Development of Molecular Tools for Stressor Identification in Sediment Toxicity Tests

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Southern California Coastal Water Research Project

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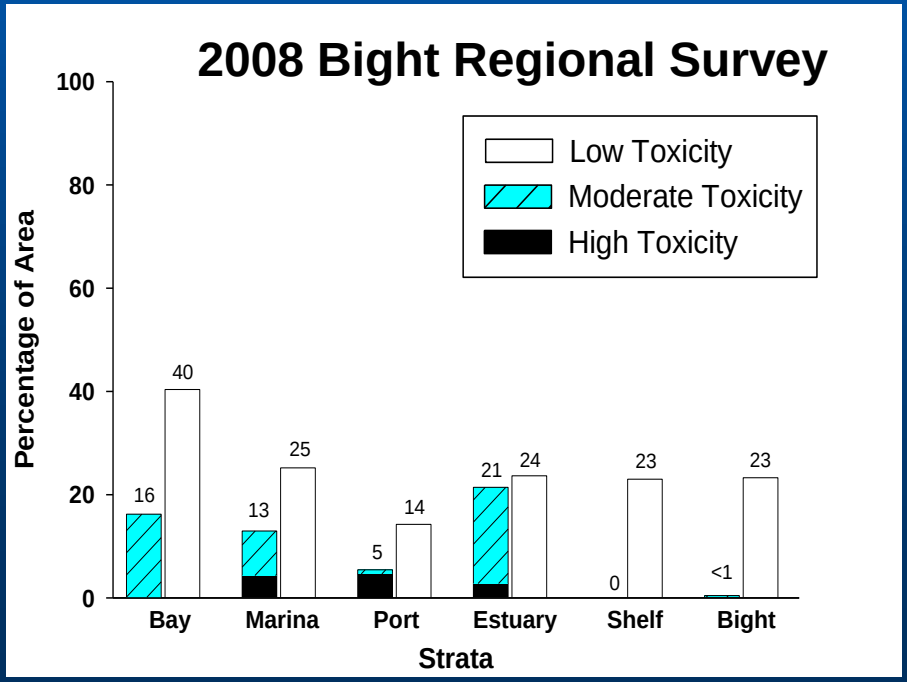
Understanding Sediment Toxicity is Essential

- **Sediment toxicity is an important factor in sediment quality assessment and management actions**
  - **30-50% of sediments in urban embayments are toxic**

- **Identifying the cause of toxicity is difficult**
  - **Complex mixtures of contaminants are present**
  - **Test endpoints not toxicant-specific**
  - **Few hotspots with strong gradients of contamination**

