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**Oxygen isotope analysis of the eyes of pelagic trilobites: testing the application of sea temperature
proxies for the Ordovician**

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Research Highlights

1. Preservation assessment shows some well-preserved Ordovician trilobite eyes
2. Ordovician trilobite eyes yield $\delta^{18}\text{O}$ values similar to Ordovician brachiopods
3. SIMS and clumped isotope results indicate diagenetic alteration of trilobites
4. Classic protocols to assess preservation may be inapt for most ancient carbonates

Abstract

The oxygen isotope composition of well-preserved trilobite eye calcite, retaining its original optical properties, represents a possible source of information on Paleozoic sea temperatures. Species of the epipelagic telephinid genera *Carolinites* and *Opipeuterella* from strata of Early to Middle Ordovician age in Spitsbergen and Australia were analyzed, and compared with benthic asaphid species. Scanning electron microscope (SEM), cathodoluminescence (CL), electron microprobe and Electron Backscatter Diffraction (EBSD) techniques were used to assess eye preservation prior to isotope analysis. Some apparently well-preserved eyes are identified from the Valhallfonna (Spitsbergen) and Emanuel (Australia) formations. The eyes show a wide variation in $\delta^{18}\text{O}$ values: -6.2‰ to -9.8‰ for the Valhallfonna Formation, -3.2‰ to -10.4‰ for the Emanuel Formation, and -3.6‰ to -7.4‰ for the Horn Valley Siltstone (Australia). Intra-eye Secondary Ion Mass Spectrometry (SIMS) isotope results reveal an even larger range in $\delta^{18}\text{O}$ in some specimens ($\delta^{18}\text{O}$ of -2.4‰ to -10.4‰), suggesting that the trilobite eyes have undergone cryptic recrystallization. A sub-set of trilobite cuticle from the three formations were analyzed for their carbonate clumped isotope compositions (Δ_{47}), and yielded crystallisation temperatures above 50°C , consistent with diagenetic alteration. The SIMS and Δ_{47} results suggest that classic preservation assessment protocols for the stable isotope study of deep-time carbonate samples may be insufficient, especially for these techniques. There is a need for extensive microstructural characterisation of lower Paleozoic biogenic carbonates, by techniques including EBSD, SIMS and Δ_{47} , before their stable isotope signatures can be used with certainty in paleoclimate studies.

Index terms: Stable isotope geochemistry; marine geochemistry; instruments and techniques; biomineralization; petrography, microstructures, and textures

Keywords: Trilobite, oxygen isotopes, Ordovician, paleotemperature, microstructure

1. Introduction

Telephinid trilobites are amongst the few unequivocally pelagic organisms preserved in Ordovician rocks that have a carbonate biomineralized skeleton. The most common telephinid, *Carolinites genacinaca*, had a global paleoequatorial distribution during the Early Ordovician (Floian) and the morphology of the *Carolinites* body plan, eye shape and eye position suggest that species were epipelagic, living near the sea surface in the mixed layer (McCormick and Fortey, 1998, 1999). The holochroal eyes of telephinid trilobites are composed of hundreds of interlocking lenses of calcite and are sufficiently large to provide sufficient material for stable isotope analysis, which in turn might conceivably yield an estimate of Ordovician sea temperature.

Trilobite cuticle has been used in Ordovician isotope paleoclimate studies in the same way as brachiopod calcite (Brand, 2004; Finnegan et al., 2011). Studies on the ultrastructure of the biomineralized trilobite exoskeleton (the cuticle) show that original features such as horizontal lamination, relict organic material, and pore canals can be preserved (Dalingwater, 1973; Dalingwater et al., 1991), although evidence for diagenetic alteration is also common (Wilmot, 1990; Budil and Hörbinger, 2007). Investigations into the original chemical and isotope compositions of trilobite cuticles indicate that they were formed from low-Mg calcite (Wilmot and Fallick, 1989; Lee et al., 2012; McRoberts et al., 2013; Teigler and Towe, 1975). Some specimens have been recorded with an intermediate-Mg calcite composition (Brand, 2004; McAlister and Brand, 1989). However, as the preservation of the cuticle ultrastructure in these latter specimens was not examined in detail, the possibility remains that the chemical composition of these cuticles may reflect diagenetic recrystallization in Mg-rich fluid. The lenses in the schizochroal eyes of phacopine trilobites have

1 been identified as being originally composed of high-Mg calcite of ~7.5 mol% MgCO₃, while the cuticle
2 comprises low-Mg calcite of ~1.4 to 2.4 mol% MgCO₃ (Lee et al., 2012).

3 As the physical properties of a functional calcite lens are well understood, even if trilobite eyes do not have
4 modern analogues, it is possible to assess their degree of preservation (Torney et al., 2014). Previous work
5 on the ultrastructure of schizochroal trilobite eyes using EBSD has revealed that the original structure of the
6 eye lenses can be identified (Lee et al., 2007, 2012; Torney et al., 2014). This finding suggests the
7 possibility that the original chemical and isotope composition of eye calcite may also be retained. High-
8 resolution SEM analysis of the lenses of phacopine trilobite eyes show that the intralensar bowl (present
9 only in schizochroal eyes) was composed of high-Mg calcite with micro-crystals of dolomite indicating
10 diagenetic alteration (Lee et al., 2007; 2012). SIMS analysis can be used to target small (less than 20 µm
11 diameter) areas of a fossil *in situ*, such as trilobite eye lenses, and SIMS analysis of δ¹⁸O has been
12 successfully applied to the analysis of conodont microfossils (Wheeley et al., 2012).

13 Biogenic proxies that have been hitherto used to provide Early and Middle Ordovician marine
14 paleotemperatures are the oxygen isotopic compositions of brachiopod calcite and conodont apatite (Shields
15 et al., 2003; Trotter et al., 2008; Wadleigh and Veizer, 1992), along with carbonate clumped isotope
16 compositions (Δ₄₇) from trilobite, brachiopod and coral calcite for the Late Ordovician (Finnegan et al.,
17 2011). However, some of these results are controversial. For instance, brachiopod isotope data from the
18 Lower Ordovician can have very low δ¹⁸O (down to -10‰). This result implies very high (up to + 60°C)
19 seawater temperatures [if it is assumed that seawater δ¹⁸O was similar to today], or diagenetic alteration at
20 higher temperatures than that of the ocean, diagenetic alteration by a different fluid, or that seawater δ¹⁸O
21 was substantially different from present (Veizer et al., 1999; Shields et al., 2003), or a combination of those
22 alternatives. In contrast, δ¹⁸O from conodont apatite from the Ordovician suggests that seawater
23 temperatures were between 30-40°C if a value similar to modern δ¹⁸O_{water} of -1‰VSMOW is used (Trotter et
24 al., 2008). Oxygen isotope data can also reflect other environmental parameters; for example, Bickert et al.

(1997) showed that the $\delta^{18}\text{O}$ values of well-preserved Silurian brachiopods reflect salinity differences due to varying fresh-water input rather than temperatures. Nevertheless, the development of proxies that can provide robust and reasonable estimates of seawater temperature for the early Paleozoic is important for many reasons. For example, such information can help constrain the environmental feedbacks or triggers of the Great Ordovician Biodiversification Event (Trotter et al., 2008; Amberg et al., 2016), and it also represents one of the few available proxies for ground-truthing General Circulation Models of early Paleozoic climate (Vandenbroucke et al., 2009; Pohl et al., 2014; 2016). Changes in seawater temperatures may have had significant effects on the ability of organisms to biomineralize (Pruss et al., 2010). The Δ_{47} paleothermometer has great potential for more accurate paleotemperature reconstruction because it is independent of the $\delta^{18}\text{O}$ of mineralising fluids (Ghosh et al., 2006). Usefully, the recrystallization temperature of diagenetic calcite recorded by Δ_{47} is thought to remain stable over hundreds of millions of years, at temperature histories below $\sim 250^\circ\text{C}$ (Ghosh et al., 2006). However, in deeply buried sedimentary deposits or carbonates having a moderate-temperature burial history ($\sim 100^\circ\text{C}$ on timescales of hundreds of million years) the closed-system solid-state diffusive reordering of atoms can modify Δ_{47} towards lower values and higher apparent equilibrium temperatures (Passey and Henkes, 2012; Henkes et al., 2014; Stolper and Eiler, 2015).

Here we test whether: (i) the unique functional morphology and microstructure of trilobite eyes allows for the accurate assessment of the preservation of their calcite lenses; and (ii) the subsequent $\delta^{18}\text{O}$ analyses of well-preserved eyes of epipelagic trilobites could serve as a sea temperature proxy. Trilobite eye microstructure and composition were compared to those of the trilobite cuticle. Two isotope methods were used on trilobite material from three sedimentary formations considered to have experienced relatively limited diagenesis: (i) conventional determination of oxygen isotope compositions, and (ii) carbonate clumped isotope Δ_{47} analysis.

2. Materials Utilised

Trilobite eyes were analyzed from three broadly coeval Ordovician formations from separate, equatorial basins: 1) Valhallfonna Formation, Spitsbergen; 2) Emanuel Formation, Canning Basin, Australia; and 3) Horn Valley Siltstone, Amadeus Basin, Australia (Figure 1). For all formations, the following materials were analyzed for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and clumped isotope Δ_{47} compositions, where possible: pelagic and benthic trilobite eyes and cuticle, sedimentary carbonate (host rock) and coarsely crystalline diagenetic calcite cement (spar). For comparison, additional trilobite cuticle was analyzed from the Upper Member of the Dalby Limestone of Västergötland, Sweden, as this basin has experienced significantly higher burial temperatures. The Supplementary Material (extended version) details further the type and number of analyses from each formation.

