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Shark spiral intestines may operate as tesla valves

Samantha C. Leigh, Adam P. Summers, Sarah L. Hoffmann and Donovan P. German

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Original submission:	11 December 2020
1st revised submission:	14 June 2021
2nd revised submission:	30 June 2021
Final acceptance:	30 June 2021

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

Review History

RSPB-2020-3056.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?
Excellent

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

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?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
General Comments
This is an interesting paper that advances knowledge and consideration about spiral intestines in animals, though I think the abstract needs to do a better job of this (more on that below). The paper provides a useful Table 1 that summarizes many examples of four types of spirals in intestines of many vertebrates and invertebrates. It maps the four varieties of these spiral intestines onto a fish phylogeny and considers the evidence that the columnar spiral intestine morphology is more ancestral. In functional tests, two of the other morphologies that are funnel-shaped were superior in producing unidirectional flow in the anterior to posterior direction, which the authors identify as characteristic of Tesla valves. I considered other claims regarding the functional measurements to be weaker or unremarkable (more on that below).

Comments on functional measurements
Lines 234-238 – It struck me as unremarkable that higher viscosity (which was defined at line 162 as higher resistance to flow) slowed flow. Please explain whether there is a reason why this is remarkable, and how the measurement at different viscosities advances the understanding.

Lines 238-243 – Comparisons of flow rate are made between plastic control tubing, proximal intestine, and spiral intestine. It seems to me that, for these comparisons to be meaningful, a more systematic presentation is in order. To begin, the equation at line 176, which essentially is flow=gradient/resistance, is simplistic, and I could not find the citation in either PubMed or Web of Science. In the simplest, expanded model, flow rate through tubes is influenced by pressure difference (this gradient was measured), and the resistance to flow is influenced by liquid viscosity (measured), and also tube dimensions such as radius and length (not presented). The authors may have measured different resistances with the simple equation, but they do not control for differences in tube length, which is a simple explanation for differences in effective resistance that may have nothing to do with resistance due to the spiral intestine. Also, for these tubes, the radius may or may not be fixed (tissue can be distensible, but this was not considered). This may or may not be a sufficient alternative model, but in any event the unmeasured/unconsidered features need to be factored in, or it must be explained to the reader why they are not important. This is why the first claim at lines 352-355 seems not strictly correct ("flow rate is slowed in the spiral intestine due to the high resistance produced by tissue folds"), and the effective resistances should be presented. However, the second claim at lines 352-355 is supported ("flow rate was slowed significantly more when the two funnel-shaped spiral intestines were subjected to flow in the posterior to anterior direction"). The reason the comparison of flow rate in two directions in the spiral intestine holds is because the spiral intestines were simply reversed and so all those tube features that determine effective resistance were held constant.

Lines 214-218 – The purpose/importance of the measures of muscle contraction should be explained and discussed in the paper. Also, if the proximal intestine should have responded to acetylcholine and it did not, then does the reader presume the tissue was dead? If that is the case, is the spiral intestine also dying, and if so, what is usefully concluded from this experiment? (related – Line 253, 256 are these standard deviations or standard errors?)

Lines 277-279 – The comment above about flow through tubes underscores how more data on the tubes would be useful. If the morphological preparations can be used to measure length, radius, surface area and volume, then present these data.

Comments on the abstract
The paper's title invokes the Tesla valve, and the abstract should mention this, linked to its best functional evidence that "flow rate was slowed significantly more when the two funnel-shaped spiral intestines were subjected to flow in the posterior to anterior direction". The abstract does make the claim that "We quantified the flow rate of material of various viscosities through the spiral intestine, demonstrating that it does slow the rate of digesta transit", though arguably that point is not strongly made without more consideration of factors influencing flow rate (see above). The abstract could state more explicitly the findings from the consideration of "the morphological data in an evolutionary, dietary, and morphological context" (line 45). Finally, the findings don't seem to inspire technological advances so much as they benefit from older, formal understanding of flow through tubes and knowledge about one particular tube design (the Tesla valve).

Minor points
Line 81 and 308 – the reference to Buddington is not in the References
Line 296-297 – this does not seem to be a sentence: "Currents flow along different paths, in different directions, and that these differences have a disproportionate effect on the resistance of the tube.
Lines 342-345 – pept1 is a transporter, but you seem to imply here that it might play a causative role in morphological development of the spiral intestine. Delete or discuss why it is also likely that its developmental appearance is simply correlated with morphological development.

