

Interactive  
Comment

## ***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.***

### **Anonymous Referee #3**

Received and published: 17 November 2010

Determination of active benthic foraminifera from dead individuals has long been discussed among foraminiferologists who were working on populational study. Rose Bengal (R.B.) staining method by Walton (1955) has widely used by scientists because of easy treatments. R.B. staining method shows obviously a lot of problems, as already pointed out by many scientists. In particular, R.B. staining is not so effective for deep-sea foraminiferal study, because organic materials including rDNA have long been preserved in test cavities after their death. This sometimes introduces over-counting of “live” foraminifera and also introduces over-estimation of foraminiferal biomass in the deep-sea.

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Interactive Discussion

Discussion Paper

BGD paper by Borrelli and others proposed FISH technique for discrimination of active foraminifera from dead specimens. I read through this manuscript with lots of interest. Exactly, FISH technique is one of candidates for determining active foraminifera. However, I feel that the authors should be required to add several additional data and explanations before publishing on BG or other journals. Many critical comments have already given by two referees. I would like to avoid overlap of comments as far as possible.

### Comments

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.

2) Photographs in figures 4 and 5 show that fluorescent parts are widely distributed in test cavity. This is quite strange. 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?

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.

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

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