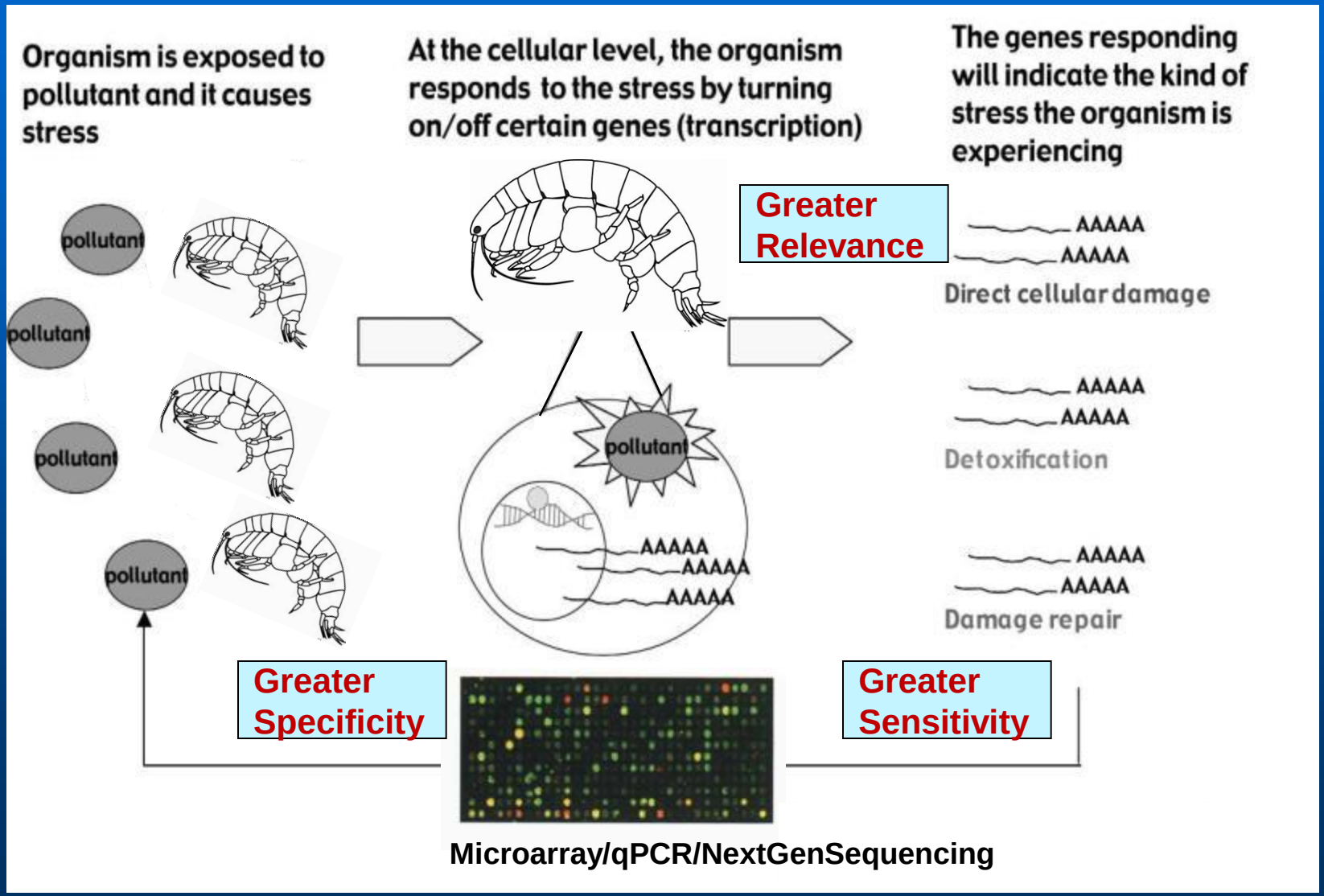


Better Stressor Identification Methods Are Needed

- **TIE results are frequently inconclusive or nonspecific**
  - **Chemical treatments have limited specificity**
  - **Chemical extraction/fractionation alters bioavailability**
- **Limited range of application**
  - **Require highly toxic sediments**
- **Limited ability to identify new types of stressors**
  - **Have to determine chemical characteristics first**
  - **Stressor-specific treatments may not be available**
- **TIEs not applicable to resident organisms**
  - **Rely on laboratory manipulations of sediment**

Can molecular methods provide a better tool?

Molecular TIE Approach



Molecular TIE Development Program

- **Focus on amphipod *Eohaustorius estuarius***
  - **Benchmark test species for Canada and U.S. monitoring programs**
- **Goal is to develop and evaluate a new approach for TIE based on gene expression**
  - **Use existing test methods (10-day survival)**
  - **Reduce need for manipulations and iterations**
- **Multiple partners**
  - **San Francisco Bay Regional Monitoring Program (RMP)**
  - **UC Berkeley**
  - **Environment Canada**
  - **NOAA (Hollings Marine Laboratory)**
  - **UC Davis Marine Pollution Studies Laboratory**

Study Questions and Design

1. **Can the microarray be used to measure amphipod gene expression?**
   - **Binding of amphipod RNA to probes and variability**
   - **Identify consistent responses**
2. **Does gene expression differ with exposure to different toxicants?**
   - **Analyze training samples and compare patterns**
3. **Is the information useful for stressor identification?**
   - **Develop a statistical method to interpret the results**
   - **Analyze independent validation samples**

