C. dubia QA evaluation study

Stakeholder Committee Meeting #5

Wednesday February 23, 2022

Agenda

1. New member introductions and review of the agenda (10 min)
2. Review and update of historical data and lab method (20 min)
3. Results from exploratory and statistical data analyses (25 min)
4. Recommendations from the Science Panel (15 min)
5. Exploring split sample design options (30 min)
6. Public comments (15 min)
7. Next steps and closing remarks

Inventory of historical data and lab methods

Our Approach

Our goal was to enroll every ELAP-accredited laboratory

- 3 public utilities ; 13 private laboratories; 1 academic labs

Initial data requests

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

Follow-up phone interviews conducted to collect critical narrative information

Goals and Accomplishments

	Goal	Achieved
Lab participation	≥ 75 %	95%
Number of tests	30 tests minimum	70% of labs had ≥ 30 tests
Audit of manually-entered data	20%	100% - twice
Completeness for test data	All information in Table 4	100%
Completeness for lab methods	All information in Table 4	90%*
Completeness for culture data	All information in Table 4	90%*

* Of available data

C. dubia Historical Data Inventory

- 17 laboratories
- 1,003 tests
- > 10,000s of data points

Lab	# of Test Controls			# of Ref tox tests (incl. control)*
	Total # test controls	# of tests with 10 reps	# of tests with 20 Reps	
A	48	48	0	31
B	48	48	0	47
C	28	28	0	28
D	19	19	0	6
E	49	24	25	30
F	45	37	8	30
G	7	7	0	22
H	0	0	0	17
I	30	30	0	30
J	7	7	0	21
K	19	19	0	15
L	27	27	0	30
M	59	59	0	34
N	30	30	0	30
O	30	30	0	30
P	80	1	79	28
Q	25	25	0	23
Total	**551**	**439**	**112**	**452**

* All ref tox have 10 replicates

C. dubia Database

We now have one of the largest and comprehensive database of its kind

For each test, supporting information was collected including:

- Culture and brood board information
- Dilution water and food recipe
- Water quality parameters

We also have general laboratory data such as lab and technician experience, test practices, etc…

Methods and Test Data Collected		Missing data*
✓ Origin of brood stock	✓ Dilution water recipe	*Measured ions concentration*
✓ Age of culture, renewal frequency	✓ Source water	*Light intensity*
✓ Age window at test initiation	✓ Dilution water shelf-time	*Culture % of males*
✓ Time to reproduction	✓ YCT vendor, shelf-time	*Culture % of adult mortality*
✓ Number of replicates	✓ YCT conc. in culture/test chamber	*Culture % of unhealthy adults*
✓ Number of neonates per female	✓ Algal species	*Culture % of neonate mortality*
✓ Survival of control females	✓ Algae vendor or recipe, shelf time	*Culture % of unhealthy neonates*
✓ Test duration	✓ Algae conc. in culture/test chamber	
✓ Reference toxicant used, source	✓ Feeding frequency	
✓ Ref toxicant LC50 and IC50	✓ Lab air temperature	
✓ Water hardness	✓ Photoperiod	
✓ Water conductivity	✓ Light source	
✓ Water dissolved oxygen	✓ Lab air temperature	
✓ Water temperature	✓ Sample volume in test chamber	
✓ Test water pH	✓ Test chamber material	
✓ Test water alkalinity	✓ Test chamber volume, diameter	* Not measured or not provided

Narrative Information Collected

- ✓ Time window and trigger to end the test
- ✓ Lab techniques for determining mortality & excluding 4th broods
- ✓ Average number of *c. dubia* tests conducted per month
- ✓ Annual percentage of test failures and reason(s)
- ✓ Known or suspected causes of test failure
- ✓ Years of experience for lead technician
- ✓ Lab experience conducting the test (i.e., number of years)

