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Extending ANOVA and ANCOVA Analyses using SEM

Jim Grace

U.S. Department of the Interior
U.S. Geological Survey

1

In this module I consider an example where randomized experiments were used to study effects. Here I try to show how SEM permits additional understanding to be developed.

An appropriate citation for this material is

Whalen, M.A., Duffy, J.E. and Grace, J.B. 2013. Temporal shifts in top-down versus bottom-up control of epiphytic algae in a seagrass ecosystem. *Ecology* 94:510-520.

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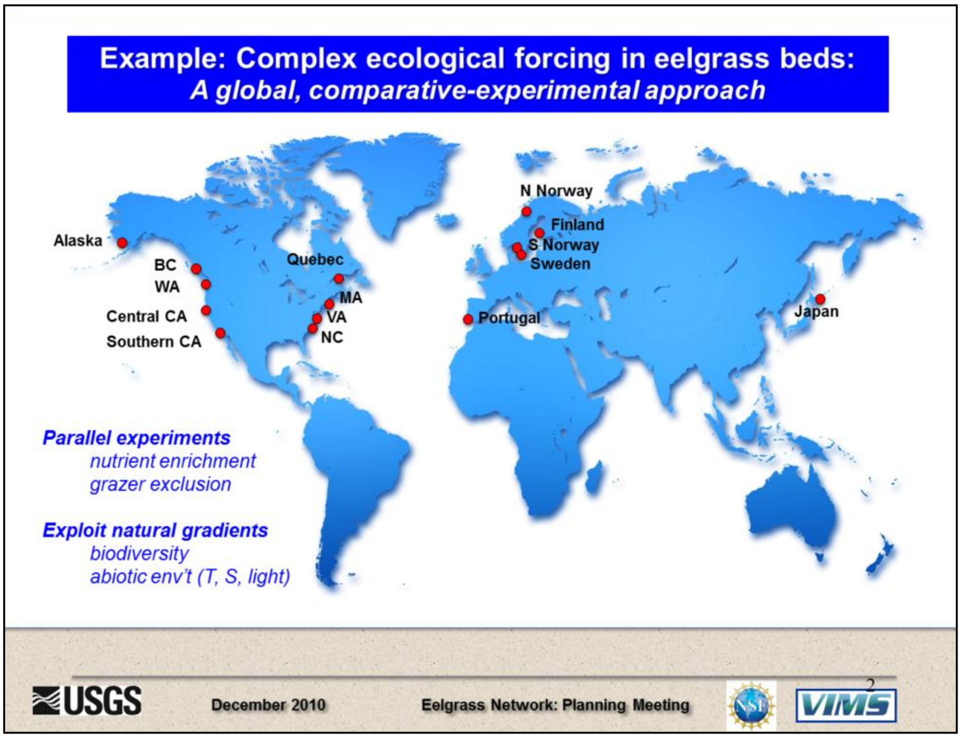
Source: https://www.usgs.gov/centers/wetland-and-aquatic-research-center/science/quantitative-analysis-using-structural-equation



These data come from a global experiment being conducted on seagrasses.