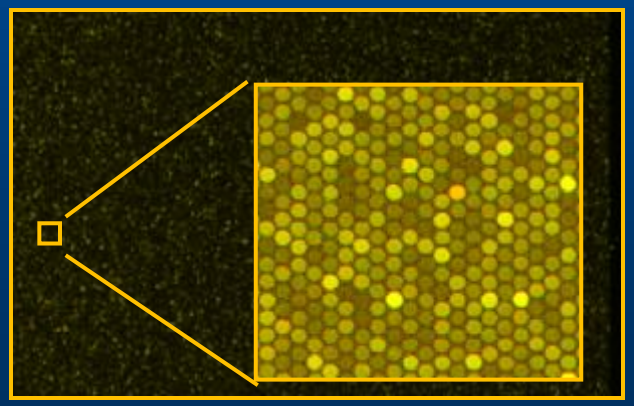
Microarray Analysis

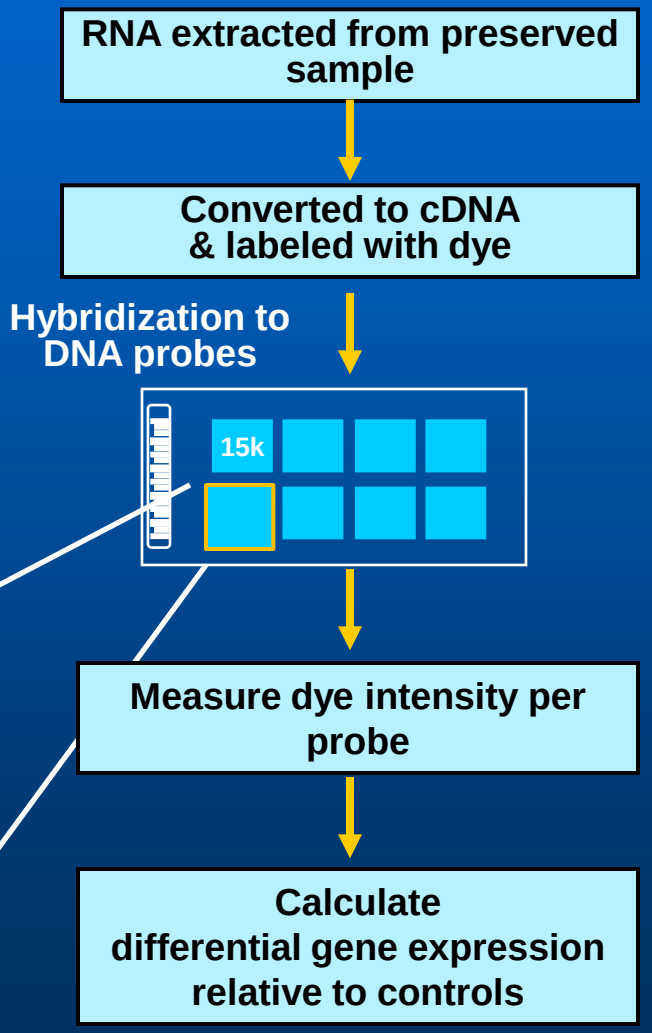
- 8,610 amphipod gene sequences
- Compare sample to control

Differential expression

= $\log_2$(sample/control)

