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Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment

Rohani Ambo-Rappe* , Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti and Inés Viana

Original Research, Front. Mar. Sci. - Global Change and the Future Ocean
Received on: 07 Dec 2019, **Edited by:** Iris Eline Hendriks
Manuscript ID: 518333
Research Topic: Seagrasses under Times of Change
Keywords: Tropical seagrass, Indo-Pacific, Traits, Growth, Nutrient content, Photosynthesis, Carbohydrates, Indonesia

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History | Editor Active | Reviewer 1 Endorsed | Reviewer 2 Endorsed

Date	Updates
21 Apr 2020	Article accepted for publication.
14 Apr 2020	Review of Review Editor 1 finalized.
06 Apr 2020	Review of Review Editor 2 finalized.
	Reviewer 2 posted new comments.
	Corresponding Author Rohani Ambo-Rappe re-submitted manuscript.
	You posted new comments.
03 Apr 2020	Reviewer 2 posted new comments.
	Corresponding Author Rohani Ambo-Rappe re-submitted manuscript.
	You posted new comments.
01 Apr 2020	Reviewer 2 posted new comments.
	Corresponding Author Rohani Ambo-Rappe re-submitted manuscript.
	You posted new comments.
25 Mar 2020	Reviewer 2 posted new comments.
13 Mar 2020	Corresponding Author Rohani Ambo-Rappe re-submitted manuscript.
	You posted new comments.
	You posted new comments.

	forum.
27 Feb 2020	Editorial Office reminded you to respond to reviewer 1 and/or resubmit your manuscript in the discussion forum.
	Editorial Office reminded you to respond to reviewer 2 and/or resubmit your manuscript in the discussion forum.
23 Jan 2020	Interactive review forum activated.
07 Dec 2019	Corresponding Author Rohani Ambo-Rappe submitted manuscript.

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Handling Editor: Iris Eline Hendriks
Received date: 07 Dec 2019
Editorial assignment start date: 09 Dec 2019
Independent review start date: 18 Dec 2019
Interactive review activated date: 23 Jan 2020
Review finalized date: 14 Apr 2020
Final validation date: 21 Apr 2020

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Manuscript submission

Version	Submitted on	Submitted by	
5	06 Apr 2020	Rohani Ambo-Rappe	Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Contribution to the field | Abstract | 518333_Manuscript.PDF | 518333_Manuscript.DOC | 518333_Manuscript
4	03 Apr 2020	Rohani Ambo-Rappe	Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Contribution to the field | Abstract | 518333_Manuscript.PDF | 518333_Manuscript.DOC | 518333_Manuscript
3	01 Apr 2020	Rohani Ambo-Rappe	Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Contribution to the field | Abstract | 518333_Manuscript.PDF | 518333_Manuscript.DOC | 518333_Manuscript
2	13 Mar 2020	Rohani Ambo-Rappe	Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Contribution to the field | Abstract | 518333_Manuscript.PDF | 518333_Manuscript.DOC | 518333_Manuscript
1	07 Dec 2019	Rohani Ambo-Rappe	Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Contribution to the field | Abstract | 518333_Manuscript.PDF | 518333_Manuscript.DOC | 518333_Manuscript

History | Editor Active | Reviewer 1 Endorsed | Reviewer 2 Endorsed

Handling Editor: Iris Eline Hendriks
Received date: 07 Dec 2019
Editorial assignment start date: 09 Dec 2019
Independent review start date: 18 Dec 2019
Interactive review activated date: 23 Jan 2020
Review finalized date: 14 Apr 2020
Final validation date: 21 Apr 2020

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Gmail

Rohani Ambo Rappe <rohani.amborappe@gmail.com>

Frontiers: Your manuscript submission - 518333

1 pesan

Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org> 7 Desember 2019 23.11
Balas Ke: Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org>
Kepada: rohani.amborappe@gmail.com

Dear Dr Ambo-Rappe,

Frontiers Marine Science Editorial Office has sent you a message. Please click 'Reply' to send a direct response

We are pleased to inform you that we have received the manuscript "Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment" to be considered for publication in Frontiers in Marine Science, section Global Change and the Future Ocean.

You can access the review forum and track the progress of your manuscript using the following link: http://www.frontiersin.org/Journal/MySubmission.aspx?stage=100

Your manuscript is now in the initial validation stage to determine its suitability for peer review. Should your manuscript be sent out for peer review, you will receive a notification once we receive the reports from reviewers and the interactive review forum is activated. You will then be able to read the review reports and exchange directly with the reviewers in the interactive review forum as well as submit a revised manuscript, if appropriate.

Best regards,

Your Frontiers in Marine Science team

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----------MANUSCRIPT DETAILS---------- Manuscript title: Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment Manuscript ID: 518333 Authors: Rohani Ambo-Rappe, Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti, Inés Viana Journal: Frontiers in Marine Science, section Global Change and the Future Ocean Article type: Original Research Submitted on: 07 Dec 2019

Research Topic: Seagrasses under Times of Change

----------ADDITIONAL INFORMATION----------

In order to enable a smooth and efficient review process, please familiarize yourself with the Frontiers review guidelines:

http://www.frontiersin.org/Journal/ReviewGuidelines.aspx?s=1595&name=global_change_and_the_future_ocean

To take part in the Resource Identification Initiative please cite antibodies, genetically modified organisms, software tools, data, databases and services using the corresponding catalog number and RRID in the text of your article. Please see here for more information: http://www.frontiersin.org/files/pdf/letter_to_author.pdf

Gmail **Rohani Ambo Rappe <rohani.amborappe@gmail.com>**

Iris Hendriks via Frontiers: Action needed: Interactive review for your manuscript has been activated - 518333

1 pesan

Iris Hendriks (Via FrontiersIn) <noreply@frontiersin.org> 23 Januari 2020 19.05
Balas Ke: "Iris Hendriks (Via FrontiersIn)" <iris@imedea.uib-csic.es>
Kepada: rohani.amborappe@gmail.com

Dear Dr Ambo-Rappe,

Two reviewers have provided extensive comments on your manuscript, both recognize the importance of the research but there are concerns about the completeness of references and methodological details. The detailed comments should be helpful to improve the manuscript.

The interactive review of your manuscript "Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment" submitted to Frontiers in Marine Science, section Global Change and the Future Ocean has now been activated.

The reviewers recommended that you make substantial amendments to your manuscript. Please respond within the next 35 days to all comments raised by the reviewers and editor in the online review forum. You can also submit a revised version of your manuscript at that time. We encourage you to submit your documents with tracked changes to highlight the revisions.
There can be more than one iteration between authors and reviewers, but only when all comments by each reviewer have been addressed successfully can the review be finalized.

To access the review forum and respond to the reviewers, please click on the following link:
http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=9f0d2a68-7e7a-4c96-bc56-098c2a251d65

Journal: Frontiers in Marine Science, section Global Change and the Future Ocean
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Manuscript title: Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment
Manuscript ID: 518333
Authors: Rohani Ambo-Rappe, Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti, Inés Viana
Submitted on: 07 Dec 2019
Interactive review started on: 23 Jan 2020

Please do not hesitate to contact us if you have any questions. Your timely response would be much appreciated. Note that if we do not hear from you by the revision deadline, the editorial office reserves the right to withdraw your manuscript from consideration for publication, as we cannot hold manuscripts in review without any updates from the authors.

With best regards,

Iris Hendriks
Associate Editor,
www.frontiersin.org

Gmail **Rohani Ambo Rappe <rohani.amborappe@gmail.com>**

Re: Iris Hendriks via Frontiers: Action needed: Interactive review for your manuscript has been activated - 518333 - request for extension

2 pesan

Rohani Ambo Rappe <rohani.amborappe@gmail.com> 26 Februari 2020 17.57
Kepada: "Iris Hendriks (Via FrontiersIn)" <iris@imedea.uib-csic.es>
Bcc: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>, Ines gonzalez viana <inesgviana@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear Iris,
with respect to the review and revision process for our manuscript number 518333, we hereby request an extension.

The reason for this is that we have had to conduct significant additional data analysis in order to respond to the reviewers comments. We are also communicating across several time zones, which further delays the process.

We would be most grateful if you could allow us a further week (until 6th March 2020) to complete our revision and compile the reply to all the (many and complex) reviewer comments.

We look forward to hearing from you

Thank you for your time and attention

Best regards

Rohani Ambo-Rappe
Corresponding author

Pada tanggal Kam, 23 Jan 2020 pukul 18.05 Iris Hendriks (Via FrontiersIn) <noreply@frontiersin.org> menulis:

> Dear Dr Ambo-Rappe,
>
> Two reviewers have provided extensive comments on your manuscript, both recognize the importance of the research but there are concerns about the completeness of references and methodological details. The detailed comments should be helpful to improve the manuscript.
>
> The interactive review of your manuscript "Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment" submitted to Frontiers in Marine Science, section Global Change and the Future Ocean has now been activated.
>
> The reviewers recommended that you make substantial amendments to your manuscript. Please respond within the next 35 days to all comments raised by the reviewers and editor in the online review forum. You can also submit a revised version of your manuscript at that time. We encourage you to submit your documents with tracked changes to highlight the revisions.
> There can be more than one iteration between authors and reviewers, but only when all comments by each reviewer have been addressed successfully can the review be finalized.
>
> To access the review forum and respond to the reviewers, please click on the following link:
> http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=9f0d2a68-7e7a-4c96-bc56-098c2a251d65
>
> Journal: Frontiers in Marine Science, section Global Change and the Future Ocean
> Article type: Original Research
> Manuscript title: Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment
> Manuscript ID: 518333
> Authors: Rohani Ambo-Rappe, Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti, Inés Viana
> Submitted on: 07 Dec 2019
> Interactive review started on: 23 Jan 2020

> Please do not hesitate to contact us if you have any questions. Your timely response would be much appreciated. Note that if we do not hear from you by the revision deadline, the editorial office reserves the right to withdraw your manuscript from consideration for publication, as we cannot hold manuscripts in review without any updates from the authors.
>
> With best regards,
>
> Iris Hendriks
> Associate Editor,
> www.frontiersin.org

Iris Hendriks <iris@imedea.uib-csic.es> 26 Februari 2020 18.12
Kepada: Rohani Ambo Rappe <rohani.amborappe@gmail.com>

Dear Rohani,

I have asked the editorial office to push back the deadline for revision. If you continue to receive reminders please ignore those. Thank you for letting me know, looking forward to receive the revised paper.

Best,

Iris

El 26/2/20 a las 10:57, Rohani Ambo Rappe escribió:
[Kutipan teks disembunyikan]

--
Iris Hendriks
IMEDEA (CSIC-UIB)
c/ Miquel Marques 21
07190 Esporles
SPAIN
+34 971611832

Gmail **Rohani Ambo Rappe <rohani.amborappe@gmail.com>**

Frontiers: New Comments in the Interactive Review Forum - 518333

1 pesan

Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org> 6 April 2020 22.02
Balas Ke: Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org>
Kepada: rohani.amborappe@gmail.com

Dear Dr Ambo-Rappe,

Frontiers Marine Science Editorial Office has sent you a message. Please click 'Reply' to send a direct response

New comments were posted by the reviewer 2.
Please visit the interactive review forum using the link below and address these comments within the coming week.
http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=9f45db02-1a20-4a82-a460-c078bcfde111&retab=2

You should aim to respond as soon as possible, directly via the online review forum, to all comments in the review reports and editor tab. If appropriate, you can also submit a revised version of your manuscript. We appreciate your timely response.

Remember that there can be more than one iteration between authors and reviewers, and only when all comments by the reviewers are addressed successfully can the review be finalized.
We wish you a successful interactive review, and remain at your disposal for any questions.

Best regards,

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Journal: Frontiers in Marine Science, section Global Change and the Future Ocean
Article type: Original Research
Submitted on: 07 Dec 2019
Edited by: Iris Eline Hendriks

Research Topic: Seagrasses under Times of Change

Gmail **Rohani Ambo Rappe <rohani.amborappe@gmail.com>**

Frontiers: New Comments in the Interactive Review Forum - 518333

11 pesan

Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org> 4 April 2020 00.15
Balas Ke: Frontiers Marine Science Editorial Office <marinescience.editorial.office@frontiersin.org>
Kepada: rohani.amborappe@gmail.com

Dear Dr Ambo-Rappe,

Frontiers Marine Science Editorial Office has sent you a message. Please click 'Reply' to send a direct response

New comments were posted by the reviewer 2.
Please visit the interactive review forum using the link below and address these comments within the coming week.
http://www.frontiersin.org/Review/EnterReviewForum.aspx?activationno=6dc3379b-87a1-4c16-9ab0-9374df0135fe&retab=2

You should aim to respond as soon as possible, directly via the online review forum, to all comments in the review reports and editor tab. If appropriate, you can also submit a revised version of your manuscript. We appreciate your timely response.

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We wish you a successful interactive review, and remain at your disposal for any questions.

Best regards,

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Journal: Frontiers in Marine Science, section Global Change and the Future Ocean
Article type: Original Research
Submitted on: 07 Dec 2019
Edited by: Iris Eline Hendriks

Research Topic: Seagrasses under Times of Change

Rohani Ambo Rappe <rohani.amborappe@gmail.com> 4 April 2020 00.56
Kepada: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>, Ines gonzalez viana <inesgviana@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear all,

Reviewer 2 again posting comment just after I submitted the revision, it is so fast. But the good thing she/he said...this is the last...hehehe...hopefully!

Please find the comment that I send you below. Because it is mostly related to the English as said in the comment...so please Mirta, you are the one.

Best Regards,
Rohani
[Kutipan teks disembunyikan]

2 lampiran

Comment-4 of Reviewer2.docx
14K

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Inés Viana <inesgviana@gmail.com> 4 April 2020 04.26
Kepada: Rohani Ambo Rappe <rohani.amborappe@gmail.com>
Cc: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear all,

I have included the couple of things that the reviewer asked for, and the version is now ready for the English editing. We cannot forget that Reviewer 1 hasn´t replied yet to our comments or accepted the publication, so she/he could ask for more changes in the text...
Best,
Inés
[Kutipan teks disembunyikan]
--
Inés Viana PhD
Faculty of Marine Sciences
University of Vigo
E-36310 Vigo, Galicia, Spain

http://www.ecotox.gal/en
http://ecocost.webs.uvigo.es/

2 lampiran

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Dr. Mirta Teichberg <mirta.teichberg@leibniz-zmt.de> 4 April 2020 18.40
Kepada: Inés Viana <inesgviana@gmail.com>, Rohani Ambo Rappe <rohani.amborappe@gmail.com>
Cc: Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

OK I will take a look this weekend and send the final english version out.
Hopefully with all these changes, the other reviewer will only suggest minor to no changes!
Best,
Mirta
[Kutipan teks disembunyikan]

--
Mirta Teichberg
Algae and Seagrass Ecology Jr. Research Group Leader
Phone: +49(0)421 238 00-53
Fax: +49(0)421 238 00-30
email: mirta.teichberg@leibniz-zmt.de
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Vorsitzender des Aufsichtsrates: Dr. Walter Dörhage
Geschäftsführung: Prof. Dr. Hildegard Westphal, Dr. Nicolas Dittert

---

Dr. Mirta Teichberg <mirta.teichberg@leibniz-zmt.de> 6 April 2020 04.13
Kepada: Inés Viana <inesgviana@gmail.com>, Rohani Ambo Rappe <rohani.amborappe@gmail.com>
Cc: Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear all,
Here is the revised version for the English editing. I actually had to change quite a bit throughout, so it was good that I did a final read through! Please see a few sentences highlighted in yellow which I changed or am still unsure about.

Ines, for the one regarding the the synergistic affects versus additive, I am not sure I agree with that. usually additive effects are considered synergist....I think the review also commented on this last time. I think i agree with the reviewer here. But maybe I dont understand the meaning? Please check again.

After these changes and clarifications, I think we can submit again.

