Reviewer´s response of the manuscript "Trophic niche of the invasive gregarious species Crepidula fornicata, in relation to ontogenic changes" Androuin et al. bioRxiv, PCI Ecology

EDITEUR

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.

Cover letter

Dr Matthew Braecken
PCI recommender

Dear recommender,

Below are our point-by-point responses (written in **bold text**) to the queries/comments/suggestions of the two reviewers. The corresponding line number for the text modification refers to the annotated version of manuscript. We also provided a new version of the manuscript, without annotation.

As you suggested, we employed a hypothesis-testing framework, which improved the structure and flow of the manuscript. Following suggestions by reviewer #1, we re-organized part of the introduction and discussion and better described the FA techniques used here. As recommended by reviewers #2, we added scientific background regarding prior works on *C. fornicata* and therefore the ecological interest of studying a possible ontogenic shift in this species. Finally, we removed parts of the discussion to focus on the main findings.

We also made minor amendments in the statistical method, as well as in the way we described the results of FA/pigment concentrations (Figure 3abc). After reorganizing the R script to make it clear and available for data repository, the normality assumption was sometimes violated, so we changed parametric to non-parametric test when appropriate. We therefore changed the corresponding sentences in the results part, without any consequences in terms of interpretations.

We paid special attention to the points and critics made by the reviewer #2 and regret that the tone was somehow inappropriate regarding the work done or the method employed. We understand that differences in carrying out scientific approaches exist, but we received some of his/her remarks as unkind subjective judgments rather than constructive criticisms. We felt that her/his comments did not follow the ”transparent, objective, and fair” line that PCI is promoting.

We hope our revisions are to the satisfaction of both reviewers and you,

Thank you,

Best regards,
Dr. Thibault Androuin

REVIEWER #1:

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

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.

Comment 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.

Reply: We agree that FA technique deserves a more detailed explanation in the introduction and amended this section accordingly. As suggested by the reviewer, we also moved information from the discussion in the introduction.

Text modification at line …: To a lesser extent, fatty acid (FA) compositions can be also specific of group of organisms, such as diatoms, bacteria, copepods or vascular plants (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). FA represents the building stock of most lipid forms. They are energy reserves (neutral lipids) but also key structural component of cell membranes (polar lipids). In molluscs as for *C. fornicata*, such lipids storages are essential for larval development and during mature ontogenic stages for gonadal development (Desloup Paoli et Heral, 1986; Leroy et al., 2013). Contrary to polar lipids,FA incorporated in the neutral fraction are directly mobilized and reflect more closely the FA composition of the diet (Langdon and Waldock, 1981; Jezyk and Penicnak, 1966; Fernandez-Reiriz et al. 2015; Waldock and Nascimento, 1979). Extracting FA from this specific class of lipids and from a tissue with a rapid turnover (e.g., digestive gland, gonad) allows assessing rapid changes in the diet (McCutchan et al., 2003). Recently, the combined use of SI, FA and pigments improved our understanding of trophic pathways from the sources of

particulate OM to benthic primary consumers (Lavaud et al., 2018; Majdi et al., 2018).

Comment 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.
Reply: Unfortunately, we think the manuscript is already long enough with many figures. We hope the reader will refer to internet images to satisfy its curiosity.

Comment 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.
Reply: We added more details about standards utilization. As mentioned in the manuscript, Sigma standard S37 were used, but also PUFA1 and PUFA3 that contain 16C PUFA, and BAME that contain branched FA. Our samples were analyzed in a lipid platform where both GC-FID and GC-MS tools are available, and lab-made standards using characterized (GC-MS) and published samples were also used, for example for NMI FA.

Text modification at line …: FAMEs were identified using two different capillary columns (ZBWAX 30 m × 0.25 mm i.d., 0.25 µm thickness, Phenomenex®; and ZB-5HT 30 m × 0.25 mm i.d., 0.25 µm thickness, Phenomenex®) by means of a standard FAME mix (S37, PUFA1, PUFA3, BAME, Sigma Aldrich®) and other lab-made standard mixtures with previously characterized and published FA composition (e.g., non-methylene interrupted FA, Kraffe et al., 2004; Le Grand et al., 2013)

Comment 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.
Reply: We agree that the first paragraph of the discussion does not fulfill its function, i.e., highlighting the main findings. As suggested by the editor, we adopted a hypothesis-testing framework which better structures the manuscript. We therefore slightly modified the objectives of the manuscript in the introduction and highlight the main findings at the beginning of the discussion.

