

EVALUATING AVOIDANCE AND OTHER BEHAVIORS IN DEVELOPMENT OF A SEDIMENT AVOIDANCE TEST FOR ASSESSING REMEDIATION OF CONTAMINATED SEDIMENTS.

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DISCLAIMER

*DISCLAIMER: THE VIEWS EXPRESSED IN THIS
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ACKNOWLEDGEMENTS

SPECIAL THANKS TO:

*KIM PRESTBO & CHRIS ECKLEY (REGION 10), KIRK SCHECKEL (USEPA),
HERMAN HARING (PEGASUS)*

WHY FURTHER DEVELOP SEDIMENT AVOIDANCE TESTS?

- Potential of being **fast, cost-effective** tool to screen for ecological risk at contaminated sites. **> 500,000 contaminated sites – few \$\$**. (*24-48 hrs. vs 7 (acute) – 56 (reproductive) days*).
- Previous success with an earthworm avoidance test. Now an international standard (ISO 17512-1).
- Some successes with sediment avoidance tests. However, **much less data** / literature. **No standardized test** for fresh water sediments.
- U.S. EPA works at a wide variety of sites. Has the opportunity to comprehensively characterize the relationship between avoidance and toxicity for a range of chemicals and soil/ sediment types.

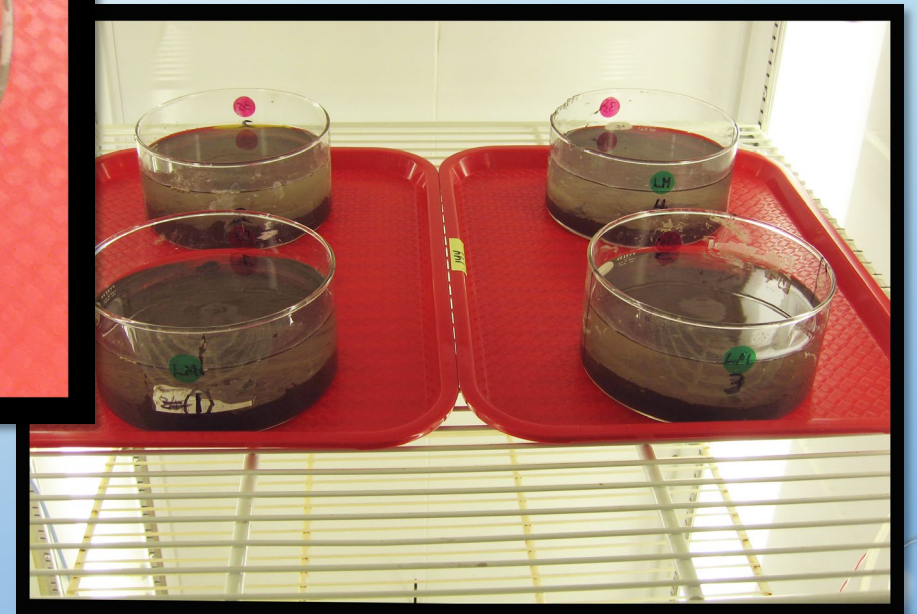
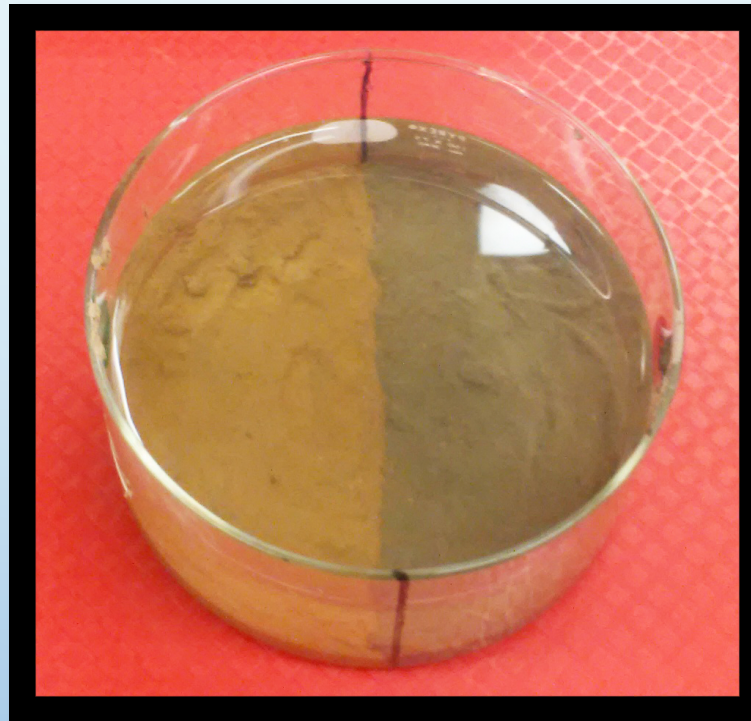
TEST ORGANISMS

