C. dubia QA evaluation study

Expert Science Panel Meeting #4

Wednesday October 20, 2021

AGENDA

1. Opening Remarks and Review of the Agenda
2. Approval of minutes of Science Panel Meeting #3
3.* Description of the State lab accreditation process by Steve Boggs (~15 min)
4. Inventory of the historical data and lab methods (~30 min)
5. Exploration of data collected (~20 min)
6. Data analysis (~20 min)
7. Next steps and closing remarks

Inventory of historical data and lab methods

Our Objectives

We seek participation from all 18 ELAP-accredited laboratories

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

Data requested

- Minimum of 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 IC50 for reproduction**	Culture % of adult mortality
Algae conc. in chamber	**Time to reproduction**	Culture % of unhealthy adults
Feeding frequency		Culture % of neonate mortality
Photoperiod	**Test water hardness**	Culture % of unhealthy neonates
Light source, intensity	**Test water conductivity**	YCT conc. in culture chamber
Lab air temperature	**Test water 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	~90% of information in Table 4
Completeness for lab methods	All information in Table 4	~70% 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
  - 4 labs use Perrier and 3 used EPA method
  - 5 labs use modified EPA method
  - 4 labs do not specify method used for a given test
  - 1 lab uses a method not described in the manual

- ✓ Feeding sources
  - 3 labs purchase both food type and 5 labs produce food in-house
  - 5 use a combination of purchase and mixture
  - 4 do not specify

Lab Methods

- ✓ Reference toxicant
  - Even split using sodium chloride or copper
  - 1 lab uses zinc

- ✓ Test set up
  - Test chamber size and materials
  - Age window at start initiation
  - Photoperiod
  - Light intensity*
  - Air temperature*

Control and Ref. Tox. Test Data

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

- ✓ Daily pH, temperature and DO
- ✓ Hardness/alkalinity
- ✓ Conductivity

Culture Information

Brood board information was inconsistent among labs

- Parameters to evaluate health of organisms and neonates are also very uneven
- Most information recorded in the database is qualitative

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

Parameters and frequency of water quality measurements are also inconsistent

Missing Data

- Measured ionic composition of dilution water
- Feeding frequency in chambers/culture
- Quantifiable health parameters in brood boards (% males, % unhealthy animals, % mortality)

- Lab techniques for determining mortality & excluding 4th broods
- Annual percentage of test failures and reason(s)
- Number of times lab had to restart culture in the last 3 years

- Training protocols
- Years of experience
- Experience reducing test variability and improving performance

Next steps to collect missing information

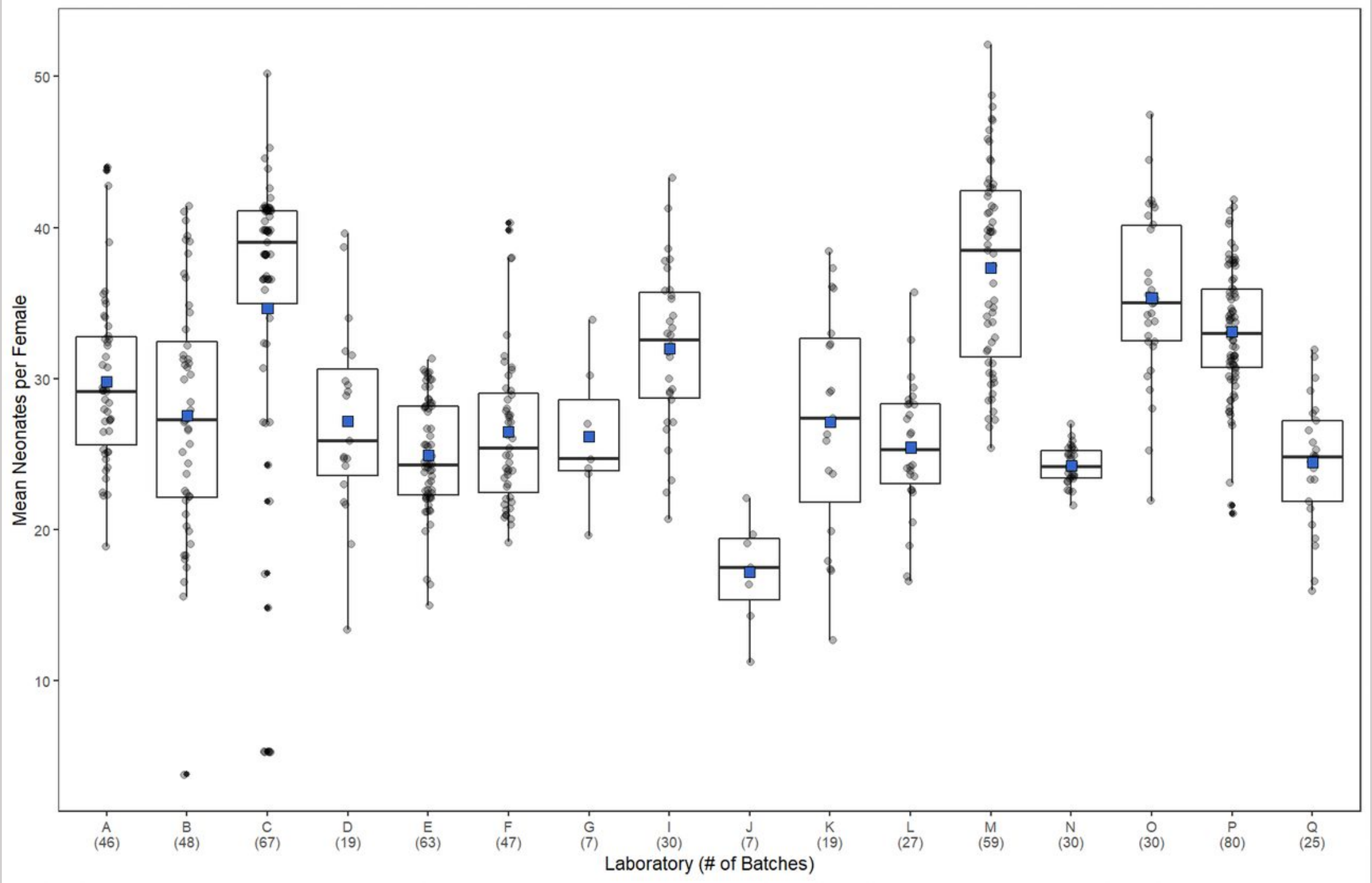
- **Measure ionic composition of dilution water**
- **Record feeding frequency in chambers/culture**
- **Quantify health parameters in brood boards**