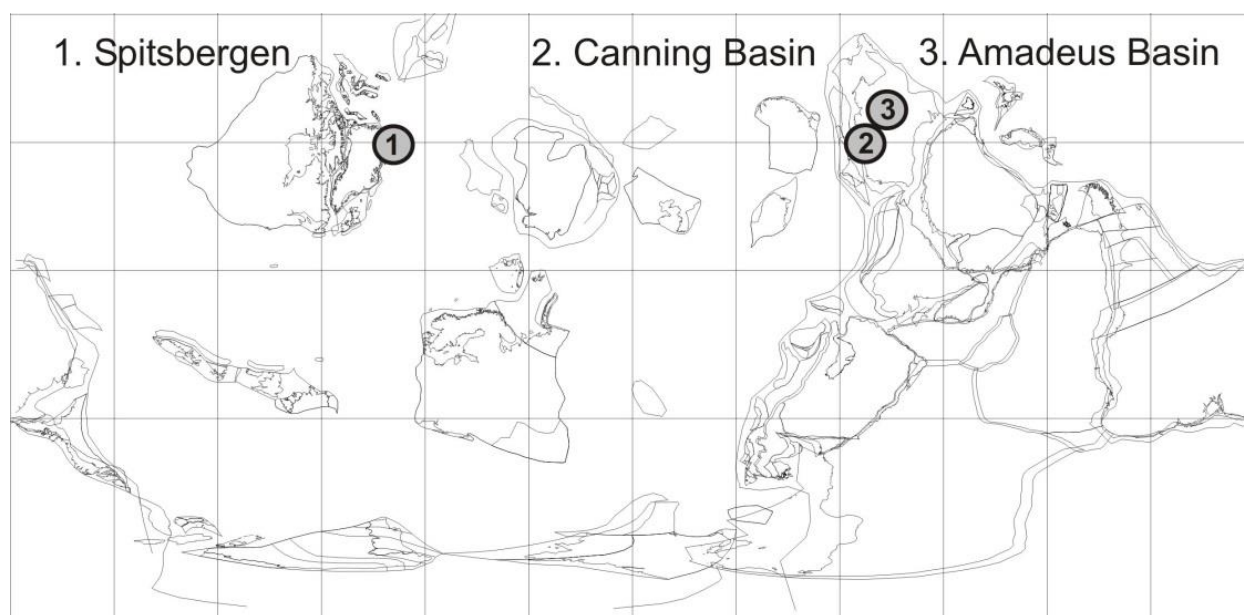


Figure 1. Paleogeographical map of trilobite sample sites: 1) Spitsbergen, Norway (Valhallfonna Formation); 2) Canning Basin, Western Australia (Emanuel Formation); and 3) Amadeus Basin, Northern Territory, Australia (Horn Valley Siltstone). Paleogeographic reconstruction for the Middle Ordovician, 470Ma, Galls Projection, cropped at latitude 30°N, BugPlates: www.geodynamics.no.

2.1 Valhallfonna Formation

Forty-three trilobite eyes were examined from eleven samples collected from the Floian (Arenigian) Valhallfonna Formation of Ny Friesland, Spitsbergen (Fortey, 1975; Fortey and Bruton, 1973). Four species of the telephinid *Carolinites* were studied (*C. angustagena*, *C. genacinaca*, *C. nevadensis* and *C. sibiricus*), along with cuticle (but no eyes) from benthic olenids (species indeterminate). The depositional environment is interpreted to be a marine shelf setting (Fortey and Barnes, 1977). The Ny Friesland succession is thought to have undergone shallow burial, with Conodont Alteration Index (CAI) values of 1 suggesting maximum temperatures of 90°C (Bergström, 1980). Calcite veins and calcite spar cements within the cavities of ostracod fossils and in pore spaces in the limestone indicate evidence for some diagenetic alteration.

2.2 Emanuel Formation

Trilobites were studied from the Floian (Bendigonian) Emanuel Formation of the Canning Basin, Western Australia (Laurie and Shergold, 1996). Fifty-one trilobite eyes were examined from the telephinid *Opipeuterella* sp. and benthic asaphids (species indeterminate), occurring in five samples from the type section of the Emanuel Formation. The burial history of the Ordovician Canning Basin indicates low thermal maturation, with a CAI index of 1 (Nicoll et al., 1993) and Apatite Fission Track Analysis (AFTA) indicating temperatures of ~100°C during the Late Devonian/Early Carboniferous (Arne et al., 1989).

2.3 Horn Valley Siltstone

Trilobites were studied from the Floian to Dapignian (Bendigonian to Yapeenian) Horn Valley Siltstone of the Amadeus Basin, Northern Territory, Australia, which can be partly correlated to the upper part of the Emanuel Formation from the Canning Basin (Laurie, 2006). Over 100 trilobite eyes from *Carolinites genacinaca* and benthic asaphids (species indeterminate) were examined from three samples collected from a field section at Mt Olifent (fig. 6 in Laurie, 2006). The burial history for the Amadeus Basin has been determined by AFTA and organic maturity data that indicate maximum burial during the Late Carboniferous, with Ordovician strata subjected to maximum temperatures of 140°C (Gibson et al., 2007).

2.4 Västergötland

Two test samples of *Telephina* cuticle and its host rock were analyzed for Δ_{47} from the Upper Member of the Dalby Limestone, Sandbian (Late Ordovician), in Västergötland, Sweden, introducing contrasting data from the opposite end of the preservation spectrum. The Västergötland rocks have been heated by local Permian intrusions, giving CAI values of 6 to 7 and temperatures of over 300°C (Bergström, 1980).

3. Methods

3.1 Preservation Assessment Protocol

In total, 198 trilobite eyes were examined under reflected light using a binocular microscope and by SEM, which allowed an initial assessment of eye lens integrity and preservation. Of these, 34 specimens representing a range of preservation states were selected for thin section analysis, based on the eye size (greater than 1.8 mm length), to enable a detailed preservation assessment. Polished thin sections were examined by cathodoluminescence (CL) microscopy and the geochemical variation in eye specimens was quantified using electron microprobe facilities at the universities of Lille (France) and Leicester (UK). Trilobite eye specimens were imaged by SEM under high vacuum using Secondary Electron and Back Scattered Electron detectors. Electron Backscatter Diffraction (EBSD) analysis of polished thin sections was undertaken at the University of Glasgow to examine intra-lens variations in crystallographic orientations (Torney et al., 2014).

3.2 Isotope Analyses

A total of 182 isotope analyses were undertaken on trilobite eyes, cuticle and host rock from all three formations (Appendix C). $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and Δ_{47} analyses were performed using three methods: 1. Conventional isotope analysis of carbonate powder, with lens/cuticle extraction using both a hand-held dental drill and automated micro-mill, at the British Geological Survey and at the University of Erlangen, Germany; 2. Secondary Ion Mass Spectrometry (SIMS) analysis of 15 μm diameter areas of thin sections (Figure 2) at

1 the Centre de Recherches Pétrographiques et Géochimiques (CRPG-CNRS) facility in Nancy, France; 3.
 2 Clumped isotope analysis (Δ_{47}) and $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ analysis of carbonate powder at the Institut de Physique
 3 du Globe de Paris (IPGP, Stable Isotope team). The extraction of material by dental drill is subject to human
 4 error and samples likely contain some underlying sediment matrix material. Micro-mill extraction is
 5 accurate to an error of $\sim 5\text{ }\mu\text{m}$, however it is possible that underlying sediment is sometimes sampled. SIMS
 6 analysis is the preferred technique in this context because it can accurately sample intra-lens material from
 7 thin sections. For a more detailed account of the methods, refer to the Supplementary Methods (extended
 8 version) section.

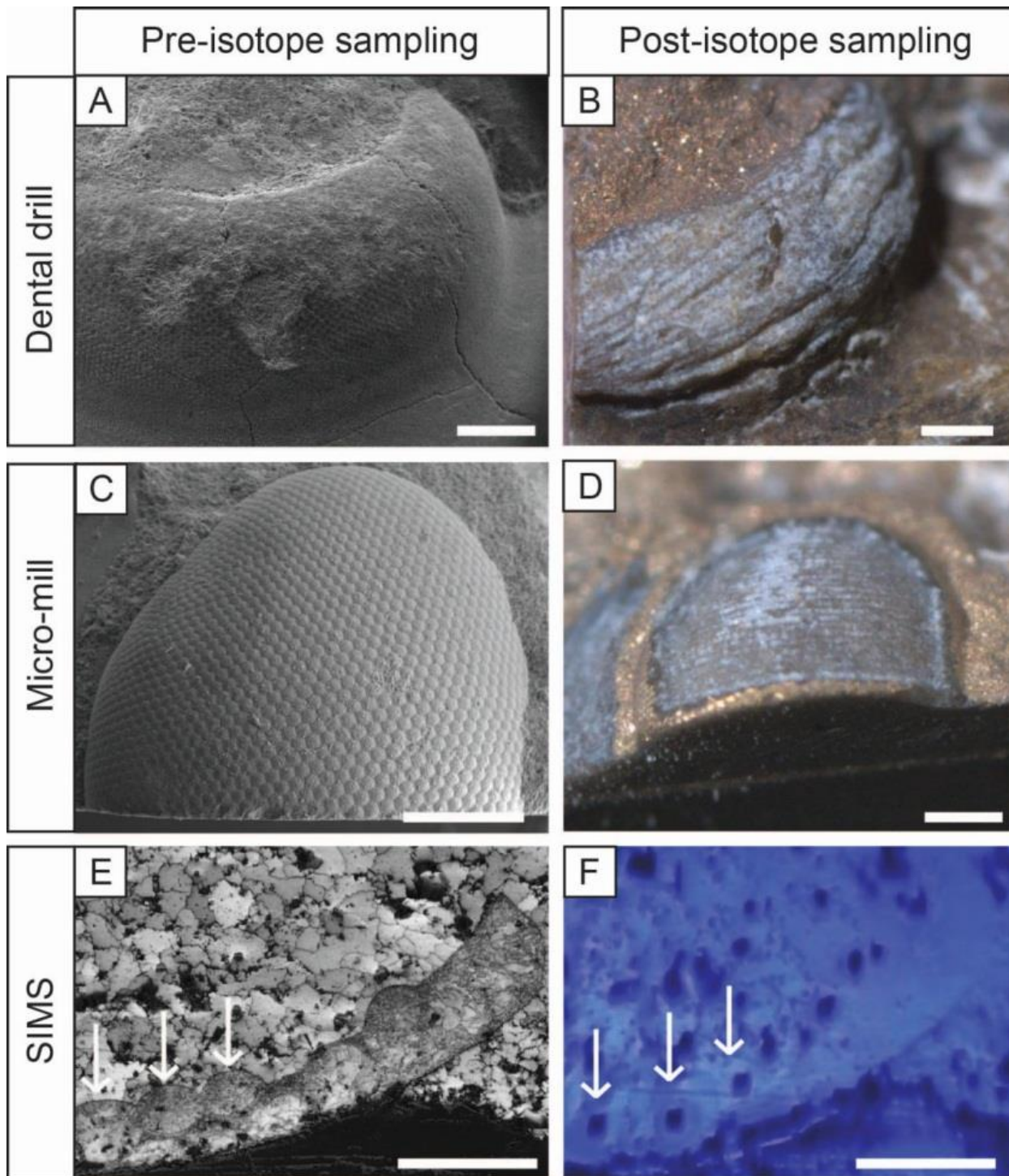


Figure 2. Lens extraction methods for isotope analysis. A-B) Hand drilling of lenses using a dentist drill for conventional isotope analysis (specimen HV_sp2, SE and light microscope images, respectively); C-D) Micro-mill automated drilling for conventional isotope analysis (specimen T_178_sp10, SE and light microscope images, respectively); E-F) Secondary Ion Mass Spectrometry (SIMS) analysis of a well-preserved eye (specimen A_178_sp1, EBSD image quality map and light microscope image, respectively).

