Technical Note: Comparison of storage strategies of sea surface microlayer samples

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We would like to thank the reviewers for their valuable suggestions and positive comments as to the welcome nature of this work. We agree that studies such as this are often neglected in the literature and that identifying “potential pitfalls” for sample storage is a useful addition.
In response to the reviewers concerns, we have provided detailed comments and incorporated a number of revisions as detailed below.

Reply to reviewers’ comments

Reviewer #1

The first reviewer stated that “notes on potential pitfalls in storage sampling are welcome”, which we find supportive. However the referee was also concerned that “the manuscript does not provide anything new and due to the length also does not serve as review on SML methodology and sampling storage, which I would consider an important exercise”. In an earlier draft of the manuscript we did include a review of SML sample storage methodologies but we removed it from the initial submission because we were concerned that this might make it too long for a Technical Note. Upon reflection and following the reviewer’s comments we have now reinstated this section. We have also clarified the novel aspects of the study and highlighted that that this manuscript, to our knowledge, is the first concerning storage of SML samples in particular, and the first one which looks at surfactant activity (SA) and CDOM at the same time. Although CDOM storage experiments exist in the literature, we believe that confirming these protocols for often unique SML samples is a useful contribution. The referee also referred to the fact that “only the Garrett screen has been used, but quite some studies use glass plate samplers”. We do direct the reader to papers (including work of our own) which discuss biases of the various SML samplers currently in use. We are of course aware that different samplers sample different SML depths but our firm view is that there are also spatial and temporal differences in SML composition that can far exceed differences introduced by choice of sampler. For the purposes of this comparative study we selected the Garret Screen because it is widely used in the literature and is easy and reproducible in use. We now make these points clear in the revised manuscript. The reviewer also states that “the current manuscript confirms some findings of the past” but importantly does not seem to consider this a disadvantage, and suggests that we should “weave the current findings into a manuscript that describes and interprets the ‘unbiased’ SML data the authors certainly have”. We find this statement somewhat ambiguous but if we have interpreted it correctly we believe that we have addressed it. Please refer to above regarding the novelty. We think that the findings may get lost in a manuscript containing significant other findings and we would not be able to discuss these results in such detail elsewhere.

Specific comments

page 2837, line 7: what are the logistical reasons, I suggest to be more specific. We have rephrased this passage to make this much clearer.

page 2838: I miss a general description how the samples have been taken. How much sample has been collected per sampling, how long did the sampling take, what precautions have been taken to avoid contamination of the samples?

We have expanded this description to take account of these comments and make it much clearer.

page 2838, line 5: The treatments are listed in Table 1. ! This comes rather surprisingly and actually you mean the storage treatments.

The section has been rephrased and made clearer.

page 2838, line 17: What peristaltic pump? What tubes? As mentioned above a proper description of the sample handling is warranted.
We now include the details as suggested.
page 2838, line 26: All others, meaning treatment number 7 or the samples of treatment number 7?
We changed it to make it unambiguous.
page 2838, line 27 – page 2839, line 28: I suggest to reference to established protocols and than describe the method briefly as is done. That way the reader may better judge whether the methods were done properly and where modifications have been made.
Done
page 2840, line 2: I would be interested in the comparison of glass versus plastic also visually.
We have added an additional figure which shows selected responses of glass and plastic.
I did not quite understand what statistical tests have been done in the end, with what software and how. I think some clearer writing is necessary here. E.g. : : :whether any of the factors were zero: : : What factors did the authors mean here (glass versus plastic, treatments: : :?). It was unclear to me whether the statistical treatment of triplicates was done on triplicates per sampling date or that the 3 sampling dates were considered triplicates.
We have now rewritten our description of the statistical procedures to cover these concerns and we believe that this is now clear.
Table 2: Instead of the many markers I suggest to introduce a 4th column for the citations.
Done

