Development of Molecular Tools for Stressor Identification in Sediment Toxicity Tests

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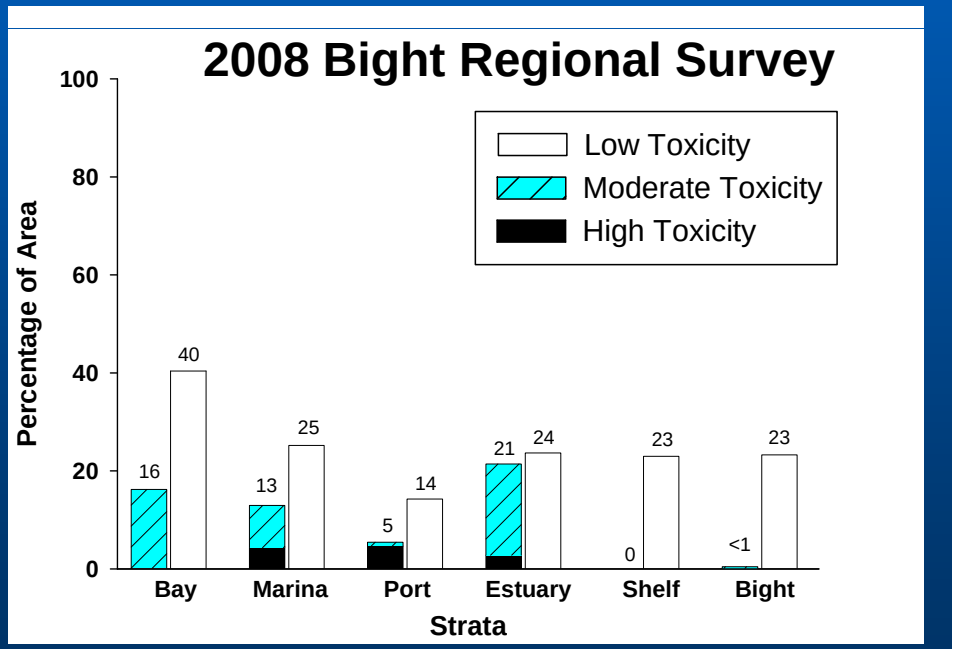
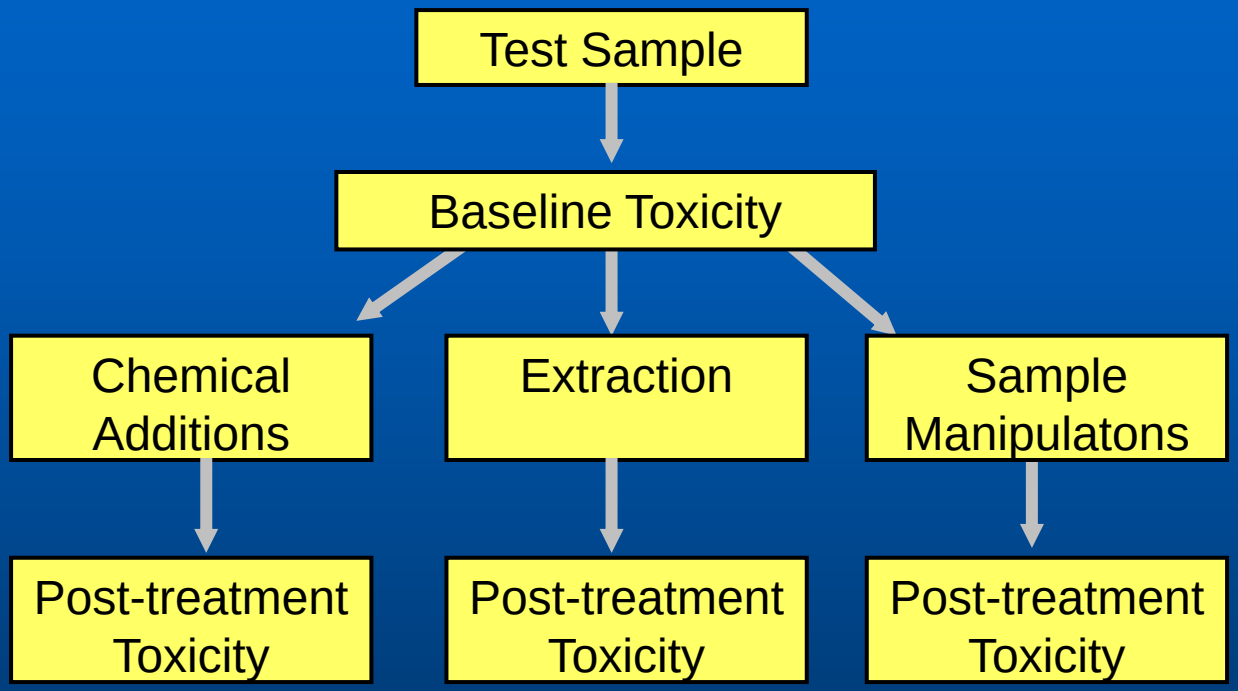
Understanding Sediment Toxicity is Essential