Arrows indicate examples of lenses that were analyzed for oxygen isotopes. Scale bars 500 μm (A-D) and 100 μm (E-F).

4. Results

4.1 Preservation

The majority of the trilobite eyes examined had intact lenses that look superficially well-preserved. The hexagonal lenses are arranged in a single layer, which is on average 70 μm in thickness. Individual eye lenses are on average 50 μm in diameter, with no mineral partitions between them. Lens size varies between species, with telephinids having significantly larger lenses than asaphids, and moreover, a different eye morphology. Here we describe the results of the preservation assessment protocol from 34 specimens examined in thin section. Specimens are classified as ‘well-preserved’ or ‘poorly preserved’ based on their microstructural and geochemical properties (Table 1).

1 Table 1. Trilobite eye preservation

Specimen	Trilobite Species	CL	SEM Observations	EBSD Observations	Preservation
<i>Valhallfonna Formation, Spitsbergen</i>					
1.1A_sp1	<i>Carolinites sibiricus</i>	n/a	lenses fractured, silicified	n/a	poor
1.1B_sp1	<i>Carolinites sibiricus</i>	L	pervasive porosity, recrystallized, lens boundary overgrowths	lenses undefined, major recrystallization	poor
1.1B_sp2	<i>Carolinites sibiricus</i>	L	pervasive porosity, recrystallized, fractured lenses	no lens boundaries preserved, major recrystallization	poor
1.2C_sp1	<i>Carolinites genacinaca</i>	L	pervasive porosity, recrystallized, zoned calcite, micro-dolomite	lenses defined, major recrystallization	poor
1.2C_sp2	<i>Carolinites genacinaca</i>	L	pervasive porosity, recrystallized, zoned calcite, micro-dolomite	lenses undefined, major recrystallization	poor
2.1B_sp1	<i>Carolinites genacinaca</i>	NL	lenses defined, no apparent recrystallization	lenses defined, minor recrystallization, trabeculae?	good
2.1B_sp2	<i>Carolinites genacinaca</i>	SL-L	pervasive porosity, recrystallized, micro-dolomite crystals	lenses defined, recrystallized internally, trabeculae	poor
2.6_sp1	<i>Carolinites angustagena</i>	NL	recrystallized, large crystals of dolomite, euhedral pyrite	n/a	poor
2.6_sp2	<i>Carolinites angustagena</i>	NL-L	recrystallized, zoned calcite-dolomite	n/a	poor
2.6_sp3	<i>Carolinites angustagena</i>	L	recrystallized, zoned calcite-dolomite, euhedral pyrite	n/a	poor
2.6_sp4	<i>Carolinites angustagena</i>	NL-L	recrystallized, calcite-dolomite, euhedral pyrite	n/a	poor
<i>Emanuel Formation, Canning Basin, Australia</i>					
T_178_sp1	<i>Opipeuterella</i> sp.	NL	lenses defined, no apparent recrystallization	lenses defined, minor recrystallization at base of lenses	good
T_178_sp3	<i>Opipeuterella</i> sp.	L	lenses defined, crystal zoning in centre of lenses	lenses defined, minor recrystallization at lens boundaries	poor
T_178_sp4	<i>Opipeuterella</i> sp.	L	lenses defined, crystal zoning in centre of lenses	lenses defined, minor recrystallization	poor
T_178_sp10	<i>Opipeuterella</i> sp.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization, trabeculae	good
T_205_sp3	<i>Opipeuterella</i> sp.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization at cornea, trabeculae	good
T_205_sp5	<i>Opipeuterella</i> sp.	L	recrystallized, fractured lenses	n/a	poor
A_159_sp1	Asaphid indet.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization, trabeculae	good
A_159_sp4	Asaphid indet.	SL	lenses defined, no apparent recrystallization	n/a	good
A_178_sp1	Asaphid indet.	NL	lenses poorly defined, internal porosity	lenses defined, trabeculae, cuticle recrystallized	good
A_205_sp1	Asaphid indet.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization, trabeculae	good
A_205_sp3	Asaphid indet.	NL	recrystallized, lens boundaries poorly defined	lenses undefined, recrystallized	poor
A_205_sp5	Asaphid indet.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization, trabeculae	good
A_205_sp10	Asaphid indet.	NL	lenses defined, pervasive porosity, micro-dolomite crystals	lenses defined, minor recrystallization	good
<i>Horn Valley Siltstone, Amadeus Basin, Australia</i>					
HV_sp2	Asaphid indet.	L	lenses distinct, recrystallized	lenses defined, calcite twinning across all lenses	poor
HV_sp4	<i>Carolinites genacinaca</i>	SL-L	lenses undefined, recrystallized, euhedral pyrite within lenses	lenses defined, major recrystallization	poor
HV_sp5	<i>Carolinites genacinaca</i>	SL	lenses undefined, some recrystallization, pyrite within lenses	lenses defined, major recrystallization	poor
HV_sp6	Asaphid indet.	L	lenses fractured, partly recrystallized, pyrite within lenses	lenses undefined, recrystallization, calcite twinning	poor
HV_sp20	Asaphid indet.	L	lenses defined, partly recrystallized, pyrite within lenses	lenses defined, calcite twinning across all lenses	poor
HV_sp27	Asaphid indet.	L	lenses undefined, recrystallized, internal porosity	no lens boundaries preserved, major recrystallization	poor
HV_sp37	<i>Carolinites genacinaca</i>	NL	lenses undefined, recrystallization, euhedral pyrite within lenses	lenses defined, recrystallization, calcite twinning	poor

HV_sp43	<i>Carolinites genacinaca</i>	SL-L	lenses defined, porosity and crystal zoning within lenses	lenses defined, recrystallization, calcite twinning	poor
HV_sp94	<i>Carolinites genacinaca</i>	SL-L	lenses defined, crystal zoning and pyrite within lenses	lenses undefined, major recrystallization	poor

Trilobite eye preservation assessment data table, for specimens examined in polished thin section. Some specimens were not observed under EBSD due to their lenses being heavily dolomitised, silicified, or fractured/broken. Note that only specimens from the Valhallfonna Formation were analyzed on the electron microprobe (see Appendix 1). Abbreviations: CL = cathodoluminescence; L = luminescent; NL = non-luminescent, SL = slightly luminescent; SEM = Scanning Electron Microscope; EBSD = Electron back scatter diffraction.

1 Well-preserved lenses are composed of a single calcite crystal, which is non-luminescent. The outer 1-2 μm
2 of the lens surface consists of the cornea, which is micro-crystalline (Figure 3B) and luminescent. EBSD
3 maps show that calcite had a uniform crystallographic orientation within each lens, with the c-axis of the
4 calcite parallel to the lens axis (Figure 3D). Micro-scale pitting (Figure 3C) and sub-crystal boundaries
5 (Figure 3D) are observed in some lenses. In some specimens the lenses contain trabeculae, which are
6 microcrystallites oriented perpendicular to the lens surface and are thought to be an original structure
7 (Clarkson et al., 2006; Schoenemann and Clarkson 2011). The trabeculae can also be seen in SEM images of
8 broken lens sections, and can be identified in EBSD maps by slight differences in the crystallographic
9 orientations of the constituent sub-crystals (Figure 3E). Apparently syntaxial calcite cements occur next to
10 the interior lens surface of some specimens, but EBSD shows these cements have a different
11 crystallographic orientation to the lens calcite.