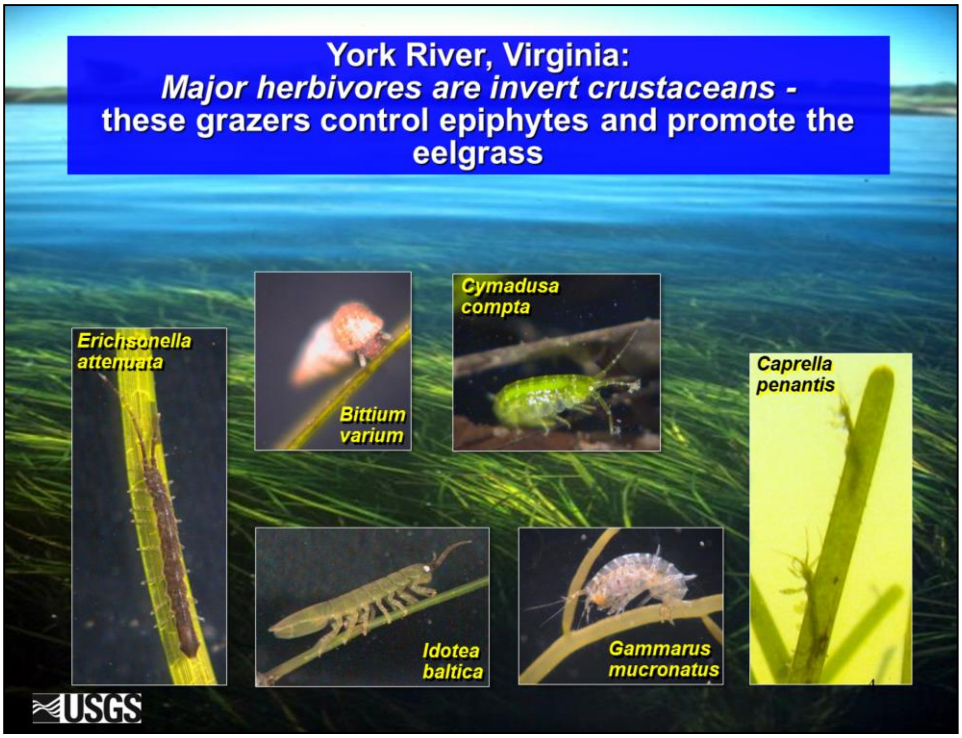
Data from:
Field-based Experimental Study of the Importance of Small Herbivores in a Seagrass Ecosystem:

Matthew A Whalen and J Emmett Duffy

Whalen, Duffy, and Grace, 2013. *Ecology* 94:510-520.
(http://www.esajournals.org/doi/abs/10.1890/12-0156.1)

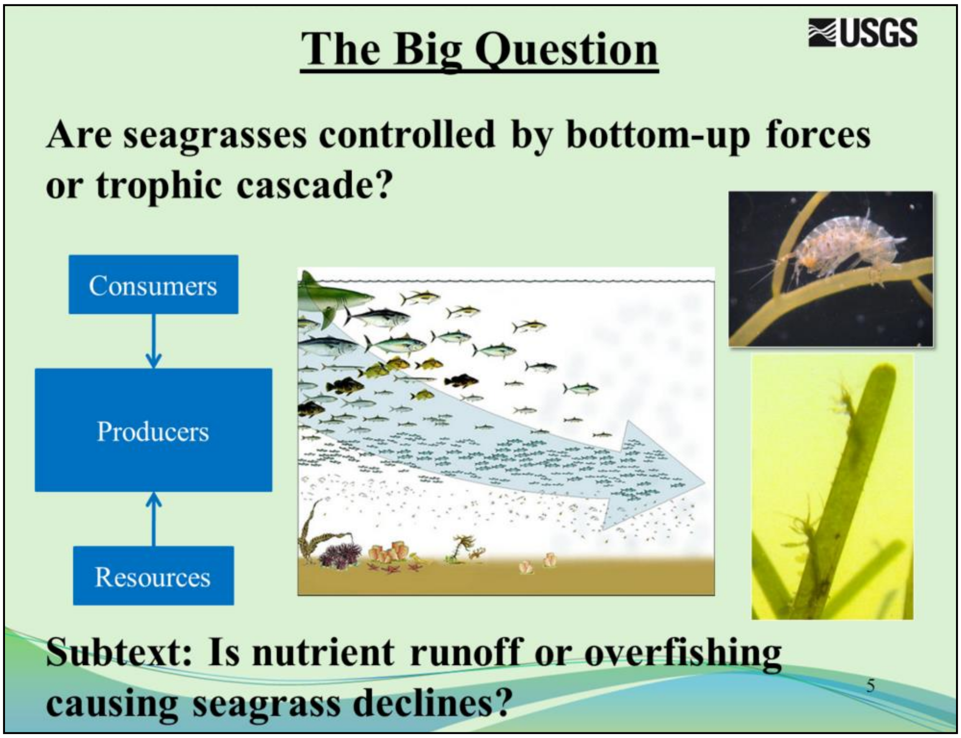
3

More specifically, these are from a study in Virginia.