Dilution Water and Food Preparation

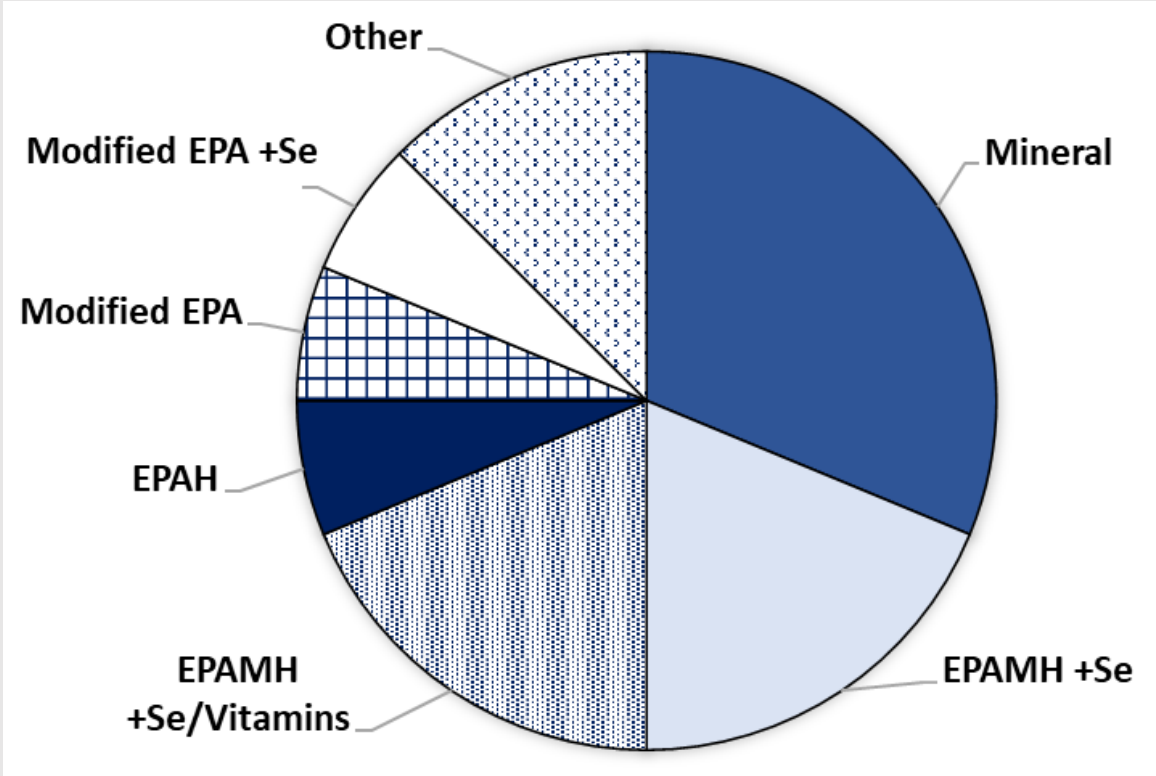
Labs using mineral water formula are fairly consistent

- All but one uses Perrier
- No other chemicals added

Labs making reconstituted water are highly variable in formulation

Similar observations were made for YCT and algae

- Labs have different combinations of purchased vs in house food sources



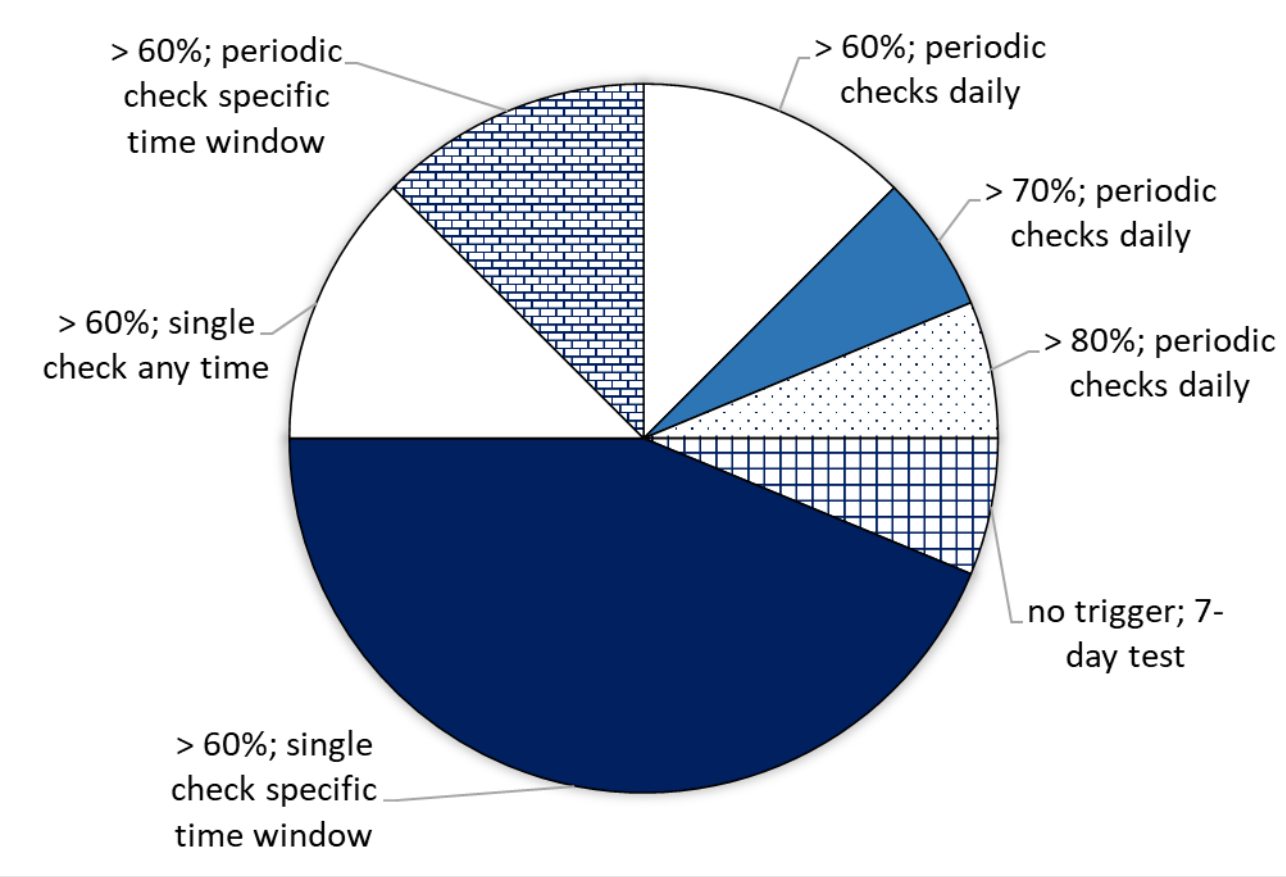
Time Window and Trigger for Test Breakdown

