

## Influence of water content on mechanical behaviour of gastropod taenioglossan radulae

Wencke Krings, Alexander Kovalev and Stanislav N. Gorb

### Article citation details

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### Review timeline

Original submission: 18 September 2020

1st revised submission: 21 December 2020

2nd revised submission: 3 February 2021

Final acceptance: 4 February 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

## Review History

### RSPB-2020-2324.R0 (Original submission)

#### Review form: Reviewer 1

##### Recommendation

Major revision is needed (please make suggestions in comments)

**Scientific importance: Is the manuscript an original and important contribution to its field?**

Excellent

**General interest: Is the paper of sufficient general interest?**

Good

**Quality of the paper: Is the overall quality of the paper suitable?**

Good

**Is the length of the paper justified?**

Yes

**Should the paper be seen by a specialist statistical reviewer?**

No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**

No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**

N/A

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

### **Comments to the Author**

Review RSPB-2020-2324: Influence of water content on mechanical behaviour of gastropod radulae

General comments.

This is a very useful and needed scientific contribution to the field of functional morphology of the molluscan radulae. I highly appreciate that the authors decided to do material tests under wet and dry conditions, which is rarely seen in such rigorous and methodologically innovative manner. Therefore, I recommend this paper for publication. Mostly, I have some minor points about the structure and some missing information in the Materials & Methods that I hope the authors consider improving the readability. These are points that can easily be added/adjusted/re-sorted to improve structure, clarity and the scope of the manuscript. In addition, some results are mixed with interpretations and, in the results section I found some minor inconsistencies in the figures, which easily can be sorted as well. Please see my detailed comments below.

Detailed comments

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Throughout: minor grammatical errors, particularly with pluralisation and tense. Engaging a proof-reader might be useful to help iron these out.

References:

Lauder, G.V. 1995. On the inference of function from structure (in) J.J. Thomason (ed) Functional morphology in vertebrate paleontology, 1-18, Cambridge University Press

## Decision letter (RSPB-2020-2324.R0)

06-Nov-2020

Dear Ms Krings:

I am writing to inform you that your manuscript RSPB-2020-2324 entitled "Influence of water content on mechanical behaviour of gastropod radulae" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Reviewers and the Associate Editor were not convinced that this was a paper of interest to a broad audience of biologists, rather than gastropod/mollusc specialists. Revisions need to make this more convincing, if possible.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

- 1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
- 2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
- 3) Line numbers in your main document.
- 4) Data - please see our policies on data sharing to ensure that you are complying (<https://royalsociety.org/journals/authors/author-guidelines/#data>).

To upload a resubmitted manuscript, log into <http://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Please note that this decision may (or may not) have taken into account confidential comments.

In your revision process, please take a second look at how open your science is; our policy is that \*ALL\* (maximally inclusive) data involved with the study should be made openly accessible, fully enabling re-use, replication and transparency-- see:

<https://royalsociety.org/journals/ethics-policies/data-sharing-mining/>

Insufficient sharing of data can delay or even cause rejection of a paper.

Full data and code/scripts to enable reuse/replication/repurposing are what this policy intends. It has been noted in review that these data are lacking, and that must be amended if the paper is to be considered further.

Sincerely,

Dr John Hutchinson, Editor

mailto:proceedingsb@royalsociety.org

Associate Editor

Board Member: 1

Comments to Author:

Thank you for the opportunity to read this study. Herein the authors examine the influence of water content on the mechanical behaviour of gastropod radulae by applying material testing approaches under both dry and wet conditions. These experiments are systematically conducted and provide novel data, although I defer to the judgement of the expert reviewer (which is largely positive) on exactly how important this data is to our understanding of gastropod biology. As a non-gastropod worker, I did find parts of the manuscript difficult to follow, and often where attempts were made to emphasise the importance of the results it wasn't immediately clear to me why or exactly how important they actually were. Both reviewers have similar criticisms and recommend major editorial changes to the manuscript, which I agree with. The authors should also make their raw data available with the paper to improve reproducibility and examination of the results by reviewers/readers.

Reviewer(s)' Comments to Author:

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References:

Lauder, G.V. 1995. On the inference of function from structure (in) J.J. Thomason (ed) Functional morphology in vertebrate paleontology, 1-18, Cambridge University Press

## Author's Response to Decision Letter for (RSPB-2020-2324.R0)

See Appendix A.

RSPB-2020-3173.R0

## Review form: Reviewer 1

### Recommendation

Accept as is

**Scientific importance: Is the manuscript an original and important contribution to its field?**

Good

**General interest: Is the paper of sufficient general interest?**

Good

**Quality of the paper: Is the overall quality of the paper suitable?**

Good

**Is the length of the paper justified?**

Yes

**Should the paper be seen by a specialist statistical reviewer?**

No

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**

No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**

Yes

**Is it clear?**

Yes

**Is it adequate?**

Yes

**Do you have any ethical concerns with this paper?**

No

### Comments to the Author

Congratulations to the authors. The manuscript was improved a lot. The authors did a very good job and revised all points I addressed in my review. I highly recommend the manuscript for publication.

