1.1 Fluid inclusion studies

Fluid flow efficiently redistributes chemical constituents and heat throughout the Earth’s crust and hydrosphere and, therefore, acts as an important driver of innumerable geological processes, including ore-deposition, biogenic calcification, metamorphism, diagenesis and structural deformation (Bredehoeft and Norton, 1990). The dynamics of subsurface fluid flow in sedimentary basins is tightly linked to the emplacement of economically important petroleum accumulations, mineral deposits and water resources. From a societal point of view, an understanding of the behavior of crustal fluids is key in enhancing our capacity to assess geohazards (e.g., earthquakes), groundwater quality and opportunities for the safe disposal or sequestration of anthropogenic CO$_2$ emissions and nuclear waste in the subsurface (e.g., Bachu et al., 1994; Dublyansky and Spötl, 2010; Matter et al., 2016). Adequate methods for characterizing inorganic and biogenic fluid systems are, thus, of great relevance in a wide array of research fields in geology.

Highly useful records of (paleo-)fluid systems are provided by minerals, which may precipitate under the right conditions, this is, whenever solutes in a fluid reach a state of supersaturation. The chemistry of such minerals stands in close relation to the parent fluid. Mineral deposits are known to form across numerous settings in nature. For instance, the percolation of groundwater into caves is associated with the precipitation of calcium carbonate and the development of speleothems. At deeper crustal levels, fluid flow through fractures and faults may produce vein deposits that – depending on fracturing intensities and fluid types – range from modest mm-scale networks to impressive meters-thick ore bodies. Apart from inorganic processes, certain marine organisms are capable of biologically mediating the precipitation of calcium carbonate. Although coral reefs are perhaps the most eye-catching expression of biogenic mineralization, algae and foraminifera fix even higher quantities of calcium carbonate (Schiebel, 2002; Balch et al., 2007). Biogenic mineral deposits in the marine realm are of unmistakable significance in the Earth’s sedimentary record.
Minute fluid quantities typically get incorporated during mineral growth within microscopic imperfections in the crystal lattice (Roedder, 1984; Figure 1.1). These micrometer-sized fluid inclusions may act as time capsules capturing geochemical signatures, temperatures and pressures of ancient mineral-forming fluids as first postulated by Sorby (1858). Fluid inclusion studies find a widespread application for characterizing fluid flow patterns, diagenetic processes and thermal histories of rock formations (Goldstein and Reynolds, 1994). Microthermometry is arguably the best-established methodology in fluid inclusion studies and reveals paleo-fluid salinities and temperatures. Crush-leach analysis of bulk fluid inclusion contents is an alternative method to acquire paleo-fluid salinities. The development of new analytical techniques is ongoing, and the potential of innumerable fluid inclusion records is left to be explored.

This thesis prospects the use of hydrogen and oxygen isotope ratios (δ²H and δ¹⁸O) of fluid inclusions in vein minerals and coralline skeletons to gain insight into subsurface fluid systems and biogenic mineralization. Water is the principal component of any mineral-forming fluid in nature, and therefore, its isotope composition evokes a particular interest. Isotopes are atoms that belong to the same element but differ in the number of neutrons contained in the nucleus. For instance, naturally occurring isotopes of oxygen include ¹⁶O with 8 neutrons, ¹⁷O with 9 neutrons and ¹⁸O with 10 neutrons. Isotope values are generally expressed as delta values relative to a standard (e.g., Eq. 1.1 for oxygen).

\[
\delta^{18}O = \frac{^{18}O/^{16}O_{\text{sample}} - ^{18}O/^{16}O_{\text{standard}}}{^{18}O/^{16}O_{\text{standard}}} \times 1000 \quad \text{(Eq. 1.1)}
\]

Each isotope of a given element has a distinct mass and, thus, distinct energetic characteristics as follows from the direct relation between energy and mass (E=mv²) (Urey, 1947). For this reason, the propensity of a water molecule to be involved in a chemical reaction depends partially on its isotope composition. Natural processes that fractionate isotopes are responsible for a heterogeneous distribution of hydrogen and oxygen isotopes in water throughout the Earth’s system (Figure 1.2). One of the best-known examples is the isotope offset between seawater and meteoric terrestrial...

![Figure 1.1](image) Example of micrometer-sized fluid inclusions in a 4 mm-wide calcite vein.
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water, which originates from Rayleigh-type fractionation dynamics in the hydrological cycle (Lewis and Cornish, 1933). Isotope signatures of meteoric water are known to systematically vary according to the distance to sea, altitude, precipitation intensity and temperature (Dansgaard, 1964). Metamorphic and magmatic fluids are generally characterized by strong enrichments in $^{18}\text{O}$ and depletions in $^2\text{H}$ (e.g., Bortnikov, 2006; Pope et al., 2012). Apart from Rayleigh-type fractionation processes, multiple other alteration mechanisms are capable of shifting hydrogen and oxygen isotope signatures in basically any direction (Horita, 2005; Figure 1.2), thereby enlarging the isotope variability of water in nature even further. The entire isotope variation in natural waters is about 250‰ for $\delta^2\text{H}$ and 100‰ for $\delta^{18}\text{O}$ (Mook, 2000).

Hydrogen and oxygen isotope ratios of natural fluids are highly variable and, therefore, constitute an ideal tracer for discriminating between fluid types and tackling fluid dynamics in geological case studies (Taylor, 1974; Sheppard, 1986). Water isotope characterizations are commonly used as a powerful tool to assess the origin of fluids in modern sedimentary basins (e.g., Engle et al., 2016). Extracting similar data from ancient fluids trapped as inclusions in vein minerals may provide excellent insights into ancient basinal fluid systems. As for biogenic mineral deposits, isotope data of fluid inclusion water could possibly be exploited as a proxy for past oceanographic environments and elucidate the calcification mechanism in corals. Calcification in corals remains strongly debated and is receiving an ever-increasing interest as it holds direct implications for the resilience of corals to anthropogenic global change.