- **Sediment toxicity is an important factor in sediment quality assessment in bays and estuaries**
  - **Cleanup targets are often based on reducing toxicity**
- **Identifying the cause of toxicity is difficult**
  - **Complex mixtures of contaminants are present**
  - **Ammonia, pesticides, and PAHs often present at levels of concern**
  - **Response characteristics (mortality, growth) not toxicant-specific**



Toxicant Identification Evaluation (TIE)

Traditional Approach



Various contaminant-specific treatments applied to sample

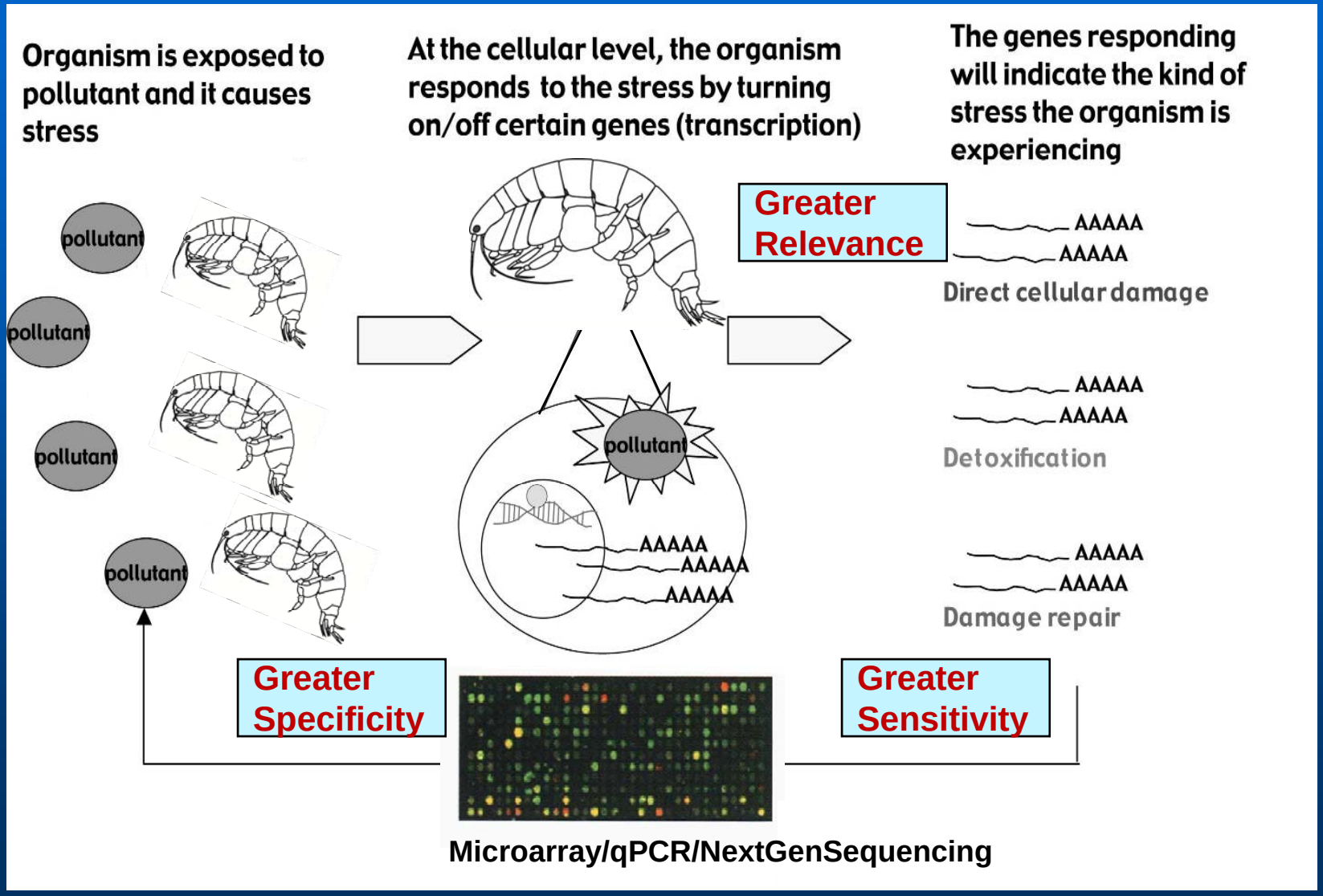
Changes in toxicity following sample treatments indicates type of toxicant