Review form: Reviewer 2

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?
Excellent

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?
No

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

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?
Yes

Comments to the Author
Here the authors test for whether the spiral valve intestine in sharks functions as a tesla valve. They utilize iodine enhanced computed tomography scans and a clever experimental design to test for whether the spiral vales encourage flow in one direction and resist it in another and indeed find evidence to suggest that these intestines work in a similar fashion to tesla valves. The authors also examine the evolution of the spiral valve across sharks and conclude that spiral valve morphology does not closely track diet.

I think that the experimental design and the many of the findings of this paper are interesting and exciting. The authors set up an important question and answered it with elegant simplicity. So, on that end, I don't see very many issues with this part of the manuscript. However, I do believe that the manuscript gets substantially weaker at the phylogenetic comparative method component. Generally, I'm not sure that the authors need it at all. The paper is plenty informative and interesting without it. However, I list some of my concerns below should the authors seek to retain this section.
1) The phylogeny. The authors do not state in the methods where they got their phylogeny from, or what transformations were made from the original phylogeny (e.g. pruning taxa). I also think that the authors should make the phylogeny ultrametric and perform an ancestral state reconstruction for diet and spiral valve morphology.
2) One the subject of diet, the authors do not state where they got their dietary information from.
3) The authors find no significant relationship between diet and valve morphology using a PGLS analysis. I would recommend pairing this analysis with a regular GLS or ANOVA to determine whether or not there is a general relationship (without considering phylogeny) and if this relationship happens to be phylogenetically structured.

Minor point
(Lines 336-349) I don't think that the authors really need the genetic discussion here.

Decision letter (RSPB-2020-3056.R0)

03-Feb-2021

Dear Dr Leigh:

I am writing to inform you that your manuscript RSPB-2020-3056 entitled "Shark Spiral Intestines May Operate as Tesla Valves" 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. Reviewers found the study to be a good potential fit to Proc B, but give constructive critiques that warrant careful attention and substantial revision to the MS itself.

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.

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.
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Sincerely,
Dr John Hutchinson
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Thank you for submitting your manuscript to Proceedings B. Both reviewers comment favourably on the interest of the paper to our readership and the scientific importance to the field. However both also have concerns regarding the comparative data analysis, both from a phylogenetic and a biomechanical standpoint. Please give thought to their suggestions, particularly the ability to control for tube length and/or radius in the flow models.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
General Comments
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morphology is more ancestral. In functional tests, two of the other morphologies that are funnel-shaped were superior in producing unidirectional flow in the anterior to posterior direction, which the authors identify as characteristic of Tesla valves. I considered other claims regarding the functional measurements to be weaker or unremarkable (more on that below).

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Referee: 2
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3) The authors find no significant relationship between diet and valve morphology using a PGLS analysis. I would recommend pairing this analysis with a regular GLS or ANOVA to determine whether or not there is a general relationship (without considering phylogeny) and if this relationship happens to be phylogenetically structured.

Minor point
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Author's Response to Decision Letter for (RSPB-2020-3056.R0)

See Appendix A.

RSPB-2021-1359.R0

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list 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?
Excellent

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

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Changes have addressed reviewer concerns. I have only one suggested editorial change to improve clarity.

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Decision letter (RSPB-2021-1359.R0)

29-Jun-2021

Dear Dr Leigh

I am pleased to inform you that your manuscript RSPB-2021-1359 entitled "Shark Spiral Intestines May Operate as Tesla Valves" 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.

The revisions requested in this case are quite simple, but useful nonetheless.

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:
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• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
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Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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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

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s).
Changes have addressed reviewer concerns. I have only one suggested editorial change to improve clarity.

Lines 253-254: The text here does not reflect the author's stated intent in their response to reviewers: "The authors did not actually compare the different viscosities to each other, rather the flow rates through the different intestine morphologies were compared to each other at each of the different viscosities individually". Suggest revise to read "Volumetric flow rate (m3/s) measured at two resistances (Mpa*s/m3) was compared across intestine sample type in all four species (Fig. 4).

Author's Response to Decision Letter for (RSPB-2021-1359.R0)

See Appendix B.

Decision letter (RSPB-2021-1359.R1)

30-Jun-2021

Dear Dr Leigh

I am pleased to inform you that your manuscript entitled "Shark Spiral Intestines May Operate as Tesla Valves" 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.

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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.

