



PERGAMON

Computers & Geosciences 25 (1999) 1159–1166

COMPUTERS  
GEOSCIENCES

# Advances in micromilling techniques: a new apparatus for acquiring high-resolution oxygen and carbon stable isotope values and major/minor elemental ratios from accretionary carbonate

Christopher M. Wurster\*, William P. Patterson, Michael M. Cheatham

*Department of Earth Sciences, 204 Heroy Geology Laboratory, Syracuse University, Syracuse, NY 13244, USA*

Received 1 January 1999; received in revised form 1 May 1999; accepted 1 May 1999

## Abstract

A computer-controlled micromilling apparatus that permits discrete sampling of accretionary biogenic carbonate specimens with micron-scale resolution has been developed for the purpose of acquiring high-resolution  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values, and major/minor elemental chemistry. Secular variation in stable isotope ratios and major/minor elemental composition records inter-annual and intra-annual changes in the environmental parameters or animal behavior for extant and extinct species.

A polished specimen is attached to a stage beneath a fixed micro-milling head, and viewed on a large-screen monitor via a color digital camera. Growth bands (analogous to tree rings) are generally a result of variable accretion rates in biogenic carbonates. These growth features are first digitized in real-time as a series of three-dimensional coordinates. To better characterize complex growth features, intermediate coordinates are interpolated using a cubic spline fit through the digital points. Intermediate sampling paths, which mimic less visible daily growth banding, are in turn calculated between digitized curves. Sampling path arrays serve to guide three high precision actuators, which position the sample stage relative to the fixed micromilling head. A fourth actuator provides vertical control of the digital color camera (compensating for vertical movement of the  $z$ -axis stage actuator) keeping the specimen image focused. This new micromilling device permits high-resolution sampling of complex internal structures via a user-friendly program interface. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Life history; Otolith; Paleoclimate; Microsample; Stable isotopes

## 1. Introduction

Accretionary biogenic carbonates are secreted by a variety of organisms such as gastropods, bivalves, corals, and in most bony fishes as otoliths (*literally* 'ear-

stones'). These  $\text{CaCO}_3$  structures are common in both modern environments and the geologic record, with well-preserved material dating back at least to the Jurassic (Patterson, 1999). As skeletal material precipitates, it stores a record of contemporary environmental and biotic conditions as variation in  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values and major/minor elemental chemistry. Significantly, such carbonate fossils recovered from geological and archaeological sites allow for reconstruction of past environments and life history.

\* Corresponding author. Tel.: +1-315-443-2672; fax: +1-315-443-3363.

*E-mail address:* chwurste@syr.edu (C.M. Wurster).

Accretionary carbonates have been sampled for seasonal variation in temperature and  $\delta^{18}\text{O}$  values of ambient water using corals (Linsley et al., 1994; Beck et al., 1997), bivalves (Dettman and Lohmann, 1993; Klein et al., 1996a), and fish otoliths (Patterson et al., 1993; Smith and Patterson, 1994; Patterson, 1998). Partition and analysis of high-resolution samples from accretionary biogenic carbonate permits interpretation of inter-annual and intra-annual variation in  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values as seasonal changes in environmental conditions because oxygen isotope ratios record variables such as temperature (e.g. Kalish, 1991; Dettman and Lohmann, 1993; Patterson et al., 1993; Beck et al., 1997; Patterson, 1998), while the isotopic signature of carbon may be used as a proxy for metabolism, reproduction, and/or trophic level (Tanaka et al., 1986; Patterson, 1998; Patterson, 1999). Similarly, various aspects of water chemistry (such as salinity) are recorded by major/minor elemental composition (e.g. Secor et al., 1995; Klein et al., 1996a,b; Secor and Piccoli, 1996).

Recovery of such high-resolution records is critical for the reconstruction of past environmental and physiological conditions. However, limitations relating to discrete physical partitioning and the ability to analyze small masses of skeletal material have traditionally impeded recovery of detailed seasonal records. These limitations only permitted seasonally deposited carbonate to be extracted from relatively large structures such as those produced by corals and large species of bivalves.

Determination of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values from as little as 20  $\mu\text{g}$  of carbonate is now relatively routine because of recent advances in automated carbonate preparation devices coupled to gas stable isotope ratio mass spectrometers (Merritt and Hayes, 1994). This technology enables detailed studies of secular variation of stable isotope ratios within accretionary materials on a sub-weekly time scale if such samples can be physically isolated.