- *Chironomus dilutes*, *Hyalella azteca*, *Lumbriculus variegatus*. Large adults.
- Same aquatic macroinvertebrates used in EPA std. ecotoxicity methods (100.1, 100.2, and 100.3).
- Allows easy comparison with acute and reproductive toxicity.
- Widely found in sediments, ecologically relevant.



TEST CHAMBERS

- Same chambers used in the earthworm avoidance tests.
- 150 x 75 mm crystallizing dishes.
- 4 reps put incubator.



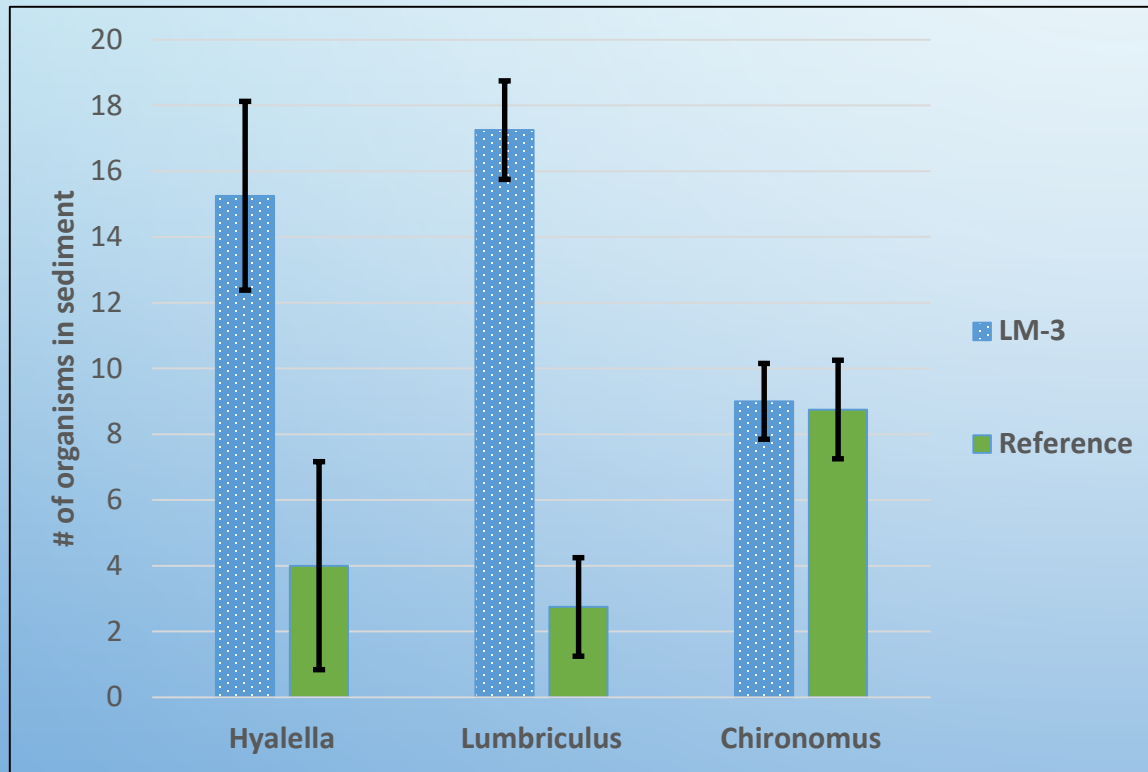
TEST CONDITIONS

- 24 - 48 Hours (organism-dependent).
- 20 organisms/ test chamber.
- Incubator: 23°C ($\pm$ 1°C), 16 hours light: 8 hours dark.
- 125 ml sediment per side. 500 ml overlying water (moderately hard).
- Test end – Sieve sediments, count organisms in test and reference sediments.



