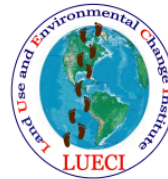


The Role of Sulfate as a Driver for Mercury Methylation in the Everglades – What Does Statistics Really Have to Say?

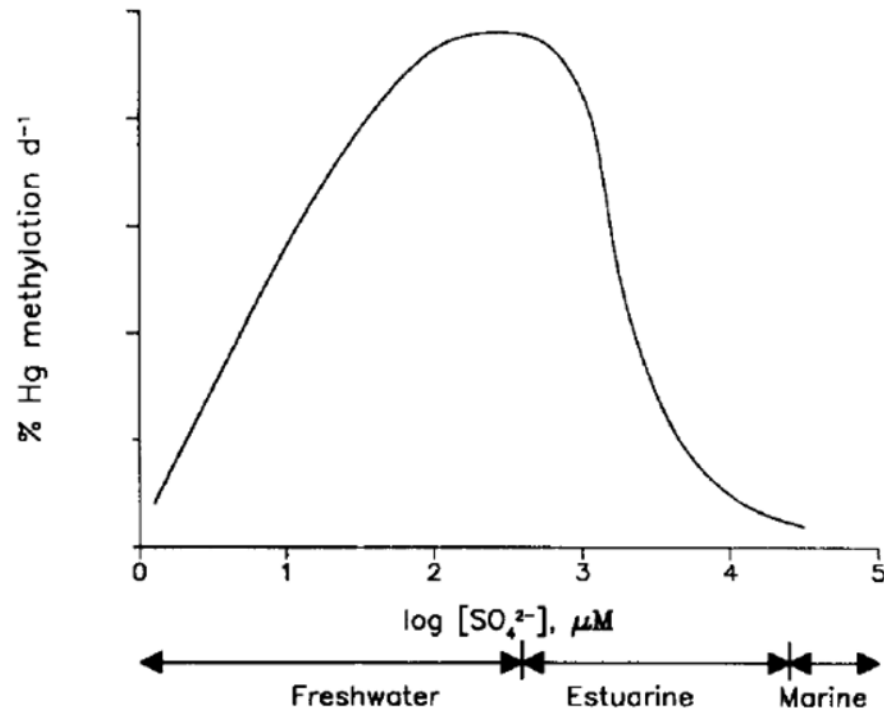
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The Conceptual Mercury-Sulfate Model

Traditional and most widely accepted model of the relationship between sulfate concentrations and mercury methylation rates is **non-linear** and **unimodal**, with an optimum sulfate concentration that results in maximal net mercury methylation.



First developed by Gilmour and Henry (1991)

First referred to as part of the “Goldilocks” hypothesis *vis-à-vis* the Everglades by Orem (2001)

Accepting the Conceptual Mercury-Sulfate Model

Large burden of proof:

1. Model needs to be consistent with the prevailing understanding of sulfate-sulfide production, sulfide-labile Hg thermodynamics, and the role of sulfate reducing bacteria as Hg methylators.
2. Model needs to be consistent with results from experimental studies, including manipulation studies, conducted at different spatial scales:
 - Laboratory or microcosm studies
 - Mesocosm studies
 - Large scale studies (*e.g.*, STA's)
3. **Model needs to be consistent with empirical data.**

Goals of this Paper

Unimodal “Goldilocks” conceptual model has been both embraced and rejected by different researchers working in the Everglades, often on the basis of statistical analysis and interpretations of the same fundamental data sets.

Goals of this paper are thus to:

1. Examine several of the most recent publications that attempt to either support or refute the Goldilocks hypothesis and provide a more rigorous data analysis perspective on the what the data show; and
2. Show results from an alternative approach – structural equation modeling (SEM) – applied to the USEPA Regional Environmental Monitoring Assessment Program (R-EMAP) to provide a quantitative assessment of the role of sulfate in driving methyl mercury production in the Everglades and its subsequent bioaccumulation in *Gambusia* (mosquito fish).

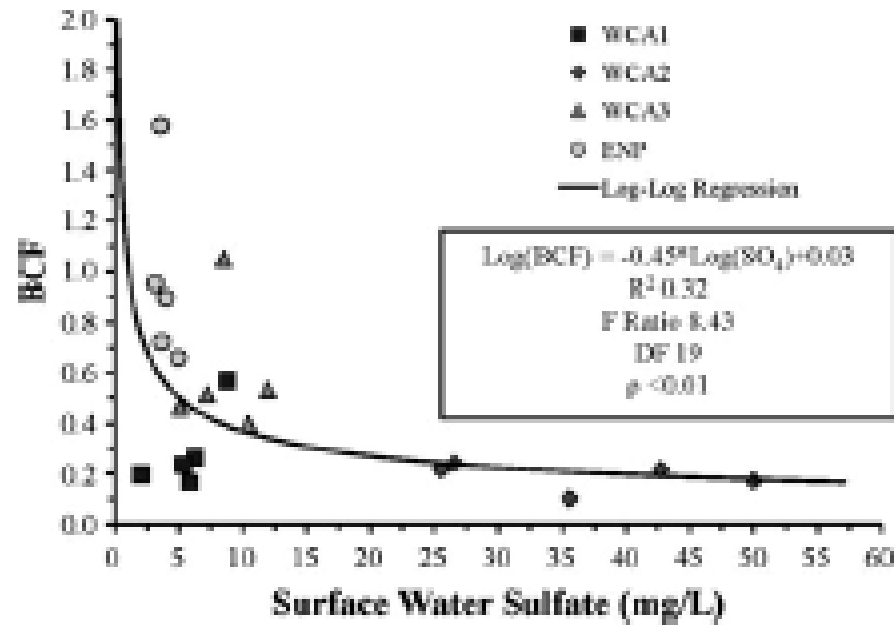
Recent Landscape-Level Empirical Analyses of Sulfate – Fish Tissue Hg Relationships

