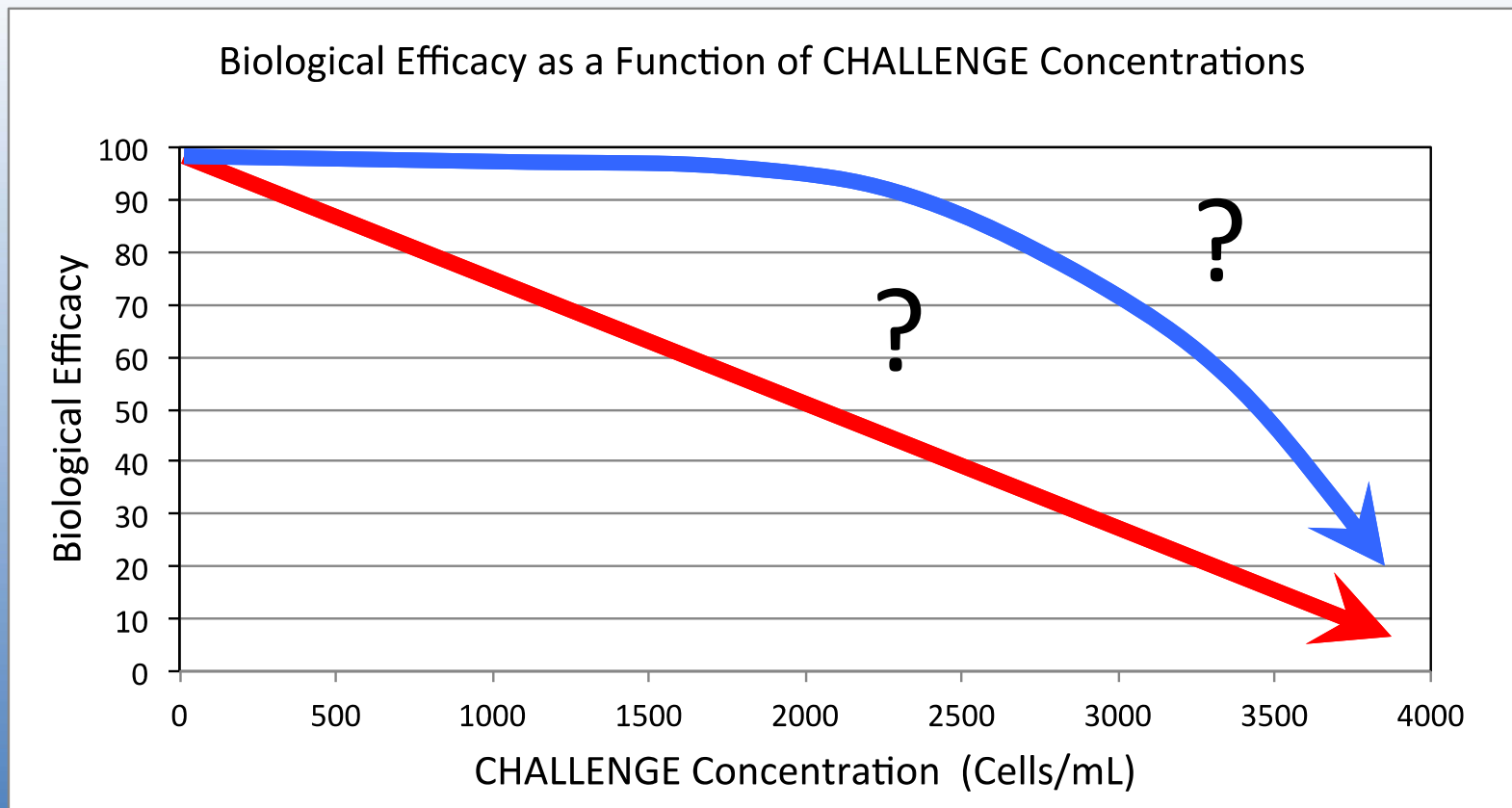


# “Ballast Water Treatment Testing: Conceptions and Misconceptions”

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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  $\mu\text{m}$  size fraction?