Trigger to end the test include

- ≥60%
- ≥70%
- ≥80%
- Standard test duration

For labs using ≥60% trigger, time window to evaluate trigger is also variable

- Which can influence likelihood of exceeding 60% trigger



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1. New member introductions and review of the agenda (10 min)
2. Review and update of historical data and lab method – TASK COMPLETED
3. **Results from exploratory and statistical data analyses (25 min)**
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Approach for Exploratory Analyses

Summary statistics were generated for biological response and water quality data

- Focus is on mean, standard deviation, CV as measures of variability

We have produced distribution plots to visualize intra- and inter-variability

We also performed simple correlations to evaluate relationship between test methods and reproduction data (i.e., neonates per female)

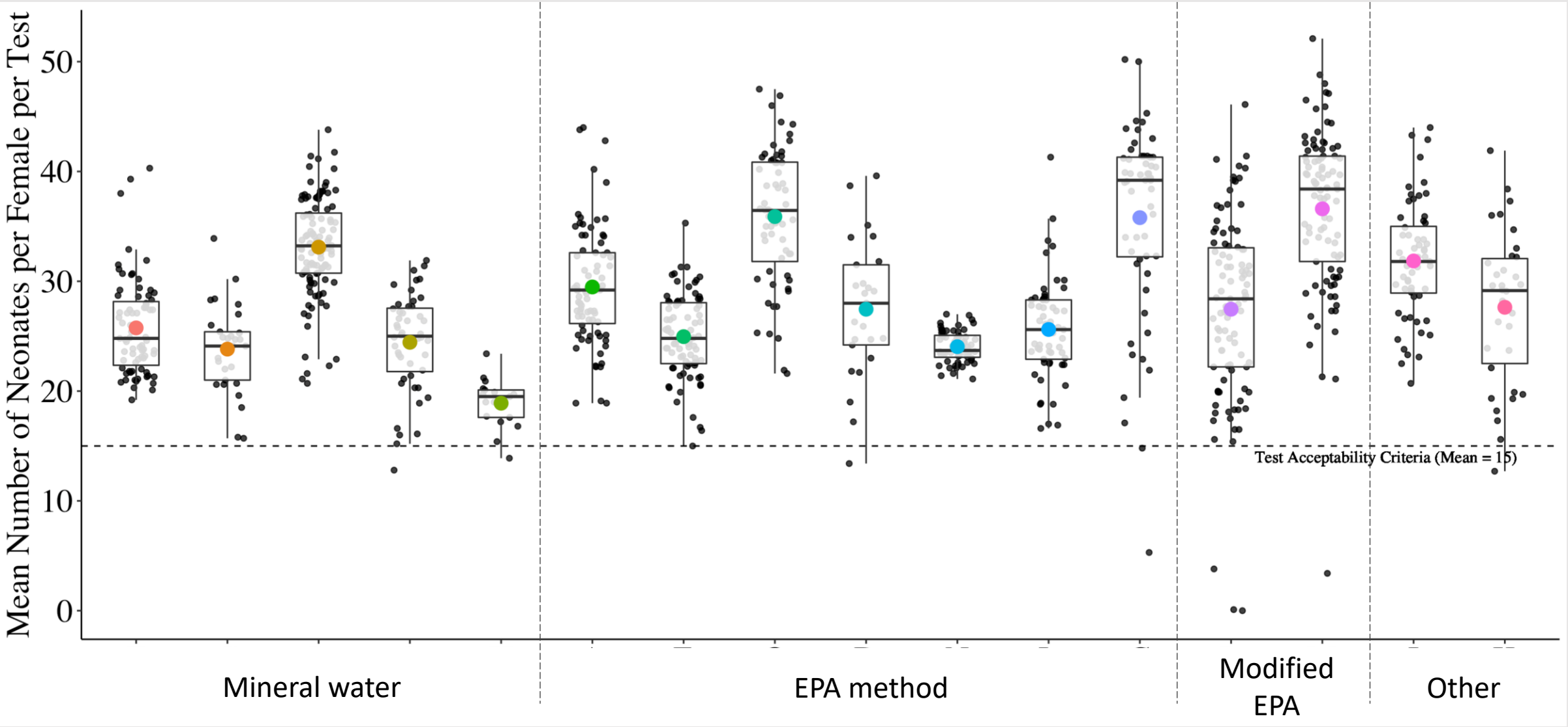
Results of Exploratory Analyses

No two labs in our database are conducting the test in exactly the same manner

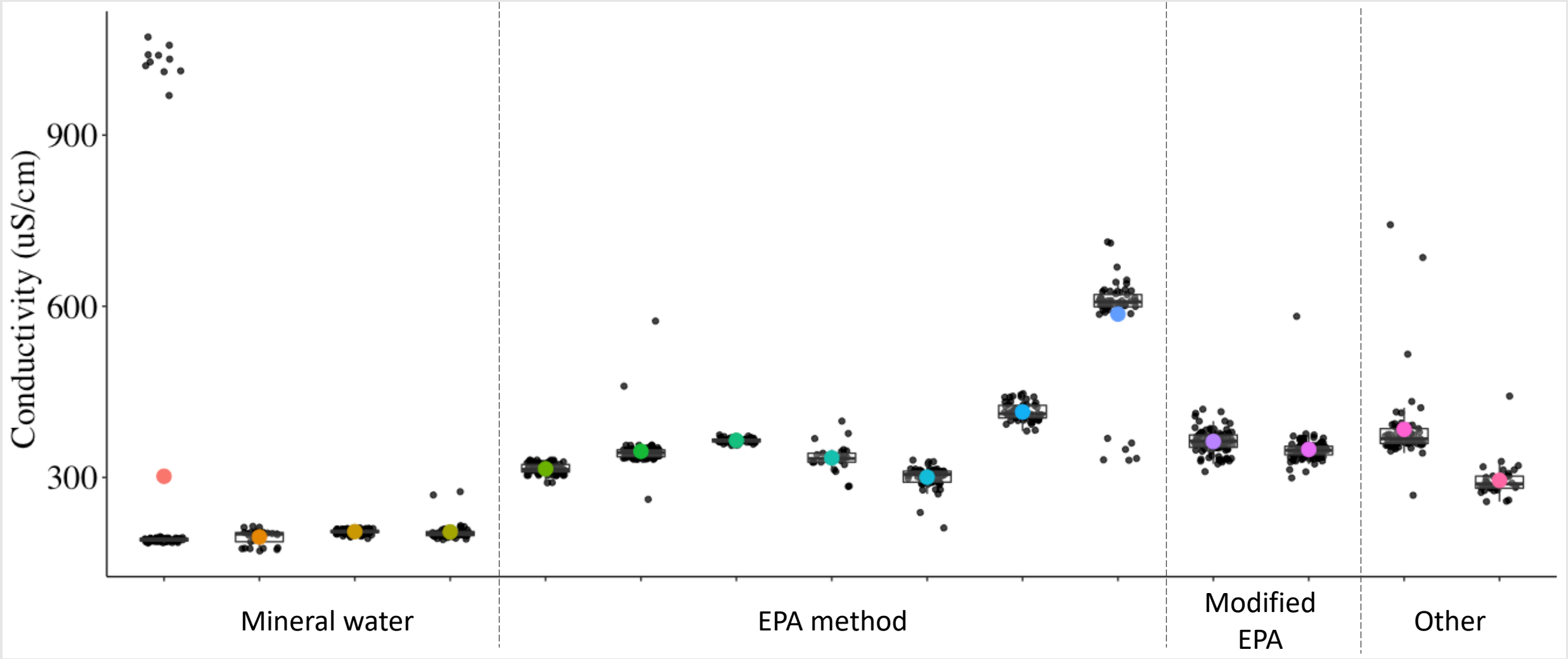
We also found high intra- and inter-laboratory variability in biological responses and water quality parameters

Confounding factors reduced the statistical power and our ability to identify key test variables