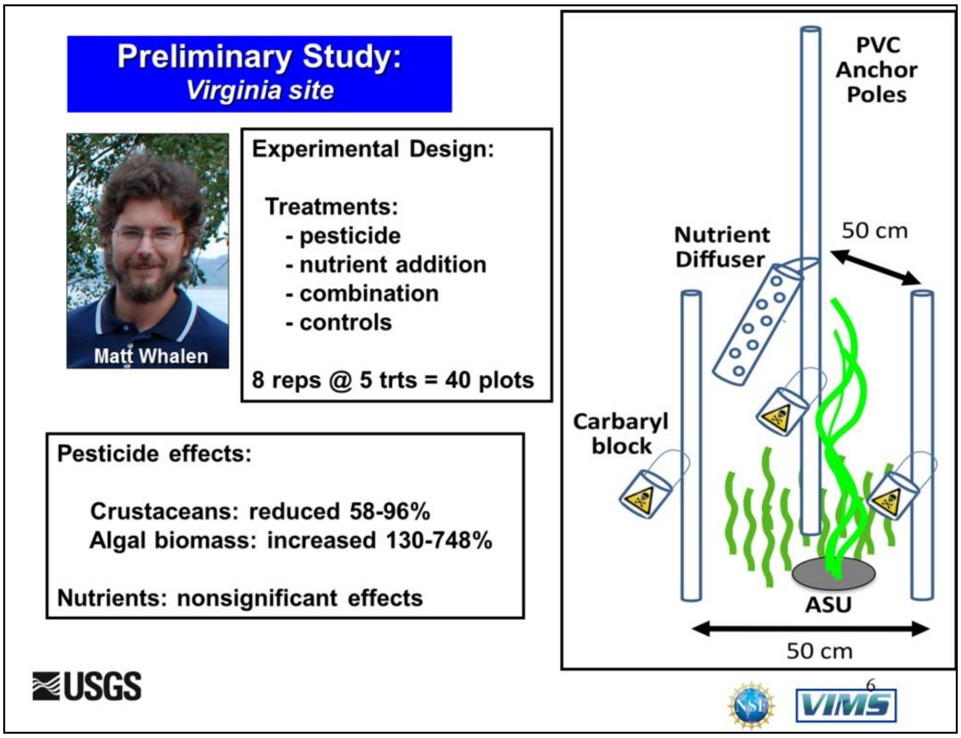


It is all about microcrustaceans grazing on the epiphytes that live on eelgrasses, a particularly important seagrass.

If grazers don’t keep epiphytes grazed down, they lead to the death of the seagrasses, causing the base of the ecosystem to collapse.

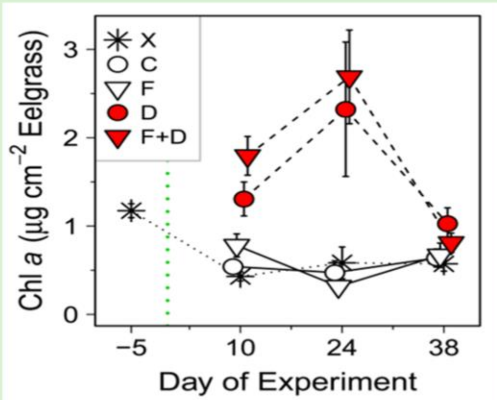


Part of the big deal is a question of what may be causing eelgrass declines worldwide and the broader implications of this issue.



Here is the part of the experimental study discussed in this example.

