

# **Dating of lacustrine molluscan subfossil assemblages using AAR techniques to contextualize the introduction of Zebra mussels to a Wisconsin lake**

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## **Abstract**

In order to accurately track paleoecological change, researchers must first gain detailed knowledge of respective sample dates and thus the degree of time-averaging among those samples. Understanding the degree of time-averaging allows us to more accurately interpret data on paleocommunities as part of a larger environmental investigation. Here, we present a source and application of age estimations of surface and core-collected molluscan subfossil assemblages from Shadow Lake, a remediated lake in Wisconsin. In order to estimate the degree of time-averaging in our core's assemblages, we selected gastropods from the upper, middle, and lower sections of the core for amino acid racemization (AAR) dating with radiocarbon calibration. AAR is complete, and an age model based on radiocarbon calibration will be correlated with other proxy records to inform and improve future conservation efforts. For now, we estimate the timing and means of introduction of zebra mussels, an invasive species, into the lake based on historical information but will verify our estimations using an AAR-based age model.

## **Introduction**

The majority of environmental conservation studies focus on recent conditions, overlooking past ecosystem states. However, the emerging field of conservation paleobiology

changes this approach by using the geologic record to establish environmental and biological baselines from which to analyze deviation. Paleoecological and historical reconstructions of lacustrine ecosystems have proven particularly informative for assessing changes in environmental quality. Although they are outnumbered by marine studies, lacustrine assessments are more accessible to local communities and can be effectively utilized by grassroots organizations and government alike to directly reduce and prevent negative anthropogenic impacts on lakes.

Shadow Lake is located in Waupaca County, Wisconsin (Figure 1). European settlement near this lake occurred in 1850. It was treated with aluminum sulfate (alum) in 1978 to reduce eutrophication and thus minimize acidity and algal growth, which can cause odorous conditions displeasing to residents (Garrison and Knauer 1983). Because phosphorous and nitrogen levels remain high, community members have taken action to improve the environmental quality of Shadow Lake and surrounding lakes by adding riparian buffers to minimize runoff and increasing fish habitat through the addition of dead trees. In addition, chemical and biological monitoring by Friends of Mirror and Shadow Lakes and the Lake Management Planning Committee is mandated by local government action (McNelly and Turyk 2012). Finally, immediate boat cleaning rules have been enforced to reduce risk of introducing invasive species.

With knowledge of Shadow Lake's current status accumulating, we aim to fill the historical knowledge gap through a paleobiological approach, putting a greater emphasis on bioindicators as part of larger ecosystem dynamics and as aids in other studies (a mindset similar to Laprida et al. 2014). We used both surface sampling and coring methods to obtain molluscan subfossil assemblages for the assessment of anthropogenic impacts on the molluscan communities of Shadow Lake through time. If lacustrine remediation efforts have been effective

in reducing and eliminating anthropogenic pressures, then one should see a deviated molluscan community return to its original baseline composition, pre-environmental change. However, interpreting paleocommunities requires an initial understanding of the degree of time-averaging, which reflects how accurately subfossil assemblages represent living communities.

In order to estimate the degree of time-averaging in our core's assemblages, we dated *Promenetus umbilicatellus* subfossils utilizing amino acid racemization (AAR) and radiocarbon calibration techniques. Time-averaging accounts for fossil mixing, in which, fossils from different time periods are found in the same strata, impacting data resolution. AAR dating and radiocarbon calibrating shells from minimized core increments throughout the core help one understand the time-averaging resolution of a study, particularly for younger time frames (<1,000 year-old samples according to Simonson et al. 2013). Amino acids are dominantly of the "L" isomer configuration while an organism is living. They racemize to the "D" configuration once an organism dies. Thus, the ratio of D/L isomers can provide an age estimate of a fossil. However, AAR dating is a rather new technique, and it is dominantly used for marine samples—making this limnologic application a pioneer study. It is essential to pursue this because with the time-averaging knowledge from one core, analysis of other cores similarly collected but not dated can progress.

## **Methods**

### *Field collection*

To determine the coring locations that avoid steep slopes and the impacts of near-shore storm surges on lake bottom settling, we examined the bathymetry of Shadow Lake overlain

Google Earth images. The cores used in this study (SAEQ-SHOW16-1A-1P-1 and SAEQ-SHOW16-3A-1P-1, from now on referred to as SHOW-1A and SHOW-3A) were taken as part of a transect and was located at the deepest and shallowest, respectively, coring locations for Shadow Lake (Figure 1). We used a Griffith corer, 7cm diameter polycarbonate tubes, and the piston coring method (Wright et al. 1984), driving the core into the sediment with drive rods.

