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Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions

Joan M. Bernhard,[1] Dorinda R. Ostermann,[1] David S. Williams,[2] and Jessica K. Blanks[3]

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[1] The conventional method to distinguish live from dead benthic foraminifers uses Rose Bengal, a stain that reacts with both live and dead cytoplasm. CellTracker Green CMFDA is a fluorogenic probe causing live cells to fluoresce after proper incubation. To determine the more accurate viability method, we conducted a direct comparison of Rose Bengal staining with CellTracker Green labeling. Eight multicore tops were analyzed from Florida Margin (SE United States; 248–751 m water depths), near Great Bahama Bank (259–766 m), and off the Carolinas (SE United States; 220 and 920 m). On average, less than half the Rose Bengal–stained foraminifera were actually living when collected. Thus, while Rose Bengal can significantly overestimate abundance, combined analyses of CellTracker Green and Rose Bengal can provide insights on population dynamics and effects of episodic events. Initial stable isotope analyses indicate that the CellTracker Green method does not significantly affect these important paleoceanographic proxies.

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1. Introduction

[2] The extensive use of fossil foraminifera as paleoclimatic proxies requires an equally extensive study of modern analogues to determine the validity of these proxies. It is therefore necessary to study living foraminifera to assess accurately their ecology and test (i.e., shell) geochemistry. The standard method used to distinguish living from dead benthic foraminifers is the protein stain Rose Bengal. Rose Bengal can also react with proteins in necrotic cytoplasm, erroneously indicating that these specimens were live at the time of collection (see *Bernhard* [2000] for review). To date, while some investigators acknowledge that Rose Bengal is not necessarily an accurate method to distinguish live from dead foraminifers, the magnitude of standing stock overestimates is unknown and an alternative viability assay has not yet been accepted by the scientific community. A candidate technique utilizes CellTracker Green CMFDA. The present study compares Rose Bengal and CellTracker Green CMFDA as benthic foraminiferal viability assays.

[3] Rose Bengal, which was first used as a means to distinguish living from dead foraminifers in the 1950s [*Walton*, 1952], adheres to proteins, producing a magenta coloration of the specimen. Rose Bengal remains the stain of choice among paleoceanographers and benthic ecologists because it is inexpensive and easy to use [*Murray and Bowser*, 2000]. Thus the existing data set on Rose Bengal–stained benthic foraminifers is extensive.

[4] There are, however, a number of negative aspects regarding the use of Rose Bengal as a viability assay for benthic foraminifers. For example, the reaction of Rose Bengal with protein defines it as a nonvital stain; that is, it will adhere to dead as well as living cytoplasm [*Bernhard*, 1988]. Rose Bengal can stain the organic lining of foraminiferal tests [*Walker et al.*, 1974] or bacteria attached to or located inside the test [*Martin and Steinker*, 1973]. Also, foraminiferal tests containing living cytoplasm do not always stain with Rose Bengal, possibly because apertural blockage prevents stain penetration into the test cytoplasm [*Martin and Steinker*, 1973]. In addition, Rose Bengal staining in opaque specimens such as certain agglutinated or miliolid foraminifers is difficult to visualize [*Bernhard*, 2000]. Furthermore, investigators with red-green color blindness have difficulty assessing Rose Bengal–stained cytoplasm (J. H. Lipps, personal communication, 1982). Finally, the lack of a standardized procedure (e.g., different solvents; variations in staining times, staining intensity and the number of stained chambers; assessment using wet versus dried specimens; see *Bernhard* [2000] for a discussion) may yield inconsistencies when comparing the results of different studies.

[5] CellTracker Green 5-chloromethylfluorescein diacetate (CellTracker™ Green CMFDA; Molecular Probes, Invitrogen Detection Technologies) is a vital fluorogenic probe that was developed to stain living, and only living, cells. A fluorogenic probe is a compound that fluoresces

[1]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA.

[2]Department of Geography, Geology, and Anthropology, Indiana State University, Terre Haute, Indiana, USA.

[3]Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, South Carolina, USA.

