# **Supplementary Materials for:**

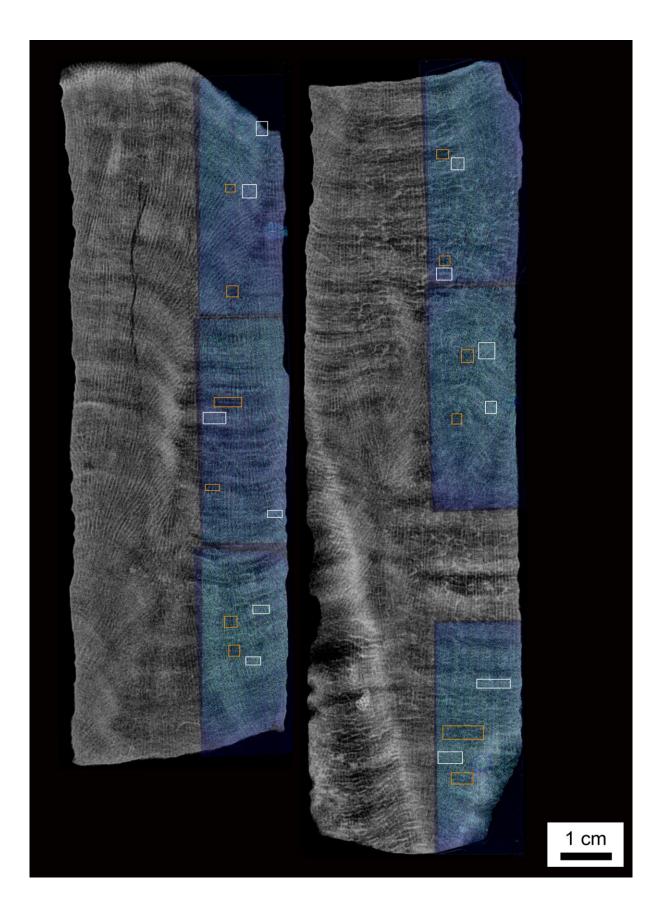
# Correction Factors for $\delta^{18}$ O-Derived Global Sea Surface Temperature Reconstructions from Diagenetically Altered Intervals of Coral Skeletal Density Banding

Mayandi Sivaguru, Kyle W. Fouke, Lauren Todorov, Michael J. Kingsford, Kaitlyn E. Fouke, Jeffrey M. Trop and Bruce W. Fouke<sup>\*</sup>

