Carbon and Oxygen Isotope Trends in Freshwater Mussels from the Cannon River, MN

Kelly Hereid, Micah Johnson, Dan Jones, Rebekah Lundquist

Abstract

As they precipitate calcium carbonate to make their shells, bivalves record geochemical information on surrounding environmental conditions. Carbon and oxygen isotopic signatures in freshwater mussel shells result from surrounding water chemistry and temperature. This study sampled carbon and oxygen isotope data, using a mass spectrometer, from growth banding throughout the life of one *Lampsilis* shell and one Pink Heelsplitter. Significant differences in $\delta^{18}O$ between the light and dark bands in the *Lampsilis* shell revealed that a light-dark band pair is precipitated annually. A heavier $\delta^{18}O$ signature from the light band is due to cool water temperature and possibly a different source of precipitation to the river during cooler months. Variation is observed in $\delta^{18}O$ and $\delta^{13}C$ as the mussel aged, however, data on river conditions during mussel growth was unavailable, so no climate correlations could be attempted. However, *Lampsilis* has potential to be a good source of paleoclimate information, and bivalve shells may prove to be sources of information for high resolution paleoclimate reconstructions.

Introduction

The shells of aquatic bivalves record geochemical information on surrounding environmental conditions. As bivalves grow, layers of calcite or aragonite are added to their shells. These layers appear in patterns of alternating dark and light laminations at different scales (fig. 1). The largest-scale banding pattern, easy to see in thin section with the naked eye, is commonly interpreted as annual or seasonal (Wurster and Patterson 2000), and smaller weekly to daily scale laminations are included within the larger bands (Schone et al 2002). The amount of material that is added for a given increment, resulting from the rate of bivalve growth, is a result of many factors. These include water temperature, age and reproductive cycle, and nutrient availability (Goodwin et al 2003). Goodwin et al. (2003) concluded that the dominant factor affecting yearly growth, the banding scale that our study is most concerned with, is water temperature. Freshwater bivalves do not grow shells in water temperatures colder than about $12^{o}C$, and shell growth peaks in temperatures around $24^{o}C$ (Dettman 1999, Schone et al. 2002). Thus, more growth typically occurs in the warm summer months, resulting in thicker banding, while growth is slow in the late fall and spring and ceases once water temperatures become too cold. Goodwin et al. (2002) found that a wide light band is precipitated during the summer and a narrower dark band is formed in cooler months.

As shell material is added, carbon and oxygen are incorporated in the calcium carbonate in isotopic ratios that reflect certain environmental conditions. The ratio of ^{18}O to ^{16}O recorded in the bivalve shell is influenced both by the isotopic signature of the surrounding water and by temperature fractionation effects. The isotopic signature of the surrounding water changes on a seasonal scale as the source of river water inputs change. Dettman and Lohmann (2000) found in one study that river water $\delta^{18}O$ changed from spring to summer as the river changed from snowmelt fed to fed by low-elevation rainfall. Large storm events can also affect river water $\delta^{18}O$, but this is generally on a weekly

scale (Wurster and Patterson 2001, Dettman et al. 1998). Temperature fractionation of oxygen isotopes between river bicarbonate and its incorporation into calcium carbonate occurs on a variety of scales, and a lot of work has been published on this effect. Most notably, Schone (2002), Goodwin (2003), and Dettman (1998) have attempted to model this effect. Their general conclusions are that mollusk shells become more enriched in ^{18}O during cold months, for bivalve shells that form in equilibrium with the surrounding water. This is due to higher magnitude fractionation at lower temperature (Faure 1986, Walther 2005). Brey and Mackensen (1996) show that an annual $\delta^{18}O$ cycle resulting from this effect can be used to prove that shell laminations are annual.

A carbon isotopic ratio of ^{13}C to ^{12}C is also preserved in bivalve shells. This is partially a result of the isotopic signature of dissolved inorganic carbon (DIC) in the surrounding water (Wurster and Patterson 2001, Veinott and Cornett 1997), which is due to river inputs and outputs of carbon. Inputs include atmospheric CO_2, organic carbon breakdown, and dissolution of carbonate rocks. Outputs include photosynthesis and precipitation of carbonates (Dettman 1998). Each of these has a different isotopic signature, and the isotopic signature of river water changes as these different inputs and outputs vary in magnitude. However, river water isotopic signature is often difficult to discern in bivalve shells. Dettman et al. (1998) concluded that although $\delta^{13}C$ seasonal cycles often exist, no simple relationship exists between DIC and shell $\delta^{13}C$ ratios. Veinott and Cornett (1997) and Dettman et al. (1998) suggest that metabolic carbon may be incorporated in shell material, depending on the metabolic activity of the organism. This offsets the isotopic ratio because metabolic carbon is strongly depleted in ^{13}C.

The purpose of this project was to sample carbon and oxygen isotope ratios and look for seasonal and annual trends from the shells of two species of freshwater mussels, Pink Heelsplitter and *Lampsilis*, from the Cannon River, MN. Any trends in $\delta^{18}O$ would likely reflect a changing river water input source or seasonal water temperature changes. Any trends in $\delta^{13}C$ would possibly reflect a change in carbon sources and sinks to the Cannon River over the life of the mussel.