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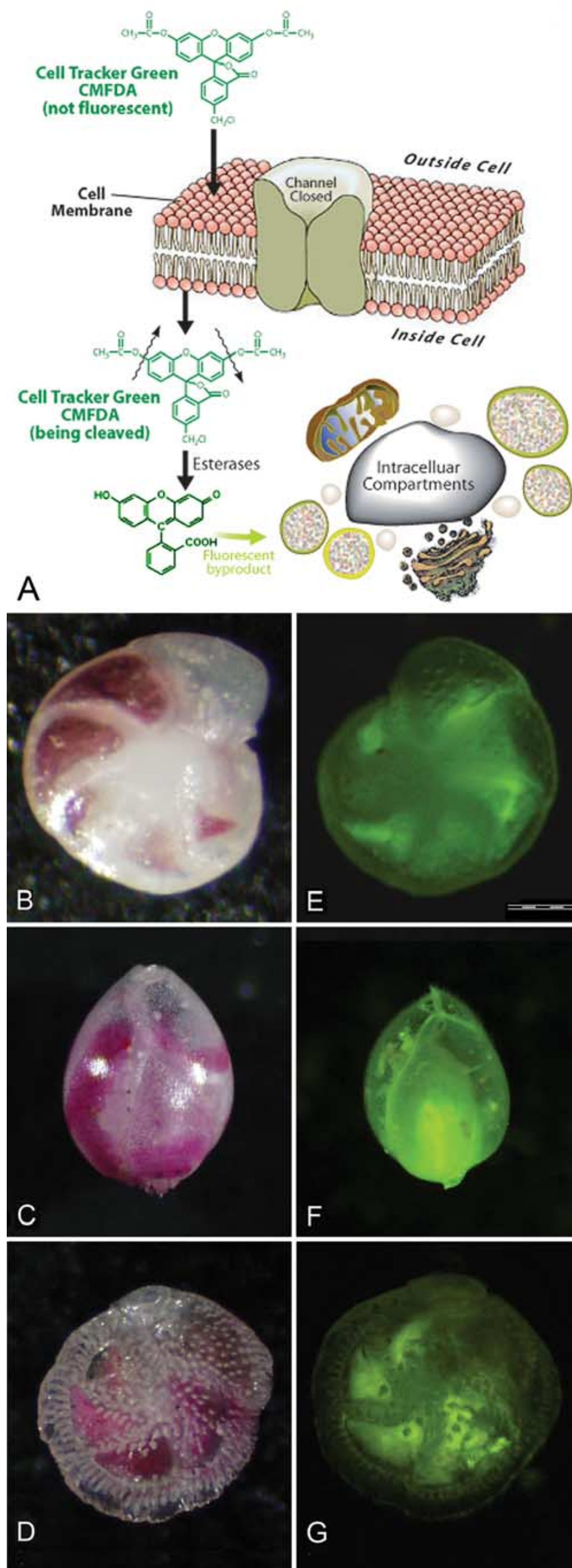

only after modification of the original molecule. Fluorogenic probes differ from fluorescent probes, which are always fluorescent. When live cells are incubated in a fluorescent probe such as fluorescein diacetate or a fluorogenic probe such as CellTracker Green CMFDA, the probe passes across the cell membrane, into the cytoplasm (Figure 1a). In the case of the fluorogenic CellTracker Green CMFDA, nonspecific esterases, which are hydrolytic enzymes in all live cells, cleave the fluorescent moiety of the probe to produce the fluorescent compound fluorescein that can be microscopically viewed after excitation at the appropriate wavelengths (Figures 1b–1g). Unlike some fluorogenic and fluorescent probes, CellTracker Green CMFDA will not leak out of the cell via ion channels in the cell membrane once it is incorporated inside the cell. In addition, CellTracker Green CMFDA is aldehyde fixable, so both living and chemically fixed populations can be examined for fluorescence.

2. Methods

[6] Sediment samples from the Florida Margin (~250–750 m) and adjacent to the Great Bahama Bank (~260–770 m, Figure 2) were collected in January 2002 using an MC-800 multicorer, which provides 10-cm-inner-diameter cores. In addition, a core was obtained from the Charleston Bump in June 2001 and another was collected from the North Carolina slope in April 2002 (Table 1 and Figure 2). Cores were maintained near ambient bottom water temperatures prior to and during sectioning. For this study, only the top 0.5-cm interval of each core was prepared and analyzed. Each sample was placed in a wide-mouthed Nalgene® jar, and covered with an approximately equal volume of chilled seawater. Samples were incubated at temperatures approximating bottom waters in either a refrigerator or environmental room (Table 1) without light for 10–19 hours in CellTracker Green CMFDA (1 μM final concentration in ambient seawater). After incubation, samples were fixed in 3.8% Borax®-buffered formalin.

[7] In the laboratory, samples were sieved over a 90-μm screen; the coarse fraction was examined using an epifluorescence stereomicroscope equipped with optics appropriate for fluorescein detection (i.e., 485-nm excitation, 520-nm emission; Leica MZ FLIII or Olympus SZX12). All foraminifera that fluoresced brightly in at least half of their chambers (i.e., CellTracker Green–labeled specimens) were wet picked, air dried, sorted by species, mounted on micro-

Figure 1. (a) Schematic showing how the fluorogenic probe CellTracker Green CMFDA is transported across the cell membrane and modified by live cells. (b–g) Micrographs of benthic foraminifers showing examples of Rose Bengal staining and CellTracker Green labeling. Rose Bengal-stained specimens are viewed with reflected light (note red coloration from Rose Bengal); CellTracker Green-labeled specimens are viewed with appropriate epifluorescence microscopy (note green coloration from CellTracker Green). Figures 1b and 1e are *Cibicidoides* sp.; Figures 1c and 1f are *Globobulimina affinis*; and Figures 1d and 1g are *Siphonina reticulata*.