## Review form: Reviewer 3

### Recommendation

Accept with minor revision (please list in comments)

**Scientific importance: Is the manuscript an original and important contribution to its field?**  
Acceptable

**General interest: Is the paper of sufficient general interest?**  
Good

**Quality of the paper: Is the overall quality of the paper suitable?**  
Acceptable

**Is the length of the paper justified?**  
Yes

**Should the paper be seen by a specialist statistical reviewer?**  
Yes

**Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.**  
No

**It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.**

**Is it accessible?**  
Yes

**Is it clear?**  
N/A

**Is it adequate?**  
N/A

**Do you have any ethical concerns with this paper?**  
No

#### **Comments to the Author**

The manuscript by Krings et al. presents results from biomechanical experiments on the radulae of *Spekia zonata* (Woodward, 1859). This study focuses on the endurance of teeth under dry and wet conditions; and follows the previous works of the main author on this species:

Krings, W., Kovalev, A., Glaubrecht, M. Gorb, S.N., 2019. Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. *Zoology* 137, 125713;

Krings, W., Marcé-Nogué, N., Karabacak, H., Glaubrecht, M., Gorb, S.N., 2020a. Finite element analysis of individual taenioglossan radular teeth (Mollusca). *Acta Biomaterialia*, 115, 317-332;

Krings, W., Brütt, J.-O., Gorb, S., Glaubrecht, M. 2020b. Tightening it up: Diversity of the chitin Anchorage of radular teeth in paludomid freshwater gastropods. *Malacologia* 63(1), 77-94.

The manuscript is well written, and the chosen methods are appropriate. I have only minor concerns regarding phrasing, illustration and references. Somewhere word choice looks not very proper for me, and the entire text needs grammar revision (please, see the attached pdf file).

Some specific comments:

Lines 119, 121 – How did you determine the feeding position of the radular teeth? As I have read in your previous works, you only suppose the position. Please, mention it in these sentences.

I personally recommend examining the odontophore under SEM (as shown for, e.g., *Clione limacina* (Phipps, 1774) in Vortsepneva, 2020) for future research. Anyway, it is not necessary for this manuscript, as this does not affect the present results.

Lines 168-172 – Teeth show gradients... – this sentence seems to fit more in the discussion.

Figure 1 – is the tooth membrane a synonym of the radular membrane? If so, please use 'radular membrane' as it is a generally accepted term.

Figure 1b – the areas of broken teeth are marked not only by letters but also by the dashed ellipses. Also, I recommend using white contour instead of the red one, as it will look more contrast both in online and printed versions.

Figure 1c – please, use whole terms instead of abbreviations in figure description (central tooth instead of CT, etc.) as it is a bit confusing for me.

Figure 1d – what do the dashed borders mark?

As a morphologist I also think that this manuscript will benefit if you add an SEM picture with the painted broken area as you describe in Material and Methods. Probably, you may divide the Figure 1 in two with the first one illustrating methods (experimental set-up); and the second one illustrating results (intact radulae, radulae with the painted broken area, diagrams, etc.)

Tables – Could you use small 'n' instead of the capital one for the number of experiments not to confuse it with 'N' used as a force unit?

Table 1 – What do you call an experiment in your study? Is it a single measurement of the force?

Also, please, check your in-text references and reference list:

Bendsoe, 1989; 1995; Bendsoue and Kikuschi, 1988; Hickman, 1984; Thompson and Czernuszka, 1995 are not present in the list. In the text Andersen, 1996 should be Andersen et al., 1996, as well as Vincent and Wegest, 2004 should be Vincent and Wegst, 2004.

Reference:

Vortsepneva E.V. (2020). Radula morphology of *Clione limacina* (Phipps, 1774) (Gastropoda: Heterobranchia: Gymnosomata) // *Invertebrate Zoology* 17(3): 291–309

## Decision letter (RSPB-2020-3173.R0)

01-Feb-2021

Dear Ms Krings

I am pleased to inform you that your manuscript RSPB-2020-3173 entitled "Influence of water content on mechanical behaviour of gastropod taenioglossan radulae" has been accepted for publication in *Proceedings B*. Congratulations!!