Methods

Two mussel shells were collected in the early 1990s from the Cannon River near Northfield, MN (fig. 2), one *Lampsilis sp*. and one *Potamilns alatus*, known commonly as Pink Heelsplitter. The shells were found after the organisms had already died, presumably within 5 years of the collection time due to the good condition they were found in. Two thin sections from the *Lampsilis* shell were taken, and one thin section from the Pink Heelsplitter. From thin sections, multiple samples of shell carbonate were extracted using a Dremel MultiPro hand-drill @7500 RPM from the Pink Heelsplitter shell. A Merchantek Leica GZ6 MicroMill was used for the *Lampsilis* shell because Lampsilis thin sections were much less thick and required more drill precision. From the Pink Heelsplitter**,** 4 samples total were taken (fig**.** 4b). From the *Lampsilis***,** 13 samples from dark bands only were taken along a transect from the first thin section (fig. 3C), and 14 samples were taken, alternating light and dark bands, from the other thin section (fig. 1B). Samples were weighed to a mass of 30 – 60 μg using a Mettler Toledo AX 26 DeltaRange Microbalance. Samples were reacted with 100% H_3PO_4 under positive He pressure at 90° C for seven minutes. The resulting CO_2 was cryogenically purified. Isotopic analysis for both carbon and oxygen was carried out using a Finnigan MAT 252

dual inlet mass spectrometer. Samples were compared against the UU – Carrara (> 140 mesh) carbonate standard. Results were analyzed statistically by regression analysis, and t-test paired two sample for means.

Results

The average $\delta^{13}C$ of shell carbonate from the *Lampsilis* was -10.33‰ (standard deviation of 1.44‰), and the average $\delta^{18}O$ was -6.18‰ (standard deviation of 0.53‰). $\delta^{18}O$ and $\delta^{13}C$ ratios from *Lampsilis* dark bands did not change significantly with shell age (regression, p=0.78 for $\delta^{13}C$, p=0.89 for $\delta^{18}O$), however, there was some variance (fig. 5). There was no significant difference between in $\delta^{13}C$ between *Lampsilis* light and dark bands (t-test paired means, p=0.39)(fig. 6). However, there was a significant difference in $\delta^{18}O$ between light and dark bands from the same *Lampsilis* sample (t-test paired means, p=0.02)(fig. 6 and 7). Dark bands averaged -6.25‰ (0.31‰ standard deviation) and light bands averaged -5.68‰ (0.40‰ standard deviation).

The average $\delta^{13}C$ from the pink heelsplitter was -8.89‰ (0.39‰ standard deviation) and the average $\delta^{18}O$ was -3.46‰ (1.40‰ standard deviation). Both the average $\delta^{18}O$ and $\delta^{13}C$ ratios are significantly different from those from the *Lampsillis* (t-test, two sample assuming equal variances, $p = .04$ for $\delta^{18}O$, $p > 0.01$ for $\delta^{13}C$).

Discussion

When examined in cross-section, the *Lampsilis* shell revealed alternating light and dark bands (fig. 3). A $\delta^{18}O$ cycle is present in these bands: lower $\delta^{18}O$ in a dark band to higher $\delta^{18}O$ in the bordering light band and back to lower $\delta^{18}O$ in the next dark band. This cycle implies that a light-dark band pair is one annual cycle, with the heavier $\delta^{18}O$ precipitating during late spring and early fall in cooler water temperatures, and the lighter $\delta^{18}O$ precipitating during the summer. Therefore, we conclude that the dark bands were precipitated in the summer months and the light bands were formed in the winter. In addition, the dark bands are visibly thicker than the light bands (fig. 3C), which implies that they were formed in the warmer summer months when the mussel shell supposedly undergoes more shell growth. This conclusion is supported by a similar study by Brey and Mackensen (1997) in marine bivalves, who concluded that a light-dark band pair represents one annual cycle due to a repeating $\delta^{18}O$ cycle reflecting seasonal water temperature differences. Their conclusion is backed by growth monitoring experiments on live specimens, which we were unable to perform. In contrast, the Brey and Mackensen study showed that light bands were formed in the summer, and dark bands in the cooler seasons. This was also true of a study in freshwater by Goodwin et al. (2001), which concluded light bands to be summer and dark to be fall or spring precipitations based on a lighter $\delta^{18}O$ signature in the wider light bands. The only difference between our conclusion and that of the aforementioned studies is the color of cool water vs. warm water banding; other evidence agrees. The banding colors may be result from different crystal forms of the calcium carbonate that makes mussel shell.

