Biogeosciences Discuss., 7, C3779–C3782, 2010
www.biogeosciences-discuss.net/7/C3779/2010/

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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Received and published: 11 November 2010

This manuscript published in BGD addresses an important and often underestimated problem in foraminiferal research. It is long recognized that distinguishing between living and dead foraminifera is very difficult and therefore, established methods may introduce biases in standing stocks, survival rates, etc. For this reason, a method that allows accurate discrimination between living and dead foraminifera is of vital importance to a wide variety of experiments and field studies.

The authors of the submitted manuscript have shown how an rRNA hybridization technique may be used to discriminate between living and dead foraminifers. The pre-

sented work, however, contains a number of serious issues that should be dealt with before the manuscript would be fit for publication. Implementation of all issues I found (listed below) may completely change the content and organization of the manuscript so that new reviews are likely to be necessary before acceptance in Biogeosciences.

Sincerely,

Lennart de Nooijer

Major concerns:

1. I don't think that the optical test for determining whether foraminifera are alive or dead yields accurate results. For species like Ammonia, we have often observed living cells that only filled up a few chambers (e.g. $\sim$5 out of $\sim$25: the other 20 were devoid of cytoplasm). Such living individuals display no pseudopodial activity unless they are kept for a number of days at high temperatures with abundant fresh food. Such observations and previous reports suggest that (some) foraminifera may go into some sort of inactive mode. Particularly with foraminifera collected in winter, this could introduce serious miscalculation of the living vs dead ratio of the population (fig 6). Results from the 'starvation experiment' also show that the FISH-method may be biased by foraminifera that are in this possible 'hibernation mode'.

For the agglutinating species, visual clues may be even more problematic since, for example, the color of their cytoplasm is easily distorted by the particles that make up their 'shell'.

The authors should be aware of such errors and include comparison of their FISH method with an established one (i.e. CTG or BCECF-AM) to check if their results produce sensible living/dead ratios. Alternatively, their presented method may be used to determine the metabolically active, rather than the living fraction of collected foraminifera. In that case, please omit discussion of living vs dead fractions.

2. Use of 'brightness' from pictures is problematic (section 3.4; fig 5). The fluorescent

signal that the authors recorded depends on a number of things including the position of the focal plane, thickness of the shell wall, position of the foraminifer, presence of fluorescent stain in the surrounding medium, etc. Instead, pictures taken may be used to illustrate variability in fluorescence intensity or general differences between groups of foraminifers.

3. The number of individuals for the 'washing' and 'dessication' protocols is too low to allow conclusions to be drawn: particularly with occasional hybridization (table 2).

4. It is unclear what the difference is between the data presented in tables 1 and 3. Are these (partly) the same foraminifers? If there are two probes tested, which is used for the experiment presented in table 1?

5. The discussion relies partly on results (lines 18-28, page 7487) that are not mentioned elsewhere in the manuscript.

6. The main conclusions (i.e. that the presented method serves as a reliable way to discriminate between living and dead foraminifera; lines 24-29, page 7489) are not supported by the results.

Minor concerns:

Figure 1 can be deleted.

Describe in Methods what a 'Autofluorescence assay' is (table 1).

How were the results of the two methods (optical and FISH) from table 1 in relation to each other? I.e. were all 21 'living' milliolids also identified as living by FISH? Or was there a larger mismatch between the two methods?

Could the authors include more agglutinants in their analysis (talbe 1)? Would there be fluorescence from 'optically dead' specimens?

I don't understand the added value of the SEM images (fig 2). Is it possible to test for organic remains by SEM? I doubt whether the complete organic lining(s) are dissolved

within one hour if the shells were not crushed before immersion in NaOCl.

Why would the probe EUK1209R (line 2-3, page 7485) stain particles and organic material in agglutinating foraminifera?

Most of section 3.5 should be in the Methods and not in the Results.

The Discussion contains large parts that belong to the Introduction. For example, disadvantages of previously developed methods is the rationale for the experiments presented by Borelli et al. and need not to be repeated in the Discussion. Instead, the Discussion should address uncertainties in the proposed method and e.g. the applicability to (large) volumes of sediment.

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