Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-207-AC1, 2020

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Reproducible determination of dissolved organic matter photosensitivity" *by* Alec W. Armstrong et al.

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Received and published: 1 October 2020

We appreciate the thoughtful and thorough comments, which we believe can be grouped into three substantial points – our use of a nitrite actinometer, our suggestions for improving reproducibility and comparability in photochemical degradation studies of DOM, and the novelty of our contribution in terms of its utility vs. existing approaches and our suggested interpretation of the data we generated.

We used a nitrite actinometer to illustrate the importance of controlling photon exposure and the potential limitations of modeling degradation processes as a function of time, shown distinctly in Figure 3. As the referee states, "Any photosensitivity study should include a detailed documentation of the optical setup and exposure measure-

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ments such that the results can be expressed either as a function of time or cumulative exposure. Studies do range in how thoroughly exposure conditions are documented [emphasis ours]." We contend it is often difficult to calculate cumulative exposure from reported time alone in the published literature, making comparability difficult, and show that using a nitrite actinometer to calculate and report exposure directly is one way to improve this situation. We agree that other means of calculating and reporting cumulative exposure, such as measuring radiation across the UV spectrum directly, can only address this issue when irradiation design allows for accurate photon dose quantification with a spectroradiometer (e.g. irradiating quartz vessels/spectrophotometer cells with a known pathlength, cells are isolated from each other, ensuring that light is collimated/off axis photons do not hit samples, etc). Many labs have successfully designed systems that allow for very accurate photon dose quantification using solar simulators. For example, Powers and Miller (2015, MarChem, 171) irradiated 10 cm cells vertically in a black water-cooled aluminum block that maintains cells at a set temperature and allows no transfer of light between samples. Additionally, a gray 0.5" PVC plate with holes matching the spacing/interior diameter of the spectrophotometer cells was placed over the block and positioned under various 2" diameter Schott long-bandpass cutoff filters (280 nm to 480 nm) that were evenly spaced in a tray located at the bottom of the solar simulator exposure chamber. Due to the reflective exclusion of high angles at the surface of the Schott glass filters, reflectance at the face of the quartz cell is minimized. Furthermore, the gray plate reduces the entrance diameter and absorbs any off axis photons within the "gershun-like" tube formed by the gray plate, which also removed (or greatly minimized) reflectance at the face of the quartz cells by collimating the light. However, as mentioned in our manuscript, this type of experimental design allows for a limited number of samples, and therefore a limited number of measurements. Because the system described in Powers and Miller (2015) irradiates samples in 10 cm cells, even samples with relatively low CDOM values are not optically thin. While Powers and Miller (2015) used the methods of Hu et al. (2002) to correct "photons absorbed by CDOM, Qa(λ)" for shelf-shading (i.e. Qa(λ) = E0(λ)$\times$S(1-exp(-ag(λ)$\times$L)$\times$t, where

E0(λ) is the measured spectral irradiance entering each cell, S is the irradiated surface area, ag(λ) is the CDOM absorption coefficient, L is the pathlength, and t is irradiation time), it is difficult to verify that these corrections are sufficient. It is possible that there are concentration effects but this is difficult to evaluate due to changes in sample matrix when diluting samples. Furthermore, samples in such a system must also be irradiated with no headspace because bubbles are terrible for optics, leading to oxygen loss during irradiations. While this may not be a problem for short exposures, many photoproducts of interest (i.e. CO2) require long irradiations to generate a measurable product and it is possible that rates decrease at lower oxygen concentrations (e.g. Gao and Zepp, 1998). Lastly, this system allows for no pH control during irradiations, even though it is well known that pH can change during irradiations (Gao and Zepp, 1998) and photodegradation rates change at different pH values (Timko et al. 2015; Song et al. 2017). While some researchers have chosen to buffer samples to overcome this issue (e.g. Powers and Miller, 2015), to our knowledge it is still unknown how buffers may or may not impact photodegradation rates.

