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Preservation effects on the isotope signatures in the biomass of a marine protist

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Dietary preferences, nutritional needs as well as food uptake rates can be identified via the natural carbon and nitrogen isotopic signatures in the biomass of an organism or by quantifying tracer uptake (e.g., 13C, 15N) into the biomass after feeding on an isotopically enriched food source. Often, the specimens to be examined have to be stored and preserved before stable isotope analysis. Unfortunately, information about the specific effects of preservation methods on the stable isotope composition is lacking many times, which is especially true for single-celled organisms. In a highly replicated study we preserved natural as well as 13C and 15N-enriched specimens of a marine protist (the benthic foraminifer Ammonia sp.) under different conditions. The applied preservation methods included freezing, air-drying, formalin and ethanol with and without the stain Rose Bengal (RB). The preservation period ranged between 14 and 240 days and the biomass of control and preserved specimens was analysed for its elemental and isotope composition. Significant amounts of carbon and nitrogen were lost from the biomass during preservation and storage, regardless of preservation method and storage duration. Shifts in the C:N ratio were also observed. The δ13C and δ15N signatures in preserved natural specimens were significantly different to unpreserved specimens, with strongest shifts caused by freezing and formalin with RB. The longer the preservation period, the more the δ13C signatures shifted away from control signatures, except for ethanol-preserved specimens. Preserving 13C and 15N-enriched specimens in ethanol for 30 days resulted in significantly lower carbon uptake estimates, while freezing isotopically enriched specimens yielded in lower nitrogen uptake estimates. This study provides detailed information on the specific effects of 7 different preservation methods on a marine protist, which will help choosing the least affecting method of preservation with regard to future studies. Our observations also show that care should be taken when comparing isotope signatures or uptake rates of specimens that were differently preserved, as differences might not derive from natural variation but from alteration of the cytoplasm during preservation.