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Analytical Artefacts Preclude Reliable Isotope Ratio Measurement of Internal Water in Coral Skeletons

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Internal water in cold-water and tropical coral skeletons was extracted and measured for its oxygen and hydrogen isotope ratios. Water was extracted by crushing pieces of coral hard tissue in a percussion device connected to either a cavity ring-down spectroscopy (CRDS) system or an isotope ratio mass spectrometry (IRMS) system. Despite most samples yielding sufficient water, each analytical system produces distinct isotope patterns. Experiments show that several characteristics specific to biominerals give rise to discrepancies and analytical artefacts that preclude the acquisition of reproducible isotope data. The main complication is that internal water in biogenic carbonates is distributed in an open interconnected micro-network that readily exchanges with external water and potentially facilitates interaction with hydration water in the finely dispersed organic matrix in the coral skeleton. Furthermore, only an isotopically fractionated part of the internal water is released from the coral skeletons upon crushing. Altogether, isotope ratio measurement of internal water in corals with bulk crushing techniques does not give primary fluid isotope ratios useful for (palaeo-)environmental or microbiological studies. As the resulting isotope patterns can show systematic behaviour per technique, isotope data may be erroneously interpreted to reflect the original calcifying fluid when using only a single technique to isotopically characterise internal fluids in coral skeletons.

Keywords: isotope ratio measurement, fluid inclusion water, biogenic minerals, corals, IRMS, CRDS.

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Fluid inclusion water trapped in inorganic minerals is commonly analysed for hydrogen (δ^2H_w) and oxygen ($\delta^{18}O_w$) isotope values for palaeo-fluid reconstructions. For instance, isotope ratio measurement of fluid inclusions can be applied on speleothems for (palaeo-)rainfall reconstructions (Arienzo *et al.* 2013, Affolter *et al.* 2014, Uemura *et al.* 2016) or on vein deposits for studying subsurface fluid flow dynamics (Baatartsogt *et al.* 2007, Wilkinson 2010, De Graaf *et al.* 2017). Biogenic carbonates also contain minor amounts of water (Gaffey 1988) but have not yet widely been analysed for water isotope ratios. In case that the internal water in biogenic carbonates preserves the initial calcifying fluid, then its isotopic composition has the potential to provide insight into the calcification process and palaeo-environments (Lécuyer and O'Neil 1994) and could, therefore, provide a valuable new proxy record.

Amongst the most important biogenic calcifiers in terms of global carbonate production are corals, foraminifera and bivalves (Vecsei 2004). Geochemical records of coral

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aragonite are commonly used as palaeo-oceanographic proxies (Goreau 1959, Todd 2008, Krief *et al.* 2010, D'Olivo and McCulloch 2017). However, the skeleton does not precipitate directly from the seawater but rather inside extracellular cavities, the composition of which is regulated by the physiology of the coral. Therefore, the coral may exert a major control on the geochemistry of the coral skeleton and obscure environmental signals; these are commonly referred to as 'vital effects' (Cohen *et al.* 2006). The precise nature of the vital effects is unclear, as the mechanism of coral calcification remains a topic of debate. Especially for asymbiotic cold-water coral types, non-equilibrium isotope fractionation is so strong that it is presently difficult to develop meaningful geochemical proxies for such corals (Weber 1973, McConnaughey 1989, Wefer and Berger 1991, Cohen and Gaetani 2006, Gagnon *et al.* 2007).

Traditional biogenic calcification models for corals are based on precipitation of skeletal aragonite through ion-by-ion precipitation from an extracellular fluid (ECF) layer at the skeleton-tissue interface (Allemand *et al.* 2011, Tambutté *et al.* 2011, Saenger and Erez 2016). In recent times, models that involve skeletogenesis starting with hydrated amorphous calcium carbonate (ACC) precursor particles have gained increasing popularity (Rollion-Bard *et al.* 2010). The ACC particles could develop in a hydrous organic matrix and transform into crystalline aragonite rapidly after attachment to the skeleton. This way of calcification could be more efficient than ion-by-ion precipitation (Raiteri and Gale 2010) and agree better with spectroscopic observations (Mass *et al.* 2017, Von Euw *et al.* 2017). Whether biogenic carbonate grows through the attachment of ACC particles or through ion-by-ion precipitation from an ECF (Figure 1) may have major relevance for predicting the response of biogenic calcifiers to ocean warming, pollution and acidification (Hoegh-Guldberg *et al.* 2007, Kayanne 2016, Mass *et al.* 2017, Hughes *et al.* 2018).

An unknown factor in biomineralisation is the nature and composition of the calcification fluid, which has commonly been assumed to be seawater-like (Gagnon *et al.* 2012, Tambutte *et al.* 2012). Several decades ago already, it was established that corals incorporate minor amounts of free water into their skeletons (Gaffey 1988, Cuif *et al.* 2004). As this internal water potentially represents the calcifying fluid, the analysis of its isotope ratio could yield valuable information for existing biomineralisation models. A pioneering set of experiments was published by Lécuyer and O'Neil (1994), who used a thermal decrepitation technique to extract water from biogenic aragonite and calcite at extraction temperatures above 200 °C. Their work showed that up to 3% *m/m* of water could be extracted from biogenic carbonates with isotope ratios that are rather distinct from the ambient water of the calcifying organisms. Lécuyer and O'Neil (1994) proposed that it could be a significant fraction of metabolically derived water that contributed to the observed isotope offsets.

Since this early work, there have been significant advances in our understanding of the distribution of water in biogenic carbonates. Thermogravimetric experiments gave insight in the water release from coral aragonite at different temperatures (Cuif *et al.* 2004). Such experiments confirm the overall $\sim$ 1.5% *m/m* water content of coral aragonite and further show that the majority of that water is associated with the thermal decomposition of a hydrated organic matrix (Cuif *et al.* 2004). Distinct water losses occur at 60–75 °C (removal of absorbed water) and 180–210 °C (release of hydration water from organics), but most water is released at temperatures between 270 and 335 °C (decomposition of the organic matrix).

