LIMITS ON BENTHIC FORAMINIFERAL CHEMICAL ANALYSES AS PRECISE MEASURES OF ENVIRONMENTAL PROPERTIES

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ABSTRACT

The “practical precision” of benthic foraminiferal Cd/Ca analysis (defined as the reproducibility on replicate picks of foraminifera from the same sample) is poorer than the analytical precision (defined as the reproducibility of replicate solutions analyzed during a 6-year period). Conversely, the reproducibility on splits of homogenized fragments (obtained by gently crushing foraminifera into ~50 fragments each and mixing the fragments) is close to the analytical precision. This experiment rules out variability in sample-to-sample handling as a cause of imprecision, and it strongly implicates individual-to-individual specimen variability as the major factor limiting reproducibility in foraminiferal Cd/Ca analysis. This inference is confirmed by a pilot study using a new method for the analysis of single specimens of benthic foraminifera: individual benthic foraminifera from a sample show large variability from one individual to the next. Hence, the fundamental factor limiting the effective precision of foraminifera Cd/Ca analysis is not analytical precision or laboratory sampling procedures, but rather the number of individuals available for analysis.

INTRODUCTION

Minor and trace element concentrations in coralline and foraminiferal calcium carbonate are used to determine variability of the oceanic environment on time scales of decades to millions of years (Beck and others, 1992; Boyle, 1992; Boyle and Keigwin, 1982; Delaney and Boyle, 1987; Delaney, 1990; DeVilliers and others, 1994; Graham and others, 1981; Shen and Boyle, 1987; Shen and others, 1987; Lea and Boyle, 1990a, b; Lea and others, 1989). Sr/Ca, Pb/Ca, Cd/Ca, and Ba/Ca have been used to a significant extent, and other elements are being actively explored. The precision and accuracy of calcareous elemental ratios as measures of environmental properties must be assessed in tandem with paleoenvironmental investigations. Laboratory analytical technology is an obvious limitation but may not be the primary limiting factor. Sr and Nd isotope measurements are routinely reported to six significant figures, and thermal ionization isotope-dilution mass spectrometry can measure some element/calcium ratios to 0.1% or better. Other analytical methods in use (e.g., atomic absorption or quadrupole plasma mass spectrometry) attain a laboratory precision of a few percent. But for all of these measurement procedures, we must determine whether the measured ratio reflects paleoenvironmental properties as precisely as the laboratory instrumentation reflects the chemical composition of a particular sample. In other words, are fossil foraminifera as precise analytical chemists as are geochemists?

There are indications that measurement accuracy may not be the limiting factor in interpreting some paleoenvironmental minor and trace element data. The ultimate test of the reliability is established by comparing analyses of real samples formed under similar environmental conditions. For example, DeVilliers and others (1994) showed that precisely-determined Sr/Ca ratios of coral bands are controlled not only by (primarily) temperature, but also by extensional rate (or other correlated secondary factors). Hence the ability to interpret Sr/Ca as a paleothermometer may depend less on the analytical capability than it does on the ability to control for secondary factors. Similarly Shackleton and Opdyke (1973), Shackleton and others (1983), Boyle (1984), and Boyle and Rosener (1990) have argued that bioturbation of sediments may limit the useful precision of δ¹⁸O, δ¹³C, and Cd/Ca measurements on foraminifera from ocean sediment cores, because the mixture of specimens that grew at different times introduces noise into measurements based on a limited number of individual specimens. As evidence for this mechanism, they noted the poorer reproducibility of replicate picks of foraminifera compared to replicate analyses of standards. Analysis of individual specimens of benthic foraminifera for δ¹⁸O and δ¹³C shows significant variability between specimens from the same Nordic Atlantic samples (Vogelsang, 1990). Lohmann and Lohmann (manuscript in preparation) have also analyzed single specimens for δ¹⁸O and δ¹³C in low-sedimentation rate cores from the South Atlantic and see large differences that they ascribe to biological mixing of glacial and interglacial specimens.

The purpose of this study is to establish the relative roles of analytical precision, laboratory procedures, and variability between specimens in limiting the meaningful precision that can be assigned to estimates of oceanic Cd from measurements in benthic foraminifera. As will be seen, variability between specimens in a sample—and the difficulty of finding enough specimens over which to average—is the principle limitation on the “useful” precision with which foraminiferal Cd/Ca can be used as a paleoenvironmental tool. Hence the reliability of estimates for oceanic Cd in ancient oceans relies far more on understanding and controlling factors controlling individual variability than it does on improving laboratory measurement precision.