Therefore, our primary methodological objectives were to establish a reproducible design that would 1. Irradiate samples under “optically-thin” conditions, even at relatively high CDOM values. We therefore chose the quartz spiral flowcell with a 1 mm pathlength, which allows for 100x high CDOM values when compared to a 10 cm spec cell. One very surprising result was that even though SRNOM with DOC concentrations ranging from 10 to 50 mg L-1 degraded at similar rates, there appears to be a concentration dependence at low SRNOM DOC concentrations. This is a subject that we hope to evaluate more in future work. 2. To prevent oxygen loss, the sample is continually returned to an equilibrator, where it is mixed and re-oxygenated. 3. While we did not control samples for pH in this study (as mentioned no pH changes when irradiating our samples at pH 3), pH control is possible with a microtitrator and has been described in a previous study (Timko et al. 2015). We realize with this design we have sacrificed accurate photon dose quantification with a spectroradiometer that is possible in designs with very well defined and controlled optics. However, with the goal

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of reproducibility in mind and limiting problems that occur when irradiating sealed vessels, we believe this system is a valuable tool to use when evaluating “photosensitivity”. For instance, it will be a great tool to evaluate some of the questions/issues mentioned above (e.g. do buffers impact photodegradation rates?). Unfortunately, many studies do not control optics or do not use reproducible designs, e.g. irradiating samples in curved vessels on their sides or in flasks. While we cannot calculate spectral photon doses in our system, we thought that using nitrite actinometry as a broadband measurement of UVA photon dose was better to use than irradiation time as mentioned in the manuscript (lines 211-220, 259-268, Figure 3). We thank the reviewer for bringing up these valuable discussion points. A revised manuscript would discuss in detail the tradeoffs we made between radiometer-quantifiable optics and sample control.

The referee raises a few points related to our other suggestions for improving comparability and reproducibility when measuring photodegradation kinetics, stating “I quite agree with these recommendations which are straightforward to implement. For others, it is not clear that they will always contribute to reproducible, or more importantly, interpretable, results.” Here we stress that we do not claim our approach provides a universally applicable prescription to ensure reproducible results – rather we hope to illustrate several obstacles to reproducing and comparing studies of photodegradation kinetics, and demonstrate some progress towards improving or at least identifying these sources of uncertainty. The manuscript text can be revised to convey this distinction more clearly. Specifically, the referee notes “[w]hile extracts are inherently more stable than raw filtrates, the authors demonstrate differences in the photosensitivity kinetics of extracts vs the original filtrates, the reasons for which are not well understood. The authors advise preparing extracts in some situations but not others, this leaves open the question of comparability.”

This is in fact exactly our intention – to illustrate obstacles to reproducibility that need to be explicitly addressed when conducting or at least when comparing studies of photodegradation kinetics. As the referee notes, the differences between extracts and fil-

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tered raw water samples are not well understood and worthy of future study. Until these issues are resolved, researchers will have to weigh the advantages and disadvantages of extracts vs. raw water. Here we chose to use only extracts in subsequent trials in order to compare samples collected at different times that were extracted for stable storage, and to limit our inference to differences in photodegradation kinetics arising from DOM composition and not matrix differences. Other studies will have to choose between using extracts vs. raw water in accordance with study priorities. The differences we demonstrate (Figures 6 and 7, Table 1) illustrate underappreciated limitations to reproducibility and identify opportunities for further research into the effects of extraction on photodegradation kinetics and mechanisms of action (e.g. effect of matrix vs. fraction of DOM extracted). Along similar lines the referee notes "The authors also advise using a standard optical density in the sample, yet also show there is a concentration dependence to the kinetics, even for an optically thin exposure conditions. Again, a decision will have to made of whats more important, knowing how the DOM behaves at its natural concentration or being able to compare it to other sources." Again, we are in agreement with the referee here, and believe one of our manuscript's contributions is highlighting these underappreciated sources of variability in DOM photodegradation kinetics. This issue in particular is vexing, as differences between DOM concentrations under conditions meeting conventional thresholds for optically thin solutions are not explained well by currently published literature. We will revise the manuscript text to make it more clear that our goals were not to show only reproducible results but to identify underappreciated sources of variability that need to be controlled, and to use these insights to conduct limited trials with comparable results.

The referee's final point concerns the utility of our approach in a wider sense – "In the end, investigators will need more information to decide whether the additional insight gained from defining photosensitivity kinetics will be worth the efforts needed to perform all these standardization steps. This report documents experimental tests that show what are sources of variability in the procedure but does not show what new understanding is actually being gained from the results (vs what can already be gained

from other optical/chemical measurements typically made on DOM)." We agree that our approach is not trivial, and some applications may not really benefit from this level of effort. Our primary research goal was to compare the sensitivity of different DOM sources to photochemical degradation processes at environmentally relevant temporal scales. Measuring kinetics of DOM photodegradation changes seemed the best way to pursue this, but we found existing approaches to describing DOM photodegradation kinetics allowed for possible uncertainties that were barriers to meaningful comparison (as discussed in detail above). We believe the methodological suggestions we present are useful to other researchers in this area pursuing similar goals, and our research findings (seasonal and spatial differences in wetland DOM photo-sensitivity, with implications for understanding DOM composition and the other ecological processes that affect it in this setting) demonstrates the potential utility of this approach and illustrates phenomena worthy of further investigation.

