

## Reviewer 2

General comments:

This is a well-written and well-structured article that makes an important contribution to the growing field of study around the potential impacts of climate change on macroalgae in the Arctic region. The authors investigated the physiological and transcriptomic responses of four kelp species to the combined effects of increased temperature, reduced salinity, and reduced light availability in a six-week mesocosm experiment. The authors describe their methodology clearly. They provide evidence that the species studied here were able to acclimatize effectively to the experimental treatments, and suggest that these species will likely be resilient to future environmental changes in the Arctic. I have some minor questions around the study's methods (chiefly the low number of replicates, and the fact that different species were analyzed for the physiological and transcriptomic portions of the paper), and I think it would be useful to provide a more detailed list of differentially expressed genes. Overall, this is a strong article that makes a novel and valuable contribution to science, and I would be very happy to see it published. The abstract is generally well-written, but a bit too long. Perhaps the experimental treatments, results and discussion could be described more briefly?:

**We thank the reviewer for their analysis and have addressed their concerns accordingly in the revised version. We have addressed the outlined concerns above with our responses below. We have reduced the length of the abstract as recommended (342 vs 330 words in the revised vs the initial abstract).**

The introduction is concise, and does a very effective job of explaining how environmental conditions in the Arctic are predicted to change, and why it is valuable to investigate how these changes might impact kelp species. The knowledge gap that this study addresses is identified clearly. A little more information on the study species, and any previous findings about their environmental tolerance, would have been useful.:

**We have included a bit of information regarding the taxonomic details of the studied kelp in response to reviewer 1. We have also added details about the environment we chose to replicate noting that these kelp are found mixed at similar densities between 5 to 10 m. These details have been included in the revised manuscript. We have added content and references in the introduction to better describe the response to temperature and salinity of the species investigated.**

The methods are logical, and explained clearly enough to be replicable. Can you explain why physiological measurements were not taken for *Hedophyllum nigripes*, and why gene expression was not investigated in *Laminaria digitata* and *Alaria esculenta*?:

**We were unable to specifically identify *Hedophyllum nigripes* pre  $T_{\text{final}}$  which limited our ability to target this species for physiological measurements. Only upon destructive sampling could we properly identify *H. nigripes*. We chose to only focus on the gene expression patterns in *S. latissima* and *H. nigripes* because *S. Latissima* was the most abundant in terms of biomass in the sampling area and appeared to be in good physical**

**health upon visual inspection at  $T_{\text{final}}$ . *H. nigripes* was chosen because it is an endemic Arctic species. We have added this reasoning to the revised manuscript in section 2.8.**

The results section is concise and clear.

**We thank the reviewer for acknowledging this. We have, however, added a few more details based on our response to Reviewer #1.**

The discussion section places the results well within the wider context of research on stress responses in kelp. Some reasonable hypotheses are provided regarding the cellular and physiological mechanisms that could underlie the observed changes in chlorophyll a content, C:N ratio, and gene expression. There are some good suggestions for other physiological responses that could be measured in follow-up studies to validate these hypotheses. The low number of independent replicates (i.e. mesocosms) used, as well as the low sample size for *L. digitata*, make it harder to be confident about the significance of some trends. This is an understandable choice, given the high spatial and economic costs of mesocosm experiments, but should be made clear in the discussion.:

**We agree with the reviewer and have acknowledged this in the discussion section of the revised manuscript.**

Specific comments:

Abstract:

Line 19-22 - The rationale behind the experimental treatments is explained very clearly in the Introduction and Methods sections, so perhaps it isn't necessary to go into so much detail here.:

**We have slightly reduced this part of the abstract but believe it is important to mention the different treatments here.**

Line 26-27 - Nitrate concentration was not one of the factors deliberately manipulated in this experiment, so be more specific about which experimental treatments were linked to this change in C:N ratio.: **This was rephrased: “*S. latissima* showed a lower carbon:nitrogen (C:N) ratio under SSP5-8.5 multifactorial conditions, suggesting tolerance to coastal erosion and permafrost thawing.”**

