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Reply to "Mercury Bioaccumulation and Bioaccumulation Factors for Everglades Mosquitofish as Related to Sulfate: A Re-Analysis of Julian II (2013)"

Paul Julian II

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Let me begin by thanking Pollman and Axelrad (2014) for their contribution to Everglades mercury (Hg) science in their thorough reanalysis of Julian (2013) and their discussion of concepts on Hg cycling. Pollman and Axelrad (2014) do address some important points and provide thoughtful criticisms, yet these authors also did not interpret the objectives of Julian (2013) correctly and misconstrued aspects of the paper and Hg cycling in general that merit responses. This reply will address key aspects of the Pollman and Axelrad (2014) commentary and provide revised analyses as a means of clarifying concepts of general scientific interest. The series of three articles on Hg in Everglades fish illustrate the very real challenges inherent in using monitoring data to test hypotheses involving multiple factors. On balance, these efforts all support the inherent complexity of the Everglades Hg problem and provide strong evidence that surface water sulfate cannot be used as a means to ameliorate Hg bioaccumulation.

Pollman and Axelrad (2014) begin their commentary noting that atmospheric deposition of Hg, sulfate in surface water and available dissolved organic carbon (DOC) are generally thought to underlie elevated methyl mercury (MeHg) in marshes of southern Florida. They state, "*Of these factors, sulfate may represent the best option to reduce methyl Hg in the Everglades…*" I agree that the potential control of sulfate to reduce MeHg production is attractive intuitively and has been suggested by Corrales et al. (2011), Orem et al. (2011), Pollman (2012), Axelrad et al. (2013) and Gabriel et al. (2014). Pollman and Axelrad (2014) go so far as to assert that after reviewing evidence on the role of sulfate in Hg cycling, "*the idea of mitigating high Hg concentrations through sulfate controls becomes obvious.*" With this obvious idea comes an imperative for clear demonstration of effectiveness in complexities of natural settings. I remind the reader that extensive empirical analyses including some being presented in this series of articles have not been able to demonstrate a quantitative relationship between sulfate and Hg accumulation that rises to the level of being useful for environmental management in south Florida (Julian et al. In Press). As a result of this lack of proven evidence, proposed management strategies for sulfur/sulfate control called for by these authors simply cannot be justified as a viable option for controlling MeHg and fish Hg concentrations in the Everglades (Julian et al. 2014, 2015, In Press). With information available at this time, control of Hg inputs to the Everglades would ultimately limit fish total Hg (THg) and MeHg concentrations (Axelrad et al. 2013) and this fact is why the state of Florida has initiated a state-wide Hg Total Maximum Daily Load (TMDL) (Florida Department of Environmental Protection 2012). Furthermore, an international treaty has been signed by the United States as well as 102 signatories (*circa* September 2014) in an effort to protect human and environmental health due to adverse effects of Hg deposition (http://www.mercuryconvention.org/; United Nations Environmental Programme 2013).

I agree with the introductory review of Pollman and Axelrad (2014) that Hg inputs, organic carbon and sulfate are involved with Hg methylation. As noted by these authors under their specific concern #3 and in other places in their article, it is also true that other chemical factors, as

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well as site-specific biological and physical characteristics also drive Hg methylation and evidential Hg accumulation. Chemical factors include the availability of dissolved Hg^{2+}, concentration of DOC, pH, chloride, dissolved oxygen and others play some role in the Hg methylation process (Skyllberg 2008; Julian et al. 2014). Biological factors including presence of Hg methylating microbes can influence MeHg formation. With this complexity in mind, I must agree with the authors that simple correlation between Hg concentrations and any single variable will generally fail to explain a large fraction of the fish tissue Hg concentrations. I must disagree with the authors, however, that any Hg control strategy can be justified without solid empirical linkages between the stressor and the environmental responses.

