C. dubia QA evaluation study

Stakeholder Advisory Committee Meeting #4

Wednesday September 29, 2021

AGENDA

1. Opening Remarks and Review of the Agenda
2. Public Comments
3. Approval of minutes of Stakeholder Committee Meeting #3
4. Inventory of the historical data and lab methods collected (~45 min)
5. Exploration of data collected (~25 min)
6. Proposed data analyses plan (~20 min)
7. Next steps and closing remarks

Our Objectives

We seek participation from all 18 ELAP-accredited laboratories

- 3 public utilities ; 13 private laboratories; 2 academic labs

Data requested

- 30 tests within the last 1.5 to 3 years
- Control data associated with test samples
- Reference toxicant tests conducted concurrently
- Brood board culture matching data submitted

We also asked for lab SOPs

Our Objectives

TABLE 4 OF WORKPLAN- METHODS AND TEST DATA TARGETED		
Dilution water recipe	Age window at test initiation	Origin of brood stock
Source water	Reference toxicant used	Culture water hardness
Dilution water shelf-time	Number of replicates	Culture water conductivity
Measured ions concentration	Daily neonate counts per rep	Culture water temp, pH, DO
YCT vendor, shelf-time	Number of neonates per female	Culture photoperiod
YCT conc. in chamber	Number of broods per female	Culture light source, intensity
Algal species, vendor, shelf-time	Reference toxicant LC50	Culture % of males
Algal culture media	Ref toxicant IC25 for reproduction	Culture % of adult mortality
Algae conc. in chamber	Time to reproduction	Culture % of unhealthy adults
Feeding frequency		Culture % of neonate mortality
Photoperiod	Daily water hardness	Culture % of unhealthy neonates
Light source, intensity	Daily conductivity	YCT conc. in culture chamber
Lab air temperature	Daily pH, temp, DO	Algae conc. in culture chamber
Sample volume in test chamber		
Test chamber material		
Test chamber volume, diameter		

Accomplishments

	Goal	Achieved
Lab participation	≥ 75 %	100 %
Number of tests	30 tests minimum	70% of labs had ≥ 30 tests
Audit of manually-entered data	20%	100%
Completeness for test data	All information in Table 4	~80% of information in Table 4
Completeness for lab methods	All information in Table 4	~60% of information in Table 4
Completeness for culture data	All information in Table 4	~60% of information in Table 4

C. dubia Database

n= 17 labs

Lab #18 excluded; has <15 tests total

Lab code	# Controls tests	# Ref. toxicant tests
A	46	28
B	48	50
C	67	25
D	17	6
E	60	29
F	45	30
G	7	22
H	*	15
I	27	26
J	7	21
K	19	15
L	27	30
M	59	34
N	30	29
O	30	30
P	80	28
Q	25	23

Lab Methods

- ✓ Dilution water recipes - no obvious patterns
  - 4 labs use Perrier and 3 used EPA method
  - 5 labs use modified EPA method
  - 4 labs do not specify method used
  - 1 lab uses a method not described in the manual
- ✓ Reference toxicant
  - Half used sodium chloride, the other used copper.
  - 1 lab uses zinc
- ✓ Feeding sources - no clear pattern for culture vs purchase of YCT and algae

Control and Ref. Tox. Test Data

- ✓ Number of neonates per female
- ✓ Number of broods/female*
- ✓ Time to reproduction
- ✓ Reference toxicant LC
- ✓ Reference toxicant IC for reproduction
- ✓ Time to end of test

- ✓ Daily pH, temperature and DO
- ✓ Daily hardness/alkalinity (*information missing from 50% of labs)
- ✓ Conductivity but not all labs measure daily

Brood Board Information

Brood board information was inconsistent among labs

- Most information recorded in the database is qualitative

Each lab used a different method to count and report # neonates

Parameters to evaluate health of organisms and neonates are also very uneven

Missing Information

TABLE 4 OF WORKPLAN- METHODS AND TEST DATA TARGETED		
	Age window at test initiation	Origin of brood stock
Measured ions concentration		
YCT conc. in chamber/culture		
Algae conc. in chamber/culture		
Feeding frequency		Culture % of males
		Culture % of adult mortality
Photoperiod		Culture % of unhealthy adults
Light source, intensity		Culture % of neonate mortality
Lab air temperature		Culture % of unhealthy neonates
Sample volume in test chamber		
Test chamber material		
Test chamber volume, diameter		

Example of follow-up questions

- What are your procedures for determining mortality?
- What is the feeding frequency and concentration during the test?
- What is your procedure to exclude 4th broods?
- What is your annual percentage of test failures and for what reason(s)?
- How frequently are cultures monitored to assess general health?
- What is your experience with regards to reducing test variability and improving performance?
- How many times has your lab had to restart your culture in the last 3 years?
- What is your percentage of data audited by the QA officer?
- How many years has your lab conducted the WET test?
- How many years of experience does your lead technician have?
- How many tests has your lead technician conducted?
- For new technician training, how many practice tests are required as part of the training process?