Recent advances in motion controllers now permit seasonal extraction from a much greater range of specimens. For example, specimens with carbonate secretion rates ranging from 0.5 to 2 mm per year for bivalves, and up to approximately 1 mm per year for some sagittal otoliths have been sampled for secular variation  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values (Dettman and Lohmann, 1993; Patterson et al., 1993). Such studies requiring accurate micromilling of the material parallel to growth banding with path widths less than 20  $\mu\text{m}$  in order to obtain weekly or subweekly time-averaged samples have only been attempted in studies since 1993 using a microsampling device developed at the University of Michigan by Dettman and Lohmann (1995). This microsampling device was used by Patterson et al. (1993) to sample otoliths cut into

transverse sections to reveal annual growth structure. However, only the transparent portion of the otolith could be used to digitize points, because a photomicrograph using the otolith as a negative was required. Furthermore, Patterson et al. (1993) were restricted to milling arcs of comparatively simple geometry. This greatly restricted the volume available for sampling.

This paper presents a new micromilling computer controlled apparatus featuring a user-friendly program for the partitioning of accretionary materials with an emphasis toward acquisition of discrete  $\sim 20$   $\mu\text{g}$  samples. This device is capable of recovering samples quicker, more accurately, and with greater stage resolution than other systems. The program was developed in the G programming language using the LabVIEW<sup>®</sup> program development application. The flexible control panel has several functions that permit detailed sampling of a variety of complex geometrical patterns. This micromilling apparatus now permits routine partitioning of sample paths with widths of at least 15–20  $\mu\text{m}$ , thereby increasing available specimens from which details of climate and life history can be obtained.

## 2. Micromilling

The micromilling apparatus consists of a movable specimen platform positioned beneath a fixed micromilling bit driven by three linear actuators (Fig. 1). Linear actuators for  $y$ - and  $z$ -axes have a stepper resolution of 0.05  $\mu\text{m}$  (monotonic inaccuracies to within 0.005%, and bidirectional repeatability to 1.0  $\mu\text{m}$ ), while the linear actuator for the  $x$ -axis has a resolution of 1  $\mu\text{m}$ . Linear actuators are driven by a motion controller using an IEEE-488 interface. The specimen is attached to the stage and viewed in real-time via a color digital camera with a focal depth approximately 9.2 cm, a maximum field of view approximately  $9 \times 12$  mm (at low magnification), and a minimum field of view approximately  $1.4 \times 0.8$  mm (at highest magnification). A fourth linear actuator providing programmatic and motorized focusing drives the camera position. The apparatus is computer-controlled via a newly developed program written in the G programming language using the LabVIEW<sup>®</sup> program development application to control all aspects of the sampling procedure. The full  $x$ - $y$ - $z$  range of motion for the specimen platform is  $17.78 \times 7.62 \times 4.54$  cm.

Micromilling requires five distinct steps which are presented below: (1) preparation of specimens; (2) digital characterization of the internal structure; (3) cubic spline interpolation of digital characterization; (4) calculation of intermediate sampling paths; (5) micromilling and recovery of carbonate.