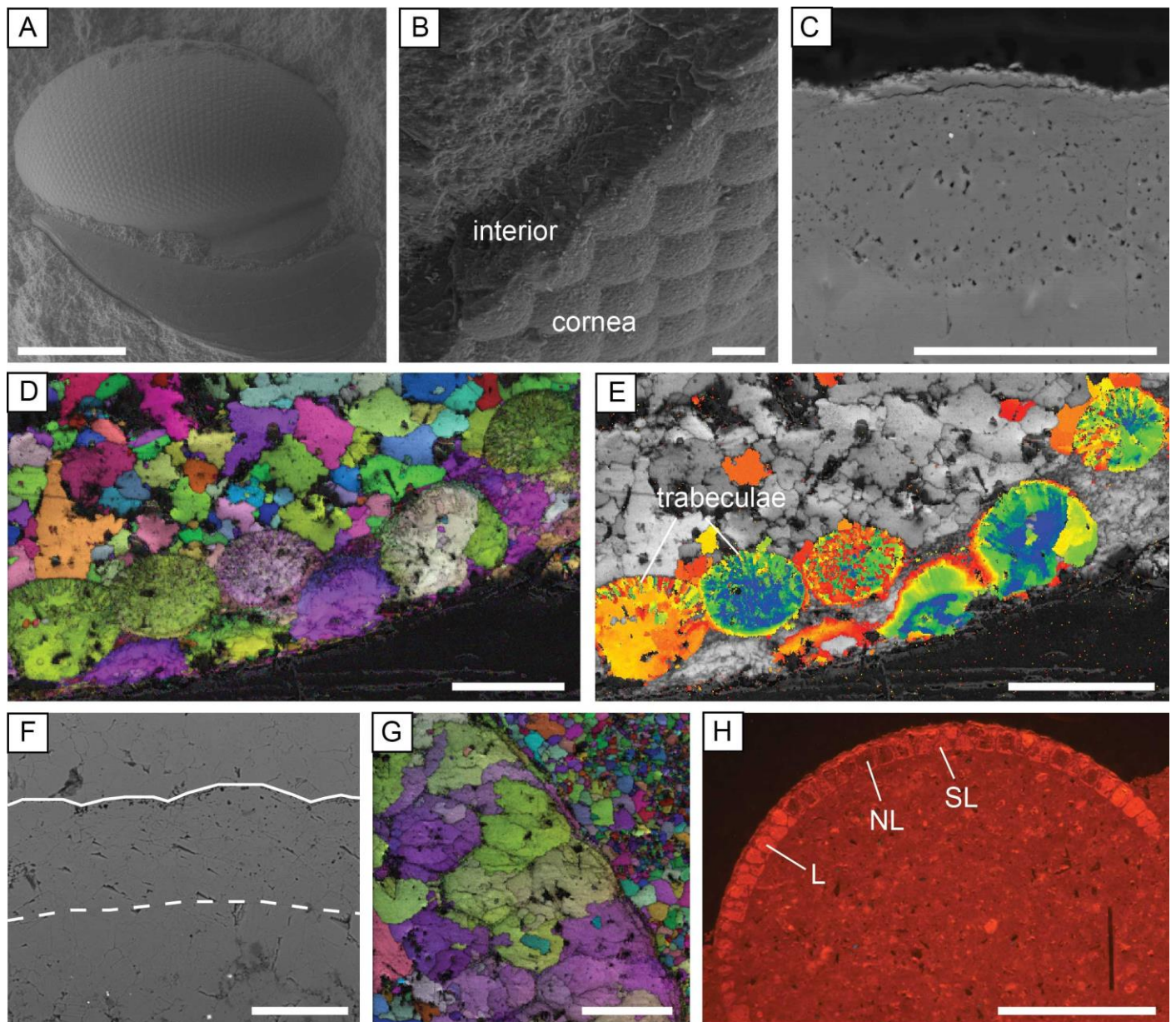


Figure 3. Trilobite eye preservation. A: *Opipeuterella* sp., with intact lenses and librigenal cuticle (specimen T_205_sp4, SE image). B: The recrystallized cornea and interior calcite of the eye lenses (specimen 2.6P, SE image). C: A highly polished telephinid eye lens that contains micro-crystalline dolomite crystals and micro-pitting (specimen T_178_sp10, BSE image). D-E: EBSD images of an asaphid specimen in thin section showing crystallographic continuity within the lenses (D) and the preservation of radial trabeculae structures (E) (specimen A_178_sp1). Image D is an inverse pole figure map overlain on an image quality map, and image E is an orientation tolerance map overlain on an image quality map. F: Lenses that are completely recrystallized, top and base of lenses marked by white lines, the basal boundary is unclear due to

crystal overgrowths (specimen 1.1B_sp1, BSE image, thin section). G: EBSD inverse pole figure map (overlain on an image quality map) of recrystallized lenses, with calcite crystals overlapping the lens boundaries (specimen 1.2C_sp2, thin section). H: Eye specimen with varying luminescence of the lenses under CL; L = luminescent; NL = non-luminescent, SL = slightly luminescent (specimen HV_sp43, thin section). Scale bars 500 μm (A, H), 50 μm (B-G).

In those specimens classified as poorly preserved, the eye lenses exhibit major alteration and lack pristine microstructures such as trabeculae. The microstructural evidence for extensive alteration of calcite lenses is as follows: 1. indistinct lens boundaries where the calcite crystals cross the lens-sediment boundary, form sub-crystals, or exhibit calcite twinning; 2. extensive pitting, resulting from the loss of small crystals and the presence of micropores, indicating that the lenses are recrystallized (Figure 3F); 3. a wide range of crystallographic orientations within a lens, indicating large-scale recrystallization (Fig. 3G); 4. The lens calcite is luminescent (Figure 3H). Minor alteration of lens calcite is evident by variable or partial luminescence due to trace amount variations in Mn and Fe, which can be detected by SEM and electron microprobe analysis. Under CL, recrystallization can be recognised by zoning in the lens calcite crystals and a luminescence intensity that is high, and similar to calcite spar cements in the host rock. Lenses that are more significantly altered can contain diagenetic quartz, dolomite and pyrite crystals.

Micro-dolomite crystals are present within the lenses of nine eye specimens (some well-preserved and others poorly preserved), and are associated with a pervasive microporosity/micro-pitting (Figure 3C). In some specimens, celestine can be recognised in BSE images as small white (i.e., high mean atomic number) crystals. Micro-crystalline dolomite is absent from the eyes of the Horn Valley Siltstone Formation trilobites, which are the most highly altered, and also does not occur in the cuticle. Micro-crystalline dolomite of a similar crystal size, celestine and a microporous texture are also features of the eyes of phacopine trilobites (Lee et al., 2007, 2012).

Trilobite eyes from the Valhallfonna Formation were analyzed using the electron microprobe to test for geochemical variation. The range in trace elements for calcite eyes is 0.6 - 1.9 wt% MgCO_3 , 0 - 0.9 wt% FeCO_3 and 0 - 0.5 wt% MnCO_3 . Trilobite eyes that are luminescent (and classified as poorly preserved) have slightly elevated Mn concentrations relative to non-luminescent lenses. There is no significant difference in the composition of eye calcite compared to that of the associated trilobite cuticle, or carbonate in the host sedimentary deposit (micrite and calcite spar) (Appendix B). Eyes containing micro-crystalline dolomite do not exhibit higher levels of Mg compared to those without micro-crystalline dolomite.

Specimens of trilobite cuticle from the Valhallfonna Formation were examined (Appendix A and B). Eight of the 44 studied had an internal structure comprising two layers of aligned crystals (Figure 4A), although most specimens lacked any internal structure. The cuticle is composed of prismatic, interlocking calcite micro-spar, with a consistent crystal size (Figure 4B), and no evidence of an outer prismatic layer of coarser crystals. EBSD of cuticle associated with trilobite eyes showed that in all specimens the cuticle crystals are randomly oriented. Some specimens have small euhedral pyrite crystals on the margins of the cuticle fragment, or in the interior. Most specimens are non-luminescent, but others exhibit partial or bright orange luminescence. Cuticle from six samples was analyzed by electron microprobe, with a maximum concentration of 2.3 wt% MgCO_3 , 0.2 wt% FeCO_3 , 0.4 wt% MnCO_3 and 0.6 wt% SrCO_3 . In comparison to the trilobite eyes, the cuticles contain relatively pure calcite with low trace element concentrations. The size of the calcite crystals within the cuticle was measured from SEM images (Figure 4C), and average size does not correlate with the size of the cuticle fragment examined or its chemical composition (Appendix A and B).

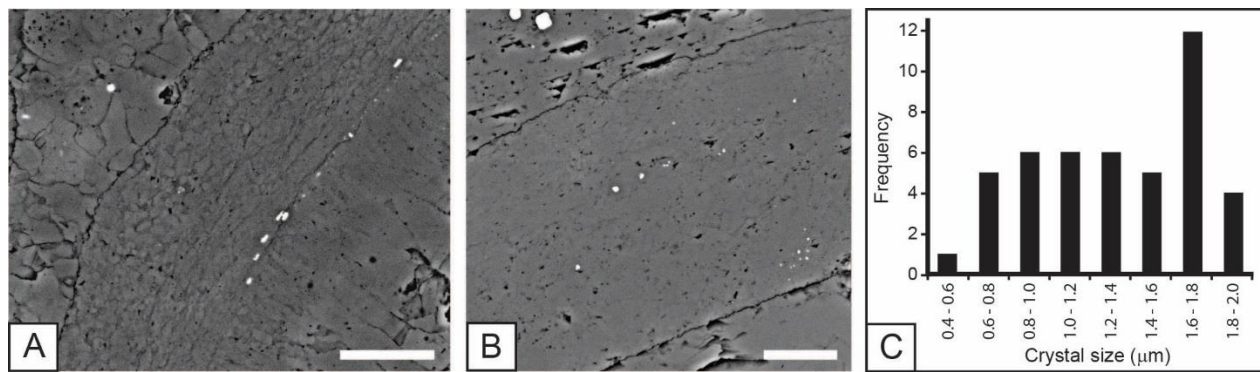


Figure 4. Trilobite cuticle preservation. A-B: Polished thin section, BSE images. A: *Carolinites sibiricus* cuticle with an internal structure of aligned crystals on the inner region (to the right), and larger crystals on the outer region. The crystal size is relatively large and small pyrite crystals are present on the internal margin of the specimen (sample 1.1B). B: *Carolinites genacinaca* cuticle with no internal structure and a relatively small crystal size (sample 1.2C_N_TS2). C: Plot of cuticle crystal size frequency. Scale bars for A and B are 25 μm.

In summary, specimens with the best-preserved eyes are from the Emanuel Formation, with nine out of thirteen eyes classified as well-preserved. Apart from one eye, all from the Valhallfonna Formation are poorly preserved. All eyes from the Horn Valley Siltstone are poorly preserved. Trilobite cuticles from the Valhallfonna Formation have variable microstructure, and EBSD shows that they lack any consistent crystallographic orientation. Well-preserved eyes have an integral preservation of lens calcite with a single crystallographic orientation and a composition of low-Mg calcite. In contrast, poorly preserved specimens exhibit multiple smaller crystals within a lens, unclear lens boundaries, luminescence, and varied chemical composition.

4.2 Stable Isotope Data

In total, 50 trilobite eyes were analyzed for isotope composition from the three formations, and where possible, the preservation state of each specimen was assessed prior to isotope analyses using the protocols described above (Appendix C). There is no difference in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between trilobite eye calcite extracted by a hand-held dental drill and that extracted by micro-mill (conventional isotope analysis

methods), or from clumped isotope analysis. For $\delta^{18}\text{O}$, there is a significant difference between conventional isotope analysis (Figure 5) and SIMS isotope results (Figure 6).