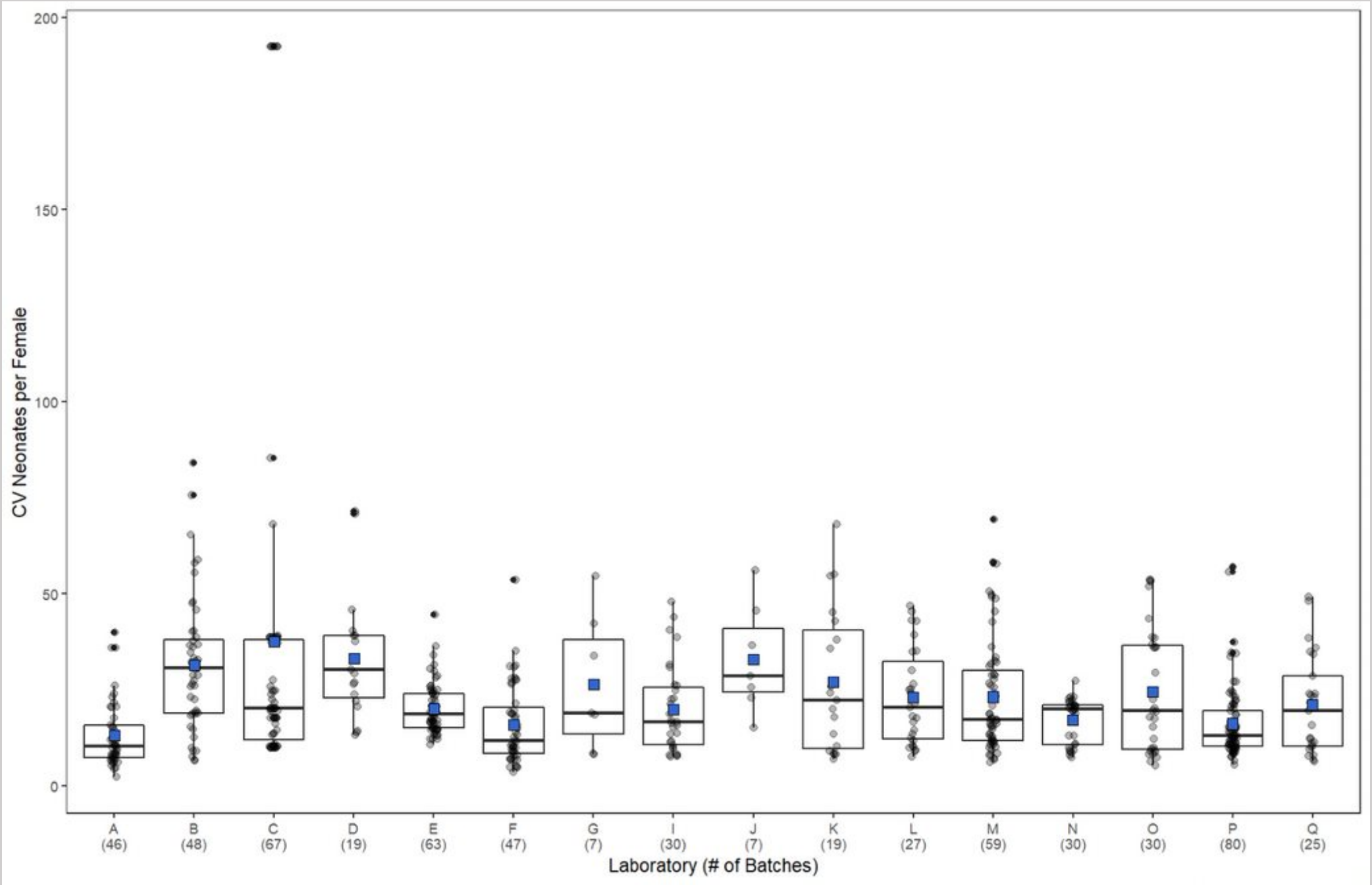
Additional testing (Task 2.3)

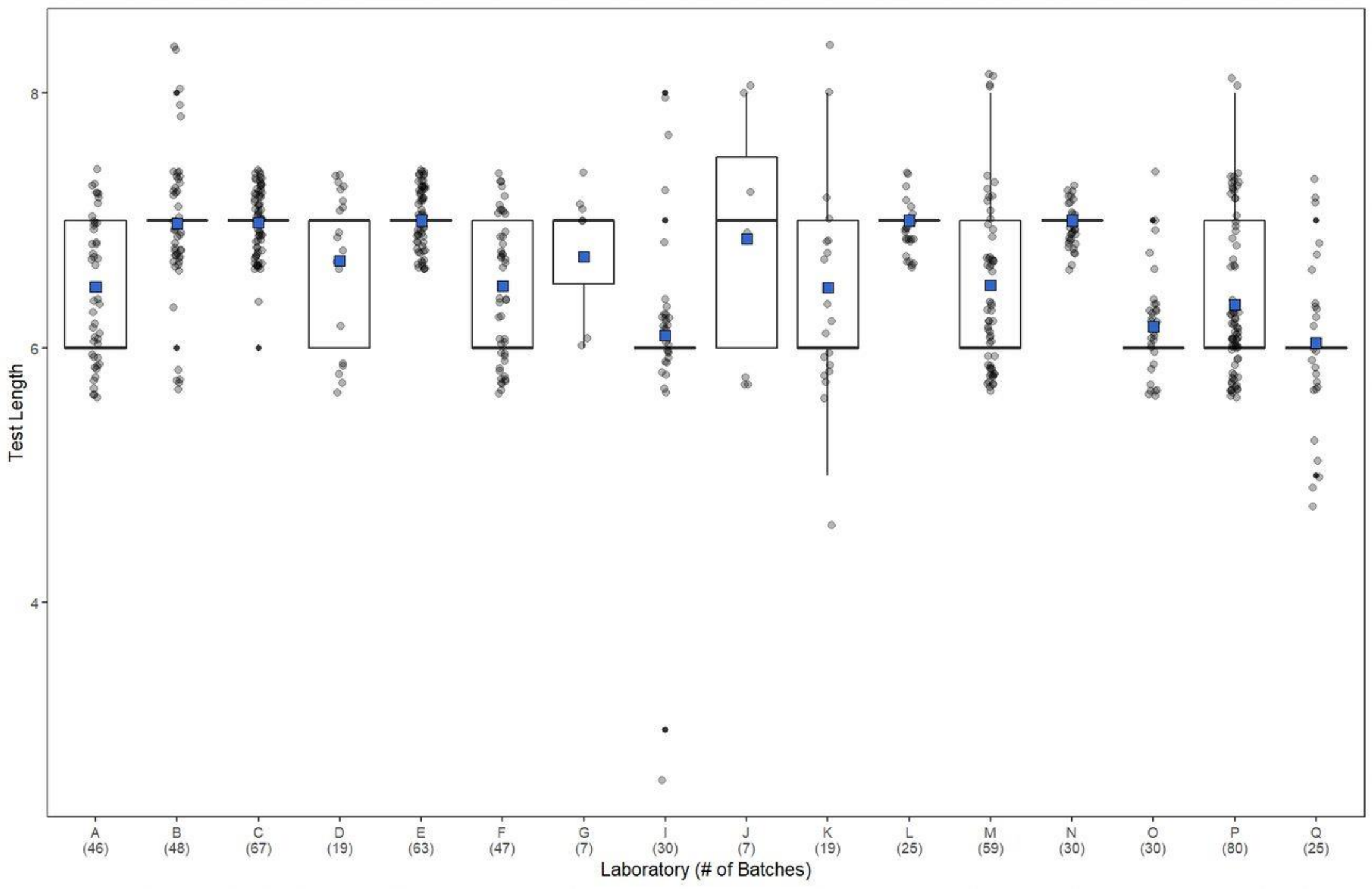
- Lab techniques for determining mortality & excluding 4th broods
- Annual percentage of test failures and reason(s)
- Number of times lab had to restart culture in the last 3 years
- Training protocols
- Years of experience
- Experience reducing test variability and improving performance