![Possible alteration pathways; see caption for processes](image)

**Figure 1.2** The variability in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water in nature, including the ranges of marine, meteoric, metamorphic and primary magmatic water. The inset (after Horita, 2005) shows some of the alteration processes that may shift hydrogen and oxygen isotope signatures of fluids. The numbers correspond to the following processes: 1. Low-temperature water-rock interaction; 2. Hydration of silicates; 3. Methanogenesis or exchange with $\text{H}_2\text{S}, \text{H}_2$ or hydrocarbons; 4. Evaporation of fresh water; 5. Evaporation of seawater; 6. Membrane filtration; 7. High-temperature water-rock interaction.
### 1.2 The history of isotope analysis of fluid inclusion water

Hydrogen and oxygen isotope analysis of sub-microliter amounts of water is no straightforward process, and the quest for finding adequate technical procedures has been ongoing for decades. The first functional techniques for bulk isotope analysis of fluid inclusion water were developed in the 70’s and 80’s (e.g., Ohmoto and Rye, 1974; Schwarcz et al., 1976; Kishima and Sakai, 1980; Coleman et al., 1982; Kazahaya and Matsuo, 1985; Ohba and Matsuo, 1988). These methodologies rely on thermal decrepitation of fluid inclusions at elevated temperatures (i.e., > 400°C). Thermal decrepitation refers to a procedure in which fluid inclusions are heated (expanded) until the internal pressure overcomes the strength of the host mineral; this breaks open the inclusions allowing fluids to escape (Scott, 1948). Water released through this procedure may be measured in a dual-inlet isotope ratio mass spectrometer for either $\delta^{18}O$ after equilibration with CO$_2$ gas, or $\delta^2H$ through off-line reduction of the water vapor most commonly using hot zinc. Thermal decrepitation techniques have been applied mostly on hydrothermal (ore) minerals to reconstruct paleo-fluid properties in basinal settings (e.g., Richardson et al., 1988; Vennemann et al., 1993; Giuliani et al., 2000; Baatartsogt et al., 2007a; Ouyang et al., 2015; Gardien et al., 2016).

Unfortunately, various analytical problems are associated with thermal decrepitation techniques. First, the high temperatures at which decrepitation is performed could entail the release of structural water that is bound at a molecular level in hydroxyl (OH-) groups. Such structural water occurs, for instance, in quartz (Knauth and Epstein, 1975; Aines et al., 1984; Ishiyama et al., 1999; Faure, 2003; Grant et al., 2003) and fluorite (Baatartsogt et al., 2007b) and is typically depleted in $^2H$ with respect to fluid inclusion water. Thermal decrepitation techniques are thought to indiscriminately release both water types and could, therefore, produce erroneously low $\delta^2H$ values (Simon, 2001). Secondly, adherence of atmospheric water to microscopic particles of CaO, which are produced during high-temperature disintegration of carbonate samples, may incur undesired fractionation effects that lead to flaws in the $\delta^{18}O$ signal (Verheyden et al., 2005). The accuracy of $\delta^{18}O$ data may also be hampered whenever fluid inclusions themselves bear significant amounts of CO$_2$ (Dennis et al., 2001). Furthermore, simultaneous acquisition of both $\delta^2H$ and $\delta^{18}O$ from a single sample is impossible, while it is precisely the combination of both isotope systems that enables an accurate fluid provenance study.

A new path for isotope analysis of fluid inclusion water was explored over the past 15 years with the development of continuous-flow crushing devices. Liberation of bulk fluid inclusion water in these set-ups is achieved through sample crushing at relatively low temperatures ($\pm 110^\circ C$), which are just high enough to vaporize the released water. The water vapor is subsequently conducted by a stable helium flow to a pyrolysis device and mass spectrometer for analysis of both $\delta^2H$ and $\delta^{18}O$. A cryogenic focusing technique is typically applied prior to entry in the mass spectrometer to produce a substantial water pulse from the sub-microliter amount of fluid inclusion...
water that is released. The relative simplicity of this on-line analytical procedure overcomes most of the problems that may throw a wrench in the works of thermal de-crepitation techniques. The first functional new-style set-up for fluid inclusion water isotope analysis was published by Vonhof et al. (2006) and was readily followed up by similar set-ups elsewhere (e.g., Dublyansky and Spötl, 2009; Arienzo et al., 2013; Affolter et al., 2014; Uemura et al., 2016; Dassié et al., 2018). Although small differences exist in i) the crushing process, ii) the transfer of water vapor to the analyzer and iii) the type of analyzer (e.g., continuous-flow isotope ratio mass spectrometry or cavity-ring down laser absorption spectroscopy), the fundamentals of these analytical procedures are alike. Besides, even simpler and more cost-effective techniques, combining off-line sample preparation with continuous flow analysis, have been developed inspired on the same basic principle (e.g., Demény and Siklósy, 2008).

To date, fluid inclusion isotope analysis following the renewed methodology has mostly been applied on speleothem deposits. Recent speleothems embody an ideal type of sample material since their fluid inclusion water is insusceptible to isotope alterations after entrapment due to the relatively stable environmental conditions in cave systems. The hydrogen and oxygen isotope record of speleothem-hosted fluid inclusions has been used extensively for making convincing paleo-climate reconstructions (e.g., Van Breukelen et al., 2008; Wainer et al., 2011; Rowe et al., 2012; Griffiths et al., 2013; Affolter et al., 2015; Labuhn et al., 2015; Meckler et al., 2015; Millo et al., 2017). Now, this obviously raises the question as to what extent the tool remains functional in other more complex and more ancient mineralization systems.