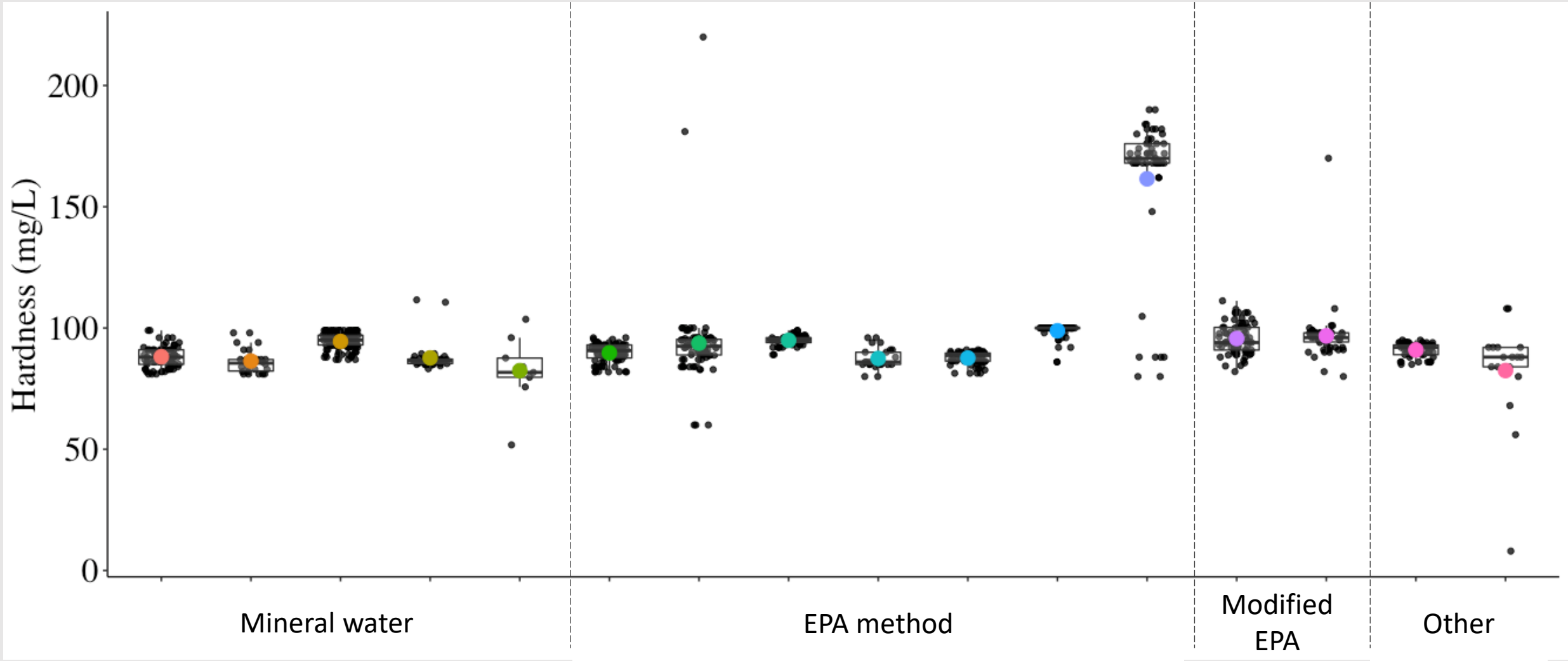
Distribution of Mean Neonates Count for Control Tests