FIRST TRIAL WITH WETLAND SEDIMENT

Lane Marsh – Bunker Hill Superfund site, Region 10 (Coeur d'Alene, ID)

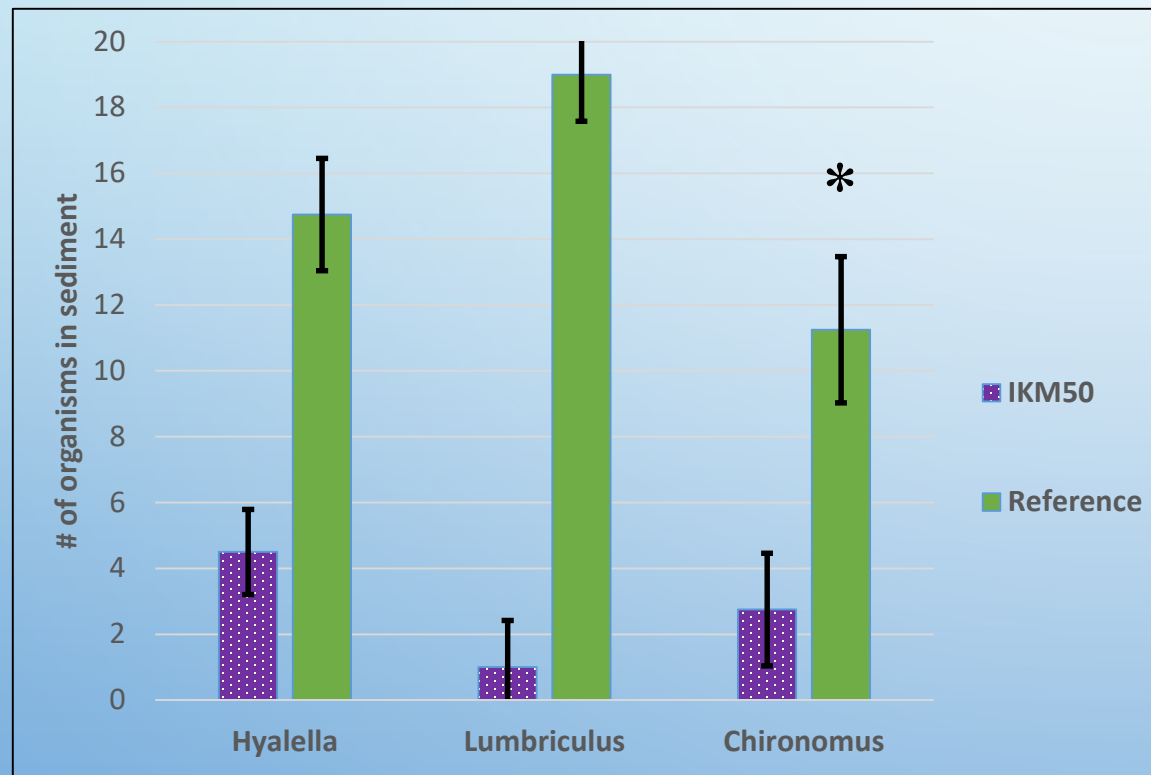


Green bars = Reference Sediment

- Site LM-3 - area of marsh with medium level of metals (Pb, Zn, Cd) contamination.
- No avoidance of test sediment, except *Chironomus*.
- Preference of metals-contaminated sediment.
- Reference sediment = Bear Skin Lake (Duluth-EPA)