- **Challenge concentrations:**

Is the concept of Type Approval '*challenge*' justified from real-world test data?

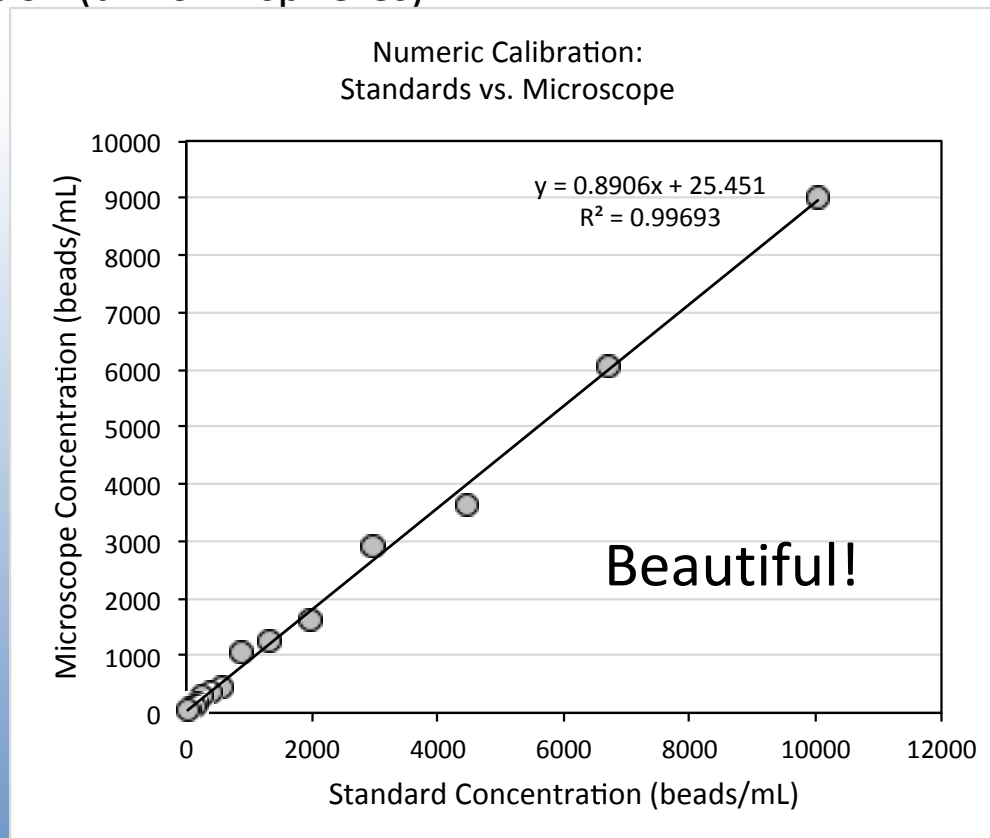
- **Treatment success:**

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)

