



Understanding Pesticide and Salt Effects on Developmental Neuroplasticity in Amphibians

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Background & Introduction

This study looked at the effect of very low doses (trace amounts) of pesticides and salt on amphibian developmental neuroplasticity.

- 10 tadpoles were placed in small pools (mesocosms) to mimic their natural environment
- Each mesocosm was given a certain combination of pesticide and/or salt

The goal of the study is to understand what effect, if any, do certain pesticides, road salts, and their interactions have on the growing brains of tadpoles, so that a better understanding of the environmental impact of pesticide and road salt runoff can be constructed.



Data Pre-Processing

Experiment 1: Pesticide
N = 56 mesocosms
(16 + 40 shared with Ex. 2),
each with 10 tadpoles

- Do pesticides alter brain shape?

Pesticides	Concentration	# of mesocosms
Imidacloprid (Imi)	Hi/Low	4, 4
Thiamethoxam (Thia)	Hi/Low	4, 4
Malathion (Mal)	Hi/Low	4, 4
Chlorpyrifos (Chlor)	Hi/Low	4, 4
Cypermethrin (Cy)	Hi/Low	4, 4
Permethrin (Per)	Hi/Low	4, 4
Ethanol	Control	4, 4
Water	Control	4, 4

Experiment 2: Pesticide and Salt
N = 120 mesocosms (80 + 40 from Ex. 1),
each with 10 tadpoles

- Road salt will alter relative brain shape
- Road salt will exacerbate the effects of pesticides on brain shape

Pesticides	Concentration	Salt	Treatment Combos
Malathion (Mal)	Hi	Hi/Low/None	3 (x 4 mesocosms)
	Low	Hi/Low/None	3 (x 4 mesocosms)
Chlorpyrifos (Chlor)	Hi	Hi/Low/None	3 (x 4 mesocosms)
	Low	Hi/Low/None	3 (x 4 mesocosms)
Cypermethrin (Cy)	Hi	Hi/Low/None	3 (x 4 mesocosms)
	Low	Hi/Low/None	3 (x 4 mesocosms)
Permethrin (Per)	Hi	Hi/Low/None	3 (x 4 mesocosms)
	Low	Hi/Low/None	3 (x 4 mesocosms)
Ethanol	Control	Hi/Low/None	3 (x 4 mesocosms)
Water	Control	Hi/Low/None	3 (x 4 mesocosms)