2 = four-fold increase in gene expression





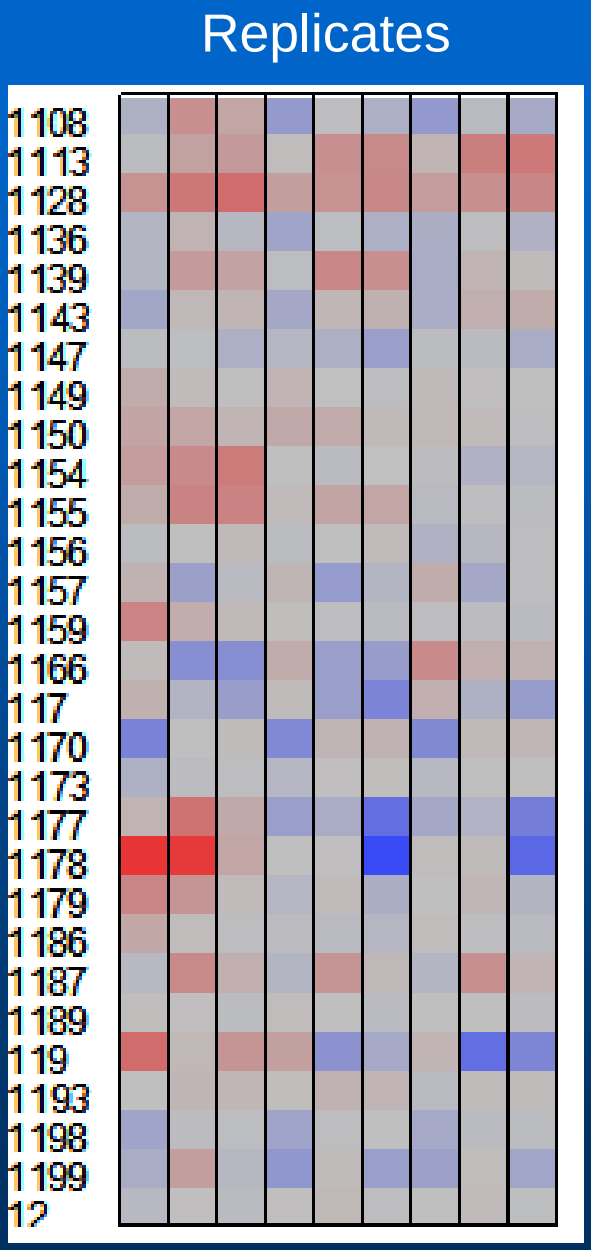
Training Data Set

- **Diverse toxicants and mechanisms of action**
  - **Pyrethroid pesticides**
  - **Trace organics**
    - **Pesticides & PAHs**
  - **Other**
    - **Ammonia & metals**
- **Focus on pyrethroid pesticides**
- **Doses near LOEC**
- **Different exposure matrices and durations**
  - **Matched controls**
- **2-3 replicates**
  - **5 amphipods/replicate**

Treatment	Concentration	Matrix	Survival (% of Control)
Bifenthrin	0.01 ug/L	Water	80
Bifenthrin	0.03 ug/L	Water	55
Cypermethrin	0.01 ug/L	Water	100
Cypermethrin	0.03 ug/L	Water	87
Cyfluthrin	0.8 ug/kg	Sediment	88
Cyfluthrin	1.6 ug/kg	Sediment	60
Fipronil	10 ug/kg	Sediment	80
Chlordane	100 ug/L	Water	58
DDE	4 ug/L	Water	80
DDT	2400 ug/kg	Sediment	58
Pyrene	10 ug/L	Water	38
Pyrene	25000 ug/kg	Sediment	90
Ammonia	100000 ug/L	Water	100
Copper	250 ug/L	Water	100
Copper	750 ug/L	Water	98
Cd	10000 ug/l	Water	83

Candidate Gene Selection

- **Not all gene probes provide consistent or relevant information**
- **Multistep process used to identify genes most likely to represent toxicant-specific response**
  - **Consistent response among replicates**