Best,
Mirta

On 03-Apr-20 22:26, Inés Viana wrote:
[Kutipan teks disembunyikan]
[Kutipan teks disembunyikan]

518333_Ambo-Rappe_Manuscript_R4-05042020.doc
1834K

Inés Viana <inesgviana@gmail.com> 6 April 2020 06.28
Kepada: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>
Cc: Rohani Ambo Rappe <rohani.amborappe@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear all,

I was confused by the reviewer´s comment on that sentence. Please, check Fig 1 of the paper attached: I understand from that figure that when stressors do not interact (so do not enhance or downscale the effect of the other stressor) they are additive, that means, the effect of both factors together are the sum of the effect of each factor separately. Now I am not sure if I am getting it wrong or if there is another way of interpreting it, so please change if you do not agree or think it is confusing,
I agree with the other highlighted sentence and I have made some other changes
Best,
Inés
[Kutipan teks disembunyikan]

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2 lampiran

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Dr. Mirta Teichberg <mirta.teichberg@leibniz-zmt.de> 6 April 2020 16.05
Kepada: Inés Viana <inesgviana@gmail.com>
Cc: Rohani Ambo Rappe <rohani.amborappe@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear Inés,

I thought additive effects were a type of synergistic effect, but I further read and that is not the case as this paper shows, synergistic effect is greater.

But for this sentence in the manuscript, you are referring to our study, correct? I dont think our results show any additive effects either, as then we would see a greater effect in the temperatureXnutrient treatments. So although they are not synergistic, they are not additive, or antagonistic either...or at least that would be the way I would interpret it. Unless you consider that if there is an only an effect of temperature then nutrient effect would be zero, and then it is "additive". It is a bit confusing.

I suggest to change the sentence to:

In our experiment, however, the effects on most traits showed no strong interactions, indicating a lack of synergistic or antagonistic effects under combined stressors. The combined treatments, therefore, can be considered additive, in which nutrient effects were generally lacking and did not add further to the temperature effect.

See attached revised version. Rohani, I think we can now submit it.
Best,
Mirta
[Kutipan teks disembunyikan]

518333_Ambo-Rappe_Manuscript_R4-05042020_iv_mt.doc
1836K

Rohani Ambo Rappe <rohani.amborappe@gmail.com> 6 April 2020 16.45
Kepada: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>
Cc: Inés Viana <inesgviana@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Hi Mirta and all,

I just submitted the revision.
Good luck for this, hopefully this format is adequate to get acceptance.

Best Regards,
Rohani

[Kutipan teks disembunyikan]

Inés Viana <inesgviana@gmail.com> 6 April 2020 16.53
Kepada: Rohani Ambo Rappe <rohani.amborappe@gmail.com>
Cc: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Hi Rohani,

Thank you for submitting this last version.
How is the CO2 paper going? Do you need anything from our side?
Best,

Inés
[Kutipan teks disembunyikan]

Dr. Mirta Teichberg <mirta.teichberg@leibniz-zmt.de> 6 April 2020 16.56
Kepada: Inés Viana <inesgviana@gmail.com>, Rohani Ambo Rappe <rohani.amborappe@gmail.com>
Cc: Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Dear Rohani,
Great! Yes, as Inés asks, we both could work on the Co2 paper again if you need our help so we can try to get it submitted this week!
Best,
Mirta
[Kutipan teks disembunyikan]

Rohani Ambo Rappe <rohani.amborappe@gmail.com> 6 April 2020 17.02
Kepada: "Dr. Mirta Teichberg" <mirta.teichberg@leibniz-zmt.de>
Cc: Inés Viana <inesgviana@gmail.com>, Suci Rahmadani Artika <sucii.rahmadaniartika@gmail.com>, Agustin Moreira Saporiti <agustin.saporiti@leibniz-zmt.de>

Hi Mirta, Inez, and Agustin,

I and Suci have been worked with the second manuscript.
I just send it to you all for review and we could continue until finalization.

Best Regards,
Rohani
[Kutipan teks disembunyikan]

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision ✓

Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment

Rohani Ambo-Rappe* , Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti and Inés Viana

Original Research, Front. Mar. Sci. - Global Change and the Future Ocean
Received on: 07 Dec 2019, **Edited by:** Iris Eline Hendriks
Manuscript ID: 518333
Research Topic: Seagrasses under Times of Change
Keywords: Tropical seagrass, Indo-Pacific, Traits, Growth, Nutrient content, Photosynthesis, Carbohydrates, Indonesia

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Reviewer 1
Independent review report submitted: 15 Jan 2020
Interactive review activated: 23 Jan 2020
Final report submitted: 14 Apr 2020

Final Evaluation

Q 1 Final comments to Author (optional):

Reviewer 1 | 14 Apr 2020 | 14:54

As I said previously, this is a very interesting and well-designed experiment. The results they have obtained will be very useful for the future conservation plans and for the research of this ecosystem. After read the new version of the manuscript and the responses of the authors to my comments, I consider that authors made a good work considering all my comments, corrections and suggestions. The new version of the manuscript is better explained and better structured. In my opinion, now they have achieved the quality that the journal requires.

Q 2 Do you ENDORSE THE PUBLICATION of this manuscript in its current form?

Reviewer 1 | 14 Apr 2020 | 14:54

– Yes

EVALUATION

Q 1 Please summarize the main findings of the study.

Reviewer 1 | 15 Jan 2020 | 11:52 #1

This article dealt with effects of temperature increase and nutrient enrichment on the seedling phase of the seagrass Enhalus acoroides, focus on the morphological, biochemical and physiological responses. It is a very interesting experiment since the specific outcome of global and local stressors on the seedling phase in seagrasses are still poorly studied.

Thank you

Q 2 **Please highlight the limitations and strengths.**

Reviewer 1 | 15 Jan 2020 | 11:52 #1

The study of changes in environmental conditions is necessary for the future conservation plans of this threatened ecosystem. Altogether, I find this study very useful. This is a well-designed study and authors provided interesting and comprehensive data and graphics to explore the effect of the two factors. However, there are some areas/issues during the text, especially in the discussion and introduction sections, that have to be developed and improved for the quality that the journal requires. My most relevant concern deals with the small amount of references and the small use of supports from previous works and the statistics used. Moreover, I also suggest some external careful English editing to improve flow, expression and clarity in some parts. My suggestions/questions are divided in the sections of the manuscript starting with the main points or concerns about. Overall, this is an interesting piece of work and it has the potential suitable to be published but there are some work needed to make this manuscript publishable in this journal.

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 09:56 #2

Thank you. We have revised the manuscript according to this comment.

Q 3 **Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns.**

Reviewer 1 | 15 Jan 2020 | 11:52 #1

#Material and methods

Although the section is well written, I think some arguments are mixed in the current version triggering an awkward reading in some parts. I suggest authors check the section to make this section more fluency. For example, after “Figure 1” a dot is necessary. Then, authors should explain that “three individual seedlings (consisting of leaves, seeds and roots at the beginning of the experiment) with a diameter of 1.02±0.02 cm and 0.70±0.03 g FW were placed in individual aquarium (n=24) (29x13x30 cm; 10 l) called incubation chamber hereinafter. The target temperature values were obtained by placing each incubation chamber in 4 larger tanks (ETs) of 250 l used as a water bath. The experiment followed a block design with nutrient treatments, nested within the 4 ETs set at the two temperatures.”

I recommend increasing the thickness of the dot and strip lines in boxes of Fig.1

Replace “20 January to 22 February 2017” by “January 20th to February 22th 2017”

At the beginning of the section, I can see the target temperature (26°C and 31°C). Target values for nutrients treatments should be necessary as well.

Water renovation rate was “4ml min-1”. I suggest using “l d-1” because the experiment period was 1 month. The water in aquarium were renovated every two days aprox. The water enter is explain but how the water go out from the aquariums? On the other hand, 4ml min-1 = 5.7 l d-1. Then, every day aprox 70 l go out from reservoirs (115l) to fill their 12 aquariums. How they filled the reservoir during the experimental period (1 month)? How many pulses of nutrients they added during the experiment?

About light, all aquariums had their own LED lamps or they were lighted by common LED lamps? Are they checked the light level in each aquarium to ensure all aquarium had similar light? It is important to ensure that author could set a homogenous field of irradiance in each chamber. What sensor was used to check the light level?

In my experience, lots days of experiment with high temperature, nutrient and light usually

Other problem in this kind of experiment is the water evaporation, which can increase the salinity inside the aquariums or reservoirs. Have the authors checked the salinity along the experiment period?

I have had some problems with the names used by authors for the equipment. At the beginning of the section they said “experimental tanks” but it is the first time they used it. They are talking about the four “larger tanks (ETs)” or the “two different reservoirs”

They collected samples for nutrients once a week in the two water reservoirs and, authors said, “random aquariums”. How many random aquariums in each week? On the other hand, check the nutrients level once a week seem a little bit taking into a count the high water renovation rate they used. Authors should explain better the frequency of nutrient pulses they made, and they should be more concrete with the water renovation rate inside aquariums and reservoirs.

There are some important stats issues that have to be revised: According to the authors, all data were checked for normality and homocedasticity. This is a common misunderstanding; what must follow normality and homocedasticity are the residuals of the linear model, i.e. of the ANOVAs, but not the data.

On the other hand, since there are not mesocosms for temperature alone, CO2 alone and combination of both, the use of two-way ANOVA is not correct. They should use a nested-ANOVA analyse.

#Results and discussion

About results, I recommend to include letters or symbols in figures to show significant differences among treatments.

I have missed any explication about ETR and photosynthetic efficiency of seedlings in discussion section.

#Discussion

They started the discussion comparing their results with previous studies. This is fine but they should try to highlight better the differences between the experiment and the previous ones (older vs young seedlings; optimum temperature increase vs higher temperature increase out of the seagrass tolerance; etc). Now the arguments are a little mixed.

I wondered why studies using older seedlings usually show negative effects under warming. Is it something related with carbon reserves levels? I suggest discuss.

They found that seedling showed more AG biomass and less allocation to BG tissues under lower temperature. Authors should discuss this under an ecological point of view. For example, the increase in AB biomass may increase community production, which would trigger more habitat, nursery and food for organisms. However less allocation to BG tissues could make the new meadow more vulnerable to high hydrodynamic forces (e.g. storms)

About the response to nutrient increase, authors explain that no significant effects were found. They assume that seedling phase does not rely on external nutrient availability. However, as they said in results, nutrient concentrations in the experimental aquaria were low in all treatments regardless of the inputs. Therefore, they should be careful not to be too exhaustive with the conclusions obtained here. I recommend to discuss why the water nutrient concentration in aquariums were less than expected. Uptake by seedlings? proliferation of fast-growing species such as epiphytes and algae? Probably this promoted the higher increase in Chl-a concentrations in the water column.

They should explain the ecological consequences of the decrease of total new root length and number under high-nutrient treatment. Probably, this can affect the establishment capacity of seedlings if they do not reach the adequate root length and for successful anchoring.

Finally they said that the combination effect (temperature + nutrient increase) was unclear, similar to studies on adult seagrass. The combination of multiple factors acting together can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors. I recommend reading more papers that used several factors and where the responses were sometimes weak or different than expected (for

Authors should be careful not to be too exhaustive in the conclusions. As their results didn't show negative responses in the experiment, they suggest that warming and nutrient enrichment not seem so detrimental and even can be positive for seedling stage. Here, they must highlighted the limitations of the methodology used (lower nutrient level than expected in the high-nutrient treatments, temperature within the optimal temperature range for E. acoroides, etc) and especially other factors related but not contemplated. For example warming can increase algae blooms, including invasive algae, which can affect seedlings; nutrient enrichment can affect the establishment capacity of seedlings and modified the palatability of new leaves and the herbivore pressure, etc.

Finally, this experiment provides interesting results but authors should also point out the large number of gaps existing in this type of research and the need to continue researching how factors related to global change affects the success in seedlings of segrass, which would help in future management plans and recovery.

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 09:56 #2

Thank you for your review in order to improve the quality of this manuscript. Please find below our responses to address each comment.

#Material and methods

Although the section is well written, I think some arguments are mixed in the current version triggering an awkward reading in some parts. I suggest authors check the section to make this section more fluency. For example, after "Figure 1" a dot is necessary. Then, authors should explain that "three individual seedlings (consisting of leaves, seeds and roots at the beginning of the experiment) with a diameter of 1.02±0.02 cm and 0.70±0.03 g FW were placed in individual aquarium (n=24) (29x13x30 cm; 10 l) called incubation chamber hereinafter. The target temperature values were obtained by placing each incubation chamber in 4 larger tanks (ETs) of 250 l used as a water bath. The experiment followed a block design with nutrient treatments, nested within the 4 ETs set at the two temperatures."

We have significantly modified this section with changes that have been made as suggested by the reviewer, including a more thorough description of the experimental nested design and to accommodate the initial categorizing we made to the seedlings into 3 sizes (small, medium, large) and also for PERMANOVA analysis requirement as suggested by Reviewer 2. Furthermore, we removed the use of incubation chamber and just use aquarium throughout the description. See in particular changes in p 6 lines 195-214:

We conducted a full-factorial experiment combining two water temperatures (26°C and 31°C) representing the minimum and maximum temperatures within the home region that seagrasses are currently exposed to, and two nutrient treatments (low nutrient concentrations of 2 µM of NH4NO3 and 0.1 µM KH2PO4 and high nutrient concentrations of 20 µM of and 1 µM of NH4NO3 and KH2PO4). This yielded in 4 experimental treatments: low temperature and low nutrient concentrations, low temperature and high nutrient concentrations, high temperature and low nutrient concentrations, and high temperature and high nutrient concentrations. The experiment was conducted under laboratory conditions in an indoor flow-through system at the MAREE (ZMT, Bremen) with 24 individual aquaria with 29x13x30 cm dimensions and 10 l volume (Figure 1A). Seedlings (consisting of incipient leaves, seeds and no roots at the beginning of the experiment) were categorized as small (diameter between 0.6-1.0 cm), medium (diameter between 1.1-1.5) and large (diameter between 1.5-1.7 cm). One seedling of each size was distributed in each aquarium, making a total of three seedlings per aquarium. The aquaria additionally contained adult seagrasses from 3 different species (Viana et al., in prep).

The target temperature values were obtained by placing aquaria in larger experimental tanks (ETs) of 250 l that acted as water baths maintaining a constant water temperature. Six aquaria were placed in 4 different ETs following a split-plot experimental design with nutrient treatments nested within the 4 ETs set at the two temperatures.

I recommend increasing the thickness of the dot and strip lines in boxes of Fig.1

Changes have been made as suggested by the reviewer, please see p.7 Line 242-243.

At the beginning of the section, I can see the target temperature (26°C and 31°C). Target values for nutrients treatments should be necessary as well.

Changes have been made as suggested by the reviewer. The text now reads: "…and two nutrient treatments (low nutrient concentrations of 2 µM of NH4NO3 and 0.1 µM KH2PO4 and high nutrient concentrations of 20 µM of and 1 µM of NH4NO3 and KH2PO4). ", please see p.6 Line 197-199.

Water renovation rate was "4ml min-1". I suggest using "l d-1" because the experiment period was 1 month. The water in aquarium were renovated every two days aprox. The water enter is explain but how the water go out from the aquariums? On the other hand, 4ml min-1 = 5.7 l d-1. Then, every day aprox 70 l go out from reservoirs (115l) to fill their 12 aquariums. How they filled the reservoir during the experimental period (1 month)? How many pulses of nutrients they added during the experiment?

The subsection Experimental design and setup has been substantially changed. The information suggested by the reviewer has been included. We know clarify how the water reservoirs were filled and how the circulation in the aquaria was. Please see Experimental Design and Setup subsection at p.7 line 227-238:

The flow was maintained at a constant rate at ~5.8 l d-1 ensuring total water renovation inside each aquarium every ~1.5 days. Water reservoirs were manually emptied from any remaining water and refilled with fresh ASW every other day. Nutrients to the high nutrient treatment reservoir were added in a previously dissolved form from stock solutions of NH4NO3 and KH2PO4 (Merck, Germany). Once in the water reservoir, ASW was gently mixed and an air pump was placed in each water reservoir to ensure further aeration and mixing. Air pumps were placed in each aquarium to ensure water aeration and mixing by moving water from the bottom to the top. Water constantly overflew from the aquaria to the water bath of the ETs ensuring water renewal. At the same time, ETs were drained of the surplus water flowing out of the aquaria.