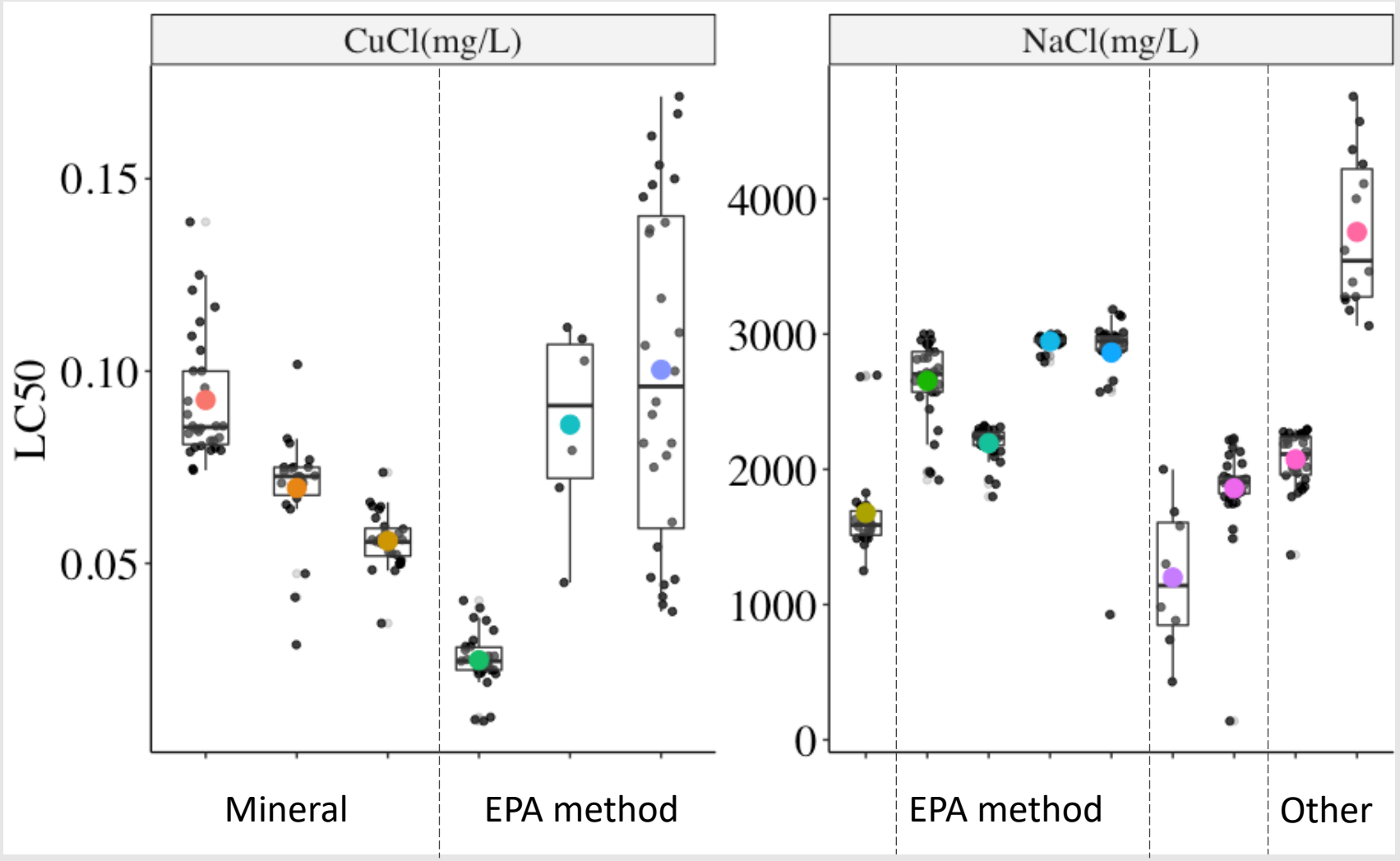
Distribution of Mean Water Conductivity for Control Tests



Distribution of Mean Water Hardness for Control Tests



Distribution of Reference Toxicant Mortality Data



Statistical Data Analysis

Overall goal is to identify a handful of test factors that may cause intra-lab and inter-lab variability

Key questions to address

- **Do water quality parameters affect *c. dubia* reproductive endpoints?**
- *Do specific lab techniques affect c. dubia reproductive endpoints?*
- *Do brood board/culture parameters affect c. dubia reproductive endpoints?*