Author(s) and Year	Journal	Data Set	Fish Species	Supports Unimodal Relationship?
Julian (2013)	<i>BECT</i>	R-EMAP	<i>Gambusia</i>	No
Julian (2014)	<i>BECT</i>	R-EMAP	<i>Gambusia</i>	No
Pollman and Axelrad (2014)	<i>BECT</i>	R-EMAP	<i>Gambusia</i>	Yes
Gabriel <i>et al.</i> (2014)	<i>Env. Management</i>	FDEP/SFWMD 1990-2009	<i>Gambusia</i> Sunfish Largemouth Bass	Yes
Gabriel <i>et al.</i> (2015)	<i>Env. Management</i>	FDEP/SFWMD 2005-2013	<i>Gambusia</i> Sunfish Largemouth Bass	Yes
Julian <i>et al.</i> (2015)	<i>Env. Management</i>	FDEP/SFWMD 2005-2013	<i>Gambusia</i> Sunfish Largemouth Bass	No
Julian and Gu (2015)	<i>Ecotoxicology</i>	FDEP/SFWMD 2005-2013	Largemouth bass	Yes
Pollman (2014)	STOTEN	R-EMAP	<i>Gambusia</i>	Yes

Analysis Problems Inherent in Julian (2013) & (2014)

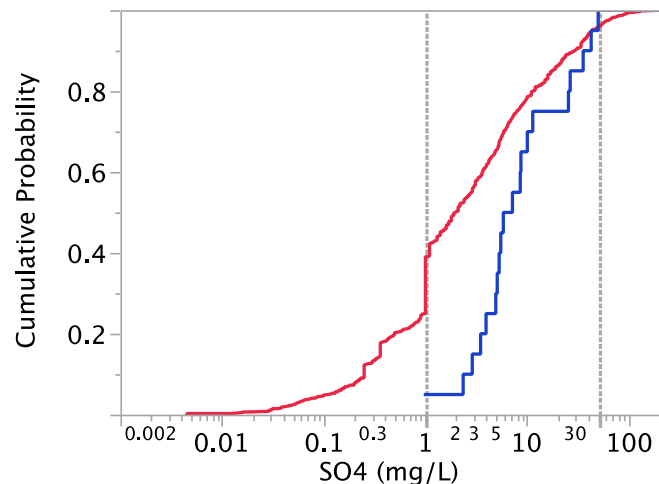
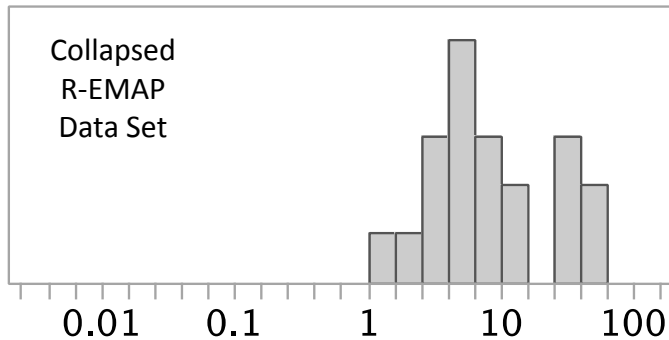
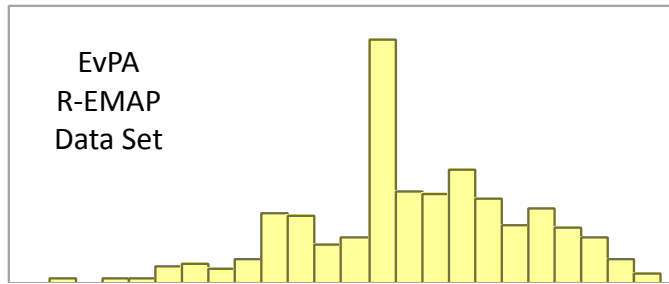
- Inappropriate data aggregation
- Analysis is not consistent with or does not support conclusions
- Model misspecification
 - Form of the model constructs not appropriate for the data
 - Missing variables

Julian (2013)



1. Analyzed relationship between log-transformed **BAF** and log-transformed sulfate.
2. Concludes “. . .that the relationship between marsh surface water sulfate and mosquito fish **Hg concentration** on a regional scale is a log–log relationship . . .”