Text modification at line …: In this study, we investigated the extent of the trophic niche of *C. fornicata* and hypothesized that intra-specific differences exist in diet, associated with ontogenic behavior changes (i.e., motile male to sessile female). We expected that ontogenic trophic shift happen within stacks of *C. fornicata*, with a higher contribution of biofilm to motile males than to sessile males and females. To test our hypothesis, we conducted a field survey and characterized potential OM sources by their SI, FA and pigments compositions and inferred their assimilation in *C. fornicata* tissues using both SI and FA trophic markers.

Text modification at line …: Contrary to our hypothesis, and while the OM sources were well discriminated by their pigments, FA and SI compositions, trophic markers measured in *Crepidula fornicata* suggested an overall similar trophic niche across its ontogenic stages. Our results confirmed that the slipper limpet is an opportunistic suspension-feeder that exploits both pelagic and benthic particulate OM in varying proportions according to the season and sources availability. Although differences in FA composition, and to a lesser extent in SI composition, were noticeable between ontogenic stages at each sampling date, we think they likely reflect ontogenic physiological changes linked to differential growth rate and energetic demand rather than profound changes in diet.

Comment 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 delC values (Fig. 4) – the isotope bi plot shows that they are different due to delN, 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 finder 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.

Reply: As pointed out by the reviewer, and since our data come from the field, we cannot exclude that we may have missed an unexpected food source. However, we paid a great attention to collect the most realistic food sources (i.e., those that actually can be ingested, in terms of size and availability at the sediment-water interface), and to sample them the most adequately. Moreover, when looking at the different trophic markers, differences were mostly due to SFA (greater in motile males) and PUFA (greater in sessile individuals) at the beginning of the survey. As biofilm has globally more 16:0 and 18:0 than SSOM and SPOM at the two first sampling dates as well as higher concentrations of total FA and pigments, one could argue a potential influence of this biofilm on the diet of motile males at this time. However, as 16:0 and 18:0 cannot be considered as specific biomarkers, we think this hypothesis was too

speculative and removed from the discussion. Macroalgae was also proposed as a potential contributor to the diet, but it varies with time and not with ontogeny. As the discussion was already long, we decided to keep the most likely explanation, i.e. the covariation of both SIA and FA across ontogenic stages.

Text modification: none

Comment 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.
Reply: We agree that there is still a lack of knowledge about FA metabolism and FA trophic transfer in limpet as for in marine invertebrates in general. As we worked here on neutral lipids and a single species, we think this kind of bias linked to physiological regulation should not have hampered our main findings. Nevertheless, in order to keep in mind this potential shortcoming, we added a few sentences in the discussion arguing the lack of knowledge and the need for more experimental work dedicated to limpet.

Text modification at line …: FA dietary biomarkers in consumers are subject to physiological regulation (e.g., specific retention, *de novo* synthesis) during uptake and trophic transfer, and can be species-specific (Galloway and Budge, 2020). Although the use of neutral lipids theoretically limits this shortcoming, dedicated experimental studies on FA metabolism and FA trophic transfer in marine limpet specifically are still needed (Zhukova 2019; Galloway and Budge, 2020).

Specific Comments

Comment 7: 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.
Reply: we agree and added the term slipper limpet.

Comment 8: 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?
Reply: The paragraph has been split as the depth range.

Text modification at line …: The slipper limpet *Crepidula fornicata* is a non-indigenous and invasive gastropod originating from the East coast of the US (Hoagland, 1985). This species lives in the infralittoral zone but can be found from intertidal down to 60 m depth. It has extensively colonized shallow soft bottom

habitats of European coasts, from Norway to the Mediterranean Sea (Blanchard, 1997).

Comment 9: Ln. 69. Wow I was unaware that these limpets achieve such high densities! (2000/m2)
Reply: yes there are!

Comment 10: Ln. 81. Can remove 'as mentioned earlier'.
Reply: "as mentioned earlier" has been removed.

Comment 11: Ln. 107. I suggest that the authors also describe the benthos of this study site. Is it rocky, cobbles, or sedimentary, etc?
Reply: we added some information about the substrate in the study site.

Text modification: The Bay of Brest (Brittany, France) is a 180 km² semi-enclosed marine ecosystem. The sampling site is located near the Elorn estuary (48°23'N, 4°23', average depth: 10 m) in a dense *C. fornicata* beds (~2000 ind. m^{-2}) (Guérin, 2004) dominated by gravelly mud sediment (Gregoire et al., 2016).