Not enough overlap among labs to assess reliably

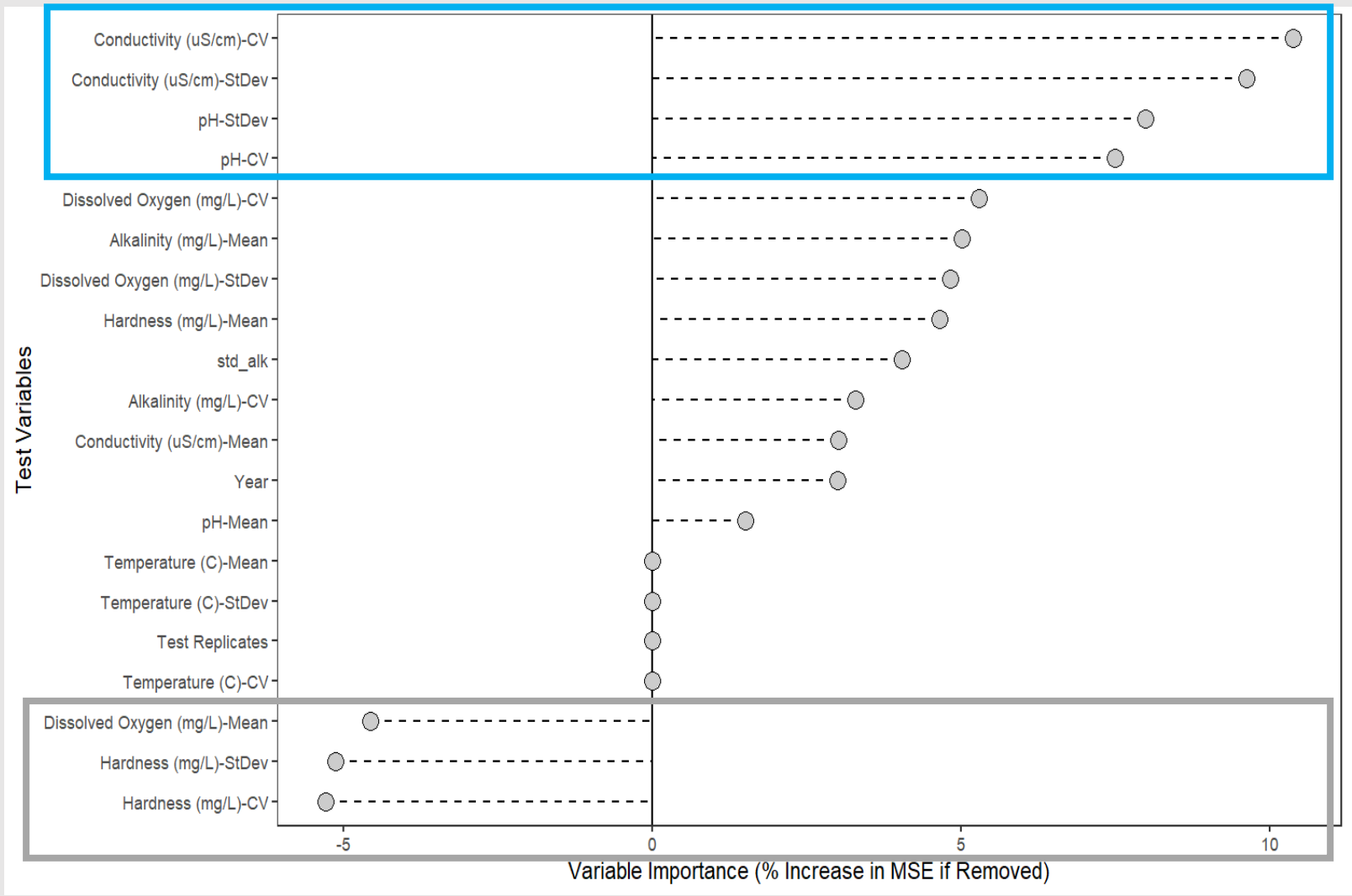
Not enough data to address

Statistical Data Analysis

- Random Forest (as described in workplan)
  - Consensus-based modeling approach
  - Calculate variable importance measures to identify key test variables
- Generalized Linear Model
  - Continuous neonate data fit for different linear distributions (gaussian, gamma, etc)
  - Modelled response as a factor of tox test variables for each lab
- Logistic & Multinomial Regression
  - Categorized neonate data as typical/atypical for each lab
  - Modelled category as a factor of tox test variables for each lab

Random Forest

- Deemed most appropriate approach
- Provides ranking of most important variables influencing test outcomes
- Can also help to eliminate variables with no effect on test outcome



Statistical analyses did not consistently identify the most important variables

CV neonates/females

Lab	Rank 1	Rank 2	Rank 3	Rank 4
G	Conductivity	pH	<-	<-
A	Conductivity	<-	pH	<-
E	Conductivity	pH	<-	Alkalinity
B	Conductivity	Temperature	<-	<-
O	Conductivity	<-	Brood Age	<-
C	pH	Conductivity	DO	<-
P	pH	Conductivity	<-	<-
H	pH	<-	DO	<-
I	pH	<-	Alkalinity	Brood Age
Q	DO	Conductivity	<-	pH
L	DO	<-	Brood Age	<-
D	Year	DO	<-	<-
F	Brood Age	<-	Alkalinity	DO
K	Temperature	<-	DO	<-
M	Temperature	DO	<-	<-
N	Hardness	<-	Year	Conductivity

Mean neonates/females

Lab	Rank 1	Rank 2	Rank 3	Rank 4
G	Conductivity	DO	<	Brood Age
A	Alkalinity	Conductivity	<	<
P	Alkalinity	pH	DO	<
E	DO	pH	<	Hardness
H	DO	<	pH	<
B	DO	Temperature	<	Year
I	DO	Temperature	Alkalinity	Hardness
Q	DO	Year	Temperature	<
L	Brood Age	<	pH	Year
D	Brood Age	<	Temperature	<
F	Brood Age	<	pH	<
K	Temperature	<	<	pH
C	Temperature	Year	DO	pH
M	Year	Conductivity	<	<
N	Hardness	Year	<	Alkalinity
O	Hardness	pH	Temperature	Year

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Science Panel Recommendations

Based on critical review of summary statistics, distribution plots, random forest and other modeling results, the Panel concluded that:

- Statistical analyses alone will not help identify key test parameters to optimize
- Combination of narrative information, data analysis output and best professional judgement is needed
- Any additional testing should assess both lab technique and water quality test parameters

Split Sample Testing Design

Two categories of test variables are recommended by the Science Panel for consideration to assess inter- and intra-lab comparability