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

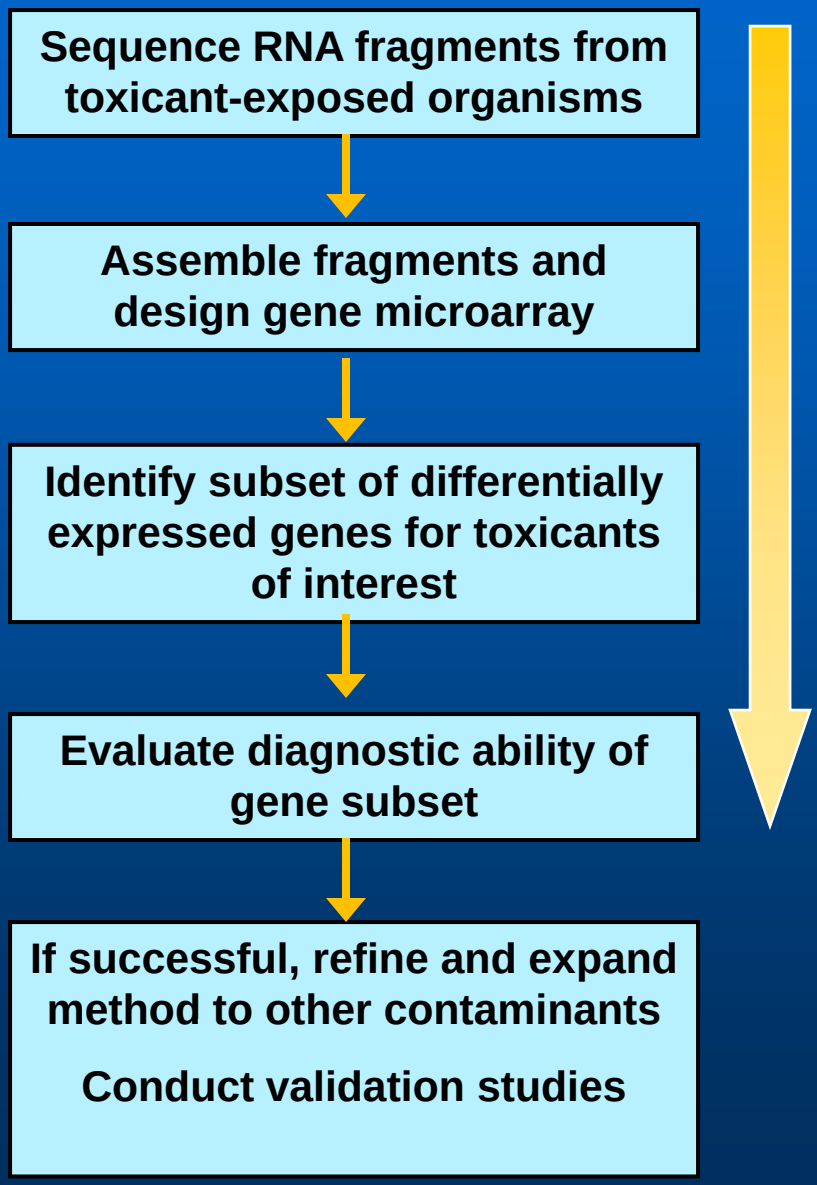


Molecular TIE Development Program

- **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 Estuary Institute**
  - **UC Berkeley**
  - **Environment Canada**
  - **NOAA (Hollings Marine Laboratory)**
  - **UC Davis Marine Pollution Studies Laboratory**

