

# Freshwater Bioassays

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May 7, 2014

# Introduction

- The floating percentile model (FPM) used to develop the new Washington State freshwater sediment standards (WAC 173-204-563) used input from both acute (10-day *Chironomus* and *Hyalella*) and chronic (28-day *Hyalella*) tests.
- The resulting standards were based on the most sensitive test, which was often the chronic 28-day *Hyalella* growth bioassay.

# Problem Statement

- Currently, the Sediment Evaluation Framework for the Pacific Northwest (SEF 2009) and the DMMP User Manual (DMMP 2013) require only short-term bioassays (10-day) using either *Hyalella* or *Chironomus*.
- The SEF and the DMMP User Manual should be consistent with the standards in requiring at least one chronic exposure measuring a sublethal endpoint

# Proposed Modifications

Requirements for freshwater sediment:

- Two different test species
- Three endpoints
- One chronic test; and
- One sublethal endpoint

Typical combination:

- One 10-day mortality test
- One chronic test with mortality and growth

Species, biological test, and endpoint	Acute effects biological test	Chronic effects biological test	Lethal effects biological test	Sub-lethal effects biological test
Amphipod: <i>Hyallela azteca</i>				
10-Day mortality	X		X	
28-Day mortality		X	X	
28-Day growth		X		X
Midge: <i>Chironomus dilutus</i>				
10-Day mortality	X		X	
10-Day growth	X			X
20-Day mortality		X	X	
20-Day growth		X		X

# Proposed Modifications

## Bioassay protocols

### Acute Effects Tests

- Hyalella azteca 10-day mortality: ASTM E1706-05 (2010)/EPA Method 100.1 (US EPA, 2000)
- Chironomus dilutus 10-day mortality: ASTM E1706-05 (2010)/EPA Method 100.2 (US EPA, 2000)
- Chironomus dilutus 10-day growth: ASTM E1706-05 (2010)/EPA Method 100.2 (US EPA, 2000)

### Chronic Effects Tests

- Hyalella azteca 28-day mortality: EPA Method 100.4 (US EPA, 2000)
- Hyalella azteca 28-day growth: EPA Method 100.4 (US EPA, 2000)
- Chironomus dilutus 20-day mortality: EPA Method 100.5 (US EPA, 2000)
- Chironomus dilutus 20-day growth: EPA Method 100.5 (US EPA, 2000)

# Proposed Modifications

## Bioassay protocols

- Unlike marine biological criteria, the freshwater biological criteria are based on a comparison to control treatments
- Due to the lack of established freshwater reference sites in Washington.
- Not necessary to collect reference sediments for freshwater bioassays.
  - Dredging projects wishing to use reference sediments must have the reference location approved prior to collection of the reference sediments.

# Proposed Modifications

## Bioassay Performance Standards

Biological Test/ Endpoint	Performance Standard	
	Control*	Reference
<i>Hyalella azteca</i>		
10-day mortality	$M_C \leq 20\%$	$M_R \leq 25\%$
28-day mortality	$M_C \leq 20\%$	$M_R \leq 30\%$
28-day growth	$MIG_C \geq 0.15 \text{ mg/individual}$	$MIG_R \geq 0.15 \text{ mg/individual}$
<i>Chironomus dilutus</i>		
10-day mortality	$M_C \leq 30\%$	$M_R \leq 30\%$
10-day growth	$MIG_C \geq 0.48 \text{ mg/individual}$	$MIG_R/MIG_C \geq 0.8$
20-day mortality	$M_C \leq 32\%$	$M_R \leq 35\%$
20-day growth	$MIG_C \geq 0.60 \text{ mg/individual}$	$MIG_R/MIG_C \geq 0.8$

M = Mortality; C = Control; R = Reference; T = Test; F = Final; MIG = Mean Individual Growth at time final; mg = milligrams.

\* ASTM is currently considering updating performance standards. Ecology will adopt the new performance standards once they are released.

# Proposed Modifications

## Hit definitions: 2-hit and 1 hit interpretive criteria

Biological Test/ Endpoint	Sediment Cleanup Objective for each biological test (2 hit criteria)	Cleanup Screening Level for each biological test (one hit criteria)
<i>Hyalella azteca</i>		
10-day mortality	$M_T - M_C > 15\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )	$M_T - M_C > 25\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )
28-day mortality	$M_T - M_C > 10\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )	$M_T - M_C > 25\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )
28-day growth	$(MIG_C - MIG_T)/MIG_C > 0.25$ and $MIG_T$ vs $MIG_C$ SD ( $p \leq 0.05$ )	$(MIG_C - MIG_T)/MIG_C > 0.40$ and $MIG_T$ vs $MIG_C$ SD $(p \leq 0.05)$