There are two unresolved issues with this conclusion. The first is that it assumes that *Lampsilis* shells are precipitated in equilibrium with the surrounding water. This is common in marine and some freshwater bivalves, and is a reasonable assumption for *Lampsilis*. The water in the Cannon River water is very rich in calcium because it flows through areas of limestone bedrock, so it is unlikely that *Lampsilis* would need a

mechanism to actively precipitate shell material, forcing it out of equilibrium with the surrounding water. An additional line of evidence is that Cannon River water in 2005 had a mean $\delta^{18}O$ value of -8.6 ppm, which would mean that a shell precipitating in equilibrium in 2005 would have similar $\delta^{18}O$ ratios to those values measured in Lampsilis (slight enrichment of about +3‰ $\delta^{18}O$ compared to river water is expected for equilibrium precipitation of calcium carbonate **(source!!!!)**). However, water $\delta^{18}O$ data is from more than 10 years after the specimens were growing, and any correlation should be made cautiously. The other issue with our conclusion is that no water temperature data from the Cannon exists for the interval that these mussels were growing. This data is important to our conclusion and would be required in future studies. Despite this lack of data, however, our conclusion that a dark-light band pair represents a year and the heavy band was a cool water deposition agrees well with a number of other studies (**sources sources**).

Studies by Dettman et al. (1999), Goodwin et al. (2001), and Veinott and Cornett (1998) showed $\delta^{18}O$ seasonal variation in bivalve growth bands of up to 5 ‰. This is a much larger value than our study, which was an average variation of 0.57 ‰ between light and dark bands. One explanation for this is that in the aforementioned studies, banding was sampled at higher resolution than our study; they took multiple samples per band on an intra-weekly scale. We only took one sample per band, a seasonal scale. Thus, in our study, seasonal extreme isotopic values would be averaged out. Another possible explanation is that the source of precipitation is different between the winter and summer months due to a weak monsoon cycle that affects Minnesota and the rest of the Midwest. Winter precipitation originates from the Pacific Ocean and travels across the Rocky Mountains before it reaches Minnesota, while the summer air originates in the Gulf of Mexico (David Fox, UMN, Personal Communication 5/12/05). As the winter air mass rises over the Rocky Mountains, ^{18}O is preferentially condensed out, so any precipitation left will be enriched in ^{16}O. Because the summer air mass does not travel the distance or through the elevation changes that the winter air mass does, summer precipitation would likely be isotopically heavier. This would dilute the temperature fractionation effect, and could explain the smaller than expected difference in $\delta^{18}O$ between light and dark bands.

Bivalve shells are useful tools in paleoclimate studies. Because Lampsilis records annual to interannual geochemical data, this species of mussel has potential as a high resolution indicator of paleoclimate. $\delta^{18}O$ and $\delta^{13}C$ are plotted against time in figures 5 and 7. However, we were unable to find a source for Cannon River discharge or temperature data for the years that the Lampsilis specimen was growing, so we are unable to compare isotope trends with any climate data. Still, it is possible to infer a few characteristics about the environment that these mussels were growing in. First of all, we know from the $\delta^{18}O$ differences between light and dark bands that the Cannon River varies seasonally by water temperature and possibly source of precipitation. Each mussel lived for approximately 20 years; this age cannot be exact because the annual light-dark bands get too small to discern in the outermost layers. Because there is no overall significant change in the $\delta^{18}O$ and $\delta^{13}C$ by time, we can hypothesize that the climate did not undergo major changes during the period of mussel growth from the 1970s to the early 1990s, at least none that influenced either Cannon River temperature or carbon sources. However, a larger sample size spanning a longer time period would be required

to determine if climate trends could be observed in Lampsilis shells. This could have important implications for high-resolution paleoclimate reconstructions. Because Lampsilis provides inter-annual geochemical data, paleoclimate changes on very precise scales could be potentially measured. Paleoclimate data from valved organisms could potentially be measured as far back as the Cambrian; inarticulate brachiopods preserved during this period have been observed to have visible banding patterns (C. Cowan, personal communication, 5/17/05).

The *Lampsilis* had a significantly different average $\delta^{18}O$ and $\delta^{13}C$ ratios than the Pink Heelsplitter. However, only 4 samples were taken from the pink heelsplitter, from very close to the umbo rather than over the entire shell length, as in the Lampsilis. This observed isotopic signature could be due to fractionation differences between the two species; however, without more data, no reasonable conclusion can be drawn.

Studying Lampsilis and other freshwater mussel species is a promising area of research of paleoclimate studies. However, a much larger sample size and supporting data on river conditions during shell growth will be necessary for a complete study. Growth rate data from live specimens would enhance any future studies. It would also be useful to compare data from freshwater bivalve shells with another regional proxy, such as nearby tree ring records, to determine how regionally restrictive a freshwater bivalve climate reconstruction would be.

Conclusions

Lampsilis shells in cross section show an alternating light and dark banding pattern that is easily visible to the naked eye. Of these bands, a light-dark pair likely represents one year, based on a $\delta^{18}O$ cycle. In this annual light-dark pair, the dark band likely represents summer calcium carbonate precipitation, and the light band represents precipitation during the spring and late fall when water temperatures are cooler but still warm enough for the mussels to grow. Lampsilis, and bivalves in general, have potential as a high-resolution paleoclimate record. The Lampsilis sampled in this project recorded isotopic variation throughout its growth period, but no major changes, implying a relatively stable regional climate from the 1970s to the 1990s. Lampsilis should be studied in more depth in the future as a potential for paleoclimate information, but more specimens spanning a wider time period will be needed, and data on the river conditions when the mussels were growing will be essential.

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Figure Captions

Figure 1: Compiled photomicrographs of a **Pink Heelsplitter** shell showing growth bands. Collected from the Cannon River, MN.