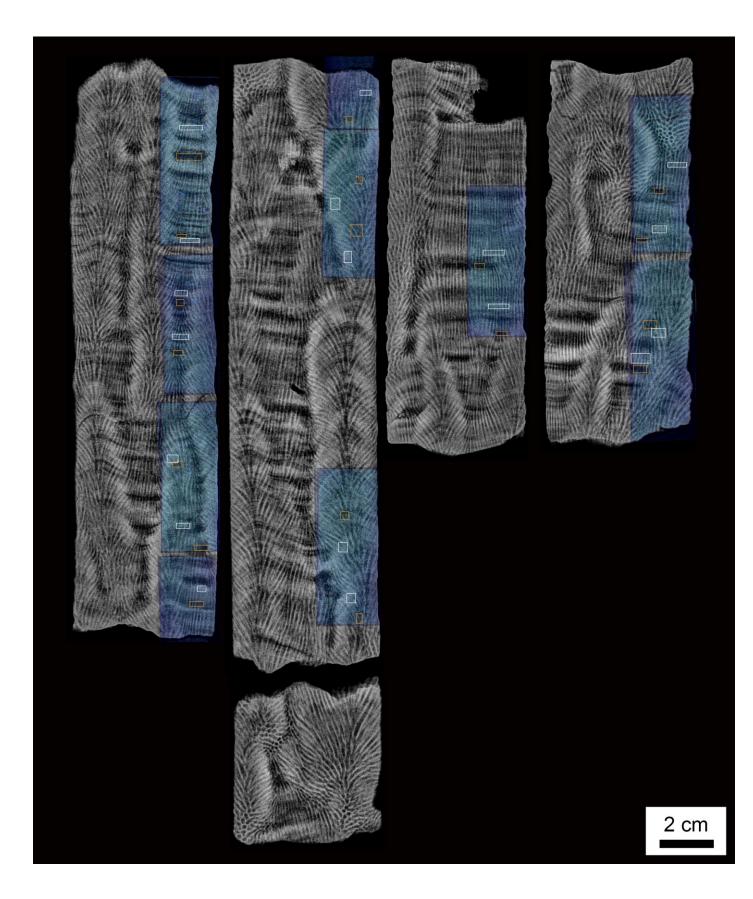
\*Corresponding Author

#### This PDF file includes:

Figs S1-S7 Supplementary Data Movie Captions Supplementary Extended Methods Supplementary Data 1 and 2



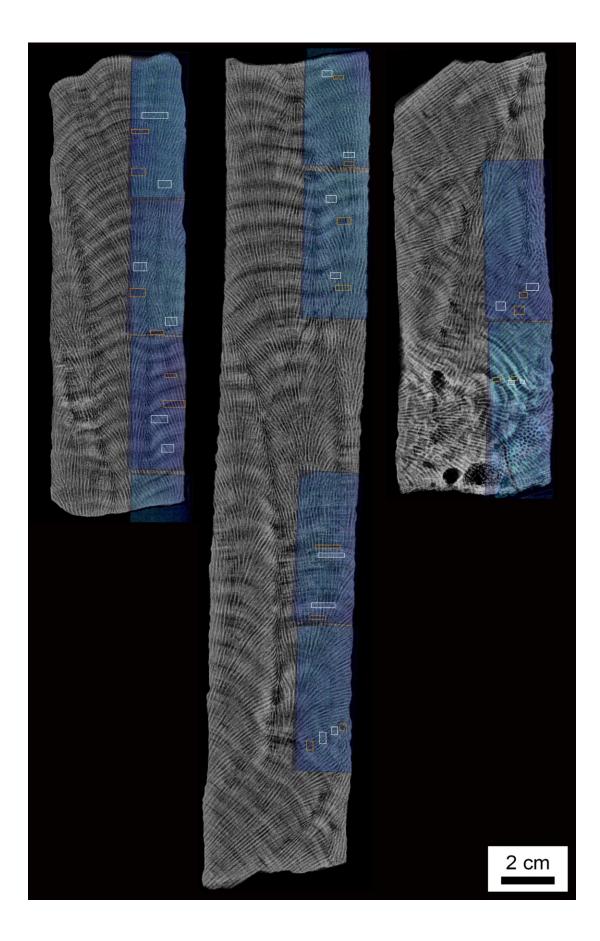
**Fig. S1.** Core 1 bright field (BF) thin section scan (blue) overlaid on XRD image (black is LDB and white is HDB), which permits high-resolution microscope analyses of the precise location of high- and low-density bands (HDBs and LDBs) within each thin-section. These precise HDB and LDBs positions are the locations where of the porosity measurements shown in **Fig. 4**, which were correlated with Nanozoomer BF images for porosity quantification.