Options to collect additional information

Questionnaire

Phone interviews

Group discussion with the Science Panel and participating laboratories

Split sample testing

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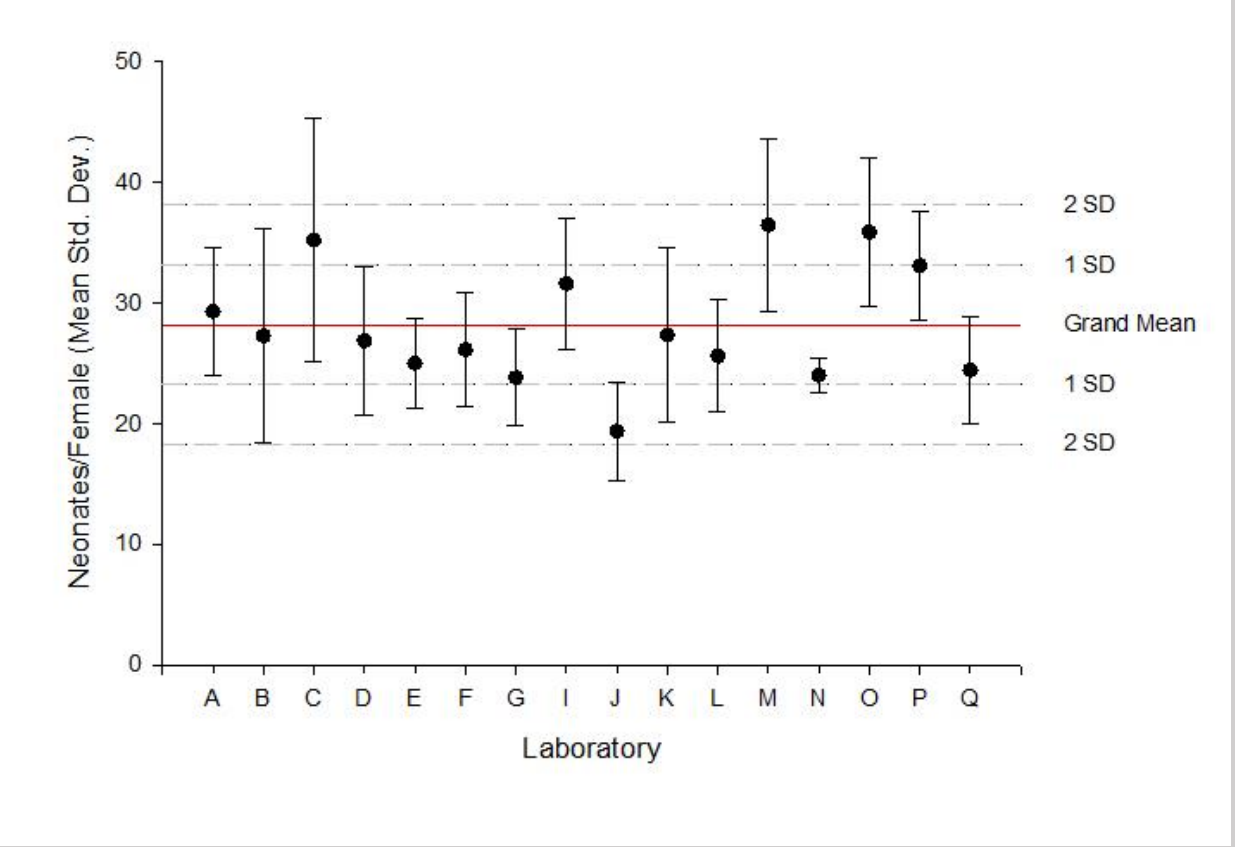
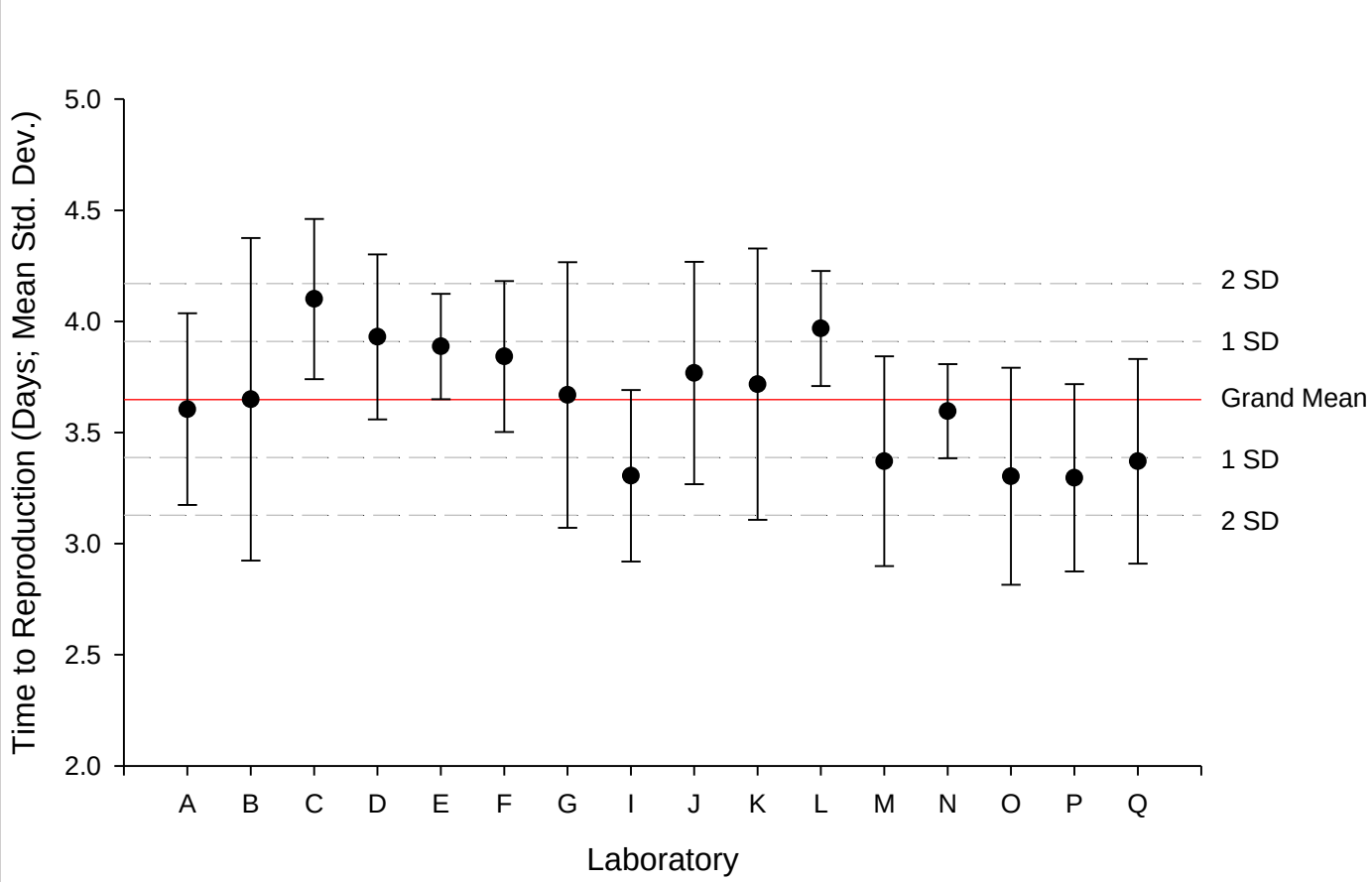
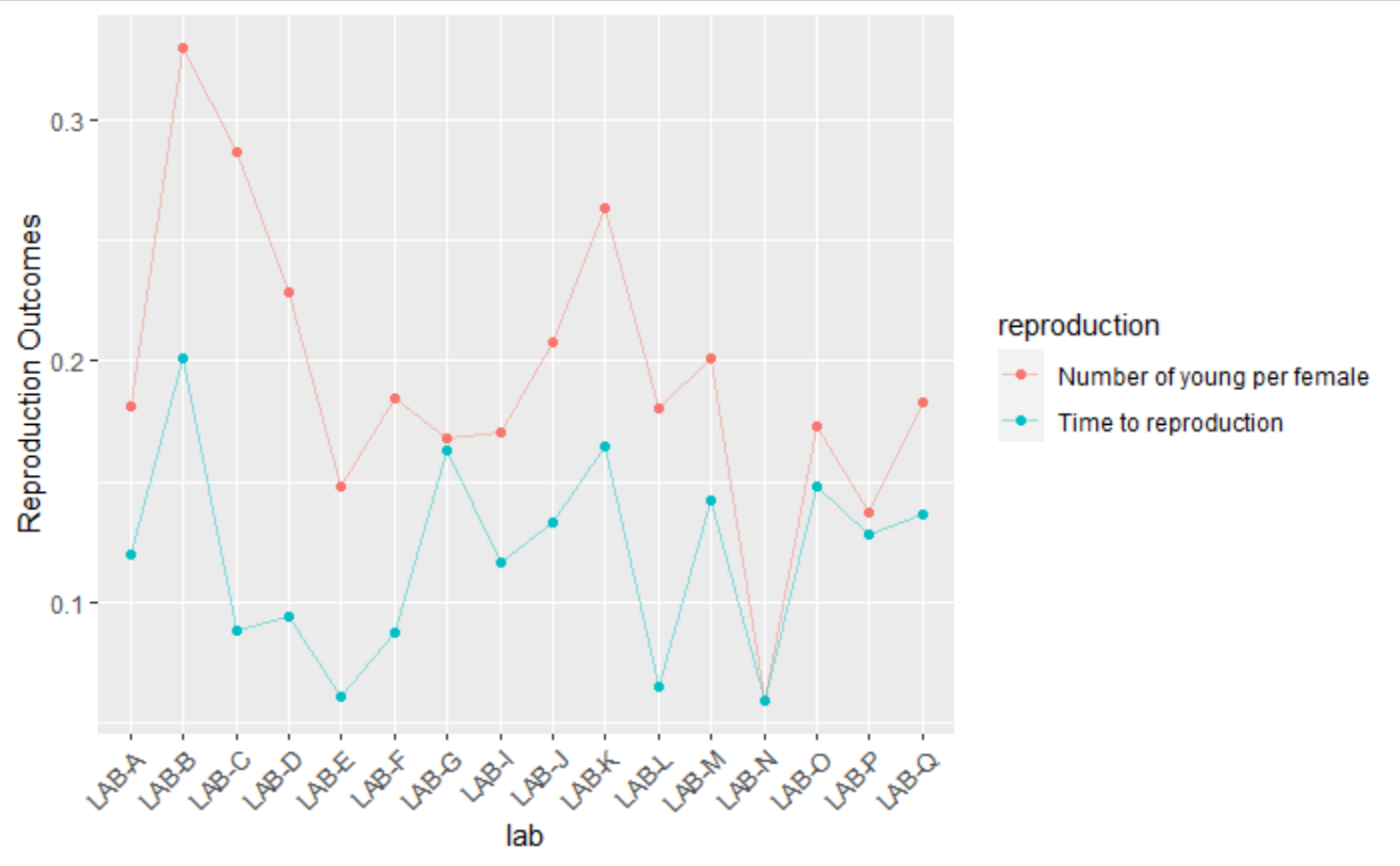
Control Mean Neonates per Female