Figure 2: A map of the Cannon River and surrounding watershed. Dots mark location where samples were collected. Figure courtesy of the Cannon River Watershed Partnership.

Figure 3: The Lampsilis shell that most data was collected from. A) photo of the intact Lampsilis shell. B) photo of one thin section of the shell in A. Samples were taken from both light and dark band along the length (drill marks can be faintly seen). The light layers appear as very bright, and the dark layers in between. Note that dark layers appear much wider than light ones. C) photo of the other thin section, professionally made. The first view is of the microdrill taking a sample, and the second view shows the drill marks clearly. Note that only dark bands were drilled in this thin section, and that dark bands are wider than light bands. Not every dark band was drilled, only those which were wide enough.

Figure 4: The **pink heelsplitter** shell. Only 4 samples total were taken from this shell, enough data to compare it to the Lampsilis in figure 3, but not to discern any trends. A) photo of the intact **pink heelsplitter** shell. B) photo of the thin section. Dots mark where samples were taken (4 samples from the top section).

Figure 5: Trends in $\delta^{18}O$ and $\delta^{13}C$ from dark bands along the length of the Lampsilis shell. Sample site denotes the band which the carbonate was drilled from, starting from the youngest band. Thus, age is increasing on the x-axis.

Figure 6: A bar graph comparing average $\delta^{18}O$ and $\delta^{13}C$ of light versus dark bands from Lampsilis. The $\delta^{18}O$ between light and dark bands is significantly different by t-test paired means ($p=0.02$), and the $\delta^{13}C$ is not different.

Figure 7: A line graph showing $\delta^{18}O$ of light and dark bands. Sample site denotes a light-dark pair, starting from the youngest bands. Thus, age is increasing on the x-axis. Note that dark bands are isotopically lighter on average than the light bands.