Example – Data Aggregation

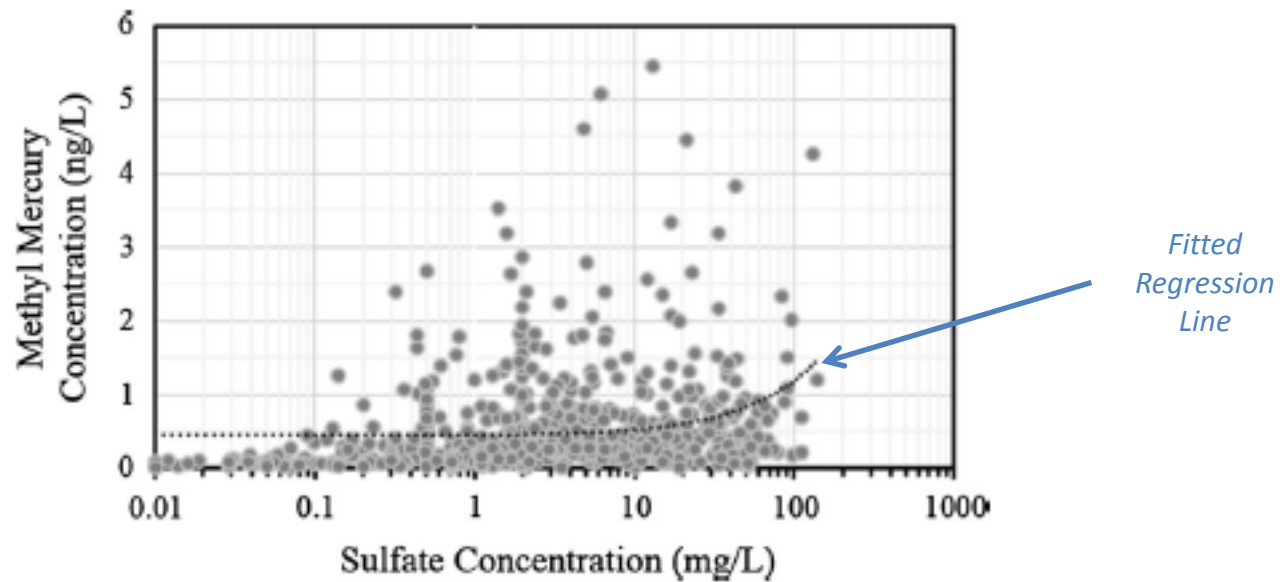


Analysis based on R-EMAP data for the EvPA, collapsed across major hydrologic unit and water year.

654 → 20 observations

Truncates and severely distorts the data distribution. If the intent was to evaluate hydrologic unit effects, then including hydrologic unit as a categorical variable, or better yet, conducting mixed or multilevel/hierarchical models would be more useful and better approach.

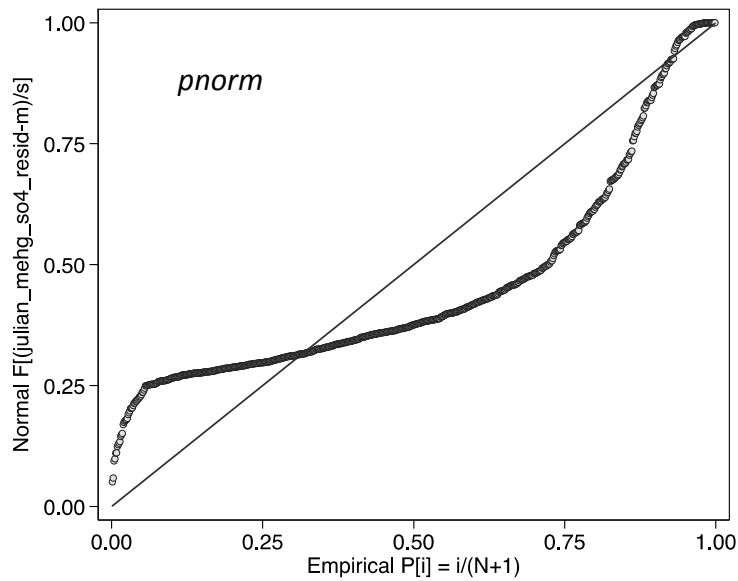
From Julian (2014)



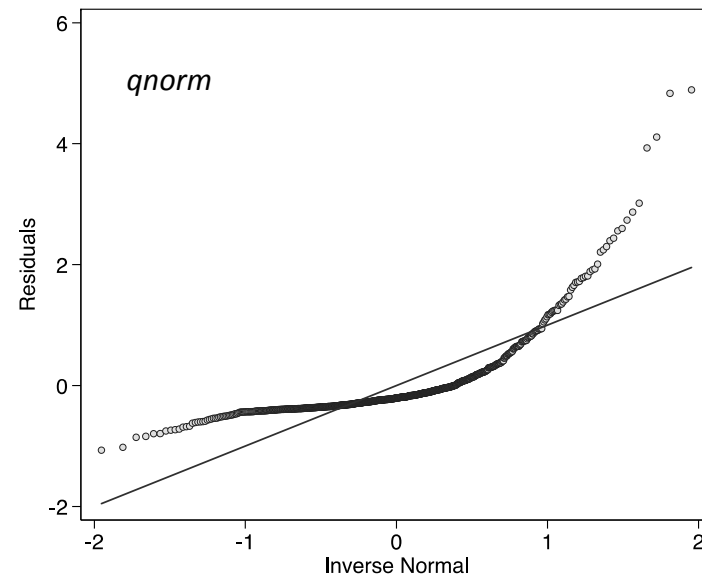
“. . . [these data] do not represent a unimodal relationship based on the shape of the quadratic regression line.”