In this thesis, the functionality of the continuous-flow set-up for fluid inclusion isotope analysis as presented in Vonhof et al. (2006) is explored for vein deposits and coralline skeletons. The processes that are at stake in these two mineralization systems differ considerably. Whereas fluid mixing and water-rock interactions (WRI) are among the processes involved in controlling isotope signatures in subsurface fluid systems, physiological (metabolic) processes play a major role in biogenic calcification. In ancient vein deposits, post-entrapment isotope alterations of fluid inclusion water due to recrystallization and in-situ exchange processes present an additional complicating factor in interpreting fluid inclusion isotope data. The exploratory research presented in this thesis assesses how isotope signatures of fluid inclusion water can be used to acquire a deeper understanding of fluid dynamics from the macroscopic scale (i.e., flow in sedimentary basins) to the microbiological scale (i.e., calcification in corals).

### 1.3 Vein systems

#### 1.3.1 Veins

The main focus of this thesis lies on ancient vein systems. Veins are defined here as mineral-filled planar discontinuities that form through channelized flow of supersaturated fluids along discrete pathways (e.g., fractures, faults or bedding
planes) (Figure 1.3). Some workers also classify small-scale mineralization in the pressure shadows of single grains as vein deposits (Bons, 2000). This mode of mineral deposition occurs in the absence of brittle deformation and is not considered in this thesis; the term ‘vein’ here is uniquely used for fracture-filling mineral bodies. Innumerable mineral types may precipitate depending on the dissolved constituents of a circulating fluid. Vein precipitation is mostly driven by changes in fluid pressure or temperature, interaction with wall rocks, fluid mixing, boiling or effervescence (Zheng and Hoefs, 1993; Henderson and McCaig, 1996). Although vein precipitation from supersaturated aqueous fluids is most ubiquitous in hydrothermal settings, veins may develop basically anywhere in the lithified part of the Earth’s crust.

Vein-forming fluids are mostly supplied to fracture networks forced by hydraulic pressure gradients along migration pathways that may reach lengths of up to tens of kilometers. Pore space in the wall rock that encloses fractures constitutes an alternative source of ion-bearing fluids (Putnis et al., 1995). Mineral precipitation may be inhibited in supersaturated pore fluids due to pore-size restrictions or high internal pressures. As soon as such supersaturated pore fluids are expelled into a larger open space provided by a fracture, the sudden drop in pressure may result in the precipitation of vein infill (Putnis et al., 1995). Fractures typically fill up through a process of repeated opening and infilling following a crack-and-seal type of mech-

Figure 1.3  Calcite veins within carbonate rock in the Ionian Zone of Albania. The aspect of veins in the field may vary considerably with respect to thickness, orientation, continuity, spacing and weathering pattern. Cross-cutting veins from different generations (see B, D and E) are highly useful for tracking hydrogeological systems through time. The vein of panel A is offset by horizontal burial stylolites, indicating that it formed early after deposition.
anism (Ramsay, 1980). Exact constraints on the time scales of vein precipitation are limited, since no experiments succeeded in precisely reproducing vein formation despite innumerable attempts (e.g., Lee et al., 1996; Bons, 1997; Hilgers and Urai, 2002). According to the experimental simulation of Lee and Morse (1999), the precipitation of a single vein could take thousands to millions of years depending on fluid properties and flow rates. Meanwhile, other models predict considerably shorter time periods required for vein precipitation in the order of decades or years (Fisher and Brantley, 1992; Jones and Detwiler, 2016; Aben et al., 2017).

The orientation of veins within the hosting rock is directly determined by the orientation of the planar discontinuities in which they form. Fractures and faults develop under differential stresses that may be installed during tectonic events or under an increasing overburden during burial (Lavenu and Lamarche, 2017). The orientations of fractures and faults are closely linked to the position of the principal stresses and may, therefore, be highly systematic (Figures 1.4 and 1.5). Tensional fractures (Mode I), for instance, form perpendicular to the smallest principal stress. The pure-opening sense of these fractures allows for extensive mineral precipitation whenever fluid flow is established. On the other hand, shear fractures (Mode II) develop at an angle of 30 to 45° to the largest principal stress and are somewhat less prone to host vein infill. A good structural eye may allow for relating events of vein formation to paleo-stress fields, thereby providing time constraints for fluid flow episodes. Wherever multiple vein generations co-exist, hydrogeological systems may be tracked through geologic time.

Figure 1.4 Systematic fracture patterns in the Jandaíra carbonate platform in northeast Brazil. The aerial photo is composed of stitched and ortho-rectified images that were taken with an Unmanned Aerial Vehicle (UAV) at a flight altitude of 50 meter. A line drawing of the fracture traces along with a rose diagram displaying preferred fracture orientations is given on the right. Image from Bertotti et al. (2017).
1.3.2 Research scope

The interplay of brittle deformation, fluid flow and vein precipitation closely controls the permeability architecture of the subsurface. Whereas fracturing creates permeability in rock formations, vein precipitation may destroy this permeability again. Rock permeabilities and fluid flow patterns are essential factors of interest in appraising the occurrence of subsurface natural resources, including petroleum accumulations, ore bodies (Cathles, 1981; Pirajno, 1992; Haynes, 1993; Barnes, 1997) and groundwater reservoirs (Lattman and Parizek, 1964). Carbonate rocks constitute a particular target of studies on fracture-controlled fluid flow in the subsurface, because early cementation may eliminate their primary matrix porosity entirely (Agar and Geiger, 2015). As a result, the permeability of carbonate rock is commonly controlled exclusively by structural deformation mechanisms (Nelson, 2001).

Depending on tectonic setting, burial depth and hydraulic stresses, rocks may be exposed to strongly different stress regimes. This has direct implications for the expected density, modality and organization of fracture networks and, thus, the expected fluid flow patterns (Ingebritsen and Appold, 2012). A wide variety of water types (e.g., meteoric, marine, magmatic, metamorphic) may circulate in basinal settings, each with distinct hydrogen and oxygen isotope signatures (e.g., Taylor, 1997; Engle et al., 2016; Figure 1.2). Isotope data of vein-hosted fluid inclusion water could, therefore, present a powerful archive for reconstructing paleo-fluid migration pathways in basinal settings through geologic time.
Although continuous-flow set-ups are highly suitable for acquiring meaningful isotope data of fluid inclusions in speleothem calcites, it is still the question whether this success can be extrapolated to ancient vein deposits. In contrast to speleothems, the elevated ages and precipitation temperatures of many vein deposits open up a wide array of conceivable post-entrapment alteration processes that may obscure original fluid inclusion isotope signatures. One of the main goals of this thesis is to explore the effects of these processes in an attempt to determine the true potential of fluid inclusion isotopes as a record for original vein-forming fluids.