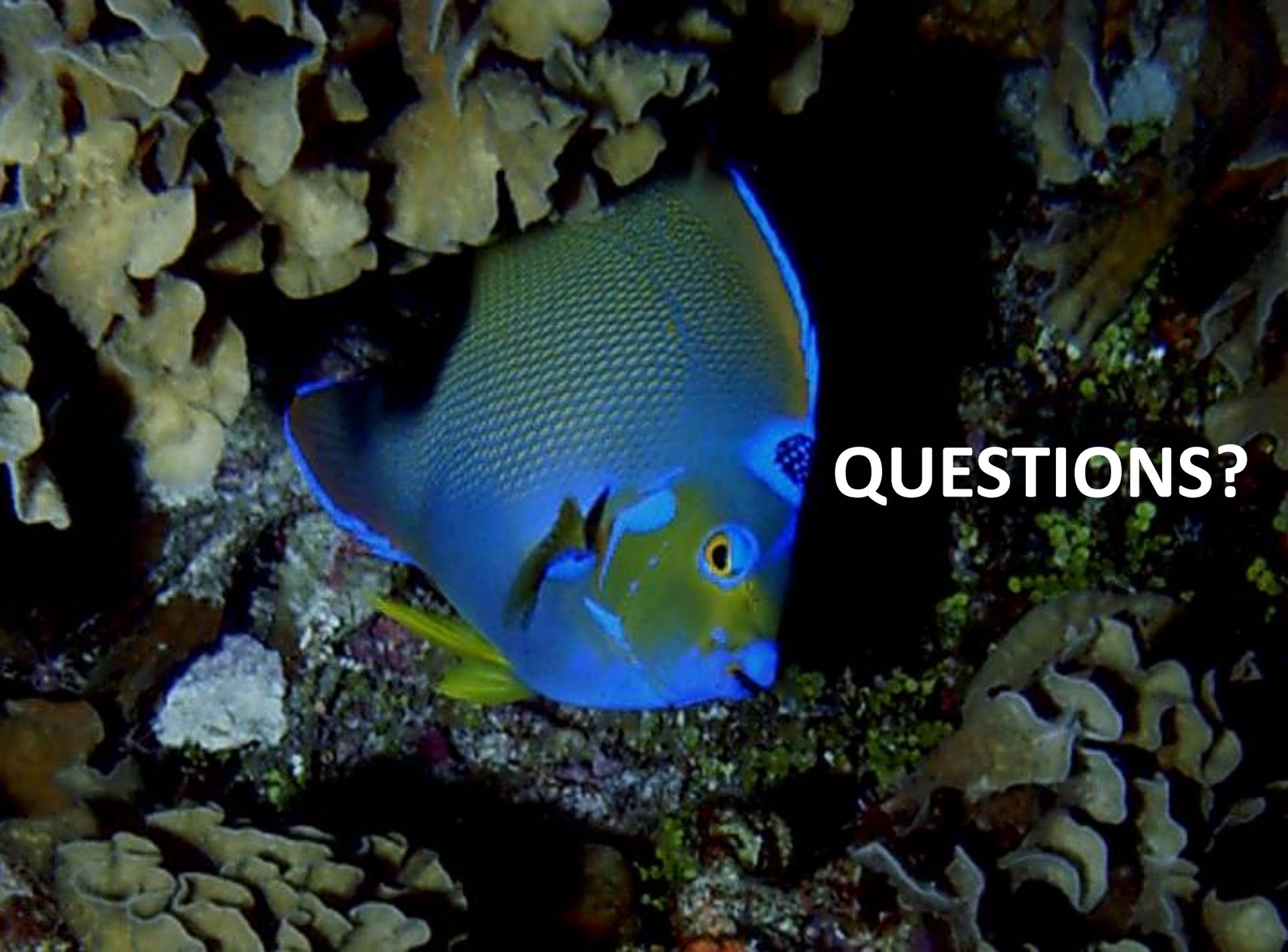
M = Mortality; C = Control; R = Reference; T = Test; F = Final; MIG = Mean Individual Growth at time final; mg = milligrams; SD=significant difference.

# Proposed Modifications

## Hit definitions: 2-hit and 1 hit interpretive criteria

Biological Test/ Endpoint	Sediment Cleanup Objective for each biological test (2 hit criteria)	Cleanup Screening Level for each biological test (one hit criteria)
<i>Chironomus dilutus</i>		
10-day mortality	$M_T - M_C > 20\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )	$M_T - M_C > 30\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )
10-day growth	$(MIG_C - MIG_T)/MIG_C > 0.20$ and $MIG_T$ vs $MIG_C$ SD ( $p \leq 0.05$ )	$(MIG_C - MIG_T)/MIG_C > 0.30$ and $MIG_T$ vs $MIG_C$ SD ( $p \leq 0.05$ )
20-day mortality	$M_T - M_C > 15\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )	$M_T - M_C > 25\%$ and $M_T$ vs $M_C$ SD ( $p \leq 0.05$ )
20-day growth	$(MIG_C - MIG_T)/MIG_C > 0.25$ and $MIG_T$ vs $MIG_C$ SD ( $p \leq 0.05$ )	$(MIG_C - MIG_T)/MIG_C > 0.40$ and $MIG_T$ vs $MIG_C$ SD ( $p \leq 0.05$ )

M = Mortality; C = Control; R = Reference; T = Test; F = Final; MIG = Mean Individual Growth at time final; mg = milligrams; SD=significant difference.



**QUESTIONS?**