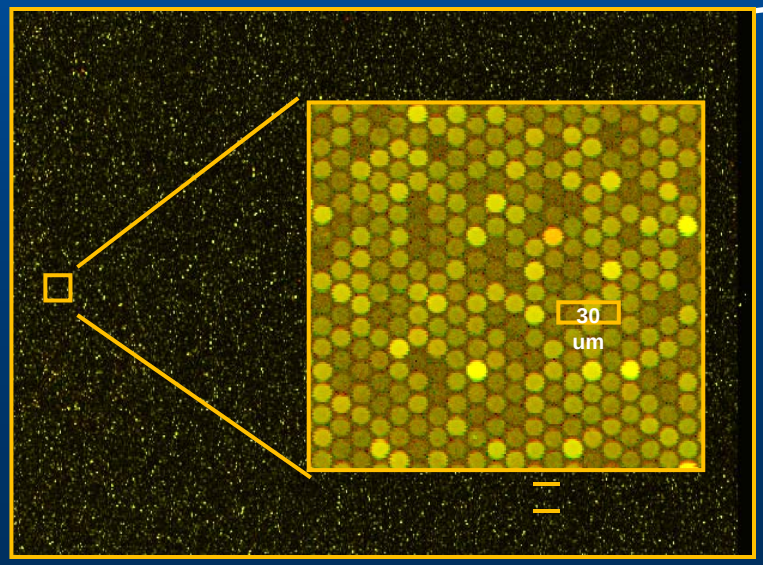
Research Program

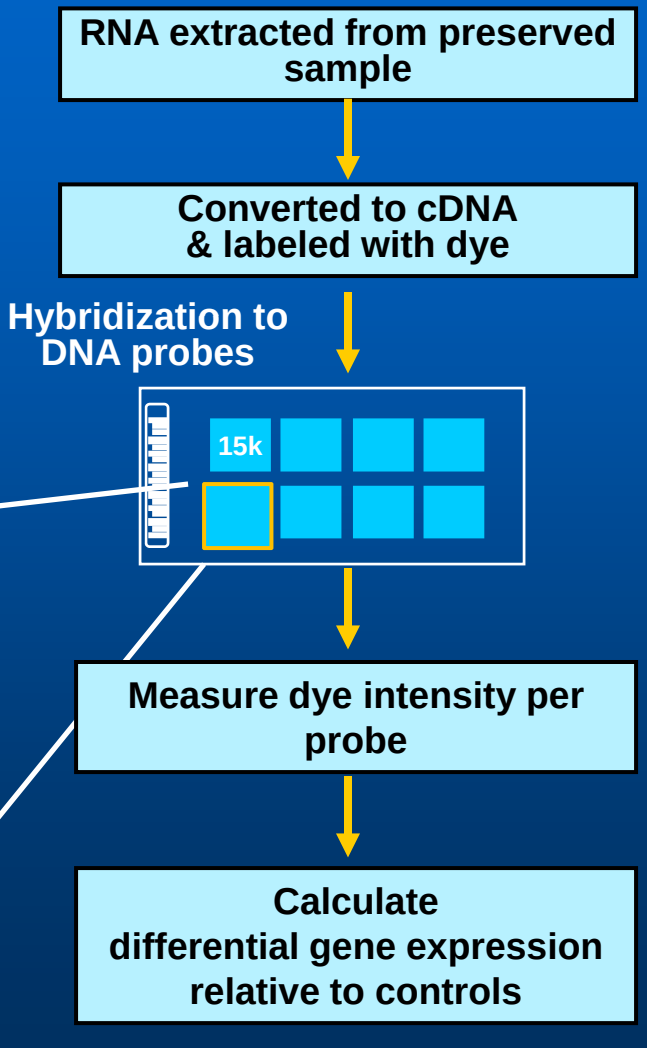
- **Substantial progress so far**
  - **Developed amphipod gene microarray**
  - **Initial demonstration of effectiveness**
- **Additional studies needed**
  - **Refinement and validation**
  - **Interlaboratory comparison**



Microarray Analysis

- **8,610 amphipod gene sequences in array**
- **8 samples analyzed simultaneously**





Preliminary Evaluation of Molecular TIE

- **Does micorarray "work"?**
  - **Binding of *E. estuarius* RNA to probes**
- **Are measurements precise?**
  - **Replicate analyses of same sample**
- **Can we detect differences among toxicants?**
  - **Compare samples exposed to different types of toxicants**
- **Can we identify toxicants in test samples?**
  - **Predict toxicant type in blind samples**

