

**Regional and local variability in the morphometric traits of two emblematic seagrass species (*Zostera marina* and *Zostera noltei*) along the French coast**

***Revision round #1***

**Decision for round #1 : *Revision needed***

**Revisions needed before publication**

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Dear authors,

First of all, I would like to apologize for the delay but it was very difficult to find two reviewers who accept to review this manuscript. The two who accepted needed extra time to complete their review but now it is done. Thanks for your patience.

Reviewers and I have found your paper very interesting but it could be improved before publication. Reviewers have proposed some corrections and I think they are relevant. Please try to improve your manuscript by considering reviewers' comments and resubmit your manuscript and your replies.

I hope you will accept to modify your work.

Best regards,

A. Vernay

by **Antoine Vernay**, 07 Jun 2024 13:45

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**Review by anonymous reviewer 1, 06 Jun 2024 15:16**

The publication is a first approach to the seasonal population dynamics of two species of eelgrass in habitats with contrasting population health and environmental characteristics. The subject is well covered, although several points need to be answered or improved in the manuscript. The dataset is indeed quite large, but the analysis could perhaps have been more statistically sophisticated to better identify the major effects and contrasts between species. The lack of an approach to the hydraulic component is somewhat frustrating.

## Introduction

line 61: delete "availability"

line 61-62: the authors assert that the species is annual, but speak about winter survival as a plant with a single leaf. Is it really an annual plant or a plant with a winter dormancy? (summergreen?). If yes, it would be better not to speak about annual plant. What is the determinant of winter survival? temperature, absence of freezing?

That was a mistake, changed to perennial.

## methods :

one may have expected that the chl<sub>a</sub> content (for assessing the part of turbidity due to phytoplankton abundance, and so assess the eutrophication pressure more accurately than nutrient content, when nutrients are in the vegetation) and water depth were surveyed (measured with a datalogger), for understanding how far submersion-emersion rhythm and turbidity rule populations (cf introduction)? This would also make it possible to measure the behaviour of the two Z marina populations, and to situate them in relation to the trajectories of the other species.

Chl<sub>a</sub>-a was measured (L 150) and considered in the analysis (L227-231), but not retained by the stepwise procedure.

## results

lines 227-228: I ask myself what is the specificity of this paper compared to the one quoted?

The reference returns to the complete raw data of the project available at <https://www.seanoe.org/data/00868/97962/>.

line 273 : the multivariate analysis method used does not seem to project the two species into the same statistical reference framework, which means that it is not possible to compare the amplitude of the statistical trajectories between species? For example, centring the dynamics data by species makes it possible to see how the two species vary within the same reference framework, and to compare the trajectories of their populations.

Possible, but it was beyond the scope of the paper to directly compare the two species. All analyses were carried out species by species and comparisons between the two are limited because our aim was rather to explore/describe/understand the dynamics of each of these species separately.

Furthermore, comparing them per se would not make much sense to us as they have completely different life history strategies and are not exposed to the same environmental variation due to their different position along the depth scale.

Figure 4 is rather difficult to understand, and would benefit from a more explicit description in the legend.

The legend has been reformulated to be more explicit.

## Discussion

line 394-395: Hydraulic stress seems to be a key factor controlling the traits and population dynamics of these species, but it has not been quantified at all. Would an indirect quantification, such as that provided by the granularity of the sediment, not have been possible, in the absence of direct, complex measurements in a partially inaccessible and turbulent environment

The plants themselves modify the current facies, and it is probable that we can have tipping effects beyond a given stem density threshold. The granulometry of the sediment at the foot of the stems is surely very informative on this point. In addition, sedimentation caused by plants can be harmful to plants in eutrophic conditions, increasing the anoxic phases of the sediment. In this context, turbulence is probably favourable to the oxidation of sediments by keeping them coarser;

It is true that hydrodynamic information is lacking (frustrating, we agree). However, the question is how good a proxy granulometry would be, as sediment properties are influenced by the interaction between the plant and the current, so the relationship may not be straightforward...

## Review by anonymous reviewer 2, 21 May 2024 09:56

This is an original piece of work with a refreshing methodology to deepen the understanding of seagrass dynamics. However, there are some issues that I consider important that the authors need to address before this manuscript is ready for publication. My review includes two parts, the questions suggested by the system and a detailed list of comments.