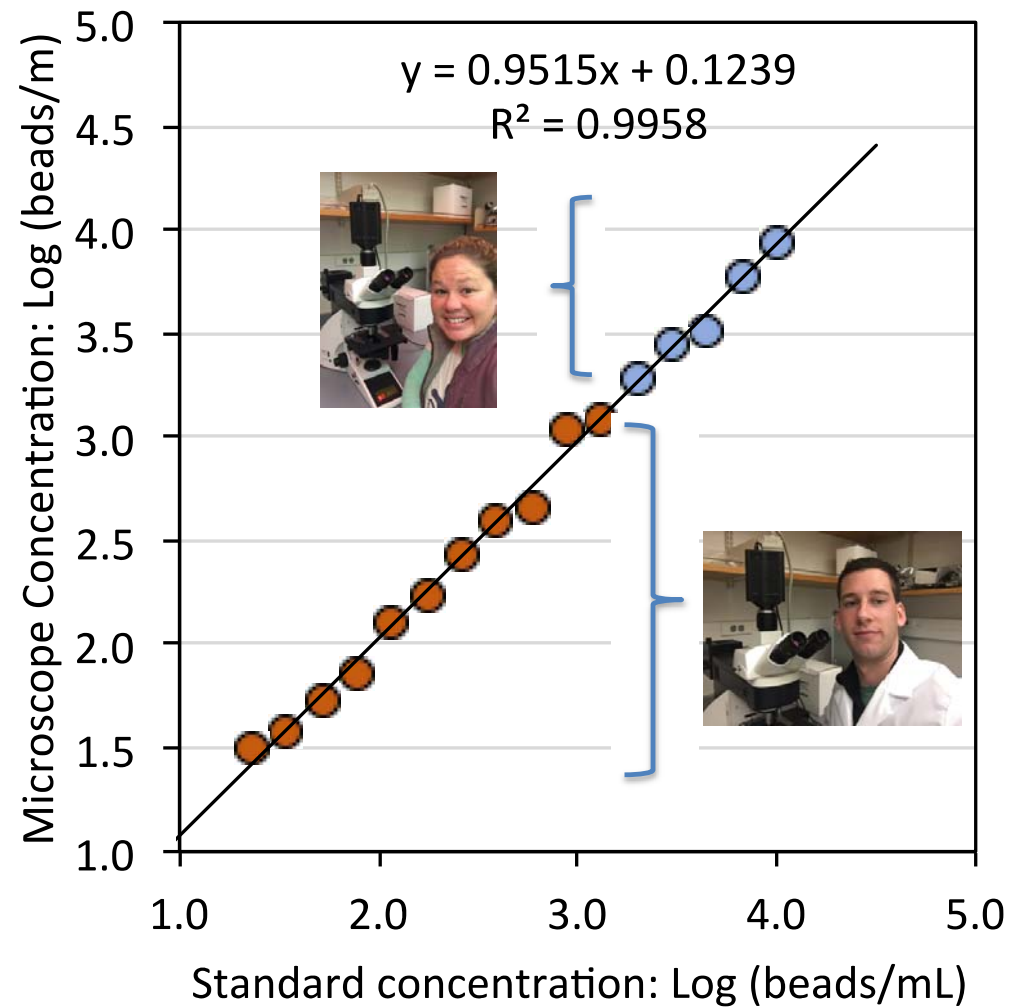


**Conception 1:** We can count live/active organisms accurately/precisely

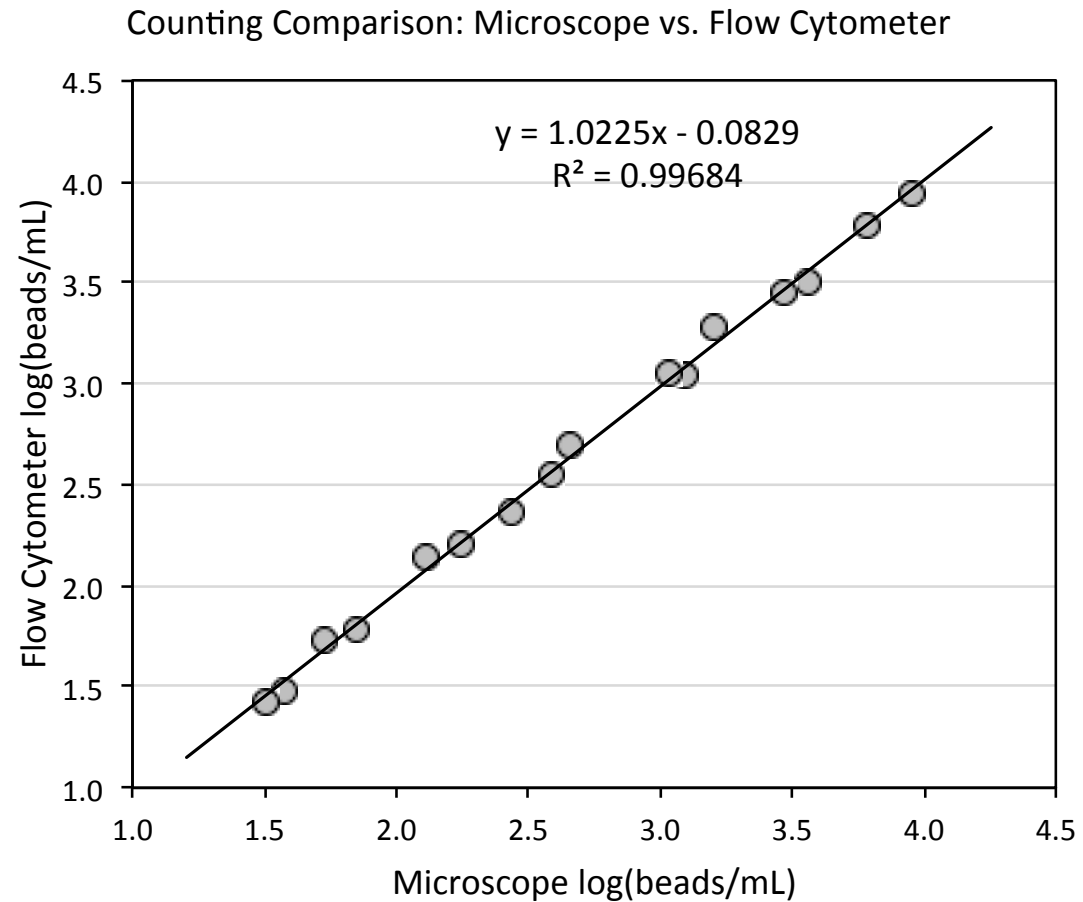
- Same data
- Expressed as log concentration
- Two independent counters

**WE (HUMANS) CAN COUNT!!**

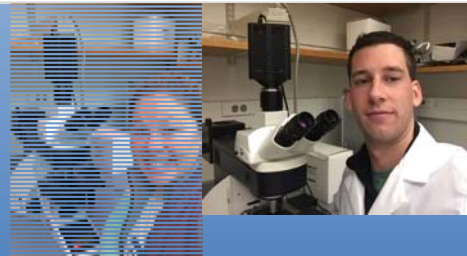
Numeric Calibration:  
Standards vs. Microscope