Table 1. Core Identification Number, Cruise Number, Water Depth, General Location, Coordinates, Bottom Water Temperature, Approximate CellTracker Green Incubation Temperature, and Benthic Foraminiferal Densities Determined Using CellTracker Green CMFDA or Rose Bengal[a]

Core	Cruise	Depth, m	Location	Coordinates °N	°W	BW,°C	Inc,°C	CTG	RB Sum
FF69	HNL0112	220	Charleston Bump	31°55.33	79°11.67	8.8	5	91.2	98.0
MC16	KNR166-2	248	Florida Margin	24°23.73	83°13.53	11.7	6	0.8	22.2
MC94	KNR166-2	259	Great Bahama Bank	24°34.12	79°13.53	18.6	10	64.2	81.7
MC84	KNR166-2	638	Great Bahama Bank	24°22.28	79°27.00	10.2	10	2.8	14.3
MC28	KNR166-2	648	Florida Margin	24°16.93	83°16.24	6.3	7	3.8	8.7
MC11	KNR166-2	751	Florida Margin	24°13.18	83°17.75	5.8	10	1.5	4.6
MC99	KNR166-2	766	Great Bahama Bank	24°34.08	79°30.57	8	10	24.7	64.7
FA24	OC376-2	920	North Carolina Slope	32°59.45	76°23.39	4.9	7	50.4	72.3

[a]Abbreviations are BW, bottom water; Inc, incubation; CTG, CellTracker Green; RB, Rose Bengal; RB Sum is sum of RB plus CTG densities (see text); HNL, R/V *Cape Henlopen*; KNR, R/V *Knorr*; and OC, R/V *Oceanus*. Densities are given as number of specimens per 10 cm^3.

paleontology slides, and counted. The densities of CellTracker Green–labeled specimens were normalized per unit volume (i.e., per 10 cm^3). A fraction of the CellTracker Green–labeled specimens were subsequently incubated in Rose Bengal prior to air drying to confirm that they stained with Rose Bengal.

[8] Following the removal of all CellTracker Green–labeled specimens, the remainder of each sample was incubated in an aqueous Rose Bengal–saturated solution for 24–48 hours. Samples were then resieved over a 90-μm screen; the coarser fraction was examined with reflected light using the same stereomicroscope. Foraminifera that stained dark magenta in at least half of their chambers were isolated, air dried, mounted on micropaleontology slides, sorted by species, and enumerated. For each sample, Rose Bengal density determinations were calculated, on a per unit volume basis, as the sum of densities determined to be stained with Rose Bengal and those that were fluorescent via CellTracker Green CMFDA. This rationale was adopted because all CellTracker Green–labeled specimens would have stained with Rose Bengal (see Results).

[9] Density differences between CellTracker Green–labeled and Rose Bengal–stained benthic foraminifera were compared by the nonparametric chi-square test and by paired two sample *t* tests assuming equal variance (α = 0.05). For our chi-square test, the Rose Bengal–stained individuals were treated as the expected values, whereas the CellTracker Green–labeled individuals were treated as the observed values.

[10] Oxygen and carbon stable isotopic analyses of foraminiferal shell carbonate were performed on three species (i.e., *Cibicidoides* sp., *Hoeglundina elegans*, *Siphonina reticulata*) using a Kiel III Carbonate Device connected to a Finnigan MAT 253 mass spectrometer system. Procedures and precision for this instrument can be found at http://www.whoi.edu/paleo/mass-spec/. In all cases, individual foraminifers were analyzed. Foraminiferal stable isotopic data is expressed relative to Vienna Pee Dee Belemnite (VPDB). Pearson correlation coefficients and linear regressions (α = 0.05 for slope) were determined to verify any correlating relationships between foraminiferal shell isotope values and their respective shell masses.

