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Discussions

Interactive comment on "Effects of *in situ* CO_2 enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*" *by* T. E. Cox et al.

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Reply to comments:

We thank Jason Hall-Spencer, Thomas Arnold, Joerg Ott, and Jon Havenhand for their insight and comments on the manuscript "Effects of in situ CO2 enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass Posidonia oceanica." by Cox, Gazeau, Alliouane, Hendriks, Mahacek, Le Fur, and Gattuso. We feel that the comments have been useful to improve the ideas and research put forth within the manuscript.

We have taken into account their comments and have revised the manuscript accord-

ingly. We believe that, with these edits, our manuscript provides critical information, which improves the current knowledge on how seagrasses will respond to future ocean acidification. The strengths of our study have been discussed in each of the comments: it is a study on an intact community, it takes into account ambient conditions and natural environmental fluctuations, it compares growth and physiology within enclosures to their natural state (reference plot), and it is the longest manipulative study to date on P. oceanica under lowered pH.

We strongly feel that the focus of this paper on P. oceanica response will have a broad appeal to carbon research community, those interested in plant physiology and ecology, and the field of coastal conservation and human impacts.

We would like to address the general concerns of all four comments by Drs. Hall-Spencer, Arnold, Ott, and Havenhand, followed by line by line responses to some of the detailed reviews by Hall-Spencer and Ott.

The discussion has focused on three main issues (outlined below) and their implications to the main findings of the study.

1) Statistical issue of pseudoreplication with the study design

2) Short-term vs. long-term effects for Posidonia oceanica, which is a long-lived species with ability to store carbon reserves

3) The enclosures may have caused stress

We have made two major changes to the manuscript to address these concerns. First, we removed statistical analyses and referred to the lack of deviation in parameters between enclosures and reference plots with the lowered pH treatment. Second, we now mention the constraints of our study design in the abstract and within a new section (Summary, caveats, and perspectives) in the discussion.

More specifically, below are our rationale and comments:

1- Issue 1:Pseudoreplication

We are aware that the study design results in pseudoreplication. Samples were collected or measured inside the plot or enclosure through time, often before and after the pH manipulation. Thus the replication is equal to one for each treatment. True replication was sacrificed at the expense of controlling pH as an offset, at the spatial scale of the plants. This was no easy task to perform a 4-month in situ study with highly controlled pH at diving depth for a natural community. The logistics of ocean acidification experiments, as Arnold discusses, often requires a tradeoff between well replicated studies or well controlled pH. The scale of the system is an additional constraint as pH-control is increasingly difficult as the scale of the enclosure increases.

The challenge of true replication is further magnified for a clonal plant that relies heavily on vegetative propagation. These plants have little to no genetic diversity throughout the Mediterranean Sea (Procaccini et al. 1996). Although advancement in molecular methods have revealed more genetic structure than in earlier works, this species is still characterized by low genetic polymorphism (Procaccini et al. 2002; Micheli et al. 2005). From DNA fingerprinting it has been estimated that a single genet can occupy more than 20 m (Procaccini et al 1996), emphasizing the difficulties of true replication in any study on P. oceanica and the need addressed by this study for multiple lines of evidence to gauge the diversity of response.

We also agree with Havenhand that just because we have pseudoreplicated does not mean data we collected do not have value and that no conclusions can be drawn. We took extra steps within the study to try to account for the limitations of our design (see initial manuscript lines 649-653 of the discussion). In contrast to statements in the comments we never stated that there was “no effect” on P. oceanica. In the initial and revised version of the manuscript, we have tempered our implications and conclude within the confines of our study design and within the context of outcomes from other studies, stating that results support “minimal benefit” and “limited stimulation” at a pH predicted to occur by 2100.