Pollman and Axelrad (2014) continue on management relevance indicating, "*With this stated goal, Julian II's results have the potential to be used by environmental managers to help determine Hg mitigation strategies.*" This statement is difficult to understand because Julian (2013) did not have a management guidance objective, and in fact, cautioned against the application of the bio-concentration factor (BCF)-sulfate relationship due to high uncertainty in the regression results. Pollman and Axelrad (2014) proceeds to address the hypothesis of Julian (2013). For unknown reasons, Pollman and Axelrad (2014) disregard the beginning of the hypothesis statement by removing "*Based on previous studies summarized and information presented within Axelrad* et al. *(*2013*),…*" the hypothesis continues with "*…I* (Julian 2013) *hypothesize that reduced Hg bioaccumulation should occur at relatively high and relative low concentrations of sulfate with a peak occurring at moderate sulfate concentrations, exhibiting a unimodal relationship.*" As stated in the original manuscript, the hypothesis was based on Axelrad et al. (2013) which summarized Pollman (2012), both authors of the subject commentary/reanalysis. In Axelrad et al. (2013), Pollman states the "*…study results indicate a unimodal response similar to that originally hypothesized by Gilmour and Henry (*1991*).*" It is worth noting that based on the BCF analysis, Julian (2013) rejects the unimodal relationship hypothesis.

Pollman and Axelrad (2014) continue their commentary and reanalysis with specific concerns and my responses are itemized below:

1. Pollman and Axelrad (2014) are concerned with my use of BCF as opposed to the bio-accumulation factor (BAF). They provide an argument against the use of surface water MeHg concentration as "*…uptake of methyl Hg by fish occurs primarily through dietary intake rather than through the gills or* via *dermal uptake…*" However, Pollman and Axelrad's (2014) definition of BCF and BAF as "*…the ratio of biota contaminant concentration relative to the concentration of the contaminate in water…*" is incorrect. Distinctions between BCF and BAF are important as

Table 1 Comparisons of BCF_{MeHg} by hydrologic unit within the Everglades Protection Area using non-parametric Steel–Dwass multiple comparisons

	BCF_{MeHg}		
Region[a]	Mean	SE	Similarity[b]
WCA-1	286.2	56.3	A
WCA-2	182.1	19.9	A
WCA-3	614.0	47.5	B
ENP	981.8	76.0	C

[a] Water conservation area (WCA) 1, 2, 3 and Everglades National Park (ENP)

[b] Similarity based on Steel–Dwass non-parametric multiple comparison

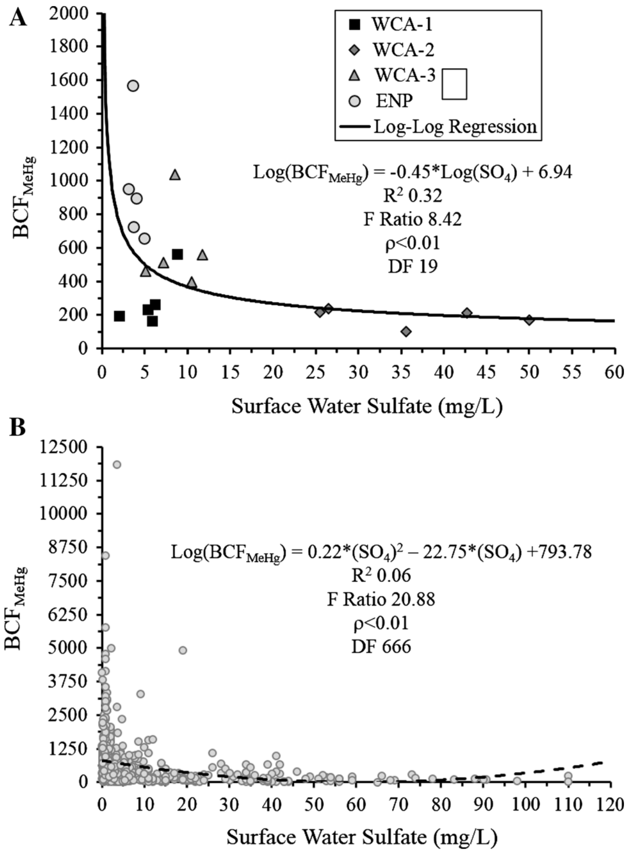

Fig. 1 **a** A revised Log–log regression of WY (May 1–April 30) mean mosquitofish bio-accumulation factor using MeHg surface water concentration BCF_{MeHg} by WY mean surface water sulfate concentration for all regions of the Everglades Protection Area. **b** Quadratic regression of BCF_{MeHg} by surface water sulfate concentration for the entire R-EMAP data set for all regions and phases