Phone interview/ group discussion

Data Exploration

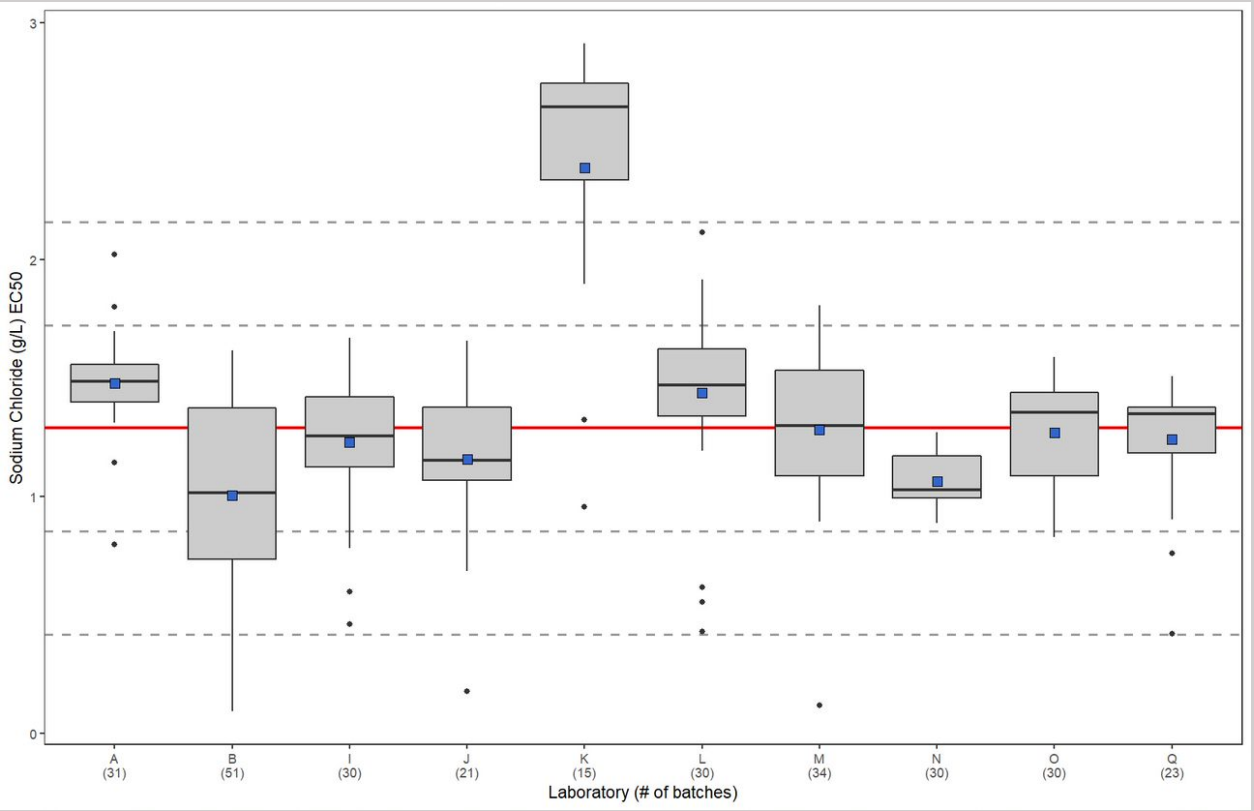

Mean Neonates per Female
50
40
30
20
10
A (46)
B (48)
C (67)
D (19)
E (63)
F (47)
G (7)
I (30)
J (7)
K (19)
L (27)
M (59)
N (30)
O (30)
P (80)
Q (25)
Laboratory (# of Batches)


CV Neonates per Female
200
150
100
50
0
A (46)
B (48)
C (67)
D (19)
E (63)
F (47)
G (7)
I (30)
J (7)
K (19)
L (27)
M (59)
N (30)
O (30)
P (80)
Q (25)
Laboratory (# of Batches)

