BGS – response to reviewers

Please note responses are in blue

Referee #1

1) The interpretation of the results is based on the assumption that clones are adapted to contrasting temperature conditions in situ, and annual averages for the sampling sites are provided. However, diatoms are microalgae that reproduce very rapidly, so in general the environmental conditions describing the sampling site cover a period of one month (three weeks before sampling and one week after). Do the authors have temperature records for this time period at the sampling sites? If so, it would be important to include them as they may impact the interpretation of the results. It would also be important to have *in situ* nutrient measurements (N, P), as these greatly affect diatom growth and physiological status.

The authors thank the reviewer for taking time to review our paper and provide the thoughtful comments and recommendations for discussion of our study.

Microalgal acclimation is indeed short lived, the result of a few generations – covering several weeks. Rather than provide a one-month temperature range, which does not typically vary greatly in the ocean, the authors have added the mean annual maximum and minimum SST for each site (see Table 1). This provides the reader with the annual range in temperature experienced by each strain, highlighting the environmental variability in temperature that diatoms experience. It also helps to place our study in context, as we grow our strains using a 6°C difference, by adding the annual ranges, we can see the broader climatology for the strains.

The authors agree with the reviewer that nutrients would also play an important role in physiology in situ, however, in Spring nutrients are often not limiting, hence diatom blooms off the coast of NSW occur typically in spring. Furthermore, the exclusion of nutrient manipulations was also a logistic one, to better control for the effect of temperature, considered the most significant driver (as was the case in Kuefner et al 2020). Given that our principal aim was to use latitudinally distinct strains to assess how temperature may influence silica production and that we grew our strains in nutrient-rich medium over time, the authors feel that the in-situ nutrient data at the time of their collection and isolation would not add to the story greatly. We have however, added some information on nutrient influences into the discussion.

2) The article's hypothesis is based on the fact that the higher the temperature, the greater the productivity. The increase in temperature thus implies a shorter generation time and impacts silicification and cell biovolume. However, there are many other environmental drivers that can affect these charactristics, including bioavailability of nitrogen, phosphorus, and iron. Increased nutrients also act by increasing generation time (see for example Kuefner et al., 2020). The article focuses only on the influence of temperature on the potential of planktonic diatoms to adapt to climate change. However, climate change also induces changes in the availability of macronutrients. The introduction suggests that "warmer surface waters can cause a shallowing of the surface mixed layer, forming a barrier to vertical exchange of nutrients from depth and constraining phytoplankton in the upper surface waters, exposing them to high irradiances and reduced nutrient availability." I therefore believe that it would have been important not only to measure nutrients at the sampling sites, but also to introduce the "nutrients" variable into the experimental design. The study would have benefited from combining temperature, irradiance, and nutrients in the experimental design. The study site and biological model chosen would therefore have been particularly interesting for advancing our knowledge of how

planktonic species adapt to rising temperatures combined with low nitrogen and phosphorus availability. Although it is too late to add this variable, I believe it is important for the authors to enrich their discussion with this aspect.

I think the discussion could be strengthened by these two points. The discussion at this stage does not appear to be sufficiently in-depth. The first paragraph of this section is, moreover, a repetition of the introduction; it would be more interesting to summarize the results obtained.

Kuefner, W., Ossyssek, S., Geist, J., & Raeder, U. (2020). The silicification value: a novel diatom-based indicator to assess climate change in freshwater habitats. Diatom research, 35(1), 1-16.

The authors' aim was to test how/if temperature influences silicification in thermally-adapted strains of the same species. The interpretation provided in the discussion is our attempt to explain the data observed. The authors acknowledge that other factors may influence cell characteristics and physiology in the environment, we feel that the interpretation needs to be limited to what was tested and manipulated, rather than extrapolate too broadly. Broad discussions around nutrients when these weren't tested or manipulated and instead held static and enriched, would be somewhat tangential to the study. That said, the authors agree with the point about the inclusion of discussion around nutrients and other potential influences and the need to test these with temperature and light in future studies, as such, we have included some points in the discussion to highlight this (see section 4.2). The section now reads:

"The low C:N ratios shown by Coffs Harbour strains grown at low light are consistent with a previous study that found light limitation caused a change in the uptake ratio and elemental composition of diatoms, specifically reduced carbon (Saito & Tsuda, 2003). In a warmer ocean, phytoplankton access to nutrients are proposed to decline, altering elemental ratios, while simultaneouly access to light for cells entrained in the surface layer will increase, reducing carbon limitation. In this study, the differences in elemental composition were not correlated to temperature or growth rates, but shifted in response to growth irradiance in the Coffs Harbour strain, suggesting that the stoichiometric plasticity is a response to low light acclimation (Sauterey & Ward, 2022; Yang et al., 2020). Therefore, under anticipated conditions caused by climate change induced stratification, our results indicate increased C:N with higher light availability could lead to decreased food quality (lower nitrogen supply) for grazing zooplankton (Finkel et al., 2010; Li et al., 2012; Marinov et al., 2010). Importantly however, the strains used in this study were grown under nutrient replete conditions, thus precluding nutrient limitation as an influencing factor (Supplementary Table S2), therefore the inclusion of nutrient manipulations in future temperature and light experiments would be important to further advance knowledge on how species adapt under the triad (temperature, light and nutrients) of critical environmental factors."

The authors are grateful for the reference to the paper by Kuefner et al. it is very interesting and nicely supports the important role of temperature on silicification in freshwater diatoms.

Referee #2

The manuscript deals with the effect of temperature and light on the morphology, physiology and elemental stoichiometry (C, N, bSi) of four strains of the diatom Leptocylindrus danicus isolated at different latitudes along the East Australian coast. The data are interesting and of good quality, as well as the manuscript that is well written. Nevertheless, some aspects would require corrections and/or more in depth interpretation before publication.

The authors thank the reviewer for their time and helpful suggestions and comments.

Introduction: the references are quite dated and ones that are more recent are required, the same holds true for the discussion.

The authors have reviewed and revised all citations throughout and have removed older citations and have updated where appropriate to a newer citation.

M and M:

-2.1: it would be necessary to add (in Supplementals) microscopic pictures of the four strains grown under the different light/temperature conditions, also for highlighting the fact that Leptocylindrus forms colonial chains.

The authors are a little confused by this suggestion, as microscopy images of all strains under their respective light and temperature conditions are provided in the supplemental (Figure S1). However, the authors agree that this is an important element for this work and have highlighted that nearly all strains grew as colonial chains in Section 2.3.

-2.1: in line with the above comment, are the four strains still forming chains under the different temperature/light condition ? Are these chains similar among strains and growth conditions (length, etc.)? These aspects are essential for further interpreting the data, especially in an ecological framework.

Yes, nearly all the strains remained chain forming during the experiment, and their sizes varied by strain, not only the size of individual cells, but also chain length – greater explanation had been provided in section 2.3 (linked with the Fig. S1) to set the groundwork for discussion on the potential role of strain morphology in the findings. It now reads:

"Of note, most cultures grew as colonial chains, except for Forster, which was solitary at high irradiance, but formed short chains under low light (Supplementary Figure S1)."

We've also added to the results section 3. - *"Growth morphology varied between strains (Supplementary Fig. S1), with long colonial chains of more than four cells for the Coffs Harbour, Maroubra and Twofold Bay strains. The Forster strain grew as either solitary cells (high light) or short chains of up to three cells (low light), with wider shorter cells. The strains from Maroubra and Twofold Bay were much thinner than those from the more northern sites."*

-2.2: strain back-ups were maintained in f/2 medium (part 2.1), why switching to f/10 medium? The authors should discuss the consequences of such a change for their experiments, if any.

The authors chose to run experiments at a much lower nutrient level, as experiments run in F/2 are highly artificial and may mask potential changes in response to treatments. For this reason, cultures were acclimated to grow in F/10 under their respective light and temperature treatments for more than 2 months (see methods section 2.2) before the measurements were obtained. Growth rates were obtained several times to ensure acclimation to experimental conditions. As such, the authors do not believe that there were any direct consequences of changing growth conditions on the results of this experiment.

-2.2: please provide the spectrum of the light source in the Supplementals; regarding the effect of blue wavelengths on diatom biology, it is important to know if the LED white spectrum is cold, neutral or warm.

The LED lights in the Climatron incubators were 4000K neutral white light, which means that there was more blue light than red, but it was not blue-shifted. While we do not have a spectrum measurement, we have now included this information in the methods to inform the reader of the light source (section 2.2).

Results:

-I found it difficult to read the graphics: it would be clearer to show a legend that follows the same latitudinal gradient as map-Figure 1, i.e. CH-FOS-MAR-TF and not the opposite (Figures 2, 3, 5); similarly it would be more 'usual' to first pinpoint 55 µmol photons data before 100, especially as TF does perform growth under 100; this is especially misleading when reading Figure 4.