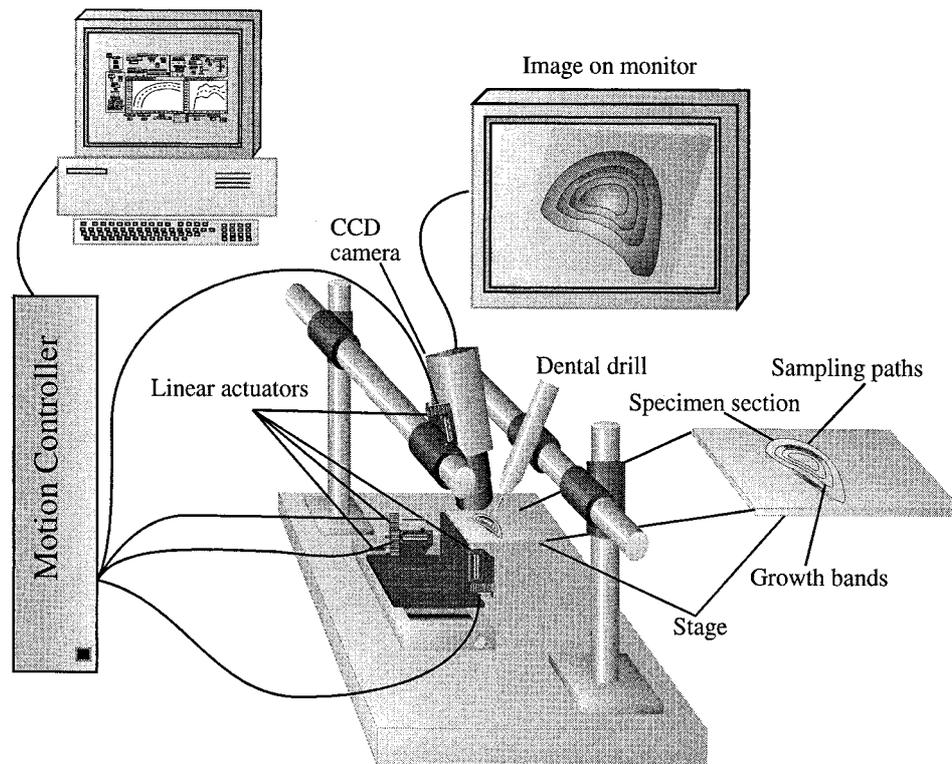


Fig. 1. Schematic of micromilling apparatus. Specimen is attached underneath a fixed dental drill and viewed on large screen monitor via color digital camera. Computer-control drives motion controller via IEEE-488 interface, and permits stage manipulation in three directions via three micropositioning actuators.

### 2.1. Preparation of specimens

Specimens are ground flat on one side, finishing with alumina powder polish (approximately 1  $\mu\text{m}$  grain size). The polished side is then affixed to a glass slide with ethyl cyanoacrylate, then the opposite side is polished so that growth features are clearly visible. The total thickness of specimens is usually 100–500  $\mu\text{m}$  (although this system can be used on materials ranging from a standard petrographic thin section up to a maximum thickness of 4.54 cm). The glass slide is fixed to the micromilling stage by nylon thumbscrews, and viewed in real-time on a monitor via a color video camera. Dual fiber optic lights are positioned for optimal visualization of growth banding during digitization and sampling via either transmitted or reflected light.

### 2.2. Digital characterization

Calculation of sample paths from which carbonate will be milled, requires that an array of  $x$ - $y$ - $z$  space coordinates be defined. Growth banding is digitized by computer-control led manipulation of the stage. Each  $x$ - $y$ - $z$  coordinate in the array is selected by positioning

the specimen such that the micromilling bit is just touching the surface with its edge along one side of the growth feature (Fig. 2A). This is visually determined using optimal magnification of the CCD camera. Generally  $z$ -axis variability is limited to less than 30  $\mu\text{m}$ , although the range of the  $z$ -axis permits micro-sampling of specimens with much greater topography. Each actuator position is read and recorded by the computer relative to a defined origin. The specimen's relative position is determined as displacement of the three actuators from a defined origin permitting digital characterization of the structure. This information is used to calculate intermediate micromilling paths.

### 2.3. Cubic spline interpolation

Organisms often secrete material in complex patterns that can not be easily described with a simple mathematical function. A solution to this problem is to linearly mill between closely spaced points along growth banding after interpolating a curved path between digitized points. We employ a cubic spline interpolation which is a robust algorithm for interpolating between sets of points which ensure that the second derivatives