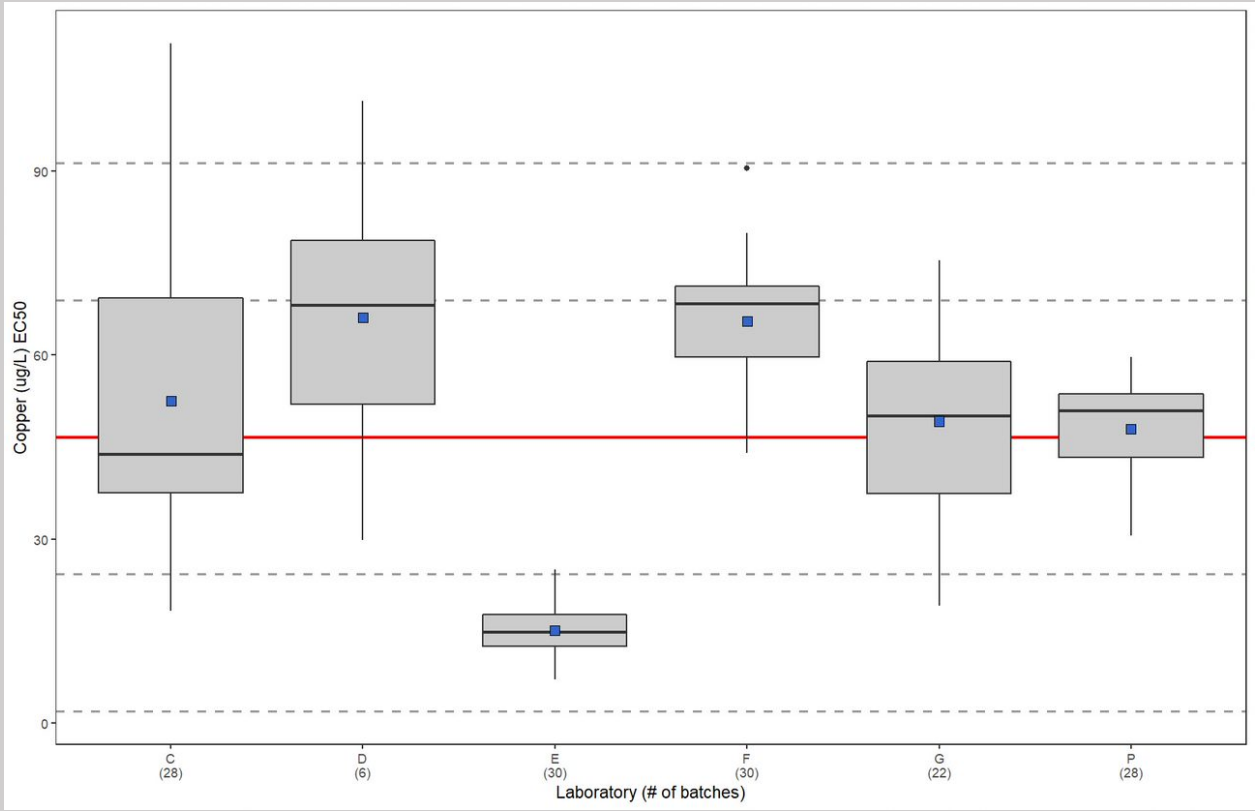

Test Length
8
6
4
A (46)
B (48)
C (67)
D (19)
E (63)
F (47)
G (7)
I (30)
J (7)
K (19)
L (25)
M (59)
N (30)
O (30)
P (80)
Q (25)
Laboratory (# of Batches)