[11] To quantify the accuracy of foraminiferal shell stable isotope incorporation, isotopic analyses were also conducted on selected seawater samples. Bottom water samples from the Florida Margin and the flank of the Great Bahama Bank were collected using a Niskin bottle attached to the multicorer frame, rigged to close as the corer tripped. These bottom water samples were analyzed for $\delta^{18}O$ using the Georgia Institute of Technology's GV Isoprime with Multiprep. The instrument was calibrated using NBS19, Vienna Standard Mean Ocean Water (VSMOW), Greenland Ice Sheet Precipitation (GISP), and an in-house standard; nine replicate analyses of an in-house standard had a 1-sigma standard deviation of 0.07 ‰ when the samples were run. Isotope results of water are presented relative to VSMOW. The $\delta^{18}O_{(calcite,\ VPDB)}$ of calcite in equilibrium with bottom water temperature and $\delta^{18}O_{(water,\ VSMOW)}$ was calculated using a paleotemperature equation and VSMOW-VPDB conversion based on *O'Neil et al.* [1969] and *Friedman*

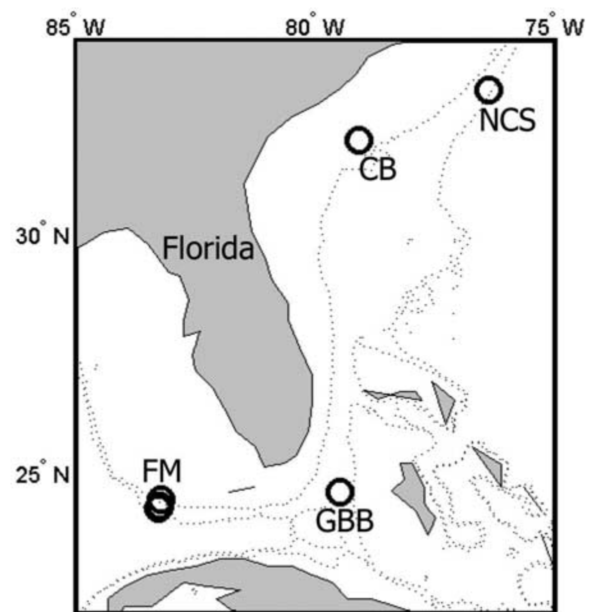

Figure 2. Map showing the sample locations. Abbreviations are NCS, North Carolina Slope; CB, Charleston Bump; GBB, Great Bahama Bank flank; and FM, Florida Margin.

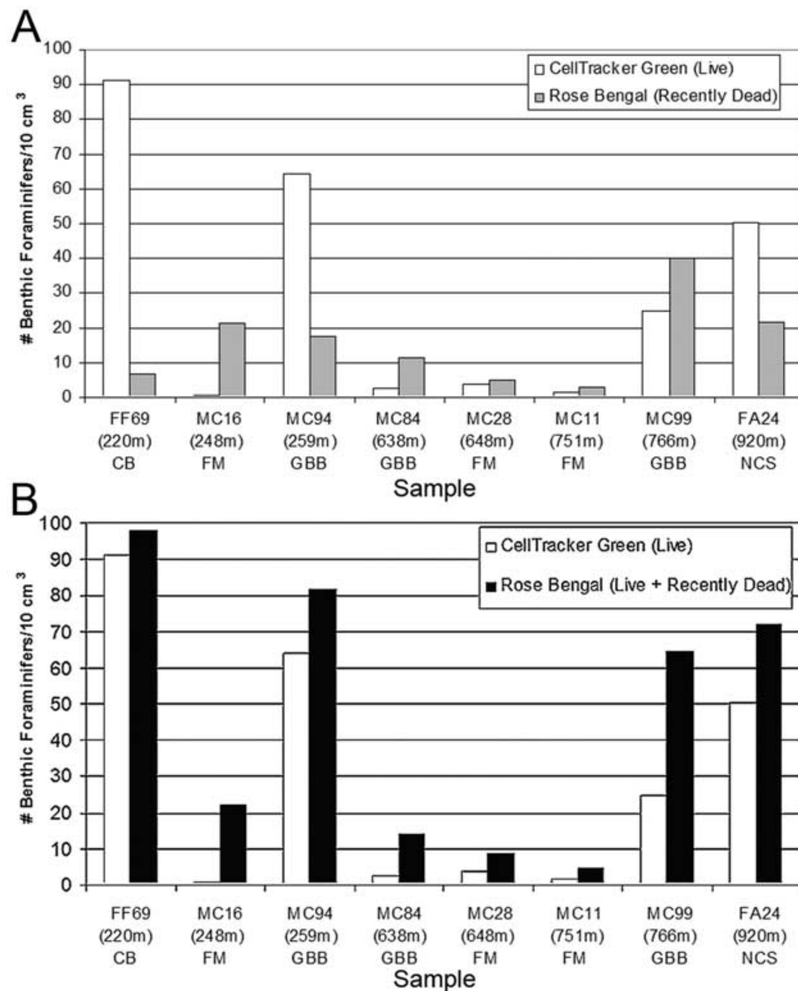

Figure 3. Comparison of benthic foraminiferal densities, presented for each core, determined by (a) CellTracker Green CMFDA direct counts (i.e., live specimens) or Rose Bengal direct counts (i.e., specimens that did not label with CellTracker Green, thus were recently dead) and (b) CellTracker Green CMFDA direct counts (i.e., live) or the Rose Bengal sum calculated from Rose Bengal direct counts (recently dead) plus CellTracker Green CMFDA counts (live). Core designations, water depths, and site locations are listed. Abbreviations are CB, Charleston Bump; FM, Florida Margin; GBB, Great Bahama Bank flank; and NCS, North Carolina Slope.

and O'Neil [1977], as described by *McCorkle et al.* [1997]. Pore waters were not collected from cores collected from the same sites, so comparisons of foraminiferal and pore water carbon isotope data are not presented here.

