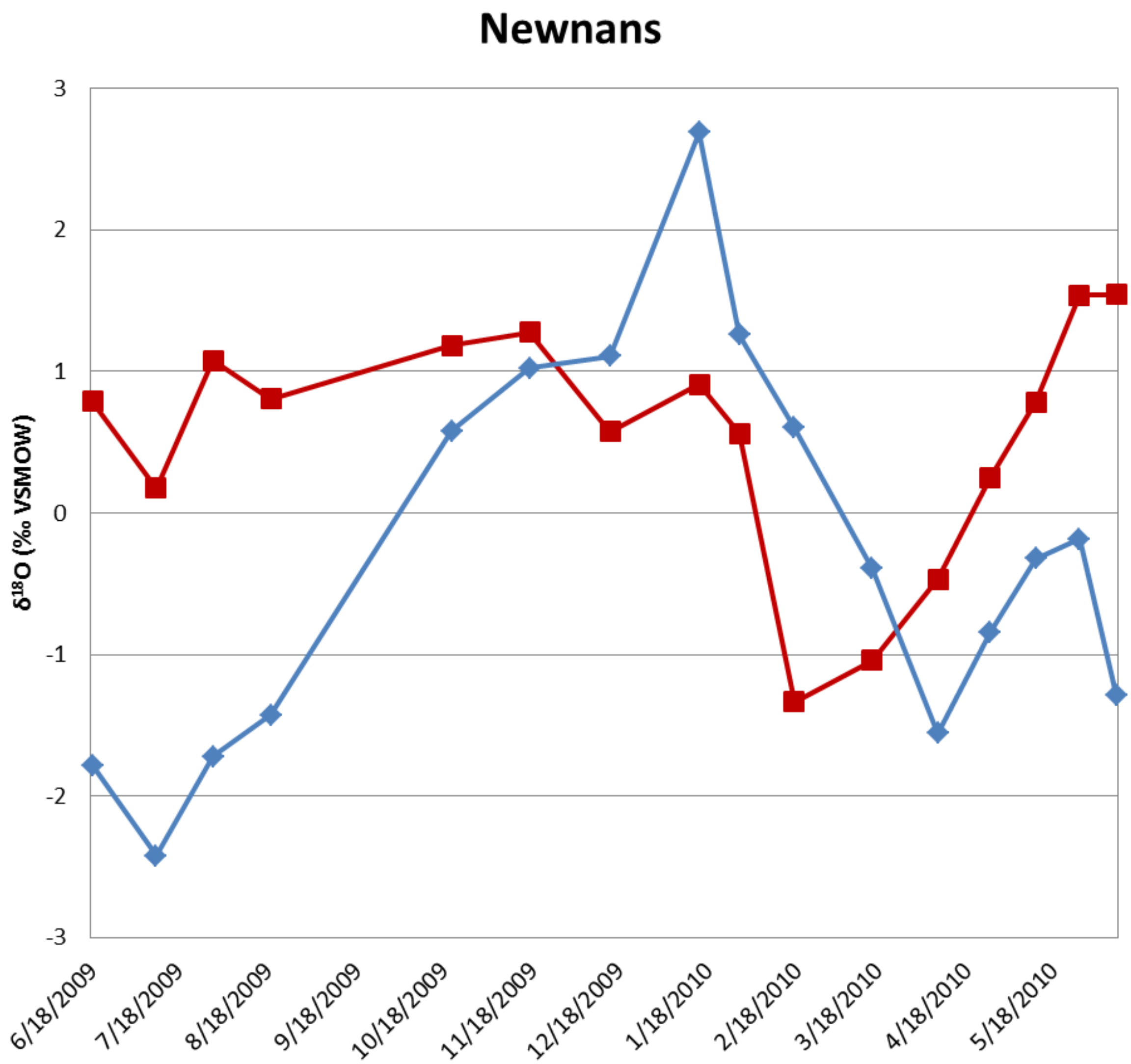


Figure 3-19. Comparison between the expected shell $\delta^{18}O$ profile generated by Grossman and Ku's equation (blue), and the surface water isotopes from Newnans Lake (red), showing the strong effect of temperature on shell isotope variations.

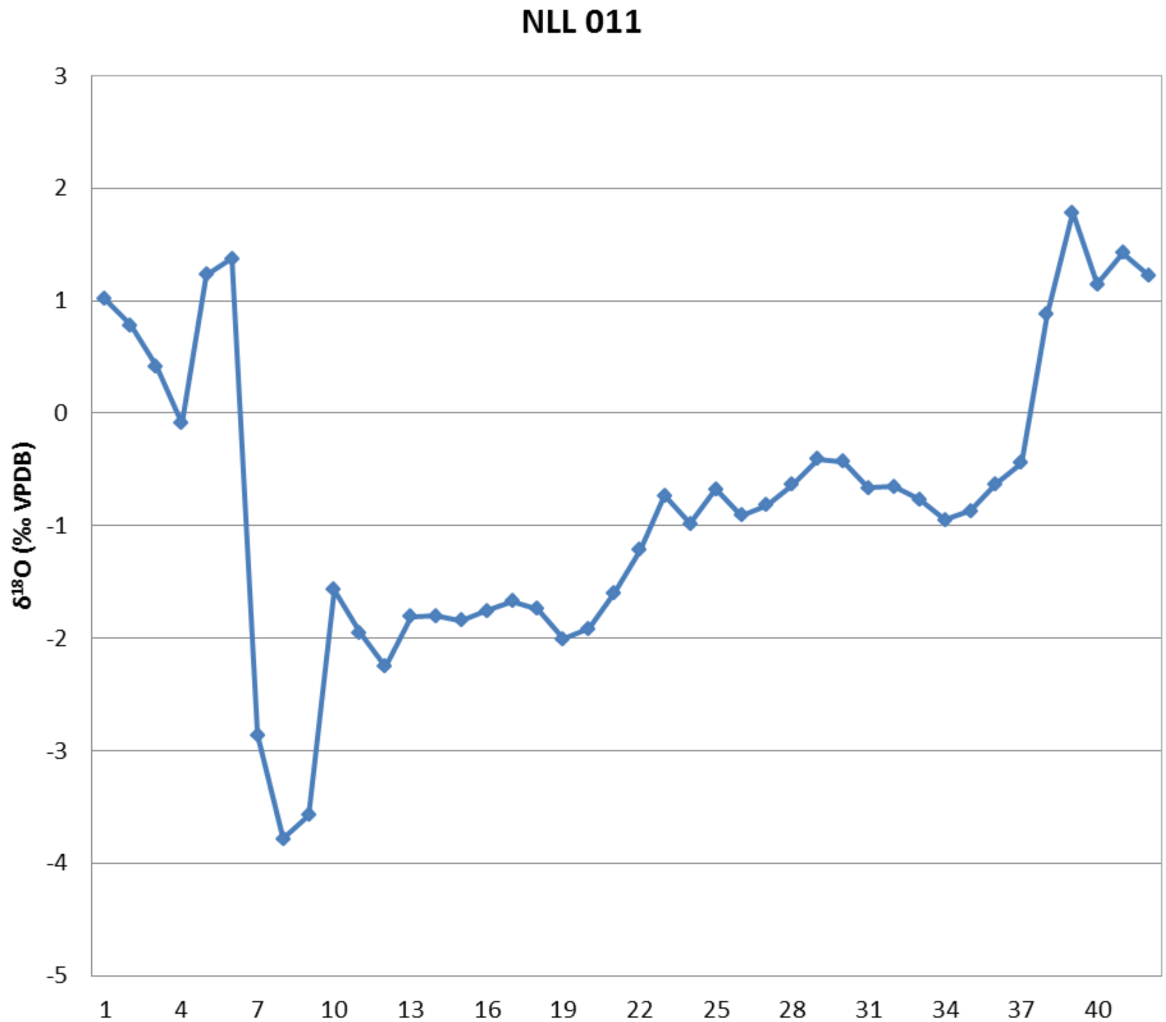


Figure 3-20. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 011. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