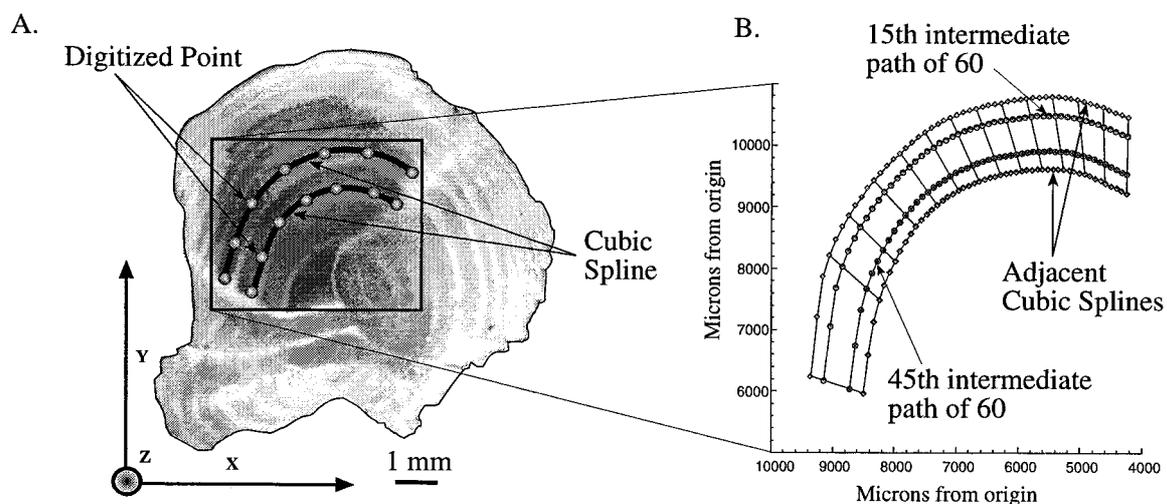


Fig. 2. Sample path generation using example otolith. (A) Two-dimensional representation of manually selected three-dimensional coordinates along growth features fitted by cubic spline. (B) Blow-up of adjacent cubic splines illustrating calculation of intermediate sample path as fraction of total distance between consecutive elements on adjacent cubic splines (determined by total number of intermediate paths).

of the interpolated function are continuous and the first derivatives are smooth (Press et al., 1992). Given a set of  $x$ - $y$  coordinates and the first derivative for the first and last two points,  $y$  positions are interpolated for any given  $x$  value. This can be repeated to calculate  $z$  values. A cubic spline interpolation program is built into the micromilling program. The cubic spline program requires a set of  $x$ - $y$ - $z$  coordinates and the first and last derivatives (chosen using a numerical slide control while viewing a graphical representation of the cubic spline), and returns a series of interpolated points. This generates sample paths, which are a more precise approximation of actual growth structures than if the sample path consisted of only manually selected coordinates with linear tracking between points (Fig. 2A). Interpolation using a cubic spline usually requires that the  $x$  value in the set of points increase in value; however, this program bypasses that requirement by calculating a series of cubic splines along the digital path and restructuring the cubic splines into a single sample path. Therefore, each path is defined as a three dimensional array of 'n' elements. The user selects the number of elements in the cubic spline, and must be equivalent for adjacent cubic splines in order to calculate intermediate sampling paths (Fig. 2B). As the number of elements for each cubic spline increases, more complex sample paths are generated which may more accurately track growth structure.

#### 2.4. Calculation of intermediate sample paths

Once growth banding is digitally characterized, in-

termediate paths between digitized arrays are calculated to mill higher-resolution time-specific carbonate. Intermediate paths are calculated by defining a line between consecutive elements of adjacent cubic splines. Intermediate path coordinates are then generated as a fraction of the total number of intermediate paths along each defined line (Fig. 2B). As the total number of intermediate paths is increased, individual sample path width (as well as sample mass) decreases. The user selects the total number of intermediate paths, usually as a function of the mass of carbonate generated.

#### 2.5. Micromilling and recovery of carbonate

The micromill uses a diamond dental drill bit to mill discrete carbonate sample aliquots from the otolith. Since the dental drill bit is much larger than the required width of the sample path, removal of discrete samples requires that the sample paths (for example 15  $\mu\text{m}$ ) are milled perpendicular to growth axes using the edge of the drill (Fig. 3). The width of the carbonate milled depends on the total number of intermediate paths calculated. The volume of sampled carbonate depends on the length, width, and depth of the sample path, and mass is calculated from the volume using the density of the sampled mineral. Current mass spectrometer technology requires a minimum of  $\sim 20 \mu\text{g}$  of carbonate when using automated carbonate preparation systems for analysis. The length function is predetermined by the morphology of the specimen; however, manipulation of both depth and width func-

