



# Peer Community In Ecology

## A lack of clear dietary differences between ontogenetic stages of invasive slippersnails provides important insights into resource use and potential inter- and intra-specific competition

**Matthew Bracken**  based on peer reviews by 2 anonymous reviewers

Thibault Androuin, Stanislas F. Dubois, Cédric Hubas, Gwendoline Lefebvre, Fabienne Le Grand, Gauthier Schaal, Antoine Carlier (2021) Trophic niche of the invasive gregarious species *Crepidula fornicata*, in relation to ontogenetic changes. Missing preprint\_server, ver. Missing article\_version, peer-reviewed and recommended by Peer Community in Ecology.

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The slippersnail (*Crepidula fornicata*), originally from the eastern coast of North America, has invaded European coastlines from Norway to the Mediterranean Sea [1]. This species is capable of achieving incredibly high densities (up to several thousand individuals per square meter) and likely has major impacts on a variety of community- and ecosystem-level processes, including alteration of carbon and nitrogen fluxes and competition with native suspension feeders [2].

Given this potential for competition, it is important to understand the diet of *C. fornicata* and its potential overlap with native species. However, previous research on the diet of *C. fornicata* and related species suggests that the types of food consumed may change with age [3, 4]. This species has an unusual reproductive strategy. It is a sequential hermaphrodite, which begins life as a somewhat mobile male but eventually slows down to become sessile. Sessile individuals form stacks of up to 10 or more individuals, with larger individuals on the bottom of the stack, and decreasingly smaller individuals piled on top. Snails at the bottom of the stack are female, whereas snails at the top of the stack are male; when the females die, the largest males become female

[5]. Thus, understanding these potential ontogenetic dietary shifts has implications for both intraspecific (juvenile vs. male vs. female) and interspecific competition associated with an abundant, invasive species.

To this end, Androuin and colleagues evaluated the stable-isotope (d13C and d15N) and fatty-acid profiles of food sources and different life-history stages of *C. fornicata* [6]. Based on previous work highlighting the potential for life-history changes in the diet of this species [3,4], they hypothesized that *C. fornicata* would shift its diet as it aged and predicted that this shift would be reflected in changes in its stable-isotope and fatty-acid profiles. The authors found that potential food sources (biofilm, suspended particulate organic matter, and superficial sedimentary organic matter) differed substantially in both stable-isotope and fatty-acid signatures. However, whereas fatty-acid profiles changed substantially with age, there was no shift in the stable-isotope signatures. Because stable-isotope differences between food sources were not reflected in differences between life-history stages, the authors conservatively concluded that there was insufficient evidence for a diet shift with age. The ontogenetic shifts in fatty-acid profiles were intriguing, but the authors suggested that these reflected age-related physiological changes rather than changes in diet.

The authors' work highlights the need to consider potential changes in the roles of invasive species with age, especially when evaluating interactions with native species. In this case, *C. fornicata* consumed a variety of food sources, including both benthic and particulate organic matter, regardless of age. The carbon stable-isotope signature of *C. fornicata* overlaps with those of several native suspension- and deposit-feeding species in the region [7], suggesting the possibility of resource competition, especially given the high abundances of this invader. This contribution demonstrates the potential difficulty of characterizing the impacts of an abundant invasive species with a complex life-history strategy. Like many invasive species, *C. fornicata* appears to be a dietary generalist, which likely contributes to its success in establishing and thriving in a variety of locations [8].

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## Reviews

### Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/2020.07.30.229021>  
Version of the preprint: 1

### Authors' reply, 05 April 2021

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### Decision by [Matthew Bracken](#) , posted 13 November 2020

#### Reviewers were split and highlight areas for improvement

In this contribution, the authors use a combination of techniques, including fatty acid profiles and stable isotope analyses, to evaluate potential ontogenetic changes in the diet of invasive slipper snails, *Crepidula fornicata*. These molluscs change sex (from male to female), habit (from more mobile to more sessile), and diet (from some grazing to all suspension feeding) as they age, and the authors were interested in whether these changes would be reflected in the species' diet. In brief, the authors found little evidence for dietary shifts with ontogeny. However, they did reveal changes associated with season and age, independent of diet. The reviewers were split in their assessments: one was very positive, whereas the other had a number of concerns. However, even the more critical reviewer indicated that this work has the potential to make a contribution to the literature. I am therefore requesting a revision that addresses the many suggestions that both reviewers provide. I concur with the more critical reviewer that there is a lot of information here that is difficult to sort through. I suggest that, in addition to carefully addressing the reviewers' comments, the authors employ a hypothesis-testing framework to structure the manuscript: hypotheses and predictions in the introduction, structured methods and results to evaluate those predictions and assess support for the hypotheses, and a discussion that begins by specifically evaluating whether those predictions were borne out in the data and the hypotheses supported. If the authors choose to submit a revision, I request that they provide a description of their edits, including a careful comment-by-comment accounting of the reviewers' suggestions and the changes that they have made in response. **\*\*Additional requirements of the managing board\*\***: As indicated in the 'How does it work?' section and in the code of conduct, please make sure that: -Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data. -Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused. -Details on experimental procedures are available to readers in the text or as appendices. -Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest

with the content of this article.” If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: “XXX is one of the PCI XXX recommenders.”