Reference Toxicant EC50

Sodium chloride

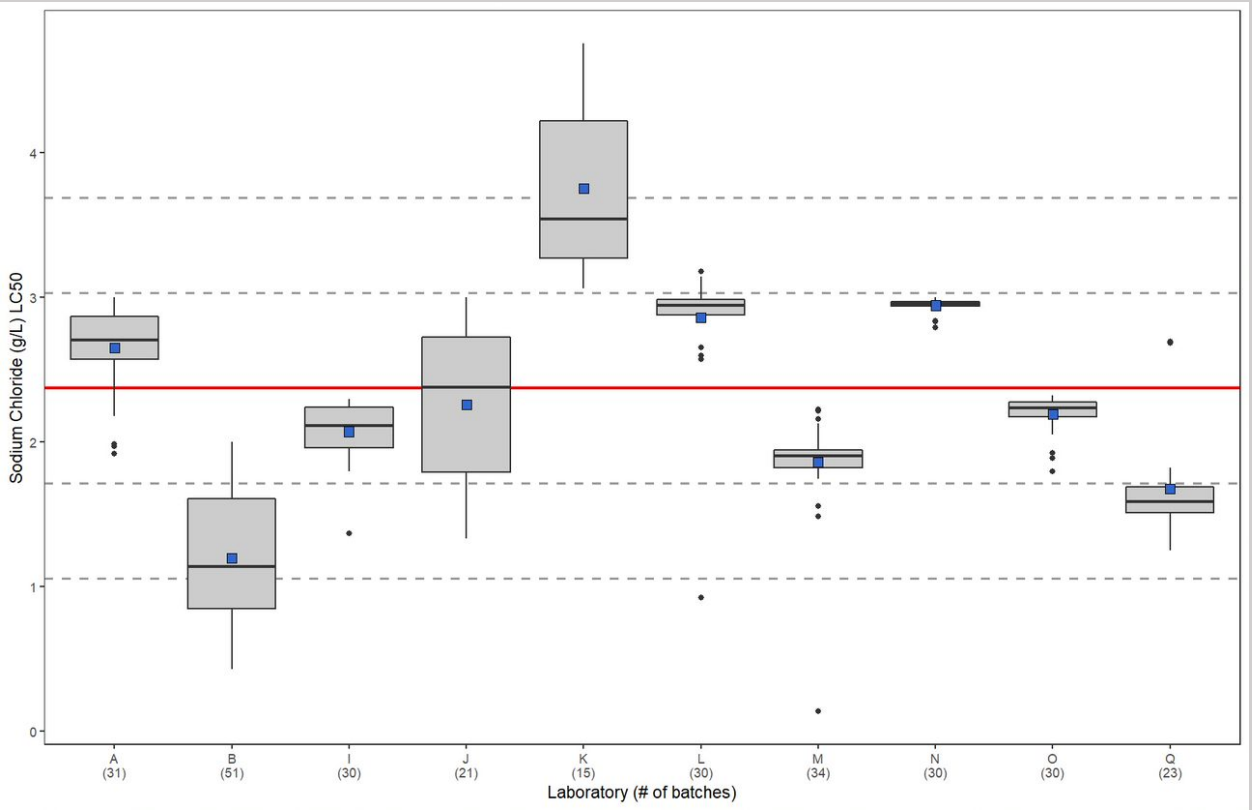


Copper

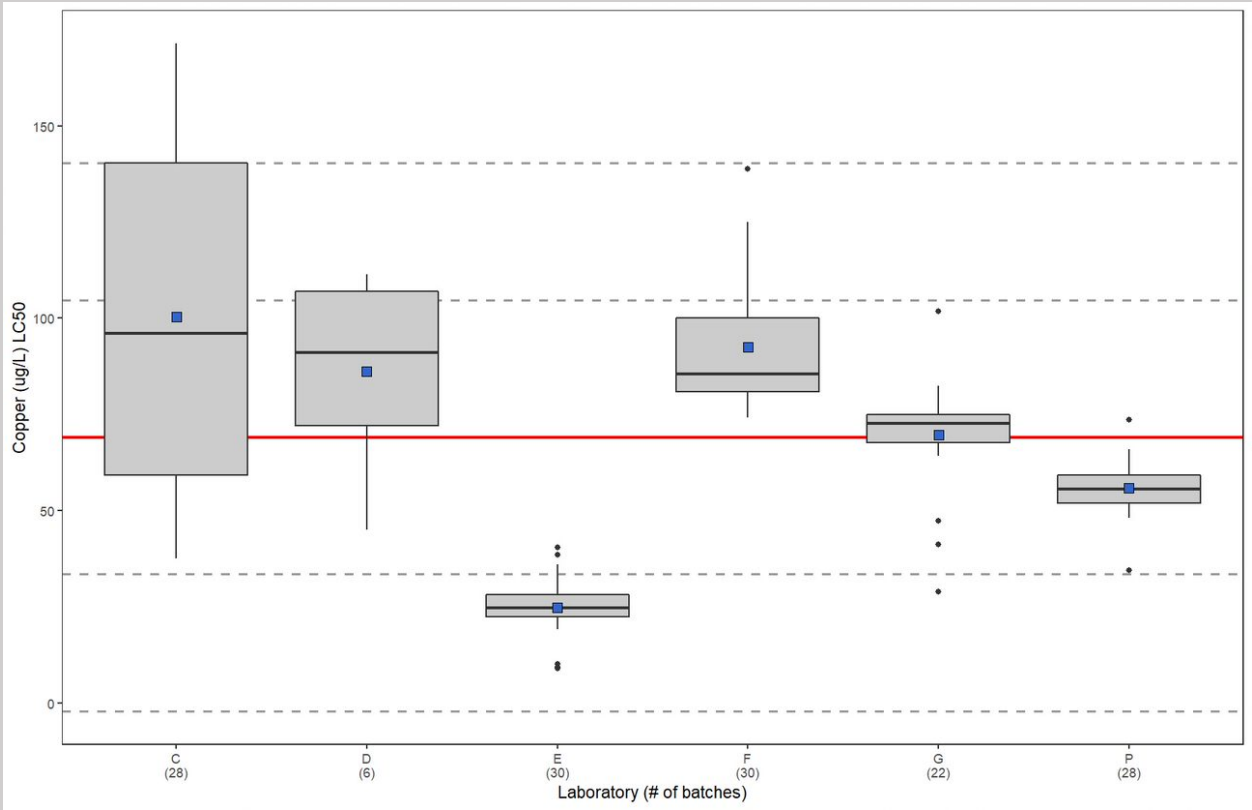


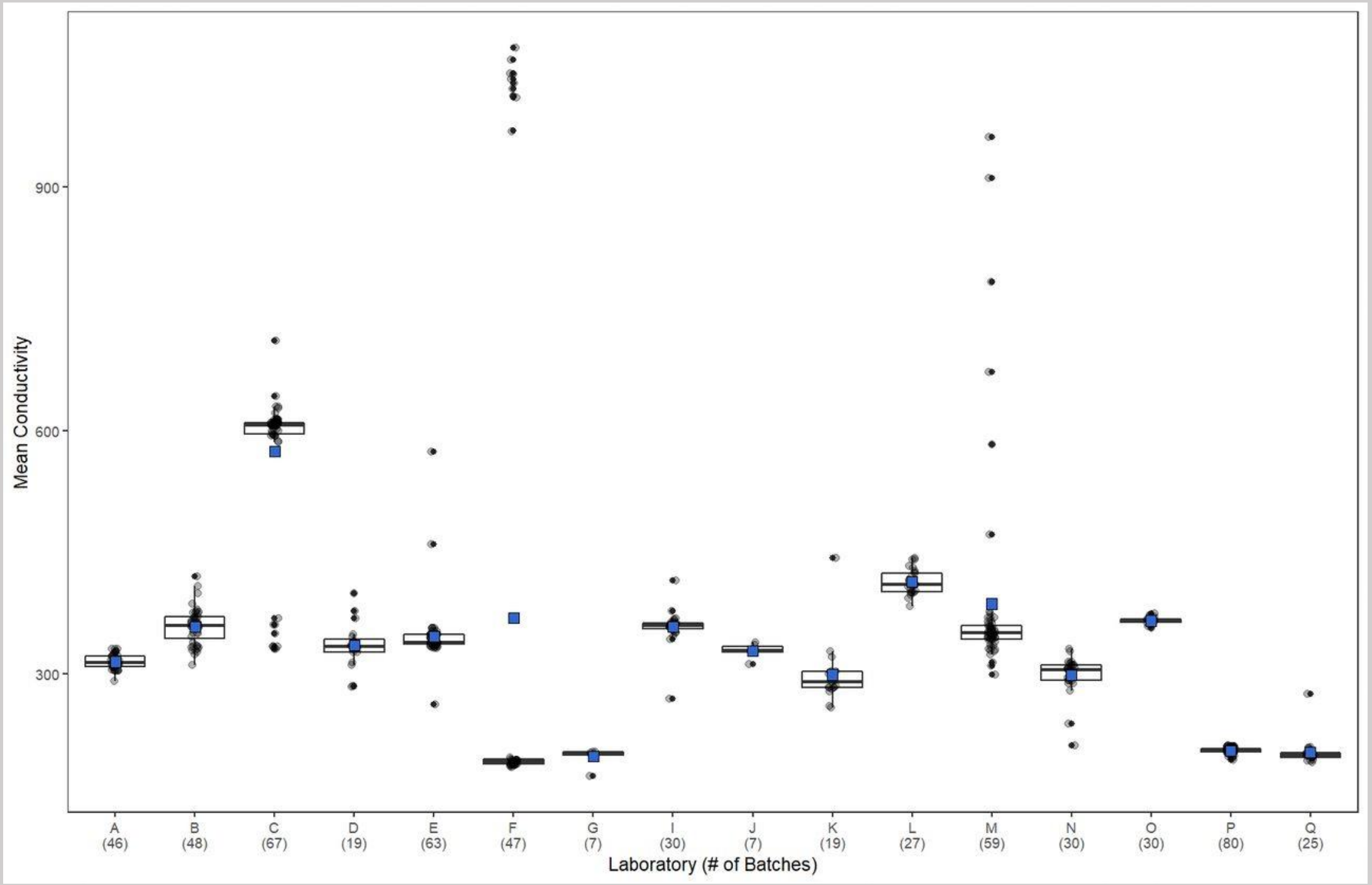
Reference Toxicant LC50

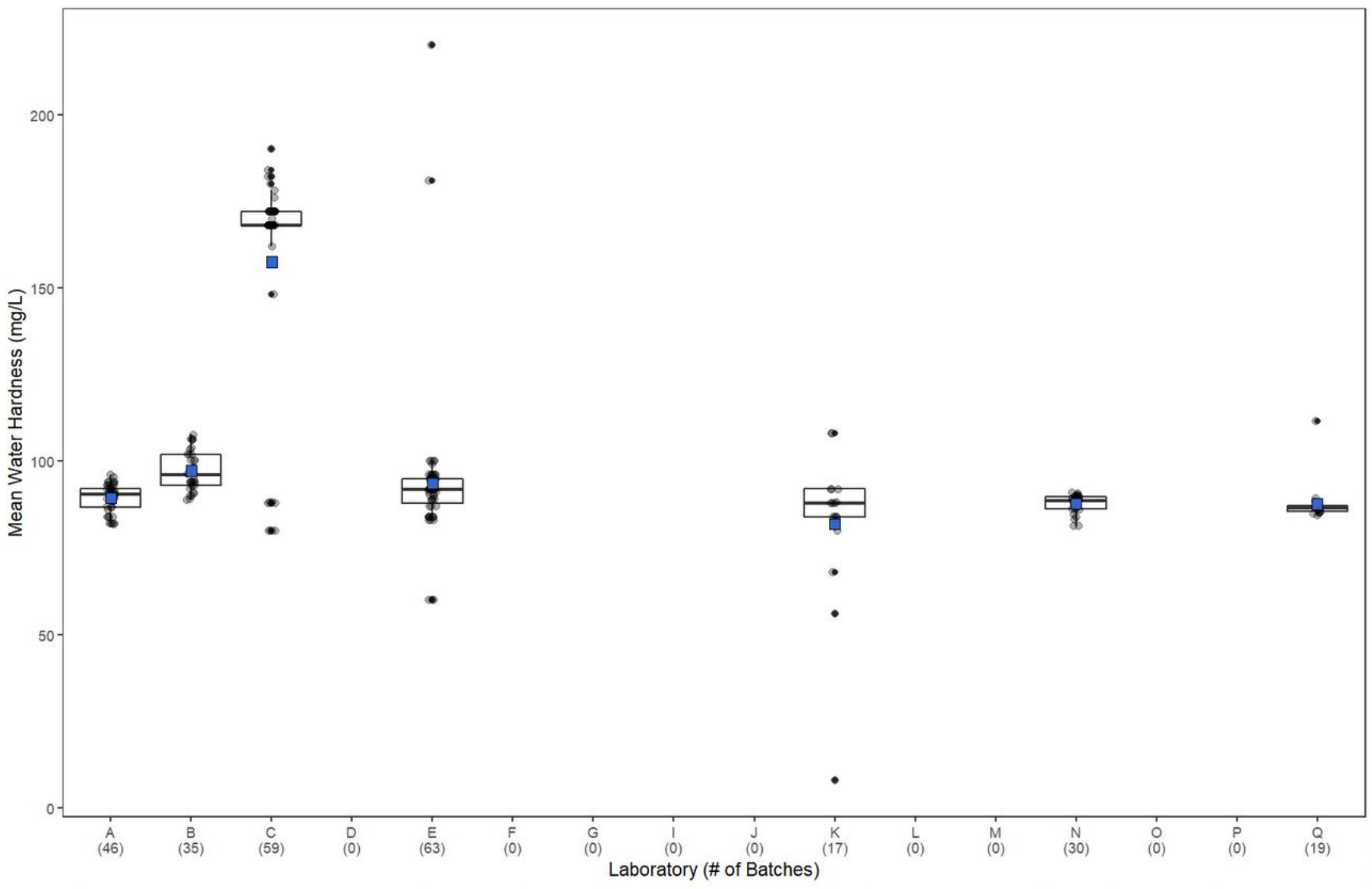
Sodium chloride

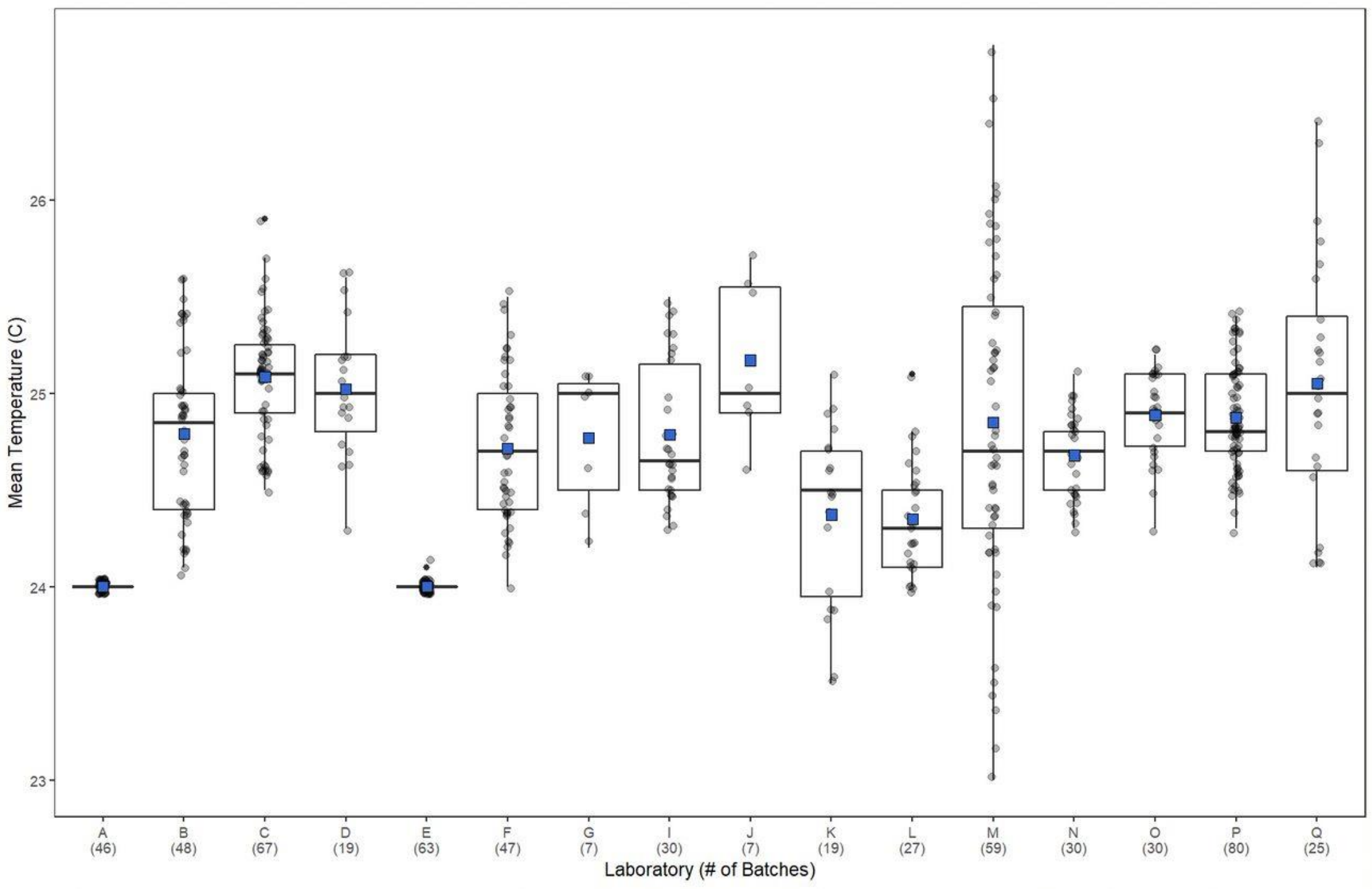


