Peer Community In Ecology

Refining our understanding how nutritional conditions affect 13C and 15N isotopic fractionation during ontogeny in a herbivorous insect

Gregor Kalinkat *based on peer reviews by* ***Anton Potapov*** *and 1 anonymous reviewer*

Samuel M. Charberet, Annick Maria, David Siaussat, Isabelle Gounand, Jérôme Mathieu (2023) Feeding and growth variations affect δ13C and δ15N budgets during ontogeny in a lepidopteran larva. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2022.11.09.515573

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Using stable isotope fractionation to disentangle and understand the trophic positions of animals within the food webs they are embedded within has a long tradition in ecology (Post, 2002; Scheu, 2002). Recent years have seen increasing application of the method with several recent reviews summarizing past advancements in this field (e.g. Potapov et al., 2019; Quinby et al., 2020).

In their new manuscript, Charberet and colleagues (2023) set out to refine our understanding of the processes that lead to nitrogen and carbon stable isotope fractionation by investigating how herbivorous insect larvae (specifically, the noctuid moth *Spodoptera littoralis*) respond to varying nutritional conditions (from starving to ad libitum feeding) in terms of stable isotopes enrichment. Though the underlying mechanisms have been experimentally investigated before in terrestrial invertebrates (e.g. in wolf spiders; Oelbermann & Scheu, 2002), the elegantly designed and adequately replicated experiments by Charberet and colleagues add new insights into this topic. Particularly, the authors provide support for the hypotheses that (A) 15N is disproportionately accumulated under fast growth rates (i.e. when fed ad libitum) and that (B) 13C is accumulated under low growth rates and starvation due to depletion of 13C-poor fat tissues. Applying this knowledge to field samples where feeding conditions are usually not known in detail is not straightforward, but the new findings could still help better interpretation of field data under specific conditions that make starvation for herbivores much more likely (e.g. droughts).

Overall this study provides important methodological advancements for a better understanding of plant-herbivore interactions in a changing world.

References:

Charberet, S., Maria, A., Siaussat, D., Gounand, I., & Mathieu, J. (2023). Feeding and growth variations affect δ13C and δ15N budgets during ontogeny in a lepidopteran larva. bioRxiv, ver. 3 peer-reviewed and recommended by Peer Community in Ecology. https://doi.org/10.1101/2022.11.09.515573

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Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology, 83(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Potapov, A. M., Tiunov, A. V., & Scheu, S. (2019). Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. Biological Reviews, 94(1), 37–59. https://doi.org/10.1111/brv.12434

Quinby, B. M., Creighton, J. C., & Flaherty, E. A. (2020). Stable isotope ecology in insects: A review. Ecological Entomology, 45(6), 1231–1246. https://doi.org/10.1111/een.12934

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Reviews

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.11.09.515573
Version of the preprint: 2

Authors' reply, 22 March 2023

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Decision by Gregor Kalinkat, posted 12 March 2023, validated 12 March 2023

Decision on your manuscript 'Feeding and growth variations affect δ13C and δ15N budgets during ontogeny in a lepidopteran larva'

Dear Samuel Charberet,

I have now received two reviewer reports on your preprint. Both are generally supportive of your work, but both also raise a couple of issues that need to be addressed. The decision on your preprint is therefore a "revise and resubmit". Please see the reviewer comments attached and try to address all of them when preparing your revised preprint.

I apologies again for the long time it took us to find a second reviewer.

Kind regards
Gregor Kalinkat

Reviewer 1

The authors present methodological study investigating the effect of nutritional conditions on 13C and 15N isotopic fractionation in Lepidoptera larvae. By exploring fractionation under different feeding intensity regimes, the authors provide support for the hypotheses that (1) 15N is accumulated under fast growth rates (probably due to intensive protein synthesis) and (2) 13C accumulated under low growth rates and starvation due to depletion of 13C-poor fat tissues. The experiment is nicely designed and analysed. The study is nicely and clearly written. However, one has to admit that the topic of starvation in isotopic ecology is not novel. Some more literature here can be mentioned. Eight studies exploring starvation (mostly in invertebrates) are compared in Fig. 5 in Martinez del Rio et al. (2009), few more are listed in Potapov et al. (2019), Table 1.