Line 29-30 - The statement about gene expression is not very specific. What patterns of gene expression were found at different temperatures, and what “ability” does this reflect? Consider giving more details, or leaving this out of the abstract.: **Modified as: “The down-regulation of genes coding for heat-shock proteins in *H. nigripes* and *S. latissima* underscores their ability to acclimate to heat stress and underline temperature as a key influencing factor.”**

Introduction:

Line 54-55: Be more specific about how temperature and salinity impact kelp physiology. Is there anything known about the tolerance ranges of these study species, and was this taken into account when developing hypotheses? **We have added content and references in the introduction to describe better the temperature and salinity tolerance of the species investigated.**

Methods:

Line 108 - Units of salinity?: **Salinity is unitless.**

Line 120 - I assume samples were taken using a scalpel or similar. What steps were taken to prevent cross-contamination between samples? **Samples were taken using a sharpened metal tube, which was used to hole punch tissue. Scissors were used for larger tissue samples. The sampling tool was wiped with paper between samples. We expect minimal cross-contamination as any residue remaining on the sampling tool would be infinitesimally small relative to the tissue sample taken for actual analysis.**

Line 136 - Mass or molar C:N ratio?: **Clarified, i.e. C:N mass ratio**

I believe it is best practice to use quotation marks when citing R packages, e.g. “EnvStats” (Line 195, 198, 201): **Added**

Results:

Line 211-212 - Units of salinity?: **Salinity is unitless.**

Line 240 - “significant differences in growth over time were only found in the T3 treatment” might be clearer.: **Agreed, modified**

It would be worthwhile to include a more detailed list of DEGs (perhaps as supplementary material): **Agreed, this was added**

Discussion:

Line 268 - “no negative impacts”: **Corrected**

Line 313 - “activity of nitrate reductase”: **Modified**

Line 350 - “involved in reducing”: **Modified**

Line 368 - “increased chlorophyll a content”: **Modified**

Line 372 - Citation would be useful.: **Added: “Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U., Huston, M.A., Raffaelli, D., Schmid, B., Tilman, D., Wardle, D.A., 2001. Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges, Science, 294, 804–808, <https://doi.org/10.1126/science.1064088>.”**

Line 410 - “barren” would be more accurate than “bare”: **This has been changed to “barren state.”**

Figures:

The figures are generally easy to interpret. Removing background gridlines would make Figures 2-5 cleaner, and reducing the width of the bar outlines in Figure 7 would make it easier to interpret : **Figs 2 to 5 and 7 have been updated accordingly.**

Figure 2 - Providing this data is helpful and transparent. There are some large fluctuations in salinity and PAR - what could have caused these? They are mostly brief enough not to affect the overall results. However, PAR increased steadily in the light-limited treatments during the latter three weeks, and did not appear to be significantly lower than T3 by the end of the experiment, which could be of concern:

**We understand the concern by the reviewer and believe the comment is extremely valid. Regarding the salinity fluctuations, this had to do with the automated regulation of the flow valves controlling the mixing of freshwater and seawater. This is thoroughly explained in Miller et al. (2024) Biogeosciences.**

**With respect to the PAR fluctuations, PAR did not increase steadily in the limited light treatments, but the overall PAR decreased as the season progressed. The display of the figure gives the appearance of an increasing PAR while in actuality the overall PAR decreased diminishing the offset from the light-limited treatments. We will remake this figure to correct this potential point of confusion. Finally, while there was variability in T3, the last few days of the experiment saw one PAR logger in the 2nd replicate of the T3 treatment give erroneous data. These were overlooked on our part and left in the plot. This was the cause of the lower T3 PAR data as the replicate with false low PAR data was incorporated into the averaged values. We apologize for this oversight and thank the reviewer for catching this mistake. This has been corrected in the revised Figure 2.**