Cuif *et al.* (2004) further observed that a small fraction of $\sim$ 0.2% *m/m* of water was released at temperatures between 100 and 150 °C, likely representing free water locked up in the coral aragonite. As it has also been demonstrated that the organic matrix is finely dispersed throughout the aragonite of the coral skeleton (e.g., Cuif and Dauphin 2005), and therewith difficult to remove with leaching techniques. The water analysed in the high-temperature experiments by Lécuyer and O'Neil (1994) may reflect a mix of free internal water and water incorporated in the organic matrix of the corals.

The central objective of the present study was to carry out a new set of experiments in an attempt to extract and isotopically analyse fluids released from coral skeletons crushed at temperatures just above 100 °C. In addition to the coral samples, a small set of samples from several other biomineralising taxa (bivalves, sea urchins and earthworm granules) were analysed to make a broader comparison.

Analytically, we applied two different continuous-flow techniques that were developed over the past 15 years for the isotope ratio measurement of fluid inclusion water. With both instruments, mineral samples are crushed in a percussion device to release internal water for stable isotope measurement at a temperature of 115 °C (Vonhof *et al.* 2006, De Graaf *et al.* 2020a). Continuous-flow techniques have already been applied successfully to obtain isotope information on cave deposits (Van Breukelen *et al.* 2008, Rowe *et al.* 2012, Griffiths *et al.* 2013, Labuhn

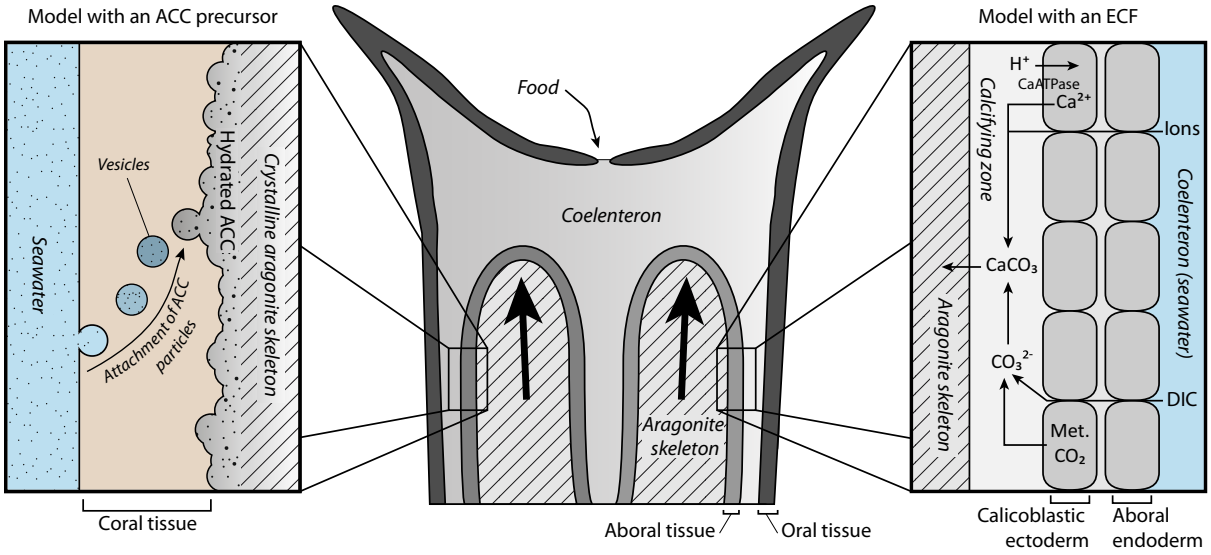

Figure 1. Schematic representation of the two main calcification models, adapted after McCulloch *et al.* (2012) and Mass *et al.* (2017).

et al. 2015, Affolter *et al.* 2019, Rogerson *et al.* 2019, Matthews *et al.* 2021, Wassenburg *et al.* 2021, Warken *et al.* 2022) and vein minerals (Bertotti *et al.* 2017, De Graaf *et al.* 2019, 2020b).

Remobilisation and alteration of water contained in biogenic samples during sample heating have been shown to affect isotope results (Pederson *et al.* 2019, Nooitgedacht *et al.* 2021). However, the temperatures at which these processes were observed (130–175 °C) are somewhat higher than the temperature to which samples are exposed in this study. Provided that we succeed in quantitatively extracting the primary internal fluid fraction, the oxygen and hydrogen isotopic composition could yield insight in the extent to which seawater and metabolic water influence the fluid pool in the calcifying organism. Therewith, this analysis could potentially provide valuable information about the calcification process because the calcifying medium could have a considerably different composition depending on the calcification model (Figure 1).

Materials and methods

Sample set

The main sample set consists of cold-water corals (*Lophelia pertusa, Madrepora oculata, Stylaster* sp. and *Desmophyllum* sp.) and tropical corals (*Porites* sp. and *Orbicella* sp.). Cold-water coral samples were retrieved from the Logachev Mound Province in the North Atlantic Ocean (Kenyon *et al.* 2003) and the Santaren Channel west of the Great Bahama Bank and included both living and fossil specimens. Tropical corals were sampled off the islands of Rodrigues near Mauritius, Curaçao in the Caribbean, and Cocos Keeling Island in the NE Indian Ocean.

In addition to the coral samples, a small set of other marine and non-marine biogenic calcifiers was sampled and analysed. Sea urchins were collected along the coast of Tanzania. Freshwater bivalves were collected from the Amazon River in Peru and the Erhai Lake in China. Marine bivalves were collected from the Logachev Mound Province. Fossil earthworm calcite granules (ECG) were retrieved from an Eemian fluvial terrace sequence in Caours, France.

An overview of the reference material waters that were used throughout all measurements and experiments is given in Table 1. The VSMOW-2 water is an international measurement standard (IAEA 2017). The other waters are internal laboratory reference materials that were calibrated against international measurement standards. The SLAP (old) water was a leftover of the SLAP-2 international measurement standard (IAEA 2017) that got isotopically enriched over time due to evaporation.