STRATEGY

In order to resolve these issues, the following three factors must be determined: (1) The analytical precision of the instrumentation. How precisely can an instrument perform on replicate analyses of precisely-controlled uniform samples? Short-term precision within a day's work is only a best-case limit on long-term precision and accuracy during many years of analyses which is invariably worse. (2) The reproducibility of analytical procedures in the laboratory. Most analytical procedures involve some manipulation: samples are dissolved, separation procedures are un-
undertaken, etc. These procedures can introduce contaminants or uncertain yields that affect the estimated composition of the original sample. (3) The heterogeneity and variability of the samples. Particularly in solids, elemental composition may vary on quite small spatial scales. Does any such heterogeneity occur for foraminifera Cd, and do sampling procedures average this heterogeneity appropriately? This problem is difficult to resolve when concentrations are low compared to instrument detection limits. In any event, it is all but impossible to determine elemental heterogeneity down to the atomic level; at fine spatial scales we must rely on indirect means for estimating heterogeneity.

SHORT- AND LONG-TERM
INSTRUMENTAL PRECISION

Most published analyses of foraminiferal Cd/Ca have been undertaken by graphite furnace atomic absorption (GFAAS) analysis of Cd and flame atomic absorption (FAAS) analysis of Ca (although other methods could be used). Other aspects of this analytical procedure are described elsewhere (Boyle, 1981; Boyle and Keigwin, 1985/6), and only those details relevant to accuracy and reproducibility are discussed here.

Atomic absorption chemical analysis is a relative method requiring the comparison of UV/visible light absorption by standards of known concentration against the unknown samples. Hence a primary factor controlling accuracy and precision is the reliability and stability of the standards used for comparison. In this laboratory, new elemental primary solution standards are made from stoichiometric solids every 5–10 years. These primary standards are then diluted and spiked into analytical-element-free solutions with compositions similar to those of the samples. In this case, foraminiferal CaCO₃ samples are dissolved in dilute nitric acid. The Cd standards are prepared by spiking diluted Cd standard into ~0.05 N HNO₃ solutions containing ~18 mmol/ml Ca(NO₃)₂ that has been previously purified of Cd by repeated batch extractions using Chelex-100. In our case, the Cd standards range from 0–5 pmol/ml; the Ca standard is a 20 μmol/ml solution that is diluted to 10 mmol/ml in a matrix–matching and interference-minimizing solution consisting of ~10⁻³ M lanthanum and ~10⁻³ M HNO₃ in 0.05 M HCl. Solutions are then stored under conditions that maximize stability. The aqueous standards are acidified and stored in acid-leached high-density polyethylene containers to minimize adsorption and leaching of elements from the container. Because polyethylene is permeable to water vapor, standard concentrations increase over time due to evaporation. In order to minimize this factor, standard bottles are stored within sealed glass jars to which a small amount of water is added to saturate the internal air. Under these conditions, evaporation is minimal (<0.1% per year) and standards are stable for years if handled properly.

Atomic absorption interferences of various types are caused by the major element components of the sample; hence it is necessary that standards and samples be matched in terms of major element composition. Because the exact concentration of samples is unknown, some mismatch of standards and sample composition is likely, so residual interferences must be known and compensated for if significant. For example, under the graphite furnace and tube surface conditions employed for this work, the cadmium absorbance signal depends slightly (±15%) on the total calcium concentration. After the calcium concentration has been determined by FAAS, the GFAAS Cd measurement is therefore corrected to account for this residual interference.

Four Cd standards comprising a 0.0–0.1 absorbance standard curve are run as duplicate 20 μl injections by GFAAS every 8 samples, and the slope of the resulting standard curves are linearly drift-corrected for samples in-between these standard curves. Sensitivity drift (mainly due to changes in graphite tube characteristics as the tube ages due to the heating cycles) is typically less than 5% between standard curves. Three aliquots of the dilute 0.075 HNO₃ (that is used to dissolve the foram samples) are run in duplicate just before and just after each group of 30 samples. In most cases, the blank is not detectable and no correction is applied. Occasionally, the acid becomes slightly contaminated from handling, in which case the blank correction is applied and the contaminated acid is discarded.

Using the matrix described above, the FAAS standard curve for Ca is reliably linear, so a single standard is run every 8–10 samples (nitrate matching relative to samples and La addition are both important for ensuring this linearity, however). The dilution matrix solution is used as the baseline solution, so no blank correction is necessary (runs of the matrix against distilled water on many occasions show that the matrix does not contain measurable Ca).