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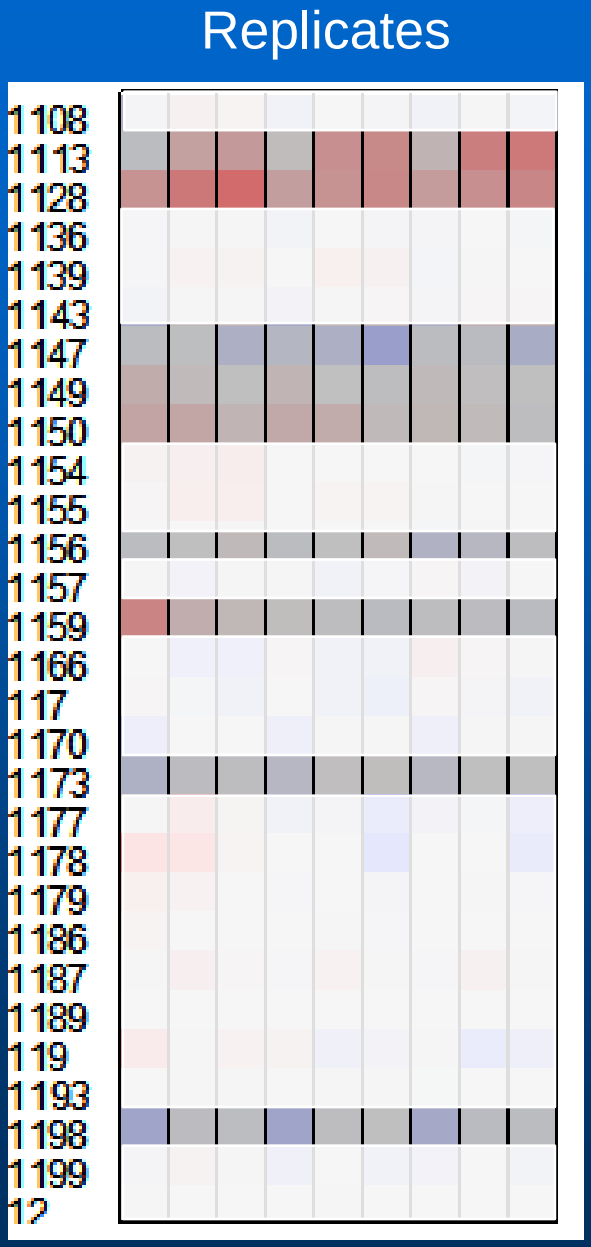