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into <https://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Before uploading your revised files please make sure that you have:

- 1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".
- 2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.
- 3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

- 4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

- 5) Data accessibility section and data citation

It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository (<https://royalsociety.org/journals/authors/author-guidelines/#data>).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:

- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such



as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (<http://datadryad.org/>) and have not already done so you can submit your data via this link

[http://datadryad.org/submit?journalID=RSPB&manu=\(Document not available\)](http://datadryad.org/submit?journalID=RSPB&manu=(Document+not+available)) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see <https://royalsociety.org/journals/ethics-policies/data-sharing-mining/> for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit <https://royalsociety.org/journals/authors/author-guidelines/>.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

Dr John Hutchinson, Editor

mailto:proceedingsb@royalsociety.org

Associate Editor

Board Member

Comments to Author:

Thank you to authors for considering the comments in the original reviews and addressing them in this resubmission. The reviewers of this version are largely happy with the resubmitted manuscript, but do recommend some minor revisions.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).

Congratulations to the authors. The manuscript was improved a lot. The authors did a very good job and revised all points I addressed in my review. I highly recommend the manuscript for publication.

Referee: 3

Comments to the Author(s).

The manuscript by Krings et al. presents results from biomechanical experiments on the radulae of *Spekia zonata* (Woodward, 1859). This study focuses on the endurance of teeth under dry and wet conditions; and follows the previous works of the main author on this species:

Krings, W., Kovalev, A., Glaubrecht, M. Gorb, S.N., 2019. Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. *Zoology* 137, 125713;

Krings, W., Marcé-Nogué, N., Karabacak, H., Glaubrecht, M., Gorb, S.N., 2020a. Finite element analysis of individual taenioglossan radular teeth (Mollusca). *Acta Biomaterialia*, 115, 317-332;

Krings, W., Brütt, J.-O., Gorb, S., Glaubrecht, M. 2020b. Tightening it up: Diversity of the chitin Anchorage of radular teeth in paludomid freshwater gastropods. *Malacologia* 63(1), 77-94.

The manuscript is well written, and the chosen methods are appropriate. I have only minor concerns regarding phrasing, illustration and references. Somewhere word choice looks not very proper for me, and the entire text needs grammar revision (please, see the attached pdf file).

Some specific comments:

Lines 119, 121 – How did you determine the feeding position of the radular teeth? As I have read in your previous works, you only suppose the position. Please, mention it in these sentences. I personally recommend examining the odontophore under SEM (as shown for, e.g., *Clione limacina* (Phipps, 1774) in Vortsepneva, 2020) for future research. Anyway, it is not necessary for this manuscript, as this does not affect the present results.

Lines 168-172 – Teeth show gradients... – this sentence seems to fit more in the discussion.

Figure 1 – is the tooth membrane a synonym of the radular membrane? If so, please use ‘radular membrane’ as it is a generally accepted term.

Figure 1b – the areas of broken teeth are marked not only by letters but also by the dashed ellipses. Also, I recommend using white contour instead of the red one, as it will look more contrast both in online and printed versions.

Figure 1c – please, use whole terms instead of abbreviations in figure description (central tooth instead of CT, etc.) as it is a bit confusing for me.

Figure 1d – what do the dashed borders mark?

As a morphologist I also think that this manuscript will benefit if you add an SEM picture with the painted broken area as you describe in Material and Methods. Probably, you may divide the Figure 1 in two with the first one illustrating methods (experimental set-up); and the second one illustrating results (intact radulae, radulae with the painted broken area, diagrams, etc.)

Tables – Could you use small ‘n’ instead of the capital one for the number of experiments not to confuse it with ‘N’ used as a force unit?

Table 1 – What do you call an experiment in your study? Is it a single measurement of the force?

Also, please, check your in-text references and reference list:

Bendsoe, 1989; 1995; Bendsoue and Kikuschi, 1988; Hickman, 1984; Thompson and Czernuszka, 1995 are not present in the list. In the text Andersen, 1996 should be Andersen et al., 1996, as well as Vincent and Wegst, 2004 should be Vincent and Wegst, 2004.

Reference:

Vortsepneva E.V. (2020). Radula morphology of *Clione limacina* (Phipps, 1774) (Gastropoda: Heterobranchia: Gymnosomata) // *Invertebrate Zoology* 17(3): 291–309

## Author's Response to Decision Letter for (RSPB-2020-3173.R0)

See Appendix B.

## Decision letter (RSPB-2020-3173.R1)

04-Feb-2021

Dear Ms Krings

I am pleased to inform you that your manuscript entitled "Influence of water content on mechanical behaviour of gastropod taenioglossan radulae" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact [procb\\_proofs@royalsociety.org](mailto:procb_proofs@royalsociety.org)

Your article has been estimated as being 8 pages long. Our Production Office will be able to confirm the exact length at proof stage.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit <https://royalsociety.org/journals/ethics-policies/media-embargo> for more information.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Editor, Proceedings B

<mailto:proceedingsb@royalsociety.org>

## Appendix A

Paper title: Influence of water content on mechanical behaviour of gastropod radulae

Reference: RSPB-2020-2324

We are extremely grateful for these two reviews and the notes from the Editorial Office, all comments were helpful and supportive and we are thankful, that researchers invested so much time and effort!