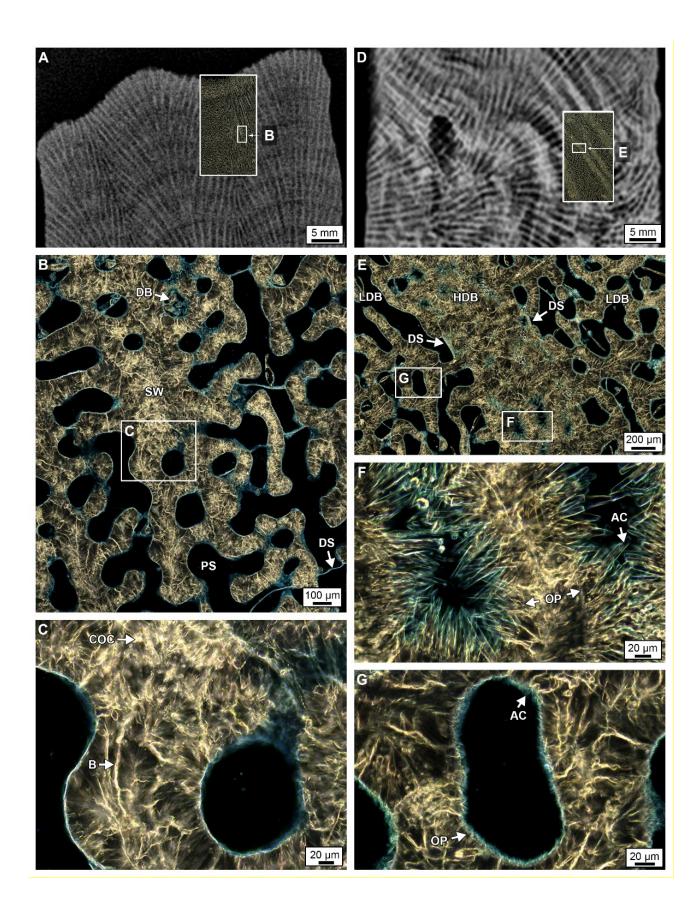
**Fig. S2.** Core 2 bright field (BF) thin section scan (blue) overlaid on XRD image (black is LDB and white is HDB), which permits high-resolution microscope analyses of the precise location of high- and low-density bands (HDBs and LDBs) within each thin-section. These precise HDB and LDBs positions are the locations where of the porosity measurements shown in **Fig. 4**, which were correlated with Nanozoomer BF images for porosity quantification.



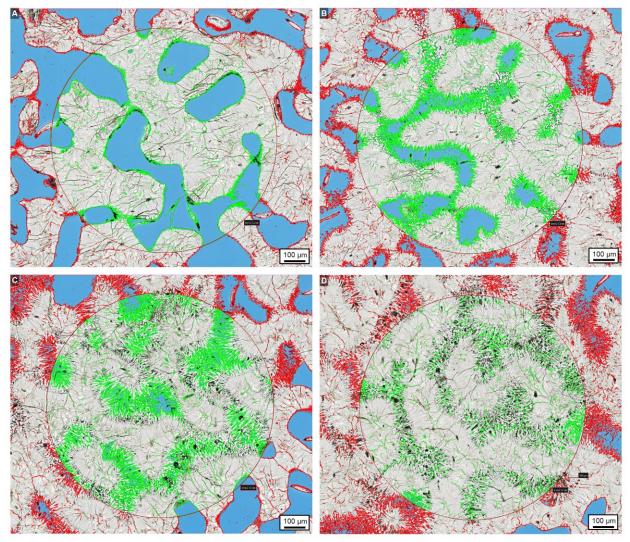
**Fig. S3.** Core 4 bright field (BF) thin section scan (blue) overlaid on XRD image (black is LDB and white is HDB), which permits high-resolution microscope analyses of the precise location of high- and low-density bands (HDBs and LDBs) within each thin-section. These precise HDB and LDBs positions are the locations where of the porosity measurements shown in **Fig. 4**, which were correlated with Nanozoomer BF images for porosity quantification.



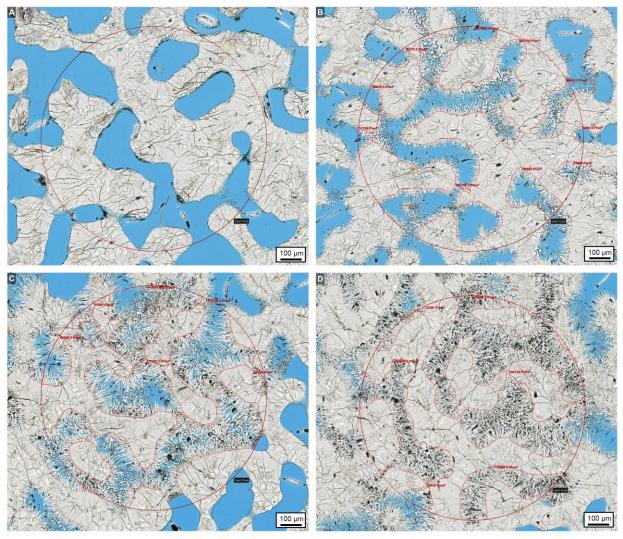
**Fig. S4.** Core 3 bright field (BF) thin section scan (blue) overlaid on XRD image (black is LDB and white is HDB), which permits high-resolution microscope analyses of the precise location of high- and low-density bands (HDBs and LDBs) within each thin-section. These precise HDB and LDBs positions are the locations where of the porosity measurements shown in **Fig. 4**, which were correlated with Nanozoomer BF images for porosity quantification.