AVOIDANCE OF IRON KING MINE (IKM) SOIL/ SEDIMENT

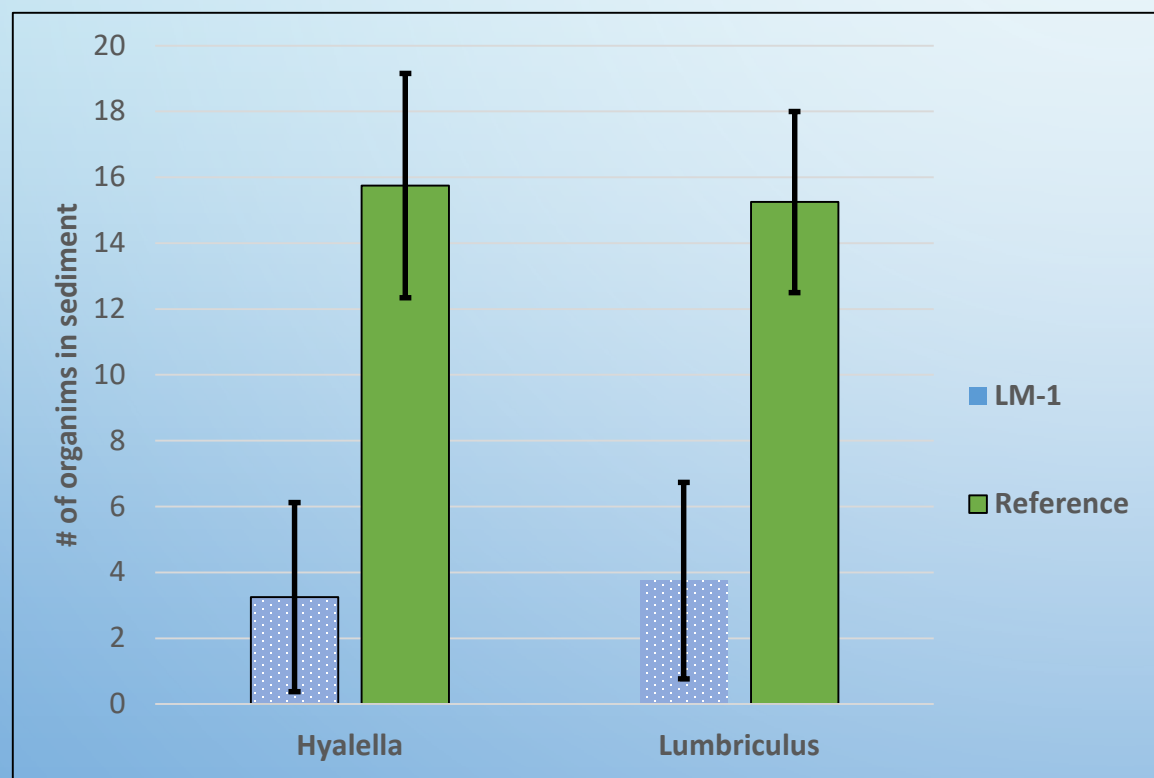
Hydrated metals-contaminated mine site soil from another project



Green bars = Reference Sediment

- Iron King Mine (AZ) – high levels of metals contamination.
- IKM50 = 50/50 mix of IKM and reference sediment.
- Avoidance, except *Chironomus* survival too low (70% *). Set acceptable level for avoidance tests @ $\geq 80\%$. Dead on surface.
- *Chironomus* - mobility and/or other issues?
- Reference sediment = Topsoil.