1.3.3 Post-entrapment fluid inclusion alteration

1.3.3.1 Alteration processes

The diagenetic stability of fluid inclusion water through geologic time is an essential factor in assessing the functionality of the fluid inclusion isotope record of ancient and high-temperature vein minerals for paleo-fluid reconstructions. Recrystallization in the presence of an external secondary fluid is a detrimental process in particular as it may overprint primary fluid inclusion isotope signals. External fluids may also infiltrate along cleavage planes or micro-cracks, which can form under tectonic or thermal strain, and thereby introduce isotopically distinct fluids into minerals after deposition (Goldstein, 1986). Not all minerals may be equally suitable for acquiring isotope signatures of original mineral-forming fluids. Depending on mineral hardness and cleavage planes, certain minerals may be more or less capable of preserving primary fluid inclusion contents and withstanding high internal pressures and strain.

Apart from fluid remobilization, there are various in-situ processes that can drive isotope alterations of primary fluid inclusion water. For instance, in oxygen-bearing minerals like calcite and quartz, isotope exchange between fluid inclusion water and its host mineral is a potential mechanism for provoking post-entrapment alterations in δ¹⁸O (Criss et al., 1987). Since the mineral phase is far dominant over the fluid inclusion water, such exchange could have a profound effect on the δ¹⁸O ratios of the latter (e.g., Schwarcz et al., 1976; Matsuhsa et al., 1978). The rates of oxygen isotope exchange have never properly been quantified due to its dependence on a variety of factors, including the time and temperature of entrapment, mineral type and fluid inclusion shapes (Naden et al., 2003). As opposed to the oxygen isotope system, hydrogen isotopes are generally assumed to be insusceptible to post-entrapment alterations even in ancient mineral deposits (e.g., Horita and Matsuo, 1986; Ohba and Matsuo, 1988). Hydrogen isotope alteration could theoretically be driven by the diffusion of hydrogen through dislocations in crystals (Mavrogenes and Bodnar, 1994; Ingrin, 2006). However, this is a low-rate process that is only feasible at high temperatures (> 300°C) in the metamorphic domain (Simon, 2001; Bakker, 2009). Furthermore, hydrogen diffusion only applies to H₂ species and not hydrogen bound in H₂O and is, therefore, unlikely to have a considerable effect on the isotope signatures of fluid inclusion water (Baatartsogt et al., 2007a).
1.3.3.2 Fluid inclusion petrography

A petrographic analysis is an essential requirement in any fluid inclusion study for determining the degree of preservation of primary fluid inclusions. Although being small (i.e., mostly < 50 μm), fluid inclusions readily show up in thin sections due to their distinct refractive index with respect to the host mineral. Fluid inclusions are commonly grouped in three different categories: primary, secondary and pseudo-secondary (Roedder, 1984; Bodnar, 2003). The primary ones are entrapped when irregularities on crystals’ surfaces are overgrown during mineral precipitation. As a result, they are typically organized along zones or bands parallel to the crystal growth faces (Figure 1.6). Since the entrapment of primary fluid inclusions occurs during crystal growth, their water contents represent a remnant of the original mineral-forming fluid.

Secondary fluid inclusions develop along fluid-filled micro-fractures that are generated under deformational or thermal stresses. The incorporation of secondary fluids within minerals occurs when such micro-fractures get sealed due to microscopic-scale precipitation – a process commonly referred to as necking down (Goldstein, 1986). Secondary fluid inclusions are formed after mineral precipitation and are, therefore, usually aligned along trails that are unrelated to the crystal growth planes (Figure 1.7). Although secondary fluid inclusions may be used for studying diagenetic processes, their presence is disadvantageous for reconstructing the chemistry of original vein-forming fluids. Pseudo-secondary fluid inclusions also develop along micro-fractures, only not after but during crystal growth. Their petrographic appearance is highly similar to secondary fluid inclusions, apart from the fact that pseudo-secondary fluid inclusion trails are typically confined within single crystals. Since pseudo-secondary fluid inclusions develop during crystal growth, their contents are representative of original mineral-forming fluids.

Figure 1.6 Primary fluid inclusions are oriented parallel to crystal growth faces and contain the initial crystallization waters. Fluid inclusions appear as minuscule black dots in these two exemplary photomicrographs, which were taken of veins from outcrops near the villages of Bodrishtë (left) and Borsh (right) in Albania.
An adequate assessment of fluid inclusion organizations is critical for knowing if primary or secondary fluids – or a mix of both – are being measured and, thus, for making correct interpretations. Nonetheless, there is always a degree of uncertainty in bulk fluid inclusion studies since a petrographic analysis of the entire fluid inclusion content of a sample is practically impossible. Migration of fluid inclusions, which may occur at high-temperatures in the metamorphic domain, could further complicate the identification of fluid inclusion types. At upper-crustal temperatures and pressures, however, migration distances are generally thought to be too low to cause a significant reorganization of fluid inclusions (Roedder and Skinner, 1968; Goldstein and Reynolds, 1994).

1.3.4 Case studies

In this thesis, the application of fluid inclusion isotope analysis on vein systems is assessed in three distinct case studies, each performed in a different geological setting. Field studies on fracture-controlled fluid flow are essential since the scale of fracture networks along which fluid flow is accommodated in nature is too large to be realistically simulated in laboratory experiments (Berkowitz, 2002). The case studies are presented in an order of increasing complexity of the geohydrological system. The case study with the least complex fluid flow system is discussed in Chapter 2 and focuses on calcite veins hosted in Late Cretaceous platform carbonates in the onshore part of the Potiguar Basin in northeast Brazil. These veins developed in a relatively quiescent tectonic regime dominated by burial stresses in a passive margin setting.

