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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 #3

1-> Number of specimens that used for the study of FISH techniques is little numbers. It is difficult to perform good statistics. The authors should add number of specimens. 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.

2-> Photographs in figures 4 and 5 show that fluorescent parts are widely distributed in test cavity. This is quite strange. Not at all, if there are ribosomes there is RNA and therefore the presence of fluorescence is not only justified but expected. Please, see

also the letter to the editor, as introduction to de Nooijer's comment answers, where a more detailed description of the rationale behind the FISH technique is presented.

3-> When I have tested FISH method for determining symbiotic bacteria in foraminiferal cells, specific parts of cytoplasm are brightening by fluorescent probe, for instance, mitochondria, nucleus and symbiotic bacteria. Symbiotic or sequestered chlorophyll-a shows fluorescent light in case of shallow water foraminifera, in particular to Ammonia beccarii. rDNA are distributed in these organelles or particles. This means that fluorescent parts are supposed to be sporadic. How do you explain all the cavity areas are brightening?

This is quite strange, and could be expected only if you used a probe different from the one we used. Please recall that we used a probe for eukaryotes while you, when searching for symbiotic bacteria, likely used a probe for prokaryotes. This can explain why you were staining also mitochondria as they contain 16rDNA. Therefore we think that the fluorescence to which the reviewer refers to is the fluorescence of bacteria within different part of the cell. This is not our case, as the two probes used are specific for the 18S rRNA of Eukaryotes (probe Euk1209R) and of foraminifera (probe S17) and have been demonstrated to produce fluorescence only from live cells. Unfortunately, we have not found in the literature the paper referring about the use of FISH to study symbiotic bacteria in foramnifera, except for the attempt made with the endobiont-bearing allogromiid from Santa Barbara basin (Bernhard et al., 2006, J of Geophysical Res., 111, G03002, doi:10.1029/2005JG000158). So that we have no elements to reply in detail to this comment. But in literature FISH has been also used to measure phagotrophy in mixotrophic protists (Medina-Sánchez et al. 2005 Appl Environ Microb).

During FISH hybridization, each probe molecule will theoretically hybridize with a molecule of rRNA. Consequently, each cell will show a more o less homogenous fluorescence signal, depending of the concentration and localization of ribosomes inside the cell. It is expected that a natural amplification of the fluorescent signal, result of

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the large numbers of ribosomes in active cell, will occur. As such, the diffused brightness may be the results of the amplification of the fluorescent signal by thousands of ribosomes in the cell.

3-> In figure 6, the authors show that only 3-6% of specimens are living by FISH techniques. How many percentages are judged to live specimens in case of conventional R.B. method or other staining methods such as cell tracker green. I would like to know the differences among methods. Otherwise, readers cannot judge which methods are adequate for ecological researches on foraminifera.

Thanks for this request. Please see our response to Reviewer #1" and "#2" and the info provided on the other methods used. In addition, we plan to perform additional analyses to produce such comparisons which will be included in the amended manuscript. We also plan to collect additional sediment samples and to compare, if needed, the results from FISH and Cell Tracker Green technique to compare the performance of these two technique in quantifying the percentage of live vs dead cells in natural marine sediments.

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