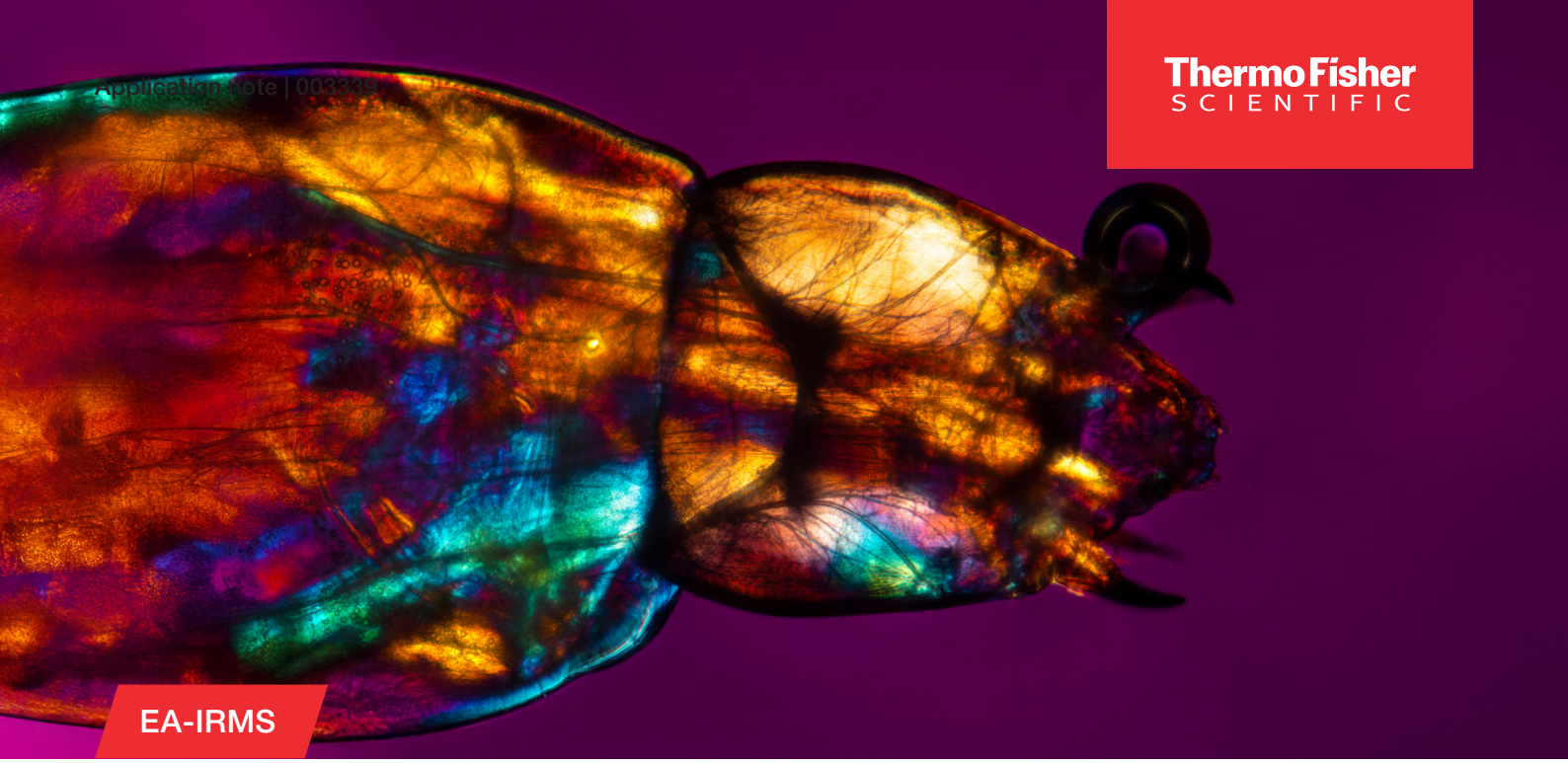

EA-IRMS

Improved lower detection limits for the oxygen isotope analysis of lacustrine chitinous invertebrate remains

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Introduction

Exoskeletons of many aquatic invertebrates (including chironomids) are composed of chitin and protein. Chitin is a biopolymer that preserves well under suitable environments and therefore head capsules released during molting and those from deceased specimens can remain preserved in sediment for millions of years. Unlike carbonate fossils, chironomids are ubiquitous in lacustrine sediments and thus are ideal stable isotope proxies for paleoenvironmental and palaeohydrological studies.

Chironomid head capsules vary in size but typically range from 1-2 µg in mass with size fluctuating between species and within species (due to food availability and environmental conditions). Previous oxygen isotope analyses of fossil chironomid head capsules have required a minimum weight of 40-50 µg. This has necessitated combining many head capsules together as a bulk sample to arrive at a sample size large enough to meet lower detection limits.