A Primary ANOVA result: Means for pesticide effect on epiphytes



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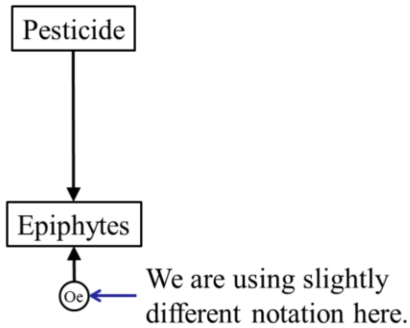
7

Anova results provide limited information.

Illustration of ANOVA-type model

```
# Read Whalen Seagrass Data
w.dat <- read.csv("WhalenData.csv")
```

```
# ANOVA Model
anovaModel <- 'epiphytes ~ pesticides'
```



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8

An anova, in its most basic form, is a very simple model. The simplicity is created by the physical control in combination with randomization.

Illustration of ANOVA-type model (cont.)

```
# Fit ANOVA Model
anovaFit <- sem(anovaModel, data=w.dat)

# Get Results
summary(anovaFit, standardized=T, rsq=T)
```

```
                     Est     SE       Z       P Std.all
Regressions:
  epiphytes ~
    pesticides   0.998  0.154  6.48  0.000 0.716

Variances:
    epiphytes   0.227  0.051               0.488

R-Square:
    epiphytes   0.512
```

9

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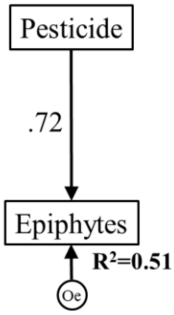
Here are the net-effect results. Note that the information extracted is similar to that obtained from an ANOVA. The main difference is that we are now treating treatment levels as points on a continuum (regression perspective) instead of simply testing for whether treatment means differ.

Results



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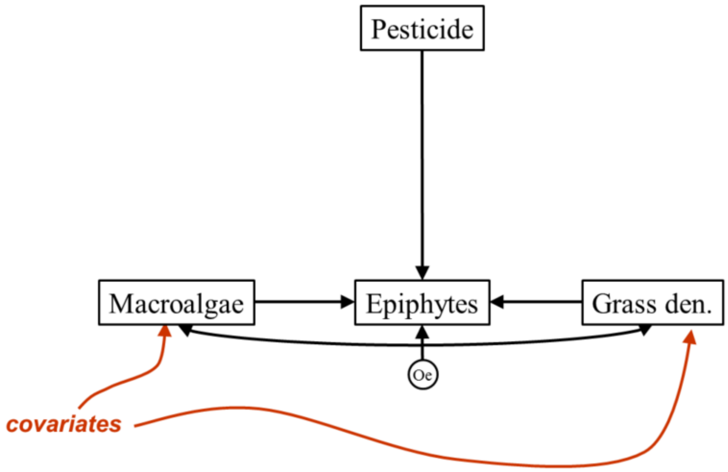
10

And shown graphically.

Illustration of ANCOVA-type model



Note: in ANCOVA, covariates are not allowed to correlate with treatment variables.

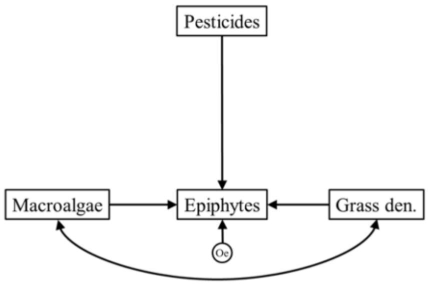
11

There are two covariates in this study, a macro alga and the density of eelgrass. We have not anticipation about what the macroalgae might do, but we expect greater eelgrass density to promote epiphytes by buffering water movement and physical damage to epiphytes.

In ANCOVA, the covariates are supposed to be uncorrelated with the treatment, which holds true in this case.