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

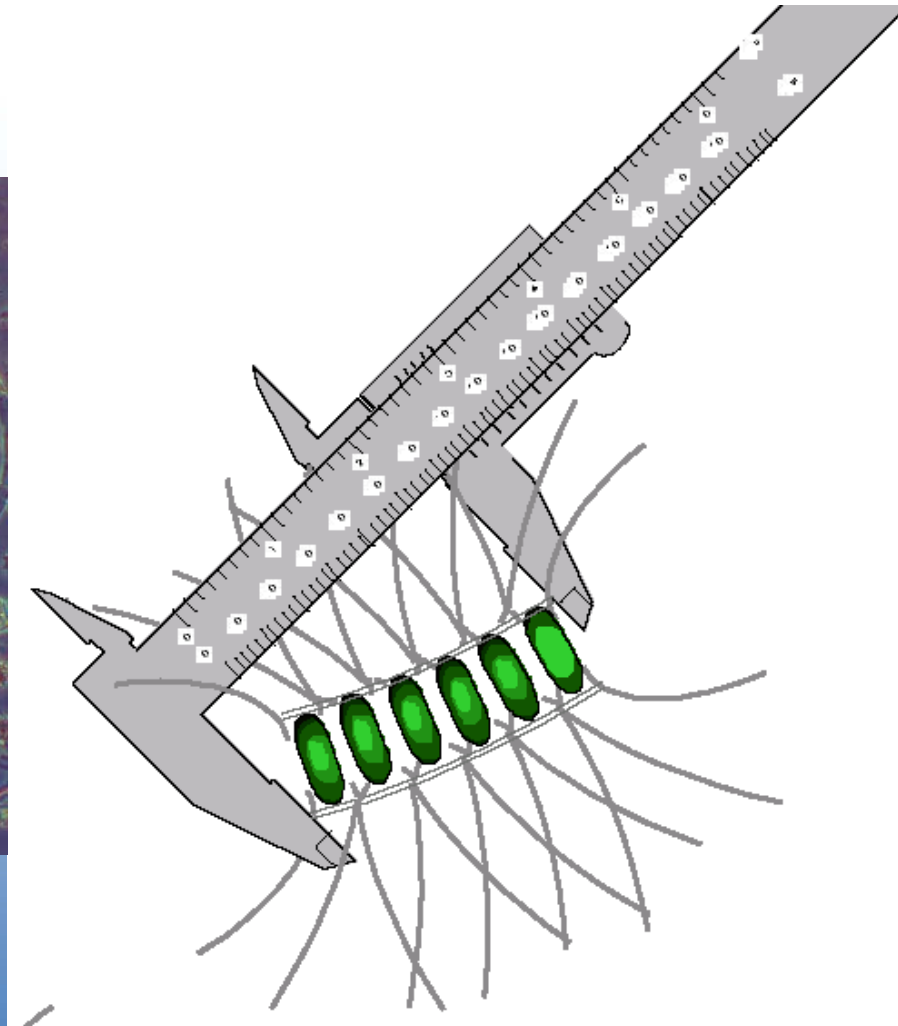
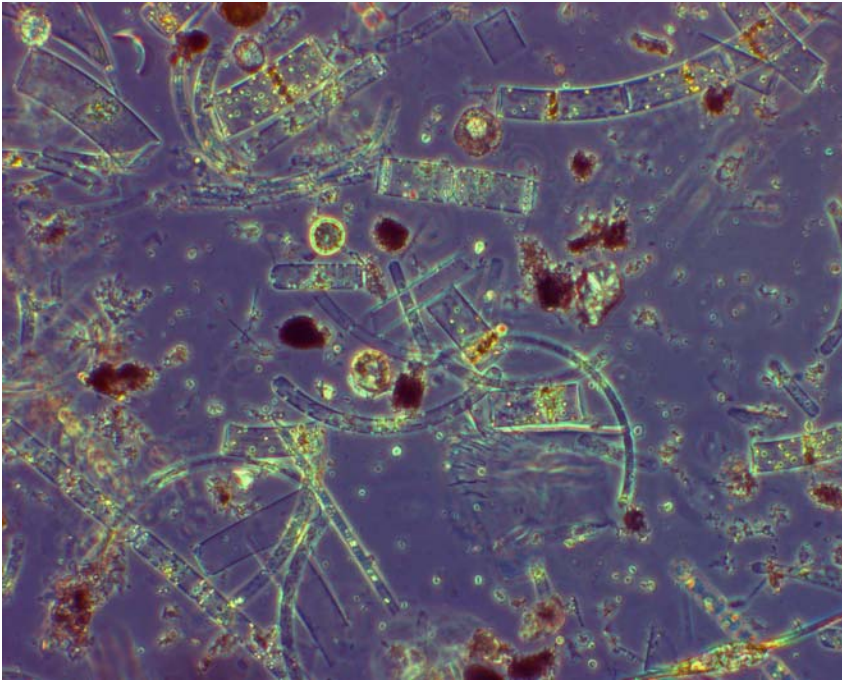


Fantastic: Humans = Machines!!



Let's count live 10-50  $\mu\text{m}$  organisms with the microscope and flow cytometer during full-scale ballast treatment testing...

What could possibly go wrong?

