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Effects of sample pretreatment and external contamination on bivalve shell and Carrara marble $\delta^{18}O$ and $\delta^{13}C$ signatures

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ABSTRACT

Stable isotope signatures of bivalve shells serve as important paleoclimate proxies. However, such data can be biased as a consequence of physical and chemical sample pretreatment and contamination during sampling. As yet, the potential isotope error induced by paleontological preservation techniques and specific methods used in bivalve sclerochronology have not been systematically assessed. To address these issues, homogenized powder of a well-preserved, aragonitic fossil shell, *Glycymeris obovata* (ca. 30 Ma old) as well as Carrara marble powder (calcite) were exposed to ultrasound, a set of different staining solutions and cleaning agents. In addition, these reference materials were artificially contaminated with increasing proportions of powder prepared from periostracum, Alcian Blue and cured adhesives. Whereas some treatments (rinsing with Milli-Q water, immersion in Alizarin Red S solution and possibly Mutvei's solution) did not affect the isotope values of the carbonate samples, severe isotope shifts were observed after immersing shell powder in Feigl's solution ($\Delta\delta^{18}O = -0.62‰$) and mixing Carrara marble powder with Gluetec Metal Epoxy (a contamination level of 41.7 wt% shifted $\delta^{18}O$ and $\delta^{13}C$ values by −2.12‰ and −1.74‰, respectively). Increasing levels of Metal Epoxy in the sample resulted in a sharp, non-linear decrease of the isotope values. The majority of tested treatments conducted with the carbonate powders and contaminants added to them caused minor to moderate, mainly statistically significant isotope offsets (bleaching with NaOCl and H_2O_2; exposure to ethyl alcohol and Rewoquat W 3690 PG, a surfactant; ultrasonic cleaning; contamination with powder of Alcian Blue, cured superglue, several different polyepoxies, methacrylates and polyvinyl butyral resins as well as periostracum). In conclusion, pretreatment of bivalve shells with an intended use for isotope analysis should be kept at an absolute minimum and contamination with adhesives etc. should be avoided. Specimens from museum collections that underwent special conservation methods may be of limited value for isotope studies, unless the history of sample treatment is known. Although only a few of the tested substances and methods strongly biased the isotope signatures, even small changes can accumulate to levels that are inacceptable for high-resolution paleoclimatology.

1. Introduction

The stable carbon and oxygen isotope composition of bivalve shells can provide important information on past seasonal to inter-annual climate variability. For example, changes in ambient water temperature are encoded in $\delta^{18}O_{shell}$ values (Williams et al., 1982; Arthur et al., 1983; Wanamaker et al., 2008) and carbon dynamics in $\delta^{13}C_{shell}$ (Mook, 1971; Chauvaud et al., 2011; Schöne et al., 2011). A major prerequisite for isotope-based reconstructions of environmental variables is that pristine isotope signals can be recovered from the shell carbonate, and sample pretreatment, preparation methods and sampling itself do not introduce any bias to the isotope data.

As demonstrated for different carbonates, cleaning procedures, contact with certain organic substances and treatment with dyes can significantly modify the original isotope signatures. To name just a few examples, immersion in formalin, a tissue fixative, shifted the $\delta^{18}O$ and $\delta^{13}C$ values of foraminifera by up to +0.62‰ and +0.80‰, respectively, and both isotope values of crushed Solnhofen limestone by ca. +0.15‰ (Ganssen, 1981). In benthic foraminifera, the stain Sudan Black B modified $\delta^{13}C$ values by +0.24‰, whereas hydrogen peroxide and ultrasonic cleaning resulted in a $\delta^{18}O$ change of −0.18‰ (Serrano et al., 2008). Furthermore, Grottoli et al. (2005) observed a negative shift of $\delta^{13}C$ values of three different coral species after treatment with sodium hypochlorite and hydrogen peroxide. Similar pretreatment-related isotope effects in bivalve shells are much less well studied. In particular, it remains unknown if specimens from museum collections that underwent preservation procedures can still serve as reliable climate archives. For example, partly solidified argillaceous or marly sediments

