TECHNICAL RESPONSE

ENVIRONMENTAL TOXINS

Response to Comment on "Models predict planned phosphorus load reduction will make Lake Erie more toxic"

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Huisman *et al*. claim that our model is poorly supported or contradicted by other studies and the predictions are "seriously flawed." We show their criticism is based on an incomplete selection of evidence, misinterpretation of data, or does not actually refute the model. Like all ecosystem models, our model has simplifications and uncertainties, but it is better than existing approaches that ignore biology and do not predict toxin concentration.

Huisman *et al.* provide a point-by-point criticism of our recent paper (*1*), to which we respond in turn. First, the model predicts total MC concentration and we equate that to toxicity, which is necessitated by limited availability of congener-specific knowledge on synthesis, function, potency, and it is common practice, e.g., WHO, EPA, ELISA, also by Huisman *et al.* (*2*). We agree that a change in N-limitation will likely affect MC congener composition and toxicity. However, the underlying mechanisms remain unclear and the evidence is not as consistent as Huisman *et al.* suggest. In the study they cite (*3*), the more toxic MC-YR also increased, and some studies found no change of composition with changing N (*4*).

Second, in the model, H_2O_2 is produced photosynthetically by *Microcystis* and other sources, including respiration and extracellular. Yes, there is increasing evidence that cyanobacteria do not produce H_2O_2 via the Mehler reaction, but photosynthetic production has been observed (*5*). In Lake Erie, H_2O_2 peaks prior to or coincident with *Microcystis* blooms (*6*, *7*), consistent with photosynthetic production. However, our more recent work also suggests most biological H_2O_2 production in Lake Erie is by heterotrophic bacteria (*7*). It would be useful to extend the model to more explicitly resolve the various H_2O_2 sources and sinks. In the meantime, the simplified representation is a reasonable approximation, and if it is *Microcystis* or associated bacteria is not critical since H_2O_2 readily diffuses across cell membranes.

Third, Huisman *et al.* question whether oxidative stress occurs at environmental H_2O_2 concentrations and cite two studies. The first study (*8*) does not support their assertion. The lowest concentration evaluated was 15 μmol/L at which effects were observed, which does not rule out effects at untested lower concentrations, which were also observed in another study by the same group (*9*). The second study (*10*) found no effect at 22 μmol/L, but the H_2O_2 was degraded within a few hours and not replenished as it was in a study that did observe an effect (*11*) and as it would be in the environment. Our more recent work also shows effects at natural H_2O_2 concentrations, though it also suggests more complex strain-level diversity of H_2O_2 sensitivity (*6*). Note that the effects in the model are sublethal and subtle, corresponding to ~20% growth rate differences. Huisman *et al.* also question whether MC binds to proteins at ambient conditions, although their own work (*12*) showed this.

Fourth, Huisman *et al.* point to a study (*13*) that showed a toxigenic strain is more sensitive to H_2O_2 than a non-toxigenic strain and suggest that this contradicts the model, but the model actually reproduces that experiment (our Fig. 3, right side). Huisman *et al.* also propose a different function for MC based on their more recent research, where MC enables acclimation to C-limitation. We don't question this mechanism and it would be useful to update the model to include it. Development of mechanistic models is a dynamic/stepwise process, and models will always lag biological understanding. There are many potential functions of MC and they are not mutually exclusive. What evidence is there to refute that MC (also) binds to proteins and protects them against oxidative damage, as it is implemented in the model?

Fifth, Huisman *et al.* suggest the ecology of toxigenic and non-toxigenic strains may be affected by competition for C, consistent with their more recently proposed biological role of MC (see above). The model, like most phytoplankton models, does not consider C-limitation, and we acknowledged above that it would be useful to extend the model. They also point to a study (*14*) that showed the toxigenic wildtype is a better competitor for N than the nontoxigenic mutant, and suggest this is opposite to the model. It is true that, in the model, the additional N required to make MC gives the toxigenic strain a disadvantage under N-limitation. However, although the mutant non-toxigenic strain used in that study is useful for exploring molecular mechanisms, it is not a good representative of wildtype non-toxigenic strains or their ecology. The deletion of the *mcyB* gene has consequences for the expression of many genes and it increases synthesis of other cyanopeptides and consequently N requirements (*14*). The model could reproduce those experiments, but it would require different parameterization of the two strains (beyond presence/absence of the *mcyB* gene) to reflect those differences.

Sixth, Huisman *et al.* criticize that the model only considers *Microcystis* and no other phytoplankton, and they suggest that nutrient reductions will lead to a shift away from *Microcystis* toward nontoxic eukaryotic phytoplankton. We acknowledge that any changes due to management or climate may result in a species shift, which would not be predicted by the model, and that adds uncertainty to our results. However, understanding and predicting species shifts is complicated, and it is not clear that nutrient reductions always or in the case of Lake Erie will lead to a shift toward eukaryotes. In Lake Erie (and many other systems), the present resurgence of *Microcystis* occurred following nutrient reductions (*15*). With the limited current understanding, assuming such a shift will not occur may be a good precautionary management approach.

Huisman *et al.* end their critique by pointing to the success of P load reductions in controlling cyanobacteria blooms, which is entirely consistent with the model (our Figs. 4B1 and 4C5) and misses the point of our paper, i.e., that it may increase toxin concentrations. The specific criticisms they provide are useful in that they point to uncertainties and potential future developments of the model. However, such uncertainties are unavoidable and inherent in all models of complex ecological systems. The model we presented is based on a large body of biological evidence, and even with its simplifications constitutes the most complete and consistent representation of *Microcystis* growth and toxin production available today. The model predicts that reducing P alone will increase toxin concentrations, which is in striking contrast to the present management approach, which assumes P load reductions will reduce toxicity. Management should be based on the best science and models available. We scientists need

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to be clear and maybe sometimes "provocative" to make this happen.

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