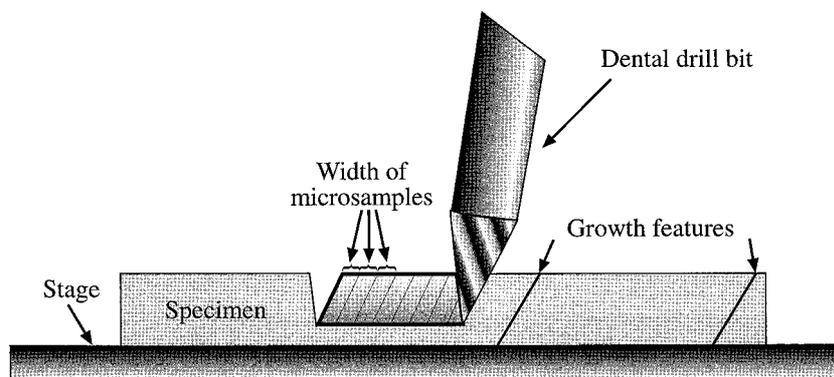


Fig. 3. Illustration showing how carbonate is extracted from sample paths with widths much less than dental drill width. Note how first sample is coarsely drilled without extracting high-resolution time-specific carbonate. High-resolution samples are later extracted by milling carbonate from specimen using dental drill bit. Note micron-scale widths of subsequent sample paths.

tions is possible. The depth function is selected by increasing or decreasing the drill depth ( $z$ -axis). The width function is determined by the number of intermediate paths between any two digitized paths (the greater the number of intermediate sampling paths, the higher the resolution and the lower the sample size and mass). Carbonate samples are manually collected with a small scalpel while viewing the specimen on the large screen monitor. Each sample is stored in a stainless steel vessel, which is placed in numbered brass convoys prior to analysis.

### 2.6. The program

The micromilling apparatus is controlled via a computer program written in the G programming language using National Instruments LabVIEW<sup>™</sup> program development application. This computer control coordinates a Newport<sup>™</sup> motion controller with high-resolution stepper motors to sample carbonate specimens using an IEEE-488 interface. The computer program consists of two control panels, which contain all necessary functions in an easy to learn and use organization. The computer program was developed to offer a user-friendly interface, which can be mastered quickly by laboratory technicians and students alike.

The main panel features the controls necessary for linear actuator manipulation and initiating sampling (Fig. 4). Controls permit the user to move and set the velocity for each axis individually. Sample path controls permit the user to select the total number of intermediate paths between adjacent cubic splines and the sample path that will program the motion controller. Other controls enable the user to change the milling depth, program the motion controller and execute each sample path. Indicators allow the user to preview sample paths, and calculate path width (which

ultimately determines sample mass) between adjacent cubic splines for each path. Abort and drop controls permit the user to quickly stop the sampling process in mid path if desired.

The digitizing panel provides controls used to digitize growth features and smooth the digital paths calculated. This control panel permits the user to move the stage and select individual points along the growth feature by viewing on a large screen monitor. Once points are selected each one can be modified, eliminated, or additional coordinates may be inserted to provide the best possible fit to actual growth structure. Cubic splines are generated for selected points and saved in a storage file, and substitution of one cubic spline for another is possible. A control to select a center point allows the user to gather carbonate for the first year of growth by calculating paths radiating from the nucleus of growth.

### 3. Sample processing and example data

Once samples have been milled, they are roasted *in vacuo* for 1 h at 200°C to remove volatiles that may interfere with  $\delta^{18}\text{O}$  or  $\delta^{13}\text{C}$  values. We present samples analyzed using the Finnigan MAT 252 stable isotope ratio mass spectrometer directly coupled to a Kiel III automated carbonate preparation device at Syracuse University. Carbon dioxide was generated by reaction of carbonate with 3 drops of anhydrous phosphoric acid in individual reaction vessels at 70°C. Individual samples are run using a micro-inlet which reduces sample 'memory' and permits analysis of  $\sim 20\ \mu\text{g}$  of carbonate.

As an example data set we sampled a 3300 year-old freshwater drum (*Aplodinotus grunniens*) sagittal otolith recovered from the Eastman Rockshelter archaeo-