Control Mean Time to Reproduction

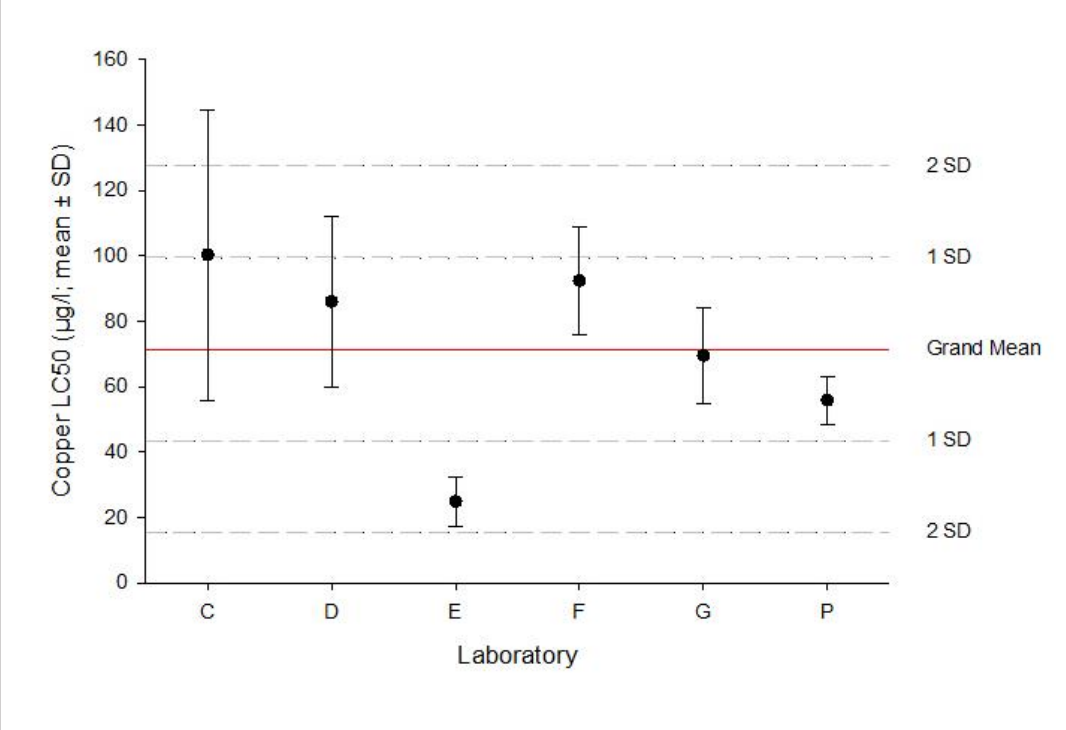


Coefficient of Variation for Reproductive Endpoints

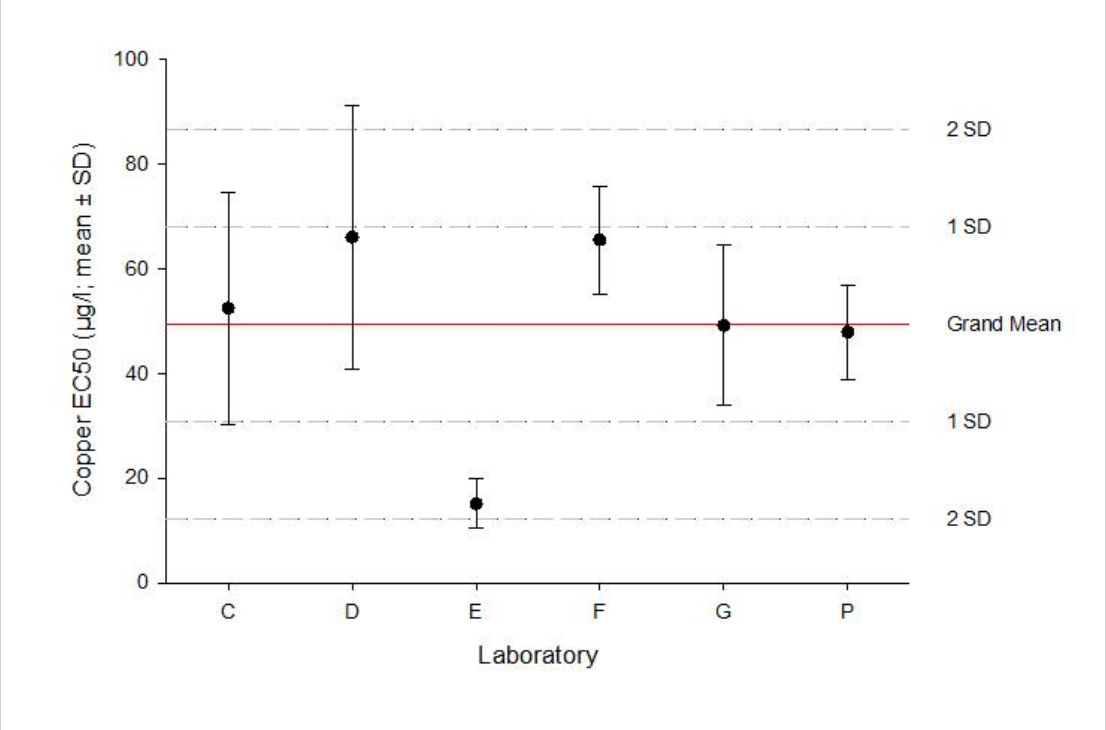


Reference Toxicant (Copper)