We agree given the comments of Arnold and Havenhand that it is better to use appropriate analyses rather than applying statistics incorrectly to analyze our data. Thus we have removed the statistical analyses in the revised manuscript and refer to the figures to interpret the scale and magnitude of the effects observed. We have revised the initial manuscript to explain this approach, replacing section 2.9. We now raise attention to the caveats of our design and thus conclusions in a new section after the discussion. The following changes have been made.

- In the abstract we edited lines 30-34 (or 33 to 39 in revised manuscript) to read: “The greatest magnitude of change in P. oceanica leaf biometrics, photosynthesis, and leaf growth accompanied seasonal changes recorded in the environment and values were similar between the two enclosures. Leaf thickness may change in response to lower pH but this requires further testing. Results are congruent with other short-term and natural studies that have investigated the response of P. oceanica over a wide range of pH. They suggest any benefit from ocean acidification, over the next century (at a pHT of ~7.7), on Posidonia physiology and growth may be minimal and difficult to detect without increased replication or longer experimental duration. The limited stimulation, which did not surpass any enclosure or seasonal effect, casts doubts on speculations that elevated CO2 would confer resistance to thermal stress and increase buffering capacity of meadows. ”

- Section 2.9 has been replaced with: “2.9 Pseudoreplication”: “Samples were collected or measured inside the plot or enclosure through time, often both before and after the pH manipulation. Thus the replication is equal to one for each treatment. True replication was sacrificed at the expense of controlling pH as an offset, at the spatial scale of the plants. Traditional inferential statistics could, therefore, not be rigorously applied and we compare results graphically, paying careful attention to any divergence in values between the enclosures and the reference plot.”

- New section at the end of the Discussion: “4.1 Summary, caveats and perspectives”: “Any benefit from ocean acidification, over the next century, on Posidonia physiology and growth appears minimal. This conclusion is supported by the similarity of measures between enclosures and in context of results from other studies. We have cautioned that the eFOCE study, like all studies, has limitations. There may be small gains in plant productivity which are masked by an enclosure effect or difficult to identify without replication or more prolonged duration. We recommend that future in situ manipulative efforts use FOCE systems to control pH as an offset, as we did, and increase replication. The field of ocean acidification and future seagrass ecology could benefit from further in situ experiments that focus on combined stressors, extended experiment duration, and differences which occur over varying spatial and temporal scales (eg. within a season promoting above-ground biomass)."

2- Issue 2: Short-term vs. long-term effects for Posidonia oceanica, which is a long-lived species with ability to store carbon reserves

The authors are not naïve to the life-history of P. oceanica and are aware of the slow ability to colonize new space and the ability to store carbohydrates (see initial manuscript lines 102, 546-548, 611-633).

Most manipulative and published studies to date investigating or modeling the impacts of lowered pH on P. oceanica have relied on hourly incubations of leaf segments (see Invers et al. 1997, 2001, 2002). Prior to the present eFOCE experiment, the longest published manipulative study was a laboratory experiment conducted over six weeks on isolated shoots (Cox et al. 2015). In all experiments P. oceanica responded in the short-term and showed major increases in productivity at a pHT of 7.3, with no detectable effect at pHT 7.7. This suggests that the plants would be expected to respond in the short-term. It is also why we are suggesting that effects may be minimal at pHT above 7.7. It is true that we do not know the long-term response to ocean acidification for the next century. The closest approximations for this at this time would be the studies conducted along CO2 vents and our study adds to the growing picture.

We have discussed the implications of our findings in terms of experiment duration and

carbon storage extensively in the initial manuscript (lines 611-633). We have added this caveat to our new proposed section "Summary, caveats, and perspectives" (see above) and we have added some text to the abstract to highlight the confines of experimental length (see above). We also addressed some of the specific concerns of Ott in respect to lag time in the line by line response below.