We obtained our *P. umblicatellus* surface samples from a 2015 Shadow Lake study. This study had multiple transects, and at each, filled a Ziploc bag with mollusks using a scoop of sediment from the lake floor 20m out from the lake shore. This sediment was sieved for samples  $\geq 2\text{mm}$ .

### *Core Subsampling*

Initial core sediment subsamples were collected in centimeter increments. In order to select the subsamples from which to obtain the mollusk shells, we divided SHOW-3A into three sections of equal length (an upper, middle, and lower). Although using samples from only a centimeter increment is ideal, we could not find a sufficient abundance of the same species within this limit and therefore extended it to a 10cm increment (except for the lower section, in which we covered the full ~34cm extent, see Figure 2). Once we selected the specific subsamples, we sieved out the shells using a 125 $\mu$  sieve. We selected *Promenetus umblicatellus* (identified using the key from <http://northamericanlandsnails.org/WIFreshwaterSnailskey/wifwsnailkey.html>) as our sole sample species based on its abundance throughout the core. We also noted the presence of zebra mussels in the core.

Once the specific specimens for AAR analysis were identified, we recorded their lengths and widths using the program Motic Images in accordance with a binocular microscope (Figure 2).

### *AAR Dating*

For the majority of the AAR process, we followed the sonicating, leaching, hydrolyzing, and reverse phase High Performance Liquid Chromatography (HPLC) guidelines of Kaufman and Manley 1998. We did not dremel the samples because they were too small and fragile. To leach them, we used 33% of the sample weight in 2M hydrochloric acid. The processed samples were placed in microvials and set in the oven for hydrolysis for six hours.

Low-precision radiocarbon calibration will aid in the development of an AAR age model.

## **Results**

We obtained D/L values for eight different amino acids: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), alanine (Ala), valine (Val), phenylalanine (Phe), isoleucine (Ile), and leucine (Leu). When plotted against one another, fit was apparent but with some scatter. The best D/L fit was Ala to Asp (Fig. 2). One sample had an L-Ser value particularly high when compared to its L-Asp value (7436/8650 respectively), indicating it is contaminated and thus will not be used in construction of the age model. Regardless, the D values increase as the shells are from further downcore, fitting expectations despite not having specific dates (Figure 3).

Zebra mussels were found in centimeter increments 12-13, 14-15, and 15-16cm.

## **Discussion**

### *AAR Dating*

Although our amino acid D/L graphs had noticeable scatter (Fig. 2), visual quality assessment of fit (guided by Kaufman et al. 1992) suggests species misidentification (interspecies variability) is not a major factor in our study. Furthermore, the various D/L trends check the integrity of the ratios. Although most studies have D/L Glu to D/L Asp as the tightest fit, our dataset shows D/L Ala to D/L Asp as the best fit (Figure 3). Therefore, our age model input ratios will be from D/L Ala to D/L Asp rather than the typical D/L Glu to D/L Asp.

AAR sample contamination is usually indicative of the introduction of modern amino acids, either in situ or in lab and handling (Kosnik and Kaufman 2008). We know one sample was contaminated because Serine degrades quicker compared to other amino acids; thus, there should be substantially higher L-Asp than L-Ser in the sample, which is not the case here.

Radiocarbon calibration of select AAR samples (Figure 3) will not only allow us to develop an age model, but it will also allow for further assessment of the broad range of D/L values in the surface samples and strengthen the calibration along the core depth.

### *Zebra mussels*

Shadow Lake is currently not listed on the University of Wisconsin Sea Grant Zebra Mussel Watch (<http://seagrant.wisc.edu/zebramussels/table.html>). However, we found a few specimens while subsampling, indicating a past invasion of this species. The centimeter increments in which the zebra mussels were found in SHOW-3A correlate with location of other proxy peaks seen in SHOW-1A and SHOW-3A, including magnetic susceptibility. Peaks in magnetic susceptibility represent high metal content, most likely due from runoff influx. Metal

concentrations can separately be determined and graphed as well. Both magnetic susceptibility and heavy metal concentrations peak from ~15-25cm downcore of SHOW-1A (Figure 4).