Comment 12: 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.
Reply: thank you!

Comment 13: 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.**
Reply: we added the duration of these two steps.

Text modification at line …: Samples were stored at -80°C during a few days before freeze drying.

Text modification at line …: Immediately after freeze-drying, tissues of *C. fornicata* were powdered and subsampled for FA analyses: between 2 and 20 mg - depending on ontogenic stages - were put in glass tubes (previously heated for 6 h at 450°C) containing 6 mL of a chloroform/methanol mixture (2:1, v:v), and extracted with a Dounce homogenizer.

Comment 14: 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.

Reply: We addressed these 2 comments above. Please refer to the justifications at comment 1 and 3, respectively.

Comment 15: 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.
Reply: We agree about the awkward wording. We rephrase the sentence and include the possibility of brown macroalgae to inter the diet of *C. fornicata*.

Text modification at line …: Finally, the contribution of FA 18:4n-3 increased over time for all ontogenic stages. According to the literature, this FA may originate from different primary producers such as dinoflagellates (Budge and Parrish, 1998), brown or green macroalgae (Fleurence et al., 1994; Kelly and Scheibling, 2012). Considering i) the absence or low temporal variation of other dinoflagellate biomarkers (peridinin pigment and 22:6n-3 FA) in the OM sources, ii) the presence of brown algae on the rocky shore, and iii) the frequent seasonal accumulation of green macroalgae in our study area (Study Centre for Algal Promotion, http://www.ceva.fr; Ragueneau et al. 2018), we can expect a seasonal trophic role of these macroalgae for *C. fornicata* at our study site, probably in the form of detrital particles.

Comment 16: 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.
Reply: Thank you! But in accordance with PCI requirements, all the data (and R script) are available in a repository.

REVIEWER #2:

Comment 1: 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.
Reply: We disagree. We investigated the trophic niche of *C. fornicata* at different ontogenic stages, and about the title as it is do not give any additional information to the reader. The fact that the trophic niche is possibly changing according to ontogenic changes is not suggested.
Alternatively, we could rephrase the title as: "No trophic ontogenic changes in the invasive gregarious species *Crepidula fornicata*". However, we feel that using the negative form overlooked an important part of the discussion and may ultimately lead to less visibility of the work.

Text modification: none

Comment 2: 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.

Reply: We don't agree with this remark as we think there are several reasons (listed below) to believe that the mode of feeding of young individuals could be different (at least partially) to those of adults. While the filter-feeding mode is well documented for adult stage, very little has been done on the juvenile stage. Hence, following the suggestion of the editor, we make more clearly the hypothesis that an ontogenic difference in diet may exist for C. fornicata, with a higher contribution of biofilm to motile males than to sessile males and females. To better support this hypothesis, we first mention the field observations by Breton and Huriez (2010) who found that isolated adults grazed their surrounding habitat (indeed, they found diatoms frustules that matched well with the diatoms living on the grazed area). These authors also found a small individual (likely motile male) on the same substrate but without sampling it. We make more clear in the revised version of the manuscript that motile males sampled for this study were collected on stacks of *Crepidula fornicata*, whatever their position among other individuals (at the apex of these stacks, or not). Even if we only considered stacked individuals we think that what is possible for isolated individuals should be true for stacked ones. We also mentioned the study by Yee and Padilla (2015) who found a more important role of the radula in smaller snails: "This small radula to body length ratio would be expected if the radula is not used for grazing, except possibly in smaller snails". In their study, "smaller snails" refer to sizes < 12.5 mm, which correspond to our small categories (10 ± 1 mm). Finally, in Shumway et al. (2014), it appears that the relative contributions of the radula to the feeding behavior of different life stages of *C. fornicata* are not as well understood as expected (Shumway et al. 2014).

Text modification at line …: Such a shifting feeding mode have also been suggested for *C. fornicata* albeit without further behavioral evidence nor quantitative measurements. Using stomach content analysis, isolated adults of *C. fornicata* have been found to graze their surrounding habitat (Breton and Huriez, 2010). Younger individuals were also observed near grazing tracks but not analyzed. By comparing the radula to body length ratio, Yee and Padilla (2015) found a more important role of the radula in small individuals (< 12.5 mm) and suggest a potential grazing behavior for those individuals. So, the relative contributions of the radula to feeding in different life stages of *C. fornicata* remain unclear (Shumway et al., 2014).

