

## ***Interactive comment on “Temperature and UV light affect the activity of marine cell-free enzymes” by Blair Thomson et al.***

**Blair Thomson et al.**

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Author response to Reviewer #2 We thank the reviewer for the constructive comments on this manuscript. We have taken them on board and our responses to reviewer comments, including modifications to the manuscript, are detailed in the following:

Reviewer #2 REVIEWER COMMENT 1 by Referee #2: This is an interesting paper, but a bit overly simplistic and seems to miss much of its potential. The fact that enzymes are affected by UV should not be surprising (they are complex organic molecules and the literature is replete with photochemistry). What are the structures of these enzymes? Since the result is different, what's different about the structures of the enzymes that suggests differences in sensitivity to UVR?

Author response: Although we agree with the reviewer that the effect of UVR on free

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enzymes could be expected, it had not been shown how marine produced cell-free enzymes were affected by UVR. We had a hypothesis based in fundamental theory and we applied that hypothesis to the marine environment, within a context where cell-free enzymes happen to be very important, and in an environment that happen to frequently fluctuate in temperature. It is difficult to tell at this point what the differences could be due to exactly in terms of the structure of the enzymes. For that we would need to perform more sophisticated protein structural research which is far from our scope here. The reality is that the majority of marine cell-free enzymes are poorly characterized and understood. There is likely to be structural differences between the glycolytic and proteinous enzymes for example which could affect their relative sensitivity to UVR, but a claim such as this would be a postulation/speculation at this point.

REVIEWER COMMENT 2 by Referee #2: Nowhere do the authors address whether the effect is on the enzyme or perhaps the substrate? What's the structure of the substrates, will they absorb UV?

Author response: We are not sure whether the reviewer refers to the natural substrates in the seawater sample or the artificial substrates used in the EEA assay. In the first instance, it is a good point that UV could affect the substrate (e.g. proteins, carbohydrates, etc), so we have now included this possibility in the discussion (p. 7, l.216-217). For the latter, it is not a problem as the incubated plates themselves (which were supplemented with the artificial substrates) were not exposed to UVR, thus the artificial substrates were not exposed to UVR directly.

REVIEWER COMMENT 3 by Referee #2: The exposure methodology is unclear, the samples were placed in glass vials but were they irradiated through the glass (blocking much UV) or left open and irradiated from the top?

Author response: They were left open and irradiated from the top. This has been clarified in the methods (p. 5, l.150-151).

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REVIEWER COMMENT 4 by Referee #2: Spectrum of the lab light source is very different from the spectrum found in seawater, lamps are a necessary evil, but a bit over simplification to say they had environmentally relevant irradiance. Why not do the incubations in situ in UV transparent containers (quartz, teflon, polyethylene?)

Author response: We agree with the reviewer that in-situ experiments would be much closer to reality in terms of a UVR dose. Our aim was to have the greater number of conditions/factors as controlled as possible to avoid other confounding factors. These experiments are part of a series which will eventually test multi-stress patterns, including both UVR and temperature; temperature being much harder to control in situ. We had already specified in the abstract that by “environmentally relevant irradiance” we mean that the authors tested and then used a dose level measured in-situ (p. 1, l.20-21).

REVIEWER COMMENT 5 by Referee #2: Finally, the discussion misses some classic literature - there were numerous papers published in the 80's from John Paul's lab on extracellular nucleases (DNase)

Author response: These papers from John Paul's lab have now been reviewed. Thank you for pointing these out; some of these references were added to the discussion (p. 8, l.231-233).

END OF REVISION

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