Refinements of Biological Test Methods

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NEWFIELDS

11.1

- **Neanthes Growth Test**
  - **Role of gut contents in growth calculation**
  - **Dry weight versus ash-free dry weight**
- **Larval Test**
  - **Entrapment in flocculent layer of fine sediments**
  - **Resuspension and screen tube modifications**
- **Microtox Test**
  - **Role of storage time in sediment toxicity**
  - **Effects of aeration on toxicity**

NEWFIELDS

11.2

Neanthes Growth Test

- Reference failures particularly with fine sediments
- Varying amounts of sediment appearing in worms
- Coarse sand used as control substrate
- Other biomass-based tests use AFDW

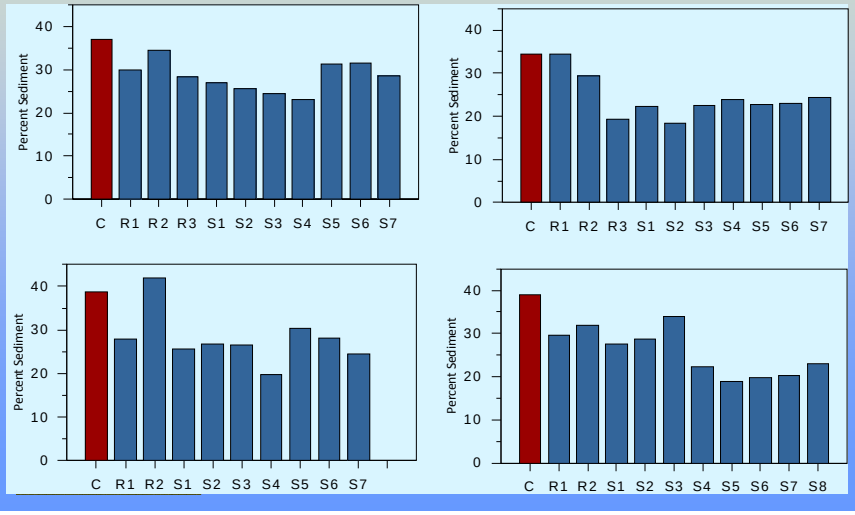
NEWFIELDS

11.3

Neanthes Growth Test

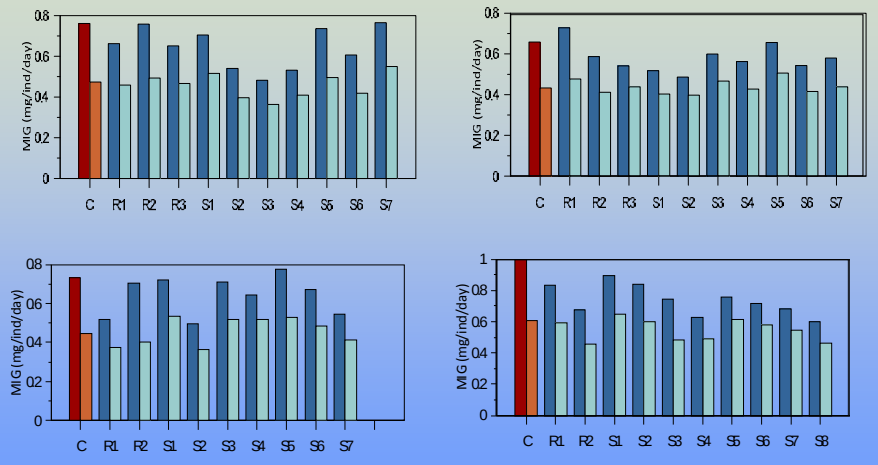
Percentage of weight that is sediment:

Controls: 34% (21% to 46%)
References: 28% (19% - 34%)
Treatments: 26% (17% to 40%)