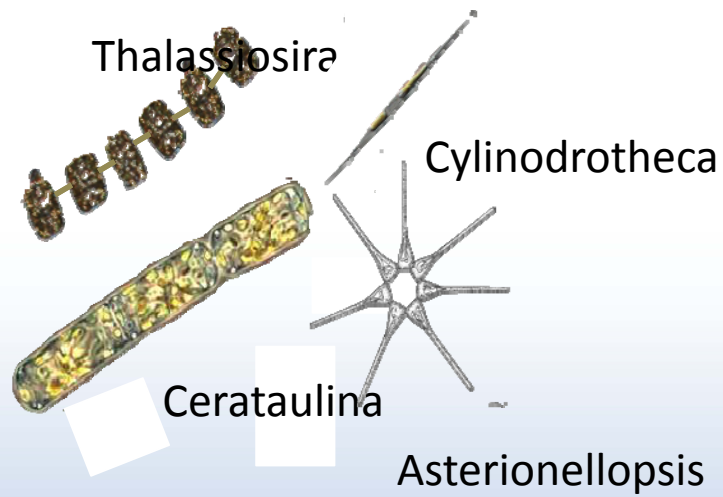


Microscope Issues:  
- Size

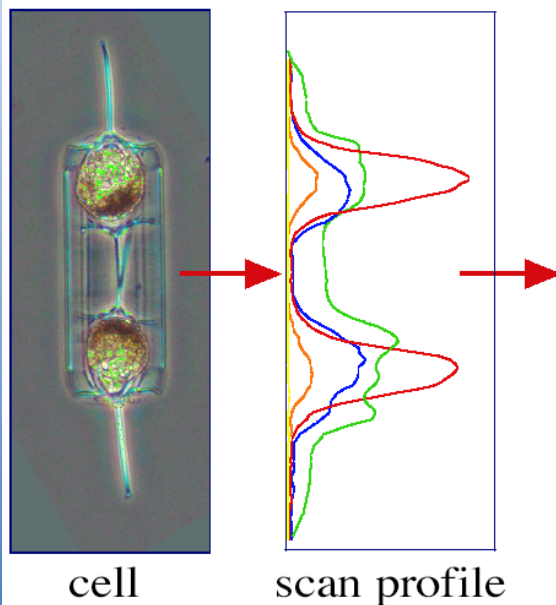
Hey Dad.... What is the  
minimum  
dimension of an  
organism  
shaped like the  
Eiffel Tower??



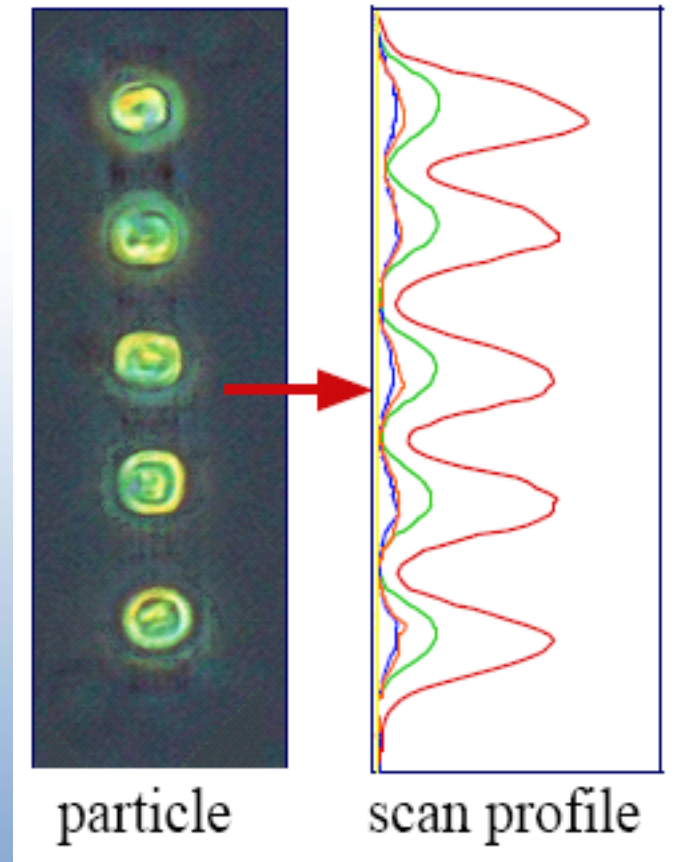
## Flow Cytometer issues: Individuals vs. entities