We choose to not only respond by changing our manuscript but to additionally respond to each comment individually. Therefore, in the text below are your comments and our responses. Whilst your comments are marked in blue, we marked all our responses in red. All lines given refer to the latest word document with all changes revealed.

Comments:

Editorial Office:

Reviewers and the Associate Editor were not convinced that this was a paper of interest to a broad audience of biologists, rather than gastropod/mollusc specialists. Revisions need to make this more convincing, if possible.

Overall, we present a new way to approach the topic of functionality on radulae. So far usually radular tooth shape had been interpreted, but we think that this is rather problematic since mechanical behaviour and material properties needs to be considered. However, teeth are so small that we can do estimation of YM only under dry condition and simulations are – without some kind of validation – only simulations. Overall, we want to establish a workflow for researchers so that the topic of trophic specialization and radular tooth functionality is more accessible.

Associate Editor Board Member: 1

Comments to Author:

Thank you for the opportunity to read this study. Herein the authors examine the influence of water content on the mechanical behaviour of gastropod radulae by applying material testing approaches under both dry and wet conditions. These experiments are systematically conducted and provide novel data, although I defer to the judgement of the expert reviewer (which is largely positive) on exactly how important this data is to our understanding of gastropod biology. As a non-gastropod worker, I did find parts of the manuscript difficult to follow, and often where attempts were made to emphasize the importance of the results it wasn't immediately clear to me why or exactly how important they actually were. Both reviewers have similar criticisms and recommend major editorial changes to the manuscript, which I agree with. The authors should also make their raw data available with the paper to improve reproducibility and examination of the results by reviewers/readers.

We hope that the manuscript has improved. We are grateful for the comments, thank you for your time. The data is available under <https://doi.org/10.5061/dryad.2jm63xsnj>.

**Reviewer(s)' Comments to Author:**

**Referee: 1**

General comments.

This is a very useful and needed scientific contribution to the field of functional morphology of the molluscan radulae. I highly appreciate that the authors decided to do material tests under wet and dry conditions, which is rarely seen in such rigorous and methodologically innovative manner. Therefore, I recommend this paper for publication. Mostly, I have some minor points about the structure and some missing information in the Materials & Methods

that I hope the authors consider improving the readability. These are points that can easily be added/adjusted/re-sorted to improve structure, clarity and the scope of the manuscript. In addition, some results are mixed with interpretations and, in the results section I found some minor inconsistencies in the figures, which easily can be sorted as well. Please see my detailed comments below.

**Thank you so much for your supportive review, we tried to address as many points as possible.**

#### Detailed comments

##### Abstract

p. 2, l. 11: case insensitivity – molluscan instead of Molluscan

**Changed accordingly.**

##### Introduction

l. 58-66: This is a super-long sentence(s) and it contains unspecific wording. Please clarify and be more specific. It is not clear what is meant with the very broad term “mechanical behaviors”. It would be helpful to specify which mechanical behaviour in particular is meant and important for the selected taxon. Further, it would be helpful to improve the structure of the manuscript towards more clarity and focus of the study (for example introduce research aims or hypotheses (sorted / labelled) to be more specific and focused regarding the aims of the study (for example: why it is utterly necessary to develop experimental setups? What ecological advantage would the animal have? How knowledge about breaking structures (of which tooth positions) could be translated into durability and resistance or even tooth wear? The authors have published already innovative work about function of the molluscan radula to build on and I would encourage the authors to help the readership a little more to follow their argumentation. The authors tried to address this aspect already at l. 74-78 in a broad sense, and it would be important to focus even more and in particular on the selected taxon. Further it would allow the reader to better see the gap of knowledge the authors aim to fulfil.

**We reworded the introduction to clarify the research approach. We have a problem with receiving wet YM data, so far we were unable to obtain such data since we were unable to create a parallel radular tooth sample (nanoindentation on such small teeth is actually overall quite problematic and very prone to artefacts, that’s the reason why we embedded them and needed a large sample size in previous studies). Additionally, when we submitted the FEA on *Spekia* reviewers had doubts if these simulations are even related to reality. Therefore we established these experiments; and they correspond to the results from the FEA and fortunately also to the hypotheses derived from experiments with dry YM. We actually want to establish this experimental set-up first and in the next step we will include more species from the Lake Tanganyikan flock with distinct ecological guilds (soft-substrate feeder, solid substrate feeder, mixed substrate feeder).**

l. 77: “allowing hypotheses...” – Unclear what exactly is meant, please specify / re-word; optionally you could introduce hypotheses.

**Changed accordingly.**

##### Material and Methods

l. 87-88/124: Six specimens were sampled; only one collection number is given ZMH 154652/999, but in Figure 1.a. ZMH 150008/999-2 is mentioned. Please refer to the complete collection numbers and add which specimens have been tested under wet and which under dry conditions.

