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# Editorial: New Frontiers in the Application of Stable Isotopes to Ecological and Ecophysiological Research

Keith A. Hobson Western University London

John P. Whiteman

Old Dominion University, jpwhitem@odu.edu

Seth D. Newsome University of New Mexico

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\*CORRESPONDENCE Keith A Hobson ™ khobson6@uwo.ca

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## Editorial: New frontiers in the application of stable isotopes to ecological and ecophysiological research

Keith A. Hobson<sup>1,2\*</sup>, John P. Whiteman<sup>3</sup> and Seth D. Newsome<sup>4</sup>

<sup>1</sup>Western University, London, ON, Canada, <sup>2</sup>Science and Technology Branch, Division of Environment and Climate Change, Government of Canada, Saskatoon, SK, Canada, <sup>3</sup>Department of Biological Sciences, College of Sciences, Old Dominion University, Norfolk, NE, United States, <sup>4</sup>Department of Biology, University of New Mexico, Albuquerque, NM, United States

bulk tissues, compound-specific analyses, C,N,O,H,S, metabolic routing, origins of nutrients, metabolism, adding tools

## Editorial on the Research Topic

New frontiers in the application of stable isotopes to ecological and ecophysiological research

Application of the measurements of naturally occurring stable isotopes in animal tissues has expanded greatly over the last few decades and has become a firmly established component of the ecologist's toolbox (Hoenig et al., 2022; Hobson, 2023). This is a rapidly evolving field and we are in the midst of new and exciting developments based on creative uses of this technique and on recent technological and computational breakthroughs that enable us to measure stable isotopes of more and more elements. However, applications to ecophysiological research have generally lagged behind more descriptive ecological investigations, despite the tremendous potential to contribute to this field. Our objectives in formulating this set of 15 papers was to provide readers with examples of new and innovative approaches in the use of tissue isotope measurements to investigate animal ecophysiology, or examples which demonstrate creative ways in which the isotope approach can be combined with other tools to provide novel ecological insights, some of which have implications for species conservation. We are delighted to present this special series of papers because they are overwhelmingly diverse and touch upon several avenues of investigation that provide the reader with an impressive update on the current state of isotopic investigations in animal ecophysiology and community ecology.

There are many hundreds of published papers that have used isotopic measurements of animal tissues to reconstruct diet, trophic position, and/or sources of nutrients fueling food webs. As these approaches have become more established, we are witnessing new and creative combinations of isotopic data with information provided by other techniques. For example, Vanderklift et al. combined multiple isotope ( $\delta^{13}$ C,  $\delta^{15}$ N, and  $\delta^{34}$ S) measurements of blood and nails of the turtle Chelonia mydas in western Australia with acoustic telemetry to evaluate ontogenetic shifts in diet that could be associated with spatial distributions and habitat use. Importantly, more information was gained using this combination of tools than could be provided by either singly. Similarly, Bloomfield et al.

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used acoustic telemetry and stable isotope measurements to link fish diet with thermal guild and space use in several species of freshwater fish during winter in boreal Canada.

Stable isotope measurements provide a unique opportunity to quickly and efficiently evaluate individual isotopic niches within complex communities that can ultimately be related to niche segregation and responses to broad environmental parameters at the landscape scale. These themes are well represented by the papers by Yohannes et al. who, using feather  $\delta^{13}$ C,  $\delta^{15}$ N and  $\delta^{34}$ S, evaluated how a group of closely related birds in a Madagascar rainforest segregate through diet and space use and by Sekercioglu et al. who examined isotopically dietary responses of forest birds to fragmentation and juxtaposition to agriculture in Costa Rica. Pereira et al. similarly evaluated the effect of land-use practices on caiman in Brazil. Their study importantly made use of varying periods of isotopic integration of different tissue types to extend dietary inferences from the short term (weeks) to many months thereby exploiting one of the key benefits to using an isotope-based approach to reconstruct species diet and habitat use.

Closer to the theme of applying of stable isotope measurements to ecophysiological questions, two papers combined the use of fatty acid profiles with bulk tissue stable isotope measurements of stored lipids. In a captive study, Anparasan et al. made use of natural abundance  $\delta^{13}$ C measurements in (C<sub>3</sub>) larval diet of a migratory moth to distinguish larval use of essential fatty acids with those derived later during (C<sub>4</sub>) consumption of nectar during the adult stage. That study showed essential fatty acids were conserved across life stages and the isotope data provided a means of identifying origins of larval versus adult diets. Genier et al. similarly quantified fatty acid composition of blood plasma in swallows to investigate their acquisition of essential (omega-3) fatty acids and used plasma δ<sup>2</sup>H as a marker of where diets were derived. Aquatic emergent insects generally have lower tissue  $\delta^2$ H compared to upland insects and this study emphasized the utility of hydrogen isotopes in examining local transfer of resources from aquatic habitats to terrestrial (riparian) communities, a key development given the importance of long-chain polyunsaturated fatty acids (PUFAs) in animal nutrition and how access to these essential nutrients may be threatened by climate change (Shipley et al., 2022).