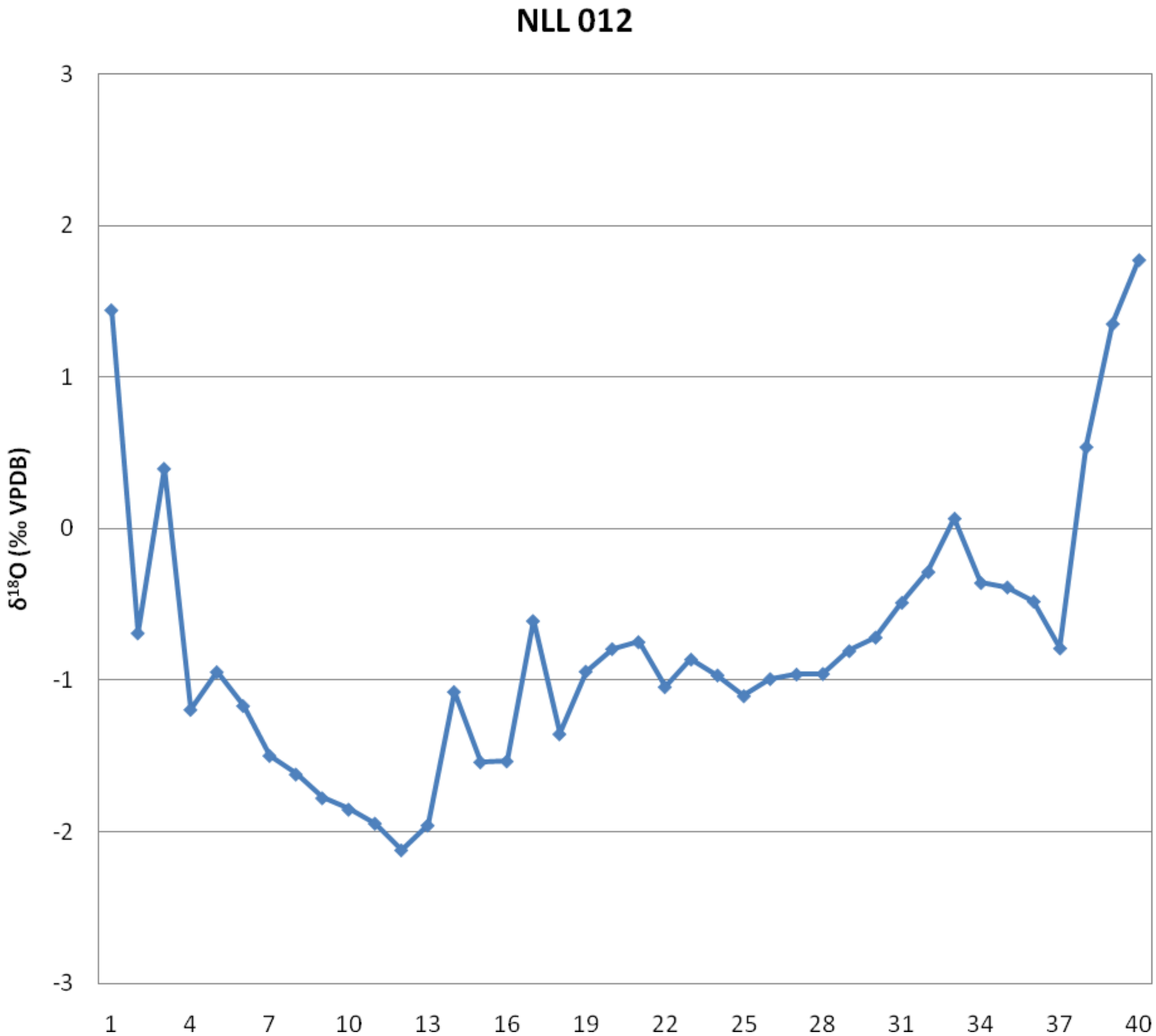


Figure 3-21. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 012. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

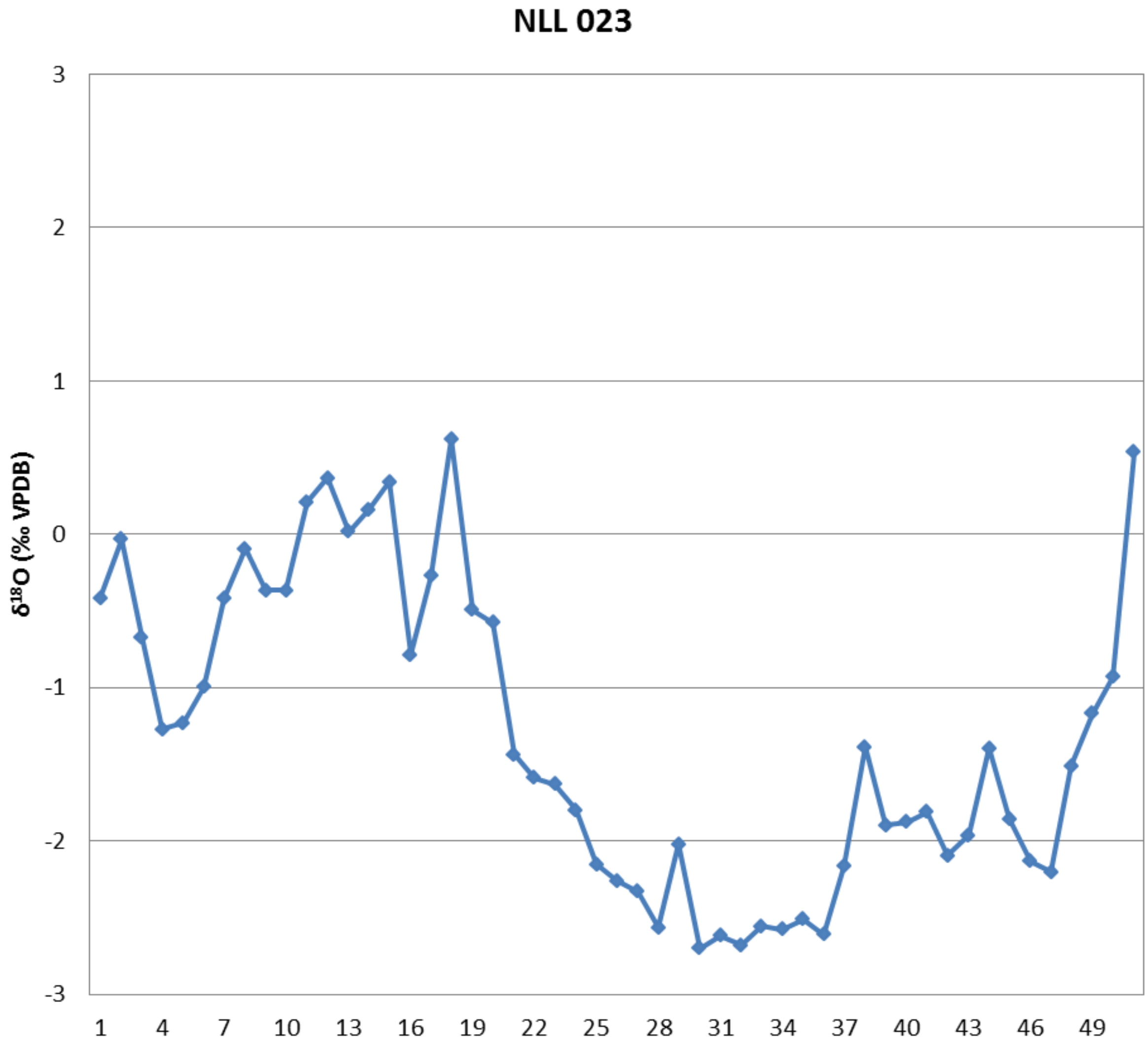


Figure 3-22. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 023. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

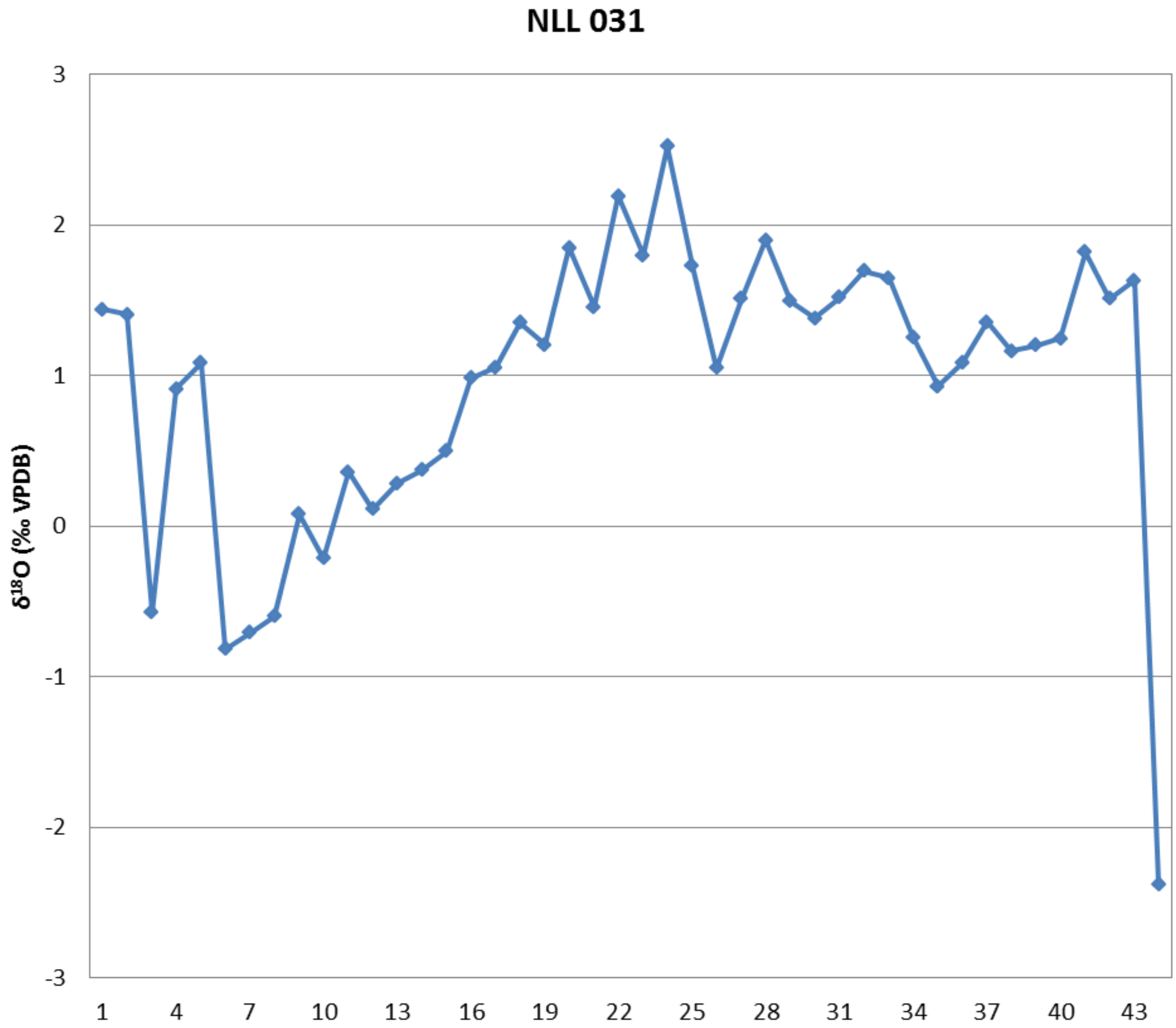


Figure 3-23. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 031. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