Chapter 3 addresses fluid inclusion isotope signatures of calcite veins from a naturally fractured carbonate rock formation in the strongly deformed Albanide fold-and-thrust belt. Fold-and-thrust belts are highly complex systems, in which

![Figure 1.7](image)

**Figure 1.7** Photomicrograph of fluid inclusion arrangements in a calcite vein crystal from sample JD2 of the case study on the Jandaíra Formation (Chapter 2). Secondary fluid inclusions (B) are aligned on a trail that is unrelated to the crystal growth faces. Such trails develop along micro-fractures, which accommodate infiltration of secondary fluids after mineral precipitation. In this particular sample, primary fluid inclusions (A) outnumber the secondary ones.
rocks are subjected to an interplay of burial and tectonic stresses that vary laterally from the foreland basin to the orogenic wedge. The studied carbonate rock formation experienced multiple events of fracturing-controlled fluid flow and vein cementation. The various generations of calcite vein infill allow for tracking fluid flow pathways in the fold-and-thrust system through time.

As opposed to the low-temperature settings of the first two case studies (i.e., < 70°C), Chapter 4 sets forth the application of fluid inclusion isotope analysis on high-temperature hydrothermal vein deposits from the Harz Mountains in Germany. Brine-like fluids and a variety of mineral assemblages characterize Mesozoic vein deposition in the Harz Mountains. Vein minerals analyzed for fluid inclusion isotope ratios include calcite, quartz, fluorite, barite and the metal sulfides galena, sphalerite and chalcopyrite. The high-temperatures during vein precipitation in the Harz Mountains (100-200°C) may have promoted post-entrapment alteration processes. The three vein-based case studies discussed in this thesis allow for assessing the extent to which fluid inclusion isotope data of veins remain meaningful as the complexity of the geological setting increases.

1.4 Fluid inclusion water in coral skeletons

Corals are capable of taking up Ca\(^{2+}\) and dissolved inorganic carbon (DIC) from seawater and fixing it as calcium carbonate in an aragonite skeleton. Geochemical records from coralline skeletons are commonly exploited as proxies for past environmental conditions (e.g., Goreau, 1959; Todd, 2008; Krief et al., 2010; D’Olivo and McCulloch, 2017). Environmental reconstructions work particularly well for tropical shallow-water corals. As for cold-water corals, on the other hand, geochemical proxies are mostly dysfunctional for paleo-oceanographic research. This dysfunctionality is partly related to the limited variation in environmental parameters in the deep-sea, which leads to a stronger expression of physiological processes in the skeletal chemistry (Saenger and Erez, 2016). These so-called “vital” processes pose a special challenge to geochemical studies on biogenic carbonates. The complex biological control on skeletogenesis in corals is still not fully understood, and discussion persists on their precise calcification mechanism.

One of the main unresolved issues is the character of the calcification fluid and its degree of connection with ambient seawater. Traditional ideas predicate calcification from an extra-cellular fluid layer at the tissue-skeleton interface (Tambutté et al., 2007a; Allemand et al., 2011). Recent ideas, however, are more inclined towards a process involving the continuous attachment of amorphous calcium carbonate precursor particles to the coral skeleton (Mass et al., 2017; Von Euw et al., 2017), which has already been reported for numerous other marine calcifiers (Weiss et al., 2002; Addadi et al., 2003; Politi et al., 2004). The question as to how skeletal aragonite precipitates finds its relevance in predicting the response of corals to anthropogenic environmental changes (e.g., ocean warming, acidification, pollution). In models where a direct connection with ambient seawater exists, the effect of
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Environmental change may be more dramatic compared to models with calcification in isolated spaces within the coral organism, where the coral has better control on the chemistry of the calcification fluid (Mass et al., 2017).

Gaffey (1988) established that coral skeletons incorporate minor amounts of unbound fluid inclusion water just like non-biogenic minerals. Isotope characterization of this fluid inclusion water, which may directly represent the calcification fluid, could help shed light on the mechanism of coral biomineralization. Isotope analysis of fluid inclusion water in biogenic minerals is an almost completely unexploited terrain. Lécuyer and O’Neil (1994) did present the first – and to date only – preliminary isotope study on water included in biogenic carbonates. Their data, however, had significant analytical uncertainties since the water releases were established through thermal decrepitation at high temperatures (> 300°C). At these temperatures, non-fluid inclusion water may get liberated through the decomposition of hydrated organic compounds, which are commonly embedded in biogenic skeletons. When releasing fluid inclusion water through sample crushing at lower temperatures following the methodology of Vonhof et al. (2006), water contained in organics remains fixed (Cuif et al., 2004), and acquired isotope data would be representative of the fluid inclusion water only.

Chapter 5 discusses the implications of fluid inclusion isotope data of a set of cold-water coral specimens, which were retrieved from actively accreting reefs in the northeast Atlantic and from a soft-sediment core that was taken in the Santaren Channel west of the Great Bahama Bank (Figure 1.8). Although Lécuyer and O’Neil (1994) suggested that considerable amounts of metabolic water are possibly captured as fluid inclusions in biogenic carbonates, prevailing ideas on the calcification process in corals are mostly based on the assumption that the calcification fluid is seawater-like. Fluid inclusion isotope data retrieved from coral skeletons might put this assumption to the test and could provide exciting new insights into biogenic calcification in corals.