Illustration of ANCOVA-type model

```
# Simple ANCOVA Model
ancovaModel <- 'epiphytes ~ pesticides
                           + macroalgae + grass'
```



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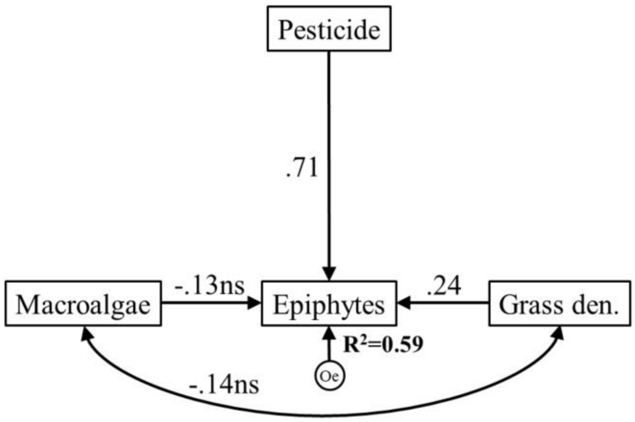
12

A simple ANCOVA here.

Results (visual)



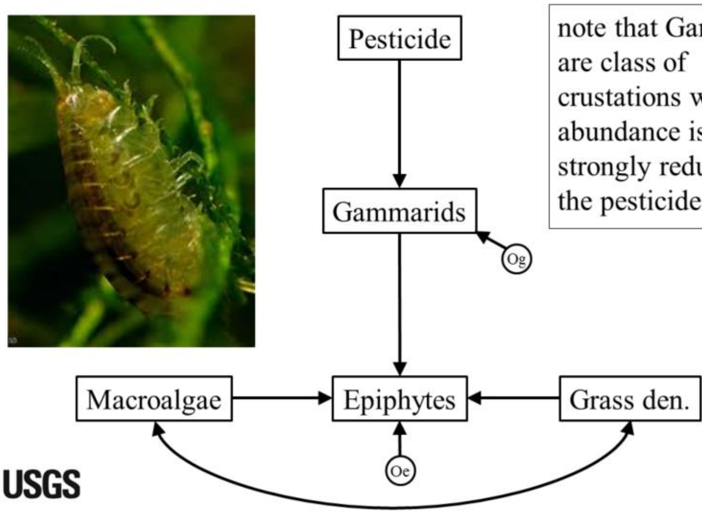
1. Variance explanation for epiphytes improves.
2. Grass density promotes epiphyte development.
3. Macroalgae have nonsignificant negative effect on epiphytes.

13

And the results

The test of mediation

Does reduction of Gammarids explain promotion of epiphytes by pesticide?



note that Gammarids are class of crustations whose abundance is most strongly reduced by the pesticide.

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14

Here we perform the test of mediation with one of the microcrustaceans, the Gammarids.

Lavaan code and results

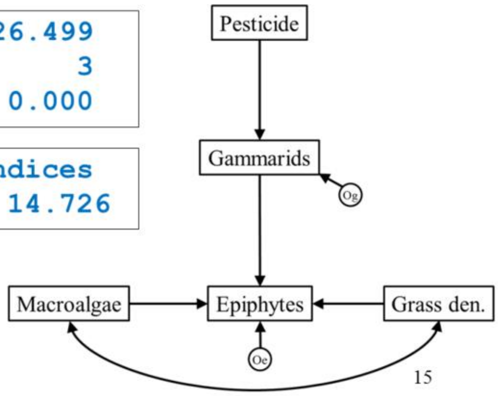
```
# SEM Model 1 "sem1"

sem1 <- 'epiphytes ~ macroalgae + grass + gammarids
         gammarids ~ pesticide'
```

```
Chi-square                  26.499
Degrees of freedom               3
P-value                      0.000
```

```
# Select Modification Indices
gammarids ~ macroalgae  14.726
```

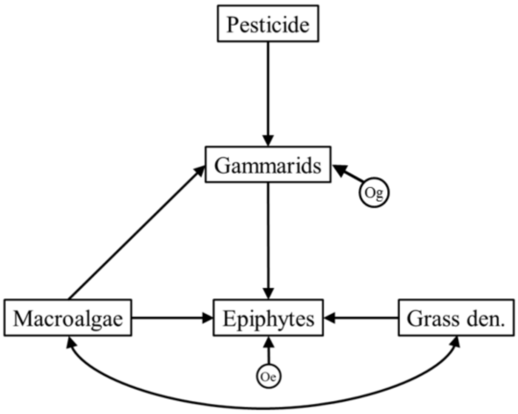
So, we should add path from macroalgae to gammarids.