The authors were following the logic of low-high temperatures, rather than north to south latitudes. However, we see the referees point about consistency in the order, as presented in Figure 1 so we have re-plotted the figures to reflect the site order by latitude on the x-axis and the associated temperature indicated in the legend. See new figures.

The authors appreciate the suggestion on presenting the low irradiance before the high irradiance for Figure 4, and the suggestion of including the Fv/Fm T0 data here and remove Table 3 to supplementary. These changes have been implemented. There was also the suggestion below about NPQ vs PAR (see response there). Given these suggestions, the authors have revisited all the PAM data for figure 4.

-Figure 4: why not computing NPQ and fitting NPQ vs. PAR curves? NPQ is an important process that strongly modulates photosynthetic productivity and I would not be surprised if showing differences among strains and growth conditions.

The authors agree that the NPQ of the strains can be informative and have determined and plotted these initially. While the NPQ vs PAR curves were not very different among growth conditions except for the Maroubra strain, we have added the NPQ vs PAR data to Figure 4 to provide a more complete picture of the photo physiological conditions of the strains.

-Any explanation for the spreading of data points for FOS (Fig 2, 3) and MAR (Fig 5) as compared to the other strains?

The authors were likewise keen to find a plausible explanation for the spread in the data for the FOS 100umol photons (Fig 2, 3). We attempted to link the differences in the growth rates (spread in Figure 2) of the replicates with cell size differences (Figure 3), but unfortunately there was no correlation between these two parameters. For the MAR 100umol (Fig 5) the spread is attributed to the difference in silicification rates between the replicates. As the cell size and growth rates were relatively similar, which meant that we were unable to find the cause of the spread.

-Table 3: a bit strange; while Fv/Fm T0 is used as a photophysiological trait (included in the PCA analysis Figure 7), Fv/Fm T24 is used a some kind of methodological index (verifying that the PDMPO treatment does not impair the photophysiology of cells): I suggest to keep Fv/Fm T0 in the manuscript and include it in Figure 4, and move Fv/Fm T24 in the Supplementals and cite it only the M and M part (2.4).

The authors are grateful for this suggestion and have included the Fv/Fm T0 data in Figure 4 and removed Table 3 to the Supplementary.

Discussion:

-4.1: see my comments for part 2.1; the fact that Leptocylindrus forms chains needs to be included here.

The authors agree that this is an important point, and have highlighted this in the discussion in section 4.1 and 4.3.

"... including differences in chain forming and chain length between strains."

"...latitudinal effect on growth rate and individual cell size (surface area and volume) for each strain, independent of whether strains were colonial or solitary."

-4.1, line 442: the temperatures used in this study reflect the Spring situation, it would be good if the authors could provide the range of year-round temperatures for each latitude in order to check how much this range is broader than the delta 4°C examined here.

This is a good suggestion. The authors have included the mean annual maximum and minimum SST for each site into Table 1. The authors agree that it helps to place the 6°C difference here in the broader climatology for the strains – see Table 1.

-4.1, line 449: division rate instead of reproductive rate ?

This has been changed to division rate.

-4.2: I found the title of this section a bit exaggerated reading the fact that the delta Temperature is only 4°C and the delta light intensity is only 45 µmol photons; what if this delta would be increased ?

This title was referring specifically to what our findings showed, where we measured stronger effects on C-allocation and elemental stoichiometry between growth irradiance rather than the 6°C difference in temperature. The authors found this somewhat surprising, as temperature is often considered the bigger driver of physiological plasticity, however, the direct role of light in photosynthesis (and given the relatively small ranges in temperature), meant that light was a stronger determinant. That said, to tone down the statement the authors have edited the sub-heading to "*The effect of growth irradiance on carbon allocation and elemental stoichiometry*", rather than quantifying it relative to temperature.

-4.3, lines 490-493: this interpretation should modulated by the fact that Leptocylindus forms chains, see my comments above.

The referee raises an important point, and while our focus was on differences in individual cell volume and silica production, the authors agree that this section needs to be modulated by the fact that we were looking at chain-forming species. We have highlighted this point to remind the reader that the patterns we saw were inclusive of differences in growth morphology (chain or non-chain).

Minor corrections:

-Line 130: space lacking between '100' and 'and'.

Done

-Figure 6 shows no colour legend.

The legend has been added.

-Line 447: through instead of though ?

Done.

-Line 482: ‘which varies within and among a diatom species’, reformulate please.

This has been re-written, it now reads: …*which varies across diatom species and strains*…