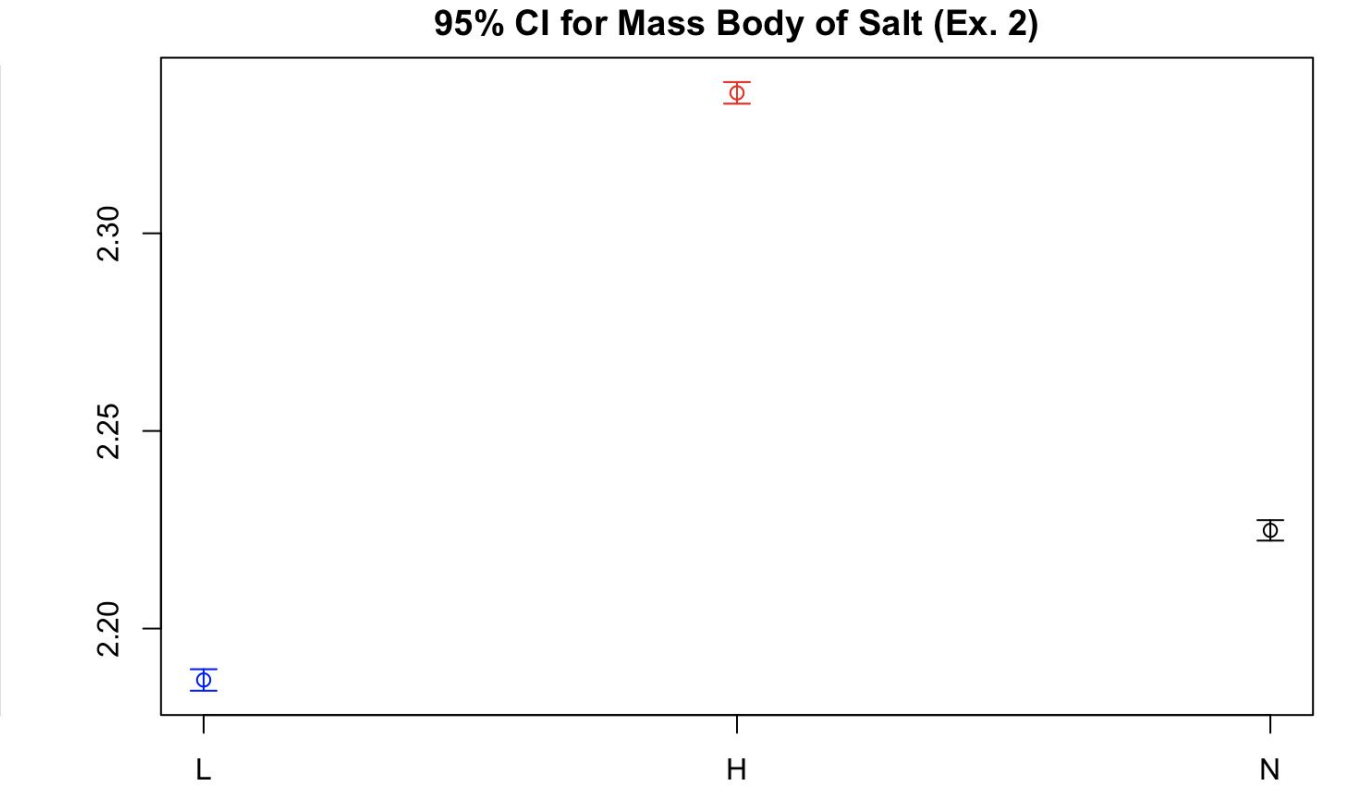
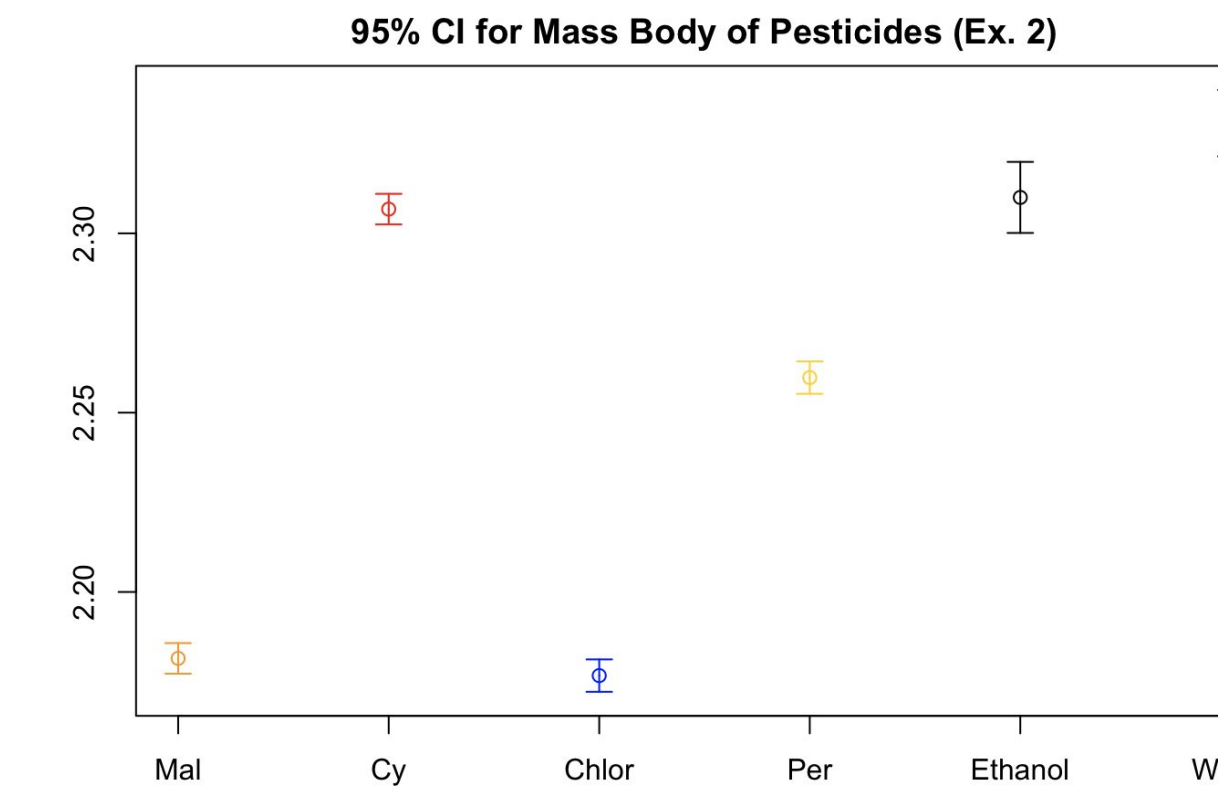