3. Results

[12] Specimens labeled initially with CellTracker Green CMFDA and later exposed to Rose Bengal did stain with Rose Bengal (n = 20; not shown), indicating that Rose Bengal abundance calculations for this study are correctly presented as the sum of those labeled with CellTracker Green CMFDA and those that subsequently stained with Rose Bengal.

[13] CellTracker Green–labeled and Rose Bengal–stained foraminifera were found in each sample (Figures 1b–1g and 3). It is beyond the scope of this manuscript to present assemblage data resultant from these two methods, but a qualitative survey indicates that both methods produced similar species' compositions. Benthic foraminiferal densities determined using the CellTracker Green approach ranged from 0.8 to 91.2 specimens 10 cm^{-3}; those determined using the Rose Bengal method ranged from 4.6 to 98.0 specimens per 10 cm^3 (Table 1). The Rose Bengal method produces significantly higher benthic foraminiferal density estimates than the CellTracker Green method because the mean densities calculated using the two methods were significantly different. More specifically, Rose Bengal significantly overestimates benthic foraminiferal abundances (chi-square, $p \sim 1.3 \times 10^{-12}$; *t* test for paired two sample for means, p = 0.008).

[14] Although the differences between density determinations using the two methods for any given core varied considerably (Figure 3), the average difference between

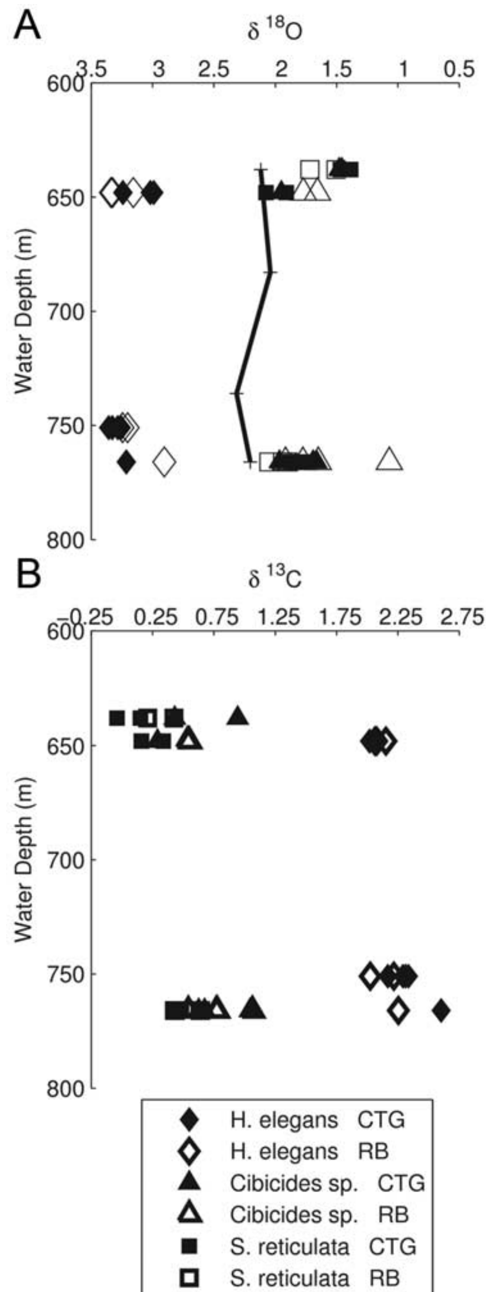

Figure 4. Plots of (a) δ^{18}O and (b) δ^{13}C for individual foraminifers, either labeled with CellTracker Green CMFDA (solid symbols) or stained with Rose Bengal (open symbols), from four cores analyzed in this study (MC11, MC28, MC84, and MC99). In Figure 4a the curve represents bottom water δ^{18}O (K. M. Cobb and T. M. Marchitto, unpublished data, 2005).

