

Effects of lugol' s fixtation on preservation of samples for carbon and nitrogen stable isotope and molecular analyses

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Recently, carbon and nitrogen stable isotope analysis and molecular analysis are commonly used as powerful tools in marine ecological studies including sediment trap experiments. In the southern ocean ecosystem studies, moored sediment trap samples are important to obtain information all-year around including winter season when we cannot observe directly by research vessels. Usually, collecting cups of sediment traps were filled with formalin for preventing degradation of samples during deployment periods, but formalin fixtation is not suitable for those analyses because of crosslinking and fragmentation of DNA and alteration of carbon stable isotope. On the other hand, lugol' s iodine solution is commonly used as fixatives of microzooplankton and phytoplankton samples, but its usefulness as a long term preservative of samples for stable isotope analysis and molecular analysis is still not examined. In this study, we compared carbon and nitrogen stable isotope ratios of zooplankton samples fixed by lugol' s iodine solution and other methods over 6 months and extract DNA from lugol fixed samples after 16 months preservation for examining the usefulness of lugol' s iodine solution. Samples were collected by ORI net during a cruise of training vessel *Umitaka-maru* in 2017. Collected samples were fixed by 5%, 10% lugol' s iodine seawater solution or 5% buffered formalin seawater or frozen at -80°C on board. Samples were sorted after 1 week, 1 month, 3 months and 6 months preservation and used for stable isotope analysis. DNA was extracted from samples after 16 months preservation and amplified by PCR. In most cases, carbon stable isotope ratios were significantly different between formalin fixed samples and other samples after 1 week preservation. Nitrogen stable isotope ratios were also significantly different between formalin fixed samples and other samples after 1 month preservation. There are no significant differences in carbon and nitrogen stable isotope ratios between samples fixed by 10% lugol solution and frozen samples in all the periods. Amplification of DNA extracted from lugol fixed samples was detected by a gel electrophoresis. Thus, lugol' s fixtation is considered to be suitable for long term preservation of samples for both stable isotope analysis and molecular analysis.

Keywords: Zooplankton, Sediment trap, Stable isotope ratio, DNA, Fixation method