From the 1930's-1950's, an inflow channel to Crystal River and an outflow channel from a wetland were dredged to control storm water drainage and grant recreational lake users access to different bodies of water (McNelly and Turyk 2012). Runoff that reaches a lake generally increases that lake's nutrient and heavy metal concentrations. Thus, increased directing of storm runoff to a lake most likely results in higher metal and magnetic values, as we suspect is the reason behind our Shadow Lake magnetic susceptibility and heavy metal concentration peaks. This advanced flow could also explain the presence of zebra mussels (Figure 5). Having inflow and outflow channels no longer makes the lake isolated and allows for the invasion of nonendemic species if those species are present in the connecting bodies of water. Our argument for the connection between Shadow lake channelization and the introduction of zebra mussels to this lake is strengthened by the matching core ranges (from ~12-25cm), the location of the alum layer in core SHOW1A (locating the year 1978 at 17cm, see Figure 4), and the recent discovery of zebra mussels in Crystal River (<http://www.wisconsinrivertrips.com/segments/crystal-river>).

## **Conclusions**

AAR methods can prove effective in reconstructing lacustrine paleocommunities as they provide detailed information for the production of an age model that can be aligned with the presence/absence of various proxies. This correlation is especially important in cases of identifying the timing of invasive species introduction. For Shadow Lake, zebra mussels most likely arrived after and due to the construction of manmade storm water runoff and recreational channels. This case exemplifies why lake-river channelization should be approached with caution by verifying species compositions of the involved bodies of water.

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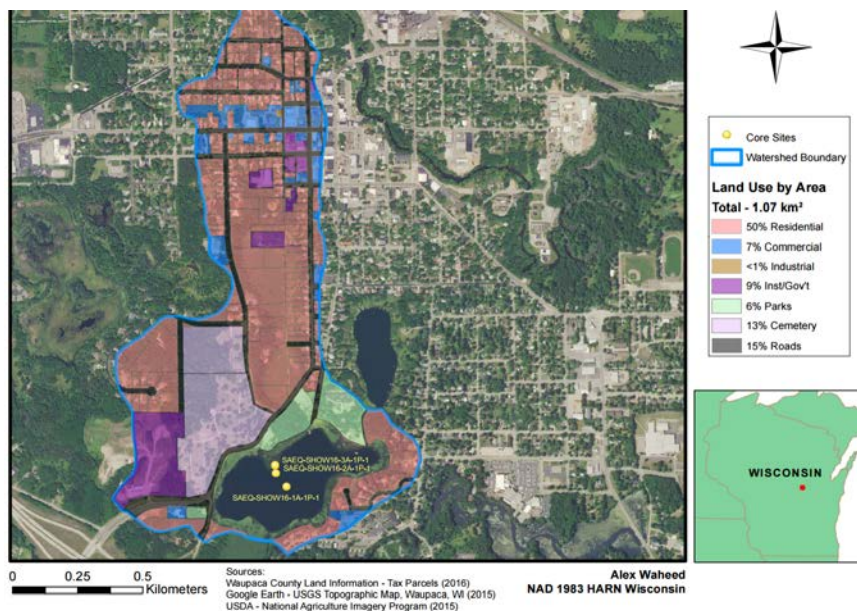
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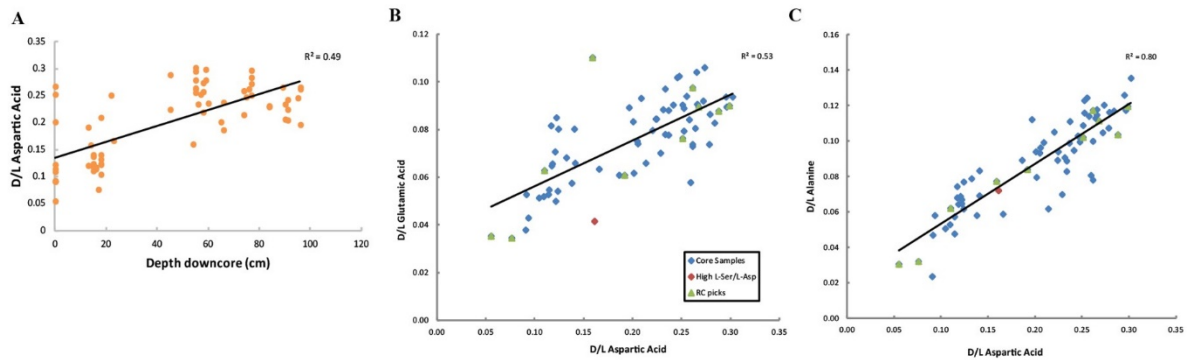
Schwab Endowed Geology Fund. Coring was guided by Amy Myrbo from LacCore. As part of Keck, initial field and lab work was conducted in a team including Andrew Conaway (College of Wooster), John Dannehl (Washington & Lee University), Sloane Garelick (Oberlin College), Taylor Mooney (Colgate University), and Khawaja Waheed (Whitman College). AAR procedures were conducted at Northern Arizona University's Amino Acid Geochronology Laboratory with great assistance from Katherine Whitacre and Darrell Kaufman.