- Water chemistry
- Lab techniques

Options for Split Samples to Evaluate the Influence of Water Chemistry

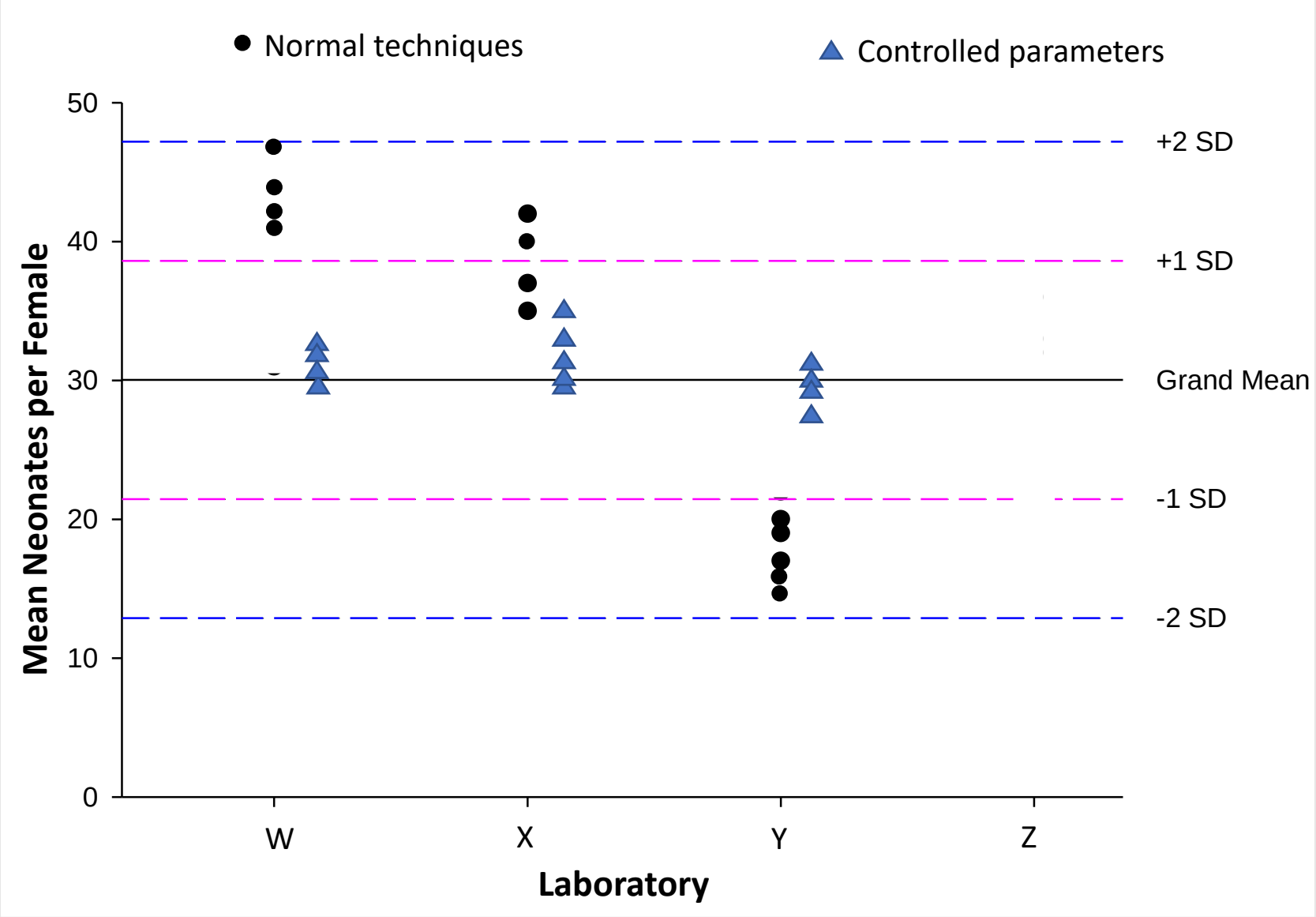
- Conductivity
- Hardness
- Alkalinity
- pH
- Minerals

Options for Evaluating the Influence of Lab Techniques

- Specific test duration
- Narrower time window for neonates at test initiation
- Reduced maximum age at test initiation
- Specific time window for daily neonate counts
- Defined age window for brood board

Note: Imposed lab techniques will be compared to normal lab techniques

Example graphic showing reduced variability when test is conducted using similar biological parameters. **Data not real!**



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We may also want to send a reference toxicant possible as “positive” control to investigate influence of control variability on calculated EC/IC 50.

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Next Steps and Tentative Schedule

Activities	Tentative Deadlines
Refine study questions and design for split sample testing	Mid March 2022
Present draft study design to Science Panel for approval	End March 2022
Develop QAP including logistics for sample preparation, shipping, lab data collection and submittal formats	Mid April 2022
Communicate with labs for outreach, training, timing and costs	Mid April 2022