Figure 1.8  Skeletal fragments of the scleractinian coral species *L. pertusa*, which were manually picked from a soft sediment core from the Santaren Channel. *L. pertusa* has a branch-like structure and lives in reef-forming colonies in the deep-sea. The background is millimeter paper; sample numbers from left to right: 120 cm, 95 cm, 141 cm, 125 cm and 160 cm (see Chapter 5).
1.5 Fluid inclusion isotope analysis

1.5.1 Analytical procedure

All isotope analyses presented in this thesis were performed in the stable isotope laboratory of the Department of Earth Sciences at the VU University Amsterdam. Isotope data of mineral-hosted fluid inclusions were acquired following the methodology developed by Vonhof et al. (2006), which allows for on-line analysis of both $\delta^2$H and $\delta^{18}$O of bulk fluid inclusion contents in mineral samples of about 0.2 to 2 grams (Figure 1.9). The first step in the analytical procedure consists of sample crushing in the Amsterdam device, which is a crusher unit connected to a continuous-flow pyrolysis furnace (ThermoFinnigan TC-EA). The sub-microliter amount of water that is liberated due to the opening of inclusions is vaporized at 110°C and transported to a TC-EA reactor tube, which transforms the water vapor into $\text{H}_2$ and CO gas as a result of reaction with glassy carbon at 1400°C. A cryogenic focusing technique is applied prior to entry into the reactor tube to generate a water pulse short enough to be analyzed. The $\text{H}_2$ gas and CO gas are separately measured in a continuous-flow isotope-ratio mass spectrometer (ThermoFinnigan Delta XP). A rapid magnet peak jump between the entries of $\text{H}_2$ gas and CO gas allows for the analysis of both hydrogen and oxygen isotopes from a single water release. Figure 1.10 presents an exemplary measurement. Isotope values are reported as $\delta^2$H$_w$ and $\delta^{18}$O$_w$ ratios relative to Vienna Standard Mean Ocean Water (VSMOW).

Prior to analysis of each mineral sample, a number of standard water measurements is performed to stabilize the mass spectrometer. Standard water is chosen that is expected to be isotopically similar to the fluid inclusion water of the mineral sample in order to minimize analytical uncertainties related to memory effects. Standard water can be injected with a micro-syringe directly into the crusher unit of the Amsterdam Device (Figure 1.9C). The loaded mineral sample is crushed and measured as soon as the mass spectrometer is stabilized. Direct calibration of isotope values of mineral samples is accomplished by comparing their recorded isotope values with bracketing standard water measurements. The volume of the standard water injections used during the stabilization series is tentatively kept equal to the water yield of the sample to rule out sample-size effects. Especially $\delta^2$H$_w$ values tend to change considerably with decreasing water yields. An example of an entire measurement sequence for a mineral sample is given in Table 3.1.

1.5.2 Determination of true isotope ratios

Two additional corrections are included in the calculation of true fluid inclusion isotope values of mineral samples. First, a correction factor for the linearity of the mass spectrometer is implemented, which accounts for a discrepancy between the isotope difference between two waters as measured by the machine compared to their actual isotope difference. Secondly, the instrument is affected by a memory effect that results in attenuated differences in isotope values between subsequent
Figure 1.9  (A) Schematic representation of the capillary network of the crusher preparation device for on-line analysis of fluid inclusion isotope ratios. The ports A, B, C and D may be opened or closed in order to manipulate the path of the helium carrier flow. During analysis of water samples, the blue pathway is used (port A and C open) so that the helium carrier is guided via the crusher unit and the cryogenic trap towards the TC/EA analyzer. The helium carrier flows through a bypass (black path; only port D opened) whenever the crusher is opened, for example when loading a sample. (B) The inner structure of the crusher unit. Mineral samples are loaded in a reservoir in the center. After sample loading, the crusher hat is placed on top to seal the unit. (C) For analysis, the helium carrier flow is guided through the crusher unit. Standard water can be injected through the septum port to stabilize the mass spectrometer. Once stabilized, sample crushing can be initiated by repeatedly moving the piston up and down. The released water is vaporized and guided to the inlet of the TC/EA for hydrogen and oxygen isotope analysis.
Figure 1.10  Exemplary isotope measurement of a water sample. After the water is vaporized and separated in H$_2$ and CO gas, the H$_2$ gas enters the mass spectrometer first. Values for $\delta^{2}$H are obtained through calibration against a H$_2$ reference gas, which is measured two times right before the sample H$_2$ gas enters the mass spectrometer. A magnet peak jump is applied as soon as the hydrogen sample peak terminates to set up the mass spectrometer for analysis of the heavier CO gas. The sample measurement for $\delta^{18}$O is calibrated against a double measurement of a CO reference gas.

Table 3.1  Example of an entire measurement series for the fluid inclusion isotope characterization of a single calcite vein sample. After three standard water measurements (DNS3), the mass spectrometer was identified to be stable, and the mineral sample was crushed and measured. After sample measurement, two more standard water measurements were performed at a similar amount as the water release from the mineral sample (in this case, an area H$_2$ of approximately 200 Vs). The measured isotope values of the mineral sample are compared to these standard water measurements to calculate the true fluid inclusion isotope ratios of the mineral sample. Area H$_2$ and Area CO are measures for signal intensities expressed in Vs.
measurements. Memory effects are of minor importance in speleothem studies, where fluid inclusion isotope values of samples can quite accurately be predicted. As for basinal fluids, however, hydrogen and oxygen isotope variations can be much larger, and a correction for the memory effect is required for accurate calculation of true isotope values of mineral samples.

Values for these two correction factors are based on a measurement series of two distinct standard waters of known isotope composition. For a calibration, one of the standard waters is measured until stable values are obtained. Subsequently, the other standard water is measured until stable values are attained. Table 3.2 presents an example of a calibration series, in which the standard waters DNS3 (−9.5‰ for $\delta^2\text{H}_w$ and −1.43‰ for $\delta^{18}\text{O}_w$) and KEILA2 (−158.4‰ for $\delta^2\text{H}_w$ and −21.2‰ for $\delta^{18}\text{O}_w$) were used. The linearity can be determined for $\delta^2\text{H}_w$ and $\delta^{18}\text{O}_w$ by comparing the difference between the stable recorded values of the two standard waters with their actual isotope difference (Eq. 1.2). The memory effect is expressed as the fraction of the difference between two subsequent measurements that is falling short (Eq. 1.3). The terms STD1 and STD2 in Eq. 1.2 and 1.3 refer to the isotope values of the two isotopically distinct standard waters used for calibration. Since the linearity and memory effect of the machine are subject to minor drift through time, it is required to regularly perform calibration series. Table 3.3 presents an overview of recorded correction factors through time.