Rose Bengal and CellTracker Green benthic foraminiferal density estimates was 47%. Thus less than half of the Rose Bengal–stained benthic foraminifers were actually living at the time of collection. The most severe discrepancy between the two viability assays indicated that only 3% of the Rose Bengal–stained specimens were determined to be living by CellTracker Green CMFDA (sample MC16, Florida Margin; 248 m; Figure 3b). Conversely, CellTracker Green CMFDA labeling indicated that ~93% of the Rose Bengal–stained population in FF69 (Charleston Bump, 220 m) was living at the time of collection. Because multiple CellTracker Green incubations were commonly conducted in the same environmental room or refrigerator, in some cases differentials between in situ and incubation temperatures were > ±4°C (Table 1). The magnitude of temperature differential did not, however, produce a significant correlation in the differential of foraminiferal densities determined by either method (α level = 0.05; p = 0.948).

[15] Preliminary stable isotope analyses of CellTracker Green CMFDA–labeled specimens compared to those from the same sample but stained with Rose Bengal indicate no significant or consistent offsets between the two viability methods for either δ^{13}C or δ^{18}O (Figure 4). In other words, the Rose Bengal–stained specimens are not consistently heavier or lighter than those labeled with CellTracker Green CMFDA. Of the three species analyzed isotopically and deemed living by either method, the δ^{18}O of *Cibicidoides* sp. and *Siphonina reticulata* were relatively close to the δ^{18}O of bottom water that was collected simultaneously from the same sites (Figure 4a).

[16] The δ^{13}C of Rose Bengal–stained specimens of the aragonitic *Hoeglundina elegans*, which is occasionally used in paleoceanographic studies [e.g., *Rathburn et al.*, 1996], was consistently lighter than that of the two analyzed calcareous species (*Cibicidoides* sp. (Figure 4a) and *Siphonina reticulata* (Figure 5b)). Although there was no significant correlation between stable isotope signature and specimen mass, which is manifest as pressure in the Kiel carbonate device after acidification but prior to analysis in the mass spectrometer, of *Cibicidoides* sp. (Figure 5a) or *H. elegans* (not shown), there was a significant positive correlation between specimen mass (i.e., pressure) and δ^{13}C for *Siphonina reticulata* (Figure 5b; p = 0.042).

4. Discussion

[17] The observation that significant populations of foraminifers stained with Rose Bengal, but did not label with CellTracker Green CMFDA, demonstrates that Rose Bengal is an inaccurate live-dead indicator. If it were an accurate live-dead indicator, then no foraminifers would have stained with Rose Bengal after removal of CellTracker Green–labeled specimens. The observed discrepancy in density determinations between the two methods shows that Rose Bengal commonly and consistently stains dead foraminifers in addition to live specimens. Our observations contradict conclusions reached by *Murray and Bowser* [2000], which promotes the use of Rose Bengal as a foraminiferal live-dead indicator, especially in well-aerated habitats. Because none of our four sample sites (Charleston Bump, North Carolina slope, Florida Margin, flank of Great Bahama Bank) are depleted in oxygen, results from our study indicate that Rose Bengal will significantly overestimate benthic foraminiferal standing stocks even in typical marine sediments from bathyal depths.

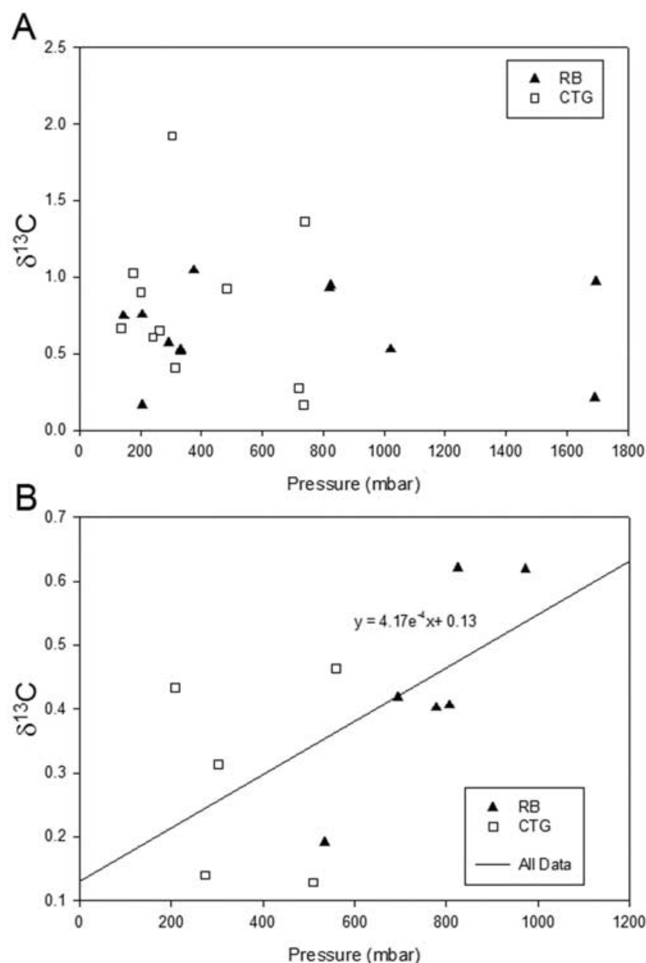

Figure 5. The $\delta^{13}C$ values versus evolved CO_2 pressure after foraminiferal acidification prior to introduction into the mass spectrometer, which is an indirect measure of foraminiferal mass: (a) *Cibicidoides* sp. and *Siphonina reticulata*. For *S. reticulata* the relationship is significant ($p = 0.042$ and $R^2 = 0.3835$).