**Changed accordingly.**

l. 94 Epoxy instead of Epoxi

**Changed accordingly.**

l. 111-112: since experiments were documented visually – Please clarify, if movies available, please add in a supplement.

I am sorry, I started documenting it for *Lavigeria nassa* and *Lavigeria grandis*, but quality was extremely low. I can submit the movies for these two species with the next manuscript that includes all tested Lake Tanganyikan species.

l. 114: breaking area was determined – please clarify, if extra software was used.

Changed accordingly.

Results:

l. 138: membrane ripping – please specify in Fig. 1c where exactly is the membrane that is meant.

Changed accordingly. This is the thin membrane embedding and holding the teeth.

l. 142: “Breaking force depends on the mechanical behaviour... and the water content.” Please re-word; this sentence is a conclusion / interpretation and would be better placed at the discussion. In addition and strictly spoken, that has not been tested in this experiment; it would require a statistical model, where you would test for example water content as a response variable to breaking force and include tooth position as dependent variable. Yet, you did ANOVA and that allows reporting on the group differences.

Changed accordingly.

l. 146: ANOVA and other test results: the output of both is missing; please add (for example as a supplement).

Changed accordingly. We added the results from ANOVA as separate Table. The connecting letters from Tukey-Kramer test are in Fig. 1e, above the boxplots.

l. 158: “The breaking stress ...depends on...” same as l. 142. Please re-word; this sentence is a conclusion and works better in the discussion.

Changed accordingly.

l. 169: “Hardness and Young’s modulus...are strongly correlated”. Same as l. 142; Please re-word or add a correlation test / model. Strictly spoken, that has not been tested in this experiment.

We rephrased the sentence.

Discussion

l. 173-182 (first paragraph): Please excuse my direct criticism, but I miss a clear argumentation line at this paragraph. The general comparison of two measuring conditions of various materials in various animal groups seemed a little arbitrary; please re-word. I highly recommend to focus either on materials with the same function in various animal groups (for example the feeding organs) or on one taxonomic unit (for example invertebrates).

We actually wanted to just present the data in a broader context to show that this principle seems to be universal. For Mollusca there are only 3 references and none of them is directly comparable since e.g. chemical compositions are different. For Mollusca there is so much lack of data.

l. 214 “solid feeding substrate”, l. 216 “obstacle”: At this point you give a good explanation regarding the function of the radula teeth during the scratching action. However, at this point as a reader, I miss some more detailed information regarding the substrate/obstacle. Would it be possible to give more material characteristics of the substrate (particle size, material properties) to relate/discuss the mechanical properties of the radula teeth in relation to the substrate?

Unfortunately we have limited data on the feeding substrate, we know that *Spekia* forages on rocks, however, which kind of rock, the surface structure and sharpness is not known (since we did not receive funding to test Lake Tanganika substrates). Our major concerns regarding

this study was the validation of the FEA. Additionally, we wanted to show, that our YM data – even though testes under dry conditions – still allows to put forward substantial hypotheses on tooth functionality.

l. 218 “to build substantial hypotheses on *Spekia’s* tooth functionality” – this reads as a big advertisement/ announcement. However in the next sentences it is not fulfilled. Contrary to those sentences you just showed what you found, but you are not introducing new hypotheses. Please either do so and propose new hypotheses or rephrase.

We rephrased this part.

l. 222-224: “In the future...molluscan evolution.” This statement appears randomly and unspecific chosen. As a reader I would rather be interested to learn more about the broader implication of what the specific research results would imply for the field of material research / functional morphology / feeding ecology.

Unfortunately studying function of radular teeth and potential trophic specialization in mollusca usually means in past studies looking at the radular tooth shape and deriving hypotheses from this. We do not want to directly criticize other researchers, especially since the functionalities are very difficult to reveal (e.g. teeth are too small, so the direct contact of tooth with ingesta cannot be documented; the precise 3-dimensional shape of radula during foraging and the interplay of the odontophoral cartilages, the buccal mass musculature, the radular membrane, and the tooth rows is not known). We are trying to approach this broad field by experimental set-ups; of course we did these experiments for more species from Lake Tanganyika and we can see distinct mechanical behaviour and resistance to force between soft, mixed, and solid substrate feeders, but we thought that this would be too much for one manuscript.

Figure 1: This is a very nice overview figure. I have only minor comments to improve readability: d) the two graphs have different scales; please adjust to make the signal better visually comparable. e) The red/blue letters are hardly visible; please improve readability, for example by using different colors for the boxplots.

Changed accordingly.

Table 1 and Table 2: In case the authors want to save space for a table with the descriptive statistics of the ANOVA and tests. It would be possible to combine Table 1 and 2 by simply give the Ns from table 2 in Table 2 directly after the SD (N). Further it would be helpful to indicate the meaning of the / (was it not measured, not available or simply not broken?).