About light, all aquariums had their own LED lamps or they were lighted by common LED lamps? Are they checked the light level in each aquarium to ensure all aquarium had similar light? It is important to ensure that author could set a homogenous field of irradiance in each chamber. What sensor was used to check the light level?

We have now specified that 2 LED lamps were placed on each ET at the same height and that the mean and standard error provided in the text refers to the variability among all aquaria. Please see p.6 line 219-222.

In my experience, lots days of experiment with high temperature, nutrient and light usually create the ideal conditions for epiphytes or algae growing although they used ASW. Authors had to deal with this kind of problems? If so, this should have been explained.

We appreciate the reviewer rises this issue. We tried to reflect the higher algal growth in the water column with the chlorophyll values, but we agree it is interesting to describe the differences between nutrient treatments. We have now included a brief explanation in the result section 3.1.Experimental water chemistry and eutrophic conditions within the aquaria, p.11 line 383-393:

Therefore, the different treatments effectively changed trophic conditions within aquaria, as indicated by the increased Chl-a and Chl-b concentrations, but also other algal blooms observed in the high-nutrient treatments. Although the abundance of these other microorganisms could not be quantified, they were observable by naked eye and could be felt as a slimy layer on the aquaria and some seagrass leaves. They also formed fluffy masses with a slimy feel which disintegrated when attempts were made to capture them. Therefore, even though nutrient concentration parameters in aquaria were low, other observable parameters suggested eutrophic conditions were occurring in the high nutrient treatments. These symptoms were especially noticeable when high nutrient were combined with high temperature, leading to a greater growth of epiphytic algae.

the salinity along the experiment period?

Salinity was regularly monitored, please see section 2.3. Water sampling, p.7 line 245-246.

We have also specified that ETs were covered with lids to dim the potential water evaporation. Please see p.7 line 223-224.

I have had some problems with the names used by authors for the equipment. At the beginning of the section they said “experimental tanks” but it is the first time they used it. They are talking about the four “larger tanks (ETs)” or the “two different reservoirs”

The terms have now been standardized. Large tanks and experimental tanks are now called experimental tanks (ET), while the small aquariums that acted as replicate are called aquarium. Changes have been made in the Material and Methods and Results section and in Figure 1.

They collected samples for nutrients once a week in the two water reservoirs and, authors said, “random aquariums”. How many random aquariums in each week? On the other hand, check the nutrients level once a week seem a little bit taking into a count the high water renovation rate they used. Authors should explain better the frequency of nutrient pulses they made, and they should be more concrete with the water renovation rate inside aquariums and reservoirs.

We have now provided more details in the Material and Methods and Results sections about the sampling and analysis. The nutrient concentrations in the aquaria were in most cases very low and could not be quantified, and therefore not included in the analysis (we have now specified it).

Low nutrient concentrations have been a common issue in other nutrient experiments with seagrass, as for example Touchette et al. (2003) reported that mean water column nutrient levels ranged from <0.01 to 1.7 µM nitrate, 0.75 to 2.45 µM ammonium and 0.7 to 1.15 µM TP, when the concentrations of nitrate provided to experimental tanks were 8 µM above ambient. This showed that “the majority of the nitrate added to the tanks (daily additions) was taken up relatively quickly by the system.” On the other side, Moreno-Marín et al. (2018) reported that HN (high-nutrient) treatments were provided with 375 µM ammonium as target concentration and had aprox 25 µM ammonium just after addition. Ontoria et al. (2019) showed that that “ammonium concentrations in the water of the moderate and high treatments were increased to the target values at the beginning of the experiment and had decreased at the end of the experiment, despite the repeated additions of ammonium and irrespective of the thermal treatment”

The water treatments were continuously provided to the aquaria with a constant water flow that was the same for all treatments. We have rewritten this subsection in the Material and Methods as we understand it was not clear. We have also specified the flow per day, and the renovation per day in each aquarium.

There are some important stats issues that have to be revised: According to the authors, all data were checked for normality and homocedasticity. This is a common misunderstanding; what must follow normality and homocedasticity are the residuals of the linear model, i.e. of the ANOVAs, but not the data. 20 µM of and 1 µM

We appreciate the reviewer has highlighted this error. Statistical analysis has been redone and the section has also been rewritten. See also comment below.

On the other hand, since there are not mesocosms for temperature alone, CO2 alone and combination of both, the use of two-way ANOVA is not correct. They should use a nested-ANOVA analyse.

We agree with the reviewer and we have redone the statistical analysis split-plot design with nesting factors and applied a PERMANOVA. Please see the new Statistical analysis section 2.5 p 10-11, Fig. 1B, and Tables 4 and 5 to check the results of the new analysis. We have selected the PERMANOVA analysis as it is based on a geometric partitioning of variation across a multivariate data cloud defined explicitly in the space of a chosen dissimilarity measure in response to the selected factors.

About results, I recommend to include letters or symbols in figures to show significant differences among treatments.

PERMANOVA do not compare the average values but the distance in the dissimilarity matrix, therefore there is not necessarily correspondence between the results of the test and what is seen in the figures and we thought it could be confusing for the reader. Moreover, results of this test are so extensive that would be too much information for the figure. Besides, we have now included in the figure 2 caption that the results are in Table 4. For all these reasons, we have decided not to follow this suggestion. We hope the reviewer agrees with our decision.

I have missed any explication about ETR and photosynthetic efficiency of seedlings in discussion section.

We appreciate the reviewer points this out. We have included some explanation in the Discussion. Please see p.15 Line 579-584.

#Discussion

They started the discussion comparing their results with previous studies. This is fine but they should try to highlight better the differences between the experiment and the previous ones (older vs young seedlings; optimum temperature increase vs higher temperature increase out of the seagrass tolerance; etc). Now the arguments are a little mixed.

Thank you for this comment. Changes have been made in the first paragraph of the Discussion following the reviewer´s suggestion.

I wondered why studies using older seedlings usually show negative effects under warming. Is it something related with carbon reserves levels? I suggest discuss.

We appreciate the reviewer points out this issue. However, we have searched for information related to this issue but could not find if this phenomenon could be supported with other data. Therefore, we could confidently say that the effects are merely due to the seagrass growing in natural conditions at different latitudes. We have added some information on this in the discussion, p.13-14 Line 513-516.[m1]

They found that seedling showed more AG biomass and less allocation to BG tissues under lower temperature. Authors should discuss this under an ecological point of view. For example, the increase in AB biomass may increase community production, which would trigger more habitat, nursery and food for organisms. However less allocation to BG tissues could make the new meadow more vulnerable to high hydrodynamic forces (e.g. storms)

Thank you for the suggestion. We have added a paragraph on this in the Discussion p.13 Line 498-509.

About the response to nutrient increase, authors explain that no significant effects were found. They assume that seedling phase does not rely on external nutrient availability. However, as they said in results, nutrient concentrations in the experimental aquaria were low in all treatments regardless of the inputs. Therefore, they should be careful not to be too exhaustive with the conclusions obtained here. I recommend to discuss why the water nutrient concentration in aquariums were less than expected. Uptake by seedlings? proliferation of fast-growing species such as epiphytes and algae? Probably this promoted the higher increase in Chl-a concentrations in the water column.

Regarding the nutrient concentrations in the aquaria, please see the response to your previous comment above. We discuss there how other studies have had the same results regarding nutrient concentrations in the aquaria. We have now better explained in subsection 3.1. of the Results section the different trophic conditions observed under different treatments, also detectable with Chlorophyll data. Based on this, we believe we can assume that the treatments had an effect on the conditions of the systems, even though these changes had no effect on most seedling responses. Besides, we have followed the reviewer´s suggestion and we have added a

matter concentrations in the sediments or lower light availability.

They should explain the ecological consequences of the decrease of total new root length and number under high-nutrient treatment. Probably, this can affect the establishment capacity of seedlings if they do not reach the adequate root length and for successful anchoring.

We have added a few sentences in the Discussion as suggested by reviewer. Please see p.13 Line 506-509.[m2] [SRA3]

Finally they said that the combination effect (temperature + nutrient increase) was unclear, similar to studies on adult seagrass. The combination of multiple factors acting together can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors. I recommend reading more papers that used several factors and where the responses were sometimes weak or different than expected (for example Gunderson et al. (2016) Annu. Rev. Mar. Sci. 8; Moreno-Marin et al. (2018) Limnol. Oceanogr. 63 1528-1544; Egea et al. (2018) Marine Pollution Bulletin, 134: 14-26). Then, authors should explain why the combined response was unclear

We have added some information related to the effect of multiple stressors in the first paragraph of the Discussion and in the section 4.3 Ecological Implications.

Authors should be careful not to be too exhaustive in the conclusions. As their results didn't show negative responses in the experiment, they suggest that warming and nutrient enrichment not seem so detrimental and even can be positive for seedling stage. Here, they must highlighted the limitations of the methodology used (lower nutrient level than expected in the high-nutrient treatments, temperature within the optimal temperature range for E. acoroides, etc) and especially other factors related but not contemplated. For example warming can increase algae blooms, including invasive algae, which can affect seedlings; nutrient enrichment can affect the establishment capacity of seedlings and modified the palatability of new leaves and the herbivore pressure, etc.

We raise this issue in last section of the Discussion, "Ecological Implications" and clarified it (see also the response to previous comments). Besides, we aimed to check the effect of nutrient enrichment per se and not the side effects of eutrophication processes, as increasing organic matter concentration in the sediment or light deprivation.

Finally, this experiment provides interesting results but authors should also point out the large number of gaps existing in this type of research and the need to continue researching how factors related to global change affects the success in seedlings of seagrass, which would help in future management plans and recovery.

We have now added a few sentences in the subsection "Ecological Implication" of the Discussion.

Q 4 **Check List**

Reviewer 1 | 15 Jan 2020 | 11:52 #1

Is the English language of sufficient quality?
- No

Is the quality of the figures and tables satisfactory?
- No

Does the reference list cover the relevant literature adequately and in an unbiased manner?
- No

Are the methods sufficiently documented to allow replication studies?
- Yes

Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication)
- Yes

Does the study adhere to ethical standards including ethics committee approval and consent procedure?
- Not Applicable

Have standard biosecurity and institutional safety procedures been adhered to?
- Not Applicable

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 09:56 #2

The quality of English language of the manuscript have been improved by having a native English to proof read before the submission, we are hoping now the manuscript is in the sufficient English language quality.

All figures and tables has been re-done in order to improve their qualities. Moreover, some new and related references have been added (some suggested by the reviewers).

Q 5 **Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List):**

Reviewer 1 | 15 Jan 2020 | 11:52 #1

This article dealt with effects of temperature increase and nutrient enrichment on the seedling phase of the seagrass Enhalus acoroides, focus on the morphological, biochemical and physiological responses. It is a very interesting experiment since the specific outcome of global and local stressors on the seedling phase in seagrasses are still poorly studied. The study of changes in environmental conditions is necessary for the future conservation plans of this threatened ecosystem. Altogether, I find this study very useful. This is a well-designed study and authors provided interesting and comprehensive data and graphics to explore the effect of the two factors. However, there are some areas/issues during the text, especially in the discussion and introduction sections, that have to be developed and improved for the quality that the journal requires. My most relevant concern deals with the small amount of references and the small use of supports from previous works and the statistics used. Moreover, I also suggest some external careful English editing to improve flow, expression and clarity in some parts. My suggestions/questions are divided in the sections of the manuscript starting with the main points or concerns about. Overall, this is an interesting piece of work and it has the potential suitable to be published but there are some work needed to make this manuscript publishable in this journal.

#Introduction

The introduction, although well structured, it needs a deep review to improve their quality and the appeal for readers. Some areas of the introduction must be extended, especially about the effects of temperature increase and nutrient enrichment in seagrasses (paragraph 2 and 3). The description about the current knowledge of these factors in seagrases is poor and in a tangential way. A more complete description of the current knowledge, with more references, is necessary. That is essential for the scientific quality that the journal requires. I will break it down below:
• First, why they explore these factors instead of others important factors related with climate change and human disturbance (e.g. acidification, changes in salinity and littoral current, etc)? Probably they know that eutrophication processes has been identified as one of the most important factors affecting productivity, community carbon dynamic and seagrass growth and one of the major threats confronting coastal ecosystems. On the other hand, probably they know lots of studies have highlighted the importance of temperature in

effects were very short and poorly explained. They should explain how the effects of nutrient enrichment depend on species-specific features, the level of nutrient surplus (moderate or severe) and local conditions. They also should indicate the effect of ammonium toxicity and how nutrient surplus can also attract to new herbivores (please see and/or mention Jiménez-Ramos et al. (2017) PLoS ONE 12(8): e0183256 and Campbell et al. (2018) J. Ecol. 106 (1), 401-412.).

• About temperature increase, authors focus on the negative impact of this stressor both warming and heat waves. However, they should also indicate that temperature increase can produce positive effect. For example, temperature increase can benefit the growth and biomass of several seagrass species and even the flowering. Indeed, a recent experiment suggest that heat waves may enhance the autotrophic metabolism of seagrass communities in contrast to previous researches suggesting solely negative effects on seagrasses (please see Egea et al. 2019 PLoS ONE 14(1): e0210386).

• Finally, authors worked hard to explore the effect of two factors under a full factorial mesocosm design. To improve the quality and the appeal for readers, authors should explain the importance of make this kind of experiments (with two or three factors). Please see recent works: Ontoria et al. (2019) Marine Environmental Research, 145 27-38 and Egea et al. (2018) Marine Pollution Bulletin, 134: 14-26. This is, in my opinion, one of the most important appeals of the experiment and it has been overlooked in the introduction. Certainly, the combined response was not significant in the most of variables in the study. They should discuss why since an ecological point of view, using the large amount of bibliography on this subject available in the discussion section.

At the same way, a more complete explanation about why biochemical, physiological or morphological responses are important to the seagrass conservation would be necessary. Authors focus on these variables as indicators. They should also discuss that changes in these variables also alter abiotic factors (such as hydrodynamic conditions, sedimentation rates, etc; see Fonseca et al. 2019 Front. Mar. Sci. 6:376) which affect the distribution of organisms within the canopies, and therefore affecting to diversity levels and even productivity. I recommend read and/or mention: Meysick et al. 2019 PlosOne 14(9):0222020; Jiménez-Ramos et al. 2019 Oikos 128: 64–76; González-Ortiz et al. 2014 PlosOne, 9(8):e104949

Finally I have missed a description about the current knowledge about seagrass conservation and restoration programs using seeds or seedlings. Planting seeds is a cost-efficient method for large-scale seagrass meadow restoration. More studies about changes in environmental conditions (such as temperature or nutrient enrichment) on this phase may enhance the restoration efforts. This is other important appeals of the experiment and it has been overlooked in the introduction. The use of recent cites about the effect of environmental conditions changes in seeds or seedlings are also necessary. I recommend read and/or cite: Pereda-Briones et al. 2019 Marine Pollution Bulletin 141: 36-45; Yue et al. (2019) Marine Pollution Bulletin 146: 848-856

Minors concerns and suggestions:

The paper is focus on tropical seagrasses but readers also must know that seagrass is one of the most widespread habitat. I suggest include it at the beginning of introduction.

About ecological services authors should include more number of references and more recent. They highlight the “high rates of primary production”. In my opinion this is not exactly a ecosystem service. For example, the service would be that seagrass fuelling coastal food webs as a consequence of their high rates of primary production. Authors also include “0.49 Gt C year-1” as data. In order to put the data in perspective, I suggest comparing this data with other ecosystems or the C emitted by human activity.