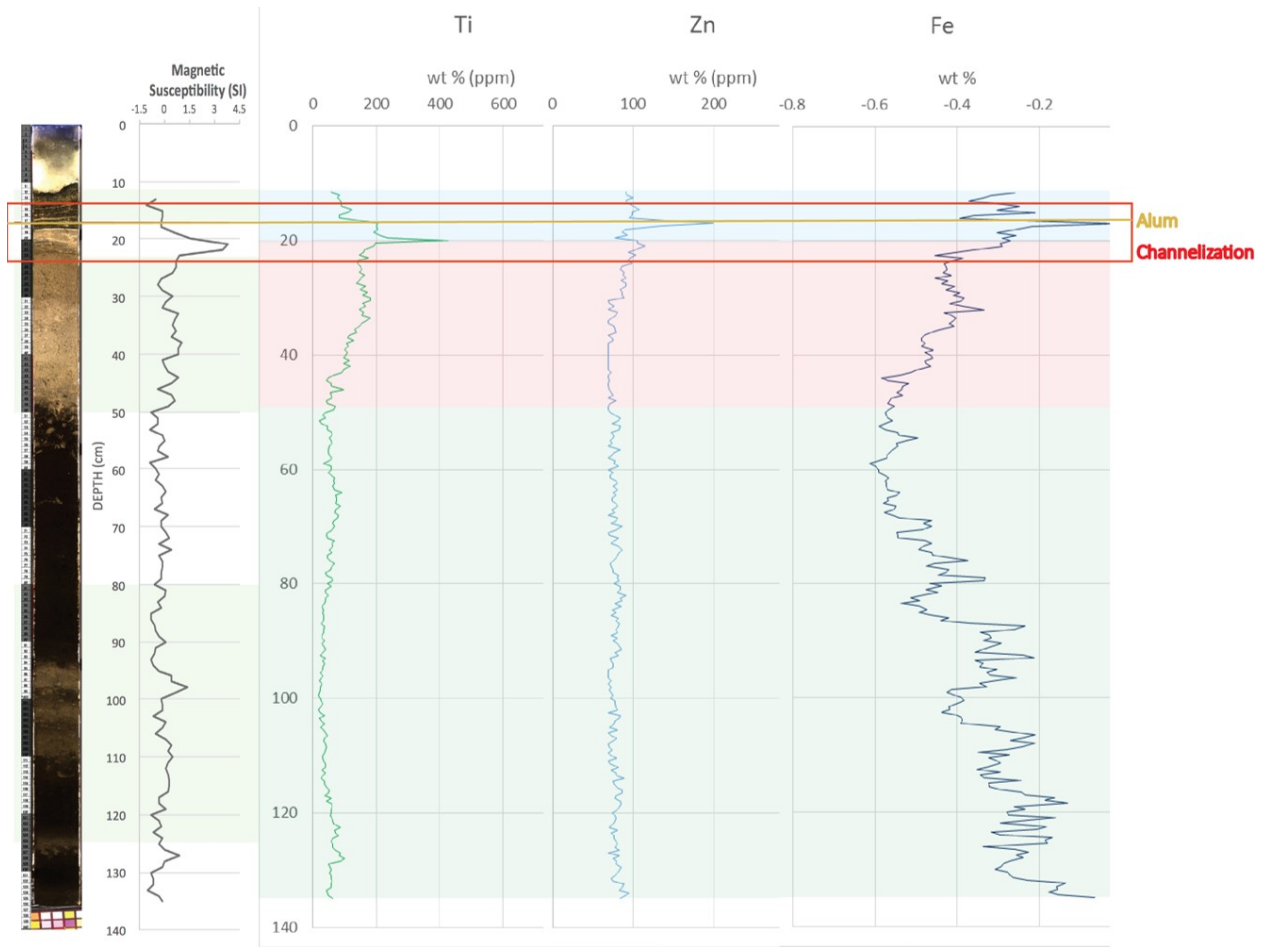
**Figure 1.** Map of study area (made in GIS by Khawaja Waheed), displaying our coring transect and the area's landuse.