3- Issue 3: Enclosures may have caused stress

We understand the concern about manipulative stress and drawing conclusions from stressed plants. However, plants were left in situ and not cut into leaf segments nor maintained outside of their natural setting to investigate the pH impact. These are greater steps that have been taken to limit stress than in any other publication that manipulated P. oceanica and pH. In our experimental design, we have compared the manipulative enclosure to a control enclosure and to a reference plot, thus we have taken greater steps than many studies to assess artifacts. In laboratory studies, the manipulative treatment is often compared to a control that is handled similarly and not compared to the response in un-manipulated, natural environmental conditions. Also, even along vents stations the stress of the habitat or organisms within control stations are often not measured. They are assumed to be at optimum at the time of study (discussion in Lauritano et. al, 2015).

To address this concern, we have added a new section entitled "Summary, caveats and perspectives" at the end of the manuscript (see above). We also clarified the issue in the abstract by adding text (see above). Briefly, we used information from studies conducted in the laboratory, in situ incubations with modeling, and along vents in comparison and concluded with caveats. We discussed combined evidence in the initial manuscript (lines 596 to 610). The combined evidence and lack of difference between enclosures supports the conclusion of limited stimulation for P. oceanica.

References cited:

Cox, T. E., Schenone, S., Delille, J., Díaz-Castañeda, V., Alliouane, S., Gattuso, J.-P.

and Gazeau, F.: Effects of ocean acidification on Posidonia oceanica epiphytic community and shoot productivity, J. Ecol., 103(6)1594-1609, 2015.

Invers, O., Romero, J., Perez, M. and Pérez, M.: Effects of pH on seagrass photosynthesis: a laboratory and field assessment, Aquat. Bot., 59(3-4), 185–194, 1997.

Invers, O., Zimmerman, R., Alberte, R. S., Perez, M. and Romero, J.: Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters, J. Exp. Mar. Biol. Ecol., 265, 203–217, 2001.

Invers, O., Tomas, F., Perez, M., Romero, J., Tomàs, F., Pérez, M. and Romero, J.: Potential effect of increased global CO2 availability on the depth distribution of the seagrass Posidonia oceanica (L.) Delile: a tentative assessment using a carbon balance model, Bull. Mar. Sci., 71(3), 1191–1198, 2002.

Lauritano, C., Ruocco, M., Dattolo, E., Buia, M. C., Silva, J., Santos, R., Olivé, I., Costa, M. M. and Procaccini, G.: Response of key stress-related genes of the seagrass Posidonia oceanica; in the vicinity of submarine volcanic vents, Biogeosciences, 12(13), 4185–4194. 2015.

Micheli, C., Paganin, P., Peirano, A., Caye, G., Meinesz, A. and Bianchi, C. N.:Genetic variability of Posidonia oceanica (L.) Delile in relation to local factors and biogeographic patterns, Aquatic Bot., 82(3), 210–221, 2005.

Procaccini, G., Alberte, R.S., Mazzella, L.: Genetic structure of the seagrass Posidonia oceanica in the Western Mediterranean: ecological implications. Mar. Ecol. Prog. Ser. 140, 153–160, 1996.

Procaccini, G., Orsini, L., Ruggiero, M.V.: Genetic structure and distribution of microsatellite diversity in Posidonia oceanica. Biol. Mar. Medit. 7 (2), 115–118, 2000

Line by Line response to reviews follows. The response is noted by a dash (-) after each reviewer comment.

In response to Jason Hall-Spencer:

Hall-Spencer wrote: The abstract mentions speculations about the potential for increased CO2 levels to confer resistance to thermal stress, yet there is no reference any published work on this in the text. Either remove it, or explain the basis of this speculation backed up with references.

-We have added the following reference and text to line 680 in the initial manuscript and now refer to the discussion by Jorda et al. (2012) (in revised manuscript lines 640-645).

- Zimmerman, R. C., Hill, V. J. and Gallegos, C. L.: Predicting effects of ocean warming, acidification, and water quality on Chesapeake region eelgrass: Predicting eelgrass response to climate change, Limnol. Oceanogr., 60(5), 1781–1804, 2015.