- Martinez del Rio, Carlos, Nathan Wolf, Scott A. Carleton, and Leonard Z. Gannes. 'Isotopic Ecology Ten Years after a Call for More Laboratory Experiments'. Biological Reviews 84, 1 (2009): 91–111. https://doi.org/10.1111/j.1469-185X.2008.00064.x
- Potapov, Anton M., Alexei V. Tiunov, and Stefan Scheu. 'Uncovering Trophic Positions and Food Resources of Soil Animals Using Bulk Natural Stable Isotope Composition'. Biological Reviews 94, 1 (2019): 37–59. https://doi.org/10.1111/brv.12434.

Nevertheless, the study has a nice replication and looks closer into the mechanisms behind the patterns. Thus, it reports some interesting and rather novel relationships. As a note of caution, the study overstating the observed difference and I think the conclusions and the abstract should be rephrased. The difference between (extreme!) feeding categories is on average 1.5‰ only (Fig. 2d). 2.5‰ is the total range of individual variation, not fully related to the growth/food intake (individual variation of isotopic composition is typically high, e.g.). As such the difference is smaller than a trophic level difference, and much smaller if we do not consider extreme nutritional situations, but non-neglectable. Revealed correlation explained 35-50% of individual variation in the laboratory conditions. I agree that this is a good argument for "when assessing trophic levels using isotopic data, the nutritional status of individuals can hardly be ignored". But at the moment I am missing the realistic application perspective of this knowledge. In most cases it is not feasible to evaluate nutritional status or growth rates of the entire population or even community in the field. For now, it is just 'one should be cautious' statement. Or maybe authors can propose something more specific? We can only hypothesize the 'V' shape of d15N – growth rate relationship. It is still to be tested for the negative growth rates, unfortunately. Therefore, I'm not sure if study anyhow contributed to revealing the 'V' shape. And I don't think it is fair to give this hypothesis again in the middle of your conclusions. As a methodological note, it is not perfectly clear if there was diet switch in the experiment or all animals fed on the same diet as before the experiment. It would be good to clearly state this.

Reviewer 2

In this paper the authors investigate the assimilation of carbon and nitrogen isotopes by larvae of Spodoptera littoralis under different conditions of starvation. They show that starvation does have an effect on isotope incorporation and that the magnitude of the effect is appreciable in the context of isotope ratio differences that are used to infer trophic level.

Overall, the work seems sound and the conclusions that the authors draw are supported by the results.

There are a few additional points the the authors may wish to consider.

1. It is my understanding that Spodoptera littoralis larvae, like many Noctuids can be cannibalistic (the congener Spodoptera frugiperda is notorious for this). If I understand the methods correctly there was no opportunity for cannibalism in these experiments because larvae were kept as isolated individuals. Neverthe-

less, the authors may want to make this more explicit. They may also wish to consider in the discussion how cannibalism may affect the inference of trophic level from isotope data.

2. The authors' focus is on the effect that starvation may have on C and N isotope ratios in the context of inferring trophic level. Stable isotopes are also used to study other aspects of Lepidopteran biology. Ratios of C and N isotopes are used to study the geographical origins of migrating moths. It would be nice to see the authors discuss the significance of their results to studies of this nature. Plants that use C3 and C4 carbon fixation mechanisms differ in their incorporation of stable carbon isotopes. Consequently, carbon isotope ratios have been used to infer the larval host plants of adult moths. Again, it would be interesting if the authors considered the implications of their results to the inference of larval diet. In this context it is noteworthy that the diet the authors used in their experiments includes both maize (C4) and soy (C3).

3. Assorted minor points:

- Page 2, line 17. Typing error, should be ”weight”
- Page 3, line 39. I do not understand this. A near-empty gut has a low food concentration by definition, no? Do the authors mean that food moves more slowly through a near-empty gut?
- Page 6, line 144. Replace ”sensible” with ”sensitive”. Note: this appears to be a case of ”false friends”. ”Sensible” (French) = ”sensitive” (English). ”Sensible” (English) = ”sérieux” (French).
- Page 7, line 163. Typing error, replace ”id” with ”is”.
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Download recommender's annotations

Reviewed by **Anton Potapov**, 19 December 2022

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