We merged these two tables into one and explained the /, thank you so much for this advice.

## Referee: 2

### Comments to the Author(s)

This manuscript compares experimental measurements of breaking stress in wet and dry specimens of snail radulae, and thus offers useful primary data. My comments below appear in the order which they appear in the manuscript. I think there are areas which can be clarified, but mostly I think that where it occurs, the lack of clarity reflects ambiguous word choice or sentence structure, rather than some scientific oversight. My main query refers to identifying whether failure reflects failure of the teeth vs failure in the radular membrane, and the need to expand on both the mechanical and biological details of this distinction.

We are grateful for the supportive review, we hope that we were able to address all of your comments.

L54: “The detection of functional gradients in taenioglossan radular teeth...” Presumably by this you mean gradients in the Young’s modulus (YM) of the teeth? These are not ‘functional’ gradients (sensu Lauder, 1995), they are materials gradients, which probably fall within the

remit of being variants in form (as opposed to function). While this almost certainly will result in differences in performance (indeed, this is supported by your previous FEA study), this is not 1) guaranteed, nor 2) the same as a difference in function.

Changed accordingly.

L56: “However, mechanical property data was achieved by testing...” Because of the ambiguity in the previous sentence, it is unclear which mechanical properties you are referring to. Suggest, “However, the mechanical property data referenced above was obtained by testing...”

Changed accordingly.

L57: “...which is not unproblematic”. You need to explain why, preferably in a new sentence. To be fair, you do this to an extent in the following sentence, but start by explaining a link between YM and performance, only mentioning water at the end. If people don’t already know that water content can affect YM, then this is really unclear. So start by explaining how YM is influenced by water, then say why YM is important in a performance context.

Changed accordingly.

L69: The punctuation here makes the sentence hard to follow, because I think what you’re trying to explain is that stress IS force per unit area, however, your commas make it look like you’re listing these as separate things. I think the force/area to break part should be in parentheses, and then you don’t need all those references about bird and mammal bite forces – they’re not particularly relevant here, and stress is a fairly fundamental physics concept that I think most people would understand without mixing bite force studies in to it. Same with your own radula force paper – it surely will come up later in the methods? Here, it’s just confusing your point.

Changed accordingly.

L79: Take out the word ‘first’

Changed accordingly.

L81: Replace “since,” with “because”

Changed accordingly.

L82: Again, I think you’re overcomplicating your point here, I think it’s enough to say that you chose this species because you have produced an existing body of work on it. Distinctive doesn’t necessarily mean unusual, and you can’t know that something is unusual without a large-scale morphological study; if the tooth’s material properties are already known, why bother testing it? (I presume you mean the dry properties are already known, which is a different question, but that’s not what you wrote).

Changed accordingly.

Edit: I realize now, having finished reading the manuscript, that you never actually test the wet YM material properties. From the introduction, I was kind of expecting that you would. It’s a shame, because these would have been useful data! Was there a reason that you were unable to do this? I appreciate that it’s a difficult experimental set-up, but it would have been interesting to have seen the YM values changing between wet and dry, rather than having to infer their change on the basis of changing breaking stresses.

We are sorry, but we have tried many times to receive wet YM data. But the teeth are too small to break them in such a way that you receive a rather parallel sample. We are not capable of fixing the wet teeth in such a way that they are unable to move during nanoindentation – we therefore have to embed and polish them. We have conducted so far dry nanoindentation on almost 30 gastropod species and detected indications for trophic specializations. Additionally, we performed FEA on 6 more species and were able to combine dry YM with morphology. However, when we submitted the FEA on *Spekia* reviewers had, of



course, doubts, if the simulations are somewhat related to reality, therefore we performed these experiments. The observed mechanical behaviour corresponds to the simulated ones, thus our past approaches seem to be a good way to approach the topic of radular tooth function.

L92: Should say “unwound”

Changed accordingly.

L99: “...radulae were rehydrated with water.” Rehydrated how? By submersion? For how long? What kind of water (filtered, municipal, saline, etc.)

We added this information. It took some time beforehand to get an idea about the time frames when handling of the radula while drying. When dealing with radulae and when you mount them on the SEM stub you usually experience that large water drops attached to radula result in a twisting of the membrane and teeth, potentially due to water surface energy. When the radula is too dry teeth become brittle.

L109: “The needle tip was positioned on the concave part of the tooth cusps at 30° to the horizontal plane. The force corresponding to the teeth/radula failure was determined using software Acq Knowledge”. Presumably in between these, you applied the force? What rate was the force applied at? Many biological materials, especially those with a high water content, exhibit rate-dependent strains (viscoelasticity), so I think it is relevant to include specific information about the way the force was applied to the tooth. In the abstract, you mention a “shear load” applied “stepwise”. You should expand the details of this here.