-Former line 680 now reads: "The speculation that increased CO2 availability would enhance seagrass production and help to alleviate thermal stress (Zimmerman et al., 2015) was not supported. Jordà et al. (2012) also draws attention to the continuing decline of P. oceanica meadows from 1990 despite the increase in CO2 as a demonstration of the limited capacity of ocean acidification to buffer seagrass vulnerability to disturbances."

Hall-Spencer wrote: Line 67 states that variability in CO2 prevents the determination of a reliable dose response relationship at seeps. This was true a few years ago but more recent work has been able to assess the CO2 dose more accurately (Boatta et al. at Vulcano, Fabricius et al. in PNG, Kroeker et al. off Ischia. Change from prevents to hampers

-We have changed line 66 (in revised manuscript line 71) to "Although studies along carbon dioxide vents allow for a whole ecosystem approach, the high spatial and temporal variability in CO2 levels hampers the determination of a reliable dose-response relationship."

Hall-spencer wrote: Line 69: work has been carried out using the FOCE approach in Chesapeake Bay by Tom Arnold; I do not know if this has been published so this is worth checking.

-We cite Arnold et al. (2012) later in the discussion (line 579 in initial manuscript, line 531 in revised). Our FOCE system differs from Arnold et al. (2012) and differs from those cited by Campbell. In Arnold et al. (2012), CO2 was bubbled directly in a free flow manner. The Campbell design delivers low pH seawater (instead of direct bubbling) but it does not control pH as a continuous offset from natural fluctuations. To be clear about our meaning we have revised the sentence at line 69 to read:

-"To the best of our knowledge, only Campbell and Fourqurean (2011, 2013a, 2014) have manipulated the partial pressure of carbon dioxide (pCO2) in a controlled manner (ie. as opposed to free flow CO2 bubbling) in situ within a Thalassia meadow to test the response of seagrass to ocean acidification."

Hall-Spencer wrote: The authors have not mentioned an in situ study of the effects of increased CO2 levels on several seagrass species by Russell et al. (2013) Mar Poll Bull 73, 463-469 which I think would augment the introduction and discussion sections, especially as this investigates net primary production and respiration alongside biometrics.

-We have added Russell et al. (2013) to the citations in the introduction 63-64, and to the discussion. Line 638 in initial manuscript (now lines 598 to 593) now reads: “In addition, at CO2 seeps in Papua New Guinea, two seagrass species (Cymodocea serrulata and Halophila ovalis) occur in mixed stands and while both species had increased productivity along the lowered pH gradient, it was only C. serrulata with dense below ground biomass that had increased abundance (Russell et al. 2013); demonstrating that outcomes may be species specific, related to the plant physiology and structure, and vary with competition.”

-Russell, B. D., Connell, S. D., Uthicke, S., Muehllehner, N., Fabricius, K. E. and Hall-

Spencer, J. M.: Future seagrass beds: Can increased productivity lead to increased carbon storage?, Mar. Pollut. Bull., 73(2), 463–469, 2013.

Hall-Spencer wrote: I found the result provided on line 401 interesting and wonder if the authors could elaborate on what they think drove the seasonal change in seawater pH in the Discussion.

-We have added a sentence to the discussion. Line 661-662 of the initial manuscript now reads: “In the current study, the decline in leaf length and 3°C difference in temperature likely contributed to the decline of ambient pHT from 8.10 to 8.01 from May to November.”

Hall-spencer wrote: Line 441 confused me a little; were the plot quadrats not placed haphazardly? Please clarify.

-We had two methodological approaches (permanent vs haphazard) for two types of measurements (shoot density vs. surface cover). First type: 3 permanent quadrats, initially placed haphazardly and left in position to follow through time in order to determine shoot density. Second type: 3 to 5 quadrats placed haphazardly at each sampling interval to determine the % change in the surface cover of benthic macroflora or macrofauna. This is explained in the methods line 226 to 232 of the initial version of the manuscript and we have added text to lines 440 and 447-448 in the results section to remind the readers of the two approaches.

