APPENDIX S2: SUGGESTIONS FOR PREPARATION OF PHYTOPLANKTON GROWTH MEDIA

1. To minimize HOOH production, buffers that are poor producers of HOOH should be preferred. By this metric, Tris and glycylglycine, already in use for several algal media recipes ([e.g., Volvox medium, ES medium, and the various formulations of ASP medium, Andersen 2005](#_ENREF_1)), may be ideal choices as they produce no HOOH. However, Tris is known to permeabilize biological membranes ([Irvin et al. 1981](#_ENREF_3)) and thus can be toxic to many microbes ([Macleod & Onofrey 1954](#_ENREF_4), [McLachlan 1964](#_ENREF_5)). Our own data suggest that *Prochlorococcus* was able to tolerate Tris, but the highly variable growth curves (Fig. S4) suggest that this buffer is not ideal. Glycylglycine actually appeared to scavenge HOOH (Figs. 1C, S2), but was toxic to *Prochlorococcus* for reasons apparently unrelated to oxidative stress (Fig. S4). Moreover, both Tris and glycylglycine have both been reported to be labile carbon sources for contaminating heterotrophic bacteria ([Harrison & Berges 2005](#_ENREF_2)). For these reasons, the utility of such buffers must be evaluated on a case-by-case basis. If these buffers cannot be used, we recommend TAPS over all other Good’s buffers. For culturing *Prochlorococcus* in a defined artificial seawater medium, we use AMP-J, a modified form of AMP-1 ([Moore et al. 2007](#_ENREF_6)), which replaces 1 mM HEPES with 1 mM TAPS and is filter-sterilized rather than autoclaved.
2. If possible, the use of artificial media may be preferable to natural seawater or freshwater, as they tend to generate less HOOH under light exposure. While the major chemical constituents (i.e., salts) were very similar in both our natural and artificial seawater media, DOC concentrations were significantly (t-test, df=13, p = 6.4 x 10-12) higher in the natural seawater (112.5 +/- 8.77 μM vs. 17.6 +/- 1.01 μM DOC in artificial). Since DOC is the primary source of HOOH in natural light-exposed water, we suspect that DOC is the “seawater solute” (e.g., Fig. 2B) involved in the HOOH production reaction, and therefore using “cleaner” artificial media should consistently reduce HOOH production.
3. If HOOH-producing buffers must be used, the lowest concentration capable of maintaining an acceptable pH should be empirically determined and used for all media recipes. In some cases, pH fluctuations may be constrained by preventing carbon limitation (i.e., by better aeration and/or reducing nutrient levels in the medium), thus allowing a lower amount of buffer (or elimination of buffer entirely).
4. Media containing Good’s buffers should be stored in the dark until inoculation.
5. In order to reduce the base HOOH concentration of the medium, avoid or minimize flaming the cultureware, and consider filter sterilization as an alternative to autoclaving.
6. When cultures must be grown under high light or with higher buffer concentrations, starting inocula should be kept high.
7. Catalase may be added in small amounts to ameliorate buffer toxicity in cases where dilute cultures are required. However, our results indicate that exogenous catalase photodegrades fairly quickly, so its protective power will not last more than a day or two. Further, we have observed in other studies that inactivated catalase is itself potentially toxic ([Morris et al. 2011](#_ENREF_7)).
8. If culture purity is not required, the best solution may be to intentionally contaminate the algal culture with a heterotrophic catalase-positive “helper” microbe to maintain a low HOOH environment ([Morris et al. 2008](#_ENREF_8)). As an example, experiments measuring growth of non-axenic *Prochlorococcus* cultures often showed much higher tolerance for the presence of HOOH-producing buffers (Fig. S4).

References

Andersen, R. 2005. Algal Culturing Techniques. Academic Press, Burlington, MA, pp. 596.

Harrison, P. J. & Berges, J. A. 2005. Marine culture media. *In* Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Elsevier Academic Press, Burlington, MA, pp. 21-33.

Irvin, R. T., MacAlister, T. J. & Costerton, J. W. 1981. Tris(hydroxymethyl)aminomethane buffer modification of *Escherichia coli* outer membrane permeability. *J. Bacteriol.* **145**:1397-403.

Macleod, R. A. & Onofrey, E. 1954. Cation antagonism of the antibacterial action of amines. *J. Biol. Chem.* **210**:193-201.

McLachlan, J. 1964. Some considerations of growth of marine algae in artificial media. *Can. J. Microbiol.* **10**:769-&.

Moore, L. R., Coe, A., Zinser, E. R., Saito, M. A., Sullivan, M. B., Lindell, D., Frois-Moniz, K., Waterbury, J. & Chisholm, S. W. 2007. Culturing the marine cyanobacterium *Prochlorococcus*. *Limnol. Oceanogr.: Methods* **5**:353-62.

Morris, J. J., Johnson, Z. I., Szul, M. J., Keller, M. & Zinser, E. R. 2011. Dependence of the cyanobacterium *Prochlorococcus* on hydrogen peroxide scavenging microbes for growth at the ocean's surface. *PLoS ONE* **6**:e16805.

Morris, J. J., Kirkegaard, R., Szul, M. J., Johnson, Z. I. & Zinser, E. R. 2008. Facilitation of robust growth of *Prochlorococcus* colonies and dilute liquid cultures by "helper" heterotrophic bacteria. *Appl. Environ. Microbiol.* **74**:4530-34.



Figure S4. Growth of non-axenic *Prochlorococcus* MIT9215 cultures in Pro99 medium supplemented with 10 mM of various buffers. Dotted lines shows the mean of 3 replicate unbuffered control cultures grown under the same conditions. Error bars are the standard errors of the three control replicates.