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**Biogeosciences Discussions**

# *Interactive comment on* "Determination of the metabolically active fraction of benthic foraminifera by means of Fluorescent in situ Hybridization (FISH)" *by* C. Borrelli et al.

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Anonymous Referee #4

Much has been written over the past several decades on methods used to distinguish live from dead foraminifera in environmental samples. Borrelli et al. propose the application of FISH as a more accurate tool for this, but several concerns need to be addressed. First, I am particularly concerned about the small sample sizes used in the study. Overall, too few individuals were used in each treatment to make the results meaningful. For example, only 5 individuals with agglutinated tests were used, and it appears that these data are repeated on Tables 1 & 3. On Table 3, it appears that only



a few specimens were assayed using the S17 probe (7 Ammonia, 6 miliolids – species not distinguished). Only 62 individuals (Table 1) or 69 individuals (Table 3) were used for all treatments and controls. This is clearly not sufficient given the range of variability, including the intensity in fluorescence.

Please see also comments to Referee #1 and #2. The amended manuscript will contain additional data from other tests in both cultures and sediment samples. Second, the discrepancy in Table 1 between the number of individuals tabulated as live/dead using optical examination (which I assume is the search for pseudopodia and cytoplasmic coloration) and the live/dead tally using FISH is troublesome. To show that this method is truly valid, only foraminifers that were verifiably alive should have been used. There is no way to tell whether individuals were counted as alive using optical methods that were actually dead (or the other way around). Again, this discrepancy is particularly troublesome given the small sample size.

(Please see also Answers to DeNooijer, point 1and Answers to referee #2, point 8). It is impossible to simultaneously test the same organism with multiple techniques (e.g., RB, CTG, calcein or MTT and FISH). As such, to discern live/dead foraminifera, we have used the only method (optical test) which was compatible with the further FISH testing of the same cell. In addition, several papers have shown that the "optical" approach for distinguishing live/dead organisms is widely utilised: Filipsson et al., 2010 (Biogeoscience, 7, 1335-1347); de Nooijer et al., 2008 (Limnol. Oceanogr. Methods, 6, 610-618 – "All specimens were screened for pseudopodial activity or presence of colored cytoplasm, indicating that individuals were alive at the start of incubation with HPTS").

Third, the results on fed v. starved individuals is interesting, and in a general way, they are in agreement with the findings of Parfrey and Katz (2010, Genome Biol. Evol., 2:678-685). However, again, the small number of individuals examined precludes drawing any solid conclusions. Overall, the results only demonstrate the potential application of FISH as a vital assay, but the superiority of this method over others was not

adequately demonstrated for the reasons cited above.

Of course are data are not based on a large dataset, but we will include more analyses on the amended manuscript, which will possibly include representatives of other foraminiferal groups.

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Interactive comment on Biogeosciences Discuss., 7, 7475, 2010.