Text modification at line …: Smallest males were not necessarily found at the apex of the stack, fitting with the fact that they were fully motile and therefore potentially able to graze the substrate around them.

Comment 3: 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.

Reply: Yes, it is one of the main results of this work, which was not *a priori* expected to our mind. Please refer to the comment 2 for justifications.

Text modification: none

Comment 4: This paper is a classic example of 'collect a lot of data and see if it tells us anything'.
Reply: no comment

Comment 5: It is also common sense that the FA profiles would be different between the males and females and sampling dates.
Reply: We are not sure to fully understand the point raised by the reviewer with this comment. FA profiles may vary with intrinsic factors (e.g., physiology) and extrinsic factors (diet), and this is precisely the point of this study. We aimed at investigating the FA variations due to the trophic niche while minimizing potential variations due to physiological changes. For example, we used neutral lipids instead total lipids that best reflect the dietary lipids, we analysed the visceral mass because this tissue has higher turnover rate and thus reflect a smaller temporal window, and we correlate our findings with the most likely food sources. Finally, we discussed the limits of this technique and highlight possible factors that may influence our findings (i.e., gonadal development, energy storage, temperature). Looking at the literature in the same topic, this is also the aim of most of publications in ecology using FA analysis.

Text modification: none

Comment 6: Abstract: what is 'opportunistic suspension feeding behaviour'? That is their natural feeding mode, they feed upon what is in the surrounding water column!
Reply: Suspension feeding is a very diversified feeding mode, including passive and active suspension feeders, generalist and specialist (Riisgard and Larsen, 2010; Ward and Shumway, 2004). So, even if suspension feeders feed upon what is in the surrounding water column, they do not all in the same manner. An opportunist suspension feeder reflects the composition of its environment (in our case organic matter coming from both the water column and interface bottom layer), without active selection (using specific organs like labials palps in mussels) but depending on its retention ability (e.g., cirri size for barnacles or mucous net for *Crepidula fornicata*). This term is defined at line … "The fact that *C. fornicata* lacks pre-ingestive mechanisms for particle selection likely explains their opportunistic trophic behaviour based on both fresh and detritic organic matter (Beninger et al., 2007). "

Text modification: none

Comment 7: 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.
Reply: Here again, we think this is an undue exaggerated criticism of the soundness of our objective. We do not understand why the nonexistence of trophic niche

difference is so obvious to the reviewer. We made a hypothesis supported by preliminary work (e.g., Yee and Padilla, 2015; Chapparo et al., 2002 for the role of the radula in young *Crepidula* sp.; Breton and Huriez, 2010 for field evidence of grazing by adult of *C. fornicata*; Androuin et al., 2018 for the stimulation of microphytobenthic biofilm on living shells; de Montaudouin and Accolla 2018 for the facilitation process through substrate availability). We tested this hypothesis and recognised that it was not verified. We think our main conclusion is a negative result, not an overkill. Please refer to the following reference discussing on the importance of publishing negative data (Fanelli 2012, doi.org/10.1007/s11192-011-0494-7; Nimpf and Keays, 2020 doi.org/10.15252/embr.201949775).

Text modification: none

Comment 8: There isn't even a clear discussion of why trophic niche differences would or could make a difference to anything tangible.
Reply: We agree that this part of the introduction was not discussed properly. We added information about the advantages of grazing for young limpet, and a sentence to mention the consequences of ontogenic trophic niche differences for *C. fornicata* population dynamics and the surrounding habitat.

Text modification at line …: In winter, when food is less available in the bay of Brest for suspension-feeders (Lavaud et al., 2018; Chatterjee et al., 2013), grazing may help young slipper limpet to avoid intraspecific competition for food. Moreover, *C. fornicata* often proliferates on muddy and turbid habitats with high suspended inorganic load, thus grazing behavior could also prevent the overloading of their digestive tract with inert matter of low nutritional quality (Navarro and Chaparro, 2002). Investigating the relationship (facilitation *vs*. competition) between age classes in a fecund invasive species like *C. fornicata* is crucial to better understand its population dynamic and so the consequences for the surrounding habitat.

Comment 9: It is also a dangerous practice to 'infer' anything, least of all assimilation of organic material (line 363).
Reply: we do not understand what the reviewer meant by "dangerous practice to infer anything". Stable isotope and FA analysis are well known and widely used techniques to infer assimilation of organic matter in the field (see the many references in the introduction). In this paper every "inference" from these techniques was supported by appropriate references. So please give example of such dangerous practice.