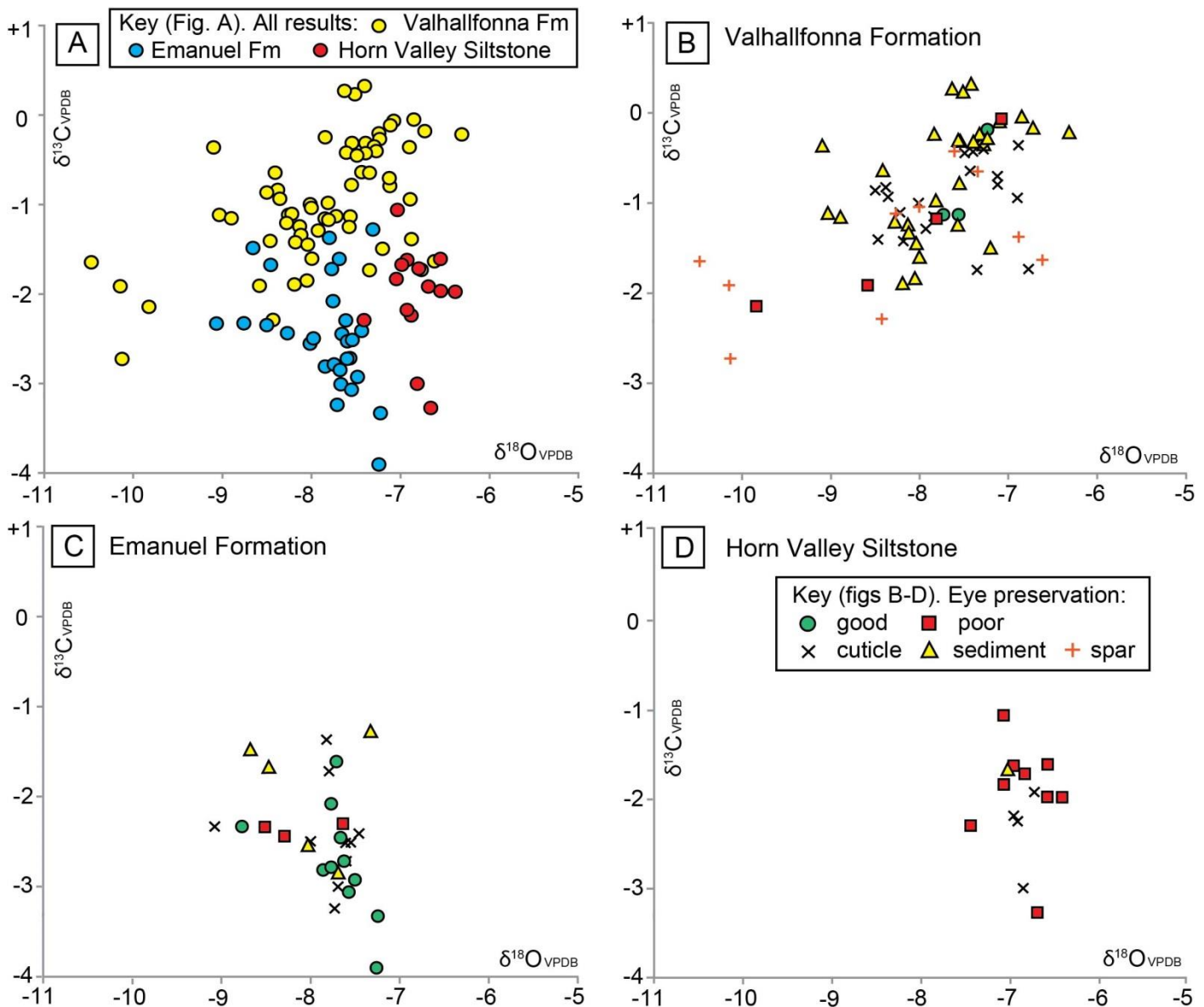


Figure 5. Dental drill and micro-mill $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ conventional isotope results for the three formations studied. A: All results (trilobite eye, cuticle and host rock), plotted by individual formation. B: Results from the Valhallfonna Formation, including trilobite eyes and other material. C: Results from the Emanuel Formation. D: Results from the Horn Valley Siltstone, note that all the eyes from this formation are interpreted to be poorly preserved. The key to symbols for graphs B-D is illustrated within D.

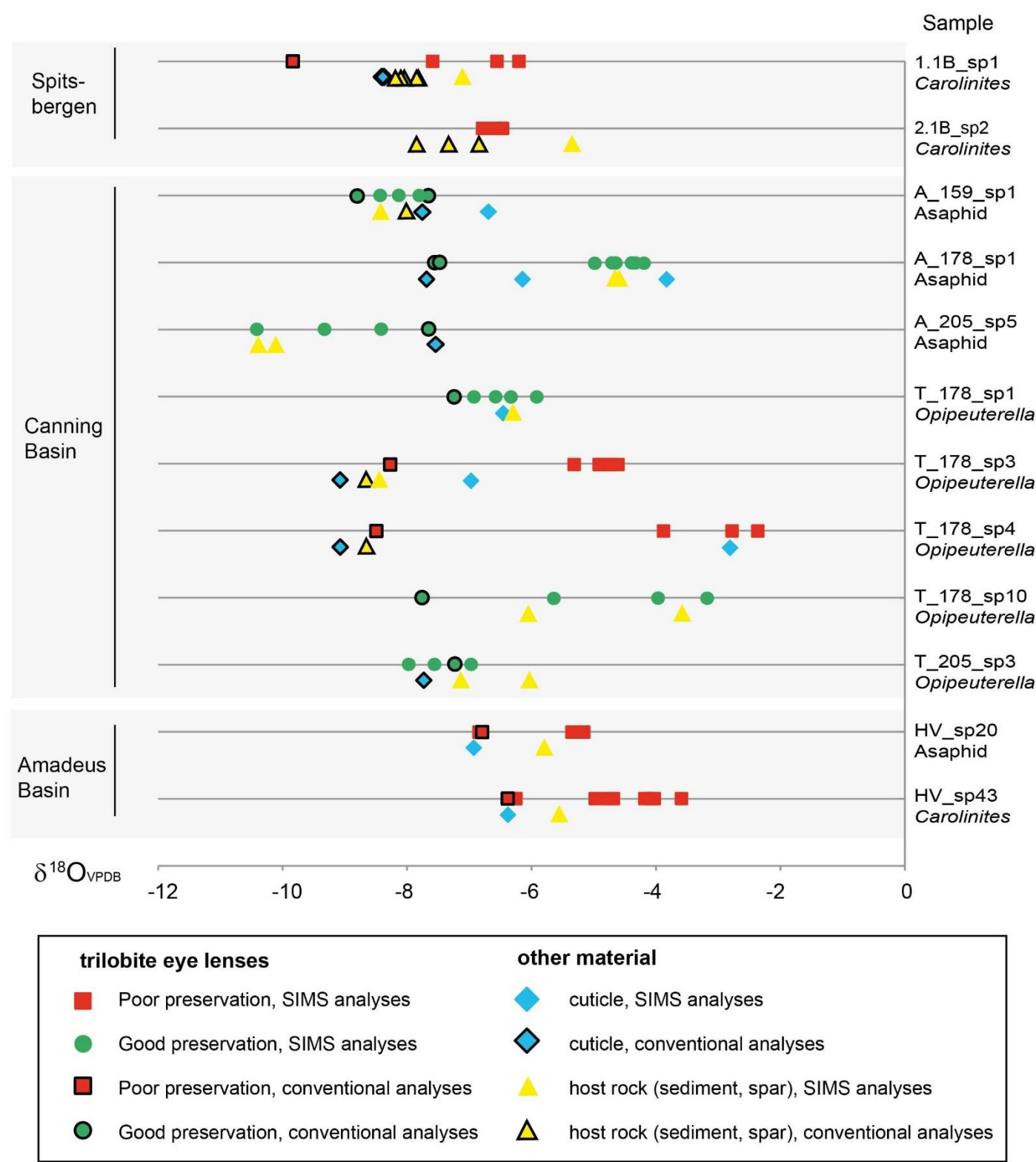


Figure 6. $\delta^{18}\text{O}$ results from SIMS analysis plotted against conventional isotope results, for individual specimens, including results from trilobite eye calcite, cuticle and rock.

4.2.1 Conventional Isotope Results

The total ranges in isotope composition for trilobite eyes (independent of preservation state), cuticle, and host rock (sediment and spar) analyzed are $\delta^{18}\text{O}$ -6.3‰ to -10.5‰ and $\delta^{13}\text{C}$ $+0.3\text{‰}$ to -3.9‰ . Each formation plots in a different, but overlapping, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ field (Figure 5A), and within each formation the trilobite eye isotope values are within the range of the results from the trilobite cuticle and host rock (Figure 5B-D).

With regards to the Valhallfonna Formation, the combined isotope results from all material (trilobite eyes, cuticle and host rock) show a low covariance between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ ($R^2 = 0.28$) with values ranging from $\delta^{13}\text{C}$ $+0.3\text{‰}$ to -2.7‰ and $\delta^{18}\text{O}$ -6.3‰ to -10.5‰ . This result is different from the other formations, which lack any covariance between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ ($R^2 = 0.13$ Emanuel Formation; $R^2 = 0.01$ Horn Valley Siltstone). Well-preserved trilobite eyes yield $\delta^{18}\text{O}$ that are on average higher than those of poorly preserved eyes, ranging from $\delta^{18}\text{O}$ -7.2‰ to -7.7‰ , although there is some overlap (Figure 5B). There is no significant difference in isotope composition between *Carolinites* species, stratigraphic units, or between the cuticle of *Carolinites* and olenid trilobites (Appendix C). The $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ variation between cuticle specimens that were identified as having relatively small or large calcite crystals is also negligible.

Results for all materials from the Emanuel Formation range from $\delta^{18}\text{O}$ of -7.3‰ to -9.1‰ and $\delta^{13}\text{C}$ of -1.3‰ to -3.9‰ . Well-preserved trilobite eyes have a similar range in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ to that of poorly preserved eyes (Figure 5C). There is no significant difference in isotope composition between the planktonic *Opipeuterella* sp. and benthic asaphid trilobites, or between different samples (Appendix C). It is possible that the outlier well-preserved eye result of $\delta^{18}\text{O}$ -8.8‰ is lower than the other well-preserved eye results due to the presence of diagenetically altered matrix material underlying the calcite lenses. Post-sampling photographs after the micro-mill had powdered the lenses do not indicate that this is the case, however the

1 imprecision of the micro-mill technique compared to intra-lens sampling by SIMS means that all micro-mill
2 samples must be considered to be subject to some sediment contamination.

3 Results for all materials from the Horn Valley Siltstone have a fairly narrow range of $\delta^{18}\text{O}$ -6.4‰ to -7.4‰
4 and a broader range of $\delta^{13}\text{C}$ -1.1‰ to -3.3‰ . All eyes examined were poorly preserved and there is no
5 difference in isotope composition between the planktonic *Carolinites genacinaca* and benthic asaphid
6 trilobites, or between different samples (Appendix C).

7 The isotope composition of lenses containing micro-crystalline dolomite does not differ from those
8 composed purely of calcite. All samples were analyzed as calcite and it is uncertain whether dolomite
9 crystals even contributed to the signal due to the small quantity of dolomite present ($<1\%$). The presence of
10 dolomite does not correspond to the preservation state of the eyes (Table 1).

11 Four larger well-preserved specimens were analyzed to test the average isotope variation across the eye. For
12 each specimen, two sub-samples were taken for isotope analysis using the micro-mill or dental drill. Two of
13 the specimens had a consistent composition in both sub-samples, while the other two showed a different
14 composition, with a variability of up to circa 1‰ in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (Appendix C).

15 Twenty-one specimens with cuticle material attached to the eye (such as the librigena or glabella) were
16 analyzed to compare the composition of the cuticle and adjacent eye lenses. In well-preserved specimens
17 from the Valhallfonna Formation, the cuticle is similar in composition to that of the eyes (with a maximum
18 difference in $\delta^{18}\text{O}$ of 0.6‰) while in poorly preserved specimens the difference is greater (increasing to
19 $\delta^{18}\text{O}$ 2.4‰) (Appendix C). In the Emanuel Formation, the composition of the cuticle is similar to that of the
20 eyes (with a difference in $\delta^{18}\text{O}$ of 0.8‰) for asaphids and *Opipeuterella* sp., with no significant difference
21 between poorly preserved and well-preserved specimens (Appendix C). However, the difference in $\delta^{18}\text{O}$
22 between eyes and cuticle from the Horn Valley Siltstone, which contains only poorly preserved specimens,
23 is less than 0.2‰ .