Analytical platforms

Isotope analysis of internal water was performed on three different set-ups. Two instruments make use of a continuous-flow isotope ratio mass spectrometer (IRMS), while the other set-up runs on a wavelength scanning cavity ring-down spectroscope (WS-CRDS). The used IRMS-based

Table 1.
Isotope values of the reference material waters used for the measurements and experiments in this study

Water type	$\delta^{18}O$ (‰ *vs.* VSMOW)	$\delta^{2}H$ (‰*vs.* VSMOW)
Turkana	6.6	47.0
KONA	1.12	3.29
VSMOW-2	0	0
DNS-3	-1.43	-9.5
ALW-2011	-8.37	-59.3
Mainz Tap	-8.82	-63.67
Chamonix	-13.7	-100.6
SLAP (old)	-47.1	-382.9

instruments are stationed at the Earth Sciences Stable Isotope Laboratory of the Vrije Universiteit Amsterdam (Vonhof *et al.* 2006) and at the Max Planck Institute for Chemistry (MPIC) in Mainz (De Graaf *et al.* 2020a).

The two IRMS-based set-ups are conceptually identical and make use of a mechanical crusher unit that is interfaced with a pyrolysis furnace (High Temperature Conversion Elemental Analyser; TC-EA; Thermo Scientific, Bremen, Germany). The crusher unit is maintained at 115 °C to quantitatively vaporise the water that is released upon crushing. Transported by helium carrier gas, water vapour is collected in a cold trap during 4 min after the sample crush. Subsequently, the cold trap is heated to generate a short pulse of water vapour. The water vapour is converted in the TC-EA into H_2 and CO gas, which are thereafter chromatographically separated. An IRMS magnet peak jump routine allows for online analysis of both $\delta^{2}H_w$ and $\delta^{18}O_w$ from a single water release. The acquired isotope values are then normalised using reference material water injections in the crusher that are run directly before and after the sample. These reference material water injections are processed identically to the crushed samples. Fluid inclusion isotope results on the IRMS platforms typically have a measurement repeatability (1*s*) of 0.4‰ for $\delta^{18}O_w$ and 2.0‰ for $\delta^{2}H_w$ for water yields down to 0.1 µl.

Analyses on the CRDS-based set-up were performed at the MPIC in Mainz. A detailed description of the used CRDS set-up is provided in De Graaf *et al.* (2020a). Sample crushing in this set-up is achieved using a spindle crusher that is maintained at 115 °C. The crushing unit is coupled online to a CRDS instrument to allow for continuous-flow isotope analyses. The nitrogen carrier gas in the system is constantly moisturised by Mainz Milli-Q water up to a water vapour concentration of 14000 ppmv. Mainz Milli-Q water has isotope values similar to our in-house Mainz Tap reference material water (Table 1). The moisturised carrier gas flow removes memory effects between samples (Affolter *et al.* 2014), while its isotope ratio does not affect the bias of the fluid inclusion isotope data (De Graaf *et al.* 2021). Upon sample crushing, a peak is generated on top of the water background. Isotope values can then be calculated by subtraction of the water background from the sample peak. The 1*s* measurement repeatability on the CRDS set-up is 0.3‰ for $\delta^{18}O_w$ and 1.1‰ for $\delta^{2}H_w$ for water yields down to 0.1 µl.

Isotope values throughout this manuscript are reported on the VSMOW-SLAP scale. Values are given in permil (‰), which corresponds to 1 mUr. For both techniques, water yields of sample crushes were estimated per sample by comparing their signal strength with reference material water injections.

Heating experiment

The main set of data was collected following the standard protocol. However, as biogenic minerals have not yet widely been used for fluid inclusion isotope ratio measurement, we executed extra experiments to test the suitability of biogenic sample material for this type of analysis.

A first experiment consisted of measuring coral samples at different crusher temperatures. The temperature of the oven in which the crusher is installed can be freely adapted but is generally maintained at 115 °C to ensure complete evaporation of released water while preventing thermally induced alteration of fluid inclusions as much as possible. Measuring samples at different crusher temperatures can provide insight into how prone the sample material is to outgassing and decrepitation of water inclusions. The *L. pertusa* sample BC-53 was used for this experiment, as it was already measured multiple times following the standard protocol. A set of sub-samples of BC-53 was measured on the CRDS set-up at an oven temperature of 155 °C and another sub-sample at an oven temperature of 75 °C.

Immersion experiment

Another experiment aimed at testing the extent to which the coralline sample material retains or absorbs external water. For this experiment, discrete ~ 1 g chips of *L. pertusa* sample BC-53 were immersed in four isotopically contrasting waters (Table 1) for 72 h at room temperature, after which they were left to dry in laboratory air for a day and then placed in the crushing cell of the CRDS set-up. The standard

stabilisation time of the instrument before crushing kept the samples at 115 °C for 15 min. For inorganic mineral samples, 15 min of pre-heating is sufficient to remove any external water adhered to the outer surface of inorganic mineral samples in order to enable an accurate analysis (De Graaf *et al.* 2020a). Subsequently, the samples were measured following the standard protocol.

Stepwise heating of coral material to 300 °C

Two sub-samples of *L. pertusa* sample BC-53 were used to determine the total amount and isotopic ratio of non-inclusion water. This experiment is comparable to the thermogravimetric experiments performed by Cuif *et al.* (2004). For the experiment, the crusher unit on the IRMS set-up was decoupled from the pyrolysis furnace and replaced by a stainless-steel tube with a length of 20 cm and an internal diameter of 10 mm. About 0.03 g of sample material was crushed into a powder and introduced into the centre of the tube. The sample was held in place by a plug of quartz wool at both ends.

Helium carrier gas was led through the tube at 115 °C for approximately 10 min until the water background recorded by the IRMS was negligible. The stainless-steel tube was subsequently stepwise heated to a final temperature above 300 °C. Before each heating step, the cold trap was placed to collect all water released. Water was collected for 4 min per heating step and isotopically analysed in the IRMS for $\delta^{18}O_w$ and $\delta^{2}H_w$ before increasing the temperature to the next step. The generated mass spectrometric peaks were recalculated to water amounts through a calibration based on peak sizes of injected reference material waters.

Results

Main data set

Nearly all biogenic samples yielded water when crushed. The amount of water extracted was roughly between 0.01 and 0.05% *m/m*, which is in the order of magnitude of the amount of free water released in thermogravimetric experiments with different coral species at temperatures below 200 °C by Cuif *et al.* (2004).

