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

2008 Bight Regional Survey

<table>
<thead>
<tr>
<th>Strata</th>
<th>Low Toxicity</th>
<th>Moderate Toxicity</th>
<th>High Toxicity</th>
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<tbody>
<tr>
<td>Bay</td>
<td>16</td>
<td>40</td>
<td>13</td>
</tr>
<tr>
<td>Marina</td>
<td>25</td>
<td>14</td>
<td>5</td>
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<td>Port</td>
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<td></td>
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<td>Bight</td>
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</table>

Percentage of Area (%)
Toxicant Identification Evaluation (TIE)
Traditional Approach

Test Sample

Baseline Toxicity

Chemical Additions
- Post-treatment Toxicity

Extraction
- Post-treatment Toxicity

Sample Manipulations
- Post-treatment Toxicity

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

Organism is exposed to pollutant and it causes stress

At the cellular level, the organism responds to the stress by turning on/off certain genes (transcription)

The genes responding will indicate the kind of stress the organism is experiencing

Greater Relevance

- Direct cellular damage
- Detoxification
- Damage repair

Greater Specificity

Greater Sensitivity

Microarray/qPCR/NextGenSequencing
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

Sequence RNA fragments from toxicant-exposed organisms

Assemble fragments and design gene microarray

Identify subset of differentially expressed genes for toxicants of interest

Evaluate diagnostic ability of gene subset

If successful, refine and expand method to other contaminants

Conduct validation studies
Microarray Analysis

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

RNA extracted from preserved sample

Converted to cDNA & labeled with dye

Hybridization to DNA probes

Measure dye intensity per probe

Calculate differential gene expression relative to controls
Preliminary Evaluation of Molecular TIE

- Does microarray “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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Matrix</th>
<th>Survival (% of Control)</th>
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<tbody>
<tr>
<td>Bifenthrin</td>
<td>0.01 ug/L</td>
<td>Water</td>
<td>80</td>
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<tr>
<td>Bifenthrin</td>
<td>0.03 ug/L</td>
<td>Water</td>
<td>55</td>
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<tr>
<td>Cypermethrin</td>
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<td>Water</td>
<td>100</td>
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<tr>
<td>Cypermethrin</td>
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<td>Cyfluthrin</td>
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<td>Sediment</td>
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<td>Cyfluthrin</td>
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<tr>
<td>Fipronil</td>
<td>10 ug/kg</td>
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<tr>
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<tr>
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<tr>
<td>Cd</td>
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<td>Water</td>
<td>83</td>
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</table>
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
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

![Graph showing dose response for Bifenthrin 0.01 and 0.03 ug/L](image)
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

### Percent of Replicates in Toxicant Category

- **T1**: 100% (N=3)
- **T2**: 100% (N=2)
- **T3**: 67% (N=3)

Legend:
- Other
- Trace Organic
- Pyrethroid
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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