Training Data Set

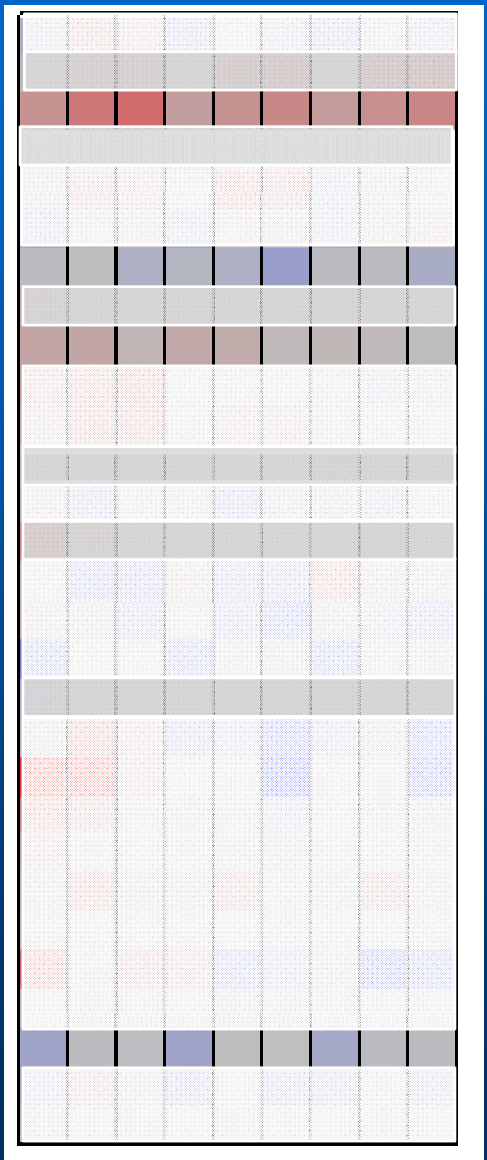
- **Diverse toxicants and mechanisms of action**
  - **Current use pesticides**
  - **Chlorinated pesticides**
  - **PAHs**
  - **Ammonia**
  - **Metals**
- **Focus on pyrethroid pesticides**
- **Doses near LOEC**
- **Different exposure matrices and durations**
  - **Matched controls**
- **2-3 replicates**
  - **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