In order to estimate the precision of this procedure, several other standard solutions—"Consistency Standards"—are run as if they were samples and the Cd and Ca concentrations of these solutions are calculated as for normal samples. These Consistency Standards are not used for calibration, but only to assess analytical accuracy and precision. The concentrations of these standards are set to be typical of foraminiferal samples (for the solution discussed here, it is equivalent to a Cd/Ca of 0.181 μmol/mol for 0.15 mg of cleaned foraminiferal calcite dissolved in 75 μl). The reproducibility of these analyses during a period of a few hours on individual days is considered to be the instrumental precision, and reproducibility of these standards during many years is considered to be the effective analytical precision relevant to real sample analysis. Figures 1 and 2 show the time-trends and histograms of all paired consistency standard analyses during a period of 6 years (June 1987–October 1993). For each group of samples run, the Cd and Ca concentrations of these consistency standards were run in duplicate (except for one group where the second replicate was not run). Excluding no analyses, the standard deviation of these 193 analyses during this period is 3.4% for Cd (at ~3 pmol/ml) and 2.0% for Ca (at ~18 pmol/ml before the 250-fold dilution with the analysis matrix). Assuming the errors are uncorrelated, the error for the Cd/Ca ratio is then 3.9%. The relative standard deviation (RSD) of the ratios for each of these pairs is 4.1%, confirming the uncorrelated nature of the errors. These statistics would be improved only marginally by rejection of a few outliers. This 4% result should be taken as the long-term precision of the entire analytical technique as it is applied.

Because each consistency standard was determined in duplicate for all but one group of samples, the short-term pre-
FIGURE 1. Cd and Ca Consistency Standards analyzed over a period of 6 years (June 1987–October 1993).
reproducibility sets a limit on the ultimate quality attainable for long-term precision. It appears that it would be possible to improve long-term precision somewhat by stricter attention to standard stability and more frequent standardization during AA runs.

Accordingly, it has been established that the reproducibility of Cd/Ca analysis on stable solutions in this laboratory is about 4% during a period of 6 years for solutions containing Cd and Ca concentrations typical of those encountered when analyzing benthic foraminifera. Obviously this precision could be improved upon, perhaps by a factor of two or so staying within AAS analytical technology, or more dramatically by using other methods such as TIMS. Nonetheless, the reproducibility of replicate picks of analyses of different groups of 10–20 individuals picked from the same sample and analyzed on the same day is considerably worse than the present level of analytical precision. Hence further analytical improvements are futile unless the factors leading to this between-sample variability can be understood and controlled.

REPRODUCIBILITY OF ANALYTICAL PROCEDURES IN THE LABORATORY

Foraminifera must be processed in various ways before they are presented as a solution to the atomic absorption spectrophotometer. Is it possible that a lack of complete reproducibility in these laboratory procedures is the major cause of the lack of analytical reproducibility on replicate samplings of foraminifera? For example, could the following two factors influence the ultimate reproducibility? (1) Foraminifera must go through a rigorous cleaning procedure to remove contaminant solutions. If this cleaning is not carried out reproducibly, perhaps some residual contaminants may remain on the sample. (2) Random contamination of samples after the cleaning procedure may occur during handling and (temporary) storage in centrifuge vials and AA cups. Indeed, an occasional contaminated sample is likely, because complete elimination of trace element contamination has never been achieved. However, experienced labs expect that handling contamination is rare and does not occur for most samples.

Because these procedures already are done carefully, it would be difficult to devise a direct experiment to test for these supposed variable procedural effects (one cannot be more careful than as careful as one can be). However, an indirect procedure rules out this factor as a significant factor in controlling reproducibility. As noted earlier, biological stirring of sediments can create a mix of individuals that lived at times of different environmental properties. Because of the scarcity of benthic foraminifera, only a few specimens (often 10–20 or less) are available from the largest practical samples. Statistically, this is like taking a handful of marbles from a well-mixed hat containing an equal mixture of black and white marbles. The rules of sampling statistics apply; repeated picks of ten marbles on average will have 5 of each color and will show a standard deviation for each of \( \pm \sqrt{10} \) for a large number of samplings. Boyle (1984) and Boyle and Rosener (1990) devised a statistical procedure for assessing the effect of this process on replication precision, and demonstrated that one should expect that it is often

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**FIGURE 2.** Histograms of these Consistency Standard analyses over a period of 6 years (June 1987–October 1993).
more important than measurement precision. Similarly, Andree (1987) demonstrated that AMS $^{14}$C analyses were severely affected by this artifact.