Along the text they use the expression “regional and global scale”. Replace “regional” by “local”

Along the text they use the expression “nutrient impacts”. Replace “impacts” by “enrichment”

They should explain what is “eutrophication process”.

They wrote: “Human development trigger in land use changes and urbanization that leads to an increase in the concentration of nutrients”. Replace “has resulted in” by “has triggered” or similar. In addition authors also should talking about aquiculture as cause of increase in the concentration of nutrients.

They wrote: “To achieve these aims, a combined temperature and nutrient enrichment laboratory”. Include “of” after “combined” and “increase” after “temperature”

#Material and methods

Section 2.1. Collection and maintenance of seagrases

As they collected fruits, the title of the section should be “Collection and maintenance of seagrass seeds” or similar

They write: “Temperatures vary in this area between 28 to 32ºC. Erase “s” in “temperatures” and add “range” after “Temperature”

Section 2.2. Experimental set up

The title of the section should be “Experimental design and setup”

Although the section is well written, I think some arguments are mixed in the current version triggering an awkward reading in some parts. I suggest authors check the section to make this section more fluency. For example, after “Figure 1” a dot is necessary. Then, authors should explain that “three individual seedlings (consisting of leaves, seeds and roots at the beginning of the experiment) with a diameter of 1.02±0.02 cm and 0.70±0.03 g FW were placed in individual aquarium (n=24) (29x13x30 cm; 10 l) called incubation chamber hereinafter. The target temperature values were obtained by placing each incubation chamber in 4 larger tanks (ETs) of 250 l used as a water bath. The experiment followed a block design with nutrient treatments, nested within the 4 ETs set at the two temperatures.”

I recommend increasing the thickness of the dot and strip lines in boxes of Fig.1

Replace “20 January to 22 February 2017” by “January 20th to February 22th 2017”

At the beginning of the section, I can see the target temperature (26°C and 31°C). Target values for nutrients treatments should be necessary as well.

Water renovation rate was “4ml min-1”. I suggest using “l d-1” because the experiment period was 1 month. The water in aquarium were renovated every two days aprox. The water enter is explain but how the water go out from the aquariums? On the other hand, 4ml min-1 = 5.7 l d-1. Then, every day aprox 70 l go out from reservoirs (115l) to fill their 12 aquariums. How they filled the reservoir during the experimental period (1 month)? How many pulses of nutrients they added during the experiment?

About light, all aquariums had their own LED lamps or they were lighted by common LED lamps? Are they checked the light level in each aquarium to ensure all aquarium had similar light? It is important to ensure that author could set a homogenous field of irradiance in each chamber. What sensor was used to check the light level?

In my experience, lots days of experiment with high temperature, nutrient and light usually create the ideal conditions for epiphytes or algae growing although they used ASW. Authors had to deal with this kind of problems? If so, this should have been explained.

Other problem in this kind of experiment is the water evaporation, which can increase the salinity inside the aquariums or reservoirs. Have the authors checked the salinity along the experiment period?

Section 2.3. Water sampling

“were monitored every other day”? What does this specifically mean?

Any reference for Hobo loggers?

I have had some problems with the names used by authors for the equipment. At the beginning of the section they said “experimental tanks” but it is the first time they used it. They are talking about the four “larger tanks (ETs)” or the “two different reservoirs”

They collected samples for nutrients once a week in the two water reservoirs and, authors

they made, and they should be more concrete with the water renovation rate inside aquariums and reservoirs.

2.4. Seedlings morphological and physiological traits

I think is a well section described. I suggest check the quality of equations because it seems a little blurred.

2.5. Statistical analysis

There are some important stats issues that have to be revised: According to the authors, all data were checked for normality and homocedasticity. This is a common misunderstanding; what must follow normality and homocedasticity are the residuals of the linear model, i.e. of the ANOVAs, but not the data.

On the other hand, since there are not mesocosms for temperature alone, CO2 alone and combination of both, the use of two-way ANOVA is not correct. They should use a nested-ANOVA analyse.

#Results and discussion

About results, I recommend to include letters or symbols in figures to show significant differences among treatments.

Replace “Treatment” by “Treatments” in Figures.

I have missed any explication about ETR and photosynthetic efficiency of seedlings in discussion section.

4.1. Seedling responses to increased temperature

They started the discussion comparing their results with previous studies. This is fine but they should try to highlight better the differences between the experiment and the previous ones (older vs young seedlings; optimum temperature increase vs higher temperature increase out of the seagrass tolerance; etc). Now the arguments are a little mixed.

I wondered why studies using older seedlings usually show negative effects under warming. Is it something related with carbon reserves levels? I suggest discuss.

They found that seedling showed more AG biomass and less allocation to BG tissues under lower temperature. Authors should discuss this under an ecological point of view. For example, the increase in AB biomass may increase community production, which would trigger more habitat, nursery and food for organisms. However less allocation to BG tissues could make the new meadow more vulnerable to high hydrodynamic forces (e.g. storms)

They wrote: “This is in contrast to other known studies on subtropical and temperate seagrass species”. Replace “other known” by “previous”

They wrote: “This indicated that the sedlings stage of this species shows resistance to increasing temperatures expected under climate change scenario”. Erase “s” in “species”. Use the past form of “show”. Rewrite “resistance to expected temperature increase”

They wrote: “In Zostera marina seedlings, growth was inhibited at temperatures higher than 30ºC, and photochemical pigments were negatively affected at 25ºC, atemperature that” Separate “atemperature”

They wrote: “To further test temperature tolerance of tropical seedlings, we would need to carry out experiments under a higher range of temperatures (above 32°C).” This kind of sentences would be better at the end of discussion to open possible ways to future experiments

4.2. Seedling response to increased nutrient

About the response to nutrient increase, authors explain that no significant effects were found. They assume that seedling phase does not rely on external nutrient availability. However, as they said in results, nutrient concentrations in the experimental aquaria were low in all treatments regardless of the inputs. Therefore, they should be careful not to be

the higher increase in Chl-a concentrations in the water column.

They should explain the ecological consequences of the decrease of total new root length and number under high-nutrient treatment. Probably, this can affect the establishment capacity of seedlings if they do not reach the adequate root length and for successful anchoring.

Finally they said that the combination effect (temperature + nutrient increase) was unclear, similar to studies on adult seagrass. The combination of multiple factors acting together can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors. I recommend reading more papers that used several factors and where the responses were sometimes weak or different than expected (for example Gunderson et al. (2016) Annu. Rev. Mar. Sci. 8; Moreno-Marin et al. (2018) Limnol. Oceanogr. 63 1528-1544; Egea et al. (2018) Marine Pollution Bulletin, 134: 14-26). Then, authors should explain why the combined response was unclear

4.3. Ecological implications

They should be careful not to be too exhaustive in the conclusions. As their results didn't show negative responses in the experiment, they suggest that warming and nutrient enrichment not seem so detrimental and even can be positive for seedling stage. Here, they must highlighted the limitations of the methodology used (lower nutrient level than expected in the high-nutrient treatments, temperature within the optimal temperature range for E. acoroides, etc) and especially other factors related but not contemplated. For example warming can increase algae blooms, including invasive algae, which can affect seedlings; nutrient enrichment can affect the establishment capacity of seedlings and modified the palatability of new leaves and the herbivore pressure, etc.

Taking into account that this study explored the effect of temperature and nutrients increase, I suggest adding how these factors can produce indirect consequences in the ecosystem (such as bloom of macroalgae or attraction of new herbivores) that can indirectly affect to the success of seedlings. Please read: Pereda-Briones et al. (2019) Marine Pollution Bulletin 141, Pages 36-45

Finally, this experiment provides interesting results but authors should also point out the large number of gaps existing in this type of research and the need to continue researching how factors related to global change affects the success in seedlings of segrass, which would help in future management plans and recovery.

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 09:56 #2

Please find below detail responses to the reviewer's comments:

#Introduction

The introduction, although well structured, it needs a deep review to improve their quality and the appeal for readers. Some areas of the introduction must be extended, especially about the effects of temperature increase and nutrient enrichment in seagrasses (paragraph 2 and 3). The description about the current knowledge of these factors in seagrases is poor and in a tangential way. A more complete description of the current knowledge, with more references, is necessary. That is essential for the scientific quality that the journal requires. I will break it down below:

• First, why they explore these factors instead of others important factors related with climate change and human disturbance (e.g. acidification, changes in salinity and littoral current, etc)? Probably they know that eutrophication processes has been identified as one of the most important factors affecting productivity, community carbon dynamic and seagrass growth and one of the major threats confronting coastal ecosystems. On the other hand, probably they know lots of studies have highlighted the importance of temperature in the seagrass metabolism, growth and survival. Authors should emphasize why changes in temperature and nutrient are essential for seagrass services, development and survival.

• About nutrient enrichment (second paragraph), I found that the explanations of their effects were very short and poorly explained. They should explain how the effects of nutrient enrichment depend on species-specific features, the level of

e0183256 and Campbell et al. (2018) J. Ecol. 106 (1), 401-412.).
• About temperature increase, authors focus on the negative impact of this stressor both warming and heat waves. However, they should also indicate that temperature increase can produce positive effect. For example, temperature increase can benefit the growth and biomass of several seagrass species and even the flowering. Indeed, a recent experiment suggest that heat waves may enhance the autotrophic metabolism of seagrass communities in contrast to previous researches suggesting solely negative effects on seagrasses (please see Egea et al. 2019 PLoS ONE 14(1): e0210386).
• Finally, authors worked hard to explore the effect of two factors under a full factorial mesocosm design. To improve the quality and the appeal for readers, authors should explain the importance of make this kind of experiments (with two or three factors). Please see recent works: Ontoria et al. (2019) Marine Environmental Research, 145 27-38 and Egea et al. (2018) Marine Pollution Bulletin, 134: 14-26. This is, in my opinion, one of the most important appeals of the experiment and it has been overlooked in the introduction. Certainly, the combined response was not significant in the most of variables in the study. They should discuss why since an ecological point of view, using the large amount of bibliography on this subject available in the discussion section.
At the same way, a more complete explanation about why biochemical, physiological or morphological responses are important to the seagrass conservation would be necessary. Authors focus on these variables as indicators. They should also discuss that changes in these variables also alter abiotic factors (such as hydrodynamic conditions, sedimentation rates, etc; see Fonseca et al. 2019 Front. Mar. Sci. 6:376) which affect the distribution of organisms within the canopies, and therefore affecting to diversity levels and even productivity. I recommend read and/or mention: Meysick et al. 2019 PlosOne 14(9):0222020; Jiménez-Ramos et al. 2019 Oikos 128: 64-76; González-Ortiz et al. 2014 PlosOne, 9(8):e104949
Finally I have missed a description about the current knowledge about seagrass conservation and restoration programs using seeds or seedlings. Planting seeds is a cost-efficient method for large-scale seagrass meadow restoration. More studies about changes in environmental conditions (such as temperature or nutrient enrichment) on this phase may enhance the restoration efforts. This is other important appeals of the experiment and it has been overlooked in the introduction. The use of recent cites about the effect of environmental conditions changes in seeds or seedlings are also necessary. I recommend read and/or cite: Pereda-Briones et al. 2019 Marine Pollution Bulletin 141: 36-45; Yue et al. (2019) Marine Pollution Bulletin 146: 848-856

We appreciate the reviewer´s suggestions. The Introduction has been rewritten trying to incorporate all the reviewer´s suggestions. We acknowledge the reviewer´s exhaustive comments and the interesting references suggested. We have followed all suggestions made by the reviewer. Please, check the changes made in the Introduction in the new version of the manuscript. We have also made changes in the Discussion raising the new topics mention in the Introduction.

Minors concerns and suggestions:

The paper is focus on tropical seagrasses but readers also must know that seagrass is one of the most widespread habitat. I suggest include it at the beginning of introduction.

Changes have been made as suggested by the reviewer. The sentence: “Seagrasses are marine flowering plants that are globally distributed and can form dense meadows in shallow water coastal environments (Duarte, 1991, 2001; Duarte and Cebrián, 1996; Short et al., 2007).” have been added in the first paragraph.

About ecological services authors should include more number of references and more recent. They highlight the “high rates of primary production”. In my opinion this is not exactly a ecosystem service. For example, the service would be that seagrass fuelling coastal food webs as a consequence of their high rates of primary production. Authors also include “0.49 Gt C year-1” as data. In order to put the data in perspective, I suggest comparing this data with other ecosystems or the C emitted by human activity.

Changes have been made in the first paragraph of the Introduction as suggested by the reviewer and new references have been included, as Hemminga et al., 1991; Duarte and Cebrián, 1996; Hemminga and Duarte, 2000; Duarte, 2001; Fourqurean et

Along the text they use the expression “regional and global scale”. Replace “regional” by “local”

Changes have been made as suggested by the reviewer.

Along the text they use the expression “nutrient impacts”. Replace “impacts” by “enrichment”

Changes have been made as suggested by the reviewer.

They should explain what is “eutrophication process”.

An explanation in the Introduction section has been included. Please see paragraph 3 of the Introduction (p.3 Line 69-94).

They wrote: “Human development trigger in land use changes and urbanization that leads to an increase in the concentration of nutrients”. Replace “has resulted in” by “has triggered” or similar. In addition authors also should talking about aquiculture as cause of increase in the concentration of nutrients.

Changes have been made as suggested by the reviewer. The aquaculture is now mentioned as a potential nutrient source, please see p.3 Line 70.

They wrote: “Seagrass responses, as biochemical, physiological or morphological changes, to
nutrients and temperature changes”. Erase “s” in “nutrients”.

Changes have been made as suggested by the reviewer.

They wrote: “To achieve these aims, a combined temperature and nutrient enrichment laboratory”. Include “of” after “combined” and “increase” after “temperature”

Changes have been made to this paragraph and corrected.

#Material and methods

Section 2.1. Collection and maintenance of seagrases

As they collected fruits, the title of the section should be “Collection and maintenance of seagrass seeds” or similar

Changes have been made as suggested by the reviewer, please see p.6 Line 175.

They write: “Temperatures vary in this area between 28 to 32°C. Erase “s” in “temperatures” and add “range” after “Temperature”

Changes have been made to this section.

Section 2.2. Experimental set up

The title of the section should be “Experimental design and setup”

Changes have been made as suggested by the reviewer.

Although the section is well written, I think some arguments are mixed in the current version triggering an awkward reading in some parts. I suggest authors check the section to make this section more fluency. For example, after “Figure 1” a dot is necessary. Then, authors should explain that “three individual seedlings (consisting of leaves, seeds and roots at the beginning of the experiment) with a diameter of 1.02±0.02 cm and 0.70±0.03 g FW were placed in individual aquarium (n=24) (29x13x30 cm; 10 l) called incubation chamber hereinafter. The target temperature values were obtained by placing each incubation chamber in 4 larger tanks (ETs) of 250 l used as a water bath. The experiment followed a block design with nutrient treatments, nested within the 4 ETs set at the two temperatures.”

The section has been substantially changed following the reviewer´s suggestions.

Replace “20 January to 22 February 2017” by “January 20th to February 22th 2017”

Changes have been made as suggested by the reviewer, please see p.7 Line 242-243.

At the beginning of the section, I can see the target temperature (26°C and 31°C). Target values for nutrients treatments should be necessary as well.

Changes have been made as suggested by the reviewer. See comments to this question above.

Water renovation rate was “4ml min-1”. I suggest using “l d-1” because the experiment period was 1 month. The water in aquarium were renovated every two days aprox. The water enter is explain but how the water go out from the aquariums? On the other hand, 4ml min-1 = 5.7 l d-1. Then, every day aprox 70 l go out from reservoirs (115l) to fill their 12 aquariums. How they filled the reservoir during the experimental period (1 month)? How many pulses of nutrients they added during the experiment?

The subsection Experimental design and setup has been substantially changed. See comments to this question above. See comments above.