4.2.2 SIMS Oxygen Isotope Results

SIMS analysis of individual lenses across trilobite eyes in thin section reveals a wide range in $\delta^{18}\text{O}$, from –2.4‰ to –10.4‰ (Table 2). The range can dramatically vary even within specimens identified as well-preserved, and is often different from the conventional isotope analysis results of the same specimen extracted using the dental drill or micro-mill (Fig. 6). For most of the 12 specimens analyzed, the lens composition is within the general range of $\delta^{18}\text{O}$ as that of cuticle and host rock. However, many of the SIMS results for trilobite eyes, cuticle and host rock yield $\delta^{18}\text{O}$ values that are substantially more positive than the conventional isotope analysis results.

Specimens identified as well-preserved and poorly preserved have SIMS $\delta^{18}\text{O}$ results within the same range, and there is no correlation with specimens that have micro-crystalline dolomite within the lenses. The average $\delta^{18}\text{O}$, and the range of $\delta^{18}\text{O}$ within a single eye, does not correlate with the state of preservation of the eyes. There is no difference between the results from telephinids and asaphid specimens from the same formation, even in well-preserved eyes.

Multiple lenses of individual specimens were analyzed using SIMS, giving an intra-eye $\delta^{18}\text{O}$ range, with the greatest range seen within a single eye specimen of 2.7‰ and the lowest range of 0.3‰. Eight specimens have a relatively high $\delta^{18}\text{O}$ range (1‰ or greater), while four have a low range in values (less than 1‰) (Figure 6).

The SIMS and conventional isotope analysis results for individual specimens show some variations. All trilobite cuticle analyses of specimens from the two methods show a high disparity (with $\delta^{18}\text{O}$ difference greater than 1‰) (Figure 6). Eleven trilobite eye specimens were assessed by SIMS and conventional isotope analysis, and of these, six specimens have a high disparity between the SIMS and conventional methods, while five specimens had similar results ($\delta^{18}\text{O}$ <1‰ difference). There is no apparent link between the analysis conditions (beam intensity, chamber pressure, measurement error, run order) and those trilobite

eye samples with a large range in $\delta^{18}\text{O}$ (see Supplementary Information). In general, $\delta^{18}\text{O}$ results obtained by SIMS are more positive (by as much as 6‰) in comparison to those obtained by conventional isotope analysis.

4.2.3. Clumped Isotope Results

Of the 12 samples examined for their Δ_{47} compositions, the cuticle and limestone analyzed from Västergötland had markedly low Δ_{47} values of $\Delta_{47}\text{CDES}_{25} = +0.383$ and $+0.396\text{‰}$, respectively [reported against the absolute Carbon Dioxide Equilibrated Scale - hereafter CDES - reference frame described by Dennis et al. (2011) and referring to CO_2 extracted by phosphoric acid digestion at 25°C]. These values correspond to very high “apparent equilibrium temperature” (over 200°C , see Appendix D) representing the “blocking temperature” with respect to diffusional resetting of the calcite clumped isotope thermometer (e.g., Passey and Henkes, 2012; Bonifacie et al., 2013).

In contrast, the range in $\Delta_{47}\text{CDES}_{25}$ values for the three other basins investigated in this study is much higher (with $\Delta_{47}\text{CDES}_{25}$ from 0.581 to 0.610‰ for Spitsbergen, 0.608 to 0.623‰ for the Canning Basin and 0.606 to 0.608‰ for the Amadeus Basin; Appendix D). These Δ_{47} values correspond to temperature ranges of 55–68°C (average 63°C) for the Valhallfonna Formation, 50–56°C (average 52°C) for the Emanuel Formation and 56–57°C for the Horn Valley Siltstone Formation, respectively (Figure 7). These temperature estimates were calculated from the Δ_{47} -T universal calibration published by Bonifacie et al. (2016) defined on all (Ca, Mg, Fe) CO_3 carbonates. Only small differences in $\delta^{18}\text{O}$ are observed between cuticle and limestone (up to 0.45 ‰; Appendix D) and there is no difference in temperatures (derived from Δ_{47} data) between those of the host rock and cuticle from the same sample. These results indicate that all fossil calcite materials analyzed have experienced (and imprinted a Δ_{47} signature characteristic of) higher temperatures than those at which they originally precipitated in Ordovician seawater.

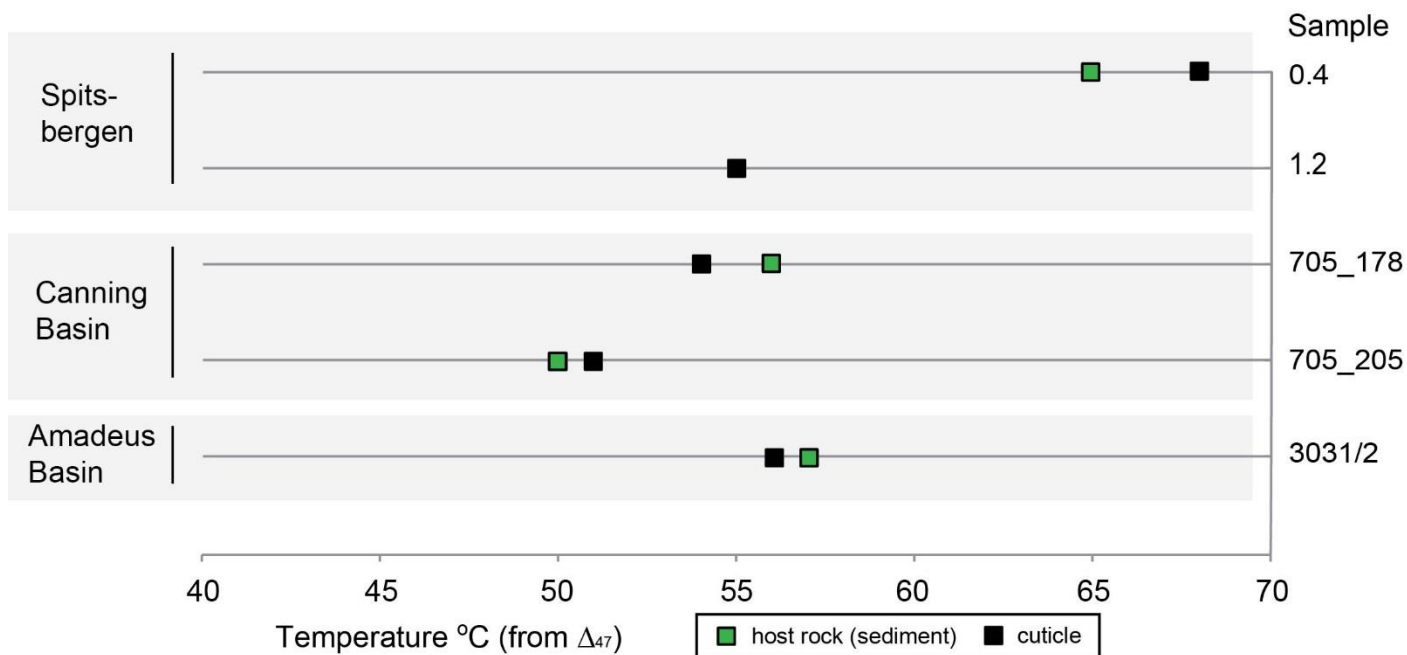


Figure 7. Clumped isotope temperature estimates from Δ_{47} for the three main formations studied. For further details including conventional isotope $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ results, Δ_{47} values and reconstructed water $\delta^{18}\text{O}_{\text{water}}$, see Appendix D.

The $\delta^{18}\text{O}_{\text{water}}$ from which the calcite in these three formations was precipitated was calculated using the temperature estimated from Δ_{47} and the $\delta^{18}\text{O}$ of the carbonate, and referenced to published Ordovician $\delta^{18}\text{O}_{\text{VSMOW}}$ estimates. They correspond to $\delta^{18}\text{O}_{\text{water}}$ of -0.4 to $+1.0\text{‰}$ (average 0.2‰) for the Valhallfonna Formation, -1.6 to -0.2‰ (average -0.9‰) for the Emanuel Formation and 0.0 to $+0.6\text{‰}$ (average $+0.3\text{‰}$) for the Horn Valley Siltstone (Appendix D). Because the Δ_{47} signatures found here do not represent those acquired over original carbonate crystallisation from seawater, the calculated $\delta^{18}\text{O}_{\text{water}}$ of the mineralizing fluid cannot be interpreted in terms of the original seawater $\delta^{18}\text{O}$, but rather likely reflects the properties of diagenetic fluids. However because the $\delta^{18}\text{O}_{\text{water}}$ results are fairly comparable to that estimated for Ordovician seawater (-1‰ $\delta^{18}\text{O}_{\text{VSMOW}}$: Trotter et al., 2008) it is probable that diagenesis may have occurred due to the flow of seawater through the rocks during burial, down to a maximum depth of two kilometres.

5. Discussion

The eye preservation assessment using a combined methodology (SEM, CL, EBSD and electron microprobe) indicates a range in degrees of alteration. The preservation of the trabeculae has been described as an indicator of good eye preservation (Schoenemann and Clarkson, 2011), but it has also been noted that trabeculae can be preserved in diagenetically altered specimens (Torney et al., 2014). The cornea (outermost layer of the trilobite eye) is likely to have been a transparent sheet *in vivo* (Clarkson et al., 2006), and its present micro-crystalline state indicates that it was recrystallized during diagenesis. The loss of *in vivo* microstructures from trilobite cuticle indicates that the majority have been recrystallized: the outer prismatic layer (Dalingwater, 1973; Dalingwater et al., 1991) is absent, the presence of pyrite within the cuticle is consistent with diagenetic alteration (Wilmot, 1990), as is the homogeneous microstructure of the calcite (Budil and Hörbinger, 2007). As the cuticle is thought to mineralize in an organic mesh-like framework, similar to that of an ostracod carapace (Teigler and Towe, 1975), the lack of correspondence between crystal size and size of the exoskeleton may indicate diagenesis. Large cuticle crystals may be expected to have incorporated trace-elements or have a different isotope composition to microcrystalline cuticle calcite. Moreover, the crystallographic orientation of the cuticle crystals (observed to be random under EBSD) has changed, with crystal c-axis perpendicular to the cuticle surface (Teigler and Towe, 1975).