11.4

Neanthes Growth Test

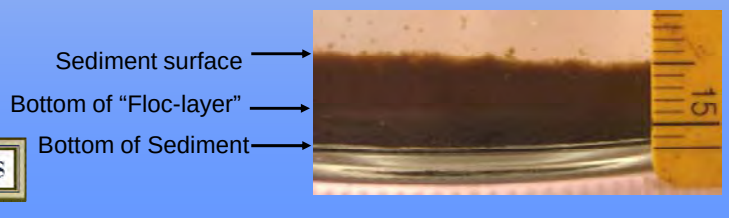


NEWFIELDS

11.5

Larval Test

- Indiscriminate failures in sediment with flocculent layers
  - High proportion of finest fines
  - Certain types of woody debris
- Low recoveries of larvae
- All larvae normal "D-shaped" larvae
- Not predictive based on percent fines

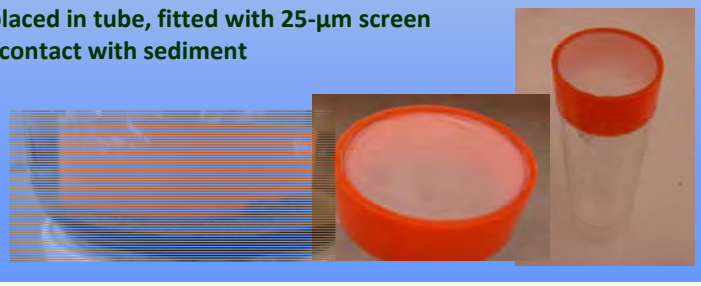


NEWFIELDS

11.6

Larval Test

- Tested three manipulations:
  - Standard PSEP larval test
  - Redisturbance
    - Termination includes homogenizing sediment and water in larval test chamber, allowing overnight settling, resampling following standard protocol
  - Screen-Tube Test
    - Method used in previous programs to separate physical and chemical effects
    - Larvae placed in tube, fitted with 25-µm screen in direct contact with sediment



NEWFIELDS

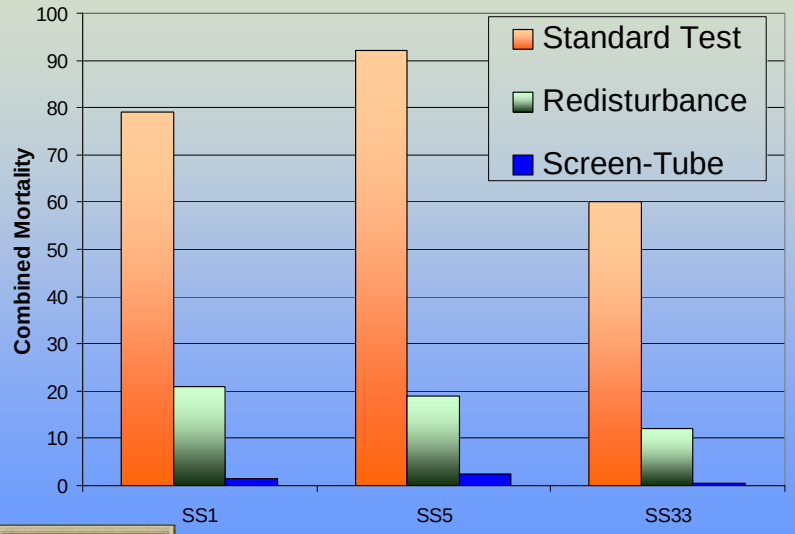
11.7

Treatments	Percent Fines	Number Normal	Combined Mortality
Control	--	271	2.5
SS-1 Standard	83.2	58	50.8
SS-5 Standard	95.1	21	61.2
SS-33 Standard	70.7	110	48.7
SS-1 Redisturbance	83.2	214	20.9
SS-5 Redisturbance	95.1	220	18.8
SS-33 Redisturbance	70.7	240	11.5
Screen Tube Control	--	214	7.1
SS-1 Screen Tube	83.2	224	1.5
SS-5 Screen Tube	95.1	235	2.5
SS-33 Screen Tube	70.7	238	0.4