Specific concerns (referee's comments bracketed by ***):

Line 140 "Solar exposure" – This was not solar exposure, but a laboratory set up. Better (?) "Total sample exposure"

We agree and will revise the manuscript accordingly

Figure 7 and Table 1 – The captions for these describe kL and kSL with units of mol photons m-2. Those are the units of P, the units of k should be the inverse, m2 [mol photons]-1.

We agree and will revise the manuscript accordingly.

***Figure 7 and Table 1 apparently show the same data – and both state the statistics of the comparison. Questionable whether both are necessary. Figure 7 caption, however, says see Fig. 5 for data. It appears that Fig. 6 has the data. ***

The referee is correct that Figure 7 caption should refer to Figure 6 instead of Figure 5, and also correct that both Figure 7 and Table 1 show the same data. Readers may find

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the graphical comparison easier to quickly parse, but the tabular presentation may be more useful to future studies seeking comparison. We think the graphical presentation is more valuable to readers in the main text, and would move Table 1 to an appendix upon revision.

Line 350-360 – Factors affecting storage of filtered water. Previous text only mentioned preparation of filtrates using 0.7 _M glass fiber filters, which allows passage of a substantial bacterial fraction. However, subsequent guidelines caution against used stored material even filtered to 0.2 _M (some bacteria can even get through those). Please clarify what filtration method was used for the stored sample, but in any case (slow) microbial degradation may be another factor resulting in changes in stored material.

We inadvertently failed to note an important step in our methods – all samples, whether raw water or solid-phase extracts redissolved in water, were filtered through syringe-mounted 0.2 μm cellulose acetate filters that were pre-rinsed with > 30 mL ultrapure C-free water. GFF filters were used as a pre-filter immediately after samples were returned to the lab, before either short-term storage or solid-phase extraction. Furthermore, since 0.7 μm filters were combusted, their true pore size is probably smaller than 0.7 μm (0.3 μm in Nayar and Chou, 2003) and therefore be comparable 0.2 μm filters (Nayar and Chou, 2003). We apologize for the omission and ensuing confusion. The referee notes that microbial degradation is still possible even when using a filter of 0.2 μm pore sizes – we agree (see Brailsford et al., 2017; Luef et al., 2015), and believe this supports our recommendation to either use extracts or keep storage time minimal and highlights the need for more research in this area. We also believe microbial degradation/contamination is minimal during exposures under the solar simulator using optically thin conditions (e.g. Stubbins et al. 2017, 122), but can revise our manuscript to mention the full range of possibilities that must be accounted for in any similar experimental set up. This would include discussion of between-sample cleaning with dilute NaOH and occasional flushes of sample lines with isopropanol (followed by extensive

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water flushes and testing for DOC residue) to prevent microbial contamination of flow lines.

Figure 10 – Caption says see Figure 6 for data, data appears to be in Fig. 9. This correction also applies to the caption for Figure A1. Figure 10 could be misleading to the reader, as a quick inspection may suggest that the spread of the multiple points for a particular sample source (and standard deviation in the table) are indications of reproducibility. Actually, only the SRNOM and three of the small wetland points are repeat determinations on the same sample. Separating the points for different sample dates would avoid this confusion, especially given that later on (section 3.3.2) the authors attribute some of the difference in the wetland results with time to possible differences in sample composition and/or previous solar exposure.