- Title and abstract
  - o Does the title clearly reflect the content of the article? Yes
    - Does the abstract present the main findings of the study? More or less. I disagree with the affirmation that they found a latitudinal pattern. The effects of differences in tidal conditions seem more relevant.

Replaced by 'site position' because local environmental conditions (due to latitude..) are also important.

- **Introduction**

- o Are the research questions/hypotheses/predictions clearly presented? Yes
- o Does the introduction build on relevant research in the field? Yes

- **Materials and methods**

- o Are the methods and analyses sufficiently detailed to allow replication by other researchers? I don't know

I do not have adequate statistical knowledge to easily follow the statistical method proposed in this MS.

- Are the methods and statistical analyses appropriate and well described? More or less

In my opinion, the methodology used is a sophisticated statistical method that is not easy to follow according to the explanations in the manuscript. I think it would reach a larger audience if they used a figure to explain the indicators used in the evaluation of the dynamics (especially for NCR and DSDSP). It would also be helpful to provide a brief explanation of what high and low values on these variables mean.

We agree that the statistical method used is unusual (but robust!). And that is what we were interested in. We would like to offer new perspectives in the interpretation of seagrass ecology. Rather than emphasising significant statistical differences between sites/modalities (and given the weaknesses in the use of anova in many publications...), we think this multivariate approach gives a more dynamic and integrated view of ecological processes, already used for terrestrial plants or benthic faunal communities. We try to add information to help non-specialists better understand the analysis:

The initial text describing NCR has been changed to give more explanation (L 201-207).

Same with DSDP L217.

However, we prefer to refer to the original figures for an explanation of the indicators (de Caceres et al. 2019, Sturbois et al. 2021) so as not to make our manuscript too long.

Personally, I do not see the advantage of using the statistical method used. The conclusion seems that it could have been similar applying anovas in figures 5 and 6.

Well, it would be harder to synthetise the changes with only anovas on individual traits and if some traits show opposite patterns, it become even harder to describe the extent of changes in one site when there is trait trade-offs. So both CTA and individual trait analysis are complementary in providing a more synthetic versus more detailed picture of the changes. We thought that if we had used ANOVA, this manuscript would have just been a catalogue of descriptions and statistical tests, without being very exciting. But it's a fair question, and one we've asked ourselves!

- **Results**

- o In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? Yes

- Are the results described and interpreted correctly? No

Figure 3 is very important to the manuscript, but the quality is terrible. The shape of the points in the left panels and the variable names in the right panels are not distinguishable. To interpret the right panels, it is also not clear when significant differences can/should be considered.

Figure 4 needs an explanation of how much difference is enough to talk about different behaviors and use a comparable scale between species (the same scale).

The quality of the figure has been improved.

We only have one trajectory per sample, so we cannot test for significance here. We could do a permanova on DSDP or anova on the trajectory metrics testing for sites but we would only have to replicates (S and D) which are quite variables on top of that. We could also test for a difference between S and D considering sites as replicates but are they?

We are the first using this approach on seagrass so we provide the first estimates and the only thing we can compare with is variation on faunal communities. For directionality, we have stronger changes than Toumi et al. on macrofaunal communities or than De caceres et al. on forest plots (for what it's worth... not sure it is comparable, but there are not that many studies yet).

The extent of trajectory length variation are quite strong in that sense for *Z. marina* if we compare with values observed in Toumi et al. but we have fairly low variation if we compare with the simulation of De Caceres et al. 2019 (fig 5).

- **Discussion**

- o Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? Yes

- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? No

The local scale *Zostera* spp. strategies do not appear to be well resolved due to a limitation of environmental data. Surprisingly, they give an explanation of the local behavior of TH that then contradicts the results of GM.

I believe that section 4.3 is not supported by the data in this manuscript; It's basically a review section.

See comment in the list of detailed comments below. We (tried to) emphasise the context-dependent effects.

## List of Detailed comments:

- Line 22. I disagree with the affirmation that they found a latitudinal pattern. The effects of differences in tidal conditions seem more relevant.

Replaced by 'site position' because local environmental conditions are also important. The 3 Atlantic sites have tides but water temperature differences are also important.

- Figure 1. An intermediate scale view would be useful to give a correct idea of each system (eg. Lines 118-119 and 123). The large scale image does not need to be that large. Small scale images need indication of North. It is necessary to improve the image quality to see the scales correctly. You should also name all figures following same pattern (Fig. vs Figure).