Survival LC50

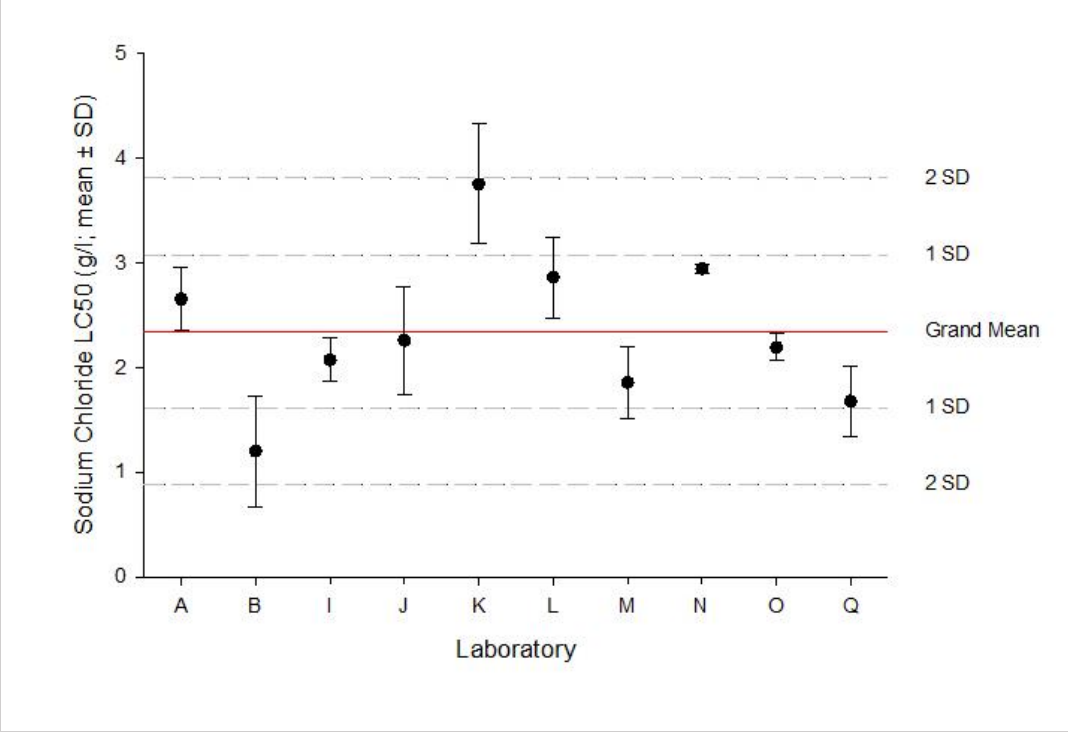


Reproduction EC50

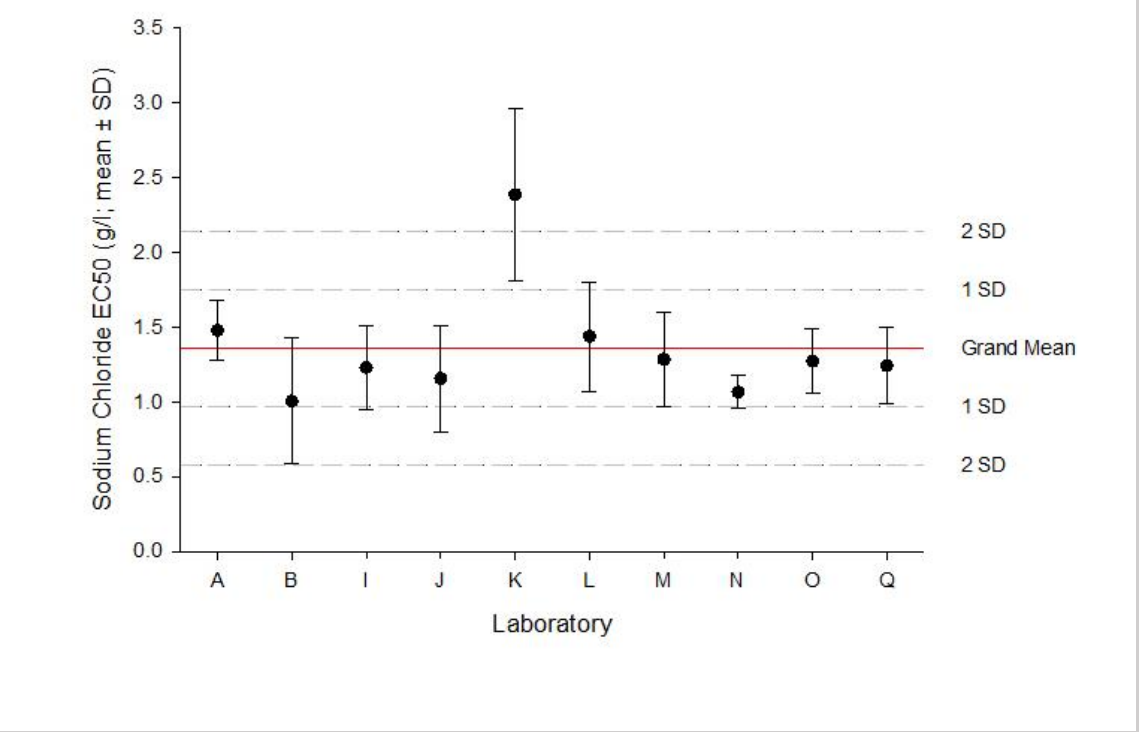


Reference Toxicant (Sodium Chloride)