\[
\text{Linearity factor (l)} = \frac{(\text{STD}_1 - \text{STD}_2)_{\text{true}}}{(\text{STD}_1 - \text{STD}_2)_{\text{measured}}} \quad \text{(Eq. 1.2)}
\]

\[
\text{Memory effect (m)} = 1 - \frac{(\text{STD}_{2\text{first}} - \text{STD}_{1\text{stable}})}{(\text{STD}_{2\text{stable}} - \text{STD}_{1\text{stable}})} \quad \text{(Eq. 1.3)}
\]

Eq. 1.4 and 1.5 are used to calculate respectively $\delta^2\text{H}_w$ and $\delta^{18}\text{O}_w$ isotope ratios of mineral samples. The subscript ‘A’ refers to the standard water measurement right before sample measurement and the subscript ‘B’ refers to a stable standard water measurement after sample measurement (see also Table 3.1). The subscript ‘STD true’ refers to the true isotope ratio of the standard water. The memory effect and linearity factor are expressed as $m$ and $l$, respectively.

\[
\delta^2\text{H}_w = (\delta^2\text{H}_a + \frac{(\delta^2\text{H}_{\text{sample}} - \delta^2\text{H}_a)}{(1 - m)} - \delta^2\text{H}_b) \times l + \delta^2\text{H}_{\text{STD true}} \quad \text{(Eq. 1.4)}
\]

\[
\delta^{18}\text{O}_w = (\delta^{18}\text{O}_a + \frac{(\delta^{18}\text{O}_{\text{sample}} - \delta^{18}\text{O}_a)}{(1 - m)} - \delta^{18}\text{O}_b) \times l + \delta^{18}\text{O}_{\text{STD true}} \quad \text{(Eq. 1.5)}
\]
Table 3.2  Example of a calibration series. This particular calibration was performed the 28th of July 2017 in the context of the project on fluid flow in the Albanide fold-and-thrust belt (Chapter 3). Area H2 and Area CO are measures for signal intensities expressed in Vs.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area H2</th>
<th>δ²H</th>
<th>Area CO</th>
<th>δ¹⁸O</th>
<th>Terms in Eq. 1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 µl KEILA2</td>
<td>159.953</td>
<td>-163.818</td>
<td>330.650</td>
<td>-41.516</td>
<td></td>
</tr>
<tr>
<td>0.3 µl KEILA2</td>
<td>148.087</td>
<td>-166.165</td>
<td>299.995</td>
<td>-41.771</td>
<td>stable</td>
</tr>
<tr>
<td>0.3 µl KEILA2</td>
<td>153.255</td>
<td>-166.858</td>
<td>309.296</td>
<td>-41.739</td>
<td></td>
</tr>
<tr>
<td>0.3 µl KEILA2</td>
<td>153.560</td>
<td>-166.272</td>
<td>310.035</td>
<td>-41.830</td>
<td></td>
</tr>
<tr>
<td>0.3 µl DNS3</td>
<td>152.603</td>
<td>-63.196</td>
<td>307.610</td>
<td>-24.406</td>
<td>first</td>
</tr>
<tr>
<td>0.3 µl DNS3</td>
<td>154.770</td>
<td>-43.756</td>
<td>312.029</td>
<td>-23.627</td>
<td></td>
</tr>
<tr>
<td>0.3 µl DNS3</td>
<td>153.081</td>
<td>-38.910</td>
<td>308.388</td>
<td>-23.494</td>
<td></td>
</tr>
<tr>
<td>0.3 µl DNS3</td>
<td>152.782</td>
<td>-35.993</td>
<td>307.521</td>
<td>-23.331</td>
<td>stable</td>
</tr>
<tr>
<td>0.3 µl DNS3</td>
<td>154.938</td>
<td>-34.614</td>
<td>313.093</td>
<td>-23.364</td>
<td>stable</td>
</tr>
</tbody>
</table>

Table 3.3  Overview of the drift of the linearity and the memory effect of the analytical setup over a period of four years. The linearity effect reflects a discrepancy between measured and actual isotope differences between samples. The memory effect accounts for an interference of previous measurements. Memory effects are especially important for the hydrogen isotope system.

<table>
<thead>
<tr>
<th>Date</th>
<th>Linearity</th>
<th>Memory effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ²H</td>
<td>δ¹⁸O</td>
</tr>
<tr>
<td>Mid 2013</td>
<td>1.504</td>
<td>1.095</td>
</tr>
<tr>
<td>11-07-2014</td>
<td>1.168</td>
<td>1.164</td>
</tr>
<tr>
<td>15-03-2015</td>
<td>1.159</td>
<td>1.086</td>
</tr>
<tr>
<td>22-09-2015</td>
<td>1.103</td>
<td>1.075</td>
</tr>
<tr>
<td>20-10-2015</td>
<td>1.139</td>
<td>1.079</td>
</tr>
<tr>
<td>02-12-2016</td>
<td>1.068</td>
<td>1.002</td>
</tr>
<tr>
<td>11-07-2017</td>
<td>1.118</td>
<td>1.055</td>
</tr>
<tr>
<td>28-07-2017</td>
<td>1.131</td>
<td>1.071</td>
</tr>
<tr>
<td>Average</td>
<td>1.127</td>
<td>1.078</td>
</tr>
</tbody>
</table>
1.5.3 Performance

Routinely measured water standards following the analytical protocol are reproducible (1σ) within 0.23‰ for δ¹⁸Oₜ and 1.30‰ for δ²Hₜ. Successful fluid inclusion isotope analysis of a mineral sample depends largely on the amount of fluid inclusion water that is released during crushing. Small water releases typically lead to unstable isotope values and unreliable data. For this reason, a cut-off value for Area H₂ of 60 Vs was employed. Although the water amount to intensity ratio may be slightly variable through time, this generally means that samples with water yields falling below approximately 0.1 µl were discarded. It is noteworthy that water yields are not representative for absolute fluid inclusion contents since sample crushing is mostly incomplete, leaving part of the fluid inclusion water behind in the crushed residue. This also means that smaller fluid inclusion fractions may possibly be overlooked. In general, fluid inclusion isotope data of speleothem calcites measured on the Amsterdam Device are highly consistent with expected meteoric signatures (Figure 1.11). Although highly saline brine-like fluids should not pose any problem for a successful analysis since dissolved salts would precipitate and stay behind in the crushing chamber, the suitability of the technique for suchlike fluids has not previously been investigated.