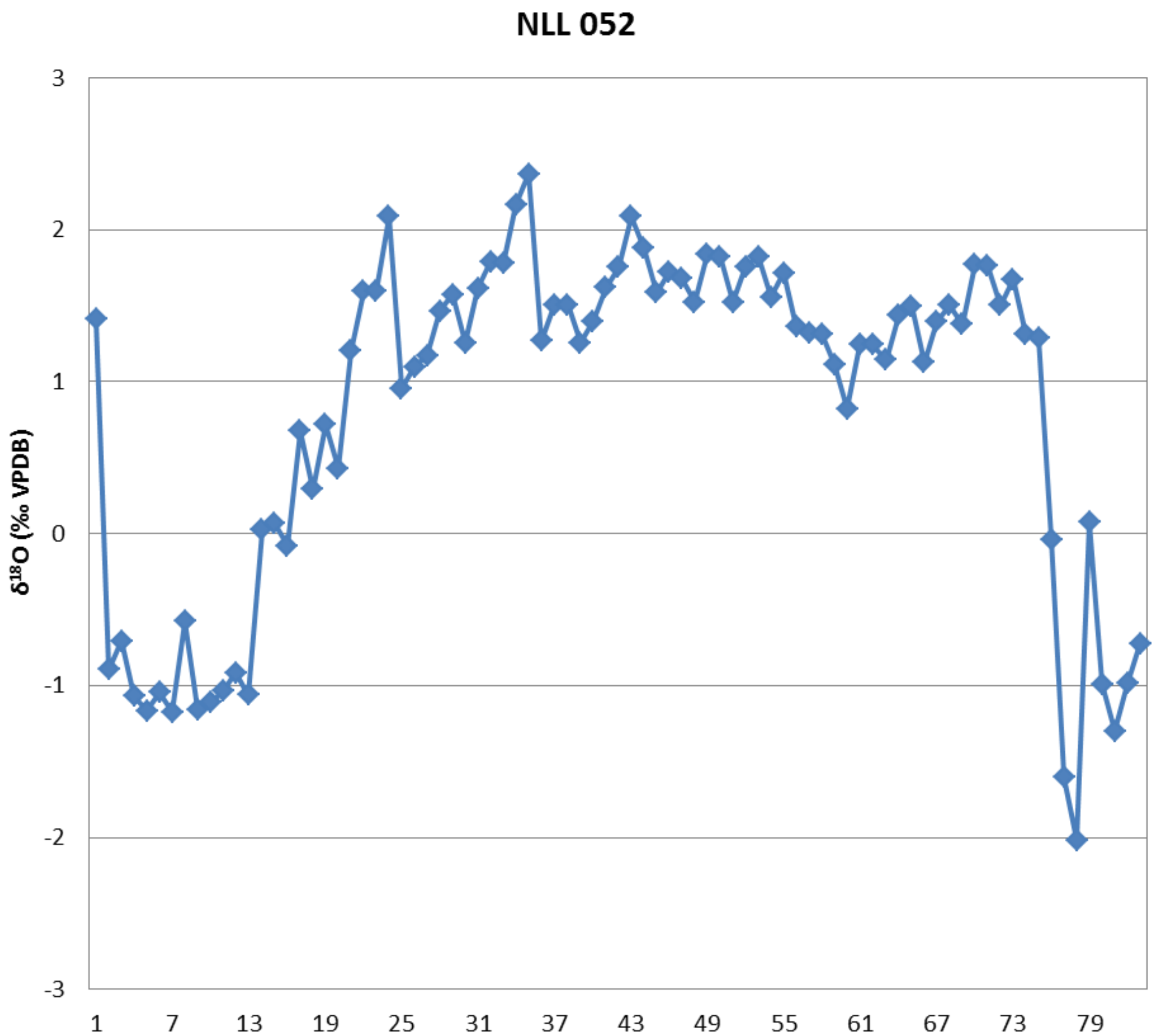


Figure 3-24. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 052. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived for approximately one year.

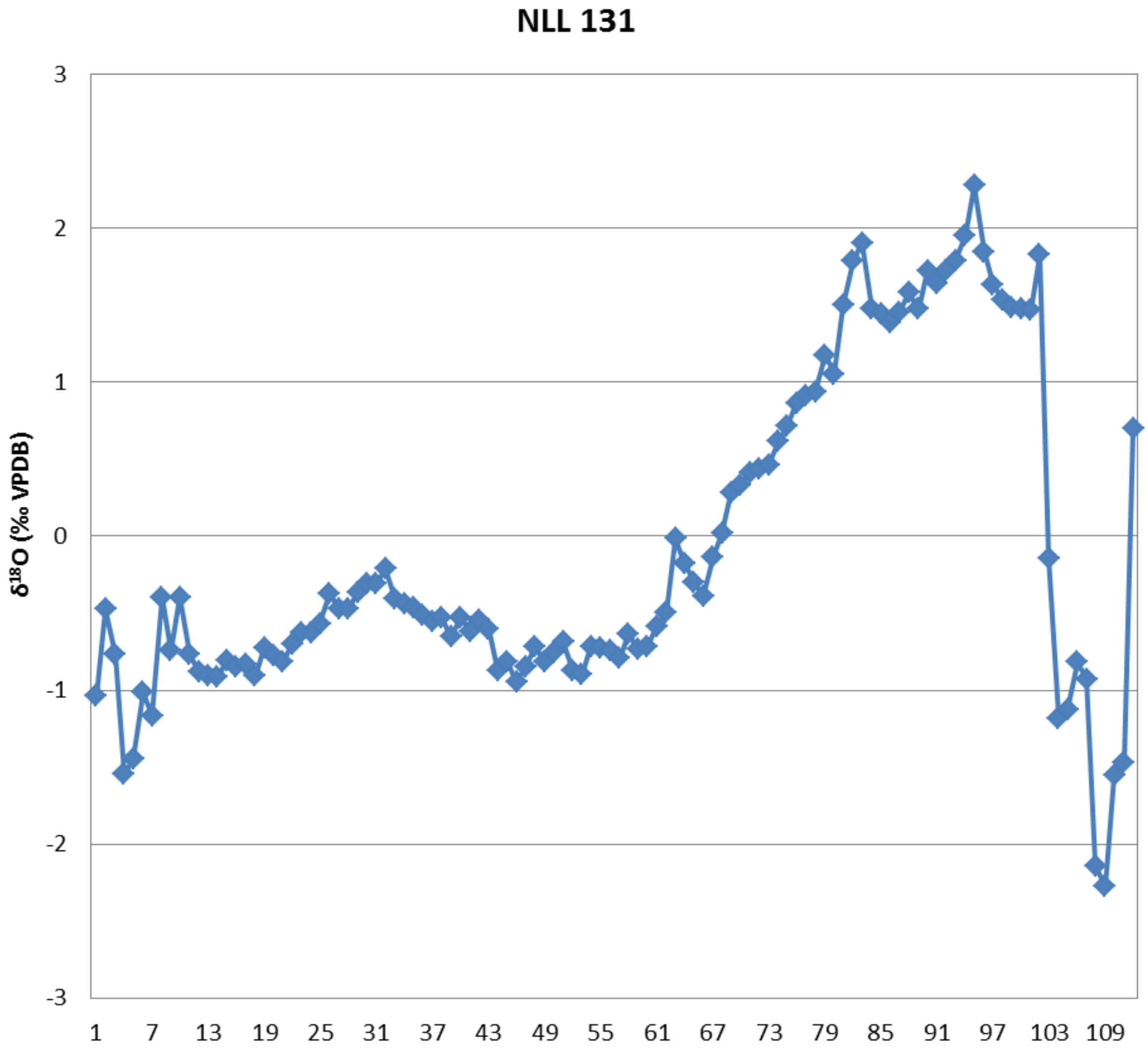


Figure 3-25. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 131. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived between one and two years.

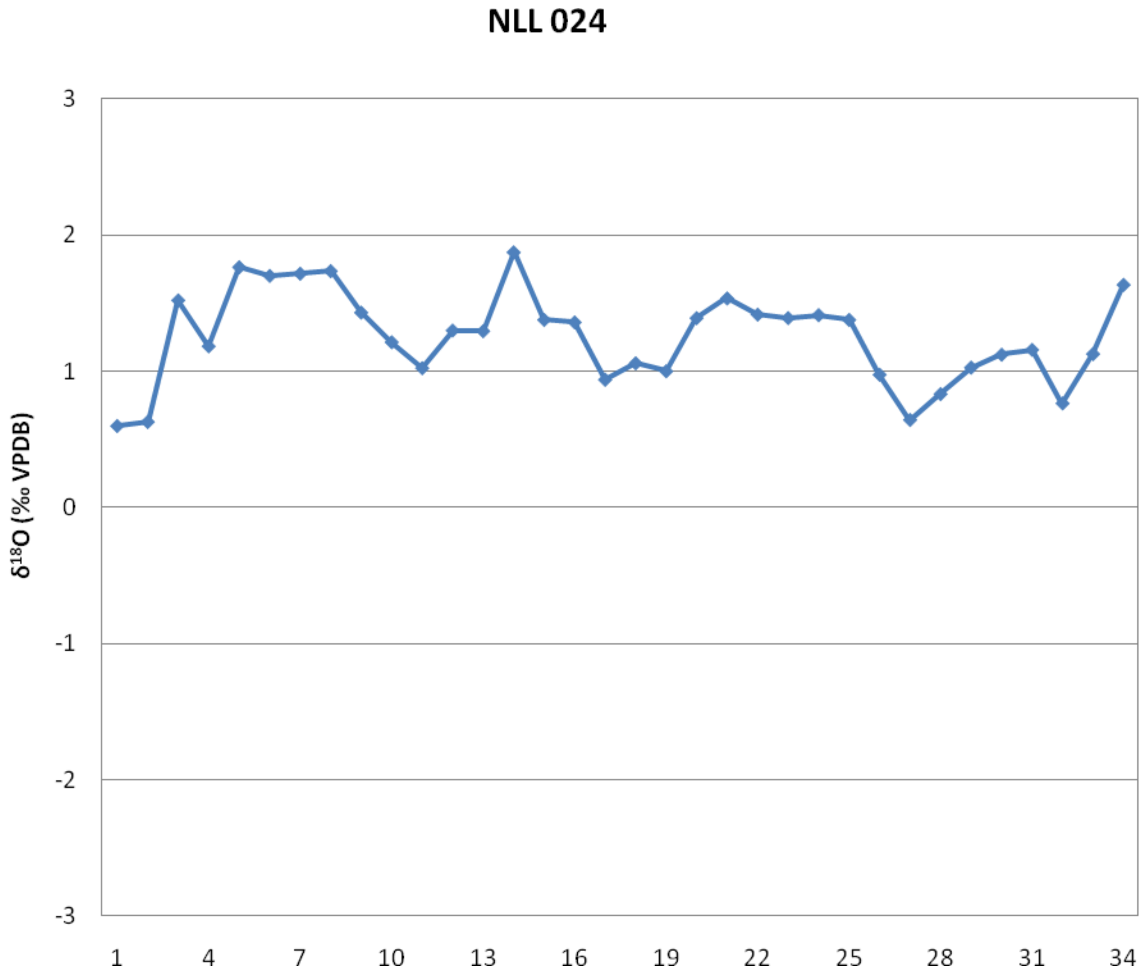


Figure 3-26. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 024. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived less than one year.