AVOIDANCE OF LANE MARSH SEDIMENT – AREA OF HIGHEST CONTAMINATION



Green bars = Reference Sediment

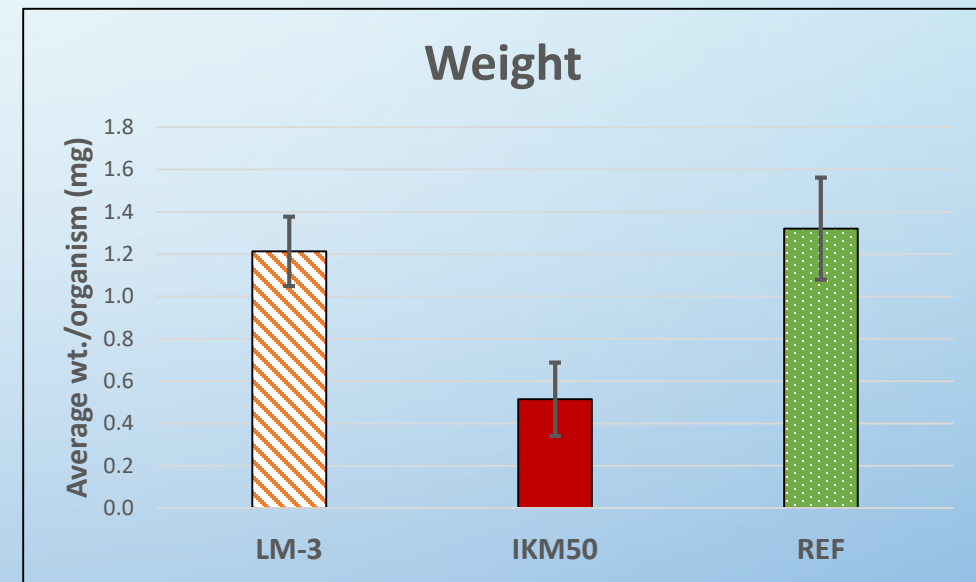
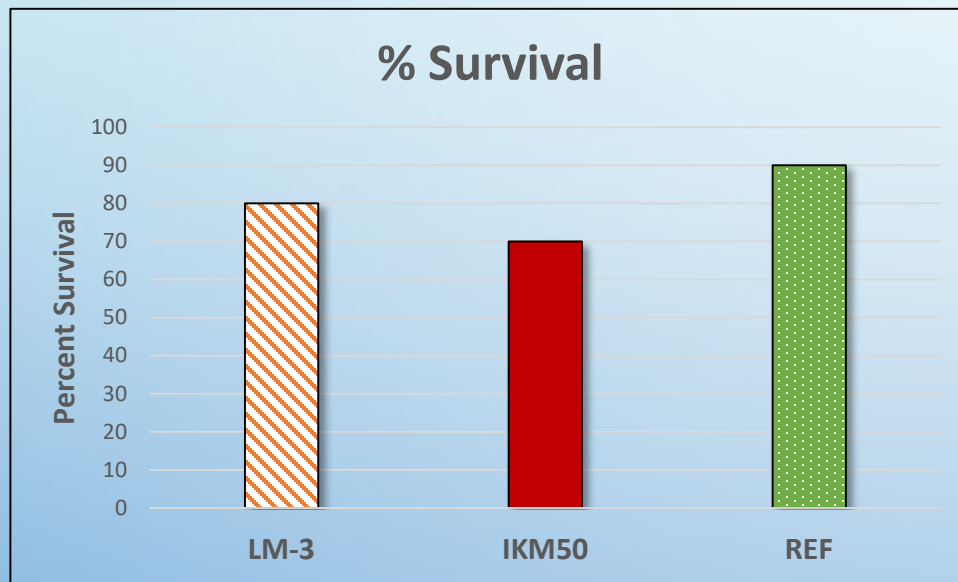
- Site LM-1 - area of marsh with highest level of metals (Pb, Zn, Cd) contamination. Avoidance of test sediment.
- *Chironomus* not used due to evidence of lack of mobility – our observations and literature.
- Reference sediment = clean wetland reference sediment from similar wetland near Lane Marsh.

IS THERE A RELATIONSHIP BETWEEN AVOIDANCE & TOXICITY?

- *Lumbriculus* and *Hyalella* will avoid 2 different metals-contaminated sediments.
- **Assess *toxicity*** of test sediments (Lane Marsh-1, Lane Marsh -3, 50% Iron King Mine) and reference (Topsoil, Bear Skin, Artificial Sediment) sediments.