NEWFIELDS

11.8

Larval Test



NEWFIELDS

11.9

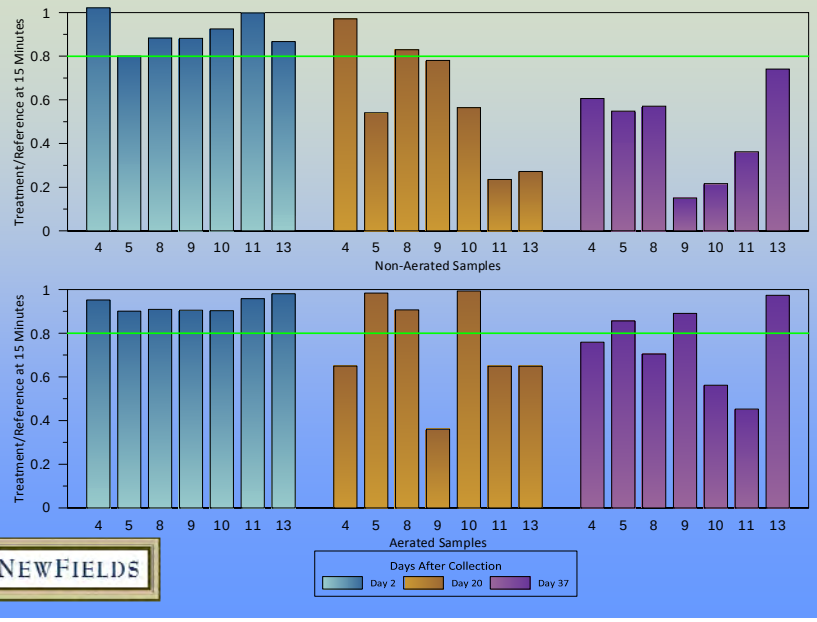
Microtox

- Sediment testing was conducted in 2008 with three standard tests (10-day amphipod, acute larval, and Microtox™)
- All stations passed SQS for the amphipod and larval tests, 7 of 13 failed the Microtox™ test
- Thirteen sediment samples were re- evaluated in 2009 based on the premise the failures may have been due to factors other than COCs

NEWFIELDS

11.10

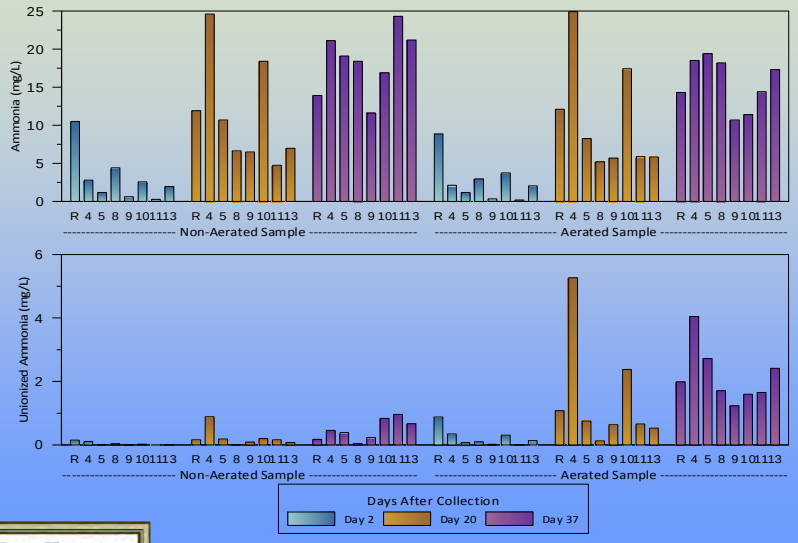
Microtox



NEWFIELDS

11.11

Ammonia Levels in Microtox Samples



NEWFIELDS

11.12

Microtox

- Sediment holding time dramatically affected the Microtox™ test:
  - All passed after 2 days holding
  - 5 stations failed test by Day 20 of holding
  - 7 stations failed by Day 37 of holding.
- Ammonia and sulfide concentrations fluctuated over time
- Sulfides tended to increase with holding time
- Aeration reduced sulfide levels
- Aeration did not decrease ammonia significantly, but did increase unionized ammonia levels
- Recommend measuring ammonia and sulfides during Microtox™ test

NEWFIELDS

11.13

Recommendations

- Neanthes Growth Test
  - Routinely collect AFDW
  - What are appropriate 1-Hit and 2-Hit criteria
  - Consider using AFDW in future
- Larval Test
  - For flocculent sediment use redisturbance or screen tube procedures
- Microtox Test
  - Minimize storage time

NEWFIELDS

11.14