15

Results suggest something missing from model.

Modifying our model: adding needed linkages




```
# New Model - SEM Model 2 “sem2”

sem2 <- 'epiphytes ~ macroalgae + grass + gammarids
         gammarids ~ pesticide + macroalgae'
```

An important discovery is an effect of macroalgae on Gammarids.

Results

```
Chi-square                    8.136
Degrees of freedom                2
P-value                       0.017
```

```
# Chi-square difference test
anova(sem1.fit, sem2.fit)

Chisq-diff = 18.363,
df-dif     =  1
p          = < 0.001
```

```
# Select Modification Indices
gammarids ~ grass      3.319
epiphytes ~ pesticide 4.205
```

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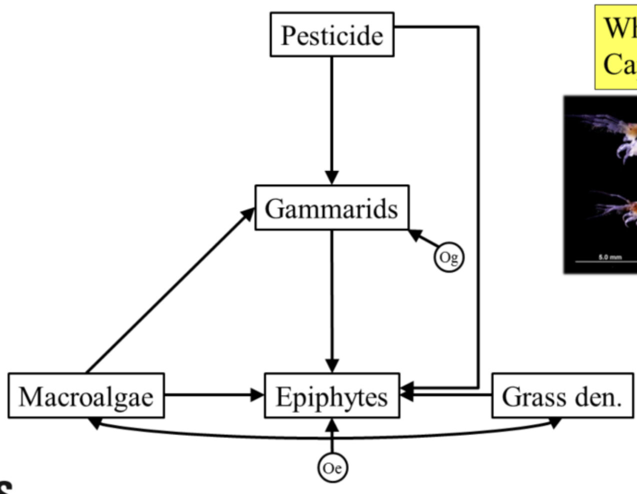
17

Model still missing another link, though the link added in model 2 definitely improved model fit dramatically. Modification indices suggest a remaining direct path from pesticide to epiphytes.

We can go further.

What is mediating the remaining effect of pesticide on epiphytes?

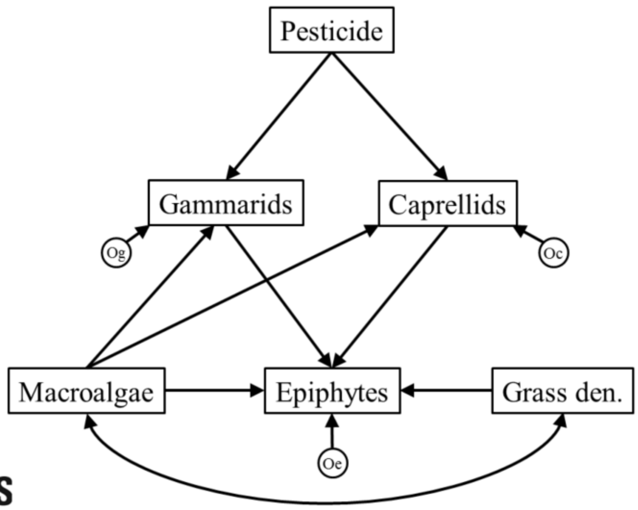


What about Caprellids?

18

Now we bring in the second most abundant type of micrograzer.

Our final model – complete mediation of pesticide.