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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

Responses to reviewer comments are included below. The original reviewer comments are in **black** and the responses of the authors are in **red**.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
General Comments
This is an interesting paper that advances knowledge and consideration about spiral intestines in animals, though I think the abstract needs to do a better job of this (more on that below). The paper provides a useful Table 1 that summarizes many examples of four types of spirals in intestines of many vertebrates and invertebrates. It maps the four varieties of these spiral intestines onto a fish phylogeny and considers the evidence that the columnar spiral intestine morphology is more ancestral. In functional tests, two of the other morphologies that are funnel-shaped were superior in producing unidirectional flow in the anterior to posterior direction, which the authors identify as characteristic of Tesla valves. I considered other claims regarding the functional measurements to be weaker or unremarkable (more on that below).
The abstract has been revised as requested (see specific comments below). The discussion of the functional measurements have also been revised and clarified (see more specific responses to specific comments below).

Comments on functional measurements
Lines 234-238 – It struck me as unremarkable that higher viscosity (which was defined at line 162 as higher resistance to flow) slowed flow. Please explain whether there is a reason why this is remarkable, and how the measurement at different viscosities advances the understanding.
The authors agree that stating that a higher viscosity leads to slow flow is not remarkable. The authors did not actually compare the different viscosities to each other, rather the flow rates through the different intestine morphologies were compared to each other at each of the different viscosities individually. Hence, the authors are showing that despite the viscosity of the fluid, the spiral intestines slowed flow (by 3.5x) when compared to proximal intestines. To make this clear, the statement “higher resistance led to significantly slower volumetric flow rate.” has been deleted (**Line 244).**

Lines 238-243 – Comparisons of flow rate are made between plastic control tubing, proximal intestine, and spiral intestine. It seems to me that, for these comparisons to be meaningful, a more systematic presentation is in order. To begin, the equation at line 176, which essentially is flow=gradient/resistance, is simplistic, and I could not find the citation in either PubMed or Web of Science. In the simplest, expanded model, flow rate through tubes is influenced by pressure difference (this gradient was measured), and the resistance to flow is influenced by liquid viscosity (measured), and also tube dimensions such as

radius and length (not presented). The authors may have measured different resistances with the simple equation, but they do not control for differences in tube length, which is a simple explanation for differences in effective resistance that may have nothing to do with resistance due to the spiral intestine. Also, for these tubes, the radius may or may not be fixed (tissue can be distensible, but this was not considered). This may or may not be a sufficient alternative model, but in any event the unmeasured/unconsidered features need to be factored in, or it must be explained to the reader why they are not important. This is why the first claim at lines 352-355 seems not strictly correct ("flow rate is slowed in the spiral intestine due to the high resistance produced by tissue folds"), and the effective resistances should be presented. However, the second claim at lines 352-355 is supported ("flow rate was slowed significantly more when the two funnel-shaped spiral intestines were subjected to flow in the posterior to anterior direction"). The reason the comparison of flow rate in two directions in the spiral intestine holds is because the spiral intestines were simply reversed and so all those tube features that determine effective resistance were held constant.

The full citation for the how the resistance was calculated (as the change in pressure divided by flow rate; $R = \Delta P/Q$; Mearin et al. 1990) is included in the reference list (**Line 486**) and has also been included below:

Mearin F, Zacchi P, Arias A, Malagelada J (1990) Quantification of resistance to flow at the esophagogastric junction in man. *Journal of Gastrointestinal Motility*, 2(4): 287-295.

Including the length and radius of the intestines was considered for analyses, but the reviewer is correct that tissue is distensible, and therefore accurate and constant measurements of these metrics are difficult to obtain. Furthermore, the existence of the internal spiral intestine structure within this gut region means that different points along the intestine may have different radius measurements and also will add additional length to the intestine that can not be observed from the outside; something with the proximal intestine and the control tubing does not have. The authors agree that this should be explained in greater detail in the materials and methods section. As such, we have added this information to **Lines 180-187**.

Additionally, we have toned down the claims in the discussion related to these results. For example, we have deleted the statement that stated, "quantitatively, that flow rate is slowed in the spiral intestine due to the high resistance of the tissue folds." Instead, just the beginning part of this statement remains (**Line 368**): "…flow rate is slowed in the spiral intestine."