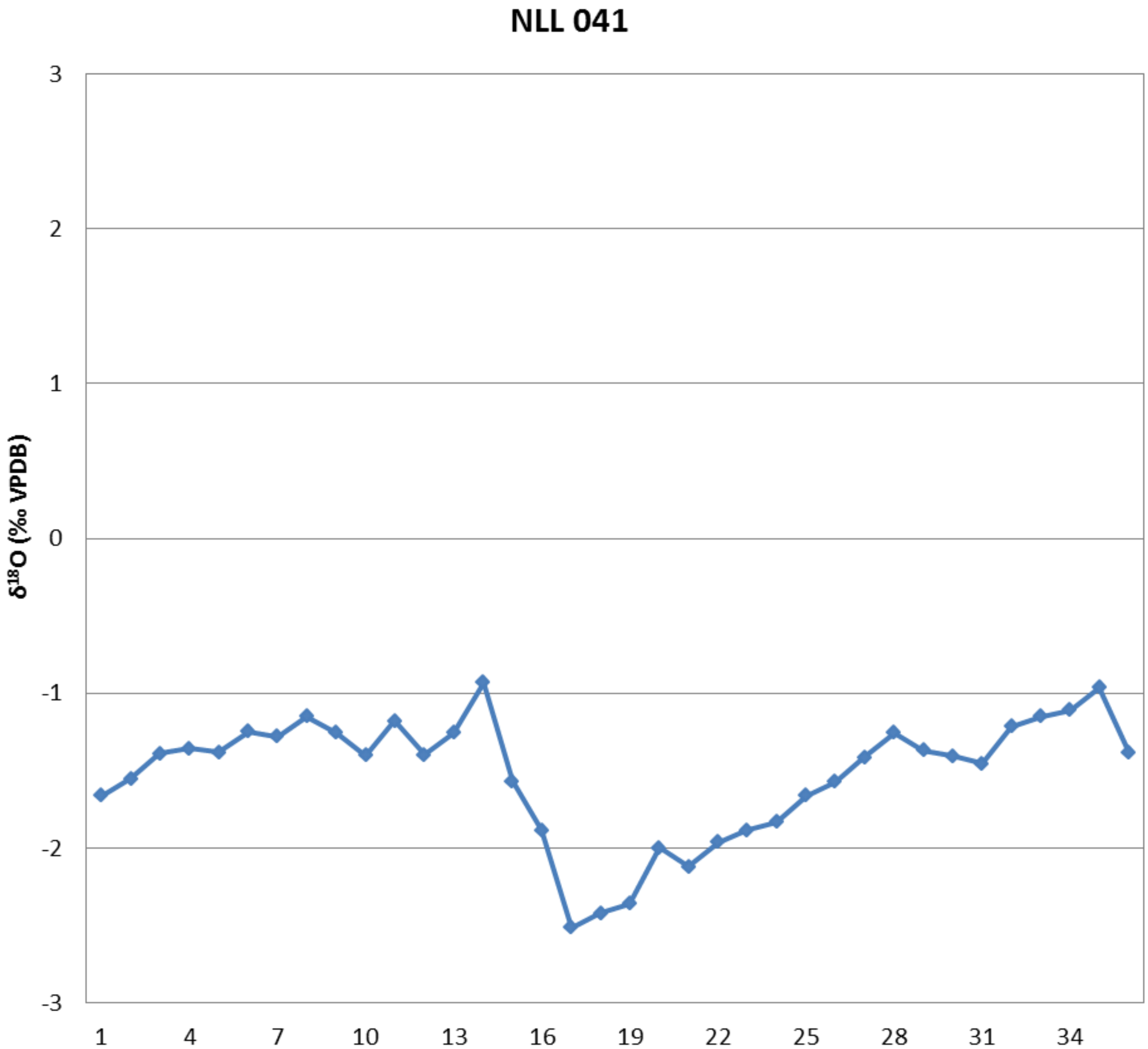


Figure 3-27. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 041. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived less than one year.

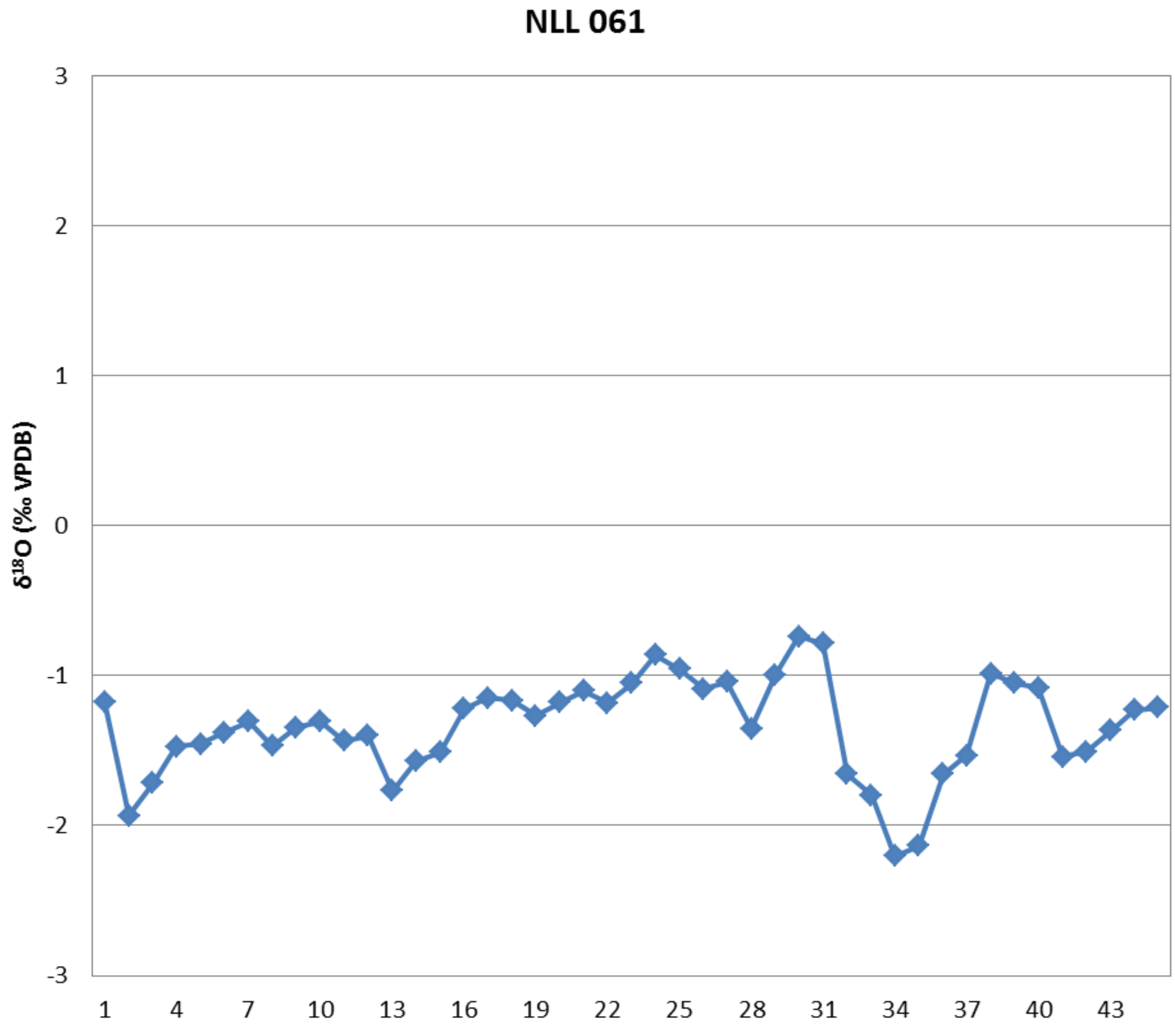


Figure 3-28. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 061. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived less than one year.

