“Ballast Water Treatment Testing: Conceptions and Misconceptions”

Nick Welschmeyer, Moss Landing Marine Laboratories, CA (CSU) and Golden Bear Facility, Cal Maritime Academy (CSU)
Presented: March 29, 2016; PBWG Annual Meeting, Sacramento CA
Topics:

• **Counting:**
  Can we count live ballast water organisms with the accuracy and precision expected of modern methods?

• **Size fractionation:**
  Are we missing much by restricting live protist counting to the 10 - 50 μm size fraction?

• **Challenge concentrations:**
  Is the concept of Type Approval ‘challenge’ justified from real-world test data?

• **Treatment success:**
  Is ballast water treatment stringent enough?
Conception 1: We can count live/active organisms accurately/precisely

Let’s start simply with plastic calibration beads (15 um dia.)
- no growth
- no death
- no shape variation (uniform spheres)

![Image of plastic calibration beads]

**Numeric Calibration:** Standards vs. Microscope

\[ y = 0.8906x + 25.451 \]
\[ R^2 = 0.99693 \]

Beautiful!
**Conception 1:** We can count live/active organisms accurately/precisely

- Same data
- Expressed as log concentration
- Two independent counters

**WE (HUMANS) CAN COUNT!!**
**Conception 1:** We can count live/active organisms accurately/precisely

\[
y = 1.0225x - 0.0829 \\
R^2 = 0.99684
\]

**Counting Comparison: Microscope vs. Flow Cytometer**

Fantastic: Humans = Machines!!
Let’s count live 10-50 um organisms with the microscope and flow cytometer during full-scale ballast treatment testing...

What could possibly go wrong?
Hey Dad.... What is the minimum dimension of an organism shaped like the Eiffel Tower??

Microscope Issues:
- Size
Flow Cytometer issues: Individuals vs. entities

Will it see 5 cells in a chain, or just one entity?

Will one cell produce two pulses?

Scan profiles from G. Dubelaar
Real-world ballast tests: Cytometer vs. Microscope

What a Mess!!

\[ y = 0.8813x + 266.23 \]
\[ R^2 = 0.32648 \]

Live Phytoplankton, 10-50 um

Epifluorescence microscope, FDA

Live Phytoplankton, 10-50 um (cells/mL): Flow cytometer, FDA

Counting Comparison: Microscope vs. Flow Cytometer

\[ y = 1.0225x - 0.0829 \]
\[ R^2 = 0.99684 \]
$y = 0.8813x + 266.23$

$R^2 = 0.32648$

$N = 142$

$N = 76$
Logarithmic plot of the same data shown previously

\[ y = 0.9889x - 0.0739 \]

\[ R^2 = 0.93894 \]

Live Phytoplankton, 10-50 um (Log (cells/mL))

Epifluorescence microscope, FDA

Flow cytometer, FDA

- Treatment
- Uptake
Live Phytoplankton, 10-50 um (Log (cells/mL): Epifluorescence microscope, FDA

Flow cytometer, FDA

Uptake

No Man’s Land

Treatment

Treatment

Uptake
**Concept 2:** Size Fractionation  
**Concept 3:** Challenge Concentration

Do we provide an accurate assessment of Numerical “CHALLENGE” in 10-50 um counting?
Phytoplankton show red fluorescence due to chlorophyll content. These particles exhibit low red fluorescence and are considered 'noise' (detritus, inorganic particles).

Conception 2. Natural organism concentrations are not challenging enough.

- Analyze beads of known diameters to generate a calibration curve.
## Comparing 10-50um size class organisms across locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean Volume (um³)</th>
<th>Equivalent Diameter (um)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seattle, WA</td>
<td>2,764</td>
<td>17.41</td>
<td>5485</td>
</tr>
<tr>
<td>Vallejo, CA</td>
<td>3,322</td>
<td>18.51</td>
<td>30325</td>
</tr>
<tr>
<td>San Francisco, CA</td>
<td>3,342</td>
<td>18.55</td>
<td>22694</td>
</tr>
<tr>
<td>Denmark</td>
<td>3,752</td>
<td>19.28</td>
<td>4229</td>
</tr>
<tr>
<td>Moss Landing, CA</td>
<td>7,204</td>
<td>23.96</td>
<td>50838</td>
</tr>
<tr>
<td>Port Angeles, WA</td>
<td>8,501</td>
<td>25.32</td>
<td>3425</td>
</tr>
</tbody>
</table>
All active (live) phytoplankton (10-50 um)  
N = 174,373 cells