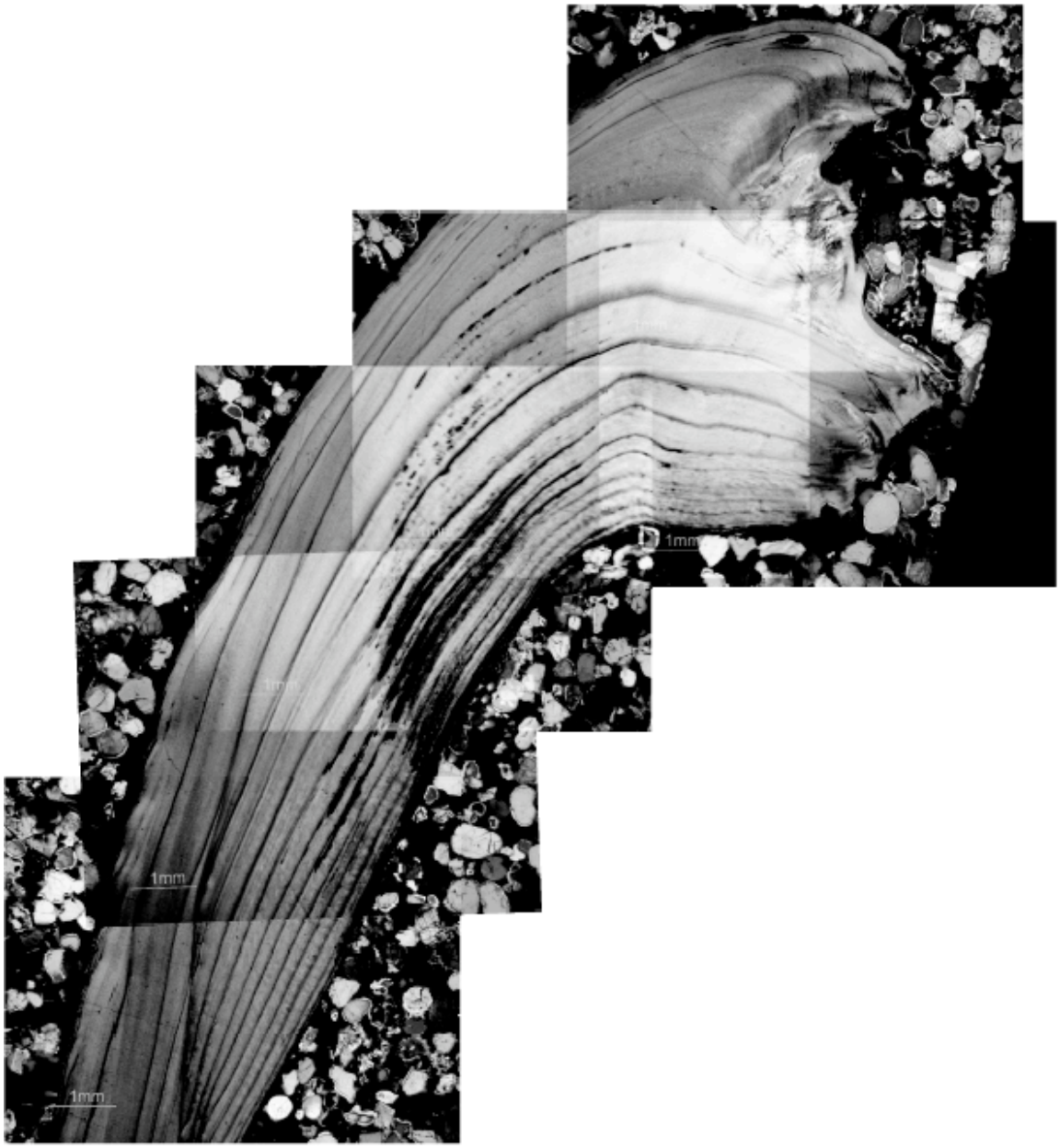

Fig. 1

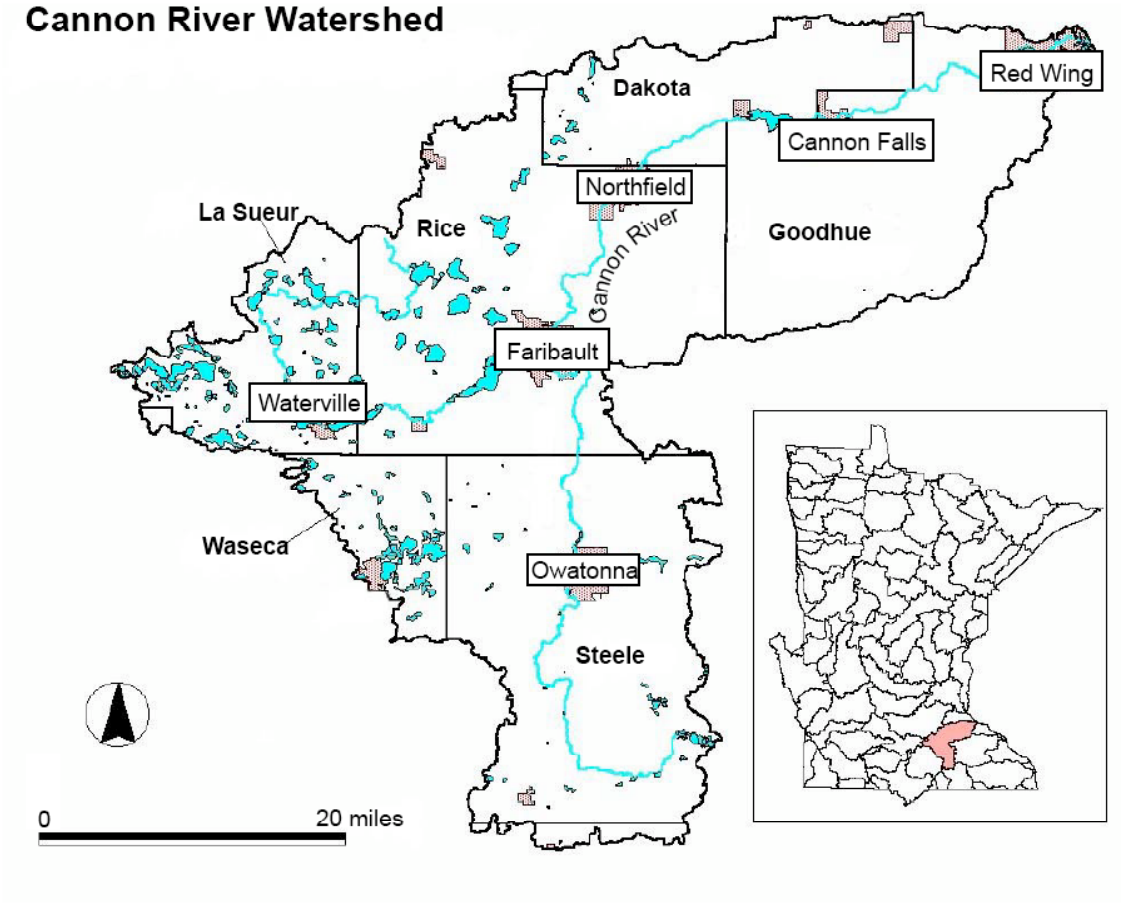
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Fig. 2

