

Interactive comment on “Shifts in organic sulfur cycling and microbiome composition in the red-tide causing dinoflagellate *Alexandrium minutum* during a simulated marine heat wave” by Elisabeth Deschaseaux et al.

Anonymous Referee #2

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The manuscript reports an experiment where a cultured strain of the dinoflagellate *Alexandrium minutum* was exposed to temperature increases of 4°C and 12°C. Growth rate, photosynthetic efficiency, oxidative stress, dimethylated sulfur compounds and bacterial community composition were measured over several days. The objective of the experiment was to study if an expected decline in growth rate resulting from impaired physiology was accompanied by up-regulated levels of dimethylated sulfur compounds, and if this matched changes in the microbiome that could be related to sulfur-utilizing bacteria. The environmental context for the lab work is the effects of

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marine heat waves on coastal ecosystems, including harmful algal blooms.

Even though the idea behind the experiments is timely and interesting, the experimental conditions chosen generate a little concern, and the actual results are only partially convincing. Perhaps the authors can provide further convincing arguments with the data at hand.

I will give my comments following the order of the manuscript:

L55: The role of DMSP as a grazing deterrent is, at the least, debatable. It is true that the works of Wolfe et al. and Strom et al. suggested deterrence, but more recent work by one of the authors and others (Seymour et al.) indicated DMSP may be more an attractant than a deterrent.

L80: acute temperature increases – should you say also “ephemeral”?

L343-349: I do not like the use of the word “driven” here. Should it be “aligned”? What the MDS analysis shows is that, in the 32°C treatment, differences in the microbiome we aligned with elevated ROS, but that the latter drove the former is just a hypothesis. The same applies to the microbiome composition and abundances in the control, and to the subsequent comparison of variables.

L374: In the case of the San Francisco Bay, MHW were characterized by “increases in temperature of about 8°C above the yearly average”. Was it +8°C of the yearly (annual?) average or of the monthly climatological temperatures? +8°C above the annual average would not be too impressive. I mention this because one of my concerns is with the experimental conditions chosen. +12°C seems quite a dramatic treatment. Is there a record of MHW in the S Australian coast where the strain was isolated from? Or perhaps this is not relevant – in any case, what are the temperature shift records of MHW in Australian coasts and elsewhere? More 20°C to 24°C, or 20°C to 32°C?

L396: The correlation is negative, not “positive”.

L421-426: I may understand, as a working hypothesis, that optimal growth (hence less

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physiological stress) could be associated with lower DMS/P/O concentrations per cell. But it is harder to understand that sulfur concentrations (per culture volume) decreased during the experiment, even with *A. minutum* being in exponential growth.

L434-438: Why do you say that algal DMSP lyases are exclusively located extracellularly? This is definitely not the case in, e.g., *Emiliania huxleyi* (works by Steinke, Wolfe, Alcolombri).

L446-451: There always is a difficulty when trying to explain and provide experimental evidence for the role of DMS in scavenging ROS: what is first, the decline in DMS or the decline in ROS? It is probably a matter of time scales and potential upregulation by metabolic synthesis. The arguments you provide here carry some assumption that must be explicated.

L492: I would replace DMSP metabolism with DMSP catabolism.

The bacterial community composition characterization was not very informative or illustrative with respect to the cycling of sulfur compounds. Very few of the OTUs that increased their abundances under warming had relatives with genes for sulfur compound transformations. I do not find it any surprising – I think it was too naïve to expect that the bacterial community associated with stressed algae relies mainly on sulfur compounds. Instead, I would expect e.g. opportunistic bacteria. So, I agree with what you say in L513-515. However, I do not agree with your statement in L509-512, at least with the wording used. Quick conversion of DMSP to DMS and oxidation of DMS to DMSO is not a reflection of preferential growth of sulfur-consuming bacteria. Actually, DMSP-to-DMS and DMS-to-DMSO are two processes that do not consume sulfur; if anything, they consume carbon or provide energy. Demethylation of DMSP does lead to sulfur consumption and utilization, and this is a competing process to DMSP cleavage.

Also, you should not base your explanation of the dynamics of the sulfur compounds on the bacterial community alone. There is a potential large role of the dinoflagellate

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itself: arrest of methionine synthase activity under growth arrest, DMSP cleavage to DMS by the algal lyases, etc.

From the figures: The (opposite) patterns of ROS and FvFm are pretty consistent. Conversely, the patterns of sulfur compounds are less convincing. The fact that the two controls (20°C) show remarkable differences makes one wonder what would have been the results from repeated perturbations. You may need an extra effort to persuade the readers/reviewers of the robustness of the observed responses with respect to the sulfur compounds.

L531: Only the “very acute” treatment elicited a response.

References: the reference Simó 2001 is repeated.

Figure 4b: The difference between treatments is essentially one time point.

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