Text modification: none

Comment 10: 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.
Reply: We disagree with the reviewer on that point. Study looking at the assimilated organic matter of *Crepidula fornicata* are numerous but they almost all used the stable isotope technique (see references in the introduction). However, in a recent study

(Androuin et al., 2019) we found inorganic carbonates in *C. fornicata* that biased these results, especially for adults. Considering this potential mistake, we think that re-evaluating the trophic ecology of this invasive species has an interest. Moreover, Dubois et al. (2014) and Leroy et al. (2103) were cited as references for FA results since they are the only references on the trophic ecology of *Crepidula fornicata* using FA. In their study, they used total lipids to investigate the diet of *C. fornicata*, whereas we used neutral lipids here, which are more relevant to study diet. Dagorn et al. (2014) also did FA analysis but not to investigate the trophic ecology.

Text modification: none

Comment 11: FA profiles would obviously be different between males and females and would vary over time, temperature, food availability, season, and other environmental factors.
Reply: The reviewer already made the same comment (see above comment #5). We think it is not 'obvious' that FA profiles vary with sex, time, temperature, food, season… For example in Beninger and Stephan (1985, https://doi.org/10.1016/0305-0491(85)90372-4**) FA profiles of two clams species reared in a common habitat were similar for both triacylglycerols (neutral FA) and phospholipids (polar FA). Please also refers to Morris (1973,** https://doi.org/10.1017/S0025315400056617**) to see that difference in FA composition can be species specific**.
However, as mentioned above in the comment 5, FA profiles may vary with biotic and abiotic factors, and this is the point of this study, investigating these variations and understand why it varies.

Text modification: none

Comment 12: 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.
Reply: We fully agree that the manuscript was too long and that some results could be removed to improve the clarity of the message. We removed parts of the discussion (20%) and rephrase some sentences to lighten the text.

Comment 13: 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.
Reply: We agree that some of the cited references were not the original works. We therefore changed Blanchard (1997) by Hoagland (1985) that used genetic to prove that *C. fornicata* was coming from the US. We also replace the review of Dalsgaard (2003) by the original works. We verified all other references and we did not find any other references of convenience.

Text modification at line …: (Blanchard, 1997) was changed by (Hoagland, 1985).

Text modification at line …: (Dalsgaard et al., 2003) was changed by (Langdon and Waldock, 1981; Jezyk and Penicnak, 1966; Waldock and Nascimento, 1979; Fernandez-Reiriz, 2015)

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.

Comment 14: I stopped making corrections, the entire manuscript needs a very careful edit.
Reply: the manuscript is now entirely corrected by a certified English editor

Comment 15: 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.
Reply: Although our hypothesis (ontogenic trophic shift) was not verified, we think our manuscript still provides new and meaningful information's regarding trophic environment of the Bay of Brest and the trophic ecology of the invasive species *Crepidula fornicata*. It definitely brings a new insight to the existing knowledge.
First, we showed that stable isotopes, fatty acids and pigments were complementary tools to described three organic matter sources, which is rarely done as a coupled approach in the litterature. We make special attention at performing an adequate sampling of these sources. Subtidal microphytobenthos (on hard and soft bottom) is often suggested to contribute for a large part of subtidal benthic fauna diet, but without being sampled (Grall et al., 2006; Lavaud et al., 2018). As the Bay of Brest is subject to strong anthropic pressure, it is obvious that these data can be extrapolated to other systems and will be of interest for a large scientific community.
Second, we describe the temporal dynamics of trophic niche of an important invasive species that has strong impacts on benthic food webs. Although many stable isotope data were available for this species, a new assessment was needed since the discovery of biochemical bias in these studies (Androuin et al., 2019). Regarding fatty acids data, our study is the first, to our knowledge, that focus on the trophic ecology using this accuracy, i.e., high frequency sampling during the spring period, using specific tissue with a high turnover rate and a lipid fraction that better reflect the diet). The fact

that there was no trophic shift with ontogenic changes in *Crepidula fornicata* in our study site is still a valuable contribution to the ecology of this species. It suggests that juveniles could be food-limited by the adults during low food availability, especially in winter when young limpets are likely in poor condition. This result support the idea of intraspecific competition that impact the recruitment and the population dynamic of this invaders (de Montaudouin et Accolla, 2018).