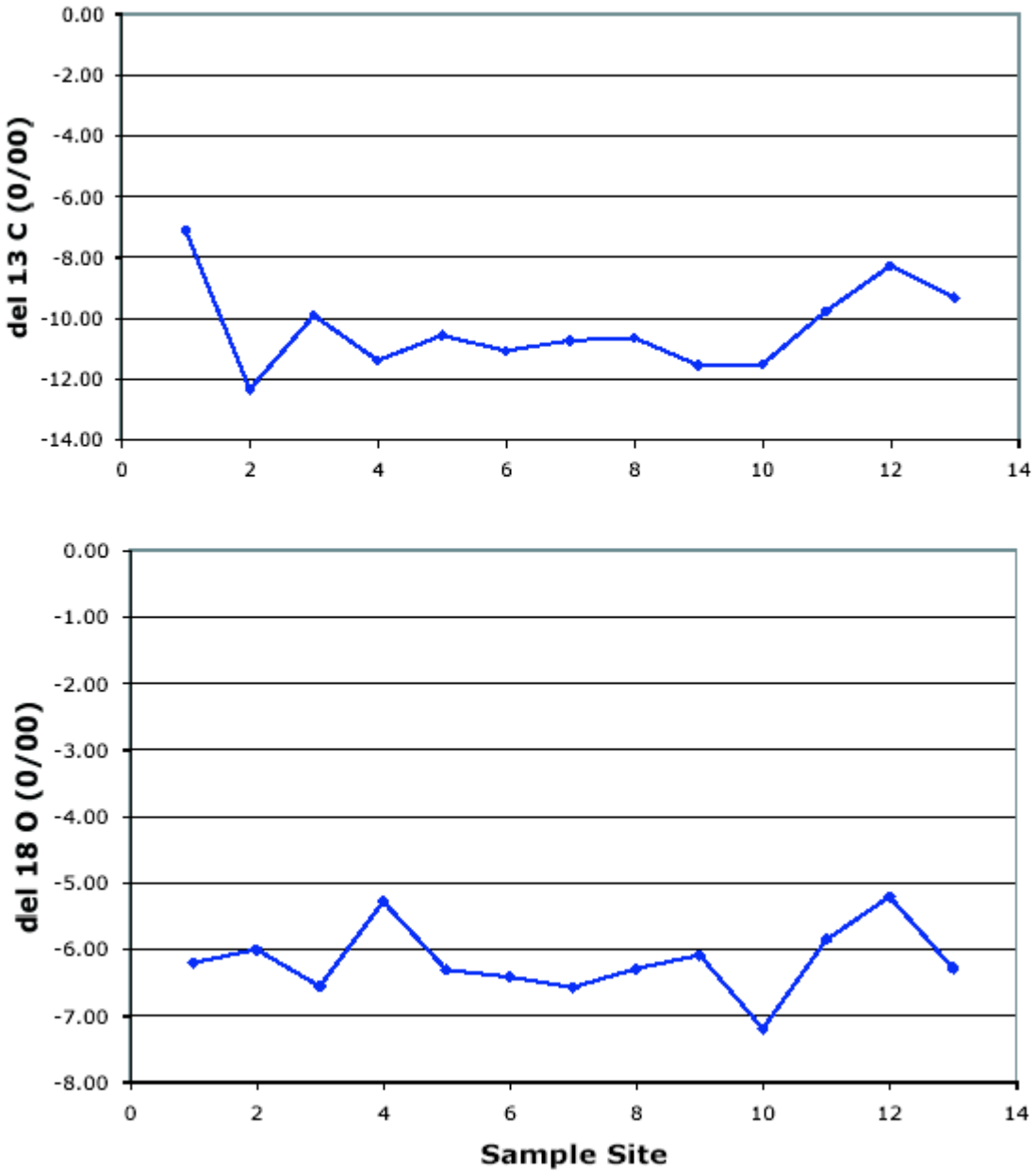
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Fig. 5