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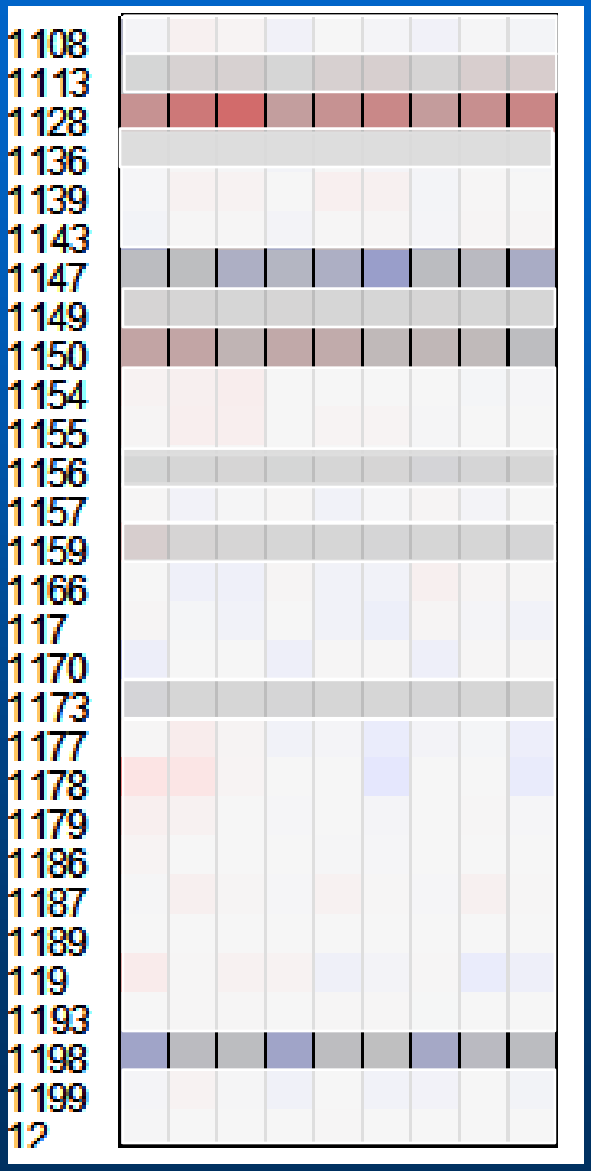
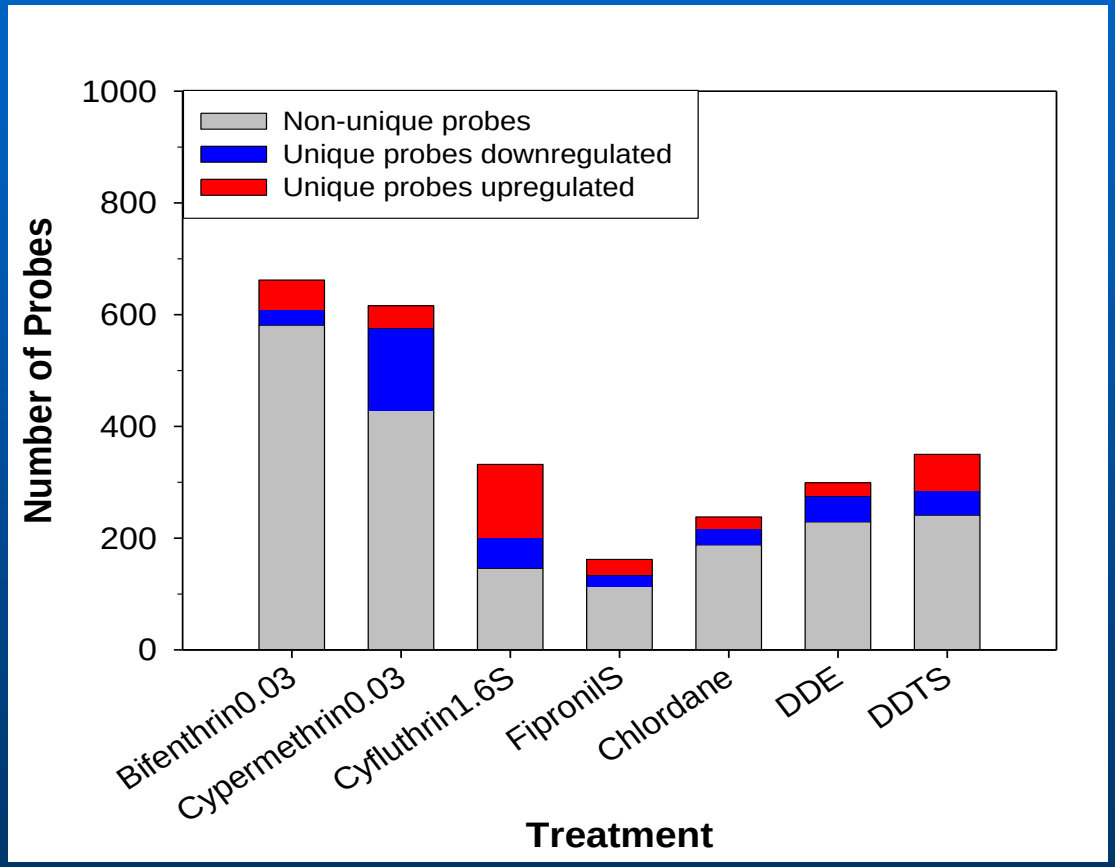
  - **Calculated mean to minimize effect of outliers**