-Lines 440 -441 (401-402 in revised) “There was no detectable change in shoot number (as determined in permanent quadrats re-sampled through time) related to the lowered pH in the experimental enclosure.”

-Lines 447-449(406 -407 in revised) “The reference plot as well as the enclosures had very low diversity of benthic macrophytes as measured by estimates conducted within haphazardly placed quadrats at each sampling interval (Fig. 2).”

Hall-Spencer wrote: Lines 444-451: when I read this section I began to understand perhaps why the findings of this study (little or no discernible effect of CO2 on seagrass in the test and control plots) differ from findings at various CO2 seeps. Posidonia oceanica in Italy, for example, tend to be heavily encrusted by Corallinaceae. At multiple Italian CO2 seeps this grass has much reduced calcareous epiphytic cover which presumably helps the Posidonia, as competitors for light and nutrients are removed. This may explain why seagrass is so abundant at CO2 seeps around the world. The results obtained in the high CO2 FOCE chamber in the current study may not be representative of what would be found in a more typical stand of Posidonia with its attendant coralline algal flora (see Martin et al. 2008 Biology Letters). Please consider this possibility in the Discussion section.

-This is an interesting point on interactions of species and how they may alter outcomes and this is a point that we have considered in an earlier publication (Cox et al., 2015) and hint at it in the discussion and introduction in this manuscript. We did assess aspects of the epiphyte-host interaction in Cox et al. (2015). In the laboratory, Cox et al. (2015) found that the loss of epiphyte competitors (at a similar percentage of leaf cover) did little to alter seagrass or shoot production. It is true that other locations could have greater epiphyte loads and thus more competition. This certainly indicates that more studies are needed throughout the Mediterranean to capture the diverse biology and interactions.

-It is currently difficult to compare the degree of epiphyte competition between locations from published studies (see discussion in Borowitzka et al. (2006)). This difficulty arises from the different methods used to quantify amounts (biomass, percent surface cover, epiphyte index) and differences in sub-sampling (i.e. measures over entire shoot, random vs. oldest leaf, random portion of leaves, distal portions of older leaves, etc.), which may cause directional biases. Furthermore, studies were conducted at different times of the year and depths. Therefore, it is almost impossible to conclude whether differences and similarities between studies in epiphyte amounts are the result of method, season, or location.

-We have edited the focus of the paragraph starting at 634 in the initial version of the manuscript to include biological and environmental variation that can alter outcomes. To specifically address this point we have added text to lines to 633, 638 to bring more emphasis to the potential variation in competition among meadows.

- 633, start of paragraph (line 583 in revised manuscript): “We caution that conclusions should not be applied to other seagrasses and that outcomes may vary with differences in community composition and environment.”

- 638 was changed to: “Biological communities and environmental conditions are variable both within (e.g. depth) and among meadows (Hemming and Duarte, 2000). For example, epiphyte coverage and thus level of competition were reported to be greater along control stations at Ischia, Italy (Martin et al. 2008) than in our study site, however, differences in methodology prevent direct coverage comparisons.”

- Borowitzka, M. A., Lavery, P. S. and van Keulen, M.: Epiphytes of seagrasses, in Seagrasses: Biology, Ecology and Conservation, edited by A. W. D. Larkum, R. J. Orth, and C. M. Duarte, pp. 441–461, Springer, Dordrecht, The Netherlands., 2006.

Hall-Spencer wrote: Line 558 has some discussion of the effects of increased CO2 on plant mechanical strength. Recent work by Newcombe et al. (2015) in Biology Letters showing that increased CO2 can weaken Acetabularia might be worthy of inclusion here.