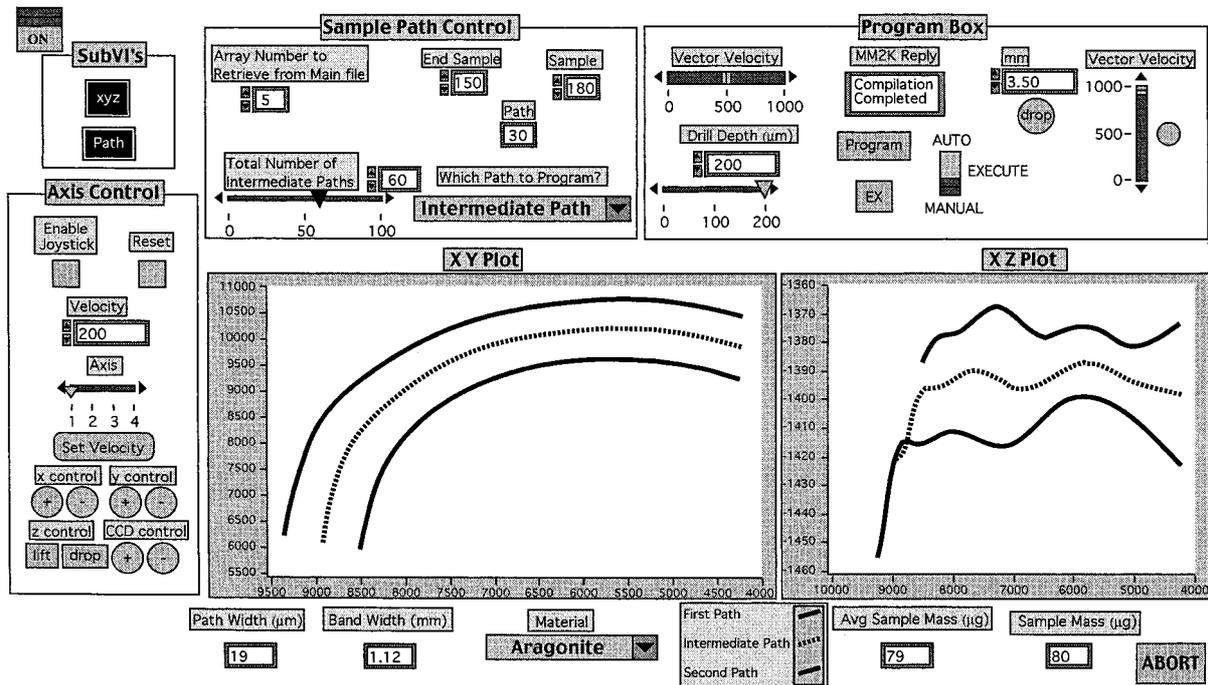


Fig. 4. Front panel control board for micromilling program. Controls enable user to perform simple axis control, and program motion controller for microsampling. Indicators show graphic representation of potential sample paths, path widths, and mass of generated carbonate.

logical site, Tennessee (dated via  $^{14}\text{C}$  and associated cultural pottery). This otolith was sampled for ontogenetic variation in  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values (Fig. 5A). Based on ecological and isotopic evidence (Edsall, 1967; Patterson, 1998) it is assumed that *A. grunniens* otoliths do not grow below  $10^\circ\text{C}$ , permitting calculation of the  $\delta^{18}\text{O}$  value of water at the beginning of the growing season using the paleotemperature equation derived by Patterson et al. (1993). This enables temperature to be calculated for the rest of the growing season from  $\delta^{18}\text{O}$  values (Fig. 5B).  $\delta^{13}\text{C}$  values co-vary seasonally with  $\delta^{18}\text{O}$  values, although they display a long-term ontogenetic trend toward higher values. These trends are apparent in all *A. grunniens* otoliths analyzed, indicating that  $\delta^{13}\text{C}$  values may be recording life history changes in trophic level, metabolism, and/or reproduction for *A. grunniens* (Wurster and Patterson, in preparation).

Two hundred seventy-four time-specific samples were milled from the first six years of the fish's life (from the origin of the otolith over a distance of 7.0 mm to the edge of the aragonite portion). Opaque portions of the otolith were avoided, which may represent diagenetic alteration. The number of growing days per year at this site for *A. grunniens* is approximately 190 days indicating a time averaging of 4 days per sample.

Better-preserved otoliths would allow for higher resolution recovery of carbonate. It is envisioned that with a modification to the internal volume of the microinlet to the Finnigan MAT 252, subdaily resolution in  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of *A. grunniens* otoliths can be extracted permitting even more detailed analysis in ontogeny and climate information. Additionally, reaction of carbonate by acidification using individual reaction vessels allows major/minor element determination on the same  $20\ \mu\text{m}$  sample. Acidified residue can be extracted, diluted and analyzed for a wide range of elements using any of a large number of standard analytical procedures and equipment such as ICP-AES or ICP-MS.