About light, all aquariums had their own LED lamps or they were lighted by common LED lamps? Are they checked the light level in each aquarium to ensure all aquarium had similar light? It is important to ensure that author could set a homogenous field of irradiance in each chamber. What sensor was used to check the light level?

See comments to this question above.
In my experience, lots days of experiment with high temperature, nutrient and light usually create the ideal conditions for epiphytes or algae growing although they used ASW. Authors had to deal with this kind of problems? If so, this should have been explained.

See comments to this question above.
Other problem in this kind of experiment is the water evaporation, which can increase the salinity inside the aquariums or reservoirs. Have the authors checked the salinity along the experiment period?

See comments to this question above.

Section 2.3. Water sampling

“were monitored every other day”? What does this specifically mean?

See comments to this question above.

Any reference for Hobo loggers?

See comments to this question above.

I have had some problems with the names used by authors for the equipment. At the beginning of the section they said “experimental tanks” but it is the first time they used it. They are talking about the four “larger tanks (ETs)” or the “two different reservoirs”

See comments to this question above.

They collected samples for nutrients once a week in the two water reservoirs and, authors said, “random aquariums”. How many random aquariums in each week? On the other hand, check the nutrients level once a week seem a little bit taking into a count the high water renovation rate they used. Authors should explain better the frequency of nutrient pulses they made, and they should be more concrete with the water renovation rate inside aquariums and reservoirs.

2.4. Seedlings morphological and physiological traits

I think is a well section described. I suggest check the quality of equations because it seems a little blurred.

The quality of the equations have been improved, please see p.8-9 Line 285, 300, 304.

2.5. Statistical analysis

There are some important stats issues that have to be revised: According to the authors, all data were checked for normality and homocedasticity. This is a common misunderstanding; what must follow normality and homocedasticity are the residuals of the linear model, i.e. of the ANOVAs, but not the data.

See comments to question above.

On the other hand, since there are not mesocosms for temperature alone, CO2 alone and combination of both, the use of two-way ANOVA is not correct. They should use a nested-ANOVA analyse.

See comments to question above.

#Results and discussion

About results, I recommend to include letters or symbols in figures to show significant differences among treatments.

Please see our response to this comment in the previous section

Replace “Treatment” by “Treatments” in Figures.

Changes have been made

I have missed any explication about ETR and photosynthetic efficiency of seedlings in discussion section.

Please, see answers to these comments above.

4.1. Seedling responses to increased temperature

They started the discussion comparing their results with previous studies. This is fine but they should try to highlight better the differences between the experiment and the previous ones (older vs young seedlings; optimum temperature increase vs higher temperature increase out of the seagrass tolerance; etc). Now the arguments are a little mixed.

Please, see answers to these comments above.

I wondered why studies using older seedlings usually show negative effects under warming. Is it something related with carbon reserves levels? I suggest discuss.

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They found that seedling showed more AG biomass and less allocation to BG tissues under lower temperature. Authors should discuss this under an ecological point of view. For example, the increase in AB biomass may increase community production, which would trigger more habitat, nursery and food for organisms. However less allocation to BG tissues could make the new meadow more vulnerable to high hydrodynamic forces (e.g. storms)

Please, see answers to these comments above.

They wrote: “This is in contrast to other known studies on subtropical and temperate seagrass species”. Replace “other known” by “previous”

to increasing temperatures expected under climate change scenario". Erase "s" in "species". Use the past form of "show". Rewrite "resistance to expected temperature increase"

This sentence was slightly modified, with including the suggestions by the reviewer where still applicable.

They wrote: "In Zostera marina seedlings, growth was inhibited at temperatures higher than 30ºC, and photochemical pigments were negatively affected at 25ºC, atemperature that" Separate "atemperature"

Changes have been made as suggested by the reviewer.
They wrote: "To further test temperature tolerance of tropical seedlings, we would need to carry out experiments under a higher range of temperatures (above 32°C)." This kind of sentences would be better at the end of discussion to open possible ways to future experiments

Changes have been made as suggested by the reviewer.

4.2. Seedling response to increased nutrient

About the response to nutrient increase, authors explain that no significant effects were found. They assume that seedling phase does not rely on external nutrient availability. However, as they said in results, nutrient concentrations in the experimental aquaria were low in all treatments regardless of the inputs. Therefore, they should be careful not to be too exhaustive with the conclusions obtained here. I recommend to discuss why the water nutrient concentration in aquariums were less than expected. Uptake by seedlings? proliferation of fast-growing species such as epiphytes and algae? Probably this promoted the higher increase in Chl-a concentrations in the water column.

Please, see the response to this comment in the section above.

They should explain the ecological consequences of the decrease of total new root length and number under high-nutrient treatment. Probably, this can affect the establishment capacity of seedlings if they do not reach the adequate root length and for successful anchoring.

Finally they said that the combination effect (temperature + nutrient increase) was unclear, similar to studies on adult seagrass. The combination of multiple factors acting together can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors. I recommend reading more papers that used several factors and where the responses were sometimes weak or different than expected (for example Gunderson et al. (2016) Annu. Rev. Mar. Sci. 8; Moreno-Marin et al. (2018) Limnol. Oceanogr. 63 1528-1544; Egea et al. (2018) Marine Pollution Bulletin, 134: 14-26). Then, authors should explain why the combined response was unclear

Please, see the response to these comments in the section above.

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They should be careful not to be too exhaustive in the conclusions. As their results didn't show negative responses in the experiment, they suggest that warming and nutrient enrichment not seem so detrimental and even can be positive for seedling stage. Here, they must highlighted the limitations of the methodology used (lower nutrient level than expected in the high-nutrient treatments, temperature within the optimal temperature range for E. acoroides, etc) and especially other factors related but not contemplated. For example warming can increase algae blooms, including invasive algae, which can affect seedlings; nutrient enrichment can affect the establishment capacity of seedlings and modified the palatability of new leaves and the herbivore pressure, etc.
Finally, this experiment provides interesting results but authors should also point out the large number of gaps existing in this type of research and the need to continue researching how factors related to global change affects the success in seedlings of segrass, which would help in future management plans and recovery.

QUALITY ASSESSMENT

Q 6	Originality
Q 7	Rigor
Q 8	Significance to the field
Q 9	Interest to a general audience
Q 10	Quality of the writing
Q 11	Overall quality of the study

REVISION LEVEL

Q 12 What is the level of revision required based on your comments:

Reviewer 1 | 15 Jan 2020 | 11:52 #1

Substantial revisions

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

Morphological and physiological responses of Enhalus acoroides seedlings under varying temperature and nutrient treatment

Rohani Ambo-Rappe* , Suci Rahmadani Artika, Mirta Teichberg, Agustin Moreira-Saporiti and Inés Viana

Original Research, Front. Mar. Sci. - Global Change and the Future Ocean
Received on: 07 Dec 2019, **Edited by:** Iris Eline Hendriks
Manuscript ID: 518333
Research Topic: Seagrasses under Times of Change
Keywords: Tropical seagrass, Indo-Pacific, Traits, Growth, Nutrient content, Photosynthesis, Carbohydrates, Indonesia

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Reviewer 2
Independent review report submitted: 15 Jan 2020
Interactive review activated: 23 Jan 2020
Final report submitted: 06 Apr 2020

Final Evaluation

Q 1 Final comments to Author (optional):

Reviewer 2 | 06 Apr 2020 | 14:03

Many thanks for your patience in considering all my comments, corrections and suggestions.

Q 2 Do you ENDORSE THE PUBLICATION of this manuscript in its current form?

Reviewer 2 | 06 Apr 2020 | 14:03

– Yes

EVALUATION

Q 1 Please summarize the main findings of the study.

Reviewer 2 | 20 Jan 2020 | 16:40 #1

The manuscript presents the results of a study about seagrass seedlings of the species Enhalus acoroides. In this study the authors perfom a full factorial experiment with two temperature treatments (26 and 31C) and two nutrient levels (high and low).
The authors find higher morphological traits (number of leaves, biomass..) and higher leaf sucrose and starch in the high temperature treatments and no differences in photosynthetic parameters. However, the water chemisty analysis performed during the experiment in the aquaria show that the high temperature treatments are also the ones with higher dissolved nitrogen and no changes in any other nutrients.

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 10:16 #2

target concentrations, it is common that concentrations once in the aquaria drastically drop. But different treatments had an effect on the trophic conditions in the aquaria, and we have described it better now in the manuscript. This has been a common issue in other nutrient experiments with seagrass, as for example Touchette et al. (2003) reported that mean water column nutrient levels ranged from <0.01 to 1.7 µM nitrate, 0.75 to 2.45 µM ammonium and 0.7 to 1.15 µM TP, when the concentrations of nitrate provided to experimental tanks were 8 µM above ambient. This showed that "the majority of the nitrate added to the tanks (daily additions) was taken up relatively quickly by the system." On the other side, Moreno-Marín et al (2018) reported that HN (high-nutrient) treatments were provided with 375 µM ammonium as target concentration and had a. 25 µM ammonium just after addition. Ontoria et al (2019) showed that that "ammonium concentrations in the water of the moderate and high treatments were increased to the target values at the beginning of the experiment and had decreased at the end of the experiment, despite the repeated additions of ammonium and irrespective of the thermal treatment"

Also, inorganic nutrient concentrations were in some cases very low to the quantification limit of the technique (we have now included a comment on this in the text) and had to be deleted from the analysis. Therefore, nutrient concentrations in the reservoirs rather than in the aquaria, and signs of eutrophic conditions, as chlorophyll concentrations in water and the presence of slimy algae (now described in section 3.1) are better indicators of the effect of the different treatments on the aquaria.

Reviewer 2 | 25 Mar 2020 | 17:44 #3

The authors added nutrients to the water that was not high in the aquaria and did not produce and increase in N in tissues. However in the same aquaria they had adult plants which can be the ones taken up all the nutrients in the aquaria due to the higher nutrient uptake surface (leaf surface). Therefore, I suggest that the authors add some data or information about if the adult seagrasses showed effects of increased nutrients in the water such as increased N in tissues, even if it is as a reference in preparation. This will more clearly point to the use of resources from seeds in the seedlings and that the nutrient addition system worked fine. Ontoria et al. saw also a reduction in water nutrients after 15 days and the correspondent increase in N, common response to N addition in seagrasses (e.g., Hernan et al 2019, Marco-Méndez 2016; Tomas 2015; Ibarra-Obando,2004; Invers 2004…), and sign that plants are actually taking the N supplied. Touchette et al also reports lower C/N in treatments where nutrients were added.

Overall, I see that the seedlings are not taking the nutrients added so there are two possibilities that should be discussed: 1) nutrients are taken by the other components of the system (i.e., adult seagrasses (probably) and epiphytes) so the nutrients available to the seedlings are not the nutrients added from the reservoir due to competition from other organisms in the system 2) these very young seedlings are not taken nutrients from the water because they rely solely in seed resources. It should be discussed this effect of competition for nutrients between adults and seedlings which is a very cool result of this work. It can be misleading to read that the seedlings were unaffected by nutrient addition when in fact nutrient addition may not correspond to nutrient availability.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #4

We appreciate this comment and the suggestion made by the reviewer. We have followed the reviewer´s suggestion and we have included some comments dealing with this aspect to the Discussion. We agree that the absence of changes in the seedling´s tissues could be to the use of the seed´s reserves but also due to nutrient competition within the aquaria. We now discuss it in section 4.2. Seedling response to increased nutrients in the Discussion. See also our response to Q4

Reviewer 2 | 01 Apr 2020 | 15:23 #5

Thanks for the changes made I have some further concerns regarding the nutrient treatment, sorry for my insistence.

Section 4.2 is a bit contradictory.

the use of internal resources that you are making in the previous sentence I would suggest. “In fact, previous studies show that seagrass seedlings in some species use internal resources on the early stages of development (Refs) while others also take nutrients from the water column (refs).”

I don’t think we can say that seedlings consume food from the seeds…

Line 552 can you say here if the adult seagrasses showed signs of nutrient addition? It would be a good way to show that there was in facts N enrichment in the aquaria.

E.g., Another possible factor to explain why external nutrients did not affect seedling´s traits could be that there were other primary producers in the experimental aquaria, including microalgae and adult seagrasses which exhibited an increased N content in leaves (or any other sign) (Viana et al., in prep), and thus might have competed for nutrients with seedlings.

Line 557. There are studies showing that seedlings use nutrients from seeds (e.g., Balestri 2009) so I think is safe to say here “Furthermore, contrary to recently germinated seedlings which can use resources stored in the seed (ref), adult seagrasses rely mainly on external nutrient concentrations for growth”

Lines 558-560 Here you are saying that seedlings were kind of forced to use internal reserves because there were not enough water nutrients meaning that your nutrient treatment failed its purpose. Are there any data of N uptake in the adult seagrass species that were also in the aquaria? So far it is unclear for the reader if the result of the nutrient treatment is because the design was incorrect or because seedlings are in fact unaffected by changes in nutrients in the water column.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #6

Thanks for the changes made I have some further concerns regarding the nutrient treatment, sorry for my insistence.

Section 4.2 is a bit contradictory.

Line 546-549 This sentence contradicts the previous phrase and besides is not used like this. It should be something like Besides using internal resources, seedlings can also use nutrients from the water column (refs). Though, to give more streght to the argument of the use of internal resources that you are making in the previous sentence I would suggest. “In fact, previous studies show that seagrass seedlings in some species use internal resources on the early stages of development (Refs) while others also take nutrients from the water column (refs).”

We have rewritten the mentioned paragraph, please see new version in L. 547-579

I don’t think we can say that seedlings consume food from the seeds…

We agree the language was a bit casual. The sentence now reads: “it is verly likey that the seedlings still use nutrient reserves in the seeds”.

Line 552 can you say here if the adult seagrasses showed signs of nutrient addition? It would be a good way to show that there was in facts N enrichment in the aquaria. E.g., Another possible factor to explain why external nutrients did not affect seedling´s traits could be that there were other primary producers in the experimental aquaria, including microalgae and adult seagrasses which exhibited an increased N content in leaves (or any other sign) (Viana et al., in prep), and thus might have competed for nutrients with seedlings.

Yes, the adult seagrass did show higher nutrient leaf contents in the high-nutrient treatments. Changes have been made as suggested by the reviewer.

Line 557. There are studies showing that seedlings use nutrients from seeds (e.g., Balestri 2009) so I think is safe to say here “Furthermore, contrary to recently germinated seedlings which can use resources stored in the seed (ref), adult seagrasses rely mainly on external nutrient concentrations for growth”

We have rewritten the mentioned paragraph, please see new version in L. 547-579. The suggested reference has been included. We appreciate the suggestion.

species that were also in the aquaria? So far it is unclear for the reader if the result of the nutrient treatment is because the design was incorrect or because seedlings are in fact unaffected by changes in nutrients in the water column.

We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #7

Thanks for the changes made I have some further concerns regarding the nutrient treatment, sorry for my insistence.

Section 4.2 is a bit contradictory.

Line 546-549 This sentence contradicts the previous phrase and besides is not used like this. It should be something like Besides using internal resources, seedlings can also use nutrients from the water column (refs). Though, to give more streght to the argument of the use of internal resources that you are making in the previous sentence I would suggest. “In fact, previous studies show that seagrass seedlings in some species use internal resources on the early stages of development (Refs) while others also take nutrients from the water column (refs).”

We have rewritten the mentioned paragraph, please see new version in L. 547-579

I don’t think we can say that seedlings consume food from the seeds…

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We have rewritten the mentioned paragraph, please see new version in L. 547-579. The suggested reference has been included. We appreciate the suggestion.

Lines 558-560 Here you are saying that seedlings were kind of forced to use internal reserves because there were not enough water nutrients meaning that your nutrient treatment failed its purpose. Are there any data of N uptake in the adult seagrass species that were also in the aquaria? So far it is unclear for the reader if the result of the nutrient treatment is because the design was incorrect or because seedlings are in fact unaffected by changes in nutrients in the water column.

We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #8

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Section 4.2 is a bit contradictory.