I am sorry about this uncertainty. We did not apply a certain force, but just moved the needle onto the cusps until failure occurred. The force needed to break structures was documented by Acq Knowledge. We tried to clarify this in the text.

L114: “the average breaking area was determined by measuring 40 areas per tooth type” I don’t understand what you’ve done here. Do you mean the area as is measured in  $\mu\text{m}^2$ , or do you just mean you examined 40 regions per tooth to see where breakage occurred? I assume it’s the latter because I assume that each tooth only failed once (at the point on your graphs (e.g. in Fig. 1d) where the force dropped). I’m confused as to how the needle could have caused simultaneous failure in 40 different locations of which you then measured the area. Or did the failure cascade through adjacent teeth once the initial tooth failed? If so, why not measure all the failed areas? Was 40 the maximum?

Thank you so much for this point. We tried to clarify this in the text.

L115 – 139: Throughout this section it is apparent that the radular membrane often failed (indeed, table 2 implies that the membrane always failed?). This makes me curious about whether the membrane ever failed before the teeth did (which would presumably appear as a drop in force on the graphs but with no evidence of tooth fracture). Are you able to distinguish this in your results and how might it have influenced your calculations of breaking stress?

Edit: to be fair, you actually address this to some extent later on, although I didn’t really understand what you were looking at until I looked at tables 1 and 2, and to be honest, I’m still not sure how you were able to distinguish the force to break the teeth from the force to tear the membrane. Also, the fact that it took me until this point in the results to pick up that sometimes your writing concerns the radula as a whole organ, and sometimes just the individual teeth, suggests that this wasn’t particularly clear from the start. I think that highlighting the fitness consequences of losing one tooth vs losing several rows of teeth, or worse still tearing the membrane, is important biological context. One could reasonably consider the breakage of the membrane to constitute failure of the organ (which

I would assume is far more likely to result in a deleterious condition for the animal than the breakage of just one tooth, given the rate of tooth replacement).

We are sorry, about this confusion. Central and lateral teeth under wet condition did not break, they relied on the adjacent teeth and only failed because the membrane ripped. The dry teeth were brittle, so they broke at their denticles. The marginal teeth however, failed individually in wet and dry condition, they thus are not capable of relying on the adjacent rows and are thus not able to resist to high forces (that's why they rather collect and not scratch). So, overall, for the components probably loosening food (central and lateral teeth), they are – due to their mechanical properties and morphology – able to resist to such high forces, that rather the membrane rips apart than teeth fail.

L163 – 170, and table 3: As far as I can tell, all you have done here is repeat the information from your earlier 2019 paper; this isn't new data or a new result, right? This is just reporting of the dry YM values and their distribution within/among teeth that you've shown before? As such, it shouldn't really be presented here in your results for this work.

We agree and deleted this table.

L176 – 182: Bone too, I believe, although I can't think of the references off the top of my head!

We added references.

L195: Should say "anchors"

Changed accordingly.

L199: Replace "is usually taking place" with "usually takes place"

Changed accordingly.

L201: Should say "finite element analysis", not "Finite-Element-Analysis" – it needs neither hyphens nor capitalization

Changed accordingly.

L204: Replace "the part were the force was exerted to" with "where the force was applied"

Changed accordingly.

L205: Replace "But overall, lateral and central ones" with "Overall, lateral and central teeth" and delete the word "to" later in the sentence

Changed accordingly.

L207: "bent", not "bended"

Changed accordingly.

L214: Replace "ones" with "teeth"

Changed accordingly.

L222: By 'more radular teeth', do you mean more in more species of mollusc?

Changed accordingly. We wanted to establish the procedure by this manuscript. But we performed these experiments already with all kinds of radulae from species with distinct ecological niches. The observed breaking is distinct between e.g. species foraging on rocks, sand, and mixed substrate, but we thought that this might be too much for one single paper.

Fig. 1: Some of the fonts here are really small. I don't expect anybody reads on paper any more, and presume there's a high-res version uploaded somewhere that hasn't been converted to my review PDF, but maybe make the labels a bit clearer? In part e, I don't understand what the red and blue letters refer to. In the caption it says 'homogeneous groups for force/stress', but I don't know what this means.

We are sorry for this confusion, these are the connecting letters from Tukey-Kramer test. We tried to clarify this. We changed the fonts.

Throughout: minor grammatical errors, particularly with pluralisation and tense. Engaging a proof-reader might be useful to help iron these out.

Hopefully we found everything.

References:

Lauder, G.V. 1995. On the inference of function from structure (in) J.J. Thomason (ed) Functional morphology in vertebrate paleontology, 1-18, Cambridge University Press.

## Appendix B

Paper title: Influence of water content on mechanical behaviour of gastropod radulae

Reference: RSPB-2020-2324

We are again extremely grateful for these two reviews and the notes from the Editorial Office, all comments were helpful and supportive. Thank you for your time!