they are key functional terms. I agree that BCF is the ratio of the contaminant concentrations in the organism relative to the concentration in the ambient medium, in this case surface water. BAF, alternatively, is the ratio of the contaminant concentration in the organism to that of the concentration in food or ingested water. Furthermore, BCFs are more important in aquatic ecotoxicology where the ambient medium (i.e. surface water) is the major source of the contaminant in question, while BAFs are more commonly used in terrestrial ecotoxicology where food items represent a major source of the contaminant (Walker et al. 2007). In the case of the Unites States Environmental Protection Agency (US EPA) Regional Environmental Monitoring and Assessment Program (R-EMAP) data being used by all authors involved here, we do not have Hg data for all terrestrial or aquatic food sources for *Gambusia*. Therefore, based on these pragmatic definitions, Julian (2013) use of BCF is reasonable and justified for the original paper or for this reply.

2. Pollman and Axelrad (2014) identified errors in the original calculation of the BCF values computed by Julian (2013). I thank Pollman and Axelrad (2014) for noticing this error and in fact, the values are orders of magnitude different due to a unit conversion error. A revised analysis is included with this reply and the fit statistics (i.e. coefficient of determination and significance) of the regression analysis are not affected, however the slope magnitude and intercept of the revised analysis are different than previously published values in Julian (2013) (Table 1; Fig. 1).
3. Pollman and Axelrad (2014) discuss four problems with Julian's (2013) hypothesis and consideration of these issues is informative. Their first problem reflects the interpretation of BCF/BAF ratios: "*BAF* (BCF in Julian 2013 and this reply, see above) *values are calculated as the ratio of two variables and that interpretation of variations in BAF in regional studies is both difficult and highly susceptible to spurious correlations.*" I agree with these cautions for interpretation while noting that Pollman and Axelrad (2014; Figs. 3, 4) are subject to the same cautions as they conducted extensive regional reanalysis using BCF/BAF relative to other water column characteristics.

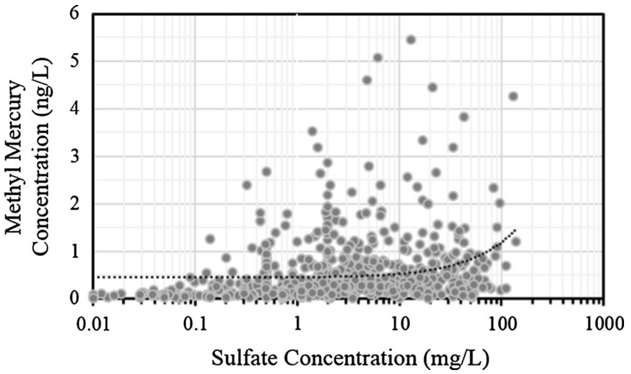

Fig. 2 Surface water MeHg concentration relative to surface water sulfate concentration for the entire R-EMAP data set. The quadratic regression line is indicated by the *dashed black line*

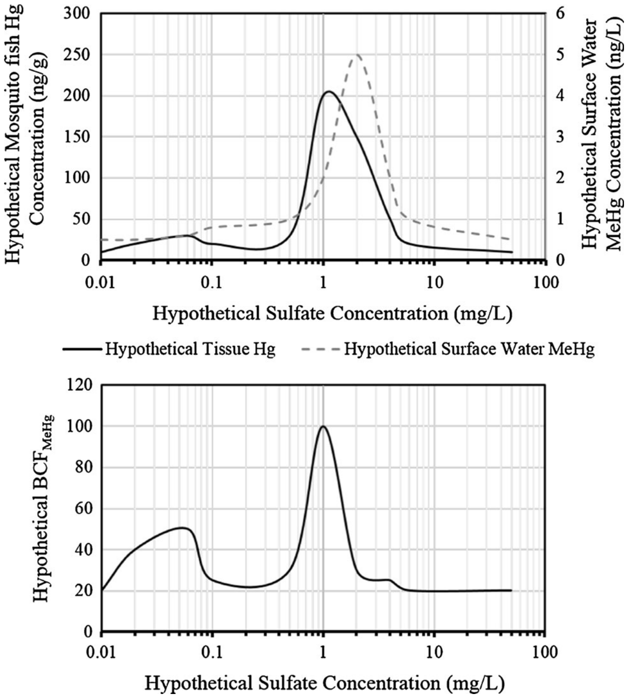

Fig. 3 Hypothetical mosquitofish THg and surface water MeHg concentration exhibiting a unimodal relationship (*upper panel*) and the resulting hypothetical BCF$_{MeHg}$ relative to a hypothetical sulfate concentration