Copper

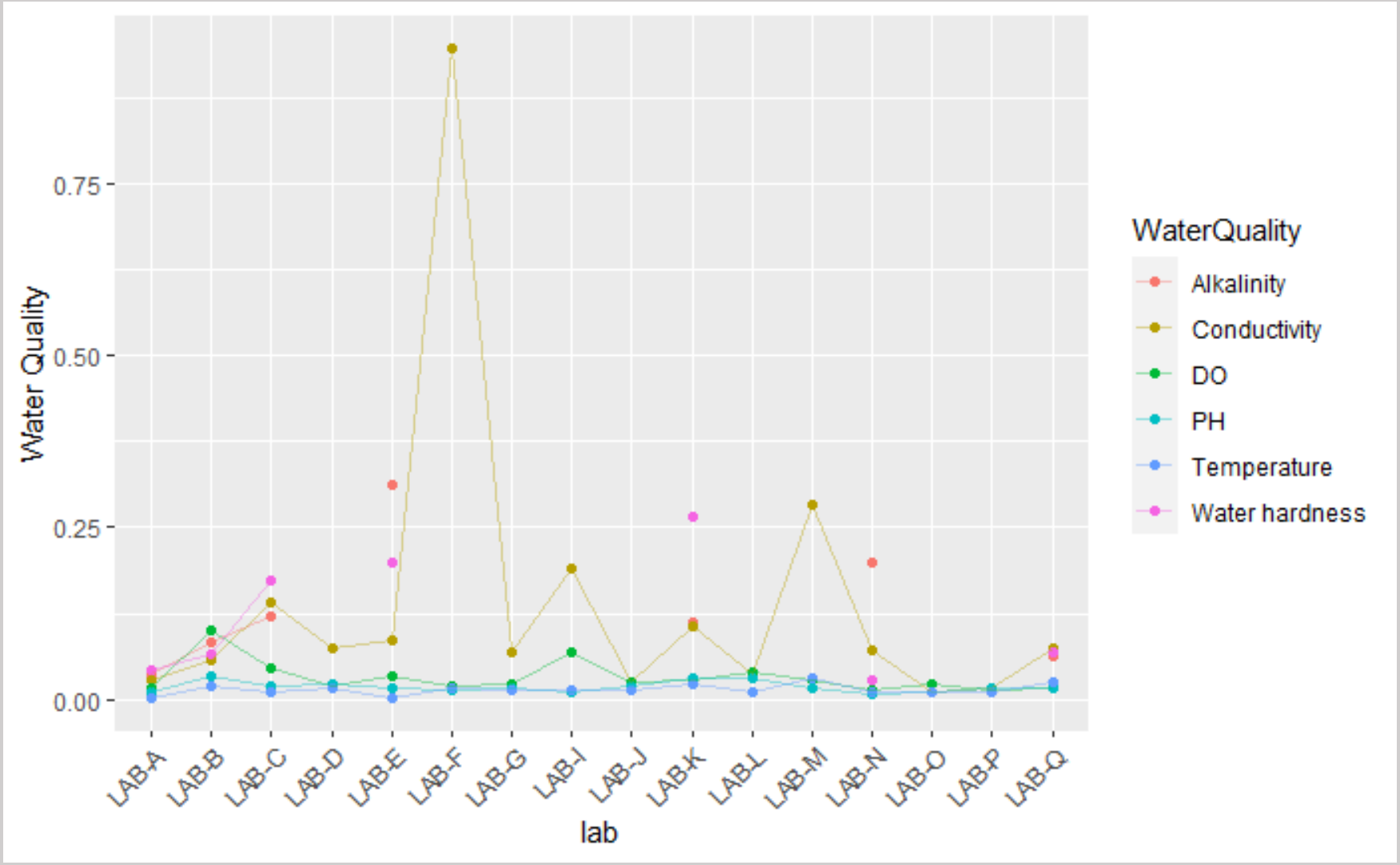



Mean Conductivity
900
600
300
A (46)
B (48)
C (67)
D (19)
E (63)
F (47)
G (7)
I (30)
J (7)
K (19)
L (27)
M (59)
N (30)
O (30)
P (80)
Q (25)
Laboratory (# of Batches)


Mean Water Hardness (mg/L)
200
150
100
50
0
A (46)
B (35)
C (59)
D (0)
E (63)
F (0)
G (0)
I (0)
J (0)
K (17)
L (0)
M (0)
N (30)
O (0)
P (0)
Q (19)
Laboratory (# of Batches)


Mean Temperature (C)
26
25
24
23
A (46)
B (48)
C (67)
D (19)
E (63)
F (47)
G (7)
I (30)
J (7)
K (19)
L (27)
M (59)
N (30)
O (30)
P (80)
Q (25)
Laboratory (# of Batches)

