Ceriodaphnia dubia Quality Assurance Study

SCCWRP COMMISSION

MARCH 3, 2023

CONTEXT AND NEED FOR THE STUDY

- Toxicity Provisions adopted by the SWRCB 12/1/2020
  - Includes numeric effluent limitations using the most sensitive species
- Stakeholders have expressed concerns about C. *dubia* chronic toxicity test reliability
  - Discussions revolved around variability within and among labs
- SWRCB delayed statewide implementation of the Toxicity Provisions for the C. *dubia* chronic test until 1/1/2024
  - Instructed SWRCB staff to conduct a study to identify and reduce test variability

THE STUDY **IS**:

- A quality assurance study to determine whether laboratory best practices might be recommended to improve laboratory performance

THE STUDY IS **NOT**:

- A method validation study to determine whether *C. dubia* should be used in California regulatory programs
- A study to estimate false positive or false negative rates using the test of significant toxicity (TST)

STUDY DESIGN

- Form a Science Panel and a Stakeholder Committee — **COMPLETED**
- Evaluate sources of variability in the C. dubia chronic toxicity test
  - Historical data analysis — **COMPLETED**
  - Split sample testing to collect new data — **COMPLETED**
- Directed laboratory testing to minimize the sources of variability — **IMPLEMENTING NOW**
- Evaluate efficacy of test method refinements to reduce variability
  - Repeat split sample testing using recommended guidance — **BY JULY 2023**

Stakeholder Advisory Committee

- Katie Fong (SWRCB)
- Amelia Whitson (EPA Region IX)
- Veronica Cuevas (RWQCB)
- Mitch Mysliwiec (Wastewater)
- Jian Peng (Stormwater)
- Sarah Lopez (Agriculture)
- Peter Arth (Private Laboratories)
- Josh Westfall (Public Laboratories)
- Annelisa Moe (NGO)

Expert Science Panel

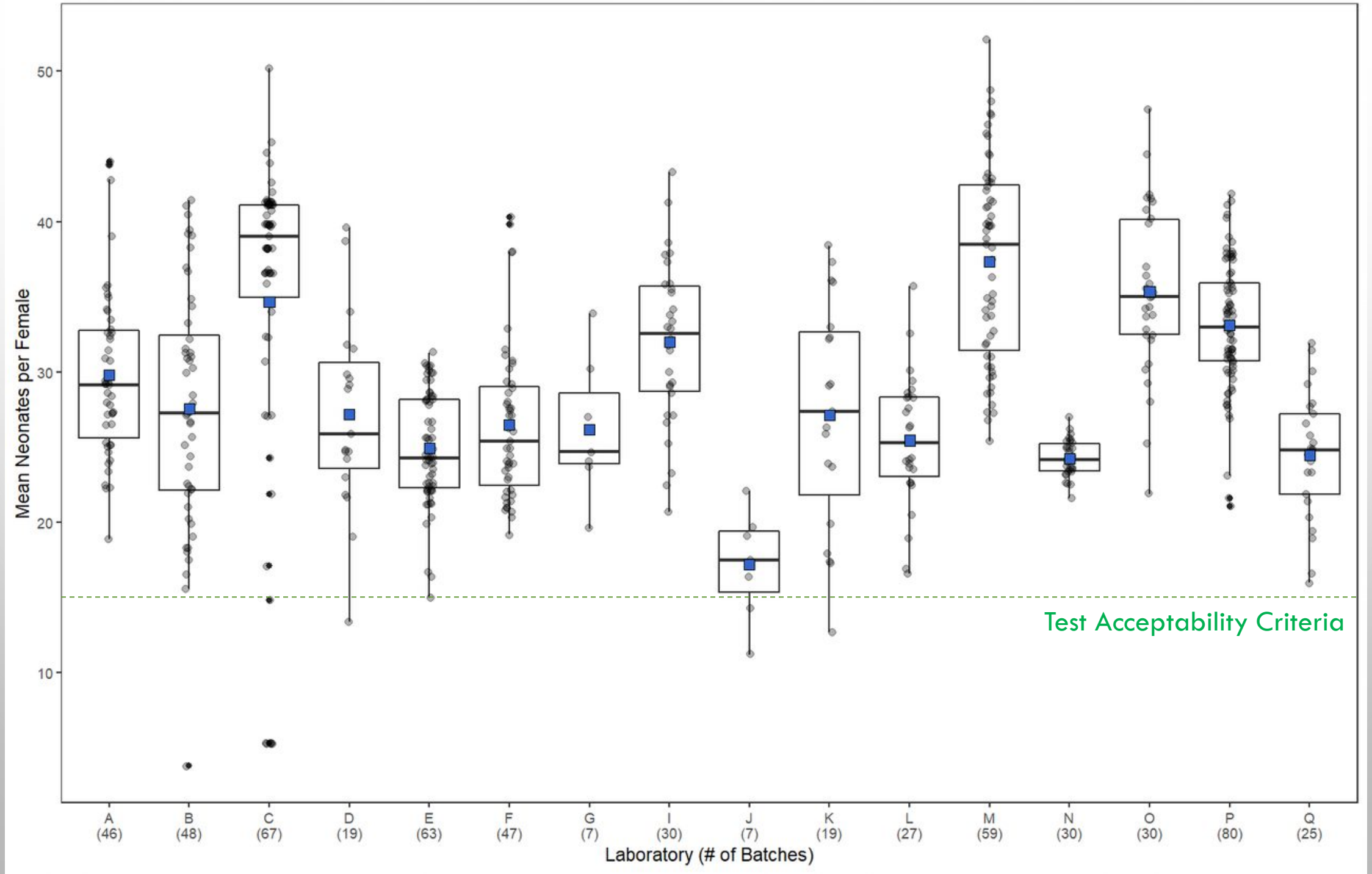
- Teresa Norberg-King (Formerly US EPA)
- Robert Brent (James Madison University)
- Howard Bailey (Nautilus Environmental)
- Leana Van der Vliet (Environment Canada)
- A. John Bailer (Miami University, Ohio)