At the start of the project, the focus was on marine calcifiers and mainly corals were measured on the IRMS-based device at the Vrije Universiteit Amsterdam. These initial isotope results covered a wide range from -4 to 12‰ for $\delta^{18}O_w$ and -60 to 20‰ for $\delta^{2}H_w$ and displayed a positive correlation (Figure 2, Table S1). Typical seawater isotope ratios were not acquired for these coral specimens. Later analyses using the IRMS-based device at the Max Planck Institute for Chemistry in Mainz produced data in a comparable isotope range, but generally with more scatter around the positive correlation between $\delta^{18}O_w$ and $\delta^{2}H_w$ ratios (Figure 2).

We next performed a series of analyses on similar material using the CRDS system, which is a fundamentally different technique for fluid inclusion isotope analysis. While IRMS and CRDS techniques give fully comparable results

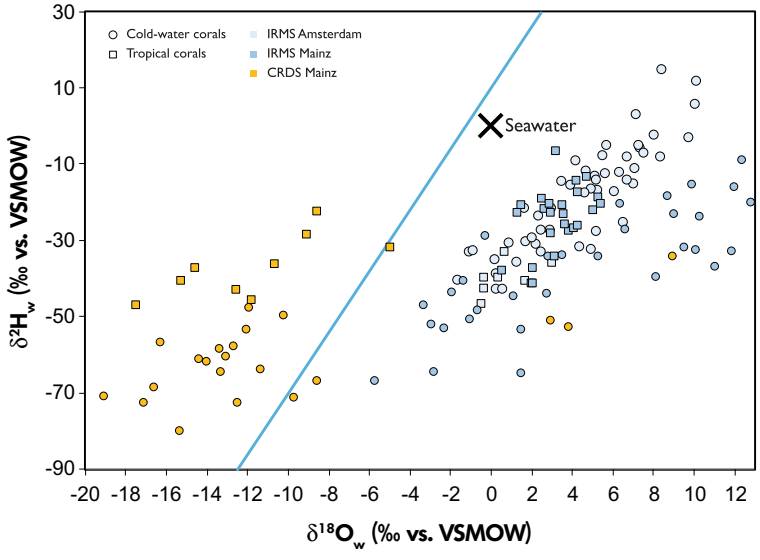

Figure 2. Isotope data of water released by crushing coral samples on three different analytical set-ups. Seawater and the Global Meteoric Water Line (GMWL) are given for reference.

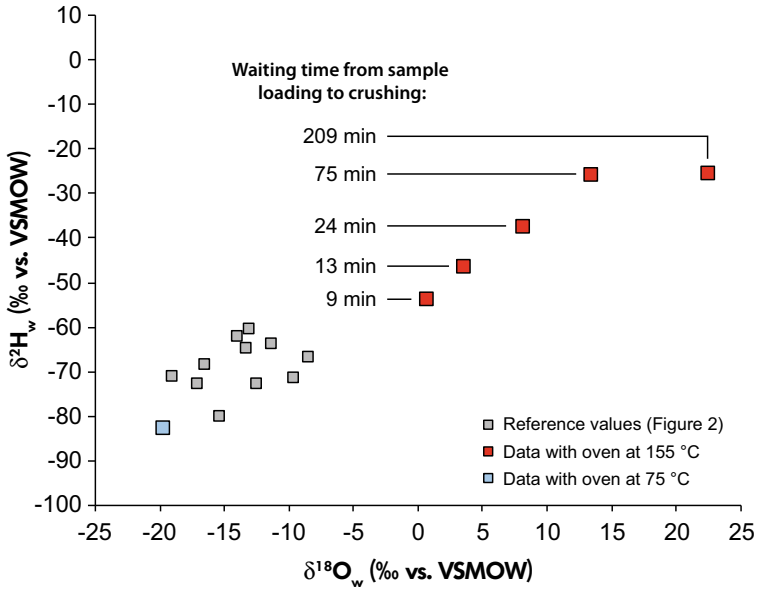

Figure 3. Sub-samples taken from a single branch of *L. pertusa* sample BC-53 were repeatedly measured on the CRDS system at different crusher temperatures and with different waiting times before crushing. A gradual enrichment of heavy isotopes occurs when a sample is left longer in the crusher before analysis. Measurements of sub-samples of BC-53 following the standard CRDS protocol with an oven temperature of 115 °C and a warm-up time of 15 min (Table 1) are shown in grey for reference.

when analysing inorganic mineral samples (De Graaf *et al.* 2020a), the CRDS-based isotope ratio measurement of water extracted from biogenic carbonates produces remarkably different isotope data compared with the IRMS-based dataset (Figure 2). Isotope values mostly range from -16 to -6‰ for $\delta^{18}O_w$ and from -80 to -30‰ for $\delta^{2}H_w$. In particular, the $\delta^{18}O_w$ values are lower on the CRDS system in comparison with the IRMS data.

To compare the data in a broader context, additional measurements were performed on several other calcitic and aragonitic biogenic calcifiers (Table S1). Earthworm calcite granules measured on the IRMS system in Mainz produce values ranging from -1.9 to -1.0‰ for $\delta^{18}O_w$ and from -64 to -56‰ for $\delta^{2}H_w$. Calcitic sea urchin shells measured on the IRMS system in Mainz give isotope values from -1.6 to 3.1‰ for $\delta^{18}O_w$ and from -40 to -38‰ for $\delta^{2}H_w$. The aragonitic freshwater bivalves from the Amazon River (Peru) and the Erhai Lake (China) were measured on the CRDS system and have an isotope range of -8.5 to -6.5‰ for $\delta^{18}O_w$ and from -69 to -52‰ for $\delta^{2}H_w$.

Isotope data of samples measured at different crusher temperatures

Measuring coral samples on CRDS set-up at different crusher temperatures compared with the standard protocol produced distinct isotope results (Figure 3, Table S2). The measurement at the lowest temperature of 75 °C resulted in lower $\delta^{18}O_w$ and $\delta^{2}H_w$ values compared with the data measured following the standard protocol. Measurements at an elevated temperature of 155 °C produced higher $\delta^{18}O_w$ and $\delta^{2}H_w$ values. Longer heat-up times in the crusher resulted in a gradual enrichment in heavy isotopes.

Isotope data of immersed samples

For the immersion experiment, sub-samples of *L. pertusa* sample BC-53 were immersed in four isotopically contrasting waters. Two sub-samples were used per water type. Overall, the isotope results of these samples reflect the water in which they were immersed (Figure 4, Table S3). Only in more detail, the isotope values of the internal water appear to be shifted to higher isotope values compared with the immersion waters for $\delta^{18}O_w$ in particular.