- Table 1. It would be great if you include the tidal range (m) in each system and (if possible) the elevation of the *Zostera* populations with respect to this tidal range. Probably inundation frequency and duration would help as environmental drivers in these dynamics.

Unfortunately, we don't have precise information on the tidal range for each experimental site. We are aware of this lack of information.

Lines 168 and 174-175. Please add the size of the quadrats and the PVC cores used for sampling.

Information added.

- Lines 170-171. Please, could you explain how a PVC sheet to cover *Z. noltei* generates the same observation conditions?

The PVC sheet allows to flatten the leaves on the substrate as if there were no water.

- Lines 204-207. Please, explain how the DSDSP should be interpreted.

Information added.

- Lines 226-227. I am confused. What means "... where in line with the general environmental description of the sites (Table 1)"? Is Table 1 the environmental data used in your statistical analysis? Or on the contrary, is your environmental data available in Lacoste et al. (2023a)? If the second, you should include a figure with your environmental data.

All environmental data recorded during this study are available in Lacoste et al. 2023. Here we give only a few of our data and the general conditions over 6 years (Table 1). Our data were used for statistical analysis.

Figure 2. Do the red stars represent the seasonal mean per site or the annual mean per site?

Corrected for: Red stars represent the average for all seasons per site (not really annual because 2 winters).

- Lines 248-249. I don't clearly see the implications of the net change in NCR. I don't understand why a higher NCR implies few changes from the starting point. Please explain better

The wording was a bit clumsy. The sentence has been modified.

- Figure 3. Very bad quality. Very difficult to see properly. Where is fig.S1?

Figure S1 should have been provided as supplementary material. It has been added in the new submission.

- Figure 4. Why don't you same scale for same variable when comparing the two species? This figure is not very intuitive.

The comparison of the 2 species is not objective. Different scales allow to emphasise the main differences between sites for the stable modality or between modalities within a site.

- Line 333. I do not see a low seasonality on Dshoot for TH. I see no seasonality at all. It is difficult to decide how much difference should be considered significant.

- Lines 338. I do not see that % cover is always higher in GM dynamic modality than in stable one. This is not right on the peak seasons (summer and autumn).

Yes, that paragraph was a bit over-interpreted, we've changed it.

## DISCUSSION

- Lines 363-364. You suggest that the subtidal location of TH population may also be advantageous given the predicted warming in the coming years. However, you do not know if they can tolerate warmer conditions than now. Please support better this suggestion of delete it.

We point out that the lack of exposure to the air at this latitude is an advantage at the moment. But there is also a warning about rising water temperatures.

Lines 386-387. I do not see the similarity in between AC and GM AND TH. AC is a type 2 (typical from light limited environments) and DH and TH have shorter leaves and less light restrictions. Please explain better.

GM and TH are intermediate with a type 2 configuration, but less pronounced, suggesting less light limitation. We have reformulated.

Line 416. Hydrodynamics is not the same as hydrodynamism. I think you should use hydrodynamic control here.

Additionally, you largely discuss the role of hydrodynamics. Perhaps you should include environmental variables to introduce these effects into your analysis (e.g. frequency and duration of inundation, incidence of waves, ...)

Unfortunately this is a weakness of our study, to not have hydrodynamic information.

- Lines 419-421. What is the validity of a hypothesis that only works in one system? I recommend reworking this part.

The point of this comment (and the paragraph below) is to show the possible misinterpretation of environmental interactions when we change scale? looking at a single site does not disprove the hypotheses, but it does not verify them either. Here we clearly show the context dependency effects....

- Lines 460-461. I do not see the relevance of mentioning genetic diversity in an isolated sentence. Please, delete it or develop this idea.

Yes, we have deleted it.

Line 471. You state that your study shows the high adaptive capacities of *Zostera* spp. to regional and local environmental conditions. However, you also indicate earlier that you don't have enough spatial resolution on your environmental conditions to demonstrate this. Please, change shows by suggest at least. **done**

- Lines 479-480. Recommendations on increasing sampling stations to a local scale should include a warning about the consequences of sampling efforts as anthropogenic pressure, since a very intense sampling effort can be quite destructive in soft sediment environments.

- Lines 487-490. I do not see the relationship of this part with this manuscript. Please, rework these lines with the last paragraph.

Yes, it was a bit out of context, so we've removed it.