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We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #9

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rely mainly on external nutrient concentrations for growth"

We have rewritten the mentioned paragraph, please see new version in L. 547-579. The suggested reference has been included. We appreciate the suggestion.

Lines 558-560 Here you are saying that seedlings were kind of forced to use internal reserves because there were not enough water nutrients meaning that your nutrient treatment failed its purpose. Are there any data of N uptake in the adult seagrass species that were also in the aquaria? So far it is unclear for the reader if the result of the nutrient treatment is because the design was incorrect or because seedlings are in fact unaffected by changes in nutrients in the water column.

We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #10

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Section 4.2 is a bit contradictory.

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We have rewritten the mentioned paragraph, please see new version in L. 547-579

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We have rewritten the mentioned paragraph, please see new version in L. 547-579. The suggested reference has been included. We appreciate the suggestion.

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We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #11

Section 4.2 is a bit contradictory.

Line 546-549 This sentence contradicts the previous phrase and besides is not used like this. It should be something like Besides using internal resources, seedlings can also use nutrients from the water column (refs). Though, to give more streght to the argument of the use of internal resources that you are making in the previous sentence I would suggest. “In fact, previous studies show that seagrass seedlings in some species use internal resources on the early stages of development (Refs) while others also take nutrients from the water column (refs).”

We have rewritten the mentioned paragraph, please see new version in L. 547-579

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We have rewritten the mentioned paragraph, please see new version in L. 547-579. The suggested reference has been included. We appreciate the suggestion.

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We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Corresponding Author: Rohani Ambo-Rappe | 03 Apr 2020 | 15:35 #12

Thanks for the changes made I have some further concerns regarding the nutrient treatment, sorry for my insistence.

Section 4.2 is a bit contradictory.

Line 546-549 This sentence contradicts the previous phrase and besides is not used like this. It should be something like Besides using internal resources, seedlings can also use nutrients from the water column (refs). Though, to give more streght to the argument of the use of internal resources that you are making in the previous sentence I would suggest. “In fact, previous studies show that seagrass seedlings in some species use internal resources on the early stages of development (Refs) while others also take nutrients from the water column (refs).”

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other sign) (Viana et al., in prep), and thus might have competed for nutrients with seedlings.

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We agree in that the message could be contradictory and we have rewritten the paragraph and added more references.

Reviewer 2 | 03 Apr 2020 | 16:14 #13

Thank you for considering my comments.

The last thing that I would suggest is some help in the **english editing** for this manuscript since some sentences read weird.

E.g., Lines 589-591. In contrast, Z. marina can survive and stay healthy after being given nutrient enrichment for 2 weeks, as under natural conditions this species is already expose to high nutrient concentrations...

586-589 For example, while nutrient enrichment usually has an effect on seagrass tissue (be more specific, what effect?) nutrient content (e.g. Ontoria et al., 2019; Viana et al., in prep), it did not have a significant effect on leaf length, leaf width and seagrass production of Thalassia testudinum (Heck et al., 2000)

Again, to be sure that nutrient concentrations were high in the high nutrient content I suggest to **add specifically if the authors found higher N, CN or whatever change they found in the adult plants of the aquaria.**

591-594Other studies that have reviewed seagrass responses to nutrients show that a number of environmental factors and intraspecific characteristics such as? influence nutrient limitation in seagrasses (Short, 1987; 594 Lee et al., 2007)

Corresponding Author: Rohani Ambo-Rappe | 06 Apr 2020 | 08:34 #14

The last thing that I would suggest is some help in the english editing for this manuscript since some sentences read weird.

E.g., Lines 589-591. In contrast, Z. marina can survive and stay healthy after being given nutrient enrichment for 2 weeks, as under natural conditions this species is already expose to high nutrient concentrations...

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A native speaker has carefully revised the manuscript and have edited the highlighted sentences and other part of the text. We hope this revised manuscript is now satisfactory in English.

Again, to be sure that nutrient concentrations were high in the high nutrient content I suggest to add specifically if the authors found higher N, CN or whatever change they

some more details have been now included in this version.

591-594Other studies that have reviewed seagrass responses to nutrients show that a number of environmental factors and intraspecific characteristics such as? influence nutrient limitation in seagrasses (Short, 1987; 594 Lee et al., 2007)

Changes have been made as suggested by the reviewer.

Reviewer 2 | 06 Apr 2020 | 14:01 #15

Thanks

Q 2 **Please highlight the limitations and strengths.**

Reviewer 2 | 20 Jan 2020 | 16:40 #1

There are few works on the effects of multiple stressors (nutrient and temperature) in seagrass seedlings and none in tropical species. The idea and experimental design are good and these more physiological experiments are also needed in order to make predictions about seagrass resilience (or tolerance) to future climate scenarios.
The highest limitation is the short length of the experiment, the temperatures chosen which are in the normal range of the species and the fact that the high temperature treatments are also high nutrient and thus it is difficult to disentangle the nutrient vs “warming” effect

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 10:16 #2

Thank you for pointing this out. Some revisions have been done to address this comment.

Reviewer 2 | 25 Mar 2020 | 17:44 #3

Thanks for adding all this information and the revisions made.

I have again expressed my concerns about the nutrient treatment in the previous question.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #4

We appreciate the reviewer´s comments that have helped to improve the previous version of the manuscript. See our responses to this issue in the queries

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #5

We appreciate the reviewer´s comments that have helped to improve the previous version of the manuscript. See our responses to this issue in the queries

Reviewer 2 | 01 Apr 2020 | 15:23 #6

Thank you for considering my comments

Q 3 **Please comment on the methods, results and data interpretation. If there are any objective errors, or if the conclusions are not supported, you should detail your concerns.**

Reviewer 2 | 20 Jan 2020 | 16:40 #1

1- There should have been some experimental error by which the system of nutrient addition did not increase P content in high nutrient treatments and N was indeed higher in high temperature treatments (0.88-1.25 for high temp vs 0.62-0.64 for low temp, table1). Also the authors don't show any statistical analysis to see if there are differences in nutrients among treatments.

2- The temperatures chosen (26-31C) are within the normal temperature range for the species (24-33C cited in the manuscript) and normal temperature range in the area where the seeds where collected (28-32C Materials and methods section 2.1).

3- Not sure if the high nutrient levels are indeed high since the authors do not provide any reference for what is normal for this species.

Less important flaws
4- The length of the experiment might be a bit short (not sure if in the length of the experiment , 1 month, they counted acclimatization) because seedlings have resources stored in the seeds to withstand unfavorable conditions that might impede normal photosynthesis and growth. More so if the temperature used is not a really high temperature for the species.

Due to some of these flaws the effects on seedlings that authors attribute to "high temperature" can be due to high dissolved nitrogen and so, data interpretation is misled by the naming of the treatments

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Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 10:16 #2

Please find below responses to reviewer in detail:

Methods seem adequate but there are major methodological flaws that affect teh interpretation of the data:

1- There should have been some experimental error by which the system of nutrient addition did not increase P content in high nutrient treatments and N was indeed higher in high temperature treatments (0.88-1.25 for high temp vs 0.62-0.64 for low temp, table1). Also the authors don't show any statistical analysis to see if there are differences in nutrients among treatments.

We have now explained the different trophic conditions observed under different treatments and run statistical analysis to compare different treatments. Please see section 3.1.

2- The temperatures chosen (26-31C) are within the normal temperature range for the species (24-33C cited in the manuscript) and normal temperature range in the area where the seeds where collected (28-32C Materials and methods section 2.1).

Climate change impact on temperature is variable in Spermonde archipelago. Studies have shown annual variability from 26-31°C and in this experiment we are testing seedling response to their current minimum and maximum range of temperature exposure. With future climate change, the seedlings will experience temperatures closer to the maximum at the longer period (daily constant). This experiment tested the interaction between nutrient and temperature within their minimum and maximum range. Further testing above the current maximum should be done in future experiments. We have included the minimum and the maximum ranges in the text in Material and methods section 2.1, line 190 to clarify.

3- Not sure if the high nutrient levels are indeed high since the authors do not provide any reference for what is normal for this species.

We have now included in the Introduction the very low nutrient concentrations in tropical seagrass meadows and the range of concentrations observed in the area.

Less important flaws
4- The length of the experiment might be a bit short (not sure if in the length of the experiment , 1 month, they counted acclimatization) because seedlings have resources

The seedlings were only kept in acclimatization conditions for enough time to have incipient leaves (a couple of weeks), i.e. to check the seeds were viable. When this happened, they were moved to the experimental aquaria. The experiment started when the desired temperature was reached in all experimental tanks (ET) and nutrients were added. We also think that seedlings might be using their stored resources to deal with unfavorable conditions, and this is one of the interesting points of this study as there are no studies on these early-phase seedlings.

Due to some of these flaws the effects on seedlings that authors attribute to "high temperature" can be due to high dissolved nitrogen and so, data interpretation is misled by the naming of the treatments

We understand the reviewer concern. We hope that the replies to the previous comments and the changes made in the manuscript help to understand the limitations of evaluating the effect of the treatments by solely looking at the nutrient concentrations data. We have now included a description of the trophic conditions observed under high-nutrient treatments that make us think that treatments had an effect on the system. Please, see section 3.1.

Reviewer 2 | 25 Mar 2020 | 17:44 #3

1- See Q1

2- Thanks for adding this information. Now it is more clear that the objective was to maintain the seedlings on the maximum temperature for a longer period.

3- I see that actually the "low nutrient" treatment has a higher N content than natural ambient level so all plants are enriched in N. In any case treatments are low-high nutrient but in both treatments plants are exposed to higher levels that natural.

4- Thanks for clarifying.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #4

Thank you for your comments. We have used for this experiment artificial seawater (see reference in the manuscript) which has small amounts of nutrients apart from the very small amount added to the low-nutrient treatment in this experiment. In tropical seagrass meadows nutrient concentrations in the water column are usually close to the detection limit, as nutrients are rapidly taken up. In this way, porewater from these oligotrophic environments become more important as a nutrient source in seagrasses as it shows higher nutrient concentrations than the water column. This compartment better reflects the nutrient availability in these environments. In an artificial laboratory experiments there are no nutrients available in the porewater, therefore those concentrations need to be added to the water column. As we were aware about the potential competition, we did not want any nutrient to be very limiting and decided to add slightly higher nutrient concentrations to the water column, also thinking about the potential algae that usually grows in this kind of experiments that would also take up these nutrients.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #5

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these nutrients.

Reviewer 2 | 01 Apr 2020 | 15:23 #6

Thank you for the clarifications

Q 4 **Check List**

Reviewer 2 | 20 Jan 2020 | 16:40 #1

Is the English language of sufficient quality?
- Yes

Is the quality of the figures and tables satisfactory?
- Yes

Does the reference list cover the relevant literature adequately and in an unbiased manner?
- Yes

Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Yes

Are the methods sufficiently documented to allow replication studies?
- Yes

Are the data underlying the study available in either the article, supplement, or deposited in a repository? (Sequence/expression data, protein/molecule characterizations, annotations, and taxonomy data are required to be deposited in public repositories prior to publication)
- Yes

Does the study adhere to ethical standards including ethics committee approval and consent procedure?
- Not Applicable

Have standard biosecurity and institutional safety procedures been adhered to?
- Not Applicable

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 10:16 #2

Thank you

Reviewer 2 | 25 Mar 2020 | 17:44 #3

I suggested some other more actual references about the effects of nutrients in adult seagrasses

Some things to change in relation to one trait analyzed (seed starch) that is discussed but there are no statistical analyses.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #4

The suggested references in Q5 have been added plus two extra references on nutrient impact on adult seagrasses.

We have decided to include the mean and standard error data of these two variables, but not the PERMANOVA results (as explained in section 2.5) as it was not possible that these variables met the assumptions required by this test. Besides, we apologize, as we

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #5

The suggested references in Q5 have been added plus two extra references on nutrient impact on adult seagrasses.

We have decided to include the mean and standard error data of these two variables, but not the PERMANOVA results (as explained in section 2.5) as it was not possible that these variables met the assumptions required by this test. Besides, we apologize, as we forget to delete the comments on these traits included in the Discussion from the previous version. We agree with the reviewer, and we have deleted them.

Reviewer 2 | 01 Apr 2020 | 15:23 #6

Thanks for the changes made.

Q 5 **Please provide your detailed review report to the editor and authors (including any comments on the Q4 Check List):**

Reviewer 2 | 20 Jan 2020 | 16:40 #1

I want to congratulate the authors for the article for the challenging experiment considering the transportation of seeds and the logistics. Experiments with seagrass seedlings in multistressor conditions are rarely performed and less so in tropical species. I really enjoyed reading this article which is in general clearly written (I suggest some parts that need clarification or change). However, there are some major flaws that need to be addressed.
1- There should have been some experimental error by which the system of nutrient addition did not increase P content in high nutrient treatments and N was indeed higher in high temperature treatments (0.88-1.25 for high temp vs 0.62-0.64 for low temp, table1). Also the authors don't show any statistical analysis to see if there are differences in nutrients among treatments.
2- The temperatures chosen (26-31C) are within the normal temperature range for the species (24-33C cited in the manuscript) and normal temperature range in the area where the seeds where collected (28-32C Materials and methods section 2.1).
3- Not sure if the high nutrient levels are indeed high since the authors do not provide any reference for what is normal for this species.
Less important flaws
4- The length of the experiment might be a bit short (not sure if in the length of the experiment, 1 month, they counted acclimatization) because seedlings have resources stored in the seeds to withstand unfavorable conditions that might impede normal photosynthesis and growth. More so if the temperature used is not a really high temperature for the species.
Due to some of these flaws the effects on seedlings that authors attribute to "high temperature" can be due to high dissolved nitrogen and so, data interpretation is misled by the naming of the treatments
It is also a bit inconvenient not to have number of lines in order to comment more easily.
Specific comments:
Page 3
1. Seagrasses provide multiple ecosystem services such as high rates of primary production (up to 0.49 Gt C year-1; Duarte and Cebrián, 1996)….

2. Enhalus acoroides is a tropical seagrass with a high tolerance to environmental changes such as temperature and nutrients (McMillan, 1984; Terrados et al., 1999), and therefore it can be used as an early warning indicator to temperature and varying nutrient fluctuations. If an organism is tolerant to changes it cannot be used as an indicator because it would not show signs of damage until later compared to other organisms in the same community
3. Nevertheless, the seed and seedling phase is a critical stage, since sexual reproduction is very variable and in general, the success of seed establishment is low (Bewley and Black, 1994), the sexual reproduction provides genetic diversity which could make seagrass populations more resistant to the current changing scenario; providing an opportunity to

The sentence is not correctly written and is too long. Nevertheless doesn't make sense in connection with the previous sentence… The opportunity of colonizing new habitats and the genetic diversity provided by sexual reproduction could make seagrass populations more resistant to the current changing scenario. In addition, due to the highly variable (frequency, flower production??) and low success of seed establishment (depending on what your reference says), sexual reproduction and seedling stages are critical phases in the life of seagrasses (Schupp, 1995; Peterson and Baldwin, 2004) However, there is a lack of research conducted on E. acoroides seeds and seedlings in order to understand their response to various environmental changes.

Page 4

4. Seagrass responses, as biochemical, physiological or morphological changes, to nutrients and temperature changes can be used as indicators of environmental stressors, so management decisions can be taken to stop further potential decline

Seagrass response to changes in nutrients and temperature can be measured through changes in biochemical, physiological or morphological traits which can be used as indicators of environmental stressors, so management decisions can be taken to stop further potential decline

5. In this way, the impact of rising temperature and increase nutrient loading effects has been studied in adult species of Zostera spp. and Cymodocea nodosa (Touchette and Burkholder, 2002; Touchette et al., 2003; Kaldy, 2014; Mvungi and Pillay, 2019; Ontoria et al., 2019) but, as far as we know, no studies have been performed in early life stages of seagrasses.