#### 4. Conclusion

An abundance of information may be retrieved through interpretation of secular variation of stable isotope ratios and/or major/minor elemental composition from accretionary materials. The method for micromilling described in this paper has several advantages over previous microsampling techniques. Firstly, digitizing is executed directly from a real-time image that allows the user to adjust the digital points if cor-

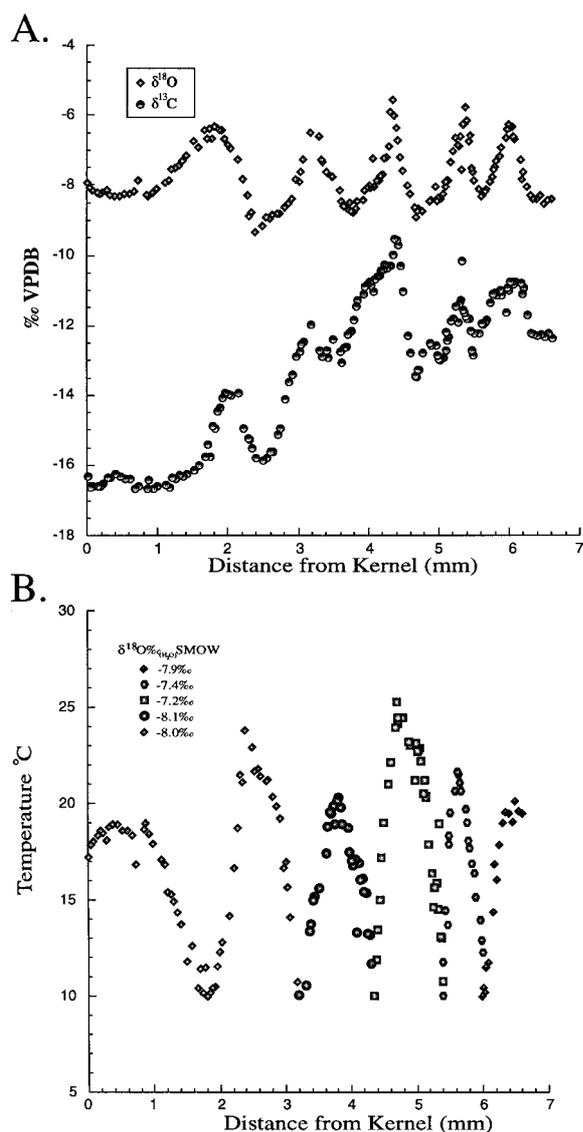


Fig. 5. (A)  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values analyzed from high-resolution time-specific carbonate extracted from 3300 year-old *Aplodnotus grunniens* otolith recovered from Eastman Rockshelter Paleo-Indian archaeological site, Tennessee. Stable isotopes show ontogenetic trend toward higher  $\delta^{13}\text{C}_{(\text{CaCO}_3)}$  values from birth to age 6. (B) Temperature calculated from  $\delta^{18}\text{O}_{(\text{CaCO}_3)}$  values assuming 10°C shutdown temperature for highest carbonate values deposited at beginning of each growing season.

rections are required. This eliminates the need to digitize from a photomicrograph with comparison to reference points. Next, further coordinates are interpolated using a cubic spline, providing accurate sample tracking along more complex growth patterns, even sampling completely around the perimeter of the specimen. Cubic spline curves generated from closely spaced

points along a complex curve permit very high-resolution milling of material.

A distinct advantage of this system is the user-friendly software. The control panel is flexible with many functions and options designed for a variety of sample geometry types. Coarse sampling work may be carried out manually, or with simple digital instructions (without using the cubic spline interpolation program). A subroutine calculates carbonate mass generated for every given sample path enabling the user to evaluate whether sufficient material will be generated for a particular analytical procedure. Sample paths are graphically displayed before sampling starts permitting the user to evaluate whether points were properly digitized. Furthermore, an abort control allows the user to quickly stop sampling at any time without damaging the specimen.

This technique permits carbonate samples to be recovered with greater resolution than ever before. The real-time imaging enables changes in lighting during digitization to achieve the clearest view of growth banding across the surface of the specimen.