In order to produce more homogeneous solid foraminiferal samples for assessment of sample treatment variability, fragments of many individuals can be homogenized. Because individual foraminifera are always slightly crushed in any event (to open up the inner portions of their chambers for cleaning), this procedure is relatively straightforward. Each foraminiferal sample is typically split into several dozen small fragments (say about 50 for the purposes of this discussion). The object is to eliminate variability between individuals as a factor in producing variability between independently processed samples. If variability in laboratory procedures (rather than variability between individual foraminifera) causes replicate picked samples to show more scatter than replicate solution samples, then fragment homogenization will not affect the reproducibility. If, on the other hand, variability between individual foraminifera is the cause of replication imprecision, then this procedure should be expected to result in a dramatic improvement in replicability ($\sqrt{50} \approx 7$-fold improvement).

The effect of this procedure on replicate precision was assessed in core TR163-31b (3°37'S, 83°58'W, 3,210 m). This core has a relatively high sedimentation rate (averaging 9 cm/kyr, with higher rates in the glacial and deglacial section than in the Holocene top). Being a deep Pacific core, the glacial/interglacial deepwater variability is minimal (because the relatively homogeneous Pacific deep waters cannot stray far from the global mean nutrient composition) with a 13% one-sigma RSD for the replicates compared to the mean Uvigerina result at each depth interval. Samples from the top and bottom halves of oxygen isotope stage 2 were analyzed (the average sample consisted of 18 ± 5 individuals weighing 32 ± 9 μg per individual, one-sigma). The top half of isotope stage 2 was analyzed as triplicate picks of individuals from the sample, and the bottom half was analyzed as splits of crushed and homogenized individuals. The results of this experiment are shown in Figure 3.

There is a dramatic decrease in the scatter of the replicates.
of the crushed-and-split samples (RSD = 5%) compared to the replicate-picked samples (RSD = 13%). The crushed-and-split reproducibility is close to analytical precision, whereas the scatter on replicate picks is significantly larger. If we consider that in these experiments the scatter is due to two factors (analytical error and between-sample variability), and assuming that we know the analytical error is 4%, then the between-sample variability can be assessed:

\[ \sigma^2_{\text{analytical}} + \sigma^2_{\text{sample}} = \sigma^2_{\text{total}} \]

According to this assessment, \( \sigma_{\text{sample}} \) improved by a factor of 4 due to the crush-and-split procedure, which is in the ballpark of the ideal factor of 7 that would result from a 50-fold increase in the number of fragments (particularly in view of the small difference between \( \sigma^2_{\text{analytical}} \) and \( \sigma^2_{\text{total}} \) and also in view of possible imperfect homogenization of fragments).

Another test of the role of laboratory procedural variability was undertaken on a large sample of a virtually pure *Uvigerina* sand provided by Scott Lehman from core Troll 3.1 (60°46.7′N, 3°42.8′E, 332 m). 0.5 g of this sample was crushed and split into individual ~0.5 mg portions. Thirteen Cd/Ca analyses of this sample are given in Table 1; the mean and standard deviation of these analyses are 0.029 ± 0.006 μmol/mol. Again, the small standard deviation shows that random contamination of samples is not a common cause of errant Cd/Ca analyses. Such a small standard deviation, even if entirely due to procedure factors, would not contribute significantly to the standard deviation of samples from the Pacific such as TR163-31b above.

From these experiments, it is evident that laboratory procedural variability is not the major factor limiting Cd/Ca reproducibility on replicate picks of foraminifera from the same sample. So neither analytical reproducibility nor analytical procedural variability can account for the irreproducibility of replicate picks. Between-individual Cd/Ca heterogeneity is a large and perhaps even dominant factor in producing the irreproducibility of replicate picks.

### DIRECT DEMONSTRATION OF INTER-INDIVIDUAL Cd/Ca VARIABILITY

Although the above indirect evidence makes a strong case for individual-to-individual variability in Cd/Ca, it would be preferable to observe this variability directly. However, previous methods for the analysis of Cd in benthic foraminifera have been limited to sample sizes >0.2 mg of initial CaCO₃. Because most individual benthic foraminifera only weigh a few tens of micrograms, until now it has not been possible to analyze individual benthic foraminifera (other than the occasional unusually large specimen). In view of the importance of understanding factors limiting foraminiferal precision and reliability, I have worked towards achieving single-benthic foraminiferal analysis. This effort was directed with the following factors in mind: (1) One problem in analyzing small samples is that small samples are disproportionately lost during cleaning (very small samples vanish entirely during the cleaning procedure). This “overhead” loss must be reduced while not reducing it below that encountered for normal samples (to avoid a cleaning bias). (2) Any new procedure must be checked against the normal procedure used on large samples and must be shown to give the same answer. In other words, the average value of a number of individuals analyzed separately must give the same value as the same individuals analyzed as a larger group.