Form of Model Not Supported by Model Residuals

Residuals should lie close to solid line depicting expected distribution

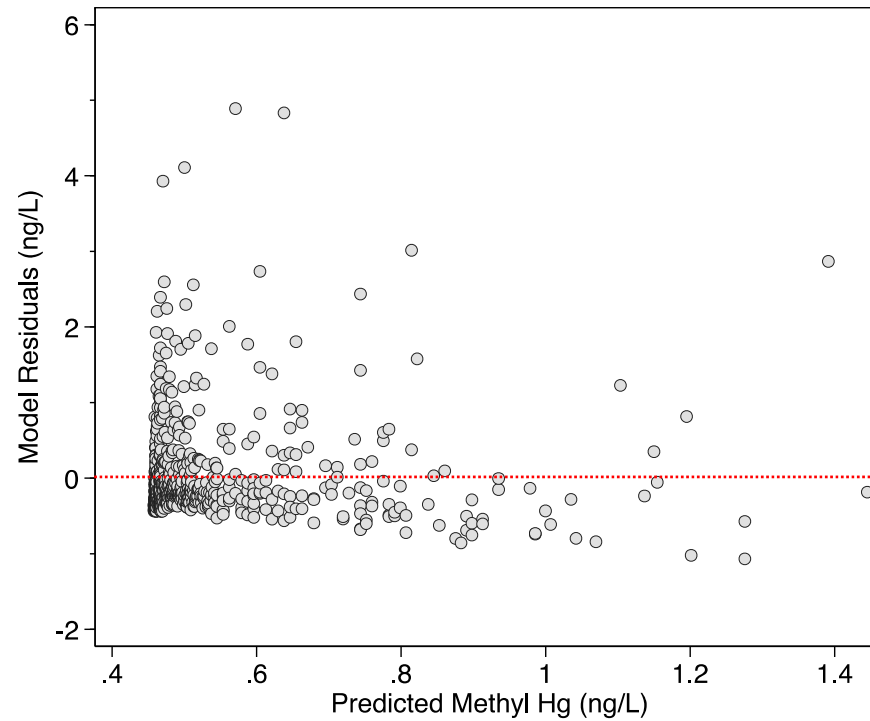


pnorm plot sensitive to deviations from particularly within the **center** the expected normal distribution



qnorm plot sensitive to deviations from particularly within the **tails** the expected normal distribution

Residuals Problems (continued)



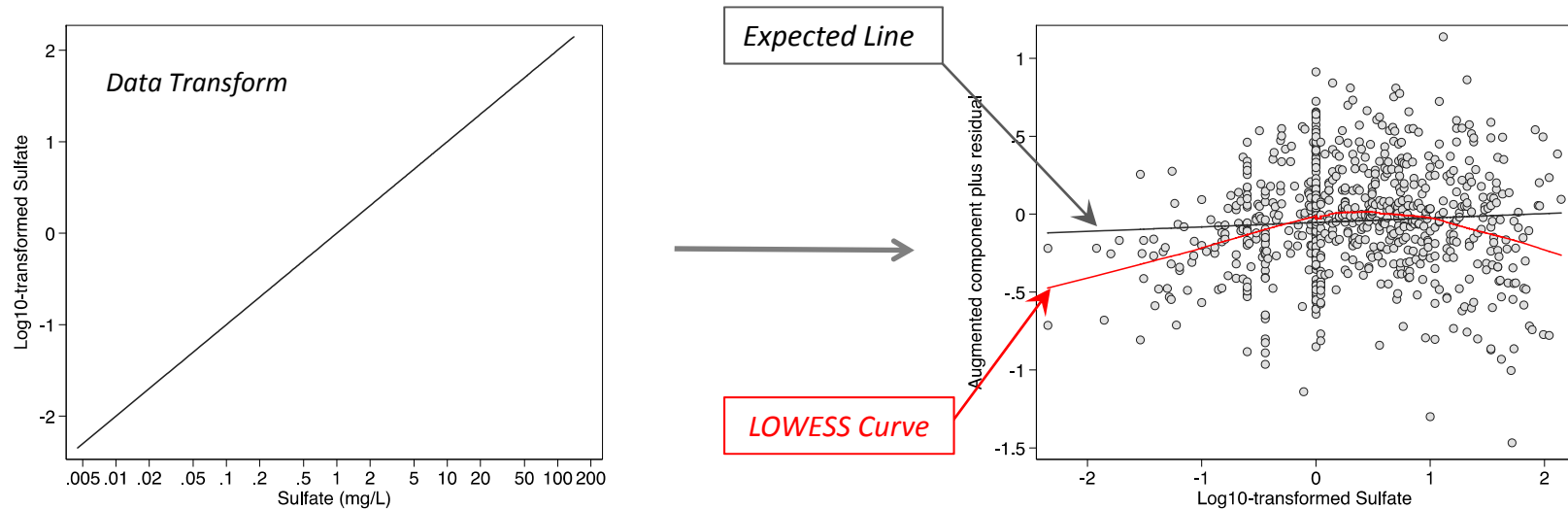
Variance of residuals should be evenly distributed (homoscedastic) as a function of predicted values

Model Misspecification

1. We know from other analyses that organic carbon influences methyl Hg concentrations significantly.
2. Construct a model of methyl Hg concentrations based on DOC/TOC concentrations in conjunction with log-transformed sulfate using R-EMAP data for the EvPA.
3. Examine ***augmented component plus residuals plots*** for model. This helps identify if there are deviations in assumed linearity dependency of methyl Hg with independent variables in model.
4. Reconstruct methyl Hg using ***sigmoidal*** transformed sulfate to evaluate whether a unimodal relationship is more strongly supported.