Count Ratio (1-50)/(10-50) = 8.2x
The outcome of 1) Whole-Water and 2) Size-fractionated (10-50 um) MPN assays (UV)

<table>
<thead>
<tr>
<th>Uptake</th>
<th>Treatment Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>KL21_UU1</td>
<td>KL21_UU2</td>
</tr>
<tr>
<td>Whole-water</td>
<td>Dilution</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

| KL22_UU1 | KL22_UU2 | KL22_UU3 | KL22_DT1 | KL22_DT2 | KL22_DT3 |
| Size-fractionated | Dilution | Size-fractionated | Dilution | Size-fractionated | Dilution |
| 1 | 6 | 36 | 216 | 1296 | 1 | 6 | 36 | 216 | 1296 |

| KL22_UU1 | KL22_UU2 | KL22_UU3 | KL22_DT1 | KL22_DT2 | KL22_DT3 |
| Size-fractionated | Dilution | Size-fractionated | Dilution | Size-fractionated | Dilution |
| 1 | 6 | 36 | 216 | 1296 | 1 | 6 | 36 | 216 | 1296 |
The outcome of 1) Whole-Water and 2) Size-fractionated (10-50 um) MPN assays (UV): *Numeric Challenge* and *Biological Efficacy* are significantly larger than we think.

### Uptake

#### Whole Water MPN concentrations

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Live cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLB1_UU1</td>
<td>3700</td>
</tr>
<tr>
<td>KLB1_UU2</td>
<td>7400</td>
</tr>
<tr>
<td>KLB1_UU3</td>
<td>1400</td>
</tr>
<tr>
<td>KLB2_UU1</td>
<td>2000</td>
</tr>
<tr>
<td>KLB2_UU2</td>
<td>3700</td>
</tr>
<tr>
<td>KLB2_UU3</td>
<td>7400</td>
</tr>
</tbody>
</table>

\[ X = 4267 \]

#### Biological Efficacy (Uptake/Discharge)\[ 46380x \]

### Treatment Discharge

#### Whole Water MPN concentrations

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Live cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLB1_DT1</td>
<td>0.094</td>
</tr>
<tr>
<td>KLB1_DT2</td>
<td>0.094</td>
</tr>
<tr>
<td>KLB1_DT3</td>
<td>0.083</td>
</tr>
<tr>
<td>KLB2_DT1</td>
<td>0.094</td>
</tr>
<tr>
<td>KLB2_DT2</td>
<td>0.094</td>
</tr>
<tr>
<td>KLB2_DT3</td>
<td>0.094</td>
</tr>
</tbody>
</table>

\[ X = 0.092 \]

#### Size-fractionated MPN concentrations

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Live cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLB1_UU1</td>
<td>240</td>
</tr>
<tr>
<td>KLB1_UU2</td>
<td>800</td>
</tr>
<tr>
<td>KLB1_UU3</td>
<td>140</td>
</tr>
<tr>
<td>KLB2_UU1</td>
<td>600</td>
</tr>
<tr>
<td>KLB2_UU2</td>
<td>240</td>
</tr>
<tr>
<td>KLB2_UU3</td>
<td>140</td>
</tr>
</tbody>
</table>

\[ X = 360 \]

\[ 4615x \]

### Biological Efficacy (Uptake/Discharge)

#### Size-fractionated MPN concentrations

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Live cells/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLB1_DT1</td>
<td>0.078</td>
</tr>
<tr>
<td>KLB1_DT2</td>
<td>0.078</td>
</tr>
<tr>
<td>KLB1_DT3</td>
<td>0.078</td>
</tr>
<tr>
<td>KLB2_DT1</td>
<td>0.078</td>
</tr>
<tr>
<td>KLB2_DT2</td>
<td>0.078</td>
</tr>
<tr>
<td>KLB2_DT3</td>
<td>0.078</td>
</tr>
</tbody>
</table>

\[ X = 0.078 \]
Conception 3 (again):

Is the concept of “CHALLENGE” meaningful in Ballast Water Treatment Testing?