[18] We believe that the use of existing and yet-to-be-reported Rose Bengal data must be approached with circumspection. Due thought should be given to the resolution of the data acquired using the Rose Bengal method. For example, since foraminifera may react with Rose Bengal after postmortem transport, it cannot be assumed that foraminifera actually lived at the collection site [*Bernhard*, 1988]. Also, seasonal studies conducted using Rose Bengal may be obscured because of the tendency of Rose Bengal to stain dead specimens for months to, potentially, years [*Hannah and Rogerson*, 1997; *Corliss and Emerson*, 1990].

[19] At this time, conclusions cannot be made regarding any possible consistency of density differences obtained using Rose Bengal and CellTracker Green CMFDA. To determine if consistent offsets occur between the two methods, analyses are required of additional material collected from different physicochemical conditions (e.g., temperature, salinity, oxygen concentration, water depth, productivity regime). It is important to heed a cautionary note: The conditions and species used in this study may not be representative of all foraminiferal species or environmental conditions. For example, incubating tropical foraminifers in CellTracker Green CMFDA at their ambient temperature and salinity for over 8 hours could be far too long an incubation to provide accurate results, while incubating polar species at their ambient temperatures of −1.8°C for less than 8 hours could be far too short an incubation to provide accurate results. Investigators are urged to conduct live-dead control experiments with their species using appropriate environmental conditions to establish the reliability of any viability method. It is also relevant to note that foraminiferal specimens that had been artificially killed 48 hours prior to incubation in a functionally similar fluorogenic probe (BCECF-AM) had detectable fluorescence, but at a significantly lower level [*Bernhard et al.*, 1995]. Additionally, this same study showed specimens that had died naturally at unknown times did not exhibit detectable fluorescence after a similar BCECF-AM incubation [*Bernhard et al.*, 1995]. Finally, while the accuracy of CellTracker Green labeling has not been established for all foraminiferal species and every environmental condition, it is important to note that the accuracy of Rose Bengal has not been established for all foraminiferal species and environmental conditions either.

[20] There are many positive aspects to using CellTracker Green CMFDA as a live-dead foraminiferal assay. CellTracker Green CMFDA relies on the activity of esterases, which are hydrolytic enzymes lacking in cells that have been dead long enough for them to degrade (e.g., hours to days, depending on environmental conditions). CellTracker Green CMFDA is relatively inexpensive compared to many cell biological and molecular reagents; the amount of time needed to pick any given sample is exactly the same time commitment as picking the sample if it were treated with Rose Bengal (the CellTracker Green CMFDA approach merely uses different microscopic techniques); opaque specimens that cannot be easily detected using Rose Bengal or CellTracker Green CMFDA can be analyzed using a functionally similar fluorescent probe and spectrofluorimetry (e.g., fluorescein diacetate [*Bernhard et al.*,1995]); CellTracker Green CMFDA offers the ability to examine specimens while they are living or postfixation (i.e., preserved; thus CellTracker Green CMFDA can be used for biological experiments as well as for analysis of large numbers of preserved samples); fluorescent specimens are easy for color-blind investigators to distinguish because a positive signal is based on light intensity, not color. In species with transparent tests, the intensity of CellTracker Green can be quantified via spot metering through a digital camera attached to the epifluorescence microscope. In this way, a standardized index based on quantitative fluorescence can be established to determine the metabolic activity, and thus health, of individuals. Such an index may be useful for certain experiments or community analyses.

[21] The cost of the necessary epifluorescence microscope attachment (~$10,000 US) may be a negative factor against the use of CellTracker Green CMFDA as a foraminiferal live-dead indicator. With the increased use of green fluo-

rescent proteins (GFP) in cell and molecular biology laboratories, the cost of epifluorescence attachments for stereo dissecting microscopes has decreased substantially over the past few years, and this type of microscopy is readily available at most academic institutions. The requirement that CellTracker Green CMFDA incubations must be conducted at ambient temperatures prior to fixation could be considered a deterrent, especially when working on deep-sea or high-latitude material. Most research vessels capable of sampling the deep sea and/or polar regions have built-in environmental rooms that can be used for the required ambient temperature incubations in CellTracker Green CMFDA. Alternatively, a typical science-dedicated refrigerator could be used for the required incubations if it can approximate in situ temperature. A third point to consider prior to employing CellTracker Green CMFDA as a live-dead foraminiferal indicator is that, at present, little data are available for direct comparison between studies. To date, although limited CellTracker Green CMFDA data sets are available [*Bernhard et al.*, 2003; J. M. Bernhard et al., manuscript in preparation, 2006), a concentrated effort to use CellTracker Green CMFDA will produce more confident conclusions about the abundances, community structure, and dynamics of benthic foraminiferal populations.