Example Misspecification Log-transformed Sulfate Model

$$\text{Log methyl Hg} = f(\text{log TOC}^*, \text{log Sulfate})$$

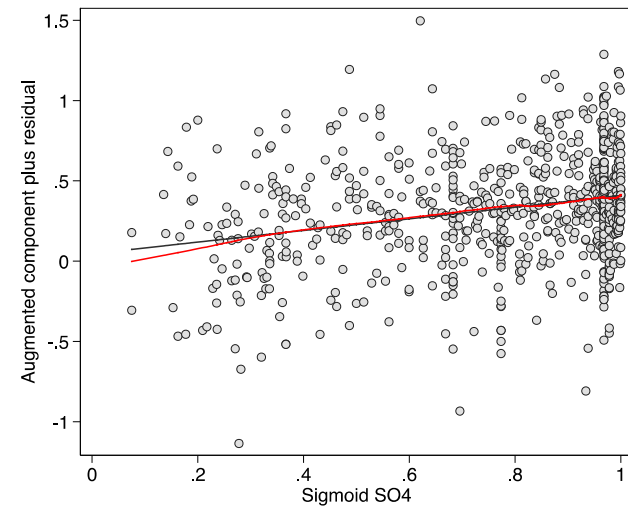
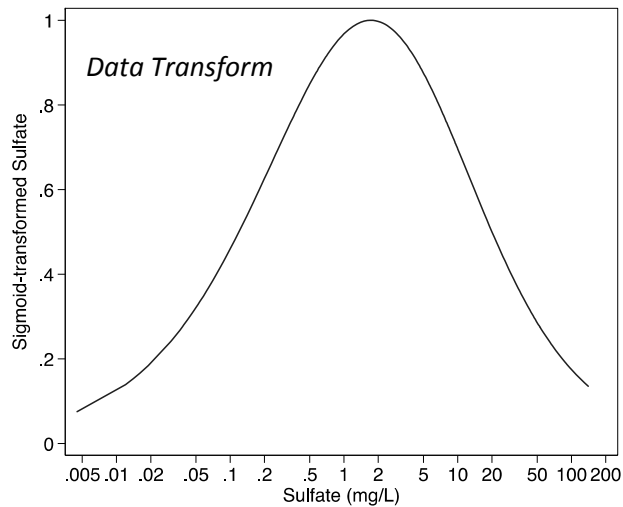


$$r^2 = 0.376$$
$$p \text{ (Sulfate term)} = 0.042$$

Example Misspecification

Sigmoid-transformed Sulfate Model

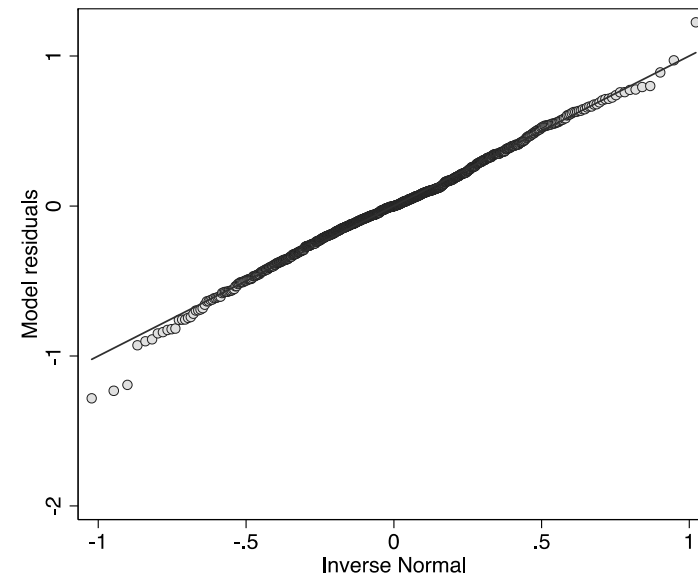
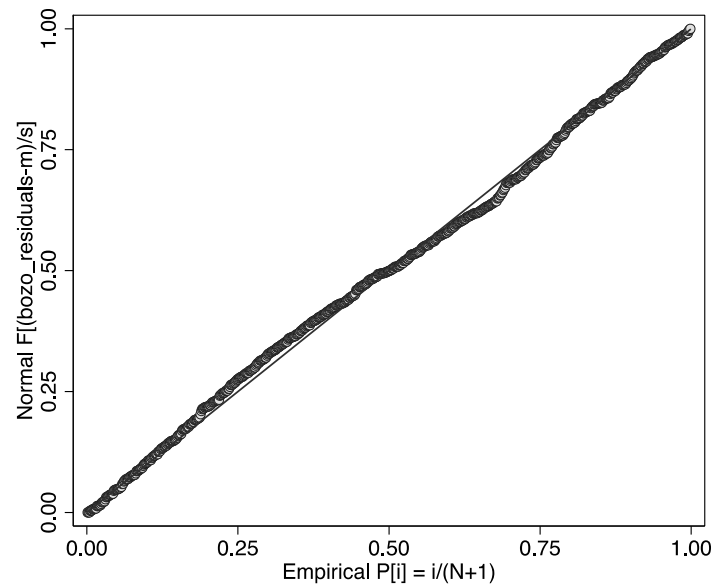
Log methyl Hg = $f(\log \text{TOC}^*, \text{Sigmoid-transformed Sulfate})$