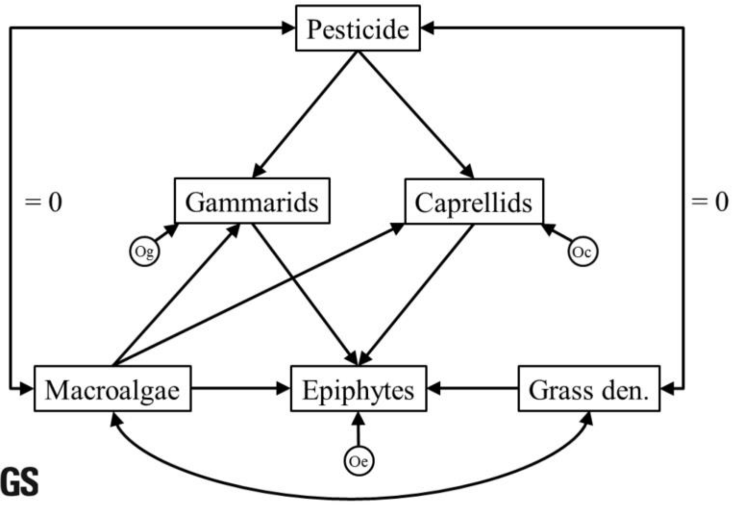


19

Finally, a fully-mediate model.

What if we wanted to include some constraits?

Here we force the correlations between treatment and covariates to equal 0.



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20

Here we simple demonstrate setting exogenous correlations to zero. this permits more pure causal attribution (if it holds).

“sem5” model and results

```
# SEM Model 5 “sem5”

sem5 <- 'epiphytes ~ macroalgae + grass + gammarids
                   + caprellids
         gammarids ~ pesticide + macroalgae
         caprellids ~ pesticide + macroalgae
         pesticide ~~ 0*macroalgae
         pesticide ~~ 0*grass'

sem5.fit <- sem(sem5, data=w.dat, fixed.x=F)
```

```
# Chi-square difference test
anova(sem4.fit, sem5.fit)

Chisq-diff =  1.363
df-dif     =  3
p          =  highly ns
```

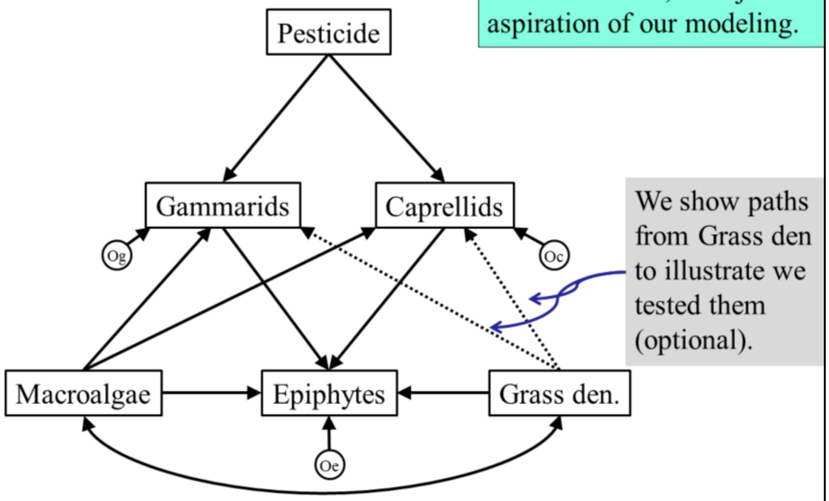
```
# note we must
declare
"fixed.x=FALSE"
to work with
exogenous
correlations.
```

21

Code in red shows how we set correlations to zero.

Final accepted model

We have now explained our treatment effect, a major aspiration of our modeling.