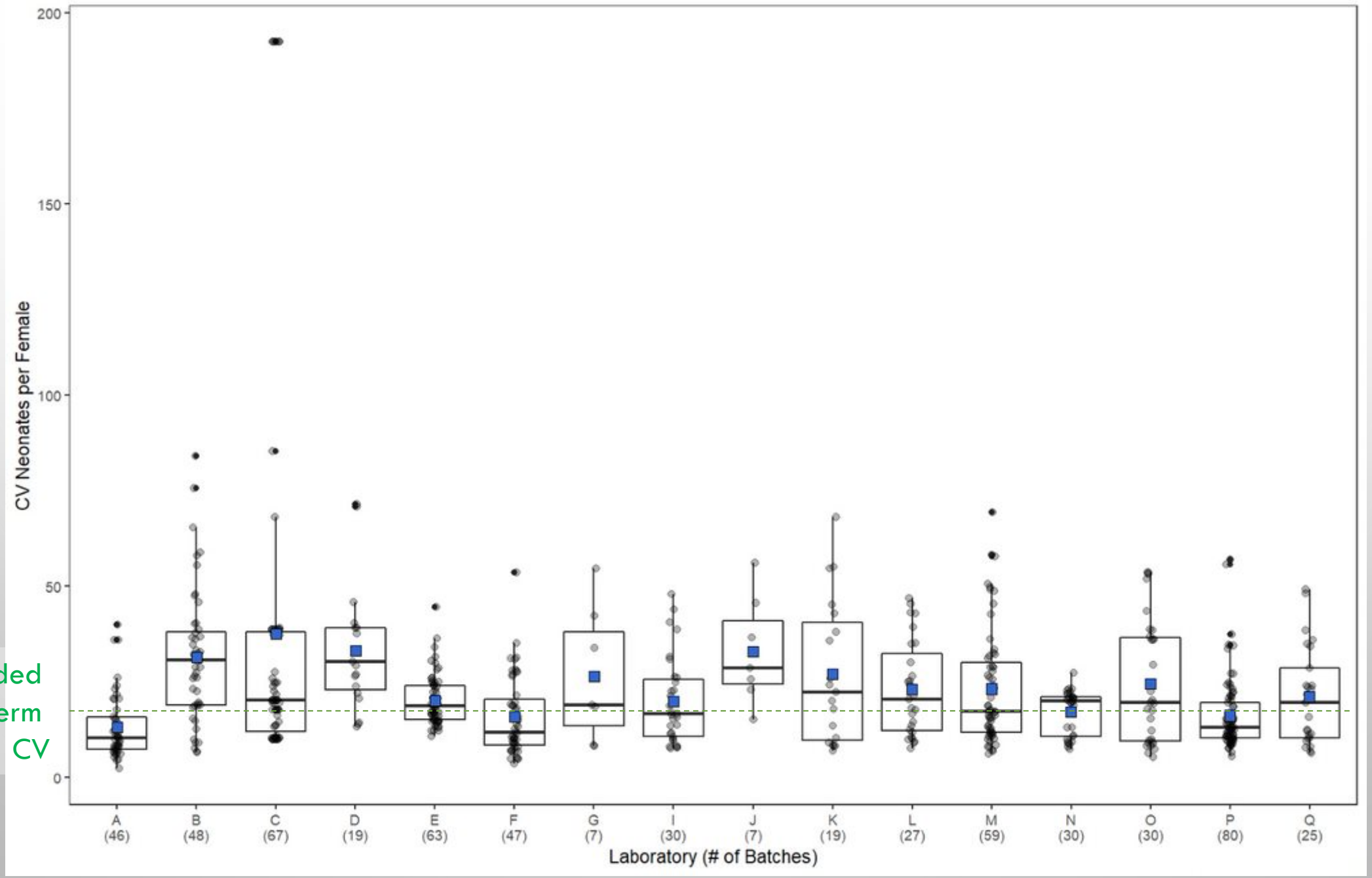
HISTORICAL DATA ANALYSIS

- All 17 California accredited labs for the C. *dubia* test
- Last 3 years of data; close to 1,000 tests
- SOPs, QA Plans, technique questionnaire and interviews

<u>Bottom line:</u>

- No laboratory runs the test exactly the same way
- No individual lab technique appeared to be the single biggest source of variability


Mean Neonates per Female
50
40
30
20
10
Test Acceptability Criteria
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)