The most important factor creating loss of sample during cleaning for samples of all sizes is loss of fine fragments due to crushing and ultrasonic removal of fine detritus. In the normal cleaning procedure, about half of the sample is lost during this step. This loss is unavoidable to some extent because the crushing and fine-removal steps are an important aspect of the cleaning procedure. However, when foraminifera are crushed individually, the size of the fragments can be maximized by observing the crushing process through a microscope, crushing them only sufficiently to open up the individual chambers (but no more). This step reduces the sample loss due to crushing and ultrasonic removal of fines by about a factor of two.

The second major cause of sample loss during cleaning is the chemical dissolution of sample that occurs during certain cleaning steps. The two steps in which dissolution is significant are (1) the reductive cleaning procedure (where CaCO₃ is dissolved because of Ca-complexation with citric acid, which is a function of the pH of the cleaning solution), and (2) the final dilute acid rinses of the cleaned samples. For normal-sized samples (0.3 mg or more), the effect of this loss is minor—perhaps an additional 15% of the original sample weight before cleaning. However, for very small samples, the effect of dissolution during cleaning is proportionately larger.

In the reductive cleaning step, two factors are important in controlling the extent of dissolution: the amount of citric acid in the cleaning solution and the pH of the solution. In view of the goal of maintaining a constant relative amount of dissolution, sample dissolution due to calcium complexation can be met by reducing the total amount of citric acid encountered by the sample in proportion to the sample size. This reduction in citric acid could be achieved by reducing either reagent volume or reagent concentration. The former method is preferable, but its extent is limited because reagent volume cannot be reduced below 20 μl or so because of difficulty in manipulation. However, simply reducing reagent volume introduces another problem, because as the ratio of reagent to void space in the cleaning vessel decreases,

### Table 1. Cd/Ca in splits of crushed-and-split standard foram sample.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cd/Ca Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.028</td>
</tr>
<tr>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>3</td>
<td>0.029</td>
</tr>
<tr>
<td>4</td>
<td>0.033</td>
</tr>
<tr>
<td>5</td>
<td>0.025</td>
</tr>
<tr>
<td>6</td>
<td>0.020</td>
</tr>
<tr>
<td>7</td>
<td>0.025</td>
</tr>
<tr>
<td>8</td>
<td>0.037</td>
</tr>
<tr>
<td>9</td>
<td>0.023</td>
</tr>
<tr>
<td>10</td>
<td>0.028</td>
</tr>
<tr>
<td>11</td>
<td>0.029</td>
</tr>
<tr>
<td>12</td>
<td>0.027</td>
</tr>
<tr>
<td>13</td>
<td>0.004</td>
</tr>
<tr>
<td>Mean</td>
<td>0.029</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.006</td>
</tr>
</tbody>
</table>
es, more ammonia (which maintains high pH) is lost to the void space. Hence a reduction in reagent volume must be accompanied by a reduction in void volume. This reduction has been accomplished by fabricating inserts that fit inside the 0.5 ml micro centrifuge tubes and restrict the void space to a small section near the bottom. Finally, a further reduction in sample dissolution is achieved by decreasing the reducing reagent concentration by a factor of four, and by forgoing (or shortening) the ultrasonic treatment during the final dilute acid rinses. Using this new treatment for small samples results in a similar extent of dissolution for very small samples as for normal samples, so smaller samples can be cleaned without disappearing.

The second aspect that needs to be addressed in analyzing smaller samples is to improve the detection limit of the analytical method. Steady progress in elemental detection limits occurs on decadal time scales—both by improvements to existing techniques and in new instrumentation developments. In this case, an improvement in Cd detection limit has been achieved by using a Hitachi Z8100 DC-Zeeman graphite furnace atomic absorption spectrophotometer with a Photon high-intensity Cd superlamp. Using a time constant of ~0.2 sec on this setup gives an instrumental noise level of <0.0002 absorbance units, allowing for detection limits of <1 femtomole for Cd.