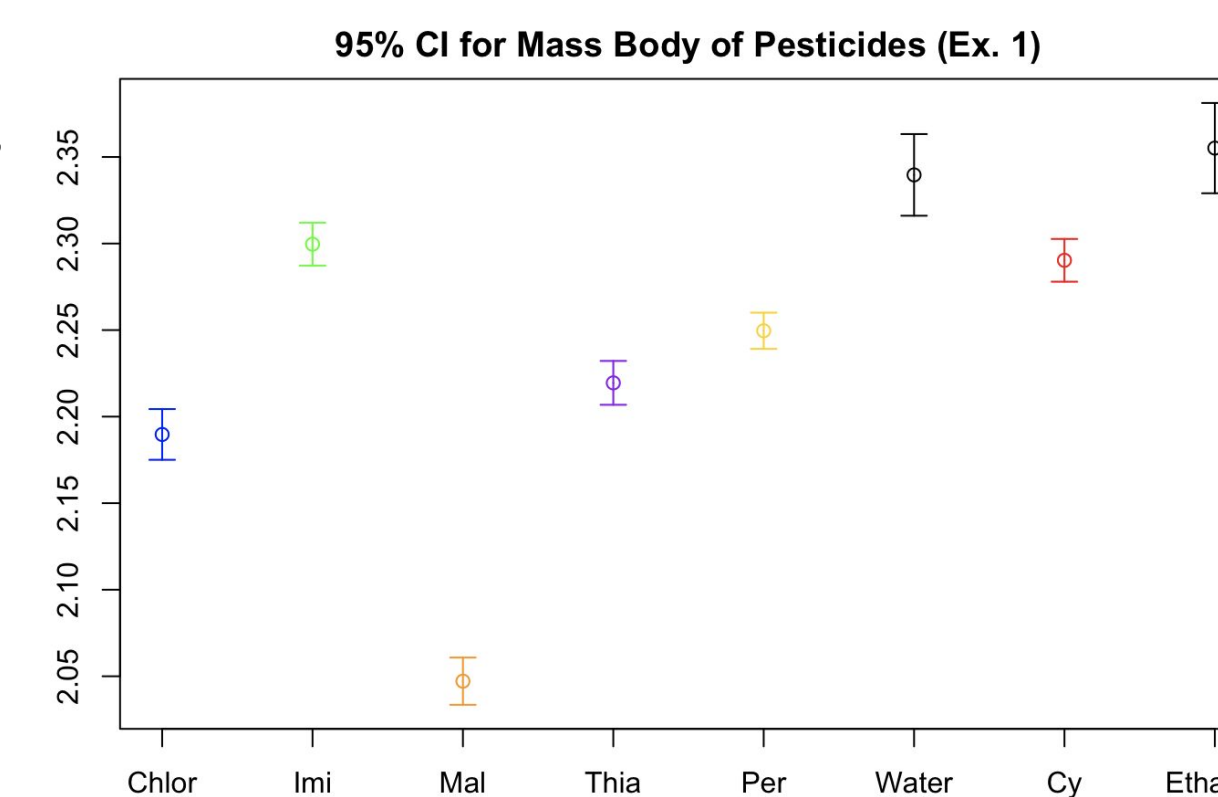
Methods