There is indeed one study that I can think of Burnell et al 2013 Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification

6. This study aims to assess the responses of morphological, physiological and biochemical traits to increased temperature and nutrient enrichment on seedling stages of E. acoroides

7. To achieve these aims, a combined warming? and nutrient enrichment laboratory experiment was conducted using seedlings of E. acoroides

8. I am not sure if it would be high temperature stress if they do not cause stress because this species is adapted to high temperature.

Would like to read somewhere in the introduction which is the natural temperature range of E. acorioides and why the authors chose 26C and 31C.

MATERIALS AND METHODS

Collection and maintenance; so the seedlings were kept at a lower temperature than what they have in their natural environment?

At this point it is unclear if they are testing the effect of rising temperatures, which is what the reader understands from the introduction, because the authors expose the seedlings to two temperatures that, according to the materials and methods (2.1), are below and within the temperature range of this seagrass in present natural conditions.

2.2.

It should be clearly stated somewhere that the experiment is full factorial or specifically which are the experimental treatments. "Different combinations of temperatures and nutrients" is very vague and doesn't state which were the experimental treatments.

Page 5

The explanation of the experimental desing is confusing. It is more clearly explained in the figure legend (Fig. 1)

Page 6

2.4

The authors use a glass spatula to cut and separate the different tissues of the seedling? Did they clean the epiphytes or there was no epiphyte growth on them?

Pag 7

Equations are fuzzy

2.4.4

Leaf, seed and root nitrogen (N) and carbon (C) content (%N and %C respectively) was analyzed on previously dried and powdered (60°C, 48 h) seagrass tissue samples.

RESULTS

3.1 Experiemntal water chemistry.

Did the authors do statistical analysis of the nutrient contents among aquaria? According to table 1, the 31C treatment had higher contents of DIN than the 26C. There is no difference in the other nutrients, Why?

If I understand well table 1, the nutrients DIN are indeed higher at 31 than at 26. Hence the responses the authors attribute to higher temperature can be due to the higher DIN content also at 31C.

3.2

1. The high-temperature and low-nutrient treatment had 5 leaves, while all other treatments had 4 leaves per seedling.

High temperature low nutrient has in fact the highest DIN (1.15 uM)

According to table 1 higher temperature had higher DIN so it can be expected that under higher DIN you have higher growth (leaf legth and biomass).
Seedlings in this treatment also exhibited higher leaf SA, AG biomass and seed diameter.
3. Overall, the total seedling biomass was the highest in the high-temperature treatments
I can't see the results of the statistical analysis on total seedling biomass.
Figure 2. add letters or asterisk to identify the statistically different groups.
4. In the low temperature treatment, seed biomass was the highest followed by AG, and lowest in BG biomass
The sentence is not clear In the low temperature treatment, most of the total seedling biomass corresponds to seed biomass followed by AG, and BG biomass
This is not sustained by the results since according to table 2 there are no differences in seed biomass. There are no results of statistical analysis on AG and BG and is difficult to infer that from figure 2.
5. In contrast, under high temperatures, AG and BG biomass were similar (add statistical results)
3.3.
6. Furthermore, when separating seedling by their initial seed size, we found that initial seed size mattered in the seedling response to increased temperature, with larger seeds increasing leaf SA at a faster rate than smaller seeds (Figure 3)
Where are the statistical results for this? At 26 C I don't see difference and at 31C high nutrients small and large are close.
7. In contrast, %N of the seeds and roots were lower under the high-temperature treatments (P-value<0.05), but did not change in the leaves. C:N ratio of leaves, therefore, showed no significant differences, while C:N ratio in seeds and roots showed noticeable significant differences with temperature (Pvalue<0.05). C:N ratio in seeds and roots were the highest under the high-temperature and low-nutrient treatment (Table 3).
In contrast, %N of seeds and roots was lower in the high-temperature treatments (P-value<0.05), but did not change in leaves. Therefore, C:N ratio of leaves showed no significant differences, while C:N ratio in seeds and roots was significantly lower under high temperature (Pvalue<0.05). (according to statistical analysis there are no differences among "nutrient"treatments)

8. The lowest concentrations of sucrose were observed at the high-temperature treatments, while starch concentrations in the seeds were the lowest in the high-temperature and highnutrient treatment and the highest in the low-temperature and high-nutrient treatment showing an interaction between temperature and nutrients (P-value<0.05)
The lowest concentrations of sucrose in seeds were observed in the high-temperature treatments, while starch concentrations in the seeds were the lowest in the high-temperature and high nutrient treatment and the highest in the low-temperature and high-nutrient treatment showing an interaction between temperature and nutrients (P-value<0.05)
DISCUSSION
There is a problem in the interpretation of results, first the temperatures used (26C-31C) are indeed in the natural temperature range found in the area where seeds were collected (28C-32C. Hence, it is not surprising that they are tolerant to the high temperature and maybe 26C is even a suboptimal temperature.
Also, according to the results of nutrient analysis shown in table1 the high temperature treatment aquaria also had higher DIN and the rest of nutrients are similar among treatments. Statistical analysis are needed here (two-way anovas or similar) to know if there are indeed statistical differences in nutrients among "nutrient" treatments.
I guess this is why the high temperature treatment has higher growth, it has higher nutrients and the temperature is within its natural range.
Maybe the higher sucrose found under low temp in seeds could be related to lower growth due to lower nutrients and thus lower use of resources stored in the seed.?
1. However, in our study we did not intend to find optimal temperature for growing, but the temperature treatment aimed to test effect increase temperature expected under climate change
If this is the case the authors need to say which is the mean temperature that E. acorioides has in the natural environment during "norther hemisphere winter months" (January-february) which is when the experiment is performed. Also, considering that the experimental temperature is at the higher end of the natural temperature range and it only lasted 1 month it would make it difficult to find symptoms of stress since seedlings could use seed reserves to withstand unfavorable conditions. Also I am guessing that the authors do not count in this 1-month experimental time the acclimatization period in which they gradually increase the temperature.
2. In our study, the decrease in %N and sucrose content of the seed also indicates that growth and biomass allocation was supported by internal nutrient and energy stores found in

Pereda-Briones 2019, Hernan et al 2017) because seedlings under high temperature had higher respiration (Guerrero messeguer et al. 2017, Hernan et al 2017) and use seed reserves.
4.1
1. Our results showed no significant effect of nutrient treatment on E. acoroides seedling morphology and physiology…
This likely is because the "nutrient treatments" have in fact similar water nutrient values. Also, which are the normal environmental nutrient levels for this species?, How about CN content in adult E. acoroides?

Corresponding Author: Rohani Ambo-Rappe | 13 Mar 2020 | 10:16 #2

I want to congratulate the authors for the article for the challenging experiment considering the transportation of seeds and the logistics. Experiments with seagrass seedlings in multistressor conditions are rarely performed and less so in tropical species. I really enjoyed reading this article which is in general clearly written (I suggest some parts that need clarification or change). However, there are some major flaws that need to be addressed.

We appreciate reviewer´s comment

1- There should have been some experimental error by which the system of nutrient addition did not increase P content in high nutrient treatments and N was indeed higher in high temperature treatments (0.88-1.25 for high temp vs 0.62-0.64 for low temp, table1). Also the authors don't show any statistical analysis to see if there are differences in nutrients among treatments.

We have now explained the different trophic conditions observed under different treatments and run statistical analysis to compare different treatments. Please see section 3.1.

2- The temperatures chosen (26-31C) are within the normal temperature range for the species (24-33C cited in the manuscript) and normal temperature range in the area where the seeds where collected (28-32C Materials and methods section 2.1).

Please, see comments above

3- Not sure if the high nutrient levels are indeed high since the authors do not provide any reference for what is normal for this species.

We have now included in the Introduction the very low nutrient concentrations in tropical seagrass meadows and the range of concentrations observed in the area (section 2.1)

Less important flaws
4- The length of the experiment might be a bit short (not sure if in the length of the experiment, 1 month, they counted acclimatization) because seedlings have resources stored in the seeds to withstand unfavorable conditions that might impede normal photosynthesis and growth. More so if the temperature used is not a really high temperature for the species.
Due to some of these flaws the effects on seedlings that authors attribute to "high temperature" can be due to high dissolved nitrogen and so, data interpretation is misled by the naming of the treatments

See our response to this comment in the previous section

It is also a bit inconvenient not to have number of lines in order to comment more easily.

We apologize for this error. We have now added line numbers to make following revisions easier.

Specific comments:
Page 3

Introduction has been substantially changed, and this sentence has been deleted.

2. Enhalus acoroides is a tropical seagrass with a high tolerance to environmental changes such as temperature and nutrients (McMillan, 1984; Terrados et al., 1999), and therefore it can be used as an early warning indicator to temperature and varying nutrient fluctuations.
If an organism is tolerant to changes it cannot be used as an indicator because it would not show signs of damage until later compared to other organisms in the same community

We agree with the reviewer that the statement is confusing. We meant to say that as E. acoroides is quite resistant it will still survive under unfavorable conditions and indicators could be measured (this will not be possible if the species would not be there). We have rephrased it to make this clear.

3. Nevertheless, the seed and seedling phase is a critical stage, since sexual reproduction is very variable and in general, the success of seed establishment is low (Bewley and Black, 1994), the sexual reproduction provides genetic diversity which could make seagrass populations more resistant to the current changing scenario; providing an opportunity to colonize more favorable habitats.
Sexual reproduction is very variable is a very vague sentence, does it refer to the number of seeds produce, the success of reproduction, the frequency of reproduction… The sentence is not correctly written and is too long. Nevertheless doesn't make sense in connection with the previous sentence… The opportunity of colonizing new habitats and the genetic diversity provided by sexual reproduction could make seagrass populations more resistant to the current changing scenario. In addition, due to the highly variable (frequency, flower production??) and low success of seed establishment (depending on what your reference says), sexual reproduction and seedling stages are critical phases in the life of seagrasses (Schupp, 1995; Peterson and Baldwin, 2004) However, there is a lack of research conducted on E. acoroides seeds and seedlings in order to understand their response to various environmental changes.

The paragraph has been rewritten following the reviewer´s suggestion.

Page 4
4. Seagrass responses, as biochemical, physiological or morphological changes, to nutrients and temperature changes can be used as indicators of environmental stressors, so management decisions can be taken to stop further potential decline
Seagrass response to changes in nutrients and temperature can be measured through changes in biochemical, physiological or morphological traits which can be used as indicators of environmental stressors, so management decisions can be taken to stop further potential decline

Changes have been made to this sentence. It now reads: Seagrass response to changes in nutrient and temperature conditions can be measured by changes in their trait values which are often used as indicators of environmental stress in coastal management. P 4 122-124.

5. In this way, the impact of rising temperature and increase nutrient loading effects has been studied in adult species of Zostera spp. and Cymodocea nodosa (Touchette and Burkholder, 2002; Touchette et al., 2003; Kaldy, 2014; Mvungi and Pillay, 2019; Ontoria et al., 2019) but, as far as we know, no studies have been performed in early life stages of seagrasses.
There is indeed one study that I can think of Burnell et al 2013 Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification

We appreciate the reviewer´s suggestion. Besides, this reference deals with the effect of temperature and CO2 concentrations in adult seagrasses. But following your suggestion we have included in the Introduction section some references that deal with multiple stressors and seagrass seedlings: "There are few works on the effects of other multiple stressors in seagrass seedlings and none in tropical species (Hernán et al., 2016; Alexandre et al., 2018; Pereda-Briones et al., 2018, 2019; Yue et al., 2019)."

6. This study aims to assess the responses of morphological, physiological and

We modified this sentence to read: This study aims to assess the morphological, physiological and biochemical trait responses of the seedling stages of E. acoroides to increased temperature and nutrient enrichment.

7. To achieve these aims, a combined warming? and nutrient enrichment laboratory experiment was conducted using seedlings of E. acoroides

Changes have been made to this sentence. It now reads: To achieve these aims, a laboratory experiment was conducted using seedlings of E. acoroides under the combination of increased temperature and nutrient enrichment. We choose not to use the word warming, as the reviewers point out it is still within the temperature range of the ambient conditions where the sedes were collected. See further comments below.

8. I am not sure if it would be high temperature stress if they do not cause stress because this species is adapted to high temperature.

We agree with the reviewer. We have deleted the word "stress"

Would like to read somewhere in the introduction which is the natural temperature range of E. acorioides and why the authors chose 26C and 31C.

We have included some information regarding the natural temperature range of E. acoroides in the area in section 2.1. Collection and maintenance of seagrass seeds

MATERIALS AND METHODS
Collection and maintenance; so the seedlings were kept at a lower temperature than what they have in their natural environment? .

At this point it is unclear if they are testing the effect of rising temperatures, which is what the reader understands from the introduction, because the authors expose the seedlings to two temperatures that, according to the materials and methods (2.1), are below and within the temperature range of this seagrass in present natural conditions.

Climate change impact on temperature is variable in Spermonde archipelago. Studies have shown annual variability from 26-31°C and in this experiment we are testing seedling response to their current minimum and maximum range of temperature exposure. With future climate change, the seedlings will experience temperatures closer to the maximum at the longer period (daily constant). This experiment tested the interaction between nutrient and temperature within their minimum and maximum range. Further testing above the current maximum should be done in future experiments. We have already included the minimum and the maximum ranges in the text in Material and methods section 2.1, line 178-182 to clarify.

2.2.
It should be clearly stated somewhere that the experiment is full factorial or specifically which are the experimental treatments. "Different combinations of temperatures and nutrients" is very vague and doesn't state which were the experimental treatments.

We have followed the reviewer´s suggestion. The text now reads: "We conducted a full-factorial experiment combining two water temperatures (26°C and 31°C) and two nutrient treatments (…). This yielded in 4 experimental treatments: low temperature and low nutrient concentrations, low temperature and high nutrient concentrations, high temperature and low nutrient concentrations, and high nutrient concentration and high temperature."

Page 5
The explanation of the experimental desing is confusing. It is more clearly explained in the figure legend (Fig. 1)

We have made substantial changes in this section following both reviewer´s suggestions.

Page 6

We have changed the term by "glass slide". The seedlings were cleaned with distilled water (see section 2.4). There was algae and epiphyte overgrowth, especially in the high-nutrient treatments (we have now described this in section 3.1 following reviewers suggestions). We have now specified that "Samples of the separated plant were gently cleaned with distilled water to remove any sediment or epiphytes".

Pag 7
Equations are fuzzy

The quality of the equations has been improved

2.4.4
Leaf, seed and root nitrogen (N) and carbon (C) content (%N and %C respectively) was analyzed on previously dried and powdered (60°C, 48 h) seagrass tissue samples.

Changes have been made as suggested by the reviewer

RESULTS
3.1 Experiemntal water chemistry.
Did the authors do statistical analysis of the nutrient contents among aquaria? According to table 1, the 31C treatment had higher contents of DIN than the 26C. There is no difference in the other nutrients, Why?
If I understand well table 1, the nutrients DIN are indeed higher at 31 than at 26. Hence the responses the authors attribute to higher temperature can be due to the higher DIN content also at 31C.

We acknowledge the reviewer raises this issue and we agree in that the data can confound the results. We have run statistical analysis to compare the nutrient and chlorophyll concentrations and included the results in the text (see section 3.1). No differences were observed among treatments in terms of the nutrient concentrations in the water from the aquaria. As we also explained above, we think that the effects of the treatments on changing the conditions of the systems cannot be based solely on this data, as this has been a widespread problem in this kind of experiments. We now describe the different trophic conditions observed under the different nutrient treatments so it is clear to the reader how different treatments had an effect on the systems.

3.2
1. The high-temperature and low-nutrient treatment had 5 leaves, while all other treatments had 4 leaves per seedling. High temperature low nutrient has in fact the highest DIN (1.15 uM)

We have now added the initial nutrient concentrations applied to the different nutrient treatments. As the flow was also the same for all aquaria within treatments we cannot affirm that high-temperature and low-nutrient treatment had higher concentrations based on the final concentrations in water in the aquaria, as these concentrations are in general very low and close to the quantification limit as nutrient were rapidly taken up once in the aquaria.