## Reviewed by anonymous reviewer 1, 14 October 2020

Review of “Trophic niche of the invasive gregarious species *Crepidula fornicata*, in relation to ontogenic changes” Androuin et al. bioRxiv, PCI Ecology

### Overview

This is an interesting ms investigating the trophic ecology of slipper limpets, using a complimentary biomarker approach, combining fatty acids, isotopes, and natural history. The findings are based on observational data. The writing is generally good; it may be more text in some sections than is absolutely necessary, and it may be preferable to move some text from the discussion to the introduction. The methods and interpretation of the data seem appropriate. The figures are very informative. I have a few suggestions for improving the flow and interpretation below.

### General Comments

1. The scholarship seems exemplary, and I learned quite a lot about an organism I was mostly unfamiliar with before. I didn't have time to go and look at the references, but the authors certainly tell a nice story and provide a thorough reference trail.

1. The Introduction is very light on the background for the fatty acids technique; there is basically only one sentence citing two very good overview articles (lines 91-92). I think that given the importance of this method for the paper, the authors should make a separate paragraph that is more thorough and comprehensive about this technique, particularly for trophic inference of basal consumers generally and gastropods. For example, certain parts of the Discussion (which is pretty long) might be better suited in the introduction. Lines 471-475, which justify the focus on the neutral lipids, could be added to the intro. This would make it more clear to the reader why it is later that the authors only extracted FA from this lipid class.

2. Is there any chance the authors can include a photograph of the stacked limpets in very high densities? This is fascinating and I would love to see a picture of this as one of the ms figures.

3. It is fine that the authors use GC FID (line 200) rather than MS (this is common), but the FAME standard does not include many interesting FA that may be in the samples. Did the authors have any of their samples run on a GCMS to identify the unknown peaks? If so, they should say so. For example, when I was reading the methods I wondered if there non-methylene interrupted (NMI) FA in these limpets? NMI are interesting and often in molluscs. This is actually later discussed by the authors in ln 542. but how were these FA identified? That is one example. There are other interesting FA (especially 16 PUFAs) that are diagnostic of certain producers that are not in the standard referenced and would probably only be identified with GCMS.

4. The first paragraph of the discussion is a little odd as written; it is a bit redundant. I like the start of a discussion to provide a big picture of the main findings (which is what this is set up to do) but it kind of falls short there, and just reiterates the methods. I would suggest highlighting here the main findings that bridge all of the different methods which are covered in the individual sections of the discussion below. For example, the authors could move the text at the start of 4.2 up to here... lines 427-433 kind of synthesize the primary findings of the biomarkers in the consumer. But doing this change may then require some re-organization of other parts of the discussion.

5. The key result from my perspective is that the limpet isotopes did not differ (similar trophic niche) but that the FA did differ; this is attributed by the authors as the FA reflecting physiological changes (growth rate, energetic demand) rather than differences in diet. I do agree that this is one reasonable explanation.

But on the other hand, it is also quite possible that the FA are detecting differences in diet that the isotopes did not (because they cannot). For example biofilm and SSOM do not differ in their  $\delta^{13}C$  values (Fig. 4) – the isotope bi plot shows that they are different due to  $\delta^{15}N$ , but this is different than what is being shown in the NMDS plot, where the FA of all sources differ strongly based on the multivariate FA signatures. But what if the limpets are also supported by other resources that are not well characterized by the sampling done here, or that the isotopic values of those resources is more variable through time? FA are known to provide much finer taxonomic discrimination between sources of primary productivity (there are several papers about this), whereas isotope values depend on environmental conditions and growth rates of the producers themselves. Basically, I would suggest that the authors dig into alternative hypotheses for the differences in FA as well.

6. I think that the discussion should acknowledge that we don't know much if anything about the FA metabolism or FA trophic transfer of the diets into the limpets. This limits the interpretation. The FA biomarkers the authors are referring to are not just tracing different diets but can also be the result of this consumer trophic modification (desaturation, elongation) or selective retention of certain FA for other physiological needs. It would be nice if the authors could suggest more experimental work for these consumers which would help clarify this issue in the future.

#### Specific Comments

Ln. 14. Suggest adding 'slipper limpet' in this first sentence just to make it so that readers don't have to immediately google the genus species to know what the paper is about. Then it will also make more sense when slipper limpets are used below later in the abstract on Ln. 25.

