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

Received and published: 30 January 2019

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 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.

The Reviewer makes a fair point and in fact, it is the cleavage of DMSP to DMS and acrylate that is believed to have strong deterrent properties for grazers, most likely through the presence of acrylate at high concentrations. We propose to change this sentence to read: "Many marine phytoplankton produce the organic sulfur dimethyl sulfoniopropionate (DMSP) (Zhou et al., 2009;Berdalet et al., 2011;Caruana and Malin, 2014), for which it can function as an antioxidant, osmolyte, chemoattractant and currency in reciprocal chemical exchanges with heterotrophic bacteria (Stefels, 2000;Sunda et al., 2002; Kiene et al., 2000;Seymour et al., 2010)."

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

The Reviewer makes a fair point and we will make this change.

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.

We agree with the Reviewer's comments and will amend this term accordingly throughout the Results section.

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.

The 8°C increase in temperature referred to here was indeed above the monthly average, whereby the MHW occurred during September, with surface water temperatures reaching 22.6°C, while the average temperature for this month is ~ 14°C. We will clarify this statement to read: "Large increases in

temperature of about 8°C above the monthly climatological average led to red-tides of exceptional density in San Francisco Bay (Cloern et al., 2005)".

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?

We agree with the Reviewer and in fact, the next sentence of this paragraph acknowledges this point: "While a 12°C increase in temperature constitutes an extreme scenario of MHWs, even for coastal habitats, this experimental temperature was selected with the intention to induce thermal stress in *A minutum*.". The amplitude of the temperature increase was dictated by preliminary experiments conducted at 20°C, 24°C, 28°C, 30°C and 32°C, with only a 12°C increase in temperature (32°C) leading to a physiological stress response in this strain of *Alexandrium* in culture. Although an increase in temperature of this magnitude might be rare in coastal marine systems (which we will acknowledge throughout the manuscript), this experiment presented an opportunity to investigate the biochemical and microbial consequences of thermal stress on this relevant phytoplankton in the context of MHWs.

L396: The correlation is negative, not "positive".

We thank the Reviewer for noting this typo. This will be amended accordingly.

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

The Reviewer is correct and we believe that this interpretation is due to our initially unclear description of the data. What we meant was that the DMS(O) concentrations were significantly lower than in the 20°C control, rather than that the concentrations decreased. We will clarify this point as follows: "This temperature optimum was associated with lower DMS and DMSO concentrations than in the 20°C control, although this was only evident 24h after the start of the experiment. Since algal stress responses often result in increased cellular sulfur concentrations in dinoflagellates (McLenon and DiTullio, 2012;Berdalet et al., 2011), it is perhaps not surprising that DMS and DMSO concentrations were lower under what appear to have been more optimal growth temperature conditions."

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).

The Reviewer is correct and we propose to modify our text to reflect this: "Although sporadic, the increases in DMS and DMSO observed in the 32°C treatment may have resulted from enhanced intracellular DMSP cleavage by phytoplankton (Del Valle et al., 2011) or enhanced DMSP exudation from phytoplankton cells during cell lysis (Simó, 2001), resulting in an increasing pool of dissolved DMSP made readily available to both bacteria and phytoplankton DMSP-lyases (Riedel et al., 2015;Alcolombri et al., 2015;Todd et al., 2009;Todd et al., 2007)."

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.

The Reviewer makes a good point and we propose to acknowledge the level of uncertainties in this paragraph by saying: "In contrast, 24h after the start of the experiment, increased ROS coincided with an abrupt decline in DMS and DMSO, perhaps suggestive of serial oxidation via active ROS scavenging

of both DMS to DMSO and DMSO to methane sulfinic acid (MSNA) (Sunda et al., 2002), although it is always difficult to confidently link DMS(O) and ROS dynamics unless using tracing techniques."

L492: I would replace DMSP metabolism with DMSP catabolism.

The Reviewer makes a fair point and we will amend this terminology accordingly.

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.

The Reviewer is correct and we propose to reword this section to clarify our point, which we agree was unclear: "Ultimately, the rapid changes in DMS and DMSO concentrations were potentially caused by (or led to) a shift in microbiome composition towards the preferential growth of sulfur-consuming bacteria (e.g. *Phycisphaeraceae* SM1A02) at the expense of other types of bacteria (e.g. *Seohaeicola*). Alternatively, the observed shifts in microbiome structure may have occurred independently to the biogenic sulfur cycling processes and was instead related to other metabolic shifts in the heat-stressed *A. minitum*. Notably, the temporal shift in bacterial composition under thermal stress was associated with increased cellular ROS at the end of the experiment, indicating a potential link to oxidative stress."

We also propose to acknowledge that: "the change in microbial abundance could have also been triggered by a range of other parameters that were not measured in this study."

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

We agree with the Reviewer and propose to include discussion of these potential processes as follows: "Although sporadic, the increases in DMS and DMSO observed in the 32°C treatment may have resulted from enhanced intracellular DMSP cleavage by phytoplankton (Del Valle et al., 2011) or enhanced DMSP exudation from phytoplankton cells during cell lysis (Simó, 2001), resulting in an increasing pool of dissolved DMSP made readily available to both bacteria and phytoplankton DMSP-lyases (Riedel et al., 2015;Alcolombri et al., 2015;Todd et al., 2009;Todd et al., 2007). However, it is notable that lower DMSP concentrations in the 32°C treatment than in the control only occurred on day 4, whereas the spike in DMS and DMSO were evident at the outset of the experiment (6h). Since this decrease in DMSP at 96h was not coupled with an increase in DMS, this could alternatively be indicative of a decrease in methionine synthase activity (McLenon and DiTullio, 2012) or assimilation of DMSP-sulfur by bacterioplankton for *de novo* protein synthesis (Kiene et al., 2000), with this demethylation pathway often accounting for more than 80% of DMSP turnover in marine surface waters."

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.

As described in the method, both experiments were conducted at different times and it was thus not to be excluded that the 2 controls kept at 20'C could present some physiological (Fig. 1 & 2) and biochemical (Fig. 4) differences, which perhaps reflected inherent heterogeneity in biological systems. However, the significant differences that were observed between temperature treatments in each experiment were clearly driven by the increase in temperature since both temperatures (control and experimental) were tested at the same time, on the same culture, and under the exact same experimental conditions of light and GSe medium in each experiment.
Because the turnover of DMS(P)(O) in biological systems can occur very quickly (Simo et al 2000), measured changes in DMS(O) concentrations can seem to occur sporadically. However, a clear cascading stress response emerged from these results, which is worth reporting and discussing.
We propose to better acknowledged variability and uncertainties in the discussion by saying that: "Because the turnover of DMS, DMSP and DMSO in biological systems can occur very quickly (Simo et al 2000), DMS and DMSO concentrations can change rapidly, which sometimes makes it difficult to clearly establish cause-effect relationships between physiological stress and the biogenic sulfur response."

L531: Only the "very acute" treatment elicited a response.

We agree with the Reviewer and will amend this sentence as follows: "Here, we hypothesized that a very acute increase in temperature, mimicking extreme coastal MHWs, would trigger both a physiological and biochemical stress response in the DMSP-producing dinoflagellate *A. minutum*."

References: the reference Simó 2001 is repeated.

We thank the Reviewer for picking this up. This will be amended.

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

We agree with the Reviewer, however, this reflects that differences in sulfur concentration between treatments rely on rapid changes in DMS(O)(P) concentrations, reflective of a quick turnover of DMS(P)(O) in biological systems (Simo et al 2000), which will be better acknowledged in the discussion.