Recommended Long term CV

SPLIT SAMPLE TESTING

- Labs used their existing protocols
- Mandated data collection procedures

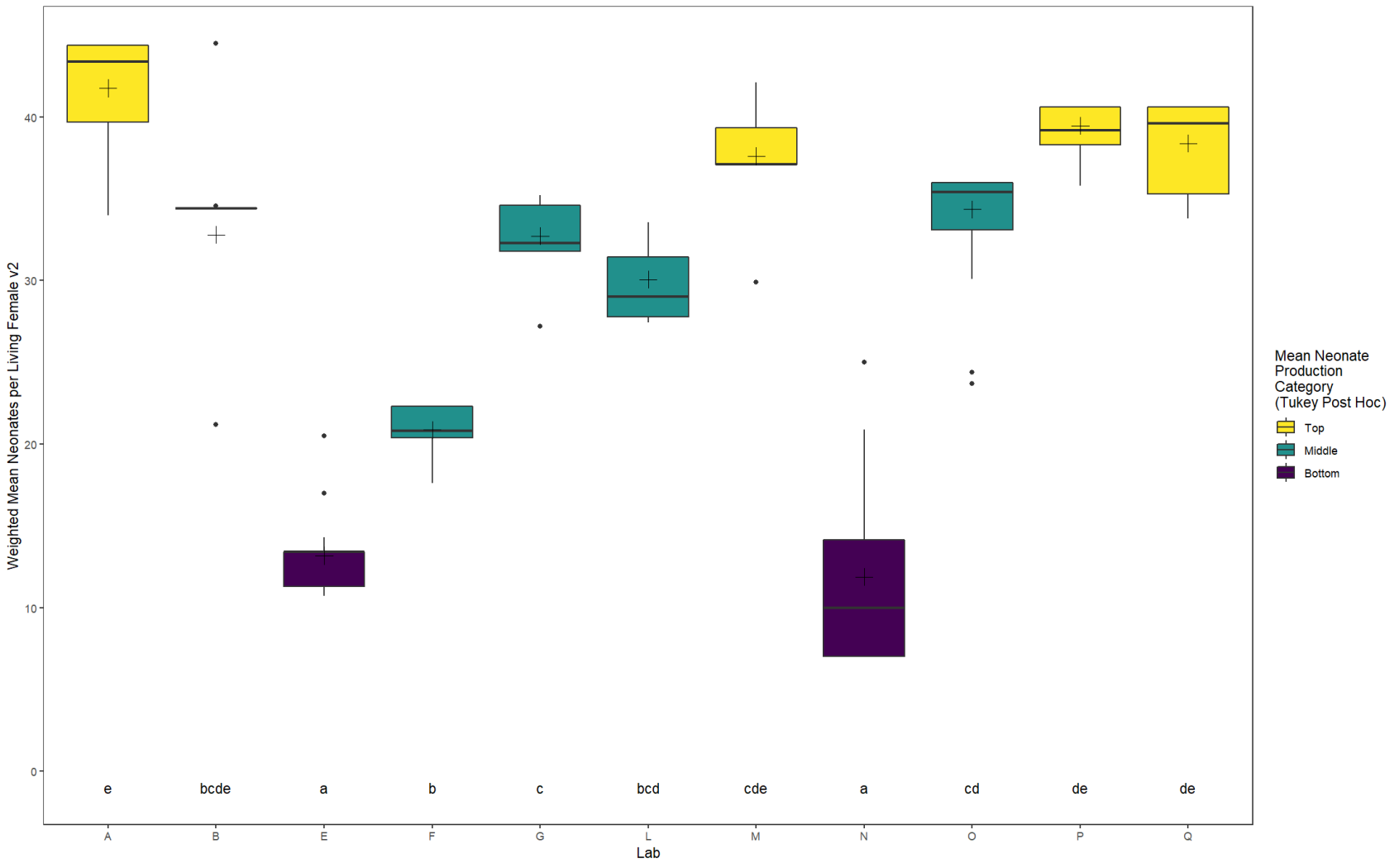
<u>Science Panel wanted to focus on three aspects:</u>

- Within and among lab control variability based on repeated testing
- Within and among lab control variability due to water type
- Differences among labs in toxic endpoint (Inhibition concentrations at 25% and 50%)