Oxygen isotopic fingerprints

Previous work has shown that the oxygen-isotope composition of chironomid head capsules is determined primarily by the composition of the environmental water in which the chironomids lived and therefore $\delta^{18}O$ values preserved in chironomids can provide an important palaeoenvironmental record where other proxies are absent (carbonates in acidic lakes for example). The $\delta^{18}O$ values preserved in chironomids can also be impacted by the oxygen isotopic composition of the dominant food source and temperature, amongst other factors (see van Hardenbroek et al., 2018, Lombino et al., 2021 and Lamb et al., 2024 for recent reviews).

Analytical Method

The following samples were analyzed using the Thermo Scientific™ EA IsoLink™ IRMS System:

a) laboratory grown *Chironomus* head capsules

b) cellulose standard (IAEA-CH-3)

Repeat measurements on these samples at a range of incremental weights were carried out. Laboratory grown *Chironomus* were grouped into samples of 1, 5, 10, 15, 20, 30 and 40 head capsules and weighed prior to analysis. It is of note that head capsules from laboratory grown chironomid larvae tend to be higher in mass (up to 4x) than fossil head capsules as preserved in lake sediments. A total of 40 samples containing head capsules from laboratory grown chironomid larvae were analyzed. The standard was weighed out at 2 µg increments between 0-60 µg, 5 µg increments between 60-150 µg and 10 µg increments between 150-300 µg. 56 samples of cellulose standard were analyzed.

For oxygen analysis, samples were weighed and wrapped into high purity silver capsules (4 mm x 3.2 mm), placed in the Thermo Scientific™ MAS Plus autosampler and introduced into the pyrolysis reactor of the EA IsoLink IRMS System. The reactor was maintained at 1450°C and consists of an outer ceramic tube and an inner glassy carbon reactor. The sample is thermally converted into H_2, N_2 and CO gases and is carried to the IRMS with continuous He flow maintained at 80 mL/min. The high temperature conversion gaseous products are separated by a gas chromatographic column. After separation, the CO gas produced was analyzed for its oxygen isotope composition by Isotope Ratio Mass Spectrometry. Analysis can be achieved in 360 seconds.

As chitin contains nitrogen, care must be taken to avoid interference from the generated N_2 on the CO peak. This was achieved through chromatographic separation of the N_2 and CO (Figure 1). Between runs the column was baked at 120°C overnight to maintain separation capabilities.