Figure 1.11 A selection of stalagmite fluid inclusion isotope data obtained with the Amsterdam Device. The data are positioned along the Global Meteoric Water Line (GMWL) and are in good agreement with present day cave drip water isotope values (grey dots). This confirms that the technique captures accurate isotope ratios over a large range. All samples shown are of Holocene age. Unpublished data from Rodrigues Island yield highest fluid inclusion isotope values. Data from the Cueva del Tigre Perdido cave (Van Breukelen et al., 2008) span the central area of the plot. Deviations towards the right of the GMWL as evident in the cluster of lowest stable isotope values are believed to be caused by evaporation of infiltration water at the cave site. This data cluster is from Huagapo Cave in the high Andes of Peru (Van Breukelen, 2009).
1.6 Stable isotope analysis of carbonate

1.6.1 Analytical procedure

The case studies presented in this thesis follow integrated approaches that combine implications from fluid inclusion isotope data with other data sources. For example, fluid inclusion water analysis of calcite and aragonite samples is easily followed up by carbon and oxygen isotope analysis of the carbonate. Crushed calcite and aragonite samples may be retrieved after fluid inclusion isotope analysis and subsequently analyzed for $\delta^{13}$C and $\delta^{18}$O isotope ratios on a Thermo Finnigan Delta+ mass spectrometer equipped with a GASBENCH II preparation device. For analysis, around 10 µg of sample material is put in a He-filled 12 ml extainer vial and subsequently digested in concentrated anhydrous phosphoric acid (H$_3$PO$_4$) at a temperature of 45˚C. The generated CO$_2$-He gas mixture is then transported to the GASBENCH II in a He carrier flow. In the GASBENCH II, water is extracted from the gas through nafion tubing, and CO$_2$ is analyzed in the mass spectrometer after separation of residual gases in a GC column. Isotope values are reported as $\delta^{13}$C and $\delta^{18}$O ratios relative to VPDB. The reproducibility (1σ) of routinely analyzed calcite standards is better than 0.1‰ for $\delta^{13}$C and 0.15‰ for $\delta^{18}$O.

1.6.2 Why isotope characterization of carbonate?

Carbon and oxygen isotope analysis of carbonate samples is a relatively low-cost and rapid method of strengthening the geochemical framework in isotope studies. Carbon and oxygen isotopes of vein calcites may give insight into the sources of dissolved inorganic carbon (DIC) species and water-rock interaction processes. This record may, thus, provide additional information on the characteristics of paleo-fluid flow and the sources and pathways of migrating fluids. Furthermore, the amount of material needed for the isotope characterization of carbonate material is much lower, which allows for identifying small-scale heterogeneities within single veins due to, for instance, partial recrystallization or the presence of multiple generations of infill. Co-variations between $\delta^{13}$C and $\delta^{18}$O in vein systems may also provide an indication whether vein cements are primary or recrystallized, and whether disequilibrium (kinetic) isotope fractionation has occurred. In biogenic minerals, carbon and oxygen isotope signatures of skeletal carbonate may relate to environmental and/or physiological signals.

In carbonate-bearing fluids, oxygen isotope values of the DIC species (i.e., CO$_2$, HCO$_3$ and CO$_3^{2-}$) gradually equilibrate with ambient water following hydration and hydroxylation reactions (e.g., Sade and Halevy, 2017). In isotope equilibrium, the heavy oxygen isotope $^{18}$O is enriched in DIC with respect to water. The combination of oxygen isotope signatures of both fluid inclusions ($\delta^{18}$O$_w$) and the carbonate of
the host mineral ($\delta^{18}O_c$) can be used to calculate fractionation factors ($\alpha$) following Eq. 1.6.

$$\alpha = \frac{1000 + \delta^{18}O_c}{1000 + \delta^{18}O_w} \quad \text{(Eq. 1.6)}$$

For isotopically equilibrated system, there is a general consensus that $\alpha$ is directly related to temperature, as first demonstrated by McCrea (1950). Since few reliable techniques exist to directly measure oxygen isotope ratios of fluid inclusion water, many studies resort to $\delta^{18}O_w$ predictions of paleo-fluids based on $\delta^{18}O_c$ values and known temperatures and assuming oxygen isotope equilibrium (e.g., Robinson, 1975; Tillman and Barnes, 1983; Zheng and Hoefs, 1993; Suchy et al., 2000; Slobodnik et al., 2006; Morad et al., 2010; Wilkinson, 2010). Fluid inclusion isotope analysis can provide direct information on $\delta^{18}O_w$ values of mineralizing paleo-fluids, giving the unique opportunity to test this generally-made assumption of isotope equilibrium. This is especially relevant considering the increasing awareness that oxygen isotope disequilibrium is common both in abiogenic (Watkins et al., 2014) and certain biogenic mineral systems (e.g., Wefer and Berger, 1991; Spero et al., 1997). In fluid systems where oxygen isotope disequilibrium is observed, the kinetic effects underlying isotope disequilibrium may be hypothesized, which could be related to fluid chemistry or precipitation rates for example. However, when fluids are isotopically equilibrated, $\delta^{18}O_w/\delta^{18}O_c$ pairs may potentially be used as a paleothermometer for mineral formation (Kim and O’Neil, 1997).