Chi-square = 5.432, df = 5, p = 0.366

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22

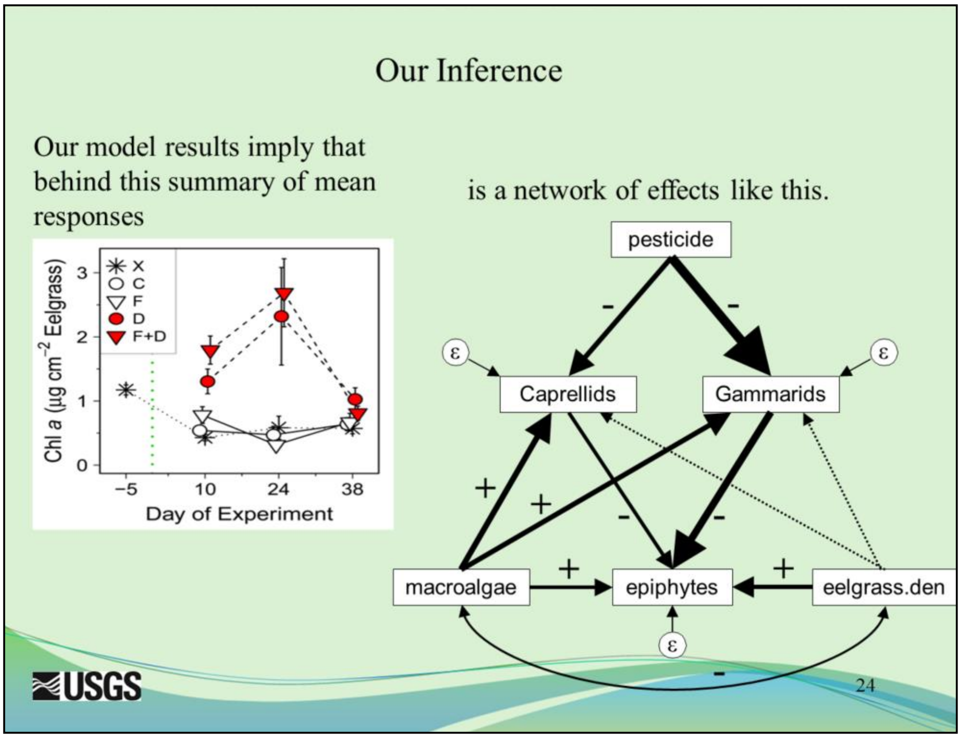
Final model.

Results

	Est.	Std.err	Z-val	P(>|z|)	Std.all
Regressions:					
epiphytes ~					
macroalgae	0.105	0.040	2.612	0.009	0.290
grass	0.405	0.100	4.034	0.000	0.389
gammarids	-0.329	0.057	-5.828	0.000	-0.663
caprellids	-0.240	0.085	-2.834	0.005	-0.335
gammarids ~					
pesticide	-2.053	0.215	-9.570	0.000	-0.748
macroalgae	0.304	0.057	5.347	0.000	0.418
grass	0.315	0.164	1.922	0.055	0.150
caprellids ~					
pesticide	-0.748	0.231	-3.239	0.001	-0.393
macroalgae	0.243	0.061	3.965	0.000	0.481
grass	0.231	0.176	1.311	0.190	0.159
R-Square:					
epiphytes	0.645				
gammarids	0.756				
caprellids	0.411				

23

Here are the details of the estimates. Shown are raw parameter estimates (Est.), their standard errors (Std.err), associated Z-values (which are like likelihood-based t-values, the probabilities associated with the Z-values (P(>\z\), and the standardized parameter estimates (Std.all).



So, behind the standard anova result (on the left), lies a network of relationships going on.

Note that there is an exercise tutorial in which you may work through the mechanics of this analysis is you like. Consult

"SEM_5_Ex1_Test_of_Mediation_Exercise.pdf" and the associated code and data.

Lessons about using SEM with experimental data

1. Test of mediation is neglected concept in biometrics.

2. Careful with classic ANCOVA; if we used mediating variables as covariates, results would indicate no significant treatment effect!

3. SEM easy to implement with simple experimental designs. With blocking, nested designs, etc., more work required for SEM analyses.

4. Recommend performing classic analyses along with SEM analyses and reporting both. Classical analyses can more easily detect interactions and in SEM you have to work to examine them (more on that later).

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25

Just a few summary points.