Lines 214-218 – The purpose/importance of the measures of muscle contraction should be explained and discussed in the paper. Also, if the proximal intestine should have responded to acetylcholine and it did not, then does the reader presume the tissue was dead? If that is the case, is the spiral intestine also dying, and if so, what is usefully concluded from this

experiment? (related – Line 253, 256 are these standard deviations or standard errors?) Measuring the rate of smooth muscle contractions in a shark has not been done to date, so the authors feel that it is important and novel information to include, but the authors do agree that more discussion of these results is necessary. As such, we have added the following (**Lines 310-317**):
"Little is known about intestinal motility in sharks. Typically, overall evacuation rate is used to estimate the length of time that digesta remains in the gastrointestinal tract of sharks (i.e. Wetherbee et al. 1987). However, by understanding contractile capabilities of the different segments (proximal, spiral, and distal intestines) of the shark digestive system separately, we can begin to establish transit rates at specific points throughout the gut. We have begun to do this by measuring the average number of contractions per minute and the average amount of time necessary to move material of a known viscosity through the spiral intestine of *S. suckleyi*."
With respect to whether or not the tissue is dying; the fact that the spiral intestines continued to contract when stimulated with acetylcholine for over 35mins after the shark had been sacrificed indicates that the tissue was not yet dead. It is possible that a thicker muscularis propria in the proximal intestine requires more stimulation than what was provided to the spiral intestine, which likely has a thinner muscular layer (as shown in Leigh et al. 2021)
Finally, **Line 262**: "standard deviation" has been added to the sentence.

Leigh SC, Papastamatiou YP, & German DP (2021) Gut microbial diversity and digestive function of an omnivorous shark. *Marine Biology,* 168(55): DOI: 10.1007/s00227-021-03866-3.

Lines 277-279 – The comment above about flow through tubes underscores how more data on the tubes would be useful. If the morphological preparations can be used to measure length, radius, surface area and volume, then present these data. This comment has been addressed above.

Comments on the abstract
The paper's title invokes the Tesla valve, and the abstract should mention this, linked to its best functional evidence that "flow rate was slowed significantly more when the two funnel-shaped spiral intestines were subjected to flow in the posterior to anterior direction". The abstract does make the claim that "We quantified the flow rate of material of various viscosities through the spiral intestine, demonstrating that it does slow the rate of digesta transit", though arguably that point is not strongly made without more consideration of factors influencing flow rate (see above). The abstract could state more explicitly the findings from the consideration of "the morphological data in an evolutionary, dietary, and morphological context" (line 45). Finally, the findings don't seem to inspire technological advances so much as they benefit from older, formal understanding of flow through tubes and knowledge about one particular tube design (the Tesla valve).

The authors have added a statement about Tesla valves in the abstract. Additionally, the authors have made clearer the findings from the evolutionary, dietary, and morphological analyses.
Lines 45-52: "The evolutionary analyses suggest that the columnar morphology is the ancestral form of the spiral intestine. Dietary analyses reveal no correlation between diet type and spiral intestine morphology. Morphological analyses determined the flow rate of material of various viscosities through the spiral intestine, demonstrating that it does slow the rate of digesta transit. Flow rate was slowed significantly more when the two funnel-shaped spiral intestines were subjected to flow in the posterior to anterior direction, indicating their success at producing unidirectional flow, similar to a Tesla valve."

Minor points
Line 81 and 308 – the reference to Buddington is not in the References
Line 387: This reference has been added:
Buddington, R.K., & Doroshov, S.I. (1986). Structural and Functional Relations of the White Sturgeon Alimentary Canal (*Acipenser transmontanus*). *Journal of Morphology,* 190: 201-213.

Line 296-297 – this does not seem to be a sentence: "Currents flow along different paths, in different directions, and that these differences have a disproportionate effect on the resistance of the tube.
Line 306: This sentence has been broken up into two as follows: "Currents flow along different paths and in different directions. These differences have a disproportionate effect on the resistance of the tube (Fig. 3). "

Lines 342-345 – pept1 is a transporter, but you seem to imply here that it might play a causative role in morphological development of the spiral intestine. Delete or discuss why it is also likely that its developmental appearance is simply correlated with morphological development.
Lines 361-365: The authors agree that there is not enough evidence to indicate a direct causative role of pept1 in the development of the spiral intestine. This has been made more clear by the addition of the following statements: "…indicating a possible correlation between an increase in mRNA expression of pept1 and the development of the spiral intestine…" as well as "Future investigations should focus on determining if mutations to these genes (*Hoxa13* and *Hoxd13*) or shifts in expression patterns of *pept1* during the developmental process can lead to changes in the morphological development of the spiral intestine in sharks."

Referee: 2

Comments to the Author(s)
Here the authors test for whether the spiral valve intestine in sharks functions as a tesla valve. They utilize iodine enhanced computed tomography scans and a clever experimental design to test for whether the spiral vales encourage flow in

one direction and resist it in another and indeed find evidence to suggest that these intestines work in a similar fashion to tesla valves. The authors also examine the evolution of the spiral valve across sharks and conclude that spiral valve morphology does not closely track diet.
I think that the experimental design and the many of the findings of this paper are interesting and exciting. The authors set up an important question and answered it with elegant simplicity. So, on that end, I don't see very many issues with this part of the manuscript. However, I do believe that the manuscript gets substantially weaker at the phylogenetic comparative method component. Generally, I'm not sure that the authors need it at all. The paper is plenty informative and interesting without it. However, I list some of my concerns below should the authors seek to retain this section.
The authors do wish to retain the information regarding the phylogenetic analyses and find the reviewers comments helpful in strengthening this section. Specific comments to reviewer comments are included below.