CV for Water Quality Parameters

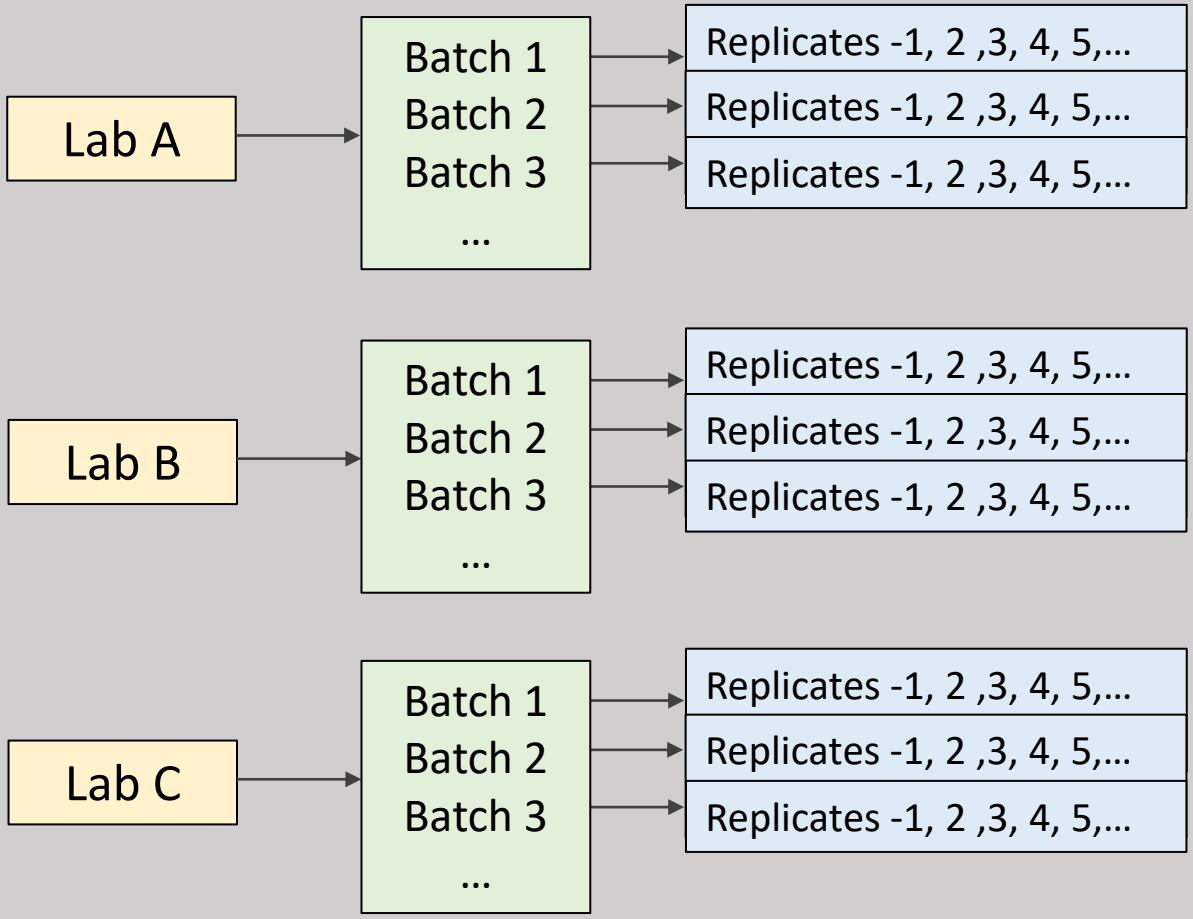


Data analysis

Data Analysis Plan

- Overall goal is to identify a handful of test factors that may cause intra-lab and inter-lab variability
- We are exploring a wide array of test variables (e.g., water chemistry, testing conditions, brood characteristics, etc.)
- Key questions to address
    - Do water quality parameters affect *c. dubia* reproductive endpoints (i.e., number of neonates per female, time to reproduction, EC25 and EC50 for ref.toxicant)?
    - Do specific lab techniques affect *c. dubia* reproductive endpoints?
    - Do brood board/culture parameters affect *c. dubia* reproductive endpoints?

Hierarchical Data Structure



Proposed Approach

- Four steps to prioritize test factors, and assess intra-lab and inter-lab variability
- Separate models to assess for reproduction and survival endpoints
- Mean and variance in control and reference toxicant as predictor variables
  - Characterizing variance as CV, SD, range
- Toxicity endpoints as response variables
  - Endpoints including # neonates/female, LC50, EC50

Proposed Approach

Step 1 – Prioritize test variables for further investigation

- Based on random forest regressions

Step 2 – Quantify batch-to-batch variability and identify test factors influencing response variables (i.e., intra-lab consistency) using structured models

Step 3 – Quantify inter-lab variability and identify test factors affecting response variables using structured models

Step 4 – Assess relative importance of intra-lab and inter-lab variables using Bayesian hierarchical models

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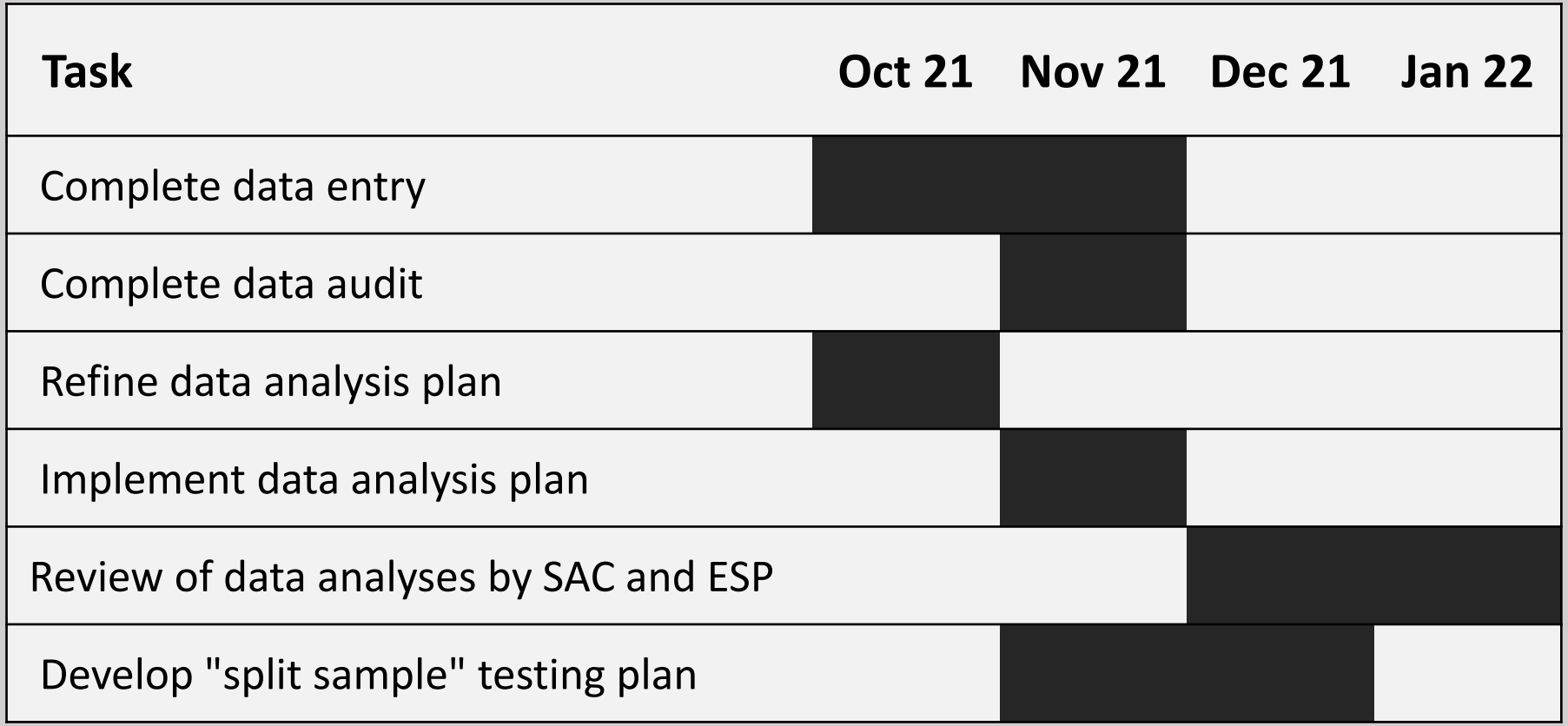
7. **Next steps and closing remarks**

Next Steps


Task
Oct 21
Nov 21
Dec 21
Jan 22
Complete data entry
Complete data audit
Refine data analysis plan
Implement data analysis plan
Review of data analyses by SAC and ESP
Develop "split sample" testing plan