Combining both of these improvements, it is now possible to measure single benthic foraminifera. For the first demonstration of this capability, I used a sample from core TR163-31p, 543–547 cm. This sample is from a high-abundance interval of oxygen isotope stage 4. From the total sample, 75 specimens were selected at random, and then crushed-and-split into five fractions as described above. Three of these splits were analyzed for Cd. From the rest of the sample, relatively large individuals (but not unusually so) were selected to be analyzed individually. Due to a defective micro balance, accurate weights were not obtained for these, but they were of the order of 50–150 µg per individual (compared to the 41 µg per average individual in the unselected crushed-and-split samples). After cleaning, the samples were 29–64 µg, representing a loss during cleaning of about 50%, comparable to the loss seen for similarly carefully-crushed multiple-individual specimens using the normal cleaning method. Figure 4 illustrates the analytical data for a single such analysis. Clearly, the signal-to-noise ratio is more than adequate for this work (and would be so for samples of less than half the size) and hence it is now possible to analyze typical single benthic foraminifera for Cd/Ca.

The 3 replicates from the 75 individuals that were crushed and split gave a mean Cd/Ca of 0.142 µmol/mol with a standard deviation of ±0.003. The 16 individuals foraminifera gave a mean Cd/Ca of 0.161 µmol/mol with a standard deviation of ±0.055 (Table 2). Two of the individuals had Mn/Ca > 200 µmol/mol, signifying excessive MnCO₃ overgrowths which are thought to artificially raise the Cd/Ca. Excluding these two individuals, the mean is 0.152 ± 0.052. Given a selection of 16 individuals from a large population containing a mean of 0.142 with a standard deviation between individuals of 0.055, a result differing from the mean by ±0.019 will occur in 17% of a large number of trials. Given a selection of 14 individuals with a mean of 0.142 with a standard deviation of 0.052, a result differing from the mean by ±0.010 will occur in 49% of the trials. In other words, at two-sigma, the 0.161 result (no rejections) is not statistically significantly different from the 0.142

![Figure 4](https://example.com/image.png)

**Table 2. Comparison of single-specimen and crushed-and-split multiple-specimen Cd analyses.**

<table>
<thead>
<tr>
<th>Weight (after cleaning)</th>
<th>Cd/Ca</th>
<th>Mn/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 individuals, crushed-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and split into 5 lots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.142</td>
<td></td>
</tr>
<tr>
<td>Std. dev.</td>
<td>±0.003</td>
<td></td>
</tr>
<tr>
<td>16 individuals, analyzed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>individually</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.161</td>
<td></td>
</tr>
<tr>
<td>Std. dev.</td>
<td>±0.055</td>
<td></td>
</tr>
<tr>
<td>Mean excl. Mn &gt; 200</td>
<td>0.152</td>
<td></td>
</tr>
<tr>
<td>Std. dev.</td>
<td>0.052</td>
<td></td>
</tr>
</tbody>
</table>
mean for many individuals run as a group, and at one-sigma
the 0.152 result (rejecting two individuals with high Mn/Ca)
not statistically significantly different from the 0.142
mean for many individuals run as a group.

This observed variability between individuals is also con-
sistent with the 13% standard deviation of analyses of rep-
licate picks of 18 ± 5 individuals from the same sample
observed in the upper section of TR163-31b (noted above).
That is, if the RSD of a population of individuals is 34%,
then when analyzed as groups of 16 individuals, the stan-
dard deviation of a number of grouped analyses is expected
to be 34 ± √16 = 9% RSD for replicates of 16 individu-
able analyzed as a group. So this new cleaning and analysis
procedure passes the test of individually-analyzed speci-
mens having the same value as collectively-analyzed speci-
men, and it also accounts for the variability of replicate
picks from the same sample.

Obviously much more work needs to be done to docu-
ment the characteristics and variability of single-specimen
Cd analyses. But the results of these single-individual anal-
yses, combined with the indirect results from the crushed-
and-split precision comparison to the precision of replicate
picks, provides overwhelming evidence that variability be-
tween individual specimens of foraminifera is the cause of
the imprecision of replicate picks from the same sample.
Hence it has been proven that improvements in analytical
precision will not affect the reproducibility of downcore
analyses of benthic foraminiferal Cd/Ca unless one can an-
alyze very large numbers of specimens.