Discussion

Data reproducibility

While there appears to be consistency in data analysed on a single instrument, the difficulties in reproducing data between different analytical systems (Figure 2) indicates potential analytical artefacts. Relatively extensive

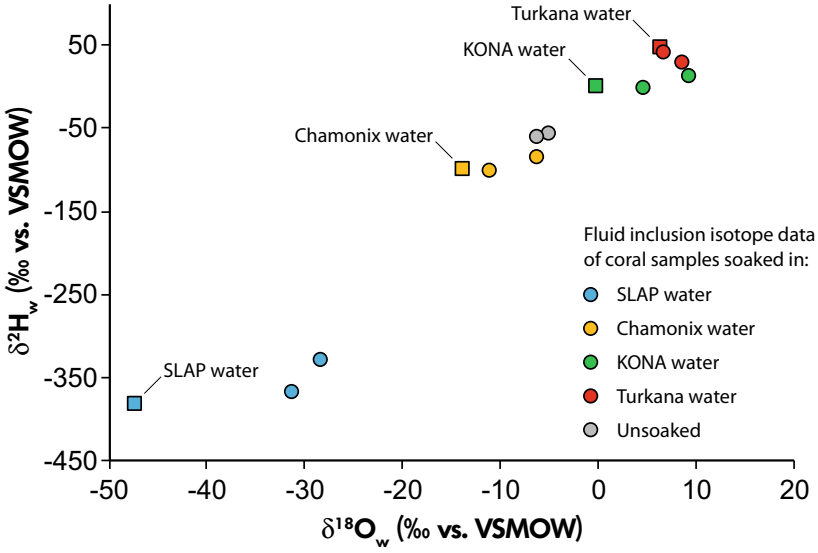

Figure 4. Isotope data of internal water in *L. pertusa* samples that were immersed in isotopically distinct waters and then analysed on the CRDS set-up. The analysed samples are sub-samples from a single branch of a *L. pertusa* coral. The isotope data reflect the water in which the samples were immersed (squares).

comparisons between the IRMS and CRDS systems at the MPIC in Mainz show that fluid inclusion isotope data compare well for inorganic samples like speleothems and fracture infilling minerals (De Graaf *et al.* 2020a). There is, therefore, reason to believe that there are analytical artefacts specifically for biogenic samples.

First, biogenic carbonates differ from inorganic samples in that they do not contain discrete fluid inclusions of the size typically observed in inorganic minerals but have free water in cavities much smaller than can be resolved in microscopic examination of thin sections. The fluids may, for example, be located in tiny spaces between crystal boundaries (Koga and Nishikawa 2014). Because we cannot microscopically inspect these fluid pockets, we also cannot rule out the possibility that they are interconnected and can exchange with external water.

Secondly, biogenic carbonate typically contains a dense network of organic tissue (Cuif and Dauphin 2005). This organic matrix is part of the calcifying matrix and is hydrated (Cuif *et al.* 2004). It is unknown to what extent this hydration water can exchange with the internal water that is released during sample analysis.

An indication to the nature of the analytical artefacts in biogenic carbonates can be found in the behaviour of the samples during crushing in each of the analytical systems used. When analysing biogenic minerals, water backgrounds typically remain elevated before and after crushing on both the CRDS and IRMS set-ups. On the IRMS set-up, the background signal on the mass spectrometer before crushing biogenic carbonates is typically 400 mV for mass 29.0, compared with 100 mV when analysing inorganic samples.

On the CRDS set-up, the recorded water amount consistently remains elevated with respect to the background by several 100 ppmv in the 30 min between loading and crushing of biogenic carbonates (Figure 5). After crushing a biomineral, backgrounds commonly remained elevated for up to several hours before coming down to the base level again. Such elevated backgrounds do not occur when analysing inorganic mineral samples like speleothems or vein minerals, indicating that water release before crushing and water retention after crushing occur specifically for biogenic sample material in the analysers used.

Analytical interferences

The data indicate that coral samples release a prolonged flux of water while warming up in the crushing cell, far exceeding the normally short-lasting flux of evaporating superficial water from inorganic samples after loading. This suggests that there is permeability in the coral samples allowing a significant fraction of the internal water to escape through diffusion and evaporation before crushing.

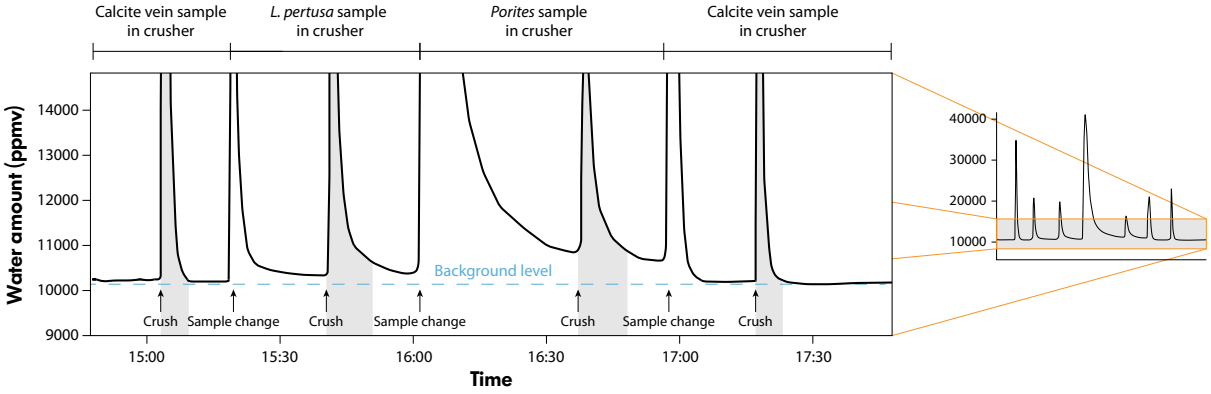

Figure 5. Water concentrations as recorded on the CRDS instrument when analysing biogenic and inorganic minerals. Peaks resulting from crushing of a sample crush are shaded grey. The other peaks are the result of sample loading, which requires the crusher to be opened and allows moisture from the laboratory air to get into the system. The biogenic sample crushes show elevated water backgrounds before and after the peak. The effect is especially strong in the porous material of the *Porites* sample. The inorganic calcite vein samples do not have elevated backgrounds before and after crushing and produce a short well-defined peak, indicating instantaneous release of all fluid inclusion water.