Reviewer #2

Reviewer #2 was concerned that "The rationale for performing this type of comparison specifically for surface microlayer samples is not well explained, raising the question on the originality of the results". We believe that we did already make this rationale clear in our introduction but in our revised manuscript we have again stressed the importance of this to specifically SML samples. The reviewer also states that "Storage protocols for CDOM samples are available, and based on previous studies the storage of filtered samples at 4 C in the dark is well accepted in the community. The authors demonstrate that this protocol also applies to surface microlayer samples. The question of how SA could change over time and with treatment is more relevant in this context, and I suggest the authors make a stronger point on this aspect." Although SA is only one of many SML components of relevance, in the revised manuscript we now stress the importance of surfactants to gas exchange in our introduction and thus feel that we give this aspect due weight. Also, storage protocols for SA are not well established and for our work it was important to have a coherent storage strategy which can be applied for all measured quantities, i.e. SA as well as CDOM and FDOM. The reviewer also believes that "The Material & Methods Section is overall well written, but the Results and Discussion Section could be more elaborate." He/she does not elucidate how this might be done but instead points to a very specific aspect: "In particular, the Figure Legends are extremely short. They do not allow to fully understand the content of the figures". We have now rewritten the captions with additional relevant information. Lastly the reviewer believes that "p. 2842 line 1-10) could be better integrated in the text". Although we were a little unsure about the specific comment, we have reformulated and clarified the section to aid reading. Finally, he/she said that "Overall, if this manuscript is to be published in BGS, I recommend the authors present a stronger message on the particularities of CDOM in the surface microlayer and thus the originality of their work, and provide a more conclusive recommendation for potential readers of their investigation." We agree with the reviewer and hope the substantial changes we have made will help to more clearly state the novel aspects of the study and place it in context with previous studies focusing upon bulk CDOM.

Specific comments

- *Only one surface microlayer device is presented here. Do the authors consider the results obtained for the Garret Screen valid for other sampling devices?*
  This point was also raised by Reviewer #1: see our response above.
- *The authors focus on the factor time for each of the treatments, by contrast, the treatment effect is very little documented. It appears that treatment is in some cases more important than time. This issue merits more attention. It would be interesting to know whether the relative change compared to the untreated sample is statistically different? The authors could then compare treatment effect and time, and conclude on which factor is more important for surface microlayer sample*.
  We believe that *both* treatment *and* storage time are important. One of the main drivers for investigating storage time is for the reasons we elucidate in the introduction; some degree of storage of SML samples is inevitable and temporal changes, whatever the treatment, are also inevitable. We have now added statistical analyses to test for treatment effects and expanded the discussion accordingly in order to give equal consideration to both storage time and treatment.
- *I strongly suggest the authors change their recommendation to a more precise conclusion from their study. Looking at Fig. 1 and 2, temporal changes appear not significant up to 15 days for most of the treatments. Thus, the term "storage times as far as practicable" could be re-phrased accordingly. Also, the statement that "SML studies should validate their chosen protocols independently" does not invite any potential reader to look more closely into the manuscript. Is this really the message the authors want to give?*
  We have now revised both of these statements in view of these comments.
- *Fig. 1 to 3. I suggest the authors explain in the Figure Legends the offset from zero.*
  We now make this clear in the legends.
- *The results from the statistical analyses could be indicated with an asteriks above each symbol, whenever the results are significantly different from time zero.*
  We have incorporated this suggestion into the revised manuscript.
- *Fig. 4. This figure would be much easier to read if the absolute values were presented, as the aim is to demonstrate that the storage effect depends on the initial SA concentrations.*
  We added a second axis to the plots which shows the absolute values.
  The comments in each graph are not clear. Should S stand for salinity?
  Yes *S* does indeed stand for salinity and we now indicate this in the captions.
  Do the numbers refer to the dates of sampling?
  Yes, and we now also indicate this in the captions.
- *Fig. 5. Again, the Figure Legend does not sufficiently explain the graph. The numbers 1 to 5, referring to 5 different fluorescence components are only briefly described in the text. Refer, for example to Table 2, to explain the fluorescence components.*
  We have incorporated this suggestion into the revised manuscript.
- *Page 2840: Line 17-18: "if so it is ...": Sentence is unclear.*
  We rephrased this sentence to avoid ambiguity.