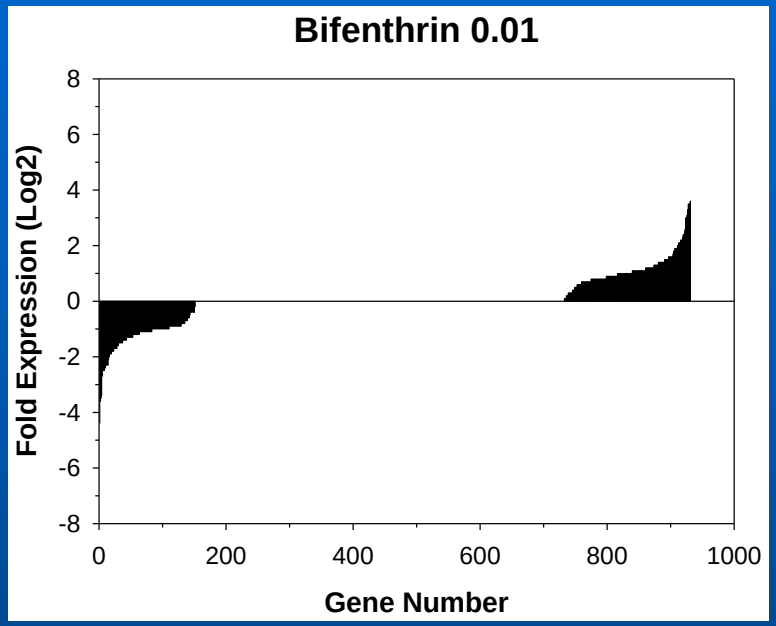


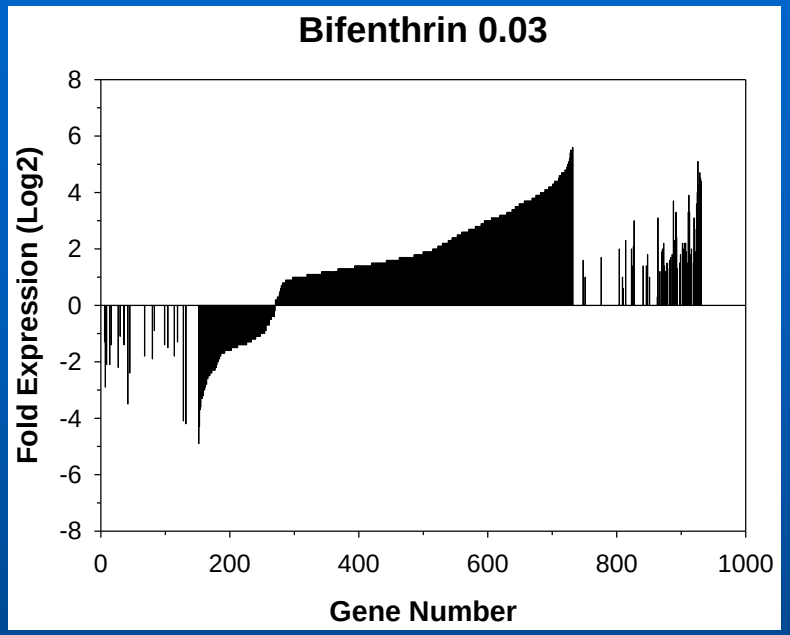
Gene Expression Differs Among Toxicant Types



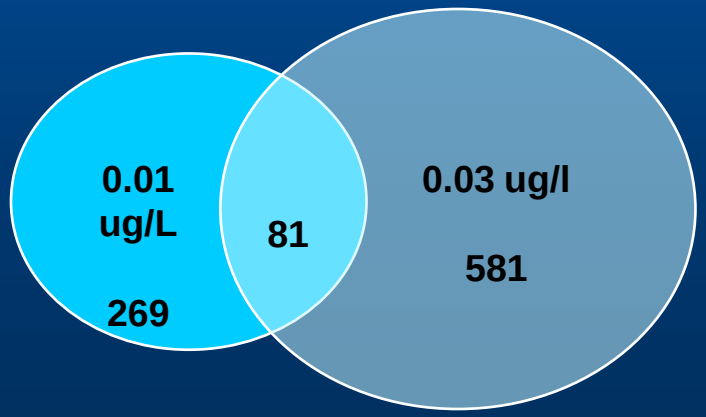
- **21-257 uniquely expressed probes for each chemical**