Diffusion and evaporation would cause a preferential loss of lighter isotopes of O and H before crushing and result in measured $\delta^{18}O_w$ and $\delta^{2}H_w$ values being too high with respect to the initial values of the internal water. Large isotope shifts are indeed shown in the experiment targeted at quantifying the effect of crusher temperature and warm-up time. A gradual increase in $\delta^{18}O_w$ and $\delta^{2}H_w$ values can be observed when heating coral samples for longer times before crushing (Figure 3). A similar shift was also observed in an experiment on the IRMS system in Amsterdam by Nooitgedacht *et al.* (2021), which showed an enrichment in heavy isotopes for a coral sample that was heated for 90 min at 175 °C prior to analysis compared with a sample that was not heated prior to analysis.

The prolonged release of water after crushing indicates that the internal water of the coral is not instantaneously and quantitatively extracted upon crushing. The long duration of the post-crush water release may point to a retarding effect of water diffusing from tiny spaces in the crushed material or potentially even from the freshly exposed organic matrix. A slowed release of water could entail a fractionation where light isotopes preferentially evaporate while leaving heavy isotopes behind.

The temperature of the crushing system thus has an important impact on the isotope results. At lower crushing temperatures, pre-crushing outgassing is lower (i.e., smaller shift to higher isotope values) and evaporation of internal water after crushing more difficult (i.e., larger shift to lower isotope values). As a result, water releases from samples measured at low temperatures are isotopically lighter than those measured at high temperatures (Figure 3).

Both effects (water loss before the crush, and water retention after the crush) should be expected to fractionate the isotope values, even when there is only a single source of extractable water in the coral aragonite. The impact of the analytical artefacts can differ between the techniques as a result of differences in analysis time, carrier gas moisture content and flow rate. For example, the pre-crushing evaporation is likely to have a less strong effect in the CRDS system, because the time from sample loading in the oven to crushing is with 30 min considerably shorter compared with the IRMS set-up (90 min). The evaporation potential in the CRDS system could also be lower due to the carrier gas being moist.

At the same time, the CRDS set-up could be more susceptible to having trouble achieving full evaporation of internal water after crushing, because the system runs on a moisturised carrier gas and at lower flow rates compared with the IRMS set-up (40 *vs.* 95 ml min^{-1}). This is illustrated, for instance, by the *Porites* material that we used, which generally has a stable internal water content and yields on average 0.16 µl g^{-1} (1*s* of 0.05) of water on the IRMS system and only 0.04 µl g^{-1} (1*s* of 0.01) of water on the CRDS system. Such differences in water yield between the two techniques do not occur when crushing inorganic

samples like veins and speleothems, which have larger size fluid inclusions (De Graaf *et al.* 2020a).

The relatively long residence time of the biogenic samples at elevated temperatures in the IRMS system could also facilitate more intense oxygen isotope re-equilibration between the internal water and the host carbonate. Although oxygen isotope exchange is assumed to be minor when heating inorganic calcite samples (Uemura *et al.* 2020), it could have a stronger impact in biogenic carbonates due to the fine dispersion of the internal water (Nooitgedacht *et al.* 2021). Oxygen isotope re-equilibration would shift $\delta^{18}O_w$ values up and may, therefore, contribute to the higher $\delta^{18}O_w$ values on IRMS set-up compared with the CRDS set-up.

Furthermore, the performance of CRDS analysers can be adversely affected by the presence of volatile organic compounds in the vaporised water sample (West *et al.* 2010), to which the IRMS set-up is less sensitive. While we do not know the precise composition of the extracted water vapour, it is not inconceivable that organic volatiles are released when biogenic samples are crushed and affect the spectroscopic performance of the CRDS system.

All of the potential analytical artefacts and fractionation processes may lead to discrepancies between the IRMS and CRDS techniques. With the currently available data, these effects are difficult to isolate or quantify, however, so that correction for the analytical artefacts remains out of reach.

Preservation of primary internal water

During coral skeleton growth, there are two conceivable primary water sources that contribute to the internal water pool, being seawater and metabolic water. Metabolic water is produced inside the living tissue through the coral's metabolism. If the growth process includes hydrated ACC as a precursor phase that subsequently transforms to crystalline aragonite (Rollion-Bard *et al.* 2010), then there may also be a significant amount of ACC hydration water in the aragonitic coral skeleton. Such hydration water would likely be isotopically fractionated with respect to the primary waters it was sourced from (Gázquez *et al.* 2017).

Whereas multiple primary water sources may be expected in corals, the considerable evaporation of water from corals during warm-up in the crushing cell raises the question to what extent corals preserve these primary fluids. The immersion experiment shows that the isotope values of internal water extracted from coral samples reflect the water in which the samples were immersed (Figure 4). After only a few days of immersion, the internal water pool is almost entirely overprinted by the external water. We take this to indicate that the coral aragonite is highly permeable and readily flushed, which makes it unlikely that the internal water pool is capable of preserving primary fluids.

Organic-bound water in the coral skeleton

Apart from free internal water, another water fraction in coral skeletons is stored as hydration water bound to the organic matrix (Cuif *et al.* 2004). The potential isotope effect of water released from the organic matrix was tested in an experiment, in which powdered samples of *L. pertusa* were heated stepwise to temperatures up to 300 °C under a continuous flow of helium. The further treatment and analysis of the water liberated from these samples was identical to the treatment of crush-liberated water on the IRMS-based systems. The results show that the water yield of powdered samples heated to temperatures above 220 °C is tens of times higher than the water yield from sample crushes at 115 °C (Table 2). This is consistent with the observations of Cuif *et al.* (2004) that water associated with the organic matrix is by far the largest fraction in the internal water pool of these corals.