Intercalibration Study Take Home Messages

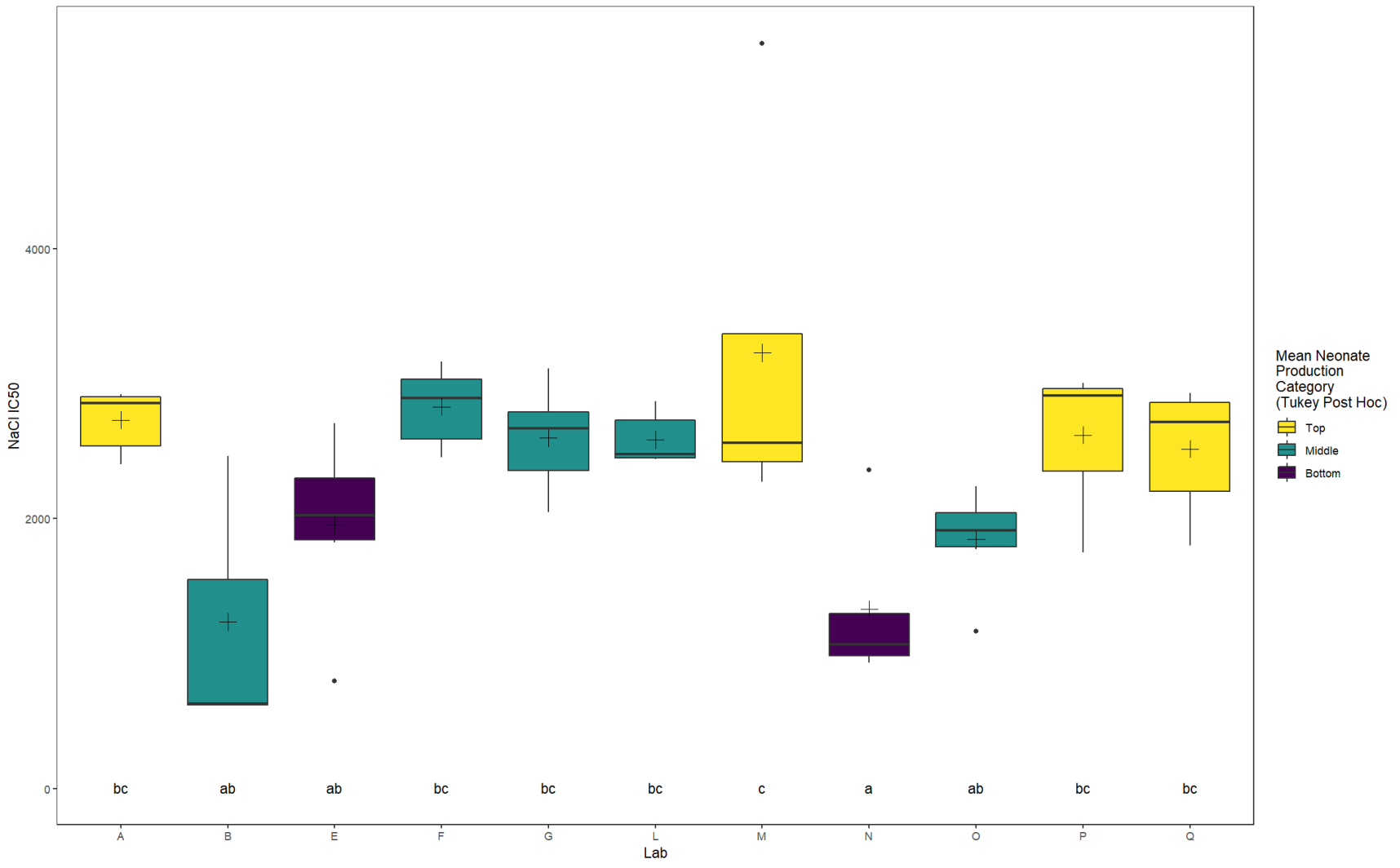
- There was a difference between laboratories for neonate production in control or "unspiked" samples
  - Some labs did not pass test acceptability criteria
- The differences between labs was not attributable to water type
- The toxic endpoint (IC50) differences between labs appeared to be less than the differences in control neonate production
- No single lab technique was strongly related to differences between labs

Comparison of mean neonates per female from unspiked samples



- Each lab N=6
- SCCWRP supplied waters
- Significant differences among labs
  - No effect of water or lab*water interaction
- Labs fall into three general groups

Comparison of IC50s Among Laboratories Using Sodium Chloride



- Each lab N=6
- Color coding based on mean neonate production from previous graph
- Significant differences among labs
  - Less differences than in neonate production

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Since There's No Clear Answer from Round 1…

- Both the Stakeholder Committee and the Science Panel recommend laboratory education and training

- Consist of lab visits by Science Panel members followed by Roundtable Workshops with all participating labs
  - Generate a list of lab techniques to potentially standardize

- Recreate the intercalibration, with the standardization that comes from the education and training

OUR NEXT STEPS

- Lab visits are scheduled for week of March 13th
- Roundtables are being scheduled for the end of March
- Second intercalibration will begin in April
- List of lab technique recommendations finalized by end of July
  - Final reports approved by end of September