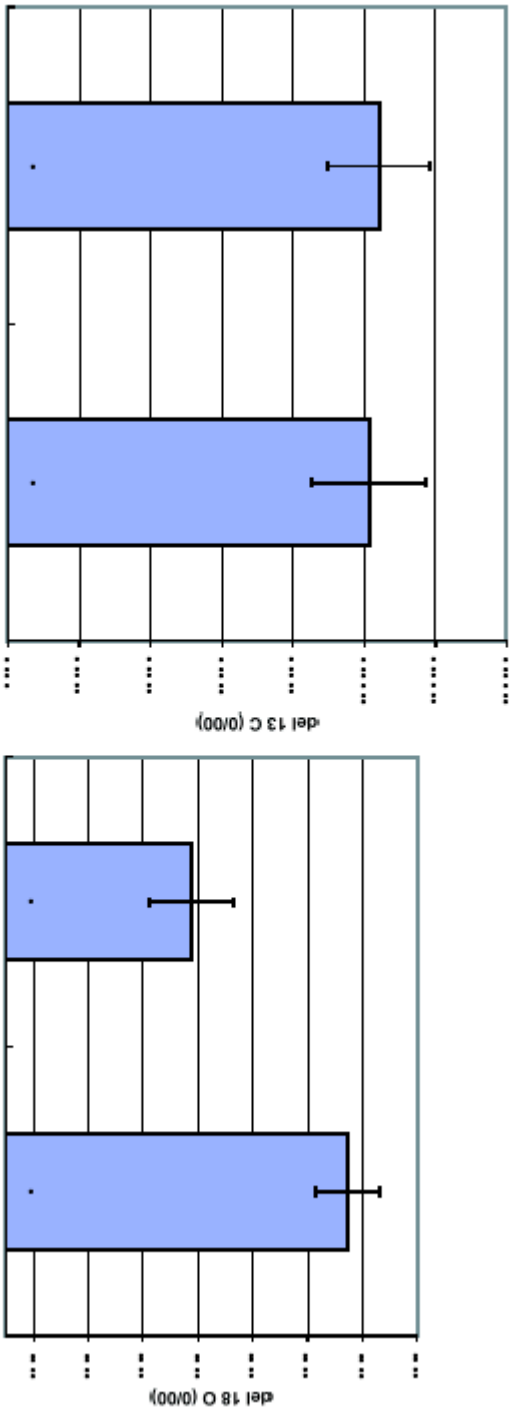

Fig. 6

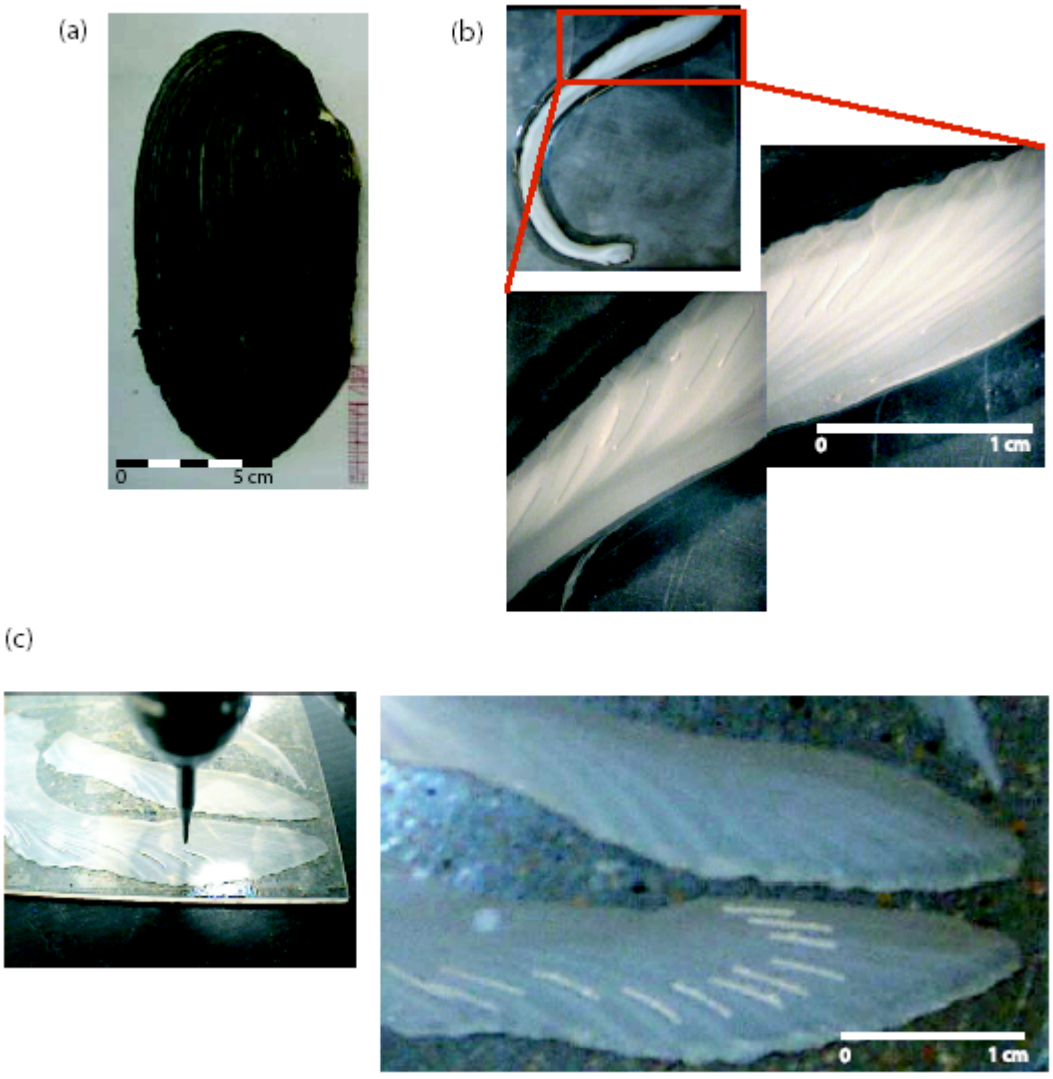

Fig. 3

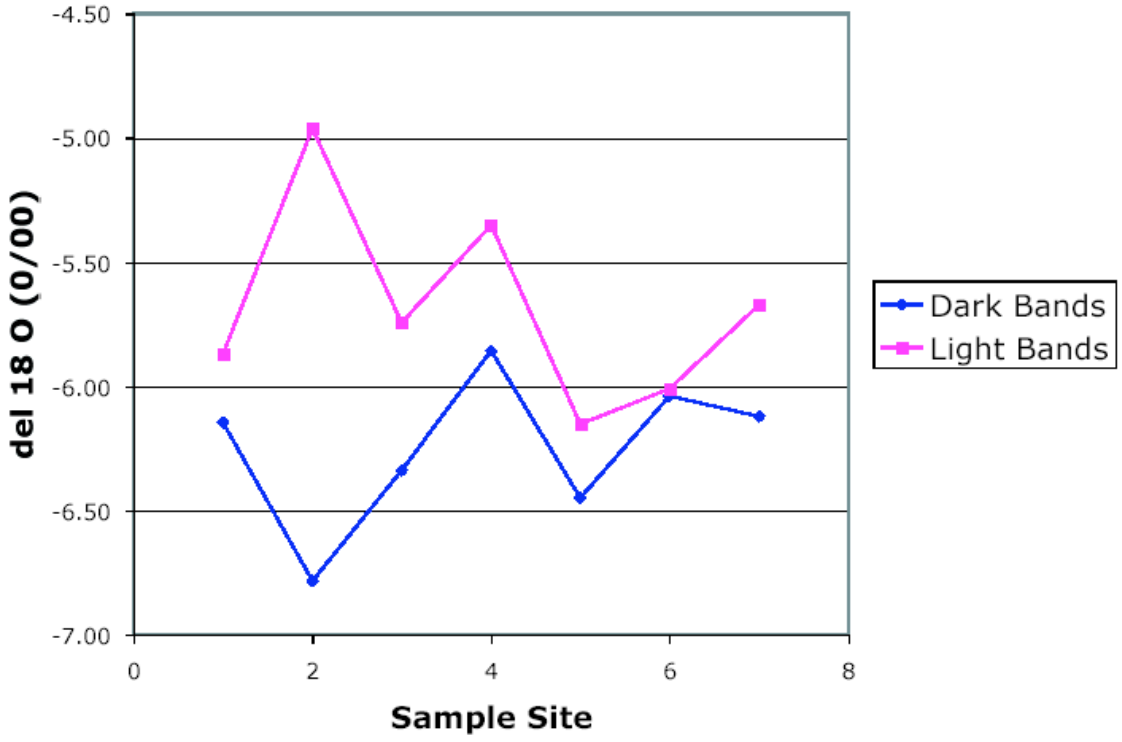