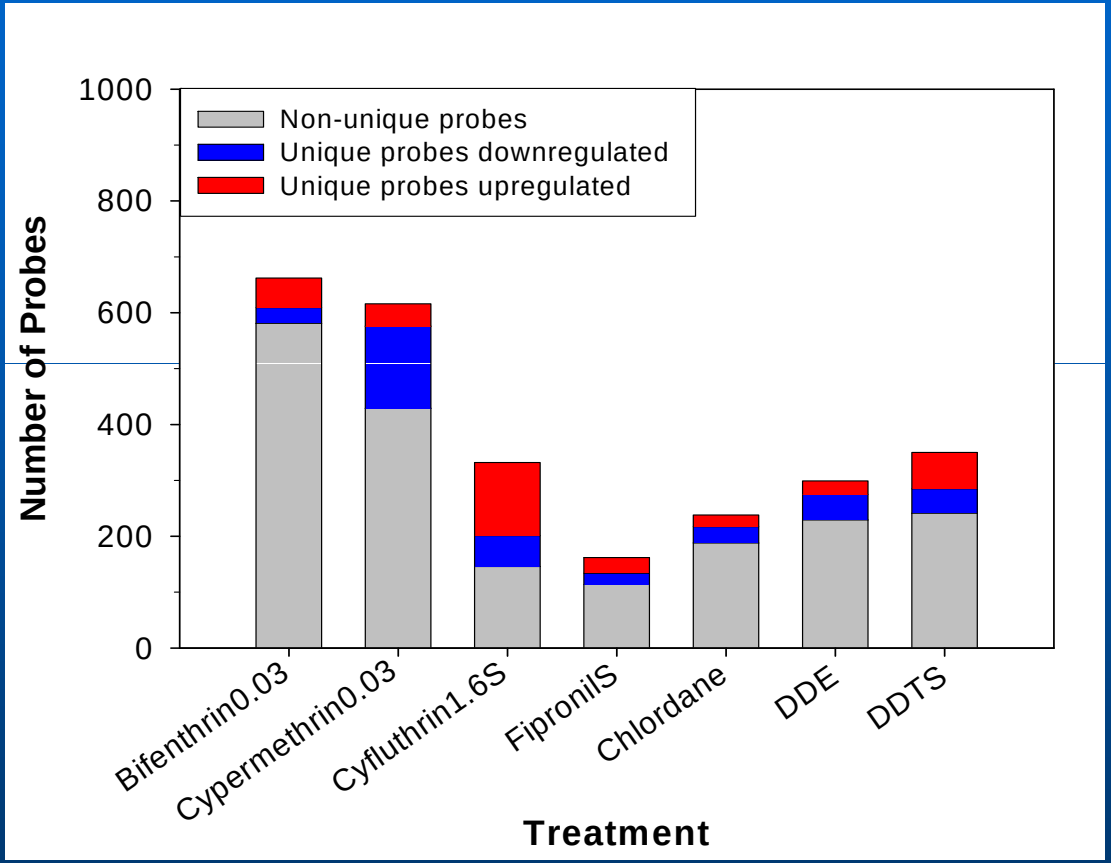
Candidate Gene Selection

- **Identify genes most likely to represent toxicant-specific response**
- **Consistent response among replicates**
- **Significant differential expression relative to control**
- **Calculated mean to minimize effect of outliers**