This result leads to “the precision paradoxes”: (1) In or-
der to obtain the most “practically reproducible” results, it
may be preferable to use a less sensitive analytical pro-
cedure that requires larger samples. (2) Improvements in sen-
sitivity will result in a degradation of “practical precision”
if used to decrease sample size (rather than to study indi-
vidual variability). These are inherent problems in the chem-
ical and isotopic analyses of fossil foraminifera.

**INTRA-INDIVIDUAL Cd/Ca VARIABILITY?**

Do individuals show as much variability within single
individuals as between individuals? It is more difficult to
answer this question than the previous one because the
available tools are incapable of analyzing parts of individual
specimens at sub-ppm levels on a spatial scale of tens of
microns or less. A blunt substitute approach can begin to
address this question however: some individuals being lar-
ger than typical, their shells can be broken into several ana-
lyzable pieces. Although fine-scale “surgery” may not be
practical, this approach provides at least a hint of whether
large variability exists between pieces of the same specimen.
The results to date provide no hint of large within-individual
variability (Table 3). The variability of individual fragments
is closer to that of crushed-and-split samples than it is to
the variability of individually-picked or single individuals.
Although this evidence does not prove there is no intra-
individual variability in elemental composition, it does show
that any such variability, if it exists, occurs on relatively
small spatial scales.

**Table 3. Cd/Ca in pieces of single large foraminifera compared to
multiple-specimen analyses.**

<table>
<thead>
<tr>
<th>Sample</th>
<th># Indiv</th>
<th>µg/indiv</th>
<th>Cd/Ca</th>
<th>Mn/Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>V17-42, 78 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoeglundina elegans</td>
<td>Single 900 µg individual,</td>
<td>0.045</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>split into 3 portions</td>
<td>0.057</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple-individual analyses</td>
<td>0.038</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>V21-46, 37 cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoeglundina elegans</td>
<td>Single 1,440 µg individual,</td>
<td>0.071</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>split into 4 portions</td>
<td>0.067</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Multiple-individual analyses</td>
<td>0.067</td>
<td>15</td>
<td></td>
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<tr>
<td>RC11-147, 51 cm</td>
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<tr>
<td>C. wuellerstorfi</td>
<td>Single 520 µg individual,</td>
<td>0.123</td>
<td>57</td>
<td></td>
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<tr>
<td></td>
<td>split into 2 portions</td>
<td>0.145</td>
<td>60</td>
<td></td>
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<tr>
<td></td>
<td>Single 320 µg individual,</td>
<td>0.127</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>split into 2 portions</td>
<td>0.134</td>
<td>50</td>
<td></td>
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<tr>
<td></td>
<td>Multiple-individual analyses</td>
<td>0.150</td>
<td>52</td>
<td></td>
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<tr>
<td>MD77-203, 408 cm</td>
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<tr>
<td>Hoeglundina elegans</td>
<td>Single 2,100 µg individual,</td>
<td>0.056</td>
<td>4</td>
<td></td>
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<tr>
<td></td>
<td>split into 5 portions</td>
<td>0.057</td>
<td>3</td>
<td></td>
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<tr>
<td></td>
<td>Multiple-individual analyses</td>
<td>0.057</td>
<td>2</td>
<td></td>
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**WHY DO INDIVIDUAL BENTHIC FORAMINIFERA FROM THE SAME
SAMPLE SHOW SUCH A LARGE RANGE IN Cd/Ca?**

Although this question cannot be answered at present,
several possible causes of this individual variability can be
identified: (1) Bioturbation creates a mixture of specimens
that lived at different times when bottom water Cd was sig-
ificantly different. (2) Differences in the characteristics of
individual foraminifera such as size, growth rate, age, ge-
etic factors, etc. affect Cd uptake. If these factors are con-
trolled for, Cd uptake may be more uniform. (3) Individual
benthic foraminifera are only imprecise analysts of bottom
water chemistry. (4) The individuals involved inhabited dif-
ferent micro-habitats with different Cd concentrations dur-
ing calcification. (5) Some (unspecified) postdepositional
process has altered the initial foraminiferal Cd to a different
extent for each individual. All of these possibilities should
be considered, and perhaps all may be significant in isolated
cases.