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Fig. 7

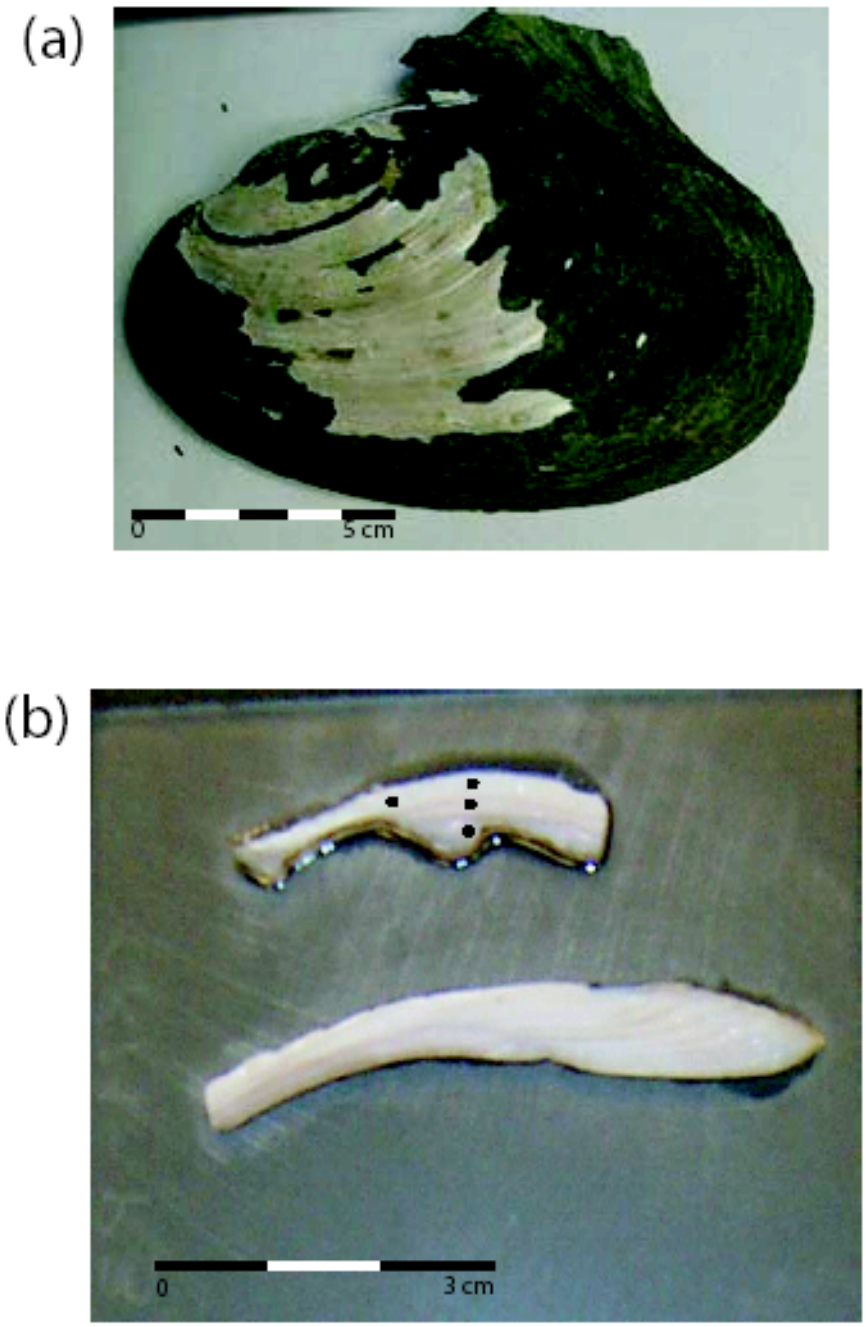

Fig. 4