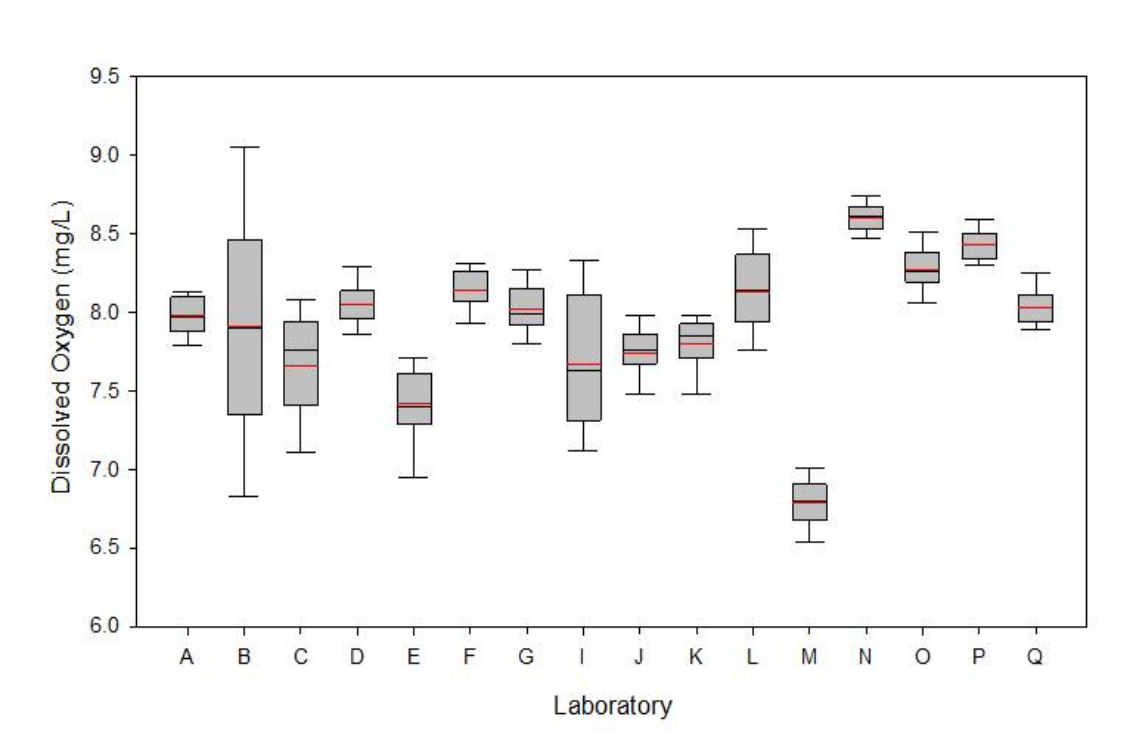
Survival LC50

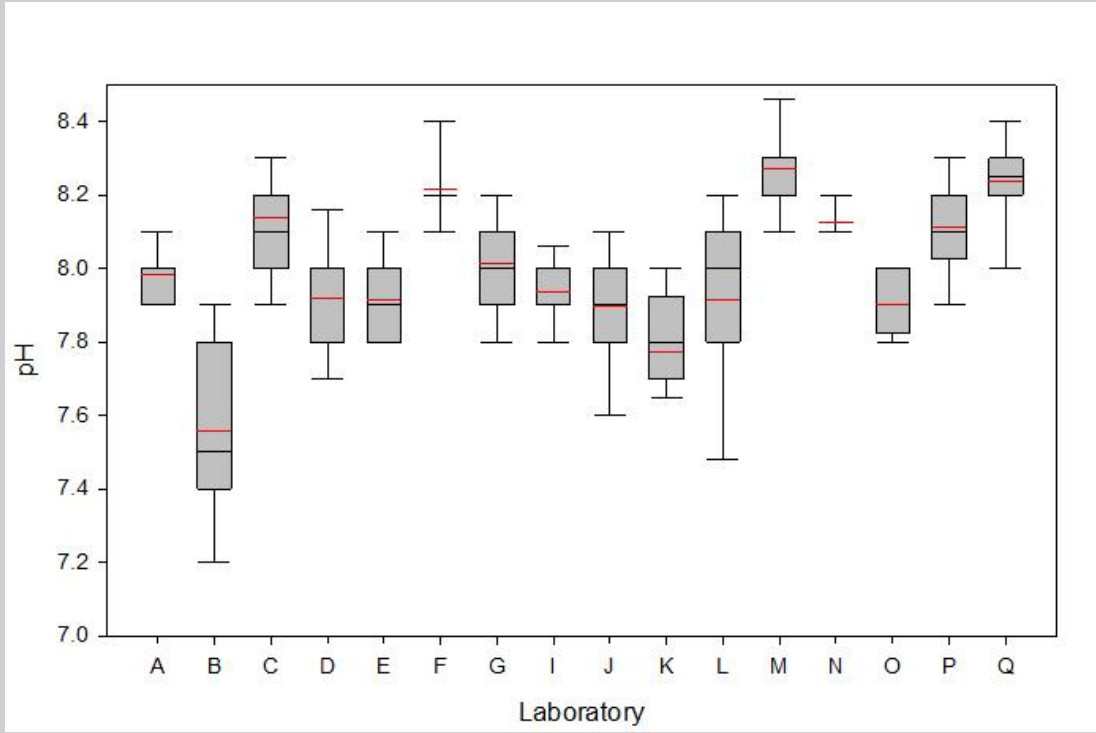


Reproduction EC50

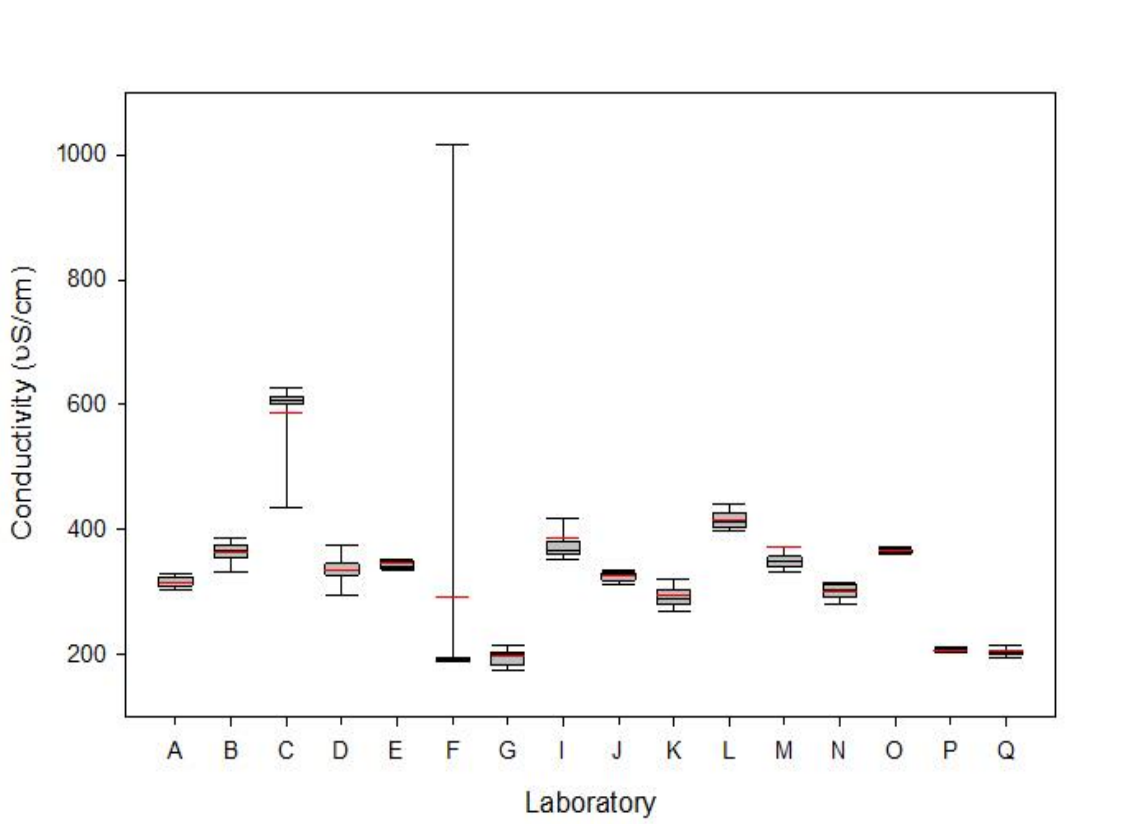


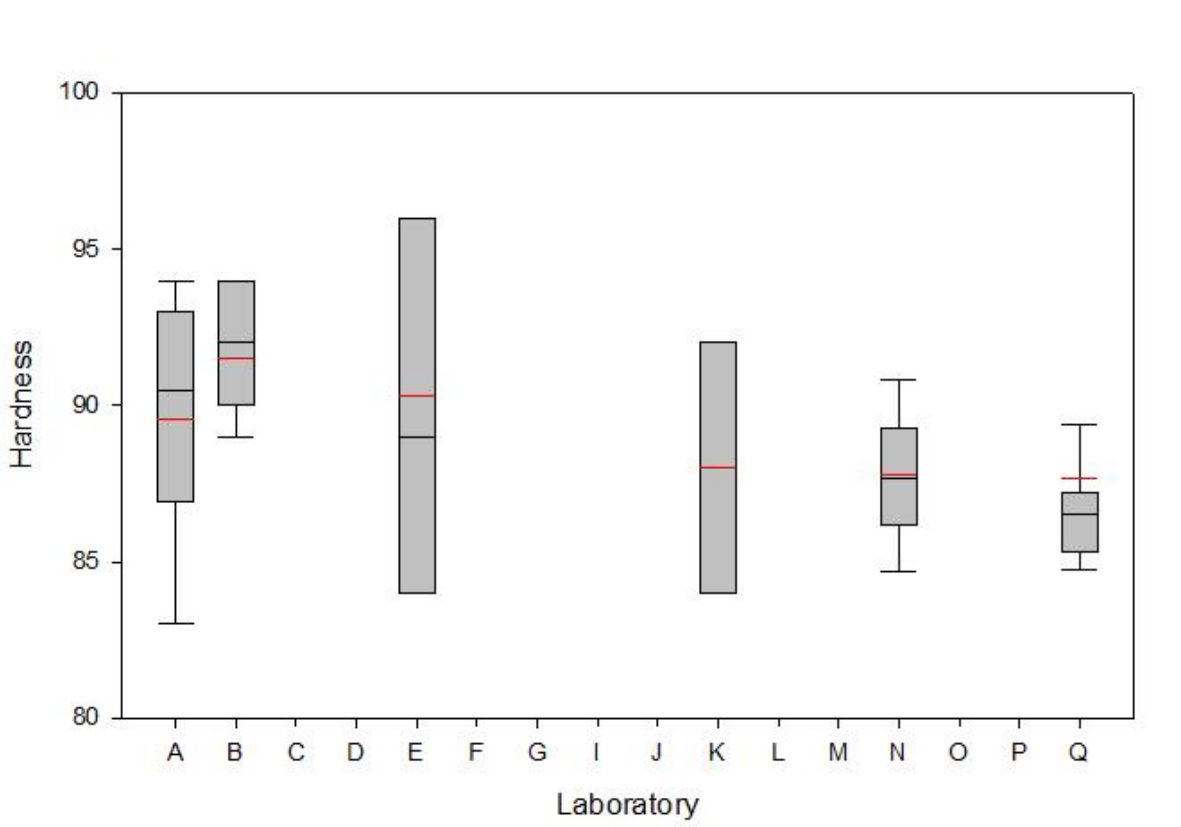
Water Quality



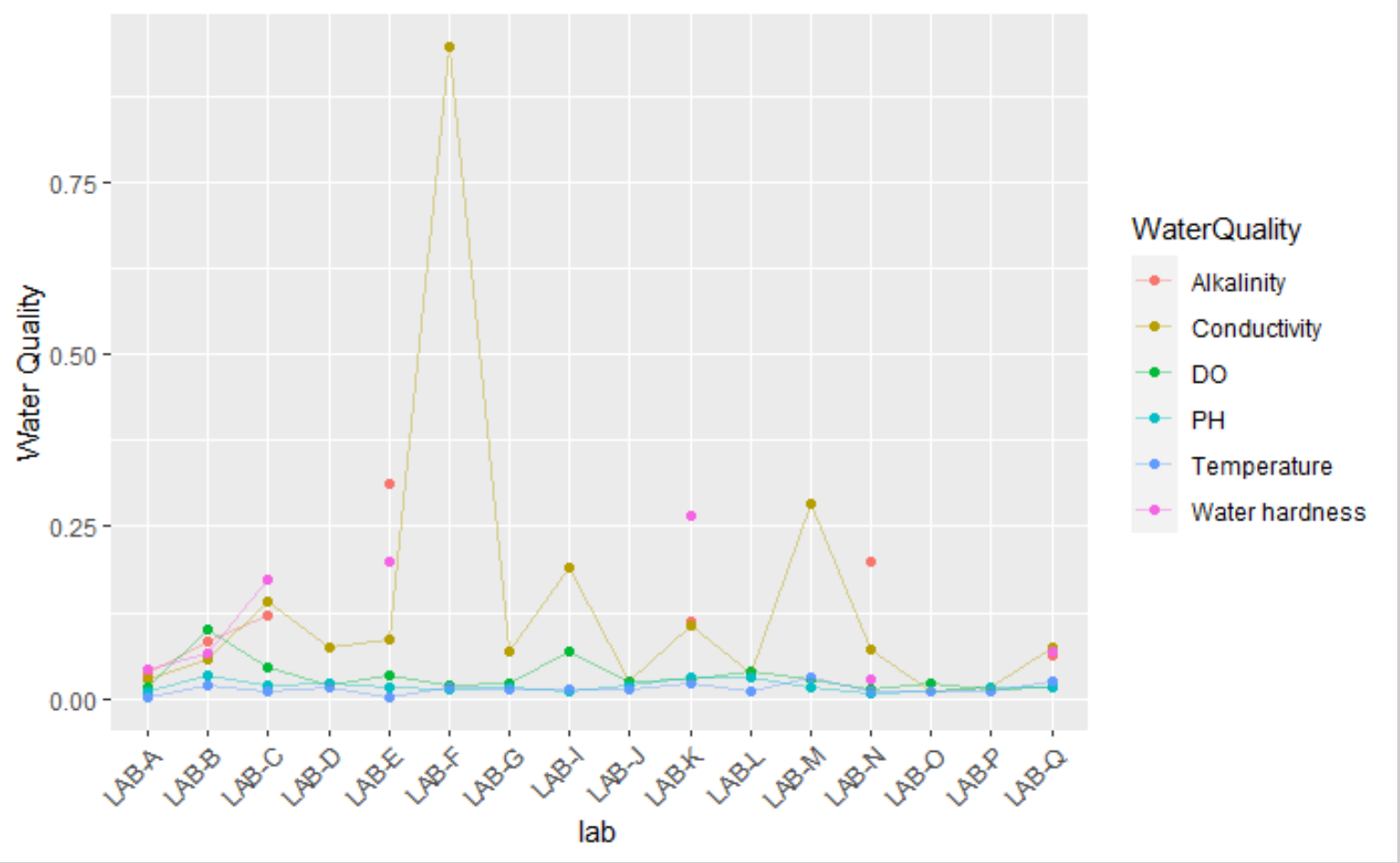


Water Quality





Coefficient of Variation for Water Quality Measurements



Data Analysis Approach

- Overall goal is to identify lab techniques that may cause variability
- Focus on mean and variance in control and reference toxicant performance