AVOIDANCE VS SURVIVAL AND GROWTH ENDPOINTS

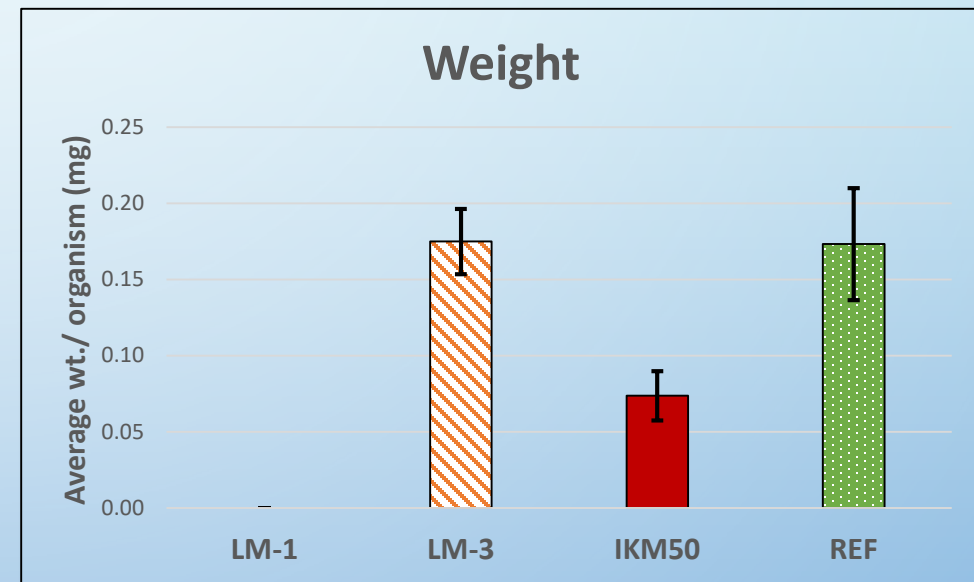
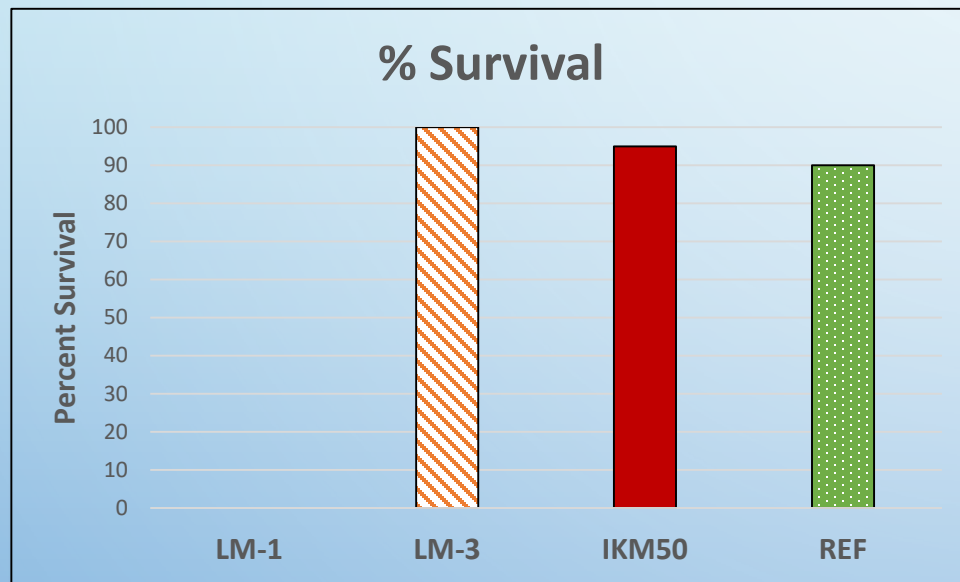
Chironomus Survival and Growth – 7 day test



- Test and Ref sediments acceptable ($\geq 70\%$) survival
 - Iron King Mine sediment with reduced growth
 - Reference sediment shown = topsoil

AVOIDANCE VS SURVIVAL AND GROWTH ENDPOINTS

Hyalella Survival and Growth – 7 day test



- LM-1 sediment 0% survival
- Other test and Ref sediments acceptable ($\geq 80\%$) survival
 - Iron King Mine sediment with reduced growth
 - Reference sediment shown = topsoil