Many studies have used animal tissue  $\delta^2 H$  and  $\delta^{18} O$  values as a means of forensically assigning individuals to origin based on the long-term Global Network of Isotopes in Precipitation (GNIP) that can be used to create tissue-specific isoscapes (Hobson and Wassenaar, 2019). Modern assignment algorithms propagate known error based on calibration relationships using tissues from known-origin individuals, but evaluating factors contributing to variance in assignments is of keen interest. Lindroos et al. evaluated variance in the  $\delta^2 H$  of monarch butterfly wings related to metabolically active structures such as hemolymph containing veins and determined experimentally that such contributions are small but can be avoided. Reich et al. similarly investigated the utility of using trace metals and strontium isotopes in monarch wings as a means of geolocation. That investigation unexpectedly uncovered the effect of sex on

wing  $\delta^{87} \text{Sr}$  values that may complicate the use of this isotope for geolocation purposes.

Koehler et al. applied isotopic mapping to estimating origins of illegally traded cheetah cubs seized in east Africa and Dargent et al. combined  $\delta^{87} Sr$  and  $\delta^2 H$  measurements to identify local versus migrant spruce budworm. Interestingly, Koehler et al. provide the first evidence that whisker  $\delta^{18} O$  measurements can provide information on the nutritional influence of nursing in these cubs, which provided a means of avoiding assignment ambiguity if those researchers had used only whisker  $\delta^2 H$ . Apparently, the different metabolic pathways involved in tissue synthesis for oxygen and hydrogen lead to isotopic fractionation differences that can be detected and used to advantage.

The work by Koehler et al. clearly emphasizes the value to ecophysiological studies involving the measurement of  $\delta^{18}O$  in animal tissues. However, the work of Navarrete et al. is an exceptional example of the kind of detailed information one can derive from expanding the measurement of this element to  $\delta^{16}O$ ,  $\delta^{17}O$ ,  $\delta^{18}O$ . This triple-oxygen isotope approach was used in conjunction with  $\delta^{13}C$  and  $\delta^{15}N$  to evaluate the sources of metabolic water to two passerine species that inhabit coastal habitats in Chile where they consume a mixture of marine and terrestrial resources. That study will have immense impact on future research into the contribution of water sources to the water balance of free-ranging birds, a topic of increasing interest as climate change modifies regional precipitation regimes.

Oxygen isotope measurements ( $\delta^{18}$ O) were also used by Jones et al. who combined these measurements with  $\delta^{13}$ C in fish otoliths to infer metabolic rates in wild populations of plaice. Although that study used an isotopic model provided by Chung et al. (2019) involving the dual isotopic measurement of aragonite in otoliths, theirs was the first application to a wild population. Jones et al. demonstrated that individual fish metabolism was clearly linked to population responses to climate change whereby fish sought out cooler deeper waters and this response had metabolic consequences.

Stable isotope applications to ecological and ecophysiological investigations have undergone a major evolution in recent years due to the more widespread use of compound-specific isotope analyses (CSIA). That work moves beyond bulk tissues to provide isotopic information based on individual fatty acids and amino acids and has provided major insights into metabolic use and origins of key nutrients to animals (Whiteman et al., 2019). Two papers in our Research Topic make use of this approach. Pilecky et al. examined  $\delta^2 H$  and  $\delta^{13} C$  values of individual fatty acids stored in the fat body of migrant monarch butterflies. Apart from indicating the extent of bioconversion of essential vs. nonessential fatty acids, of great interest was their finding that larvalderived omega-3 alpha linoleic acid (ALA)  $\delta^2$ H was correlated with wing chitin  $\delta^2$ H supporting the idea that such essential fatty acids are linked largely to the larval diet corresponding to provenance of wing formation. The other study using CSIA came out of the wellestablished CSIA laboratory of Kelton McMahon where Ramirez et al. used amino acid  $\delta^{15}N$  measurements of tissues from endangered hawksbill turtles (Eretmochelys imbricata) to establish

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relative trophic levels of two populations (Florida vs. Texas) that differ dramatically in their growth rates. A major advantage of the CSIA approach is that it can provide trophic information on individuals without the need to assay baseline food web  $\delta^{15}N$  values since tissues contain both trophic and source amino acids and the difference between these within an individual provides a self-corrected trophic estimate. This aspect of CSIA allows comparisons of community structure and sources of nutrients to animals separated spatially and temporally.

We maintain the future is bright for the continued application and development of isotopic tools in ecophysiological research. Ecologists have summarized the use of stable isotopes as "You are what you eat", reflecting an emphasis on community studies that has long characterized the field. To that, one might add "You are what you build", reflecting the increasing use of isotopes to understand exchange and transformation within (in addition to between) organisms. We predict a growing number of studies using natural abundance isotope studies that focus on evaluating field metabolic rates, water budgets, and the use and transport of fatty acids and amino acids to fuel metabolism, growth, and reproduction, especially in migratory animals. As demonstrated in our collection of papers, applications are varied and will continue to diversify.

## **Author contributions**

KH: Writing – original draft. JW: Writing – review & editing. SN: Writing – review & editing.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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