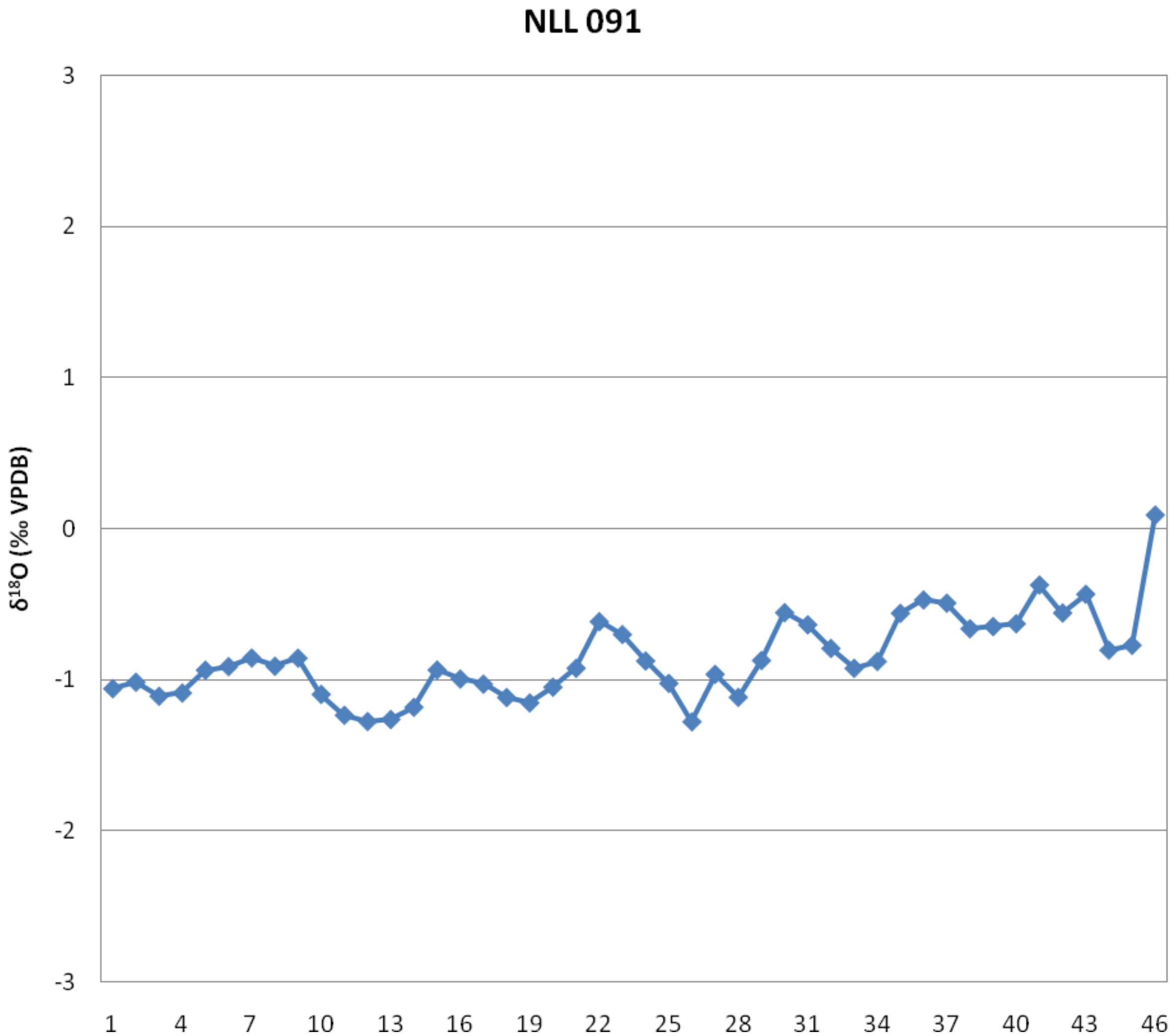


Figure 3-29. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 091. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived less than one year.

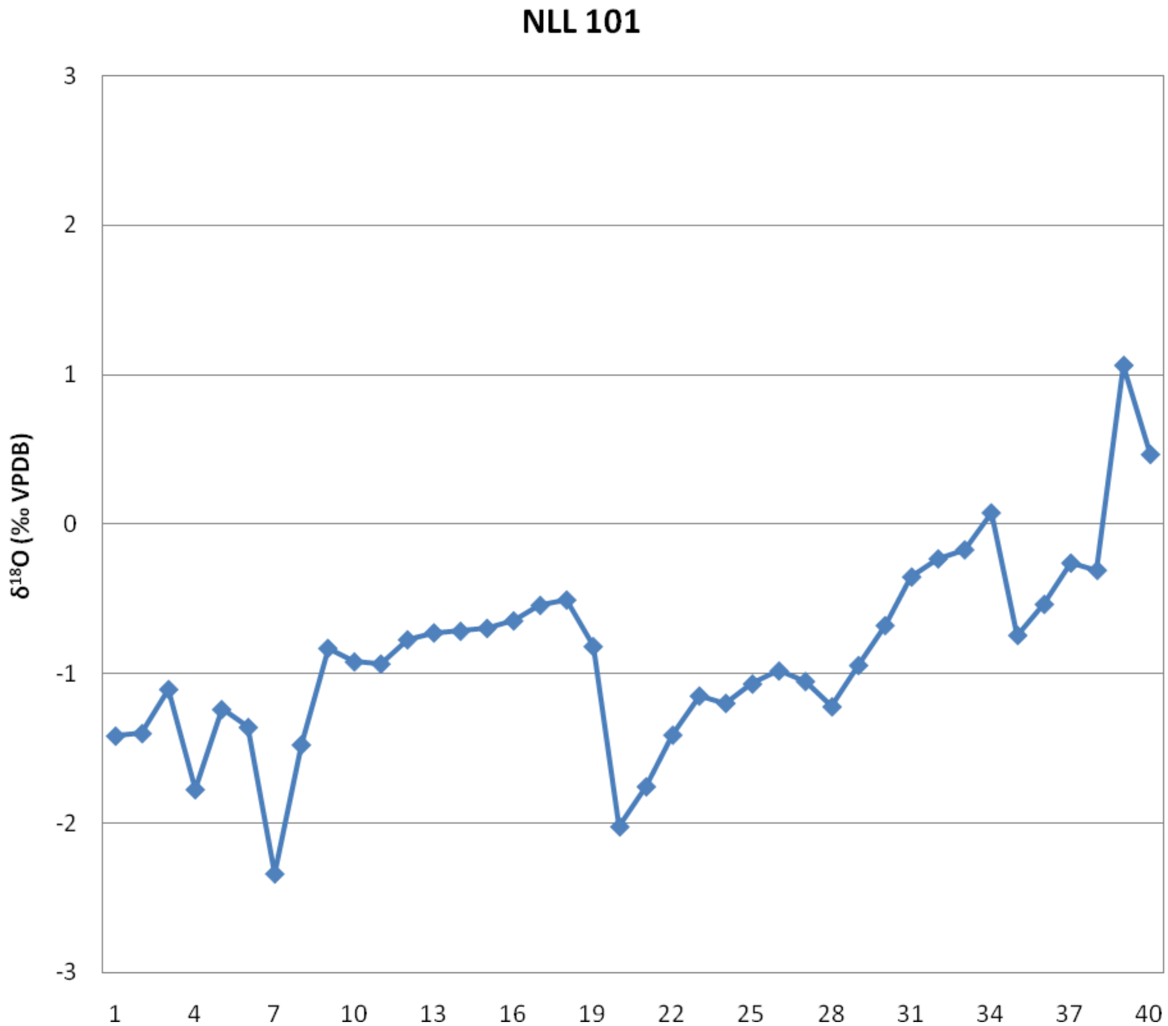


Figure 3-30. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 101. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. This snail lived less than one year.

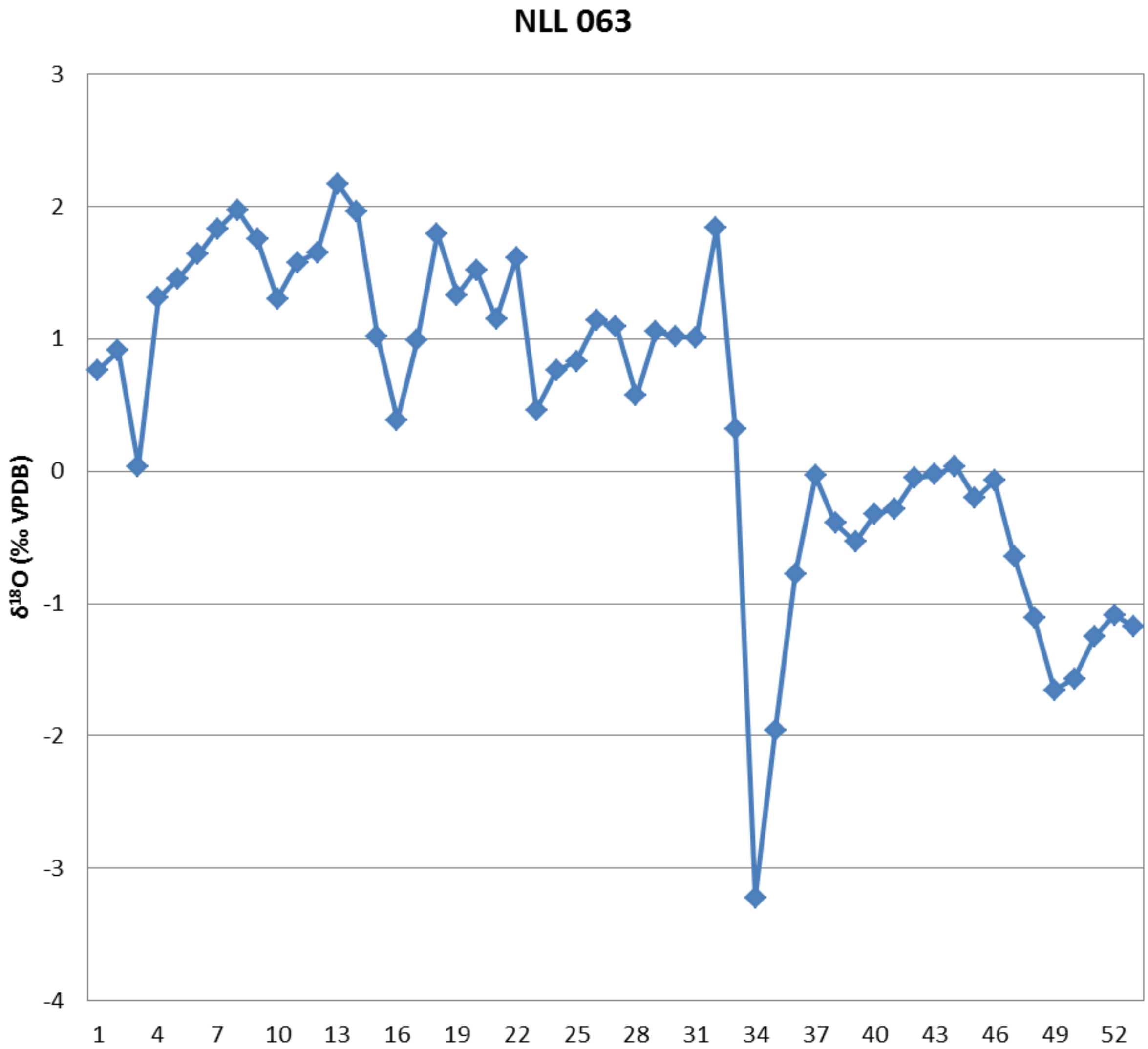


Figure 3-31. Shell profile generated by drilling along the growth axis of the Newnans Lake shell NLL 063. More positive values (enriched in ^{18}O) corresponded to winter months, while the more negative values (depleted in ^{18}O) corresponded to summer months. An annual cycle spanned one isotopic minimum or maximum to the next. The age of this snail could not be determined accurately.