The issue of bioturbation mixing of individuals has
been considered previously and shown to be a potentially
significant source of variability. Because this core is accu-
mulating at ~7 cm/kyr, an individual 3 cm depth interval
will include specimens deposited over approximately 1–2
kyr (for a ~10 cm bioturbated layer). It would be astonish-
ing to learn that the Cd composition of the deep waters of the Pacific Ocean have varied by a factor of two during this short time, and this possibility should be regarded as unlikely. It is not absolutely impossible, however, because the renewal time of the deep Pacific is about 500 years (Stuiver and others, 1983). However, this possibility will be neglected here because of its "maximum astonishment" nature: it should be proven to have happened before much is based upon it. Alternatively, could only the very nearest bottom waters have changed composition, perhaps due to large inherent fluxes of organic debris from the surface? This possibility must be considered unlikely as well. Measurements of $^{222}$Rn ($t_1$, = 4 days) show that the very most bottom waters mix up to several tens of meters depth in a matter of days (Sarmiento and others, 1976); the local flux of Cd from out of the bottom required to sustain a near-bottom chemical anomaly would have to be larger than can be easily believed. If some individuals are infaunal, it may be considered somewhat more likely that they encounter concentrations significantly different than that of the bottom water. This possibility cannot be disproved yet, but other factors should probably be considered more likely.

Present evidence argues against a significant shell size effect for foraminiferal Cd/Ca uptake. First, note that the singly-analyzed large specimens of *Uvigerina* from the TR163-3lp sample gave essentially the same result on average as the analysis of collectively-analyzed specimens averaging less than half their average weight. Second, note that the single very large specimens (that were divided into splits), which weighed 10–40 times as much as their companion specimens which were analyzed as a group, show essentially the same Cd/Ca as typical specimens from the same samples. This result indicates similar Cd/Ca uptake for small and very large individuals. Finally, in core EN 120 GGC 1 (33°40'N 57°37'W, 4,479 m), the species *Nuttallides umbonifera* was separated according to maximum linear dimension (corresponding to diameter for this symmetrical discoidal species) from a sample of particularly high abundance. The results of the Cd/Ca analyses (Table 4) show very little evidence for a significant size effect. The very smallest specimens gave slightly higher Cd/Ca. This small difference should be interpreted in view of the possibility that it is based on a very small sample (0.18 mg, analyzed before the new small sample technique was developed) and that small individuals have a higher surface area to volume ratio and hence may be more easily contaminated. Hence, although some further effort should be directed at testing this conclusion, there is very little reason to believe that shell size is a significant factor affecting benthic foraminifera Cd uptake.

The other factors are more difficult to resolve. Studies of individual living benthic foraminifera collected from the seafloor (e.g., Mackensen, 1993) or manipulated in experimental culture could shed much light on these possibilities. If factors 2–4 are *not* important, then individual living benthics should always show tightly clustered Cd/Ca values at each site on the seafloor because they have all formed in bottom water of the same composition. Laboratory cultures (or manipulated seafloor samples) may help resolve between factors 2–4. If factor 3 is important, we need to understand if the deviations are random (and hence cancel out in large populations) or if they are perniciously systematic with certain environmental characteristics. Finally, if factor 5 is important, systematic studies of foraminifera from different diagenetic environments should identify what diagenetic conditions are responsible.

### UNCERTAINTIES IN CD OCEANOGRAPHY?

Saager (1993) has argued that uncertainties in our knowledge of Cd oceanography are an important limitation in the use of Cd as a paleoceanoographic tracer. The same thing could be said of almost any other paleo-tracer; for example, it has only recently been appreciated how important gas exchange is to the global deepwater distribution of $\delta^{14}$C (Charles and Fairbanks, 1990; Broecker and Maier-Reimer, 1992). There always will be uncertainties, but the uncertainties in Cd oceanography are no worse than for other tracers, and most specifically, uncertainties in mechanisms linking Cd to phosphorus are irrelevant to the use of Cd as a paleocirculation tracer (Boyle, 1988). Furthermore, some of the arguments raised against the normal interpretation of Cd have been based on questionable data (see discussion in Boyle, 1994).

### CONCLUSIONS

Variability between individuals is the primary factor limiting the reproducibility of benthic foraminiferal Cd/Ca measurements. Although this conclusion has been demonstrated here only for Cd, it is likely to be true for other foraminiferal element/calcium ratios as well. Improvements in analytical precision cannot improve the signal-to-noise ratio of downcore records, which can only be improved by larger sample sizes. Although the cause of this individual variability is unknown, it does not appear to be related to shell size. Several possible factors for the individual variability can be identified and simple strategies for beginning to resolve between them are evident. The single most productive strategy towards this goal is to examine Cd/Ca in populations of single individuals collected alive (or very recently alive); however, laboratory manipulations and studies of individually-analyzed specimens from various diagenetic environments may also contribute.

### ACKNOWLEDGMENTS

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