   Pollman and Axelrad (2014) then raise an interesting mathematical point: "*if both variables comprising BAF follow essentially unimodal relationships with surface water sulfate…then mathematically it does not follow that BAF values plotted against surface water sulfate should produce a unimodal curve as well.*" Julian (2013) did not indicate that surface water MeHg concentrations exhibited a unimodal relationship with respect to sulfate concentrations in the first place, and in fact, they do not represent a unimodal relationship based on the shape of the quadratic regression line ($R^2 = 0.04$, $DF = 738$, $F\ Ratio = 15.07$, $\rho < 0.01$; Fig. 2). This finding is consistent with Julian et al. (2014). From a mathematical perspective using a hypothetical data set with an assumed unimodal relationship in two parameters relative to a third, a peak is apparent in the ratio of the two parameters (Fig. 3). Therefore, one could assume even if both mosquitofish (*Gambusia spp.*) tissue Hg concentrations and surface water MeHg concentrations observed a statistically significant unimodal relationship, a peak in the ratio between the two would be observed, especially when tissue Hg concentrations dominate the ratio as it does in the R-EMAP data set.

Pollman and Axelrad (2014) suggest that other water column parameters particularly DOC and total organic carbon (TOC) may confound the analysis. I agree with this concern and find their regression modeling results are supportive that organic carbon is more important in Hg accumulation and potentially MeHg formation than sulfate. Although a particular concern in their reanalysis would be the inclusion of two different chemical fractions of organic carbon (i.e. TOC and DOC), which represent different portions of the liable carbon pool and is ultimately influenced by ambient water column chemistry (Reddy and DeLaune 2008; Skyllberg 2008). DOC represents a relatively temporary pool of organic carbon available for rapid uptake by microbes whereas particle organic carbon (POC) represents a longer term pool utilized by microbes as well as, invertebrates. TOC includes all forms of organic carbon such as detrital organic carbon, POC, DOC and colloidal organic carbon. Therefore, the combination of TOC and DOC in a regression would ultimately complicate the relationship and potentially skew the results.

Pollman and Axelrad (2014) propose that aggregating data based on water year (WY; May 1–April 30) and hydrologic season is more appropriate than just WY. Unfortunately, Pollman and Axelrad (2014) only cite that "*…aggregating by WY across sampling intervals results in some data points reflecting both wet and dry season within a given year and some points reflecting wet or dry (but not both) seasons only…*". While this is an inherent limitation to the data, aggregating data using WY average BCF_{MeHg} and surface water sulfate concentration (as done by Julian 2013) include more samples per WY and region, possibly representing each region more accurately. However aggregating by region, season and WY could potentially limit the representativeness of each parameter due to limited sample size. Furthermore it seems that based on a post hoc power analysis, aggregating by WY and region results in greater power and slightly higher least significant values ($\alpha = 0.05$, $\sigma = 0.63$, $\delta = 0.41$, $N = 20$, Power = 0.78, LSV = 0.32) than the relationship developed based on WY, season and region ($\alpha = 0.05$, $\sigma = 0.36$, $\delta = 0.16$, $N = 32$, Power = 0.70, LSV = 0.30).

4. Pollman and Axelrad (2014) contend the assumption that low BCF should reflect low MeHg concentration in the water column using the data distribution of the log(BCF) – Log(MeHg) relationship as a justification. Taking Kronmal's (1993) warning regarding the use of ratios in statistical analyses aside, based on the log–log regression of BCF_{MeHg} and surface water MeHg concentration (Fig. 4), it is possible to observe low BCF_{MeHg} and relatively low MeHg concentrations. However based on this regression analysis, I agree that it is possible to observe high BCF_{MeHg} and low MeHg concentrations as well as low BCF_{MeHg} and high MeHg concentrations. Regardless, appraisal of the data should be done across its entire distribution, rather than just one region of the relationship.