Replicates



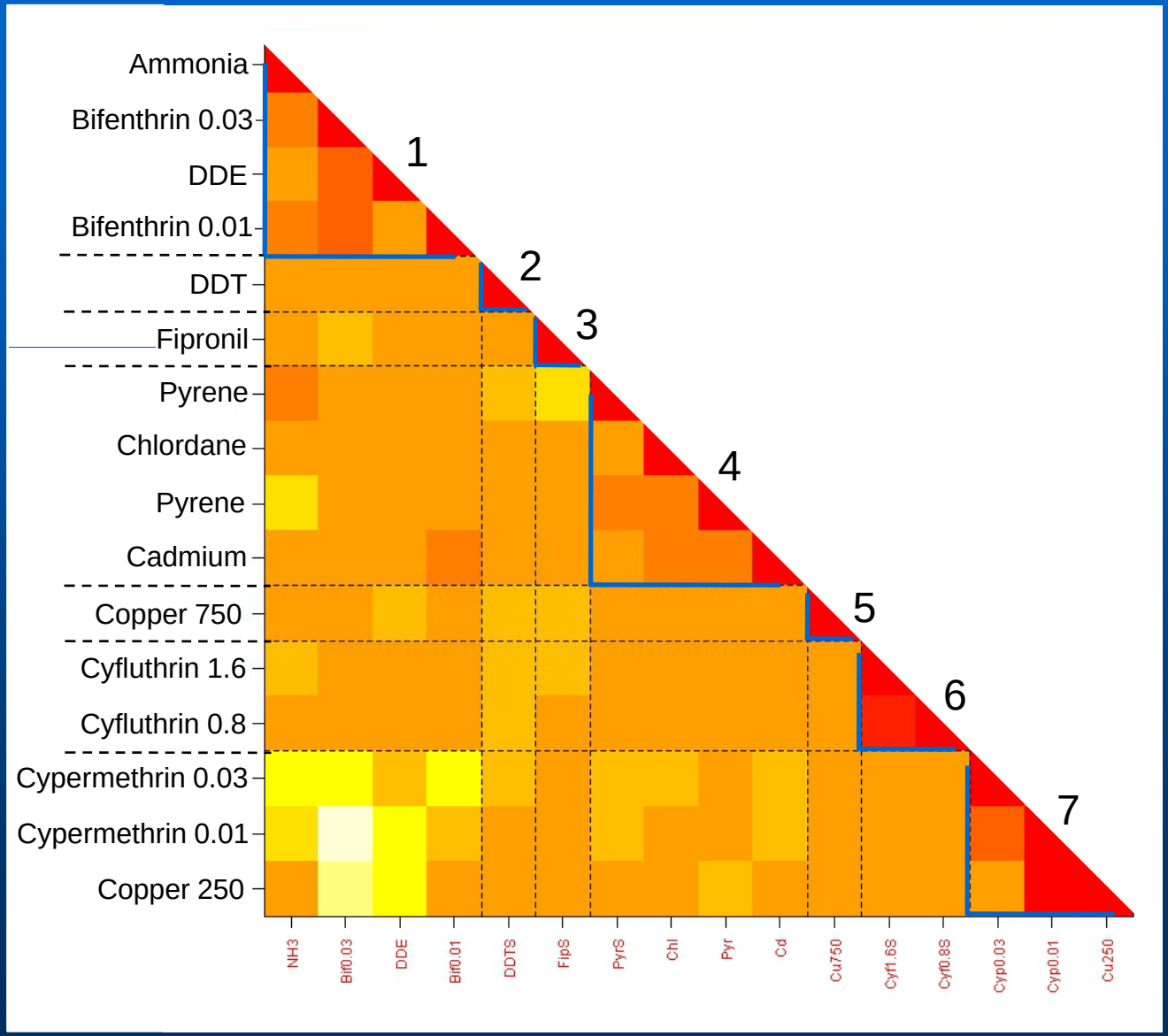
Candidate Genes: Pesticides



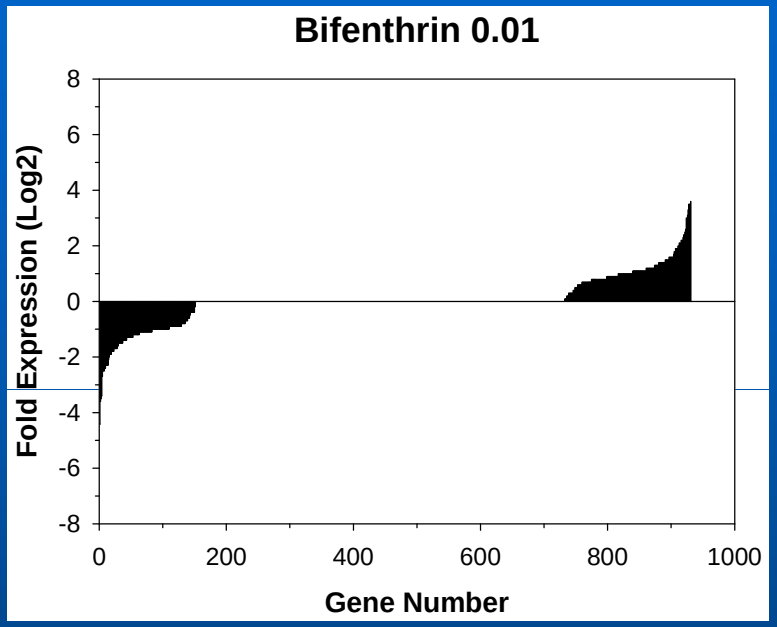
- **Approx. 100 uniquely expressed probes for each chemical**

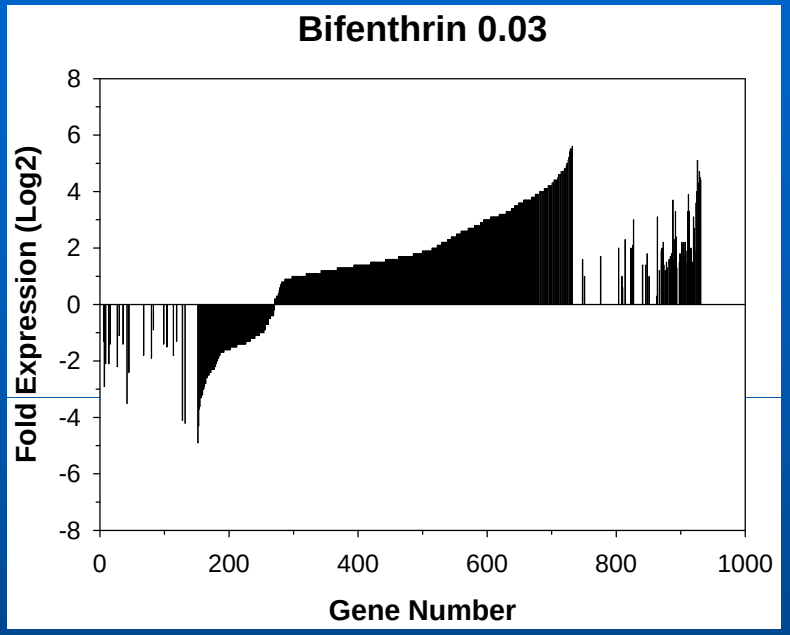
Cluster Analysis

- **Distinctive expression patterns for many contaminants**
- **Different dose levels group together (usually)**
- **Clusters show little relationship to toxicant type**