2. Maximum leaf length was the highest in the high-temperature treatments, particularly when combined with high-nutrients. This also had an impact on leaf SA and AG biomass. The only seed trait that was significantly influenced by temperature was the diameter
According to table 1 higher temperature had higher DIN so it can be expected that under higher DIN you have higher growth (leaf legth and biomass).
Seedlings in this treatment also exhibited higher leaf SA, AG biomass and seed diameter.

Statistical analysis on nutrient concentrations in water did not show significant differences among treatments. The nutrient concentrations in the aquaria during the experiment has been an issue in different experiments published on the effects of nutrients on seagrass (see comment above) and it is widespread that concentrations are low even though the initial concentrations were orders of magnitude higher. We have now included the nutrient concentrations in the reservoirs to show the different nutrient treatments applied. Therefore, differences showed by the traits in

3. Overall, the total seedling biomass was the highest in the high-temperature treatments
I can't see the results of the statistical analysis on total seedling biomass.

Following the reviewer´s comment, total biomass mean values have now been added to Table 2 and the results of the statistical analysis in Table 4

Figure 2. add letters or asterisk to identify the statistically different groups.

We have decided to change the statistical test used following the Reviewer 1 suggestion. As PERMANOVA do not compare the average values but the distance in the dissimilarity matrix, there is necessarily not correspondence between the results of the test and what is seen in the figures and we thought it could be confusing for the reader. Moreover, results of this test are so extensive that would be too much information for the figure. Besides, we have now included in the figure 2 caption that the results are in Table 4. We hope the reviewer agrees with our decision.

4. In the low temperature treatment, seed biomass was the highest followed by AG, and lowest in BG biomass
The sentence is not clear In the low temperature treatment, most of the total seedling biomass corresponds to seed biomass followed by AG, and BG biomass
This is not sustained by the results since according to table 2 there are no differences in seed biomass. There are no results of statistical analysis on AG and BG and is difficult to infer that from figure 2.

The statistical tests have been redone using PERMANOVA. The results of the PERMANOVA for morphological traits are now available in Table 4, including seed, AG an BG biomass. Fig. 2 still shows the values of all these traits. AG and BG biomass responses were significantly different under temperature treatments while seed biomass showed no effect for temperature or nutrient treatments. Seed size significantly influenced the responses of AG and BG biomass while p-value is low for seed biomass (p-value=0.08). The section has been rewritten describing these new results, please see paragraph 2 of section 3.2.

5. In contrast, under high temperatures, AG and BG biomass were similar (add statistical results)

Please see response to the previous comment

3.3.
6. Furthermore, when separating seedling by their initial seed size, we found that initial seed size mattered in the seedling response to increased temperature, with larger seeds increasing leaf SA at a faster rate than smaller seeds (Figure 3)
Where are the statistical results for this? At 26 C I don't see difference and at 31C high nutrients small and large are close.

We have redone the statistical analysis of the results and a PERMANOVA has now been applied. We agree in that the seed´s size matter for most traits and we have now included this variable as a factor in the model. Please see new Tables 4 and 5. In re-running the statistics, the effect of seed size was not significantly different for leaf SA growth trait, therefore we have decided to delete previous Figure 3 and just include SA growth data (average values of all seed sizes) in Table 3. PERMANOVA results of this trait are in Table 5. However, other traits were related to seed size such as AG and BG biomass and Total Biomass (see table 4).

7. In contrast, %N of the seeds and roots were lower under the high-temperature treatments (P-value<0.05), but did not change in the leaves. C:N ratio of leaves, therefore, showed no significant differences, while C:N ratio in seeds and roots showed noticeable significant differences with temperature (Pvalue<0.05). C:N ratio in seeds and roots were the highest under the high-temperature and low-nutrient treatment (Table 3).
In contrast, %N of seeds and roots was lower in the high-temperature treatments (P-value<0.05), but did not change in leaves. Therefore, C:N ratio of leaves showed no significant differences, while C:N ratio in seeds and roots was significantly lower under

Changes have been made as suggested by the reviewer and according to new statistical results.

8. The lowest concentrations of sucrose were observed at the high-temperature treatments, while starch concentrations in the seeds were the lowest in the high-temperature and highnutrient treatment and the highest in the low-temperature and high-nutrient treatment showing an interaction between temperature and nutrients (P-value<0.05)
The lowest concentrations of sucrose in seeds were observed in the high-temperature treatments, while starch concentrations in the seeds were the lowest in the high-temperature and high nutrient treatment and the highest in the low-temperature and high-nutrient treatment showing an interaction between temperature and nutrients (P-value<0.05)

Changes have been made as suggested by the reviewer.

DISCUSSION
There is a problem in the interpretation of results, first the temperatures used (26C-31C) are indeed in the natural temperature range found in the area where seeds were collected (28C-32C. Hence, it is not surprising that they are tolerant to the high temperature and maybe 26C is even a suboptimal temperature.

We have already included the minimum and the maximum temperature ranges in the text in Material and methods section 2.1. Studies have shown annual variability from 26-31°C and in this experiment we are testing seedling response to their current minimum and maximum range of temperature exposure. With future climate change, the seedlings will experience temperatures closer to the maximum during longer periods. We would also like to highlight that in the present study the temperature was constant (during day and night) and in natural conditions the seagrass only experience this maximum temperatures during 1-2hours per day.

Also, according to the results of nutrient analysis shown in table1 the high temperature treatment aquaria also had higher DIN and the rest of nutrients are similar among treatments. Statistical analysis are needed here (two-way anovas or similar) to know if there are indeed statistical differences in nutrients among “nutrient” treatments.

Please, see our responses to similar comments above. We have now run statistical analysis to detect differences among treatments, although, as explained above, we think that the effect of different treatments in the system should be made from a global interpretation of all variables and the description (now provided). Low nutrient concentrations in experimental aquaria has been an issue in similar experiments (please, see comments above) and a result of primary producers uptake.

I guess this is why the high temperature treatment has higher growth, it has higher nutrients and the temperature is within its natural range.

Please, see our response to related to the nutrient concentrations in the aquaria. We now show the nutrient concentrations in the water reservoirs (Table 1) to show the different concentrations that were continuously applied to the aquaria.

Maybe the higher sucrose found under low temp in seeds could be related to lower growth due to lower nutrients and thus lower use of resources stored in the seed.?

This is a good point, we have now included the seedling size as a factor in the model for analyzing the data and this factor has an effect on biomass traits.

1. However, in our study we did not intend to find optimal temperature for growing, but the temperature treatment aimed to test effect increase temperature expected under climate change
If this is the case the authors need to say which is the mean temperature that E. acorioides has in the natural environment during “norther hemisphere winter months” (January-february) which is when the experiment is performed. Also, considering that the experimental temperature is at the higher end of the natural temperature range and it only lasted 1 month it would make it difficult to find symptoms of stress since seedlings could use seed reserves to withstand unfavorable conditions. Also I am guessing

We have already included the minimum and the maximum ranges in the text in Material and methods section 2.1 to clarify. Studies have shown annual variability from 26-31°C and in this experiment we are testing seedling response to their current minimum and maximum range of temperature exposure. With future climate change, the seedlings will experience temperatures closer to the maximum during longer periods. We would also like to highlight that in the present study the temperature was constant (during day and night) and in natural conditions the seagrass only experience this maximum temperatures during 1-2hours per day maximum. Moreover, this experiment tested the interaction between nutrient and temperature within their minimum and maximum range. Further testing above the current maximum should be done in future experiments to test thermal stress.

2. In our study, the decrease in %N and sucrose content of the seed also indicates that growth and biomass allocation was supported by internal nutrient and energy stores found in the seed. Although, as far as we know, this has not been studied in seagrass seedlings, this effect has been observed in seedlings from terrestrial plants (e.g. Kennedy et al., 2004).
This has also been found in other seagrass seedling experiments under warming (e.g., Pereda-Briones 2019, Hernan et al 2017) because seedlings under high temperature had higher respiration (Guerrero messeguer et al. 2017, Hernan et al 2017) and use seed reserves.

Besides, the reviewer is right and changes in physiology and nutrient storage have been studied by the cited authors under other stressors, our intention was to highlight that no studies have aimed to assess the influence of the initial seedling size in the responses as Kennedy et al. 2004 (among others) in terrestrial seedlings. We have rewritten the paragraph so this statement is not confusing.

4.1
1. Our results showed no significant effect of nutrient treatment on E. acoroides seedling morphology and physiology…
This likely is because the “nutrient treatments” have in fact similar water nutrient values.
Also, which are the normal environmental nutrient levels for this species?, How about CN[m1] content in adult E. acoroides?

Please, see our response to previous comments. We have included information about the temperature range and nutrient concentrations in section 2.1.

Reviewer 2 | 25 Mar 2020 | 17:44 #3

ABSTRACT

Line 25 rise in average and constant temperature.

This sentence makes the reader think that there are two types of temperature treatments one with constant temperature and one with same average temperature but maybe not constant. Also in the abstract maybe the authors can try synthesize the results such as talking about increased aboveground size traits (e.g., number of leaves, leaf length, biomass and area). In general the wording of the abstract can be improved.

INTRODUCTION

There are other recent works on the effects of nutrient addition in seagrass morphological, physiological and chemical traits and palatability to herbivores (Hernan et al 2019, Marco-Méndez 2016; Tomas 2015;)

MATERIALS AND METHODS

Lines 225-230 This part is confusing.

ASW was supplied to each aquaria from two reservoirs with high or low nutrient.

There is one seedling of each size category per treatment (total three seedlings per aquaria) I see that n= 6, did the authors calculate a mean value per aquaria?, I guess that is what they did for morphological traits. Did they pooled plant material to have one value of biochemical trait or they also calculated a mean of value per aquaria?.This is not specified in any of the 2.4 subsections

Lines 342. The authors eliminate data as outliers but what data was consider outlier? How many data points were removed? There are 6 replicates calculated from one value or mean of three values hence removing data should be done carefully as it can lead to very different mean values.

Discussion

Lines 457-459 What about nutrient competition with adult seagrasses from the aquaria? It might be worth mention if the adult seagrasses exhibited an increase in N content.

Lines 463 How are effects additive if there are no effects of nutrients? In fact there is interactive

Line 548-551 One of the reasons of the no nutrient effect in Kaldy 2014 is that in natural conditions eelgrass is already exposed to very high nutrient levels in the ranges of the ones used in the experiment. Heck 2000 did not find morphological changes but they do find and increase in N in seagrass tissues.

Lines 560-562 In line 357-358 the authors say that “Starch concentration in seeds was not analyzed”so how can the authors know if starch was higher in seeds in high nutrient and high temperature.

Also, why would seed resources be consumed at a higher rate in the high nutrient and high temperature compared to the low nutrient high temperature? Resources are consumed at a higher rate with higher temperature in seedlings (e.g., Hernan 2017) but why also with high nutrients?

Line 593 what this article finds is that seagrass seedlings are tolerant and even positively affected by and extended exposure to the current ambient maximum temperature. This implies that extended exposure to warmer temperatures such as those close to the maximum as that expected under climate change, will not affect seedling survival.

Corresponding Author: Rohani Ambo-Rappe | 01 Apr 2020 | 13:22 #4

ABSTRACT

Line 25 rise in average and constant temperature.

Changes have been made as suggested by the reviewer.

This sentence makes the reader think that there are two types of temperature treatments one with constant temperature and one with same average temperature but maybe not constant. Also in the abstract maybe the authors can try synthesize the results such as talking about increased aboveground size traits (e.g., number of leaves, leaf length, biomass and area). In general the wording of the abstract can be improved.

The Abstract has been rewritten following the reviewer´s suggestions.

Q5
INTRODUCTION

There are other recent works on the effects of nutrient addition in seagrass morphological, physiological and chemical traits and palatability to herbivores (Hernan et al 2019, Marco-Méndez 2016; Tomas 2015;)

Thank you for the suggestion. The suggested references have been included in the Introduction.

MATERIALS AND METHODS

Lines 225-230 This part is confusing. ASW was supplied to each aquaria from two reservoirs with high or low nutrient. I would reword it because as I understand from the

We have made some changes in the text. We hope it reads better now.

There is one seedling of each size category per treatment (total three seedlings per aquaria) I see that n= 6, did the authors calculate a mean value per aquaria?, I guess that is what they did for morphological traits. Did they pooled plant material to have one value of biochemical trait or they also calculated a mean of value per aquaria?.This is not specified in any of the 2.4 subsections

We have deleted the phrase "…and the mean value was obtained for each aquarium…" as it was confusing (based on the new analysis of the data). As state in the manuscript, morphological and PAM data were performed in each of the seedlings (a total of 3) within each aquarium (n=6 per treatment). N=6 is the number of replicates (aquaria) not the number of seedlings. Besides, once these measurements were performed, the biochemical analysis was carried out in a pooled sample of the 3 seedlings, as otherwise we would not have had enough material for carrying out all these analyses. We have made this clearer in the manuscript

Lines 342. The authors eliminate data as outliers but what data was consider outlier? How many data points were removed? There are 6 replicates calculated from one value or mean of three values hence removing data should be done carefully as it can lead to very different mean values.

No more than one outlier was removed from each dataset, and in particular, only one outlier was removed for maximum root length and AG:BG ratio variables. We have clarified this in the 2.5. Besides, in Tables 4 and 5 the degrees of freedom of each model can be checked.

Discussion

Lines 457-459 What about nutrient competition with adult seagrasses from the aquaria? It might be worth mention if the adult seagrasses exhibited an increase in N content.

We have discussed this issue now. Please, see section 4.2. Seedling response to increased nutrients of the current version of the manuscript.

Lines 463 How are effects additive if there are no effects of nutrients? In fact there is interactive

Additive effects equal the sum of the effect of each factor in isolation, regardless there is effect or not. This means that the effect of temperature, for instance, is not enhanced nor smoothed by the presence of nutrients. If effects would be interactive, an antagonistic or synergistic effect would have been observed in some trait, which is not the case.

Line 548-551 One of the reasons of the no nutrient effect in Kaldy 2014 is that in natural conditions eelgrass is already exposed to very high nutrient levels in the ranges of the ones used in the experiment. Heck 2000 did not find morphological changes but they do find and increase in N in seagrass tissues.

Thank you for the comments suggested. We have incorporated them to that part of the Discussion. Besides, we apologize but we cannot find any information regarding nutrient content in seagrass tissues in the referred publication (Heck 2000), is it possible that the reviewer refers to other Heck´s publication? Besides, we have added other two publications which have observed nutrient tissue changes after water column nutrient enrichment.

Lines 560-562 In line 357-358 the authors say that "Starch concentration in seeds was not analyzed"so how can the authors know if starch was higher in seeds in high nutrient and high temperature.

We agree with the reviewer. We apologize, as we forget to delete this sentence from the previous version of the manuscript. We have now deleted it and the paragraph has been rewritten.

Also, why would seed resources be consumed at a higher rate in the high nutrient and high temperature compared to the low nutrient high temperature? Resources are consumed at a higher rate with higher temperature in seedlings (e.g., Hernan 2017) but why also with high nutrients?

As no interactive effect of both factors can be proofed (as statistical analysis could not be run) we have deleted that sentence from the current version of the

affected by and extended exposure to the current ambient maximum temperature. This implies that extended exposure to warmer temperatures such as those close to the maximum as that expected under climate change, will not affect seedling survival.

Changes have been made as suggested by the reviewer

Reviewer 2 | 01 Apr 2020 | 15:23 #5

Thank you for considering all my suggestions and sorry that I was mistaken about the Heck 2000 manuscript, the authors are totally right.

Please see Q1

QUALITY ASSESSMENT

Q 6	Originality
Q 7	Rigor
Q 8	Significance to the field
Q 9	Interest to a general audience
Q 10	Quality of the writing
Q 11	Overall quality of the study

REVISION LEVEL

Q 12 What is the level of revision required based on your comments:

Reviewer 2 | 20 Jan 2020 | 16:40 #1

Substantial revisions

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