The referee is correct that the captions for Figure 10 and A1 should read "see Fig. 6 for data". We agree that the representation of data in Figure 10 may be confusing. This criticism may also apply to Table 2, where group means and standard deviations for photodegradations of DOM sources with more than one trial are grouped differently – SRNOM represents three actual replicate trials using the same sample source, the three "large wetland" trials represent different samples (collected at same site but at different times), and the five "small wetland" trials represent three replicate trials using the same source and two trials from different sample sources (collected at the same site but at different times). We originally thought it was useful to make the comparison in Figure 10 in this way because of the widespread interest in differentiating DOM sources by setting. By showing fitted parameters as independent data points we thought readers could parse the different types of grouping by reading the text carefully. However, we agree with the referee's concerns – at the very least the sampling dates should be differentiated by using different symbols in the plot. Upon reflection we also believe Table 2 should be changed along these lines – our original intention was to facilitate comparisons between environmental settings, but calculating group means with these data is not appropriate given their different structures. Means for replicated trials are

sensible, and it may even be valuable to see how un-replicated trials with samples from different seasons at a site differ, as solely spatial comparisons often do not account for temporal/seasonal variation but readers may still be interested in summarizing data by site. But calculating one mean from data that includes 3 replicated trials of the same sample source and two further distinct samples does not convey useful information. We think publishing tabular data may be useful for future research, but this data would be better represented separately. For size reasons, such a table may be better suited to an appendix.

Line 456ff, section 3.3.1 Here the relative abundances of kinetic components are considered as metrics of DOM composition or other influences on photosensitivity. A problemwith the comparative use of fL and fSL is that the two parameters are not independent. Assuming a good fit to the actual time course the two fractions will always sumto approximately 1, which means that any variation in one fraction will be reflected in a complementary variation in the other. Thus, changes in, e.g. fL, with sample date or location (as later discussed in 3.3.2) could be caused by change in the concentration of labile or semi-labile fraction, or both. If the focus is on the rate parameters, this is not so important, but if the magnitudes of the fractions are to be comparative metrics, the need to be independent of each other. For example, ft could be the actual component score, score normalized to t0 total fluorescence, or score normalized to DOC.

We see the referee's point that fSL and fL are not truly independent. They were fitted as separate coefficients in the nonlinear fitting algorithm applied to the data, but should indeed sum to 1, so either could be expressed as the difference between 1 and its counterpart (i.e. fSL = 1- fL or vice versa). We expressed them separately here in accordance with their separate representation in the equation conventionally used to define the decay model (Equation 2, which Equation 3 then followed), and because they were fitted separately (which would make the choice of which to use to find its counterpart arbitrary). We agree that this causes ambiguities when trying to understand differences in these values between samples, as in the example offered by the referee.

We do believe using these values may be informative, as a major part of our claim to novelty rests on pointing out possible connections between these values and environmental phenomena (such as previous exposure to photodegradation or differences in source material composition) instead of basing inference solely on k parameters. We are not sure how to best resolve this issue. ft already represents PARAFAC component fluorescence normalized to time 0 for each sample. Further processing the data to derive a single value (like the ratio of fSL and fL) adds another layer of abstraction to a situation that already involves multiple modeling steps, and seems harder to interpret in a way that materially connects to the phenomena described. A revised manuscript would discuss these points. However, We still think it is valuable to compare fL between samples – even if differences in this value represent multiple possible causes as described by the referee, differences here represent real observed differences in photodegradation behavior. Normalization to [DOC] or to absorbance at some reference wavelength may be useful in this context. We believe this is an area ripe for future research, and view our contribution in part as pointing out the need for attention to previously unexplored dimensions of current paradigms of DOM photodegradation.

The authors offer the interpretation that the fractions correspond to "pools" of more or less reactive DOM. However, Murphy et al. (2018) interpret biexponential kinetics as possibly reflecting multiple photoreaction pathways, a fast one involving ROS and slower one related to direct photochemistry. How do the authors reconcile these differing interpretations?

We appreciate the referee's thoughtful reading of our conclusions. Our conceptualization – differences in photodegradation kinetics arising from different relative quantities of "pools" reacting at relatively fast and slow rates – may be unnecessarily general or vague. However, we think the explanation from Murphy et al. (2018) invoked by the referee is not necessarily opposed to the "pools" we ascribe to the model. We consider these "pools" in a general sense. Given the immense complexity of natural DOM, it is unlikely there are two distinct, non-overlapping compositional types of photo-sensitive

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DOM. Yet the excellent fit of biexponential models across diverse DOM sources requires some explanation. What we refer to generally as reactive pools could more specifically be the pools of DOM subject to the respective reaction pathways suggested by Murphy et al. and their associated rate constants. Or perhaps it is more appropriate to consider a single "pool" with differing capacities for two classes of reactions. We are not able to definitively conclude what lies behind the biexponential model – but believe our novel attention to the f parameters in the model equation represents the kind of exploration that is needed to make progress in this direction.

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-207, 2020.

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