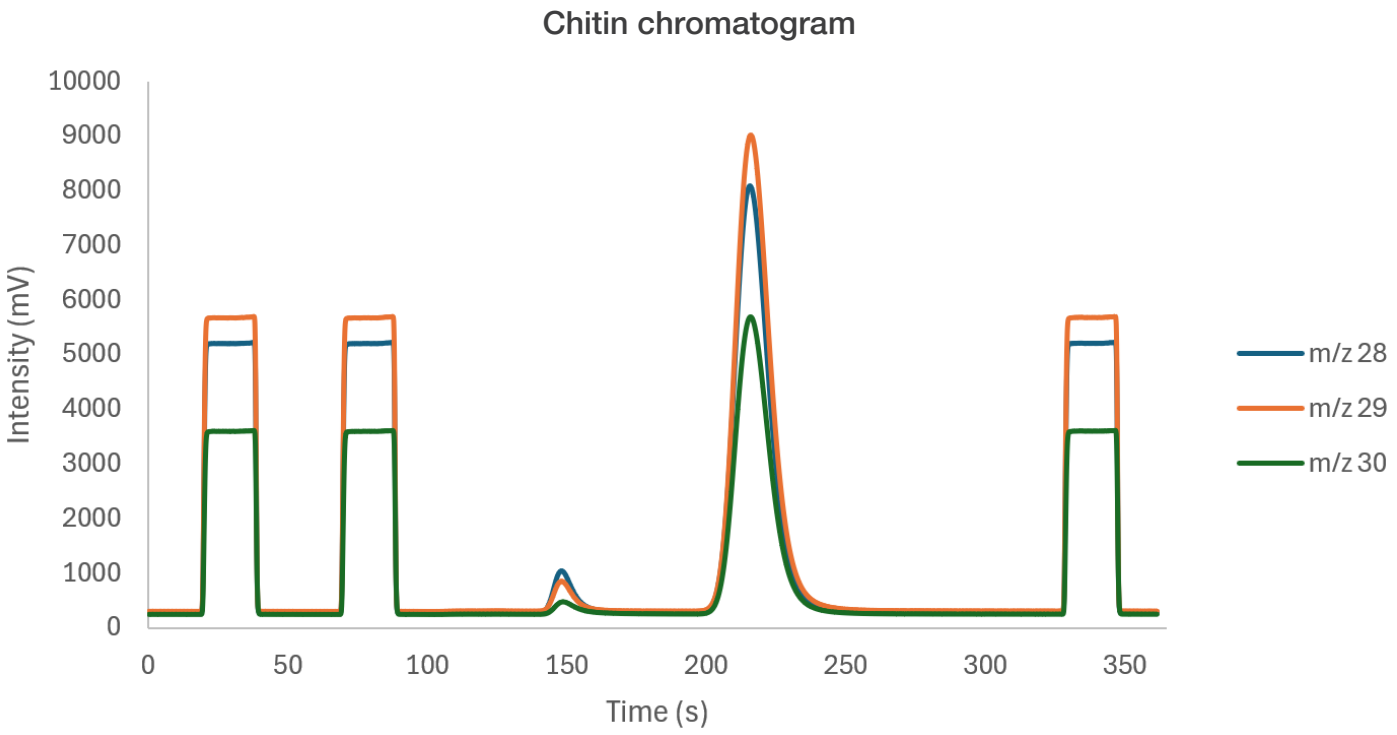

Figure 1. Chromatographic separation for N_2 and CO generated by Chitin high temperature conversion in EA IsoLink IRMS System.

Results

$\delta^{18}O_{chitin}$ measurements were normalized to the VSMOW scale using repeat measurements of IAEA-601, IAEA-602 and IAEA-CH-3 standards. The results (Figure 2; data from Lamb et al. 2024) show that there is a linearity effect on the $\delta^{18}O$ values for both materials tested. Consistent $\delta^{18}O_{chitin}$ measurements were determined using chironomid head capsule samples larger than 30 µg, resulting in an average $\delta^{18}O$ value of 17.4‰ (±0.63‰ 1SD). The results showed that a sample with a mass of 26 µg produced a $\delta^{18}O$ value that fell within the $\delta^{18}O$ average value ±1SD range. The results also show that the linearity effect is more pronounced on samples containing fewer than 10 head capsules resulting in unreliable measurements. Most samples including 10-15 large head capsules yielded consistent results that fall within the mean ±1SD range. For cellulose samples > 0.1 mg in mass, the average $\delta^{18}O$ value was 33.8‰ (±0.23‰ 1SD).

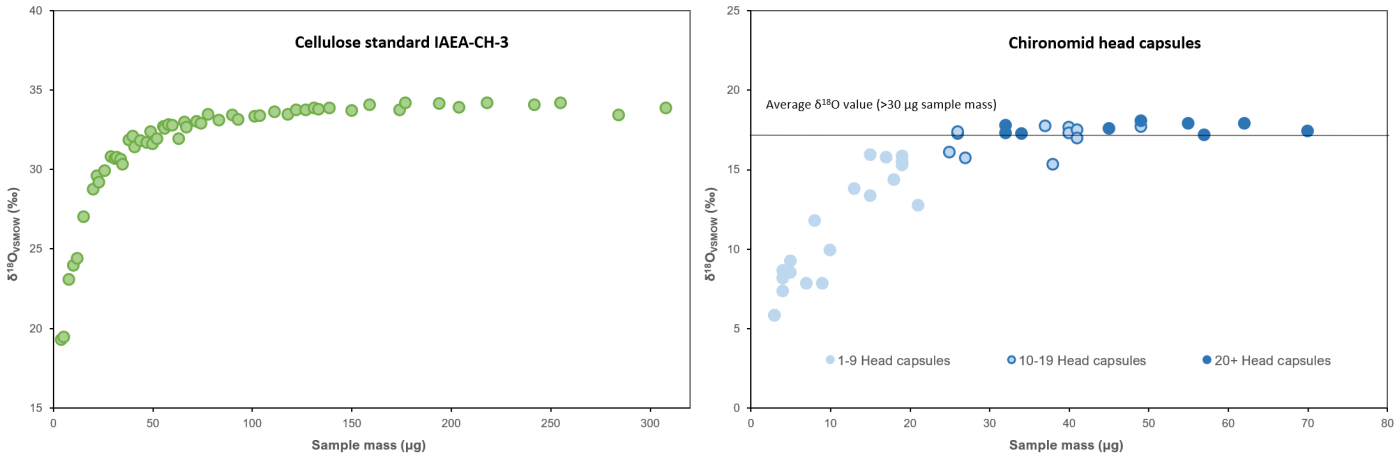

Figure 2: $\delta^{18}O$ measurements on IAEA-CH-3 – cellulose standard material (left) and on lab-grown chironomid head capsules (HCs) (right) against mass of the sample used. Symbols for the lab grown chironomid head capsules indicate the number of HCs used in an individual sample: 1-9 HCs, 10-19 HCs, and 20+ HCs. Data from Lamb et al. 2024.

Summary

The latest generation of EA-IRMS allows for samples as small as around 30 µg of chitin to be measured and that samples as small as 25 µg can potentially produce reliable results. This is a marked reduction of the amount needed previously (40-50 µg; Verbruggen et al. 2010). Small sample size requirements of ca 25-30 µg containing only 10-15 large head capsules means that taxon-specific isotopic measurements are now within reach, preventing the complexity of interpreting $\delta^{18}O_{chitin}$ measurements on samples composed of a mix of taxa. Improvements in (TC)EA-IRMS open the possibility of new areas of research that have been too time-consuming to be viable in the past.

References

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