The CHALLENGE concept:
as the concentration of challenge organisms
increases, the biological efficacy of ballast water
treatment systems will decrease.
3. **The CHALLENGE Concept**: Higher uptake concentrations yield a more ‘Challenging’ test

?? A Misconception ??

**Flow Cytometry (FDA): Phytoplankton 10-50 um**

Uptake vs. Treatment Discharge

N = 85

10-50 um
Live Phytoplankton (FDA)

**Zooplankton Uptake vs. Discharge**

N = 110

>50 um
Live Zooplankton
“CHALLENGE” in Ballast Water Treatment Testing: Conceptions and Misconceptions

Nick Welschmeyer, Moss Landing Marine Laboratories, CA (CSU)
Presented: Feb 2, 2016; ETV Tech Panel, Baltimore MD

Biological Efficacy as a Function of CHALLENGE Concentrations

[Graph showing biological efficacy declining as CHALLENGE concentration increases.]
Biological efficacy does not obey the CHALLENGE Concept in Ballast Water Testing

10-50 um
Live Phytoplankton (FDA)

>50 um
Live Zooplankton
Conception 4:
Ballast Water Treatment is not stringent enough

For perspective, let’s take a look at three of the greatest environmental successes in modern history*…

1. Vehicle Smog
2. Acid Rain
3. The Ozone Layer

*Bloomberg Report 2013;
Environmental Successes:

1. Visible reductions in Los Angeles smog
Visible reductions in Los Angeles smog... How?

Roughly... **10x** reduction in pollution emissions, even with modern 3-way converters.
Environmental Successes:  
2. Reduction in Acid Rain

Death to acid-intolerant forests

The Clean Air Act 1970

Stack-gas scrubbers: Roughly...

5x – 20x reductions in $\text{SO}_2$ and $\text{NO}_x$
Environmental Successes:
3. Reduction of the Antarctic Ozone ‘Hole’

Roughly... \textbf{10x} reduction in Fluorocarbons, ... over 30 years!!
Three of the greatest environmental successes in modern history...

1. Reduction in smog derived from automobiles
2. Reduction of acid rain
3. Shrinkage of the ‘ozone hole’

... were accomplished with reductions in the respective putative pollutants that were **approximately 10x**.

HOW ARE WE DOING IN BALLAST WATER TREATMENT?
Biological efficacy does not obey the CHALLENGE Concept in Ballast Water Testing

**Phytoplankton (10-50 um)**

- Biological efficacy as a function of CHALLENGE concentration.
- Biological Efficacy vs. CHALLENGE Concentration (Cells/mL).

**Zooplankton (>50 um)**

- Biological efficacy as a function of uptake concentration.
- Biological Efficacy vs. Uptake Concentration (organisms/m3).

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10-50 um
Live Phytoplankton (FDA)

>50 um
Live Zooplankton
Conclusions:

CONCEPTION 1: We can count accurately/precisely?  
Well... yes we can, for perfectly shaped, inert plastic beads  
but real organisms present a significant increase in variability

CONCEPTION 2: Natural organism concentrations are not challenging enough?  
Actually, for phytoplankton, the true numerical challenge concentration is  
about 10x higher than for the 10-50 um regulated size class.

CONCEPTION 3: The concept of ‘challenge’ is a well-substantiated principle  
in ballast water treatment testing?  
Actually, we have no data to substantiate that conclusion. Our results are  
opposite to common logic.

CONCEPTION 4: Ballast water treatment efficacy is NOT stringent enough.  
Actually, the current biological efficacy of ballast water treatment outpaces the  
well-documented environmental success stories by 2-4 orders of magnitude.  
A 1,000,000x reduction in zooplankton concentrations is not unusual?

WE MIGHT BE DOING A LOT BETTER THEN YOU THINK!!
Thank you!