-We have added the work and citation to line to 562 (line 512 in revised) of the initial manuscript “An increase in seagrass leaf thickness would be an opposing effect to those observed for the upright calcified alga, Acetabularia acetabulum, which lost skeletal support at lower pH (Newcomb et al., 2015).”

-Newcomb, L. A., Milazzo, M., Hall-Spencer, J. M. and Carrington, E.: Ocean acidification bends the mermaid’s wineglass, Biol. Lett., 11(9), 20141075, 2015.

Hall-Spencer wrote: Line 569 unclear meaning ‘discredits need for’?

-Changed to: “However, photosynthesis measures were not elevated by the lowered pH and thus there would be no need for increased nutrients.”

Hall-Spencer wrote: Line 617 I don’t think this paper should be drawing upon unpublished data, so the discussion of carbohydrates and carbon content can be left out for a future publication.

-We have removed and edited text to read: “In the present study, there was no indication of increased productivity as gauged by RLCs, PE curves, and measures of leaf chlorophyll. Therefore there is no available evidence that carbon availability translated into increased carbon storage as occurred for T. testudinum under elevated pCO2 (Campbell and Fourqurean, 2013a).”

Hall-Spencer wrote: Line 631 ‘are mixed in support’ meaning unclear

-We have removed the summary of conditions and “the mixed in support”. We have focused the text at line 631 (line 580 in revised) to clarify meaning. It now reads “Only two of six studies support a pulsed seasonal-pH interaction that could result in long-term gains yet, these were found at pHT < 7.7 (see Hall-Spencer et al., 2008; Invers et al., 2002).”

Hall-Spencer wrote: Line 643 For the reasons set out above I do not think that this paper provides a ”major advancement in our understanding of the response of Posidonia to ocean acidification” at all. It is a major advance in the use of the FOCE approach and can be presented as such, as in a methods paper.

-We respectfully disagree with the reviewer. This study does advance our understanding of the response of Posidonia to ocean acidification because we are addressing key needs of future perturbation experiments identified by the scientific community (see for example, Riebesell & Gattuso, 2015). The eFOCE experiment was manipulative, which is powerful to determine impacts, the duration was longer than any previous pH perturbation carried out on P. oceanica, it was conducted on the entire plant within its

natural setting, it is the first to have pH fluctuate as it would in the natural environment. It showed that in situ, when pH is manipulated the response by P. oceanica is not overwhelming. When put into perspective with other studies, the results provide a clearer understanding of seagrass response. Each study has limitations but we do not claim that they do not advance our understanding. For example, the vent stations hamper our ability to define tipping points but they have value and can provide insight into the response of Posidonia or other organisms and communities to ocean acidification. The engineering and implementation are discussed in Gattuso et al. (2014) and it is not the focus of the present paper, which addresses the biological response of Posidonia oceanica.

-Riebesell, U. and Gattuso, J.-P.: Lessons learned from ocean acidification research, Nat. Clim. Change, 5(1), 12–14.

-Gattuso, J.-P., Kirkwood, W., Barry, J. P., Cox, T. E., Gazeau, F., Hansson, L., Hendriks, I., Kline, D. I., Mahacek, P., Martin, S., McElhany, P., Peltzer, E. T., Reeve, J., Roberts, D., Saderne, V., Tait, K., Widdicombe, S. and Brewer, P. G.: Free-ocean CO2 enrichment (FOCE) systems: present status and future developments, Biogeosciences, 11, 4057–4075, 2014.

Hall-Spencer wrote: Line 680 – what speculation, where? Delete, or refer to published work on this.

-We have added the following reference and text to line 680 in the initial manuscript and now refer to the discussion by Jorda et al. (2012).

-Zimmerman, R. C., Hill, V. J. and Gallegos, C. L.: Predicting effects of ocean warming, acidification, and water quality on Chesapeake region eelgrass: Predicting eelgrass response to climate change, Limnol. Oceanogr., 60(5), 1781–1804, 2015.