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may have been removed from fossil specimens by bathing them in surfactants. Furthermore, fragile fossil shells have often been impregnated and hardened with low-viscosity glues, whereas live-collected bivalves were stored in ethyl alcohol for extended periods of time. Likewise, the potential isotope effects of specific physical and chemical pretreatment methods used in bivalve sclerochronology have never been evaluated. For example, since sclerochronological studies are typically conducted in cross-sections (e.g., Jones, 1980), the shells are either embedded in epoxy glues or wrapped in a protective layer of epoxy glue or Multi Power 3 plastic welder prior to cutting (e.g., Rhoads and Lutz, 1980; Butler et al., 2009; Marali et al., 2016). Furthermore, several different staining techniques are employed, e.g., to identify the predominant carbonate polymorphs (Feigl's solution; Leitmeier and Feigl, 1934) and better recognize internal shell growth patterns (Mutvei's solution; Schöne et al., 2005a). During sampling for isotopes, some amounts of such substances may contaminate the analyte (=shell powder) and alter its isotopic signature.

Aside from common cleaning methods, the present study evaluated the isotope bias resulting from preparation techniques specifically used in sclerochronology or methods to preserve fossils. A focus was placed on fossil bivalve shell material which is becoming increasingly exploited in bivalve sclerochronology as a paleoclimate archive. For this purpose, we selected a ca. 30 million year-old *Glycymeris obovata* specimen that exhibited signs of diagenetic overprint, i.e., increased porosity and diminished organic content, but still consisted entirely of pristinely preserved aragonite. Furthermore, a Carrara marble powder was used to evaluate the isotope effect of various different pretreatment methods applied to an organic-free calcite, simulating a stronger diagenetically overprinted, calcitic fossil shell (with pristine isotope signals still present). Results of this study can serve as a guideline for which physical and chemical pretreatments and sample preparation techniques can potentially confound isotope-based paleoclimate studies using bivalve shells and, possibly, other calcareous biominerals.

2. Materials and methods

2.1. Study materials

For the experiments, two different calcareous materials were used, (1) a Carrara marble powder distributed by IVA Analysentechnik e.K. (Düsseldorf, Germany) and (2) a single valve of a fossil bivalve (*Glycymeris obovata*) from the Oligocene strata (Rupelian stage) of the Mainz Basin, Germany, collected by F.-O. Neuffer near Langenlonsheim in 1974 and stored in the paleontological collection of the Institute of Geosciences (University of Mainz) since then. This specimen did not undergo any special pretreatments before we used it in this study, except for rinsing in tap water and physically removing dirt. After drying from air at room temperature, the shell was crushed with a hammer and then pulverized with an oscillating mill (Retsch MM200; three Milling steps for 1 min each at 20 Hz). Afterward, the powder was homogenized using an agate mortar and pestle. To determine the stable oxygen and carbon isotope signatures of these two carbonate materials (average and 1σ values) prior to the pretreatments, 20 samples of the glycymerid bivalve ranging between 60 and 118 μg and 60 IVA Carrara marble powder samples (the sample material that we used as our in-house standards) weighing between 40 and 120 μg were processed in a mass spectrometer (Table 1; see Section 2.2).

Aliquots of the Carrara marble powder and the homogenized shell powder were then mixed with different proportions of Alcian Blue powder and periostracum, dried and hardened superglue, different epoxy glues and Multi Power 3 plastic welder as well as dry impregnation agents. Furthermore, powder samples were exposed to Milli-Q water, ethanol, a surfactant, oxidizing agents, ultrasonic cleaning, and staining solutions. Dry pretreated powders were then processed in a mass spectrometer (see Section 2.2). Table 1 provides an overview of the different treatments. More details on the tested substances and treatments are provided in the following sections.

