Supplementary Material

**Protocol for the 13C enrichment of diatoms**

1. Preparation of the labeled medium:

To label the diatom cells with the stable isotope 13C, a ‘Solution 1’ is prepared by adding 336 mg of NaH13CO3 (Buchem bv. Apeldoorn, The Netherlands, sales@buchem.com) in 100 ml milliQ H2O. 5 ml of ‘Solution 1’ is added per 100 ml of the culture medium.

The culture medium used is f/2 (Guillard, 1975) made on the basis of filtered freshwater and salt or filtered seawater (GF/F, GF/C). Before adding the f2 and NaH13CO3 the water has to be autoclaved. The concentrated f/2 medium is sold by Sigma, ref. N: G9903 (with silicium).

2. Labeling of the diatoms:

Preferentially a monoclonal culture should be used. A few cells of diatoms are added into culture bottles (flat tissue bottles, company: Sarstedt) with the labeled medium. Three bottles are used for control (without the labeled medium). The benthic diatoms are grown in the ‘tissue culture bottles’ of 500 ml. The bottles are laid down, for diatoms to have the large bottom area for settling and a maximum area of irradiance. The planktonic diatoms are grown in Erlenmeyer bottles. The cultures are grown in an incubator at 18-20°C with a 12:12h light-dark period and a light intensity of 25-50 µmol • photons m-2 s-1. After approx. two weeks, diatoms underwent multiple mitotic divisions and reached high densities of cells and high levels of labeling.

3. Cleaning the benthic diatoms:

Shake the culture bottle so the diatoms dis-attach from the bottom and put the bottle vertically. Let the diatoms settle down in a few hours. Remove the up-laying water with the pipette mounted on a water pump. Leave only some water in the culture bottle. Add 200 ml of autoclaved artificial seawater and shake the bottle. Let settle the diatoms again. This washing procedure is repeated at least three times. After the last washing action, the diatoms are in a dense concentration ready to use in an experiment.

In order to estimate their δ13C signature, a drop of this concentrated solution is put into a tin cup (at least three replicates). The tin cups are put in the oven to dry.

Diatoms from the control and the labeled diatoms must be taken for isotopic analysis to measure the difference in δ13C.

4. Preparation of diatoms for the experiment (estimating density):

To estimate the density of diatom cells in the cultures, the cells are homogeneously suspended by shaking the bottles in which the cultures were grown, and then 50 µl of the cell suspension has to be transferred into a small well (of a 96-well plate). In an hour, after all the benthic diatom cells settled to the bottom of the well, their number is counted under a inverted microscope and the values obtained allow to estimate the densities in the experimental vessels. If necessary, measure the length of the cells. Also, planktonic diatoms do settle and can be counted in the same way.

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