The heating experiments further show that the extracted water at temperatures of 300 °C has comparatively high isotope values (Table 2). This could support a scenario in

Table 2.
Stepwise heating experiment of two powdered *L. pertusa* samples

Sample	Mass	Heating interval (°C)	Water yield (µl)	Peak area H (mV)	δ^2H (‰ *vs.* VSMOW)	Peak area O (mV)	$\delta^{18}O$ (‰ *vs.* VSMOW)
BC-53 #1	30 mg	0–220	0.32	253	-27.1	808	5.5
		220–300	0.27	216	-9.7	672	10.2
BC-53 #2	37 mg	0–150	0.03	27	-55.5	95	-2.3
		150–300	0.45	356	-19.7	1142	8.8

Water amounts were estimated through comparison with the signal strength of reference water injections. Below 150 °C, minimal amounts of water were released. Larger water amounts enriched in ^{18}O and ^{2}H were liberated at higher temperatures.

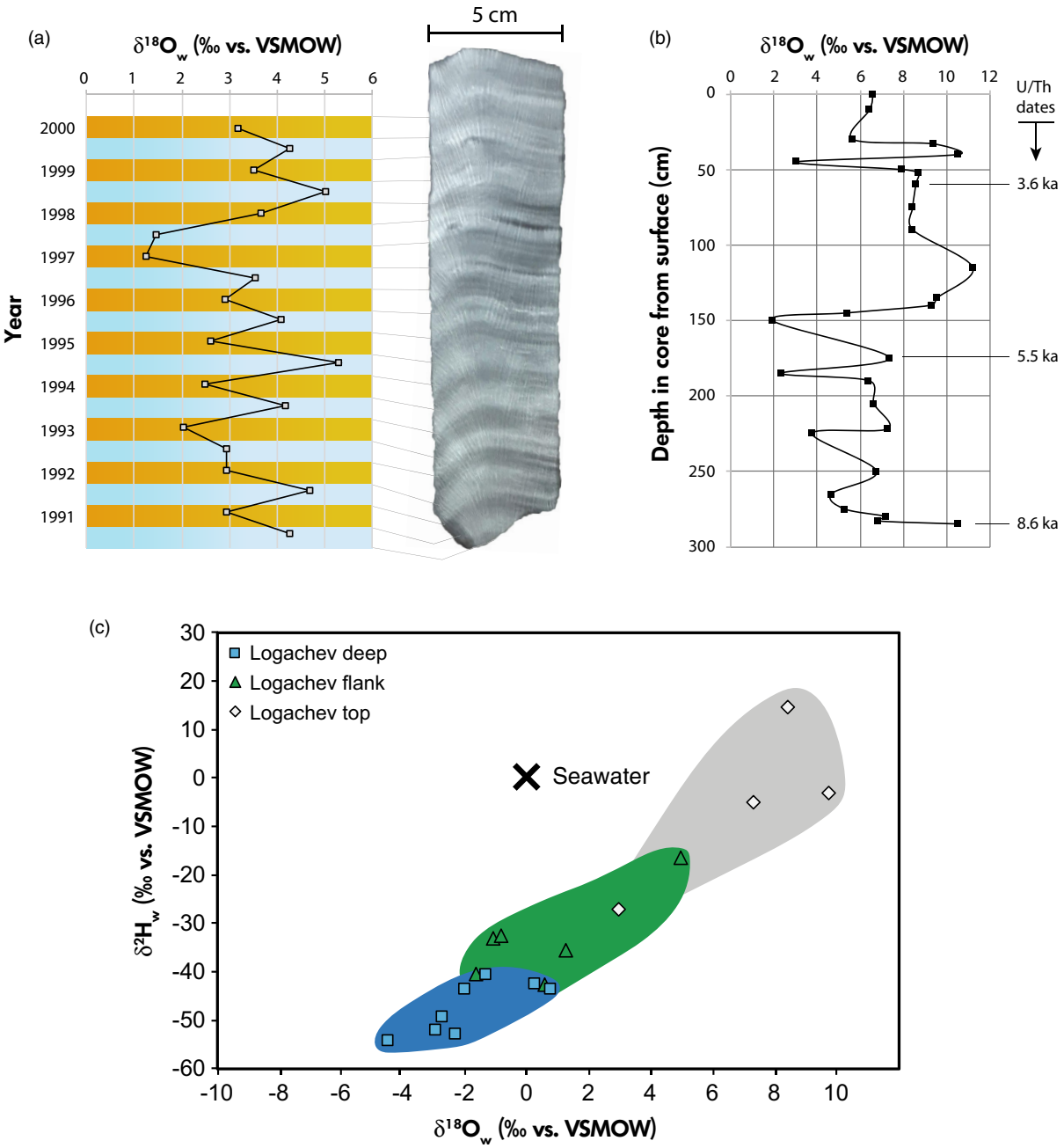

Figure 6. Isotope data of internal water from corals show an apparent relation to environmental parameters in several case studies. (a) Seasonal $\delta^{18}O_w$ variations exist between summer (orange) and winter (blue) bands within a core of the tropical *Porites* coral. (b) Depth $\delta^{18}O_w$ profile of *L. pertusa* samples from a soft-sediment core from the Santaren Channel with U/Th dates from De Graaf (2018). (c) A location-dependency of $\delta^{18}O_w$ and $\delta^{2}H_w$ values exists for living *L. pertusa* specimens from the Logachev Mound Province.

which the high isotope values for water extracted in the IRMS-based crushing experiments may in part be due to a contribution of water associated with the organic matrix. Since the heating time in the IRMS system is with about 90 min relatively long, it could potentially allow for a greater release of water from the organic matrix into the coral's internal water pool prior to crushing in comparison with the CRDS system. This would be in line with the results of the high-temperature water extraction experiments by Lécuyer and O'Neil (1994), which also yielded rather high $\delta^{18}O_w$ values.