In terms of the visual protocols used to assess preservation, the most useful method was EBSD analysis, due to the crystallographic orientation information it shows. SEM microstructural observations were also valuable and identified porosity and micro-crystals (Table 1). While CL generally corresponded to other indicators, with luminescent lenses showing an elevated trace-element profile and evidence for recrystallization, lenses that were recrystallized were sometimes non-luminescent. Thus, CL is the least reliable technique, however it does generate visual data that can be used as a starting point for more detailed preservation analysis via other techniques.

The SIMS results differ significantly from the corresponding conventional isotope analysis results in over half the trilobite eye specimens analyzed. This discrepancy may be due to presence of a fine-scale mixture of primary and secondary calcite within the lenses. In addition, little is known about the composition of

trilobite cuticle, or vital effects, in comparison to brachiopods where studies on modern specimens can be used to determine $\delta^{18}\text{O}$ paleotemperature equations (Brand et al., 2013). However, it must also be considered that the inconsistency between analytical datasets could reflect a systematic analytical error in the SIMS data in this study. Previous work has found differences in the trace element content (Mg/Ca, Sr/Ca and Mn/Ca ratios) of marine bivalves analyzed using conventional bulk dissolution and SIMS. These differences have been interpreted as reflecting variable shell organic content, crystal structures and small inter-crystalline heterogeneities in trace element concentrations (Freitas et al., 2009). It is therefore possible that the presence of organic matter within trilobite eyes, sub-crystal boundaries, or pitted surfaces could have affected our SIMS data. However, the cross-comparison of the SIMS results with the micro-crystalline characteristics of each specimen (Table 1) does not reveal a correspondence between the presence of micro-crystalline features (porosity, crystal zoning, micro-dolomite) and wide ranging or positive $\delta^{18}\text{O}$ values. The size of the ion beam used for the SIMS analysis (15 μm) may have resulted in the crossing of crystal boundaries, although most of the spots sampled the centre region of the eye lens and were verified by photographs. It is more likely that the results represent genuine intra-eye variation rather than contamination from the host rock. Further detailed work is needed to attempt to correct the SIMS data for the variables of inter-crystalline heterogeneities. The SIMS analysis has revealed large intra-eye variations of lens geochemistry that would otherwise remain undetected. Despite the limitations of the method, for example micro-scale pitting and sub-crystal boundaries that may cause analytical error, SIMS is still a useful tool for assessing diagenesis.

The range of isotope compositions within a single eye specimen (as revealed by both SIMS and conventional isotope analysis) indicates cryptic recrystallization. This means that recrystallization has likely occurred in specimens that were identified as well-preserved using the SEM, CL, EBSD and electron microprobe protocols outlined above. Ten out of the sixteen specimens examined had ranges in $\delta^{18}\text{O}$ of greater than 1‰ within the same eye. If, as seems reasonable, all the lenses in an eye were biomineralized (or indeed recrystallized) at the same time, in the same conditions, then they should have the same isotope

composition. For each formation, there is no significant difference in $\delta^{18}\text{O}$ or $\delta^{13}\text{C}$ between trilobite eye calcite or cuticle and the host rock. In addition: 1. all trilobite cuticle examined is interpreted as diagenetically altered, with a complete loss of crystallographic structure, indicating recrystallization was pervasive; 2. calcitic spar within the host rock, typically resulting from diagenetic growth, is within the same isotope range as the trilobite eye and cuticle results (Fig. 5B); and 3. the counterintuitive finding that pelagic trilobite species, which lived in relatively warm waters near the sea surface, have the same $\delta^{18}\text{O}$ as benthic species, indicates that all $\delta^{18}\text{O}$ has been reset. Therefore, most, if not all, of the material analyzed is interpreted to have been diagenetically altered, despite our original assessment of good preservation using microstructural criteria. In terms of eye preservation, there is little geochemical difference between eyes identified as well-preserved or poorly preserved, both in terms of conventional isotope analysis and SIMS results. In addition, the degree of recrystallization (complete or partial) has no significant correspondence with the isotope results.

Detailed SIMS analysis shows a large range in $\delta^{18}\text{O}$ values within a single eye specimen, even those that on microstructural criteria are well-preserved. Our preservation assessment can therefore be criticised for not identifying subtle preservation features. For example, the presence of trabeculae and lens crystallographic continuity as identified by EBSD mapping was interpreted to indicate well-preserved eyes, but the boundaries of the trabeculae were not examined in detail using TEM as they were by Torney et al. (2014). Micro-crystalline dolomite was observed in well-preserved and poorly preserved specimens, and further analysis using TEM is needed to further understand the origin of this mineral. Despite these limitations, the present study has made the most extensive preservation assessment of any trilobite material prior to isotope analysis.

The presence of micro-crystalline dolomite within the holochroal eyes examined here may indicate an original high-Mg calcite composition (as interpreted for the eyes of phacopine trilobites by Lee et al., 2012), with high-Mg calcite being diagenetically altered to low-Mg calcite. The presence of micro-crystalline

dolomite in both poorly- and well-preserved eyes, supports this interpretation. The absence of micro-crystalline dolomite in the Horn Valley Siltstone samples may be due to their more extensive diagenetic alteration. However, the lenses containing micro-crystalline dolomite do not have an elevated Mg content, as recorded in phacopine eyes, of up to 6 mol% MgCO_3 (Lee et al., 2007, 2012). This could be due to the diagenetic loss of magnesium over time. However, the sporadic occurrence of microcrystalline-dolomite - it is not present in all eyes identified as well-preserved - means that at present we cannot conclude that the holochroal lenses were originally composed of high-Mg calcite. An alternative explanation might be that the dolomite grew during diagenetic alteration and micro-recrystallisation of low-Mg calcite. Further detailed investigation of the micro-crystalline dolomite at the nanometre scale is needed to determine an original high-Mg calcite composition of the lenses.

The host rock and fossil material from each of the three basins have a distinctive range in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, which may reflect their different diagenetic histories. The Δ_{47} data reveal a minimum temperature of trilobite cuticle (re)crystallisation of 50°C , which is well in excess of the threshold for most organisms living in the surface layers of the modern oceans (Brock, 1985) and must be interpreted as resulting from the diagenetic alteration of the original cuticle. The Västergötland cuticle clumped isotope results are at the extreme temperature end (200°C), where the original Δ_{47} values (i.e. the original ^{13}C – ^{18}O bonding distribution acquired over crystallisation) have been reset due to closed-system solid-state diffusion alteration. This conclusion is consistent with independent Västergötland CAI values of 6 to 7, indicative of peak temperatures exceeding 300°C resulting from heating by Permian igneous intrusions (Bergström, 1980). In contrast, the Australian and Spitsbergen samples have lower temperatures calculated from the Δ_{47} (from 50 – 68°C ; Figure 7), which reflect early recrystallization temperatures.

The thermal history of the sedimentary formations can be linked to the fidelity of eye preservation. The Emanuel Formation was subject to the lowest burial temperatures (maximum temperatures of 70 – 80°C : Nicoll et al., 1993) and its sample set contains the best-preserved trilobite eyes. The Valhallfonna Formation

was subject to a slightly higher burial temperature (up to 90°C: Bergström, 1980) and its sample set contains just one well-preserved specimen. While the Horn Valley Siltstone experienced a much higher burial temperature (up to 140°C: Gibson et al., 2007) and yielded no well-preserved specimens.

It could be argued that the conventional $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ results for the trilobite cuticle, eyes and host rock reflect original compositions and were not affected by partial recrystallization and only the Δ_{47} composition changed. Indeed, solid-state re-ordering can alter the C-O bonds within a shell while retaining original shell microstructures and trace-element concentrations (Henkes et al., 2014). Experimental studies on brachiopod shells by Henkes et al. (2014) have shown that solid-state reordering can start to reset the original C-O bonds (and thus Δ_{47} compositions) of calcite if it experienced temperatures above 100°C for more than hundreds of millions of years. Stolper and Eiler (2015) argue that reordering can start to occur at temperatures of 75°C, and becomes more significant over 120°C. To explore this possibility further, the thermal history of each basin must be examined in terms of the duration spent at higher temperatures during burial. The detailed burial history of Ny Friesland, Spitsbergen has not yet been reconstructed. The Canning Basin experienced temperatures of 70-80°C for approximately 200 Myr in the Mesozoic, but only underwent a brief higher temperature interval of ~100°C (Arne et al., 1989) or <90°C (Wallace et al., 2002) in the early Carboniferous, making solid state diffusion unlikely. The Stairway Sandstone, which overlies the Horn Valley Siltstone in the Amadeus Basin, experienced burial temperatures of over 100°C in the Permian-Triassic (for approximately 80 million years), and Cretaceous (for approximately 110 million years) (Gibson et al., 2007). It is possible that solid-state diffusion may have affected the specimens from the Horn Valley Siltstone, although the time that the rocks experienced temperatures over 100°C is fairly short. Overall, low-temperature recrystallization, over solid-state diffusion, is the most likely mechanism responsible for altering the trilobite eyes and cuticle.

Figure 8 shows trilobite $\delta^{18}\text{O}$ data from the present study plotted against published geochemical data from trilobite cuticle and brachiopods through the Ordovician. There are no published data available for Lower

1 Ordovician trilobites, but those from the Middle and Upper Ordovician range from $\delta^{18}\text{O}$ of -3‰ to -7‰
2 (McAlister and Brand, 1989; Brand, 2004; Finnegan et al., 2011), which is within the range of that recorded
3 in the present study. Ordovician-Silurian studies comparing brachiopods and trilobites found them to have a
4 similar $\delta^{18}\text{O}$ to each other (McAlister and Brand, 1998; Wilmot and Fallick, 1989). While Finnegan et al.
5 (2011) found Upper Ordovician trilobites to have a similar $\delta^{18}\text{O}$ composition to that of contemporaneous
6 brachiopods and corals. Of all the published trilobite isotope studies, that by Finnegan et al. (2011) is the
7 only one where the preservation of the trilobite cuticle has been examined via trace element and
8 microstructural studies, prior to isotope analysis.