Ln. 56-77. This rather long paragraph may be split. I would suggest adding somewhere in this background/intro to the organism section a statement about the depth range they reside in. Are they intertidal? Subtidal?

Ln. 69. Wow I was unaware that these limpets achieve such high densities! (2000/m<sup>2</sup>)

Ln. 81. Can remove 'as mentioned earlier'.

Ln. 107. I suggest that the authors also describe the benthos of this study site. Is it rocky, cobbles, or sedimentary, etc?

Ln. 116. I like that the authors show how their sampling (red lines) fits within the natural variability of Chl-a at the study site. Most people don't do this.

Ln. 193-196. How long were the samples in the freezer before being lyophilized? How long were the samples in the freezer after being lyophilized before FA extraction? I think this info should be added.

Ln. 198-199. I think it is important to expand upon the methods of Le Grand et al. 2014 to at least explain the basics of how the authors only focused on the neutral lipids in the limpets. I think it makes sense, but the authors should explain the logic of that decision. It is a pretty important distinction that has bearing on the results but it only brought up for the first time much later in the discussion. If there is not a word limit for the journal, a little more detail would be nice. Ln. 326. The differences in branched FA are mentioned... but did the FAME standard have branched FA? How were these FA identified if not? This is why I asked if the authors also used GCMS on some samples.

Ln. 499. It is awkward wording to start this paragraph with 'Besides,....'. I am confused by that. Besides what exactly? I suggest removing this word to be more direct. Also, 18:4w3 is very common in brown algae and kelps. I won't provide a citation because I don't want to imply that that authors should cite a particular paper, but I'd suggest the authors look into the literature on brown algae FA.

I applaud the authors for including their FA data in the supplement. I would suggest including this as a CSV file in addition to these summary PDF tables. It will allow people to download the FA data for future synthesis analyses, which will then also increase the reach of this work through additional citations.

## Reviewed by anonymous reviewer 2, 02 October 2020

The title of this paper, 'Trophic niche of the invasive gregarious species *Crepidula fornicata*, in relation to ontogenic changes' leaves the reader with the impression that there are changes which is misleading as they have demonstrated none. There is no reason to believe (as they state they would expect within stacks) that there would be changes, the gastropod is a filter feeder and that is very well documented – what other mode of feeding could they engage? The discussions in prior works regarding the 'small' individuals refers to very small animals, not the well-developed animals (i.e. with gills) in stacks.

The Abstract clearly states that " the trophic niche of *C. fornicata* does not change significantly across its benthic life" which should have been the expected result. This paper is a classic example of 'collect a lot of data and see if it tells us anything'. It is also common sense that the FA profiles would be different between the males and females and sampling dates.

Abstract: what is 'opportunistic suspension feeding behaviour'? That is their natural feeding mode, they feed upon what is in the surrounding water column!

Overall, this manuscript presents a lot of data – everything they could measure – and no much in the way of synthesis or significance. In essence, it is overkill to make a nonstatement about nonexistent trophic niche differences. There isn't even a clear discussion of why trophic niche differences would or could make a difference to anything tangible. It is also a dangerous practice to 'infer' anything, least of all assimilation of organic material (line 363). Line 429 which states that ... the slipper limpet is an opportunistic suspension-feeder that exploits both pelagic and benthic particulate OM... is well known and this study did not discover that fact. It should have references. FA profiles would obviously be different between males and females and would vary over time, temperature, food availability, season, and other environmental factors.

The manuscript is excessively long and longwinded. There are some interesting data, but as presented it is just a catalog of results, many of them repeated in the discussion. The entire paper reads like a thesis with every possible data point included. It could and should be shortened by half (at least). It is a tedious read and actual results and their significance are difficult to identify.

The references in many instances are 'references of convenience', i.e. what was at hand or cited elsewhere, not the key reference for the statement. Example: Blanchard 1997 is hardly the source for noting that *Crepidula* invasions came from the US.

Minor notes:

Should not begin sentences with Latin names, abbreviations, and never with Latin abbreviations.

Line 32 of the Abstract does not make sense, something is missing.

Line 49 should be as an.

Line 59-60 watch the tenses.

Line 99 – do you mean simulated?

Line 107 Bay.

Line 402 scrapped should be scraped.

Mollusc is with a 'c', no matter what Word says.

I stopped making corrections, the entire manuscript needs a very careful edit.

My overall recommendation is that this paper is not suitable for a mainstream ecological journal as it provides no new or meaningful information regarding niche, trophic transfer or any other general ecological arena. The data presented are all expected and nothing new is presented regarding the role of *Crepidula* in food webs or with regard to their feeding. If the paper was reduced significantly it might be appropriate for a more focused or specialized molluscan journal.