CORRELATION BETWEEN AVOIDANCE AND TOXICITY

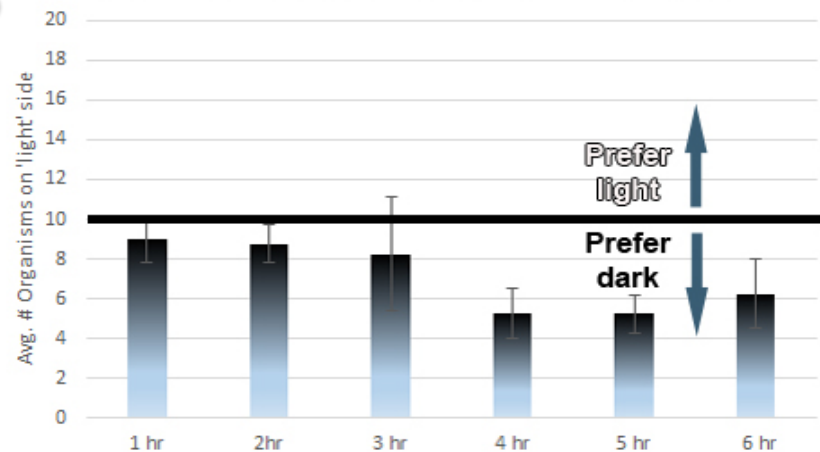
- Organisms avoid sediment (IKM50) whose only effect is reduced growth.
- Also avoid quite toxic sediment (LM-1) and don't avoid non-toxic sediment (LM-3 and several reference soils).
- Therefore, we see a ***correlation between avoidance and toxicity.***
- ***Evidence of avoidance being more sensitive*** than survival.

POTENTIAL EFFECTS OF OTHER BEHAVIORS

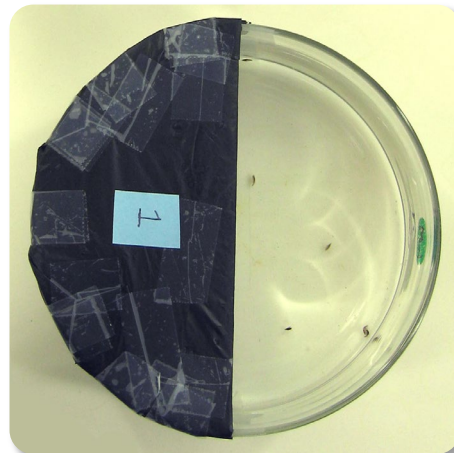
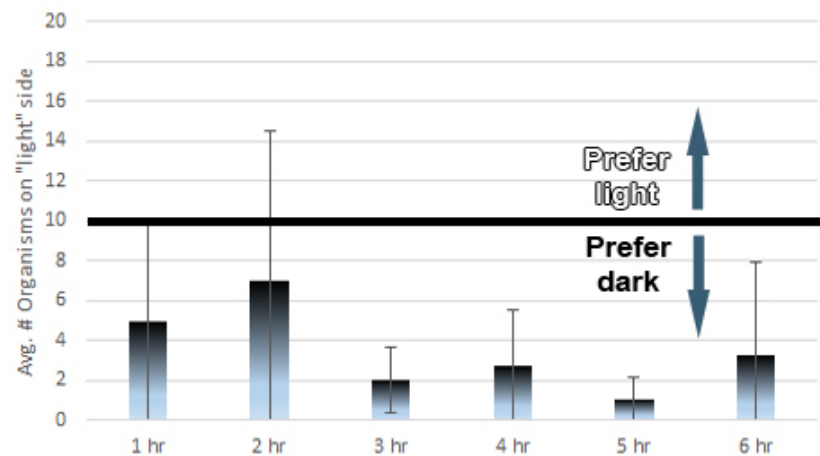
Avoidance is not the only behavior macroinvertebrates possess.

- *Movement (different modes and mobility), predator escape reflexes, food choice, sediment texture preference, phototaxis.*
- Some of these won't affect avoidance test. For others need to adjust SOP to account for (if possible). Helps interpret results.
- Some responses stronger, faster (e.g. reflexes) - predator escape. Some stimuli (and resulting behaviors) take precedence.
- Avoidance > choice of better organic content?