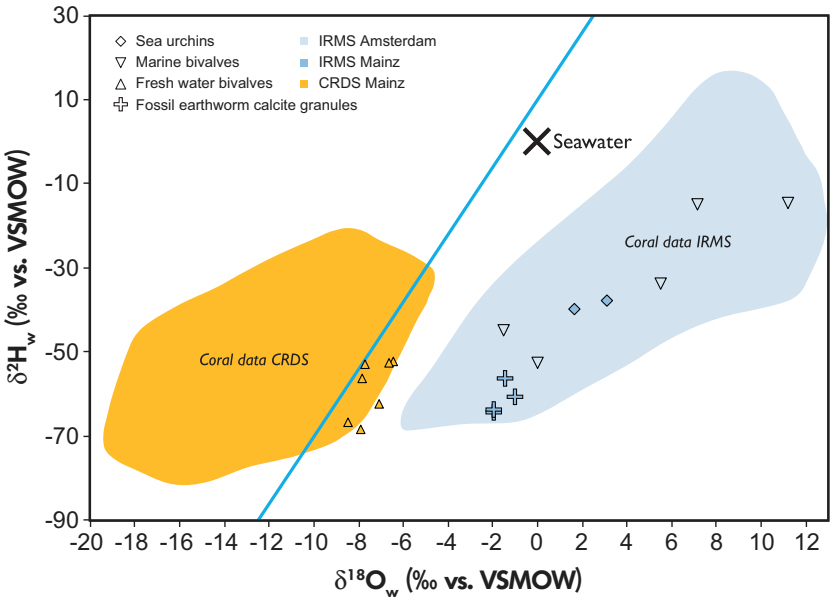

Figure 7. Isotope data of internal water from a set of sea urchins, bivalves and earthworm calcite granules. The orange and blue field, which represent the data of coral samples from Figure 2, are given for reference.

Altogether, it can be concluded that current crusher-based techniques are not suitable to quantitatively extract and isotopically analyse primary fluids from coral skeletal carbonate.

Correlation with environmental parameters

Even though our data do not reflect primary isotope signals, the isotope data of internal water of several coral sample sub-sets still show variations that appear to correlate with environmental parameters. For instance, a dataset of *Porites* specimens sampled at seasonal resolution consistently shows $\delta^{18}O_w$ data to be lower for summer growth bands and higher for winter growth bands (Figure 6a), although it should be noted that such a pattern is not obvious for the $\delta^{2}H_w$ values. Another example is the fossil cold-water coral specimens of *L. pertusa* from the sediment core from the Bahamas, which seem to display systematic isotope variations in depth, with generally higher $\delta^{18}O_w$ and $\delta^{2}H_w$ values for the younger samples (< 5 ka) compared with the older material (Figure 6b). The dataset of living *L. pertusa* samples collected at different sites in the Logachev Mound province displays a grouping based on sampling location on the coral mound with lowest $\delta^{18}O_w$ and $\delta^{2}H_w$ values for samples collected at the greatest water depth (Figure 6c). Coral growth conditions vary between the sites with optimum conditions in the deep setting and the least favourable conditions at the mound summits (Kenyon *et al.* 2003).

As coral skeletons poorly preserve their internal fluids, the apparent environmental correlations unlikely result from variability in the primary calcifying fluid, but are more likely driven by (a) environmentally-driven subtle variations in the microstructure of the coral skeleton that affect the permeability of the aragonite or (b) environmentally-driven changes in the amount and composition of the organic matrix that affect the interaction of the organic matrix with the coral's internal water pool during analysis. Such environmentally induced sample characteristics may control how strong the data are impacted by analytical artefacts and thereby cause the observed correlations.

As of such, the isotope ratios of the measured fluids have no direct relation to the physical environment in which the corals lived but are rather the result of isotope fractionation during analysis. As the impact of these analytical artefacts depends on the characteristics of the sample material, it can give the erroneous idea that the internal water released holds primary isotope signals.

Other biogenic calcifiers

A number of other biogenic calcifiers were analysed to make a first broad comparison with the coral data set (Figure 7). The isotope data of water released by crushing these other taxa have a range comparable to the coral samples (Figure 2), despite the fact that the sample set includes both marine and non-marine organisms from highly

different environments. These preliminarily results may suggest that most biogenic carbonates are subject to the same analytical effects as the coral samples in this study and are in general unlikely to reflect primary fluids.

As most samples were measured using the IRMS-based techniques and the non-marine bivalves only on the CRDS system, more extensive isotope data are still needed for the internal water of non-coral biogenic calcifiers to confirm these first findings. The freshwater bivalves, for example, produce isotope values that are rather close to meteoric water. Although this can be coincidental, it cannot be excluded that it potentially does reflect a primary signal for this particular biogenic calcifier.

Conclusions

Crushing of biogenic carbonates at a temperature of 115 °C produces sufficient water yields for isotope ratio measurement. Isotope compositions of the extracted water, analysed on IRMS- and CRDS-based instrumentation, plot over a wide range of over 100‰ for δ^2H_w and 30‰ for $\delta^{18}O_w$. However, the coral samples used in this study show discrepant outcomes between different analytical techniques. The acquired isotope data reflect analytical artefacts rather than primary isotope signals. Several observations were made when analysing biominerals:

- The internal water in coral skeletons is poorly retained and can readily by flushed by external water. The bulk extraction techniques release secondary fluids that are contained in an open system network in the biomineral.

- Long heating times and high crusher temperatures lead to higher isotope values, which indicates that water is lost from the biogenic samples prior to crushing and also potentially exchanges with the host carbonate. The diffusive water loss creates evaporation trends in the acquired data.

- Water is not instantaneously released upon sample crushing. Biogenic samples generate a peak release upon crushing but then continue to release water for a prolonged period of time, which entails fractionation processes.

- A considerable fraction of water in coral skeletons is associated to an organic matrix. This organically bound water is not integrally extracted at the temperatures at which the samples are crushed but may still affect the isotope measurements.

In summary, it appears that the internal water pool of coral skeletal carbonate, although its amount is generally sufficient for crusher-based extraction and isotope ratio measurement, does not reflect the isotope ratio of the calcifying fluid at the time of skeletal growth. The internal water appears to be easily overprinted by external water sources, possibly exchanges with water associated with the organic matrix, and is difficult to extract quantitatively in the currently used analytical systems. Apparent correlations between internal water isotope ratios and environmental parameters most likely result from changes in the aragonite crystallography or organic matrix that control the strength of the analytical artefacts. Further research may be required to test these conclusions for other biogenic calcifiers.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability statement

The data that support the findings of this study are available in the Supporting Information and on request from the authors.

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Supporting information

The following supporting information may be found in the online version of this article:

Table S1. Fluid inclusion isotope results acquired following the standard protocol on the IRMS and CRDS analytical systems in Amsterdam and Mainz.

Table S2. Isotope data of internal water of coral samples measured at different crusher temperatures.

Table S3. Isotope data of internal water of coral samples that were immersed in isotopically contrasting waters.

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