Dose Response: Bifenthrin





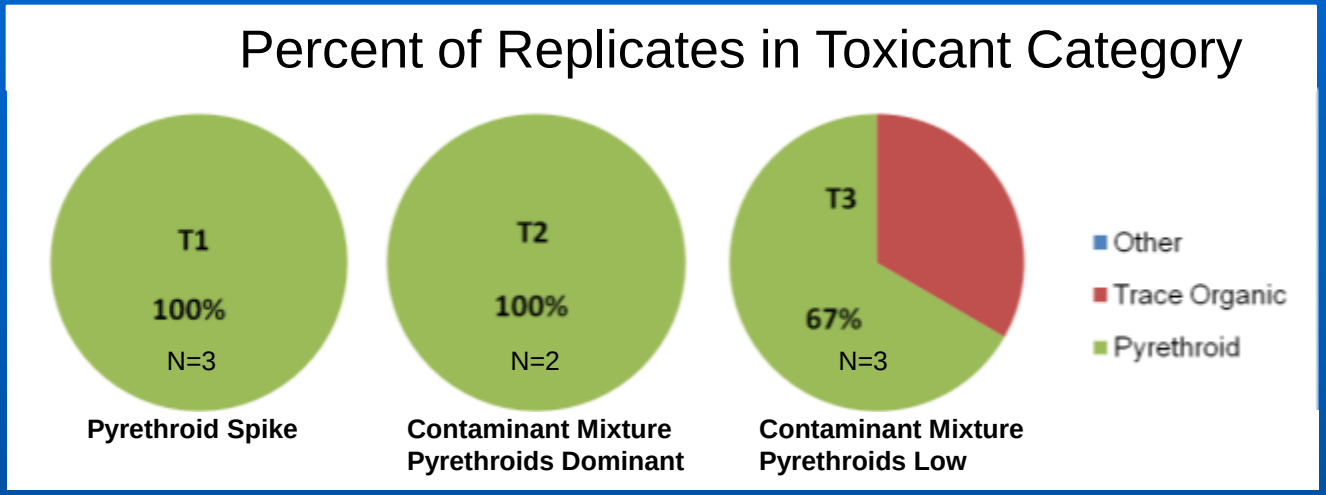
- **Concentration, exposure time, and test matrix influence gene expression**
- **Relatively few candidate genes in common between concentrations**
- **Differences among control groups also present**



Evaluation of Toxicant Identification Ability

- **Develop a statistical model to interpret (classify) results**
  - **Random Forest method used in initial studies**
    - **Training sample data used to develop prediction "trees" for each class**
    - **Trees saved and used to classify evaluation samples**
  - **Model developed for 3 classes of toxicants**
    - **Pyrethroids, Trace Organics, Other**
- **Applied model to independent evaluation samples**
  - **T1: Reference sediment spiked with cyfluthrin**
  - **T2: Ballona Creek estuary sediment (toxicity due to pyrethroids)**
  - **T3: San Francisco Bay sediment (cause of toxicity unknown)**

Evaluation Results



- **Encouraging prediction results**
  - **Correct classification for 2 samples with identified cause of toxicity**
  - **No TIE data to verify SF Bay sample results**
  - **Preliminary evaluation due to limited number and diversity of evaluation samples**
    - **Intentional emphasis on pyrethroids**

Summary

- **Substantial progress so far**
  - **Successful amphipod RNA sequencing**
  - **Microarray available for use/evaluation**
- **Initial results encouraging**
  - **Probes bind amphipod RNA successfully**
  - **Distinctive expression patterns apparent for different contaminant treatments**
    - **Dose or method variations may influence results**
- **Initial evaluation of classification potential encouraging**
  - **Additional refinement and validation needed**
  - **Specifics of approach likely to evolve with further development**

Next Steps

- **Investigate interlaboratory comparability of microarray analysis**
  - **Developing study design and partners**
- **Refine and expand stressor identification approach**
  - **Greater standardization of training samples**
  - **Additional stressors (e.g., PAHs, fine sediment)**
  - **Alternative data interpretation methods**
- **Apply approach in focused stressor identification studies**
  - **Compare to traditional TIE methods**