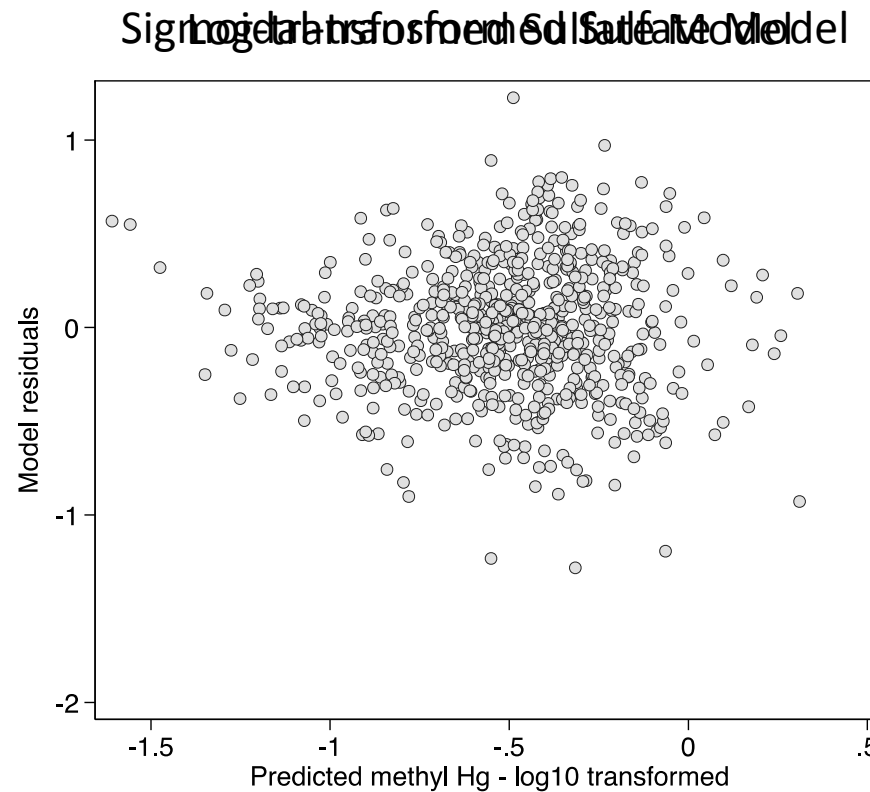
$r^2 = 0.408$
 $p(\text{Sulfate term}) < 0.001$

Inspect Residual Distribution

Signlogit transform for the Sulfate Model

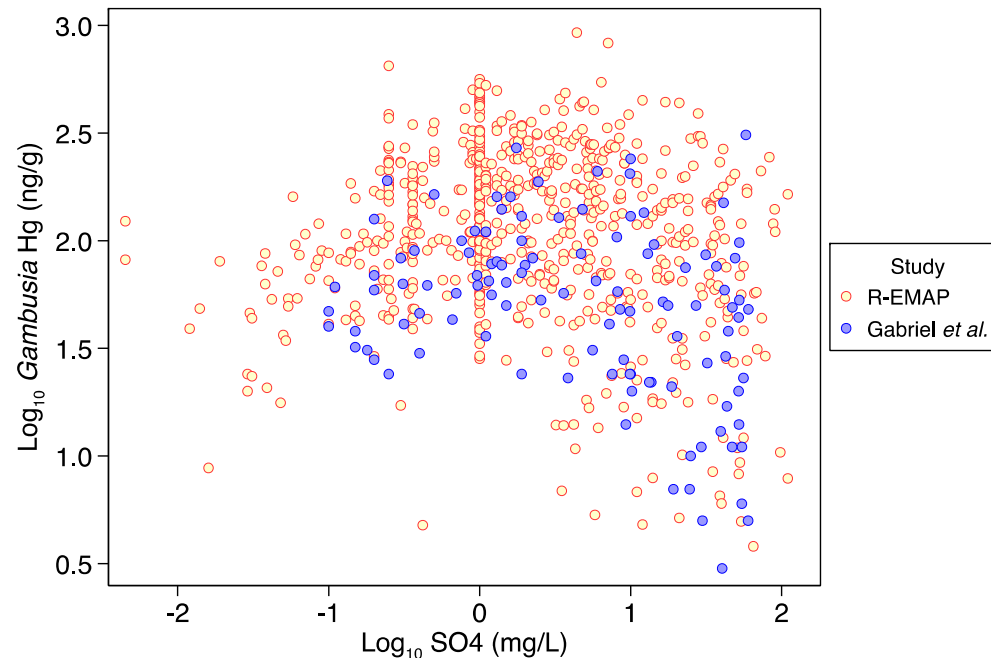


Inspect Residual Distribution (continued)