- We used various techniques to compare group means to each other to understand if there was any difference between the group means and specifically what the difference was between them. We also took into account other potential factors, like person or mesocosm, that might have impacted the measurements or the tadpoles that were not directly related to the pesticides and/or salt. Level of significance (alpha) = 0.05
 - Main methods: ANOVA, MANOVA, Tukey, Post-hoc Analysis, Mixed-Effect Models

Analysis and Results

ANOVA: The mean body mass across mesocosms are equal.

MANOVA: The mean brain measurements across mesocosms are equal.



Experiment 1 Post-hoc Test Result:

Pesticide 1	Pesticide 2	P-value	Confidence Interval Low	Confidence Interval High
Mal	Ethanol	0.0422	-0.6101	-0.005787
Mal	Imi	0.0418	-0.5	-0.004953

Experiment 2 Post-hoc Test Result:

Salt Level	Pesticide Concentration	Pesticide 1	Pesticide 2	P-value	Confidence Interval Lower Bound	Confidence Interval Higher Bound
L	H	Mal	Chlor	0.00674	0.07798	0.6628
L	H	Per	Mal	0.0101	-0.6483	-0.06353
L	L	Per	Chlor	0.000309	0.1827	0.7915
L	L	Per	Mal	0.00882	0.07169	0.6844

Experiment 1 MANOVA:

Brain Measurements	Pesticide	Pesticide Concentration	Interaction
TW	X	X	X
TL			
OTW	X	X	X
OTL			
DW	X	X	
DL	X	X	X
ODL	X		X
MW	X	X	X

Experiment 2 MANOVA:

Brain Measurements	Pesticide	Pesticide Concentration	Salt	Interaction w/ pesticide and pesticide concentration	Interaction w/ pesticide and salt	Interaction w/ salt and pesticide concentration	Interaction w/ pesticide, salt, and pesticide concentration
TW	X			X			X
TL	X		X	X			X
OTW	X	X	X	X			X
OTL			X		X		X
DW		X				X	
DL			X			X	
ODL		X				X	
MW	X	X	X	X	X		X

Mixed effect Model: Test to see whether there are significant effects of Mesocosm and Person who collected data on our model?

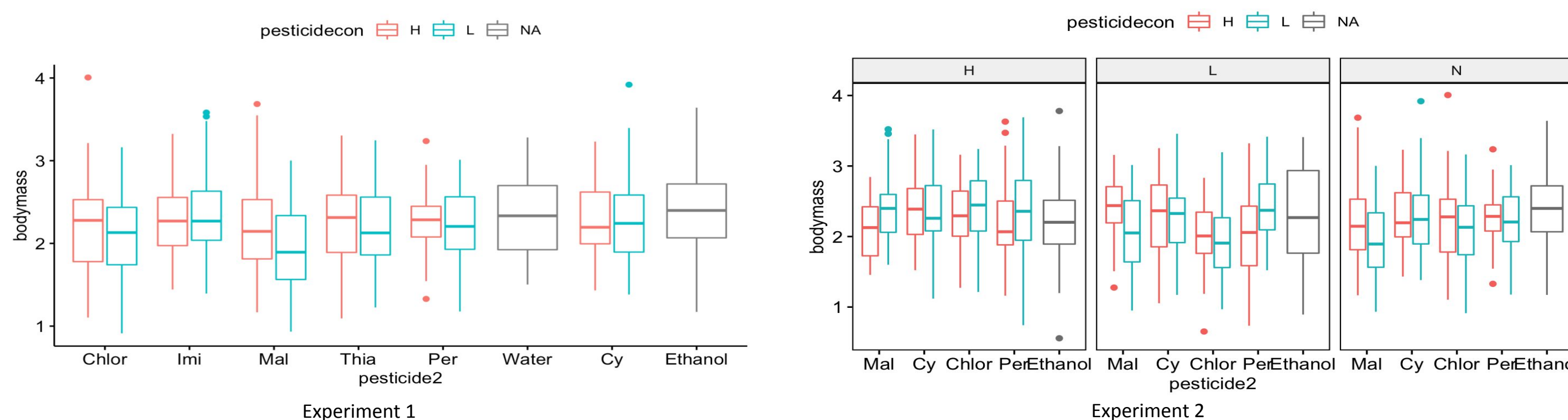
ANOVA on Mixed Effect Models versus Simple Linear Model

Experiment	Mixed Effect with Mesocosm	Mixed Effect with Person
1		
2	X (p-value = 0.01021)	

There might be random effect on Mesocosm for experiment 2, but it is negligible

Conclusions

- Experiment 1
 - ANOVA: Significant impact of pesticides on body mass
 - MANOVA: Each brain measurement differed in which variable had a significant impact on it
 - Tadpoles with Mal has significantly smaller mean body mass than the Ethanol Control group
- Experiment 2
 - ANOVA: All environmental variables had a significant impact on body mass except the pesticide concentration
 - MANOVA: Each brain measurement differed in which variable had a significant impact on it, but all were impacted by the introduction of environmental factors



EDA: Spread of bodymass for each pesticide group (exp. 1) & pesticide/salt combination group (exp. 2)