Table 3-1. $\delta^{18}O_{aragonite}$ values for the snails raised in a temperature-controlled setting. Sample locations are taken from the central portions of the growth whorl. Variable sample numbers are the result of different growth rates. All values are ‰ vs. VPDB.

Control Sample	C-001	C-002	C-003	C-004	All values
Sample location 1	-3.91	-3.29	-3.33	-3.46	-
Sample location 2	-3.55	-3.56	-3.89	-3.00	-
Sample location 3	-3.66	-3.46	-3.31	-3.31	-
Samples location 4	-	-3.64	-3.47	-3.35	-
Samples location 5	-	-3.74	-	-3.18	-
Average δ18O	-3.71	-3.54	-3.50	-3.26	-3.48
Maximum departure from avg. δ18O value within each shell	0.21	0.24	0.39	0.26	-
Average departure from mean value within each shell	0.14	0.13	0.26	0.08	-
Departure from mean value of all shells	0.23	0.06	0.02	0.22	-

Table 3-2. $\delta^{18}O_{aragonite}$ values along the shell margin (most recent period of carbonate deposition) of the snails collected from Newnans Lake. Numbers in each sample name correspond to a collection date, thus samples with identical second numbers were collected on the same day (e.g. NLL 011 and NLL 012 were collected on the same day, as were NLL 031 and NLL 032). Up to five drilling locations were selected. In some shells, certain sites were damaged and could not be drilled.

Sample Location	1	2	3	4	5
NLL 011	-	1.64	-	0.60	-0.59
NLL 012	1.21	1.22	0.77	1.14	-0.24
NLL 021	1.48	-1.29	-1.55	-1.26	-
NLL 022	-0.22	-1.75	-	-0.76	-
NLL 023	0.54	1.26	-	-0.23	-1.43
NLL 024	-	-1.33	-1.51	-	-1.31
NLL 031	-0.83	-0.70	-0.95	-0.83	-
NLL 032	-0.60	-0.73	-0.68	-0.61	-
NLL 041	-1.55	-1.25	-0.94	-	-
NLL 051	-0.54	-0.43	-0.46	-0.25	-
NLL 052	-0.72	-0.92	-1.22	-0.80	-0.99
NLL 061	-0.92	-1.12	-1.06	-0.91	-0.67
NLL 062	-0.66	-0.63	-0.60	-0.73	-
NLL 063	-1.38	-1.34	-1.10	-1.05	-1.38
NLL 064	-0.91	-0.56	-0.84	-0.82	-
NLL 071	0.57	-1.08	-	-0.74	-0.80
NLL 081	0.02	0.27	0.78	1.33	-
NLL 091	0.37	1.13	1.36	1.56	0.37
NLL 101	0.32	1.17	1.43	-	-
NLL 111	-0.55	0.45	1.14	1.36	-
NLL 121	-1.07	-0.88	-1.28	-1.21	-
NLL 131	0.701	-0.94	-1.48	-1.25	-
NLL 141	-0.23	-0.15	-0.31	0.08	-
NLL 151	-0.22	-0.28	-0.44	-	-0.32

Table 3-3. Shell growth of snails collected from Lake Verona.

Sample No.	$\delta^{18}O$ Range (‰)	$\delta^{18}O$ max (‰)	$\delta^{18}O$ min (‰)	Shell Weight (g)	Total linear growth (cm)	June-Sept. growth (cm)	Oct.-Feb. growth (cm)	Age
LVL 001	2.57	-0.01	2.58	26.14	22.0	1.5/7%	20/91%	~1 yr
LVL 002	2.36	0.41	-1.95	28.49	22.0	0/0%	21/95%	<1 yr
LVL 011	2.39	0.73	-1.66	43.23	20.4	0/0%	20.4/100%	~1 yr
LVL 012	2.51	0.67	-1.84	34.23	19.6	0/0%	19.6/100%	<1 yr
LVL 022	3.93	0.61	-3.32	36.81	23.0	2.5/11%	20/89%	<1 yr
Averages	2.75	0.482	-2.27	33.78	21.4			

Table 3-4. Shell growth of snails collected from Lake Tulane.

Sample No.	$\delta^{18}O$ Range (‰)	$\delta^{18}O$ max (‰)	$\delta^{18}O$ min (‰)	Shell Weight (g)	Total linear growth (cm)	June-Sept. growth (cm)	Oct.-Feb. growth (cm)	Age
LTD 001	2.41	0.65	-1.76	37.26	22.5	0.5/2%	21.5/96%	~1 yr
LTD 002	1.41	-0.42	-1.83	45.18	21.0	1/5%	18/86%	?
LTD 005	2.34	1.10	-1.24	56.49	27.5	0/0%	27.5/100%	~1 yr
LTD 012	2.21	0.25	-1.96	41.8	21.2	8/38%	9.2/43%	?
LTL 001	2.20	0.88	-1.32	55.32	22.0	0/0%	22/100%	~2 yrs
Average	2.11	0.49	-1.62	47.21	22.84			

Table 3-5. Shell growth of snails collected from Newnans Lake.

Sample No.	$\delta^{18}O$ Range (‰)	$\delta^{18}O$ max (‰)	$\delta^{18}O$ min (‰)	Shell Weight (g)	Total linear growth (cm)	June-Aug growth (cm)	Dec.-Feb. growth (cm)	Age
NLL 011	5.56	1.78	-3.78	17.74	22.5	8/36%	5/22%	1yr
NLL 012	3.89	1.77	-2.12	13.39	20.5	5/24%	2/10%	1yr
NLL 023	3.32	0.62	-2.70	14.59	21.6	15/70%	.4/2%	1 yr
NLL 024	1.28	1.88	0.64	8.23	14.0	0/0%	14/100%	<1yr
NLL 031	4.90	2.52	-2.38	16.56	22.5	1/4%	16/71%	1 yr
NLL 041	1.58	-0.93	-2.51	6.21	14.8	10/68%	0/0%	<1yr
NLL 052	4.39	2.37	-2.02	21.18	26.0	0.6/2.3%	14.3/55%	1yr
NLL 061	1.46	-0.74	-2.20	19.72	18.4	9.6/52%	0/0%	<1yr
NLL 063	5.39	2.17	-3.2	14.78	21.6	1.6/7%	11.2/52%	?
NLL 091	1.37	0.09	-1.28	19.21	18.8	0/0%	0/0%	<1yr
NLL 101	3.40	1.06	-2.34	6.83	12.3	2.7/22%	.3/2%	<1yr
NLL 131	4.55	2.28	-2.27	24.76	27.8	1.2/4%	6.2/22%	1yr
Averages	3.42	1.23	-2.18	15.24				

DISCUSSION

Lake Water δ^{18}O and Temperature

In south central Florida lakes Tulane and Verona, the oxygen isotopic composition of the water remained relatively stable throughout the year (J. Escobar personal communication). Surface waters in both lakes showed small seasonal differences in δ^{18}O. The δ^{18}O values were slightly higher in summer than winter, by ~1‰ in Verona and ~0.5‰ in Tulane. This seasonal shift is likely driven, in part, by relatively greater evaporation/precipitation (E/P) in late spring and early summer. Newnans Lake displayed more variable $\delta^{18}O_{water}$, with a 1.89‰ decrease from January to February, and lacked the seasonal ^{18}O enrichment identified in Lakes Tulane and Verona. The abrupt winter decline in $\delta^{18}O_{water}$ at Newnans Lake may have been driven by a rainfall source effect. During non-El Niño years, winter rainfall in north Florida is derived locally, from storms in the Gulf of Mexico (Schmidt et al., 2000). In El Niño years, precipitation over Florida originates from air masses formed over the Pacific Ocean (Ropelewski and Halpert, 1996) and the heavier isotope is preferentially rained out as the mass moves eastward over North America (Mook, 2006). By the time it reaches Florida, the rainwater is depleted with respect to the heavy isotope.

The average annual temperature range of the three lakes was 16.94°C. According to Grossman and Ku's fractionation equation, across a range of 16.94°C, the range of $\delta^{18}O_{aragonite}$ values should be 3.90‰. The average $\delta^{18}O_{aragonite}$ of all the shells collected was 3.04‰. The close approximation of these two suggests that temperature was the major driver of $\delta^{18}O_{aragonite}$ variations in the lakes.

Shell Growth Patterns

Control Snails

The four snails raised in the aquarium with constant temperature and $\delta^{18}O_{water}$ displayed relatively invariable $\delta^{18}O_{aragonite}$ along the growth axis of the shells (Fig. 3-5). The average of the standard deviations of $\delta^{18}O$ values in each shell was 0.15‰, attesting to the fact that if ambient conditions are held constant, there is little variation in the isotopic signature of shell carbonate. The snail control group did, however, display a significant offset from values expected given the isotope value and temperature of the water. All were enriched in ^{18}O relative to expected values by as much as 1.83‰, implying a vital effect. This biologically mediated offset may compromise the ability to identify precisely the season of death, but does not hinder age analysis, as discussed below.