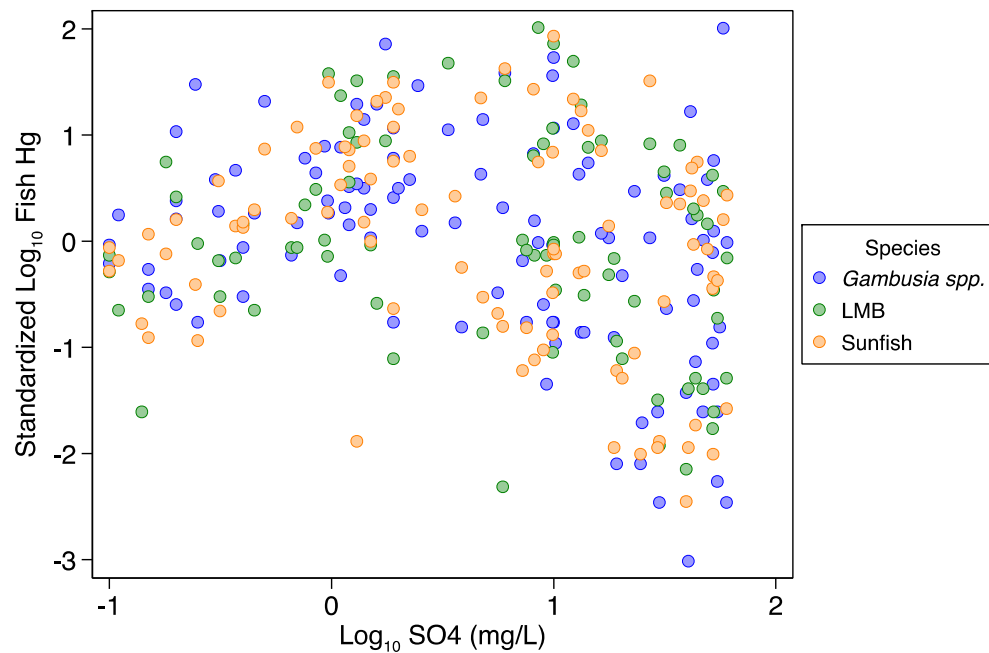
Data fit and adherence to underlying assumptions in regression modeling vastly improved by using unimodal model

Does the unimodal relationship hold for other data sets besides R-EMAP?



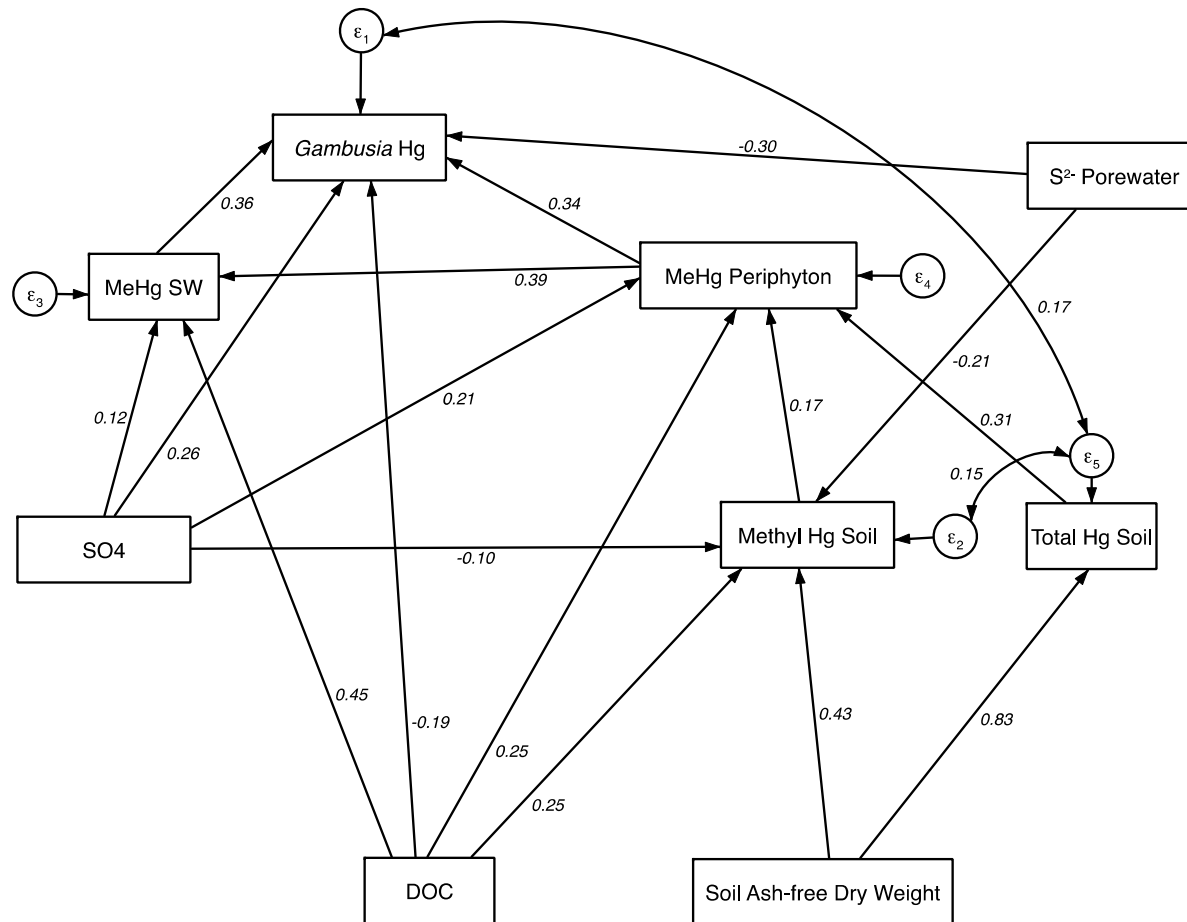
Comparison of *Gambusia* Hg concentrations as a function of sulfate concentrations for both the R-EMAP data and data compiled by Gabriel *et al.* (2014). Gabriel *et al.* data include samples collected between 1998 and 2009 at 11 stations within the Everglades (9 stations within the EvPA)