2.1.1. Adhesives

Superglue is often used in the field and laboratory to quickly fix broken fossils (Geiger et al., 2007). Depending on its viscosity and the porosity of the shell, the superglue can permeate the fossil to different depths. Inadvertently, powder samples drilled or milled from such shells contain a mixture of shell and superglue. Here, we used the colorless Superglue 2000 Universal from Gluetec Industrieklebstoffe GmbH & Co. KG which consists of 80–100 wt% ethyl-2-cyanacrylate. Although it bonds materials in seconds, it only reaches its full strength after 24-hour curing time.

Epoxy glues typically come as two individual components, a resin (polymer) and a hardener (e.g., amines, phenols, alcohols) which cross-link and cure when mixed. Of the five studied two-component glues, Araldite 2020 (e.g., Poulain et al., 2010) and Kleer-Set Type FF (e.g., Butler et al., 2009) are often used to embed fossils prior to sectioning or completing other preparative steps. The other three, quick-drying glues, 05 Epoxy, Metal Epoxy and Multi Power 3 plastic welder are frequently used in bivalve sclerochronology to wrap the shells in a protective layer prior to sectioning or mount shells to plexiglass cubes so they can be cut with a low-speed precision saw (e.g., Schöne et al., 2005b; Marali et al., 2016).

Araldite 2020 (Huntsman International LLC) is a low-viscosity epoxy glue. For ideal curing, ten parts Araldite A, a bluish resin (30–60 wt% bisphenol-A epichlorohydrin resin and 30–60 wt% 1,4-butanediol diglycidyl ether) are mixed with three parts Araldite B, a yellowish hardener (aliphatic amine).

Kleer-Set Type FF (MetPrep Ltd.), a polyester casting glue, is more viscous than Araldite 2020 and also used to embed shells. Ideal mixture proportions are 97 and 3 for resin and hardener, respectively. The resin consists only of styrene, whereas the colorless curative is made of 33 wt% methyl ethyl ketone peroxide, 63 wt% dimethyl phthalate and ca. 1 wt% methyl ethyl ketone.

05 Epoxy (Gluetec Industrieklebstoffe GmbH & Co. KG) is a fast-curing two-component glue which fully cures in less than 3 h. The resin (75–<100 wt% bisphenol-A epichlorohydrin) and hardener (80–<100 wt% mercaptane prepolymers and 10–<20 wt% polyamide resin) are mixed at equal proportions. After mixing the components, it needs to be processed within 5 min.

Metal Epoxy (Gluetec Industrieklebstoffe GmbH & Co. KG; a similar product is available under the trade name J-B Kwik Weld), a steel reinforced epoxy glue, has similar processing and curing times as 05 Epoxy. The white resin component consists almost entirely of bisphenol-A epichlorohydrin resin. The black, viscous hardener contains 70–90 wt% mercaptane prepolymers, 10–<20 wt% polyamide resin, and 1–<20 wt% 2-,4-,6-tris(dimethylaminomethyl)phenol. According to the manufacturer (personal communication), ca. 7 wt% metal oxides, ground pure metals, crystalline silica and black carbon are added to the curative. Both components need to be mixed at equal proportions. While it provides impressive strength (tensile shear strength 14–18 N/mm^2), cured Metal Epoxy is extremely lightweight (1.3 g/cm^2) and easy to grind and polish.

Multi Power 3 plastic welder (Gluetec Industrieklebstoffe GmbH & Co. KG) belongs to the group of quick-drying (processing time ca. 5 min, fully hardened after ca. 3 h) two-component methacrylate glues offering even stronger tensile sheer strength than Metal Epoxy when set, i.e., 23–25 N/mm^2. The crème white colored, viscous component consists of 80–<100 wt% methyl methacrylate, 1–<5 wt% tosyl chloride, 1–<5 wt% methacrylic acid and 1–<2.5 wt% cumolhydroperoxide. Aside from 80–<100 wt% methyl methacrylate, the other, low-viscosity, beige-colored component, i.e., the activator (hardener) contains 1–<10 wt% 3,5-diethyl-1,2-dihydro-1-phenyl-2-propylpyridine. For best results, the two components are mixed at equal proportions.

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