**Fig. S5.** (**A-C**) Crystalline structure of high- and low-density bands (HDBs and LDBs) within the uppermost 2 cm of Core 3. (**A**) Overlay of a ring aperture contrast (RAC) image on an XRD image (black is LDB and white is HDB) showing the precise location of coral skeleton density banding (CSDB) on the thin section. (**B** and **C**) RAC images of an HDB-LDB contact below the uppermost surface showing skeletal wall (SW), skeletal debris (DB), pore space (PS), dissepiment (DS), centers of calcification (COCs) and borings (B; location of **B** shown in **A** and location of **C** shown in **B**). (**D-G**) Crystalline structure of HDBs and LDBs within the lowermost 6 cm of Core 3. (**D**) Overlay of a RAC image on an XRD image (black is LDB and white is HDB) showing the precise location of CSDB on the thin section. (**E**) RAC image of an HDB-LDB contact showing that the top and bottom of the HDB is bounded by dissepiments (DS; see also **Fig. 6**). The HDB, containing aragonite cements that occlude the pore spaces, extends from the upper left center of the image to the lower right center. (**F** and **G**) RAC images showing acicular aragonite (AC) growing in from the margins of original pore space (OP) that almost completely occludes the HDB pores (**F**). However, LDB pores are only minimally occluded (location of **E** shown in **D**, locations of **F** and **G** shown in **E**).



**Fig. S6.** Brightfield (BF) images showing ~0% (A), ~21% (B), ~42% (C) and 53% (D) occluded pore space automatically traced for the blue color from the epoxy in which the samples are embedded. All images are from high-density bands (HDBs) from the bottom 6 cm of Core 3. Although all images are thresholded, the calculation of porosity is restricted to the 1 mm-diameter area (indicated by red circle and porosity edge areas traced highlighted in green) representing the diameter of a drill tip used for microsampling.



**Fig. S7.** Brightfield (BF) images showing ~0% (A), ~21% (B), ~42% (C) and 53% (D) occluded pore space in high-density bands (HDBs). Manual tracing was completed of the margin of pore spaces within the original coral skeletons (fine irregular red lines). Note the area calculated includes the remaining porosity (blue regions), which is then subtracted from the automatic traces as shown in **Fig. S6** to calculate the absolute percent of diagenetic aragonite cement. These regions are also subtracted from the 1 mm-diameter drill tip area (indicated by red circle) to obtain the area of primary skeleton. All images are from the bottom 6 cm of Core 3 HDBs.

## **Supplementary Movies Captions**

**Supplementary Movie 1a.** Movie constructed from 3D MicroCT scan of *Porites* Core 3 highlighting the complex growth geometry of corallite bundles.

**Supplementary Movie 1b.** Same as Movie 1, but showing a 3D projection of all slices of the MicroCT scan of *Porites* Core 3 highlighting individual corollum color coded with its location marked in the center showing all spiraling corallite bundles within the core.

**Supplementary Movie 2.** Movie constructed from 3D Z-stack of phase contrast (PC) images collected from the coral skeletal wall shown in **Fig. 9D**, **E** and **F** illustrating the morphology of the leading margin of diagenetic skeletal replacement.

**Supplementary Movie 3.** Laser transmitted light (BF) image sequence showing the high frequency layering of sclerodermites from a finger of etched skeleton from the top of the core 3. Extracted few 2D frames are presented in **Fig. 12**.