1) The phylogeny. The authors do not state in the methods where they got their phylogeny from, or what transformations were made from the original phylogeny (e.g. pruning taxa). I also think that the authors should make the phylogeny ultrametric and perform an ancestral state reconstruction for diet and spiral valve morphology.
The cladogram used in the current manuscript was adapted from a phylogeny that was created by using four mitochondrial and one nuclear gene to investigate the phylogenetic relationships of 229 species (all eight Orders) of sharks and is from Vélez-Zuazo & Agnarsson 2011. For the present study, the phylogeny was converted to a cladogram and only includes Families of sharks. To make this more clear, the authors have changed any mention of a "phylogenetic tree" to a "cladogram" (**Line 107 & 347**). Additionally, further details have been added regarding the methods and the data that was used in the PGLS analysis: **Lines 226-228**: "A phylogenetic generalized least squares (PGLS) test was performed using phylogenetic data from GenBank and the Barcoding of Life Project (as was done by Vélez-Zuazo & Agnarsson 2011) to determine phylogenetic relationships of shark species with respect to their spiral intestine morphology." The authors also performed an ANOVA to compare diet type and spiral intestine morphology directly (without considering phylogeny) as mentioned in **lines 233-235**.

2) One the subject of diet, the authors do not state where they got their dietary information from.
Diet information came from the citation below, which is included in the references (**Line 409**) and has been added to the Table 1 legend as well by adding the following statement: "Information regarding shark diet is from Compagno et al. 2005)."
Compagno L, Dando M, & Fowler S. (2005). *Sharks of the World*. Princeton University Press. Princeton, NJ.

3) The authors find no significant relationship between diet and valve morphology using a PGLS analysis. I would recommend pairing this analysis with a regular GLS or ANOVA to determine whether or not there is a general relationship (without considering phylogeny) and if this relationship happens to be phylogenetically structured.

The authors have added an ANOVA to compare diet and spiral intestine morphology directly (without considering phylogeny) as the reviewer suggests. This is noted in the methods section (**Lines 233-234**): "This was followed up with an ANOVA ($p<0.05$) to compare diet type and spiral intestine morphology directly (without considering phylogeny)."

The results of the ANOVA also did not find a significant relationship between diet and valve morphology. This is stated in the results section (**Lines 270-272**): "There is no significant correlation between diet type and spiral intestine morphology according to the PGLS analysis ($p = 0.4$) and ANOVA ($p = 0.09$)."

Minor point

(Lines 336-349) I don't think that the authors really need the genetic discussion here.

The authors have streamlined, clarified, and removed some statements in this section of the discussion. The authors believe that it is still important to include, since the paper discusses the evolution of these structures, and learning more about the development could lead to further evolutionary insights. The changes made include the following:

Line 361: "…indicating a possible correlation between an increase in mRNA expression of *pept1* and the development of the spiral intestine…"

Lines 362-365: "Future investigations should focus on determining if mutations to these genes (Hoxa13 and Hoxd13) or shifts in expression patterns of pept1 during the developmental process can lead to changes in the morphological development of the spiral intestine in sharks."

And finally, deletion of the following statement at **Line 366**: "Perhaps simply changing the timing of expression of some genes leads to suble changes that result in the different spiral intestine morphologies."

Appendix B

Responses to reviewer comments are included below. The original reviewer comments are in **black** and the responses of the authors are in **red**.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s).
Changes have addressed reviewer concerns. I have only one suggested editorial change to improve clarity.

Lines 253-254: The text here does not reflect the author's stated intent in their response to reviewers: "The authors did not actually compare the different viscosities to each other, rather the flow rates through the different intestine morphologies were compared to each other at each of the different viscosities individually". Suggest revise to read "Volumetric flow rate (m3/s) measured at two resistances (Mpa*s/m3) was compared across intestine sample type in all four species (Fig. 4).
The authors have changed **Lines 243-244** to read as follows: "Volumetric flow rate (m3/s) measured at two resistances (Mpa*s/m3) was compared across intestine sample type in all four species (Fig. 4).", as suggested by the reviewer.