Hyalella - light/dark - no sediment

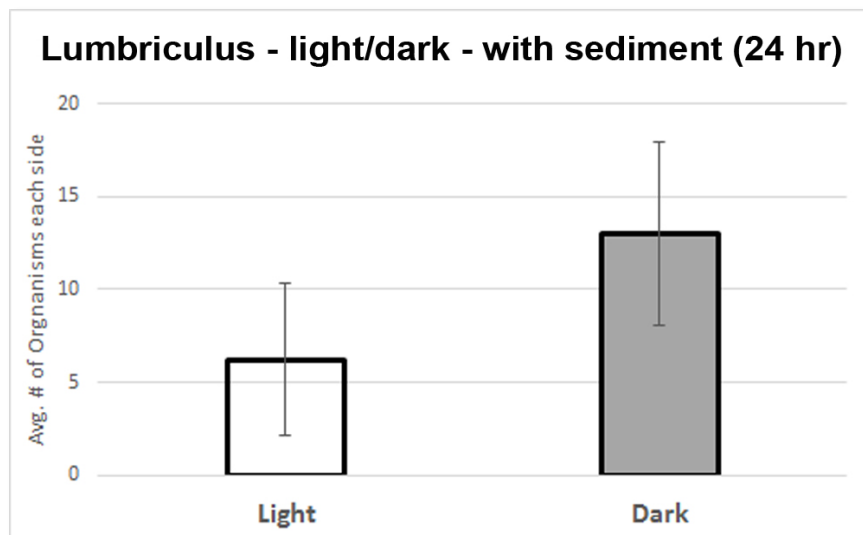
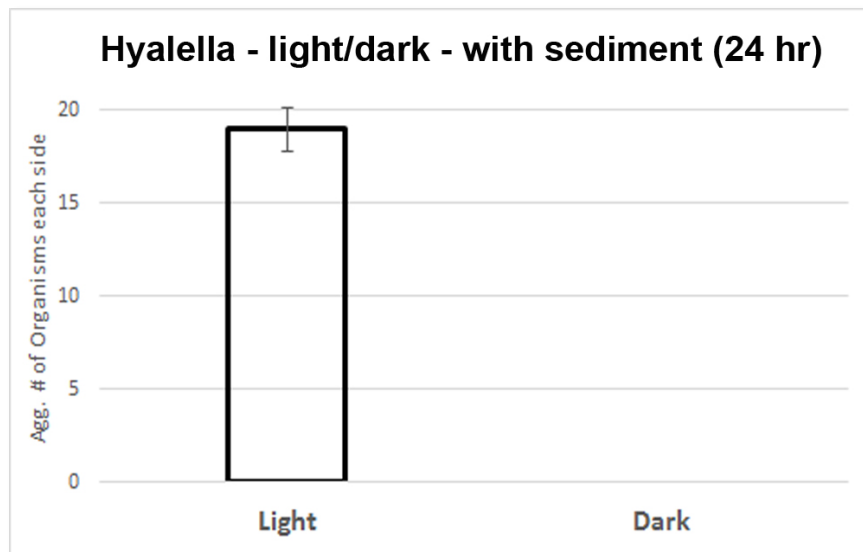


Lumbriculus - light/dark - no sediment



OTHER BEHAVIORS – PHOTOTAXIS

- Some organisms exhibit phototaxis – positive, negative
- Will incubator lighting conditions affect results?
- Time series – **no sediment - some preference for dark with time**



PHOTOTAXIS

- **Results change when sediment added**
- *Hyalella* prefer light, *Lumbriculus*, no preference
- Makes sense based on biology. *Hyalella* – ‘clingers’, burrow to shallow depth. Eyes. *Lumbriculus* deeper, more constantly in sediment.
- More research – *Hyalella* – light/shadows, both: constant light.

CONCLUSIONS

- A sediment avoidance test has potential as a screening tool for metals-contaminated sediment – Avoidance of toxic contaminants, sensitivity.
- Identification of some good candidates for test organisms - *Hyalella*, *Lumbriculus*.
- Questions about use of *Chironomus* for avoidance –from our tests & literature (limited mobility). Further testing needed.

RECOMMENDATIONS

- Consider possible effects of other behaviors (response to light, feeding, mating, predator avoidance) in method development.
- Analysis of some basic sediment characteristics (AFDM, % sand, silt clay, particle size distribution) useful for interpretation of results.

FURTHER RESEARCH

- More tests with variety of contaminants (PAHs, PCBs, PFAS) and sediments.
- Further examination of behaviors - which affect test? Account for in SOP and associated guidance.
- Test duration – 24-hour tests (some success with *Lumbriculus*).
- Reference sediments - how important are differences in sediment texture (sand, silt, clay), organic content?
- More control tests –reference sediment both sides, equal distribution?
- Avoidance tests using reference toxicants.