#### **Supplementary Extended Methods**

#### Core collection, handling 3D Micro Computed Tomography

Acquisition, visualization and analysis of MicroCT images was conducted at The University of Texas at Austin High-Resolution Computed Tomography facility. Individual intact cores were placed in an acrylic cylinder and imaged using a high-resolution microCT system (North Scientific Instrument, Feinfocus 225kV). Images of each cylindrical cores were collected with the following scanning parameters: 190 kV, 0.17 mA, aluminum filter with a Perkin Elmer detector set at 0.25 pF gain, 2 frames per second, 2x2 binning, no flip, source to object distance of 230 mm, source to detection distance of 1316.6 mm, helical continuous microCT scan, vertical extent 238.7 mm, pitch 29.8 mm, 8 revolutions, 4 sets, helical sigma 29.8378519642639, no frames were averaged, 0 skip frames, 10405 projections, 6 gain calibrations, 5 mm calibration phantom, data range [-1.0, 40.0] (grayscale adjusted from NSI defaults), beam-hardening correction = 0.25. Voxel size = 62.9  $\mu$ m. This permitted the generation of several thousand images per core. Raw data images were converted to Tiff at both 8-bit and 16-bit gray scales for visualization. The images were made from the entire 3D data sets and presented as average intensity slices.

#### Preparation of coral cores for thin sectioning

Both 2 and 5 mm-thick longitudinal slices were cut on a clean tile saw through the center of each core, ensuring that each 5 mm-thick billet was positioned at the center of each core and was directly flanked by 2 mm-thick billets on each side. All slices and billets were imaged with XRD at the College of Veterinary Medicine, University of Illinois at Urbana-Champaign Small Animal Clinic X-Ray facility. Afterwards, 2 cm-width lengthwise slices were made at the edge of each 5 mm-thick slice. From these, 6 cm-long billets were cut at the positions shown by white boxes in Figure 1 and shipped. A total of 35 biological slide-sized billets were cut (6 cm length x 2 cm width x 5 mm thick), covering a total length of 2.1 m of coral core (**Fig. 2**). The top 30 cm of each core was fully covered with thin sections, while lower down-core billets were more widely spaced (**Fig. 2**). These 35 billets were shipped to Wagner Petrographic (Linden, Utah) where they were impregnated with blue epoxy and doubly polished to a thickness of 20-25  $\mu$ m.

# Brightfield (BF), Brightfield Extended Depth of Focus (BF-EDF), Phase Contrast (PC), Dark Field (DF) Ring Aperture Contrast (RAC), Polarization (POL) microscopy of thin sections

A suite of integrated of optical microscopy techniques were applied to determine the crystalline structure of the *Porites* coral skeletons in thin section. These modalities included BF, PC, DF, RAC and POL as described before (Sivaguru et al., 2012; Sivaguru et al., 2018a; Sivaguru et al., 2018b). We have used a suite of Zeiss instruments with the appropriate optical magnifications to render an ultimate spatial resolution of 250 nm. These include: (1) Zeiss Axio Zoom.V16 microscope with a 1.0x Plan Apochromat NA 0.25 objective (for BF, BF-EDF, DF); (2) Nanozoomer whole slide scanner system with an Uplansapo 20x, 0.75 NA objective (for BF); (3) Zeiss Axioobserver Z.1; 20x Plan Apochromat 0.8 NA, 63x Plan Apochromat 1.4 NA Ph3 objectives (for BF, PC, POL); and (4) Zeiss Axioscan Z1 whole slide scanning system with 20x, Plan Apochromat 0.8 NA and 50x Plan Neofluar 0.95 NA POL objectives (for BF, POL and RAC). All optical microscopy was conducted on instruments housed in the Microscopy and Imaging Core Facility of the Carl R. Woese Institute for Genomic Biology on the University of Illinois Urbana-Champaign campus. This facility was the first North American laboratory to be selected as a Zeiss Labs@Location Partner by Carl Zeiss LLC. In addition, laser transmitted images and

Airyscan SRAF (super-resolution autofluorescence) images were obtained as described previously (Sivaguru et al., 2012) using a Zeiss LSM 880 Airyscan system.