Lake Snails

Most of the $\delta^{18}O_{aragonite}$ profiles in snails from the lakes captured, or came close to capturing, the expected seasonal range of isotope values calculated using equation 2. This implies that *P. insularum* shells preserve a record of seasonal temperature change in Florida lakes, especially in lakes for which there is little intra-annual variation in $\delta^{18}O_{water}$. There were, however, noticeable differences in $\delta^{18}O$ values from the shell growth margins of snails collected on the same date from the same lake (Table 3-2)

The oldest snail (LTL 001) was collected from Lake Tulane. Its isotopic profile showed a sinusoidal pattern with well-defined maxima and minima that suggest it lived just over two years. No other snail reached two years of age, with the majority living <1 year. Winter was the primary season of growth for the Tulane and Verona snails, i.e.

most growth occurred when $\delta^{18}O_{aragonite}$ values were relatively enriched. The bulk of the samples collected along the growth axis returned an isotopic signature that fell within the expected range of winter values. Snails from Newnans Lake apparently grew more evenly across the seasons.

The isotope derived ages of *P. insularum* are comparable to the 1-1.5 year lifespan of the native apple snail, *Pomacea paludosa* (Darby et al., 2003). The measurements obtained in this study, however, are somewhat different from the observations of Conner et al., 2008, who reported that the invasive snail lives longer than the native species. Both winter and summer growth has been found to cease in some unionid bivalves (Dettman et al., 1999). I found that carbonate deposition decreased to <2 mm/month or stopped entirely at temperatures below 11-12 ºC, in agreement with values for other mollusk species (Dettman et al. 1999; Nuemann 1993).

A biological mechanism may be responsible for the seasonal growth patterns observed in this study. The reduction in summer calcification may result from the reallocation of energy towards reproduction (Jones et al., 1986). If an individual snail averages >1 clutch per week (Barnes et al., 2008), a considerable amount of energy will be diverted from growth in the summer months. Therefore, shell growth rate likely slows during warmer times, i.e. the primary season of reproduction, and accelerates during the winter.

None of the seasonal growth patterns were adjusted for the 1.35‰ enrichment measured in the control snails. If this offset were taken into account, the most enriched $\delta^{18}O_{aragonite}$ values would correspond to the values expected for the summer months. The results from the control study are somewhat equivocal. This enrichment may be the result of evaporative loss from the uncovered tanks. Although the tank water was changed regularly, ~8% of the water was estimated to be lost from the tanks on a weekly basis. Without sampling water isotopes throughout the week, it was impossible to estimate how much of the shell enrichment resulted from preferential evaporative loss of ^{16}O.

The overall size of snails from Newnans Lake corresponded with the snail ages inferred from the $\delta^{18}O$ profile (Table 3-5). All samples >20 cm (linear distance from apex to margin) were more than a year old, while shells <20 cm had not completed a full annual cycle. Shell mass, however, did not correspond with the age of the snail. For instance, shells of young individuals NLL 061, 063, 091 weighed more than shells whose $\delta^{18}O$ profiles indicated they were older. Shells from Lakes Tulane and Verona showed no correlation between age and either shell size or mass (Tables 3-3 and 3-4).

Isotopic Offsets

Few studies have reported on isotopic fractionation in freshwater gastropods. Some reported good agreement between observed and expected $\delta^{18}O_{aragonite}$, i.e. equilibrium fractionation (Shanahan et al., 2005), whereas others found evidence for disequilibrium (Fastovsky et al., 1993). This study of *P. insularum* found isotopic offsets. Disequilibrium fractionation was apparent in measurements of the most recent growth lines, in which measured $\delta^{18}O_{aragonite}$ values were different from expected values (eq. 2-2) calculated using the $\delta^{18}O_{water}$ and temperature for the snail collection date (Table 3-2). Furthermore, carbonate samples drilled from the same growth line along the shell margin sometimes returned $\delta^{18}O$ values that varied by >3‰. Farther up the shell, multiple measures across a single growth line yielded essentially identical $\delta^{18}O$ values

(Fig. 3-6). Apparent isotopic disequilibrium and high variability in samples from the lip implies that date of death cannot be established with certainty from the $\delta^{18}O_{aragonite}$ on the shell margin.

I propose possible explanations for this apparent disequilibrium. First, carbonate along the lip may have been deposited at different times, and hence at different temperatures. At Newnans Lake, temperatures fluctuated by as much as 8°C in a 24-hour period, which might account for an almost 2‰ difference in $\delta^{18}O$ between sites along the lip. This presumes that two samples taken along the margin were deposited at the temperature extremes, which is unlikely. Furthermore, even if this does account for some of the variability along the growth margin, it fails to explain why the $\delta^{18}O$ values along growth lines farther up the shell return virtually identical values.

Second, vital effects could account for the offset. All tank-raised, control samples were enriched in ^{18}O with respect to expected values. Among the snails from the lakes, some values from the shell margin were enriched while others were depleted with respect to expected values. Isotopic offsets because of vital effects should produce covariance between $\delta^{18}O$ and $\delta^{13}C$ (Adkins et al., 2003; McConnaughey, 1989b; Shanahan et al., 2005). In this study, the two isotopes did show a strong covariance (average r=0.79).

Characteristics of the shell mineralogy may account for the unusual results detected in samples from the shell margin. During mollusk shell repair, vaterite, an unstable polymorph of calcite, is initially precipitated in damaged areas around the shell lip (Wilbur and Watabe 1963; Saleuddin and Wilbur 1969). *Pomacea* in particular, can biomineralize vaterite as calcareous spherules (Watabe et al., 1976). In snails, oxygen isotopes in vaterite are fractionated differently than are isotopes in aragonite (Kim and O'Neil 1997). This would give a recently repaired site in a shell an isotopic signature different from areas of the shell that were not repaired. My SEM analysis of shell margins revealed spherules (Fig. 4-1) similar to those found by Watabe et al. (1976). Vaterite is highly unstable and dissolves easily, only to re-crystallize later as aragonite. The aragonite will have a $\delta^{18}O$ signature similar to shell carbonate deposited at similar times. This may explain why values "even out," i.e. are similar across growth lines in older sections of the shell. In other words, it is probable that vaterite will be sampled along the damage-prone shell lip, whereas slightly "up shell" there has been conversion to aragonite. There is no satisfactory explanation for why all the values from the shell margin are offset compared to expected values calculated with eq. 2-2. Even more perplexing is why the offset was not in the same direction as that for the control snails.

Implications for Paleoclimate and Archaeological Studies

It has been proposed that $\delta^{18}O$ profiles from fossil freshwater snails could be used to make inferences about past temperature change and time of shell harvest. This assumes that temperature is the major determinant of $\delta^{18}O_{aragonite}$ variation and that the shells faithfully record the complete range of temperatures in the water body where they lived. Likewise, time of death can only be discerned if the most recent carbonate from the shell margin is deposited in equilibrium with the host water. In this study of *P. insularum*, living snails captured on the same date showed variable $\delta^{18}O$ profiles (Fig. 4-2). These snails spend most of their time in the shallow littoral zone (<1 m) where water temperature can fluctuate on short timescales. Thus, individual snails may experience different temperatures and display variable growth rates on a weekly, or even a daily basis. This may explain some of the inter-shell variability. Unlike marine gastropods, freshwater snails are exposed to a much greater range of $\delta^{18}O_{water}$ and temperatures. Reconstruction of seasonality (Andreasson and Schmitz, 2000) from freshwater mollusks is hindered by winter and summer growth cessation (Samples LTD 005, LTL 001), variable $\delta^{18}O$ profiles in coexisting snails (Samples NLL 023, NLL 024), and biological offsets that could skew results (Table 3-2).