We choose to not only respond by changing our manuscript but to additionally respond to each comment individually. Therefore, in the text below are your comments and our responses. Whilst your comments are marked in blue, we marked all our responses in brown.

### Comments to Author:

Thank you to authors for considering the comments in the original reviews and addressing them in this resubmission. The reviewers of this version are largely happy with the resubmitted manuscript, but do recommend some minor revisions.

Thank you again, we changed the manuscript according to the comments.

### Reviewer(s)' Comments to Author:

Referee: 1

Congratulations to the authors. The manuscript was improved a lot. The authors did a very good job and revised all points I addressed in my review. I highly recommend the manuscript for publication.

Thank you so much for your time and highly helpful comments.

Referee: 3

The manuscript by Krings et al. presents results from biomechanical experiments on the radulae of *Spekia zonata* (Woodward, 1859). This study focuses on the endurance of teeth under dry and wet conditions; and follows the previous works of the main author on this species:

Krings, W., Kovalev, A., Glaubrecht, M. Gorb, S.N., 2019. Differences in the Young modulus and hardness reflect different functions of teeth within the taenioglossan radula of gastropods. *Zoology* 137, 125713;

Krings, W., Marcé-Nogué, N., Karabacak, H., Glaubrecht, M., Gorb, S.N., 2020a. Finite element analysis of individual taenioglossan radular teeth (Mollusca). *Acta Biomaterialia*, 115, 317-332;

Krings, W., Brütt, J.-O., Gorb, S., Glaubrecht, M. 2020b. Tightening it up: Diversity of the chitin Anchorage of radular teeth in paludomid freshwater gastropods. *Malacologia* 63(1), 77–94.

The manuscript is well written, and the chosen methods are appropriate. I have only minor concerns regarding phrasing, illustration and references. Somewhere word choice looks not very proper for me, and the entire text needs grammar revision (please, see the attached pdf file).

Thank you so much! We corrected the text according to your pdf and tried to iron grammatical errors out.

Some specific comments:

Lines 119, 121 — How did you determine the feeding position of the radular teeth? As I have read in your previous works, you only suppose the position. Please, mention it in these sentences.

We added a description for this step.

I personally recommend examining the odontophore under SEM (as shown for, e.g., *Clione limacina* (Phipps, 1774) in Vortsepneva, 2020) for future research. Anyway, it is not necessary for this manuscript, as this does not affect the present results.

The odontophoral cartilage is highly interesting (and the paper as well) and definitely one main structure determining the mechanical behaviour of radula. We have experimented with the radular supporting structure already and hope that we are able to establish a workflow to determine mechanical properties of wet cartilages in the future (it is quite problematic and I am not sure if it is possible). Combining 3D-models and mechanical properties of teeth, of the odontophoral cartilages,

and of the buccal mass in e.g. simulations or in physical models would be ideal.

Lines 168-172 — Teeth show gradients... — this sentence seems to fit more in the discussion.

Changed accordingly.

Figure 1 — is the tooth membrane a synonym of the radular membrane? If so, please use 'radular membrane' as it is a generally accepted term.

Changed accordingly.

Figure 1b — the areas of broken teeth are marked not only by letters but also by the dashed ellipses. Also, I recommend using white contour instead of the red one, as it will look more contrast both in online and printed versions.

Changed accordingly.

Figure 1c — please, use whole terms instead of abbreviations in figure description (central tooth instead of CT, etc.) as it is a bit confusing for me.

Changed accordingly.

Figure 1d — what do the dashed borders mark?

True, this is confusing, we removed them.

As a morphologist I also think that this manuscript will benefit if you add an SEM picture with the painted broken area as you describe in Material and Methods. Probably, you may divide the Figure 1 in two with the first one illustrating methods (experimental set-up); and the second one illustrating results (intact radulae, radulae with the painted broken area, diagrams, etc.)

That is a very good idea, we included a SEM image with painted broken area.

Tables — Could you use small 'n' instead of the capital one for the number of experiments not to confuse it with 'N' used as a force unit?

Changed accordingly.

Table 1 — what do you call an experiment in your study? Is it a single measurement of the force?

Changed accordingly.

Also, please, check your in-text references and reference list:

Bendsoe, 1989; 1995; Bendsoue and Kikuschi, 1988; Hickman, 1984; Thompson and Czernuszka, 1995 are not present in the list. In the text Andersen, 1996 should be Andersen et al., 1996, as well as Vincent and Wegest, 2004 should be Vincent and Wegst, 2004.

Changed accordingly.

Reference:

Vortsepneva E.V. (2020). Radula morphology of *Clione limacina* (Phipps, 1774) (Gastropoda: Heterobranchia: Gymnosomata) // *Invertebrate Zoology* 17(3): 291–309

We have added this reference.