### Scanning Electron Microscopy

SEM was used to determine ultra-fine details of *Porites* coral skeleton crystalline architecture to complement the thin section petrography. Intact slices of coral core billets, both before and after etching with 0.1% formic acid for 30 s or 90 s (Nothdurft, 2007), were sputter coated for 70s, rinsed in water, dried in an oven (60-65° C) and coated with a gold palladium (Au/Pd) target using a Denton DESK II TSC sputter coater (Denton Vacuum, Turbo Sputter Coater, Moorestown, NJ). Samples were imaged on a FEI Quanta FEG 450 FESEM (Hillsboro, OR) at multiple magnifications to illustrate coral skeleton crystal structure across multiple length scales. In addition, a qualitative elemental analysis was performed using energy dispersive spectroscopy to confirm the identity of crystal's elemental composition. Down-core EDS analyses indicate all samples are composed of calcium, oxygen and carbon (aragonite) with rare trace elements.

### Whole Thin Section Slide Scanning and Porosity Analysis in HDB and LDB

Porosity of HDBs and LDBs was calculated from 40x Nanozoomer images of each of the 35 thin sections throughout all four cores using the Zeiss Axiovision program (version 4.9.1), with pointby-point data spreadsheets analyzed in Excel data (Supplementary Data 1, 2). A porosity analysis macro-program was created by defining a "rectangle outline" to determine the correct red, green, blue color range (8-bit gray scale) for each image. Due to slight differences in section thickness, the colors of the epoxy tones varied from light blue to dark blue. As a result, a range of blue shades were used to define and select regions of skeletal porosity. This color range was recorded for each thin section to complete the porosity calculation and determine an overall range of blue thresholds. The program calculates the total number of blue pixels (within defined thresholds) and normalizes this value to the full frame yielded porosity value for each image. A total of 420 images (12 images were collected from each of 35 thin sections) were analyzed for HDBs (n = 210) and LDBs (n = 210) 210) from top-to-bottom in all cores. The mean and standard deviation of these porosity measurements was determined by averaging all of the images within specific HDBs and LDBs defined by BF petrography. Percent porosity for a given thin section was determined by normalizing the porosity value to the full image dimension (see Supplementary Data 1). The percent porosity values and their plus-or-minus standard deviation is shown as scatter plot, with the depth in centimeters within each core (linked to middle of each thin section) is shown along the X axis. Statistics and graphing was completed with Sigma Plot 11.0 (Systat Software, NJ) and assembled in Adobe Photoshop (Adobe Systems, San Jose, CA).

### HDB and LDB Image Extraction for Porosity Analysis

Two HDB and LDB areas were chosen by overlaying XRD and MicroCT images on Nanozoomer and V16 images on each of the 35 thin sections (Supplementary Data Figs. S1-4). Areas were carefully selected to ensure they remained within single spiraling coral bundles. In addition, analysis areas were placed only in locations where the high-resolution Nanozoomer-scanned images had appropriately clear exposure, contrast, resolution and no air bubbles in the thin section. Nanozoomer opacity was raised to 100% on Photoshop for better visualization. The native NDP view (version 2.3, freeware from Hamamatsu.com) program was used to open the Nanozoomer raw image files for image capture and further analysis. Both analysis programs were lined up sideby-side for better visualization (Photoshop and NDP view). Rectangular boxes were precisely positioned on NDP View Nanozoomer images based on the exact locations of the Photoshop overlay image. This was done by determining the exact pixels and skeletal shapes. A screenshot image of this set up was taken for each thin section and is available upon request. Three images at 40X magnification (scanned resolution) were taken for porosity analyses in or around each box depending on the box size and artifacts such as air bubbles or plucked and lost skeleton as described above. All images were cross checked by in depth comparison with the XRD in order to obtain the best representation of either HDB or LDB needed. A total of six images each for HDB and LDB taken from two different bands within the thin section. HDBs can contain low density regions (due to the curving nature of spiraling corallite bundles (**Fig. 2; Supplementary Movie 1a**), which were avoided during image collection.