[22] It is beyond the scope of this contribution to discuss causes of offsets, vital effects, and calibration of isotopic proxies with regards to foraminiferal species specificity, yet a few comments can be offered regarding our isotope data. While our $\delta^{18}O$ data do not appear to indicate consistent offsets between either foraminiferal population (i.e., Rose Bengal stained or CellTracker Green labeled) and their surrounding seawater, it is important to note that our data represent a small population per species, and the variation among the analyzed conspecifics in the same sample for either viability method can be considerable (e.g., ~0.5 to 0.7‰ in *Cibicidoides* sp.; Figure 4). The slight offsets in *Cibicidoides* sp. and *S. reticulata* $\delta^{18}O$ from that of bottom water could be explained by changes in the water's $\delta^{18}O$ signature because of seasonality, as observed in some paleoceanographic studies [e.g., *Austin and Scourse*, 1997; *Scourse et al.*, 2004]. Seasonality is an unlikely explanation in our study, however, because all foraminifers analyzed for isotopes were collected from depths >600 m, which exceeds the depth of the mixed zone and thus is expected to have insignificant seasonal temperature variations. Eddy formation and propagation may perhaps account for the disequilibrium because cold-cored cyclonic (Tortugas) eddies, which are quite common and long-lived (i.e., months) in the Florida Straits [e.g., *Vukovich and Maul*, 1985; *Fratantoni et al.*, 1998], may introduce temperature decreases of over 2°C while foraminifers calcify one or more chambers of their test.

[23] Of the three species, it is apparent that the aragonitic *Hoeglundina elegans* exhibits considerable offset in both $\delta^{18}O$ and $\delta^{13}C$ compared to the two other species, which had similar isotopic signatures. Contrary to previous unpublished data of one of us (D. R. Ostermann, 2003), isotope signatures of *Siphonina reticulata* appears to agree well with those of *Cibicidoides* sp., and thus could be considered for use in future paleoceanographic reconstructions. Firm conclusions cannot be made at this time regarding any possible artifacts of additional proxies (e.g., trace or minor elements) imparted to the foraminiferal carbonate during the application of CellTracker Green CMFDA. It is known, however, that distribution coefficients for Cd, Ba, and Sr of CellTracker Green–labeled *Bulimina aculeata* are consistent with other benthic foraminiferal field and culture data [*Hintz et al.*, 2006].

[24] The combined use of CellTracker Green CMFDA and Rose Bengal could have ecologic value because CellTracker Green–labeled specimens are known to have been live at the time of collection and Rose Bengal specimens that did not label with CellTracker Green CMFDA are known to contain dead cytoplasm. Thus the two data sets provide information about cytoplasmic degradation rates and, perhaps, population turnover. In addition, the observation that stable isotope signatures do not appear to be significantly affected by CellTracker Green CMFDA may allow short-term changes in localized oceanic conditions to be elucidated by comparing the proxy data from each of the populations: CellTracker Green CMFDA (live), Rose Bengal (recently dead), and empty tests (dead for longer periods of time). Such comparisons could allow assessments of the effects of natural episodic phenomena such as algal blooms, El Niño Southern Oscillation events, and volcanic eruptions on the benthos, and thus have relevance to paleoceanographic reconstructions on recent timescales. Finally, the combined use of the two methods could provide insights into effects of anthropogenic catastrophic events such as oil spills by providing data on foraminiferal survival.

5. Conclusions

[25] On average, in the samples analyzed here, less half the Rose Bengal–stained foraminifera were determined to be live at the time of collection. As many as ~97% of the Rose Bengal–stained specimens were actually dead at the time of collection. These false positives can significantly affect ecological studies, which are used to ground truth paleoceanographic geochemical proxies. Thus we offer an alternative to Rose Bengal to distinguish live from dead foraminifers: the vital fluorogenic probe CellTracker Green CMFDA, which labels hydrolytically active (i.e., live) cells. The combined use of CellTracker Green and Rose Bengal may provide additional insights into foraminiferal ecology and effects of episodic phenomena on proxy incorporation into foraminiferal tests.

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J. M. Bernhard and D. R. Ostermann, Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA. (jbernhard@whoi.edu)

J. K. Blanks, Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC 29208, USA.

D. S. Williams, Department of Geography, Geology, and Anthropology, Indiana State University, Terre Haute, IN 47809, USA.