-Former line 680 now reads: “The speculation that increased CO2 availability would enhance seagrass production and help to alleviate thermal stress (Zimmerman et al.,

2015) was not supported. Jordà et al. (2012) also draws attention to the continuing decline of P. oceanica meadows from 1990 despite the increase in CO_2 as a demonstration of the limited capacity of ocean acidification to buffer seagrass vulnerability to disturbances."

Hall-Spencer wrote: Line 688 'amendable'? unclear and I think 'potentially powerful' is closer to the truth, given the difficulties of doing this sort of work and the limited sets of results to date.

-Because of the reviewer's concerns we have reworded the sentence- Line 710. However, we do think they are powerful tools and amendable (you can modify them to improve usability). There are difficulties in any field experiment but, each time they are implemented you learn to improve design and it becomes easier.

-Line 710: "FOCE systems are tools that can be used to investigate these types of impacts."

In response to Ott:

Ott wrote: In parts the expectations of change induced by greater availability of CO_2 appear a bit naïve. The life form of Posidonia resembles rather a "tree", than a "grass". With a life span of shoots of up to 50 years, as cited in line 611, little change in shoot density can be expected in an experiment lasting only 5 months. Furthermore, leaf growth is in part fueled by carbohydrate storage in the rhizomes, especially during the appearance of the new generation of leaves in fall and winter, rather than by photosynthesis alone (Pirc 1985 Marine Ecology, Pirc 1986 Aquatic Botany). The sequence of leaf appearance is probably an internal circannual rhythm (my paper in Mar. Biol. Letters 1, 1979). These properties may confound expected short-term changes and effects could possibly be found with a time lag after the end of the experiment (see for example the event cited in lines 683-684).

-See response to another comment above. We have edited the text and added "pro-

longed to capture any lagging effect" to line 626 which now reads in the revised manuscript: "Therefore it is possible that if the experiment were initiated earlier, in a period more conducive for biomass production, or prolonged to capture any lagging effects, the outcome may have been different".

Ott wrote: Regarding the toughness experiments, where resistance to mechanical strain was tested in the middle of the leaf length: I have rarely observed leaves being torn at mid-leaf, when still green and healthy. Leaf erosion occurs at dead tips under heavy epiphyte cover leading to a progressive shortening of leavers in the later part of their life span. Leaves that are torn off by water movement generally break at the lunula, the preformed breaking line close to leaf base.

-It would have been a better choice to measure the toughness and thickness throughout the leaf. However, the thickness and toughness were always measured at a standard location. Thus the suggestion that they were thicker with lowered pH is still valid. The implications of this relationship are still unclear. We do not want to speculate or discuss any further and we put forth the finding as preliminary.

Ott wrote: Lines 415-416: What is meant by "amplification of a metabolic signal"?

-The metabolic signal is the change in O_2 that is driven by the metabolism of the plant. When plants are enclosed this fluctuation is amplified, that is the change in O_2 is larger. We have edited the revised version of the manuscript for clarity:

-Line 415-416 changed to: "The difference in diel change between the ambient and the enclosures was due to the amplification of a metabolic signal inside a partially enclosed space (similar to the example of a larger O_2 fluctuation when a similar sized plant is contained in a relatively smaller volume of water) as was evidenced by the more similar, and greater diel change. . ."

Ott wrote: Line 465: "leaf number" instead of "shoot number"

-Changed to: "leaf number per shoot "

Ott wrote: Line 625: I dearly miss a reference to my paper in Marine Ecology 1980 where most of the annual rhythms of leaf appearance, growth and decay, as well as production have been described for the first time.

-We apologize for the oversight. We have added the reference to line 625 of the initial manuscript.

-Ott, J. A.: Growth and production in Posidonia oceanica (L.) Delile, Mar. Ecol., 1(1), 47–64, 1980.

Ott wrote: Lines 739-741: There is an error in the citation.

-Corrected.

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