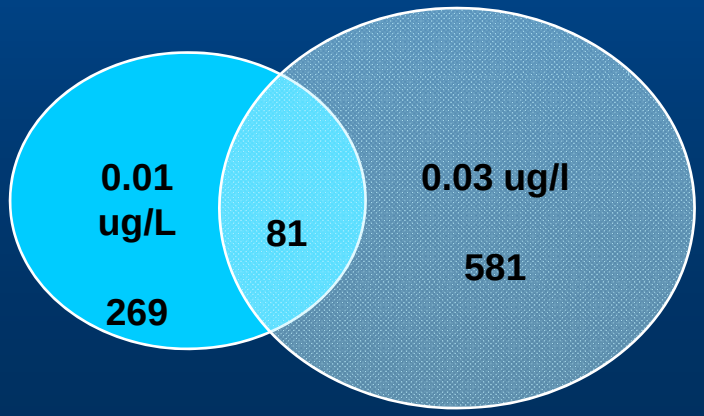


Dose Response: Bifenthrin





- **Relatively few candidate genes in common**
- **Greater differential expression at higher dose**

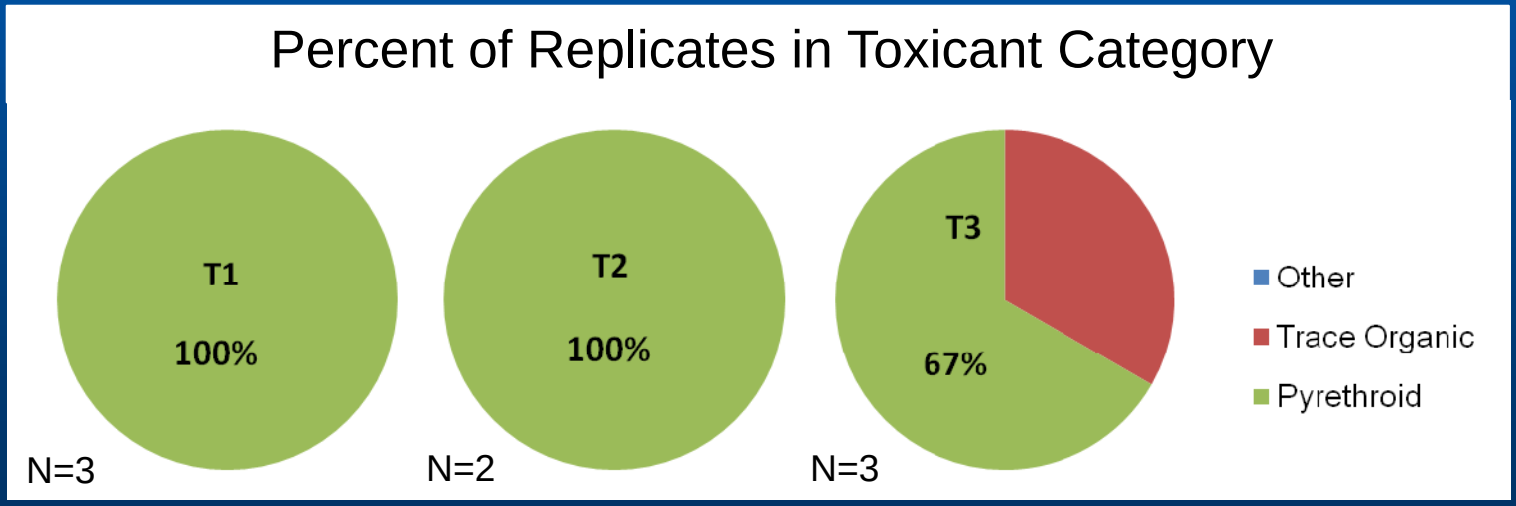


Evaluation of Toxicant Identification Ability

- **3 independent evaluation samples**
  - **Not used for training, identity unknown to analyst**
    - **T1: sediment spiked with cyfluthrin (pyrethroid)**
    - **T2: LA field sediment with toxicity due to pyrethroids**
    - **T3: toxic field sediment from SF Bay RMP BA41 (cause of toxicity not known)**
- **Developed classification model**
  - **3 classes of toxicants: Pyrethroids, Trace Organics, Other**
  - **Multivariate method: Random Forest**
    - **Selected 73 predictor genes**
    - **Used training data to develop prediction "trees" for each class**

Evaluation Results

- **Encouraging prediction results**
  - **Correct classification for 2 samples with identified cause of toxicity**
  - **SF Bay sample (T3) results cannot be verified**
  - **Small sample size**



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**

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