**Image Adjustments, Analysis and Presentation:** The techniques used to process each 3D Micro CT image is presented above. All SEM images were presented without any intensity corrections. All optical microscopy images were processed in native Zeiss Zen Black (Airyscan) or Zen Blue (all over modalities) software. The entirety of raw data collected for each image presented in this manuscript is available upon request and most of them have also been uploaded to the Figshare website as described in the manuscript. The images are adjusted after following the ethical procedures as described previously (Cromey, 2010). RGB curves were adjusted and presented as linear or with a gamma adjustment of 0.45, min/max, best mode or manually adjusted in the display properties window in the Zeiss Zen software for representative brightness, contrast and clarity. Final images are cropped, aligned, resized and in some cases the opacity has been changed to show the details of overlay of images in two different modalities, where necessary. All of these adjustments were performed in Adobe Photoshop (Adobe systems, San Jose, CA). In addition, for 3D visualization of Micro CT was completed to create 3D images and movies using Imaris 3D Visualization software (Bitplane, Zurich, Switzerland) or Avizo (Thermofisher Scientific).

**Supplementary Data 1.** Point-by-point data for graphs in figure 2. (a high resolution Excel sheet is uploaded online at figshare.com <u>https://figshare.com/s/d497f526f125406aac20</u>

	А	В	С	D	E	F
1	Core Number	Thin Section Depth (cm)	Porosity (φ %)			
2			HDB	HDB SD	LDB	LDB SD
3	Core 1	6	26.4389	6.9129	52.35	9.5459
4	Fig. 2A	12	32.0548	0.6022	69.7	4.2426
5	116.27	12	30.3797	1.0082	61.55	2.0506
6		24	39.7802	5.7947	70.3	3.2527
7		30	35.2159	7.2088	63.95	7.9903
8		37	29.8453	2.5436	60.25	2.6163
9						
10	Core 2	6	47.6	0.8485	78.75	0.9192
11	Fig. 2D	12	41.35	8.4146	68.35	3.8891
12		18	29.75	2.3335	59.35	5.869
13		24	36.95	6.2933	58.05	3.3234
14		30	24	9.6167	55.9	8.4853
15		36				
16		42	31.1	4.1012	61.85	5.869
17		48				
18		54				
19		60	30.95	5.4447	58.15	18.1726
20		67				
21		72	29.85	7.1418	67.15	3.8891
22		79	29.95	0.7778	64.8	5.5154
23						
24	Core 4	6	27.5	2.9698	58.45	8.9803
25	Fig. 2G	12	27	4.3841	69.4	5.6569
26		18	36.35	0.495	61.7	7.4953
27		24	32.05	5.7276	69.4	5.2326
28		30	29.4	3.5355	69.25	1.9092
29		36				
30		42	32.35	2.0506	64	1.1314
31		48				
32		54	25.9	5.5154	63.3	2.4042
33		60	20.45	4.4740	64.4	4.0407
34		66	30.45	4.1719	64.1	4.9497
35 36		72 78	26.7	0.9899	60.7	1.2728
37		84	20.7	0.3033	00.7	1.2720
38		90	29.35	6.0104	62.8	1.1314
39		97	26.25	4.5962	64	0.9899
40		- •				
41	Core 3	3	27.4	2.1213	52.05	0.495
42	Fig. 2J	9	30.85	5.4447	54.1	4.3841
42		15	27.7	2.5456	56.8	4.6669
43		21	25.05	6.2933	57.85	2.0506
45		27	29.25	0.6364	57.35	5.0205
46		33				
47		39	24.2	0.2828	70.85	4.879
48		45	32.55	4.879	64.2	2.2627
49		51				
50		57	23.7	4.1012	61.4	4.5255
		63	3.4	0.7071	49.85	4.4548

**Mixing Analysis Supplementary Data 2.** All data from Core 3 bottom multiple locations within HDBs showing Original skeleton, Cement, and remaining Porosity.

	Original%	Cement%	Porosity	
0	100	0	35.2	
20	53.4	21.1	25.4	
40	38.4	42.0	19.6	
60	46.9	53.1	0.0	