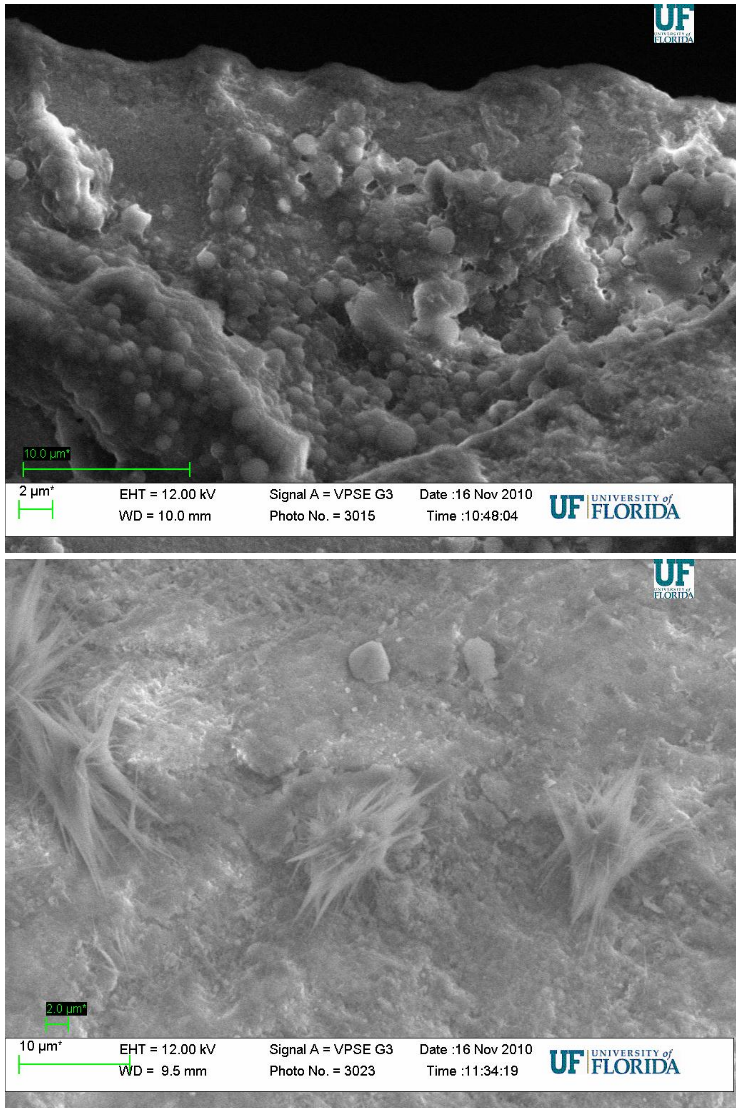
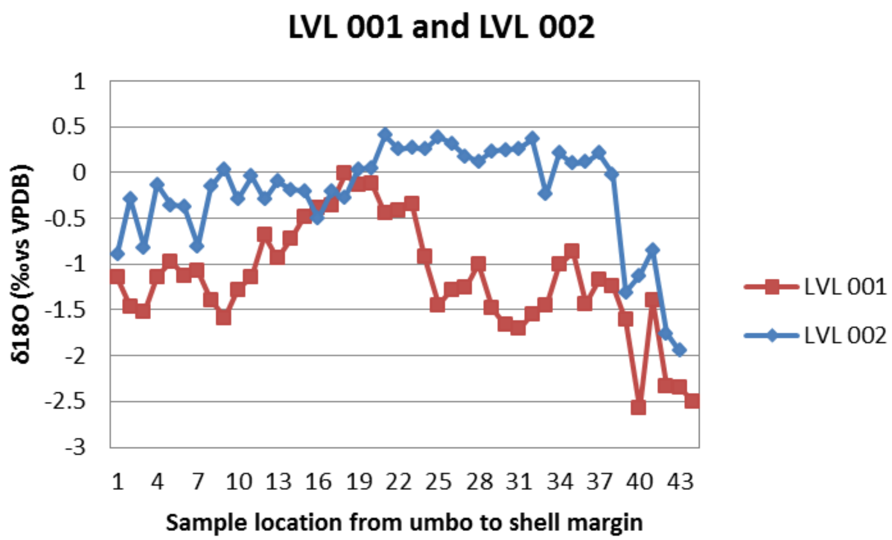
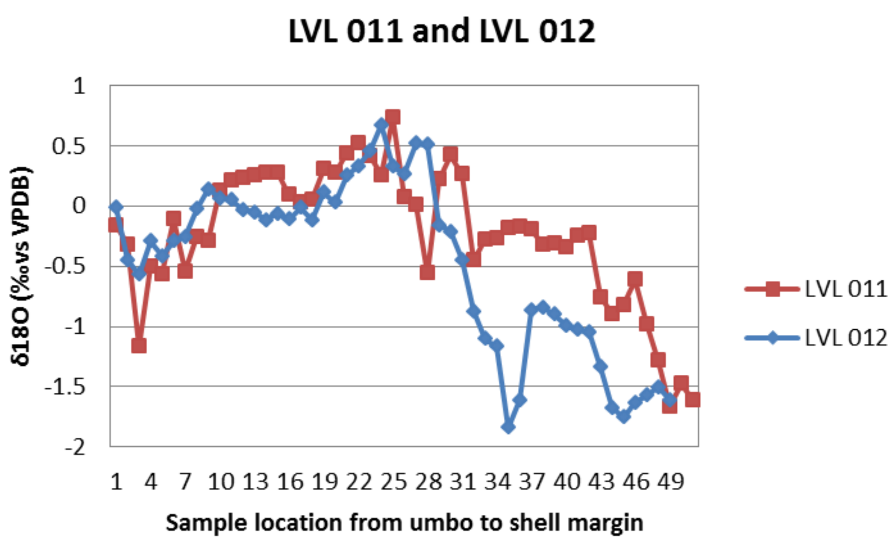


Figure 4-1. SEM scan of a *P. insularum* shell. (A) Along the margin of the shell. Two distinct crystal morphologies were present: the acicular habit of the aragonite and a spherule crystal thought to be vaterite. Image (B) may have captured the recrystalization of these spherules further up the shell (~5 mm).

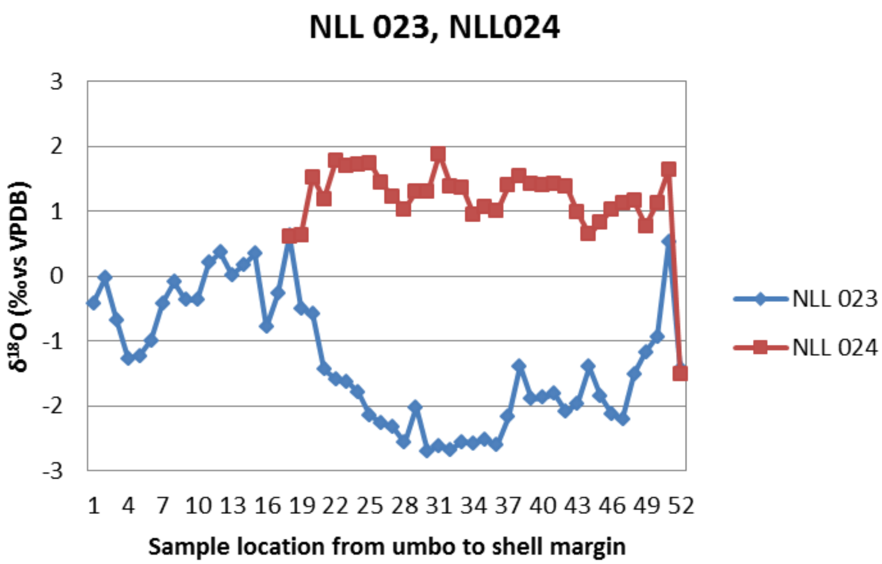


A

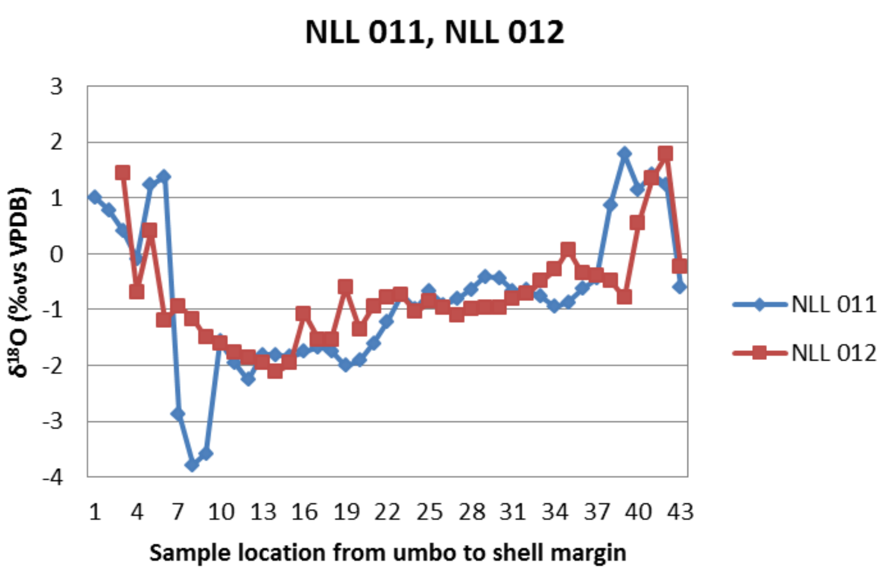


B

Figure 4-2. Comparison of the oxygen isotopic profiles of shells collected on the same dates from Newnans (NLL) and Verona (LVL). Some of the shell pairs followed similar patterns (LVL011 and 012; NLL 011 and 012), others were offset from one another (LVL 001 and 002; NLL 023 and 024).



C



D

Figure 4-2. Continued

CONCLUSIONS

Oxygen isotopic variations in shells were used to determine the ages and growth rates of freshwater bivalves and gastropods (Cespuglio et al., 1999, Jones et al., 1983, Krantz et al., 1984). In this study of exotic *Pomacea insularum* in Florida lakes, temperature was the primary environmental variable controlling $\delta^{18}O$ changes in shells. Most of the $\delta^{18}O_{aragonite}$ profiles showed a strong seasonal signal, with defined maxima and minima. The age and seasonal growth patterns of the snails were inferred by counting the number of isotope peaks and troughs that approximated the expected range of values. Of the 22 snails measured, 8 lived <1 year, 10 lived ~1 year, and 1 lived between 2 and 3 years; three of the samples yielded ambiguous results.

P. insularum is capable of rapidly reaching a large size in Florida lakes. It can deposit >27 cm of shell in ≤1 year. In Lakes Verona and Tulane, growth occurred primarily in winter. In Newnans Lake, growth occurred more evenly through all seasons. The canal in Newnans Lake was prone to dramatic temperature fluctuations, even on a daily basis. This may explain why the oxygen isotopic signature was highly variable within a single shell and across multiple individuals that lived at the same time. Variability was also detected in snails from Lakes Verona and Tulane samples, but was not nearly as extreme. Carbonate samples taken along the growth margins of individual shells showed high $\delta^{18}O$ variability. Reconstructing annual temperature range by high-resolution measurement of $\delta^{18}O$ in *P. insularum* shells, or determining season of death, using the $\delta^{18}O$ from the shell lip, will be confounded by isotopic offsets, which were identified in this study of modern snails.

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BIOGRAPHICAL SKETCH

Thomas Arnold was raised in Lancaster County, the heart of Pennsylvania Dutch Country. His life has been marked by a series of fortunate events which resulted in: starring roles in off-Broadway productions, mentoring at risk youth, and competing in the world rock, paper, scissors championship. He majored in Archaeological Sciences at Pennsylvania State University. Shortly thereafter, he served as a resident paleontologist at the Mammoth Site in Hot Springs, South Dakota. Traveling the world and meeting new people is my passion. In between his studies he has been fortunate enough to visit twenty-two countries, across four continents.