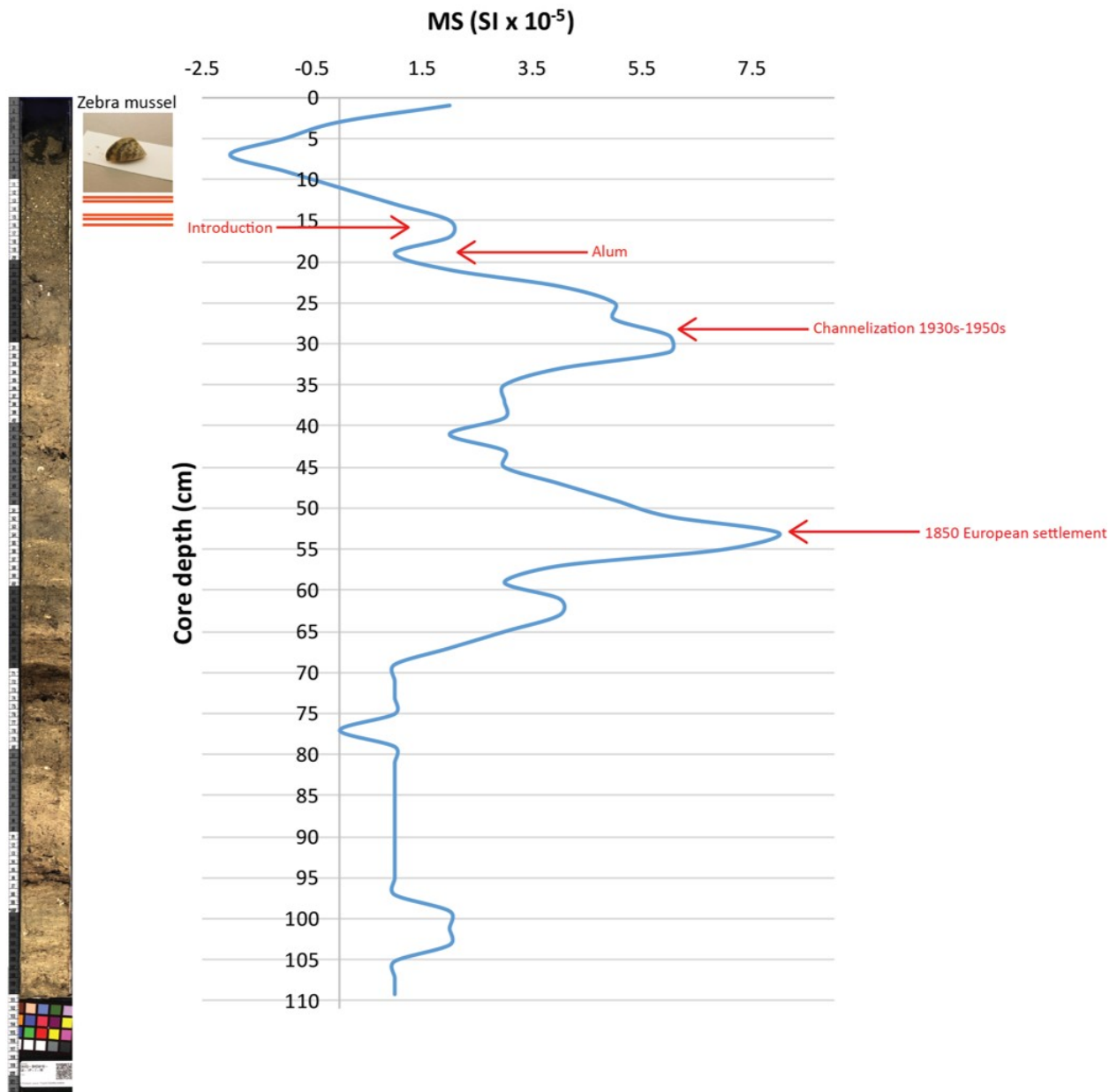
**Figure 2.** The locations of *Promenetus umbilicatellus* samples taken from throughout core SHOW3A for AAR dating, and the locations of found zebra mussels.



**Figure 3.** **A)** Without specific dates, one can see a general trend of D values increasing downcore. **B & C)** Linear regressions of two of our D/L amino acid ratios obtained after HPLC. **B)** The most commonly used ratio in other studies, but in our case, it has a lower R2 value. **C)** The best fit and thus the ratio we will use for our age model. Note: We distinguished the contaminated (high L-Serine/L-Aspartic Acid) sample from the others and noted the array of samples chosen for radiocarbon calibration.



**Figure 4.** Magnetic susceptibility and heavy metal (titanium, zinc, and iron) concentration peaks (from Taylor Mooney and Khawaja Waheed, respectively) from core SHOW-1A are featured in the red box. The yellow line signifies the alum layer, indicating that 17cm downcore marks the year 1978. We hypothesize that the above peaks are the result of storm water channelization of Shadow Lake into Crystal River and from a proximal wetland during the 1930's-1950's.



**Figure 5.** Using our subsampling results, the information from Figure 4, and historical knowledge, we hypothesize event occurrences as captured in the magnetic susceptibility (MS) of core SHOW3A.