  - Characterizing variance as CV, SD, range
- Explore a wide array of test variables that may influence variance
  - Water chemistry, testing conditions, brood characteristics, etc.
- Iterate analyses as new data become available

Proposed Data Analysis Plan

Separate models for reproduction and survival endpoints

Step 1 - Prioritize test variables for further investigation

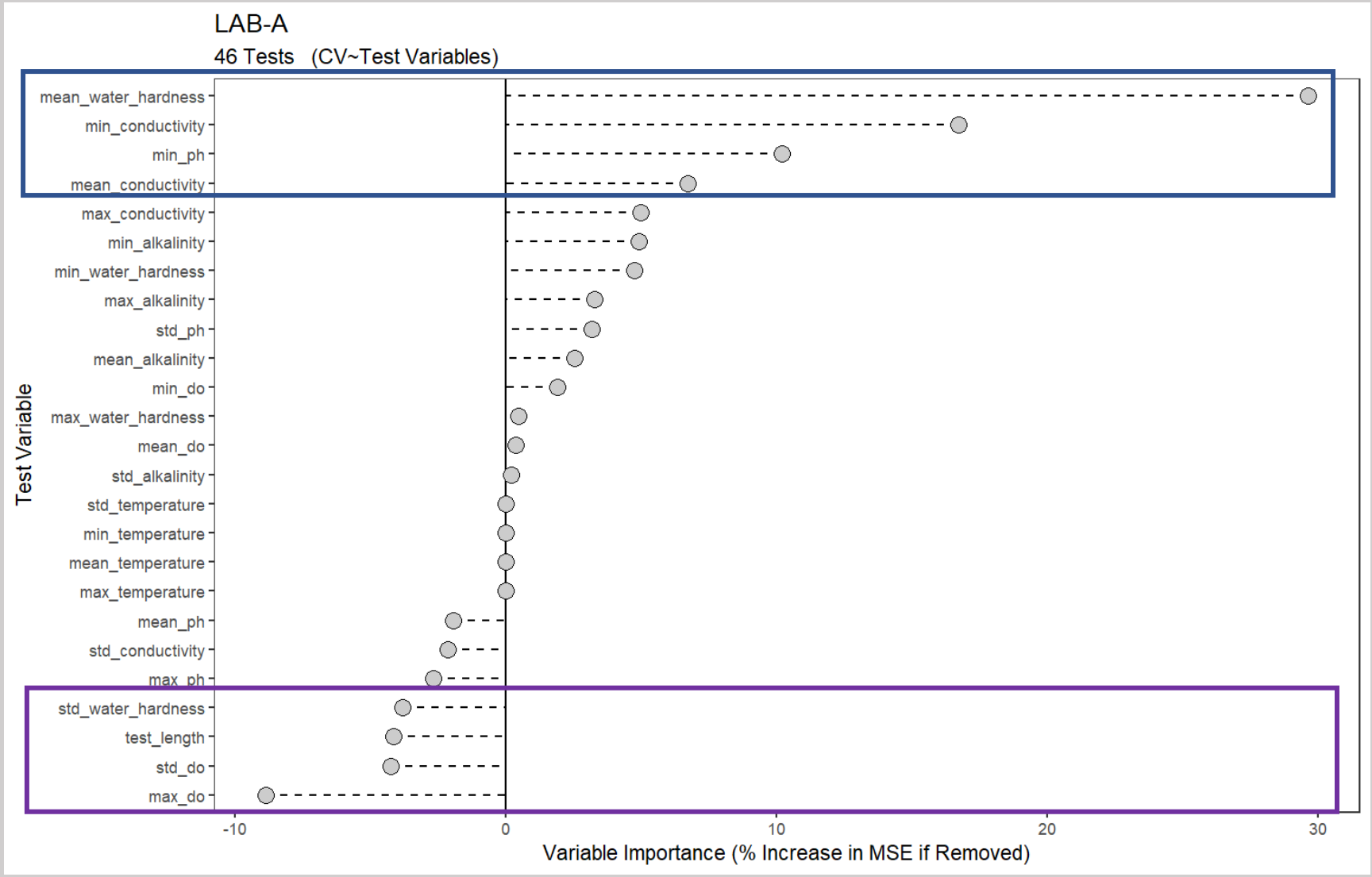
- Based on random forest regressions

Step 2 - Visualize patterns to identify the nature of each test variable response

- Direct, inverse, complex, etc

- Step 3 – Build structured models to quantify test variable influence
  - E.g. Generalized Linear Model (GLM)

Example of Factors Influencing Variability



Next steps

- Develop strategy to collect additional information
- Refine data analysis plan
- Complete step 1 (random forest) to prioritize test variables
- Present findings at the next Expert Science Panel on Wed October 20, 2021