Plot of Standardized (\log_{10}) Fish Tissue Hg Concentrations for *Gambusia*, Sunfish, and Largemouth Bass



The unimodal relationship is self-consistent across different trophic levels of fish in the Everglades

Importance of Sulfate Controlling Methyl Hg and *Gambusia* Hg Concentrations in the Everglades

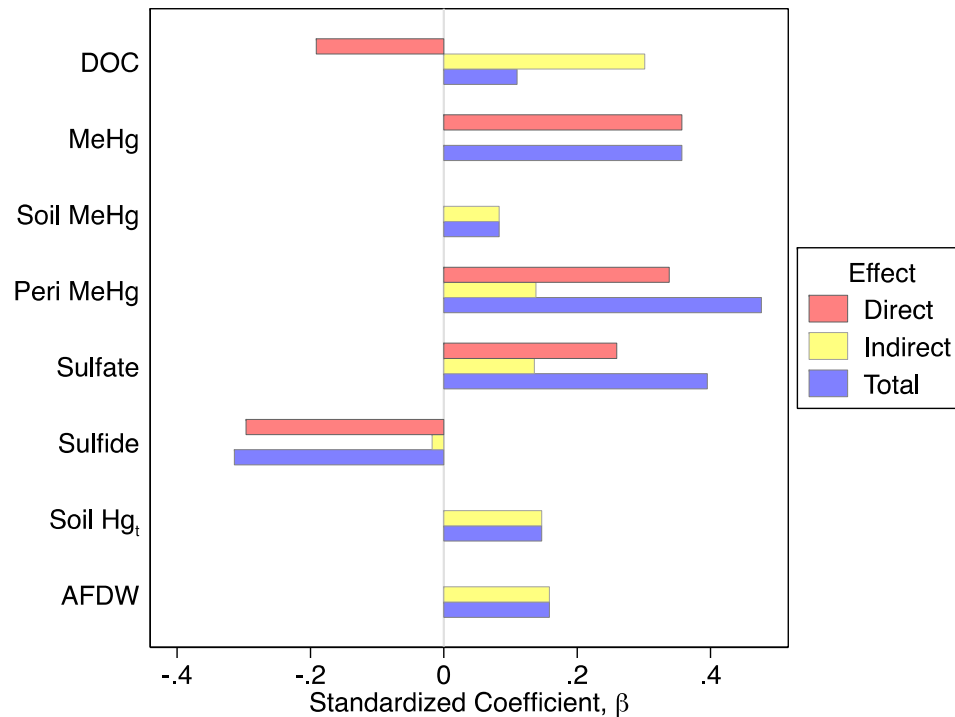


$$r^2_{\text{MeHg}} = 0.512$$

$$r^2_{\text{Gambusia}} = 0.462$$

From Pollman (2014) – *Science of the Total Environment*

Importance of Sulfate Controlling Methyl Hg and *Gambusia* Hg Concentrations in the Everglades



With respect to independent variables included in the model, sulfate is the most important with respect to the *total* effect exerted on *Gambusia* Hg concentration.

Model indicates that a 1 standard deviation change in transformed sulfate concentrations exerts ~ 0.4 standard deviation in log-transformed *Gambusia* Hg

Conclusions

Unimodal relationship between Sulfate and both methyl Hg and fish tissue Hg is statistically supportable and robust

- Model residuals
- Regression model diagnostics, including coefficient significance via bootstrapping (10,000 reps)
- Model is insensitive to outliers
- Model cross-validation
 - Jackknife (LOOCV) analysis
 - Artificial Neural Network (ANN) analysis with cross-validation
- Unimodal relationship (fish tissue Hg and sulfate) is consistent across the two key landscape level data sets (R-EMAP and Gabriel *et al.*, 2014)
- Unimodal relationship is also consistent across different trophic levels of fish.

Questions?