## Investigating the water balance of insects using triple oxygen isotope models

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The distinct oxygen and hydrogen isotopic compositions of food and water sources are reflected in the body water of animals, including insects. Previous studies have demonstrated large variations in  $\delta^2 H$ ,  $\delta^{17} O$  and  $\delta^{18} O$  which provided critical insights into water source differentiation, metabolic water contributions, and the ability of species to absorb atmospheric water vapor under extreme conditions [1]. Extracting such information from the measured stable isotope values requires a solid framework. Triple oxygen and hydrogen isotope mass balance modelling is established for vertebrates [2, 3] but published models for insects are currently lacking. Developing and testing such models requires datasets of insect body water from specimens reared under well-defined conditions. Here we report on our initial steps to fill this gap.

First, we have developed an improved methodology for extracting water from insects and their organic food compounds. Our approach builds on a published CaCl<sub>2</sub>-based extraction method [4], but eliminates the need for using the salt and vacuum conditions. Our setup mimics an elbow distil and simply maintains a ca. 60°C temperature gradient between the two sides at ambient pressures. This sub-boiling distillation approach ensures quantitative water extraction while avoiding volatilization of organic compounds.

Second, we use the data to refine body water models adapted from vertebrate models. Our findings further support that the extreme xerophilous silverfish relative *Maindronia* sp. (Zygentoma: Maindroniidae) can sustain itself through water vapor absorption at exceptionally low relative humidity levels observed in the hyper-arid core of the Atacama Desert. Adult *Antofagapraocis* sp. nov. (Coleoptera: Tenebrionidae) beetles exhibit isotopically enriched body water compositions, reflecting a strongly evaporitic food source (evaporitic leaves) or high evaporation fluxes through its cuticle.

Our methodological advancements in water extraction and isotope modelling presently focus on understanding water flux dynamics in hyper-arid environments. However, our case study is paving the way for broader applications in physiological ecology and paleohydrology research in any other environment as well.

- [1] Herwartz et al., (2024) Goldschmidt conference 2024, Chicago.
  - [2] Feng et al., (2024) GCA 365, 21-34.
  - [3] Magozzi et al. (2019) Oecologia 191, 777-789.
- [4] El-Shenawy et al. (2024) Rapid Commun. Mass Septtrom. 38, e9646

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