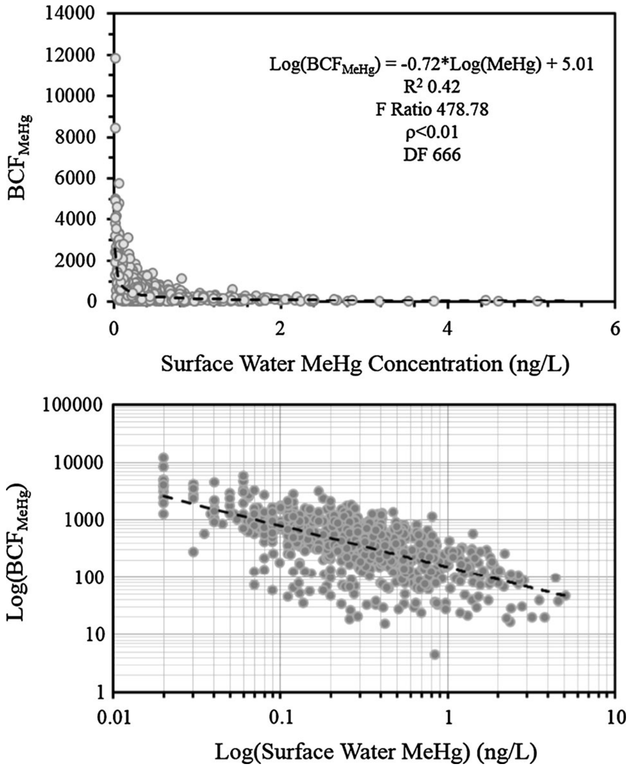

Fig. 4 Log–Log regression of BCF_{MeHg} and surface water MeHg concentration for the entire R-EMAP data set (*Upper panel*). BCF_{MeHg} – Surface water MeHg concentration on a log scale (*bottom panel*)

Pollman and Axelrad (2014) as justification also compare BCF values and MeHg concentrations across large number of lakes, stream and the Everglades Protection Area. Once again I warn the reader, interpretation of this data for or against any arguments should be cautioned. Especially since Fig. 4 of Pollman and Axelrad (2014) include data from different ecosystems (i.e. lakes, streams and marshes), which can differ biogeochemically and hydrologically as well as mixing species from different trophic positions. Pollman and Axelrad (2014) utilize both mosquitofish and largemouth bass (*Micropterus salmoides* Lacépède), both which reside at drastically different trophic positions. Largemouth bass are predominately carnivorous with their diets weighted heavily toward other fish (i.e. mosqutiofish, sunfish, mollies, etc.) and decapods (i.e. shrimp, crawfish, etc.) while mosquitofish are omnivorous consuming algae, plant detritus and invertebrate larvae (Loftus 2000). Furthermore tissue concentrations are assessed differently between these two species utilizing different tissue types that are indicative of different accumulation process. Largemouth bass are typically used to assess long-term accumulation of Hg with Hg concentrations being obtained through axial muscle fillets while mosquitofish are indicative of short-term, more localized exposure with the whole body being homogenized and analyzed for Hg.

In summary, Julian (2013), Pollman and Axelrad (2014) and this reply highlight the variability and complexity of any relationship between water column chemical characteristics (i.e. sulfate, organic carbon, etc.) and Hg in natural marshes. The revised data presented in Fig. 1, demonstrate the highly variable nature of BCF_{MeHg} across surface a variety of surface water sulfate conditions at both an aggregated level (i.e. by region and WY; Fig. 1a) and as individual observed data points (Fig. 1b). While some points presented by Pollman and Axelrad (2014) improved and clarified the mosquitofish BCF analysis performed Julian (2013). Their analysis, leaving "*spurious correlations*" aside, demonstrates that other factors other than sulfate can influence accumulation in fish tissue and that the fish tissue Hg and sulfate relationship is extremely variable. As stated by Julian et al. (In Press) and discussed extensively by Julian et al. (2014, 2015) without some quantum step in our ability to quantitatively link surface water sulfate and ambient MeHg and THg in fish tissue, there is no way to justify any ecosystem-wide sulfur strategy as a management approach to reduce Hg risk in the Everglades Protection Area.

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