High-resolution data will permit the testing of many outstanding questions in paleoclimatology (such as secular variation in seasonality), biology and evolution (such as fish migration/behavior). Ultimately micro-milling on this scale has the potential to open new frontiers in archaeology, biology, and geology in which detailed information can be recovered with a resolution unimagined only a short time ago.

#### Acknowledgements

We would like to thank Simon Catterall and Jim Lawyer for help in constructing the cubic spline interpolation program and integrating it within the LabVIEW<sup>®</sup> environment. We would also like to thank Phil Arnold for help in constructing the stage and Syracuse University for providing funding for equipment purchase.

#### References

- Beck, W.J., Récy, J., Taylor, F., Edwards, R.L., Cabioch, G., 1997. Abrupt changes in early Holocene tropical sea surface temperature derived from coral records. *Science* 385, 705–707.
- Dettman, D.L., Lohmann, K.C., 1993. Seasonal change in Paleogene surface water  $\delta^{18}\text{O}$ : fresh-water bivalves of Western North America. In: Swart, P., Lohmann, K.C., McKenzie, J., Savin, Climate Change in Continental Isotopic Records, American Geophysical Union Monograph 78, pp.153–163.
- Dettman, D.L., Lohmann, K.C., 1995. Microsampling carbonates for stable isotope and minor element analysis;

- physical separation of samples on a 20 micrometer scale. *Journal of Sedimentary Research, Section A: Sedimentary Petrology and Processes* 65 (3), 566–569.
- Edsall, T.A., 1967. Biology of the freshwater drum in western Lake Erie. *Ohio Journal of Science* 67 (6), 321–340.
- Kalish, J.M., 1991. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Marine Biology* 110, 37–47.
- Klein, R.T., Lohmann, K.C., Thayer, C.W., 1996a. Bivalve skeletons record sea-surface temperature and  $\delta^{18}\text{O}$  via Mg/Ca and  $^{18}\text{O}/^{16}\text{O}$  ratios. *Geology* 24 (5), 415–418.
- Klein, R.T., Lohmann, K.C., Thayer, C.W., 1996b. Sr/Ca and  $^{13}\text{C}/^{12}\text{C}$  ratios in skeletal calcite of *Mytilus trossulus*: covariation with metabolic rate, salinity, and carbon isotopic composition of seawater. *Geochimica et Cosmochimica Acta* 60 (21), 4207–4221.
- Linsley, B.K., Dunbar, R.B., Wellington, G.M., Mucciarone, D.A., 1994. A coral-based reconstruction of intertropical convergence zone variability over Central America since 1707. *Journal of Geophysical Research* 99 (C5), 9977–9994.
- Merritt, D.A., Hayes, J.M., 1994. Factors controlling precision and accuracy in isotope-ratio-monitoring mass spectrometry. *Analytical Chemistry* 66, 2336–2347.
- Patterson, W.P., 1998. North American continental seasonality during the last millennium: high-resolution analysis of sagittal otoliths. *Palaeogeography, Palaeoclimatology, Palaeoecology* 138 (1–4), 271–303.
- Patterson, W.P., 1999. Oldest isotopically characterized fish otoliths provide insight to Jurassic continental climate of Europe. *Geology* 27 (3), 199–202.
- Patterson, W.P., Smith, G.R., Lohmann, K.C., 1993. Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. In: Swart, P., Lohmann, K.C., McKenzie, J., Savin, Climate Change in Continental Isotopic Records, American Geophysical Union Monograph 78, pp.153–163.
- Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P., 1992. *Numerical Recipes in C: The Art of Scientific Computing*, 2nd edn. Cambridge University Press, New York.
- Secor, D.H., Piccoli, P.M., 1996. Age- and sex-dependent migrations of striped bass in the Hudson River as determined by chemical microanalysis of otoliths. *Estuaries* 19 (4), 778–793.
- Secor, D.H., Henderson-Arzapalo, A., Piccoli, P.M., 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology* 192, 15–33.
- Smith, G.R., Patterson, W.P., 1994. Mio-pliocene seasonality on the Snake River plain: comparison of faunal and oxygen isotopic evidence. *Palaeogeography, Palaeoclimatology, Palaeoecology* 107, 291–302.
- Tanaka, N., Monaghan, M.C., Rye, D.M., 1986. Contribution of metabolic carbon to mollusc and barnacle shell carbonate. *Nature* 320, 520–523.