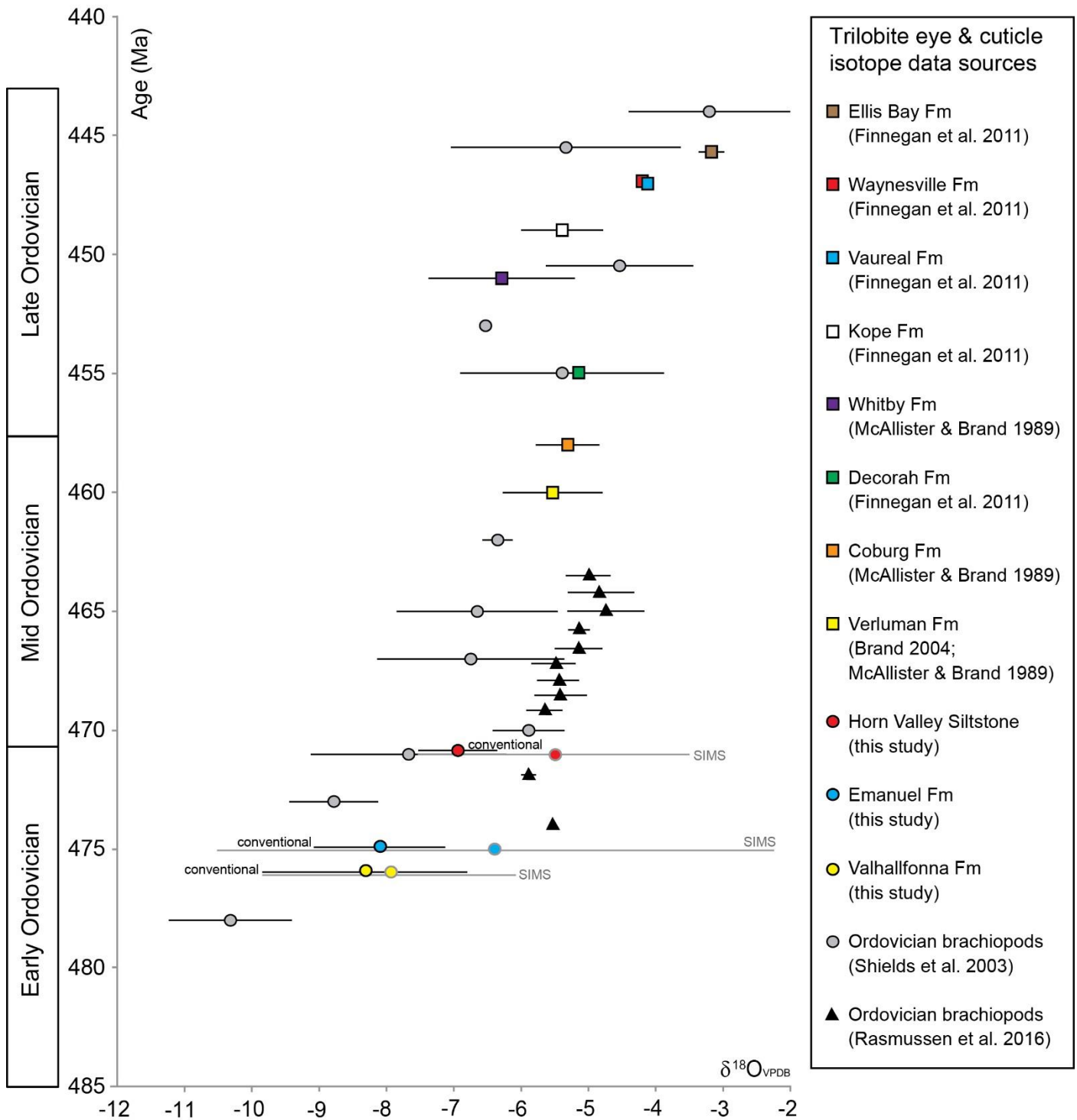


Figure 8. Trilobite eye and cuticle data from this study (conventional isotope analysis and SIMS), plotted with trilobite cuticle data from the literature, and brachiopod data (Shields et al., 2003; Rasmussen et al., 2016). Grey and black bars are isotope ranges, with the mean isotopic value indicated by the symbol in the centre.

The $\delta^{18}\text{O}$ data from trilobites in the present study are within the range of contemporaneous brachiopods, or more positive (Figure 8). The exceptions are new data from Baltica which have higher $\delta^{18}\text{O}$ values (Rasmussen et al., 2016). Given our results, this raises questions about the possibility of brachiopod calcite recrystallization from some earlier studies. Brachiopod specimens from the Lower to Middle Ordovician that were analyzed by conventional isotope techniques were assessed for preservation using trace element geochemistry and SEM and CL characterization (Veizer et al., 1999; Shields et al., 2003), but not by EBSD or SIMS. Significant cryptic diagenesis may have altered the isotope composition of these specimens: in general it is thought that only the secondary layer of the brachiopod shell is reliably unaltered and the preservation state of this layer can vary between taxonomic groups (Garbelli et al., 2012); In addition, SIMS analysis of brachiopods reveals that within certain groups, the secondary layer is only precipitated in isotope equilibrium with seawater towards the innermost part of the shell (Cusack et al., 2012). This fact implies that isotope studies where the entire secondary layer was analyzed (for example Shields et al., 2003) may reflect non-equilibrium fractionation. This is variable between brachiopod groups, for example in spiriferid brachiopods the prismatic tertiary layer is more resistant to diagenesis than the secondary layer (Grossman et al., 1993). Even in samples that have experienced low burial temperatures, recrystallisation can occur: in a study of Silurian brachiopods, Cummins et al. (2014) argued that elevated clumped isotope temperatures (up to 56°C) found in samples with $\text{CAI} = 1$ were likely resulting from diagenetic alteration due to recrystallization. This result cautions the use of apparently pristine biogenic specimens to interpret ancient paleotemperatures, without prior carbonate clumped isotope analysis. Due to the effects of solid-state reordering of clumped isotopes, which may not alter $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, analysis using the range of methods utilised in this study (SIMS, CL, SEM and EBSD) should be combined to jointly assess fossil preservation and isotope geochemistry.

Brachiopod data have been used to suggest that the $\delta^{18}\text{O}$ of Ordovician seawater was lower than at the present-day (-3‰ $\delta^{18}\text{O}_{\text{VSMOW}}$) in order to account for a reasonable paleotemperature calculation for the most negative $\delta^{18}\text{O}$ results (Veizer et al., 1999; Shields et al., 2003). Modelling of long-term seawater

geochemical composition estimates of Ordovician $\delta^{18}\text{O}_{\text{VSMOW}}$ at approximately -6‰ (Jaffrés et al., 2007), and a revised calibration of the brachiopod data gives a value of -5‰ $\delta^{18}\text{O}_{\text{VSMOW}}$ for the Early Ordovician at 475 Ma (Veizer and Prokoph, 2015). However, Trotter et al. (2008) used a value of -1‰ to interpret the temperature of formation of Ordovician conodont apatite, giving similar ocean temperatures for the Early Ordovician to that interpreted from the brachiopod data. The Ordovician brachiopod calcite, trilobite calcite and conodont apatite (Trotter et al., 2008; Veizer and Prokoph, 2015) isotope data show an increase in carbonate or phosphate $\delta^{18}\text{O}$ values over the Ordovician, which indicates either cooling oceans, changing seawater chemistry, or increasing diagenetic imprints at higher temperature with time. Conodont apatite has been demonstrated to be well-preserved (Wheeley et al., 2012) and is therefore useful as a paleotemperature proxy in the Paleozoic (Joachimski and Buggisch 2002). The results of Trotter et al. (2008) indicate that conodont apatite may be a much more reliable proxy than brachiopod calcite, as it yields more reasonable ocean paleotemperature calculations. How do the trilobite results influence this debate? The diagenetic alteration of trilobites demonstrated here reveals that the most negative $\delta^{18}\text{O}$ values in the Early to Middle Ordovician (those less than $\delta^{18}\text{O} -7\text{‰}$) are probably the result of diagenesis rather than a distinctive seawater composition. Results of the present study therefore bring into question the fidelity of some Early-Middle Ordovician brachiopod records and call for a re-appraisal. The new positive $\delta^{18}\text{O}$ brachiopod data by Rasmussen et al. (2016) for the Early Ordovician highlight the need for a review of all brachiopod data from this time. As no EBSD or SIMS work has been undertaken on Lower to Middle Ordovician trilobite or brachiopod specimens analyzed for their isotope composition prior to this study, the possibility remains that significant cryptic diagenesis may have altered these specimens. We therefore caution the interpretation of isotope results without rigorous preservation assessment and advise that SIMS analysis should be added to the tool-box of preservation studies.

6. Conclusions

- The holochroal eyes of species of the pelagic trilobite genera *Carolinites* and *Opipeuterella* from the Floian (Lower Ordovician) Valhallfonna Formation, Spitsbergen, and the Floian-Dapingian (Lower-

1 Middle Ordovician) Emanuel and Horn Valley Siltstone formations of Australia were examined to
2 assess their preservation state.

- 3 • The use of trilobite eyes for vision places strict constraints on the *in vivo* microstructure of their
4 constituent calcite lenses, and as such is a unique means of guiding our preservation assessment.
- 5 • The microstructure and chemical composition of the eyes was assessed using SEM, CL, EBSD and
6 electron microprobe. The Valhallfonna and Emanuel formations contain the best-preserved
7 specimens, based on these assessment protocols. Well-preserved eyes are composed of low-Mg
8 calcite, are non-luminescent, have clear crystallographic boundaries and retain their original optical
9 structures such as trabeculae.
- 10 • Trilobite cuticle is composed of low-Mg calcite, but the wide range of crystal size within the cuticle,
11 random crystal orientation and lack of original structures indicates that all specimens have been
12 recrystallized.
- 13 • The $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ results show a different range for each formation. However, intra-eye isotope
14 analyses using SIMS reveals a large range in $\delta^{18}\text{O}$ in some specimens, of up to 2.7‰. There is also
15 no systematic isotope difference between rock, cuticle, eyes and diagenetic cements, or between
16 benthic and pelagic trilobite eyes or cuticle.
- 17 • A sub-set of trilobite cuticles from the three formations was analyzed with carbonate clumped
18 isotope thermometry, generating a temperature range of 50-68°C and so indicating low-temperature
19 diagenetic alteration of the trilobite calcite.
- 20 • Despite rigorous preservation assessment protocols, the SIMS and Δ_{47} data both show that all
21 trilobite eye and cuticle specimens analyzed here have, at least in part, been diagenetically altered,
22 including those that were interpreted to be well-preserved using microstructural criteria. The
23 presence of sparse micro-crystalline dolomite within some eye specimens hints at cryptic
24 recrystallization processes, although the impact of the presence of dolomite on the isotope results
25 remains unclear.

- Despite clear evidence for alteration, $\delta^{18}\text{O}$ obtained in the present study are similar to those that have been previously used to infer Ordovician paleoceanographic conditions.
- We suggest that standard preservation assessment protocols may be insufficient for Paleozoic carbonates because of the difficulty in elucidating cryptic recrystallization that has taken place at low temperatures. Extensive microstructural analysis of Ordovician biogenic carbonates using EBSD in conjunction with SIMS and carbonate clumped isotope analysis is recommended before stable isotope signatures are used for paleoclimate or paleoenvironmental interpretation.

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