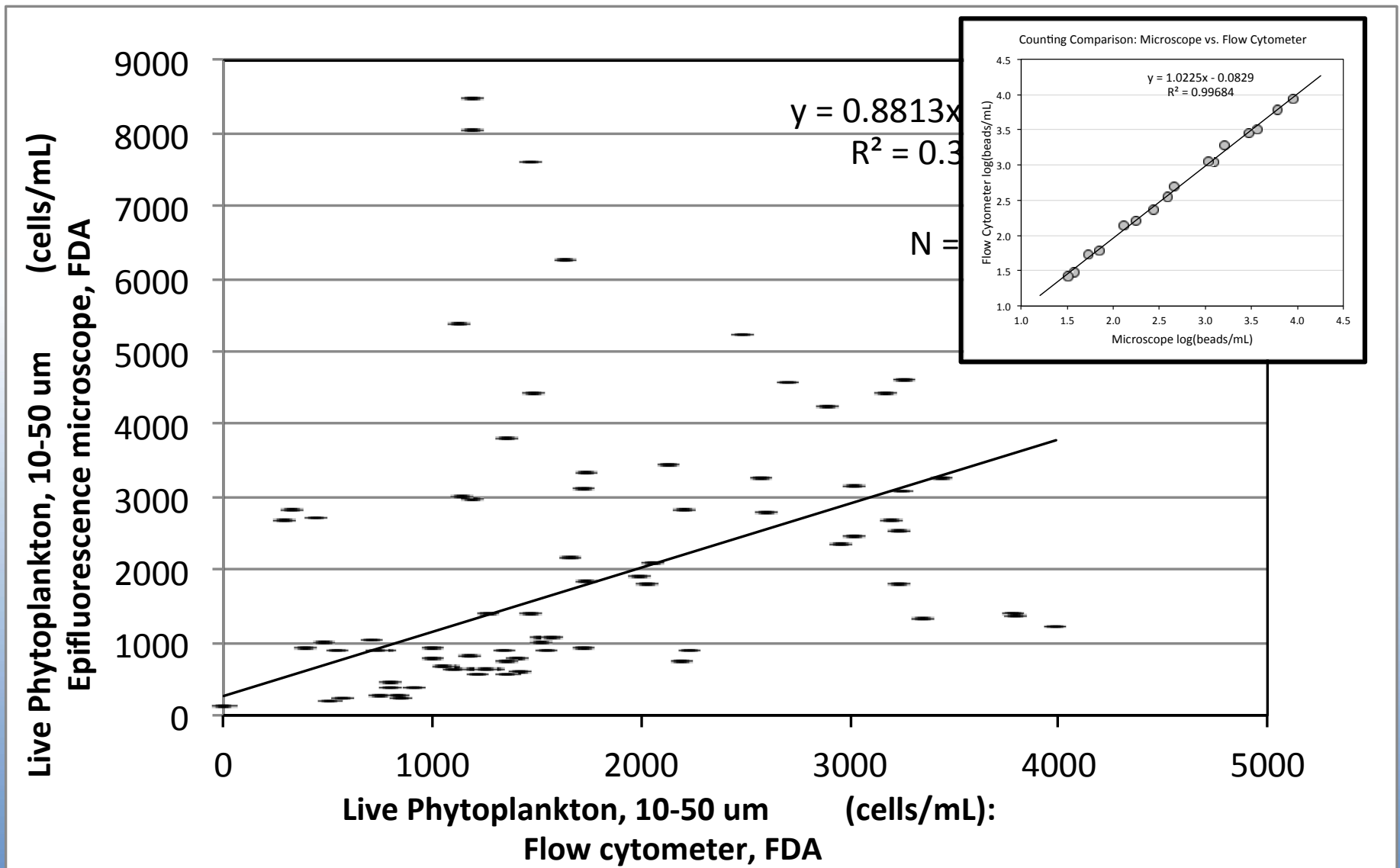
**Will one cell produce two pulses?**

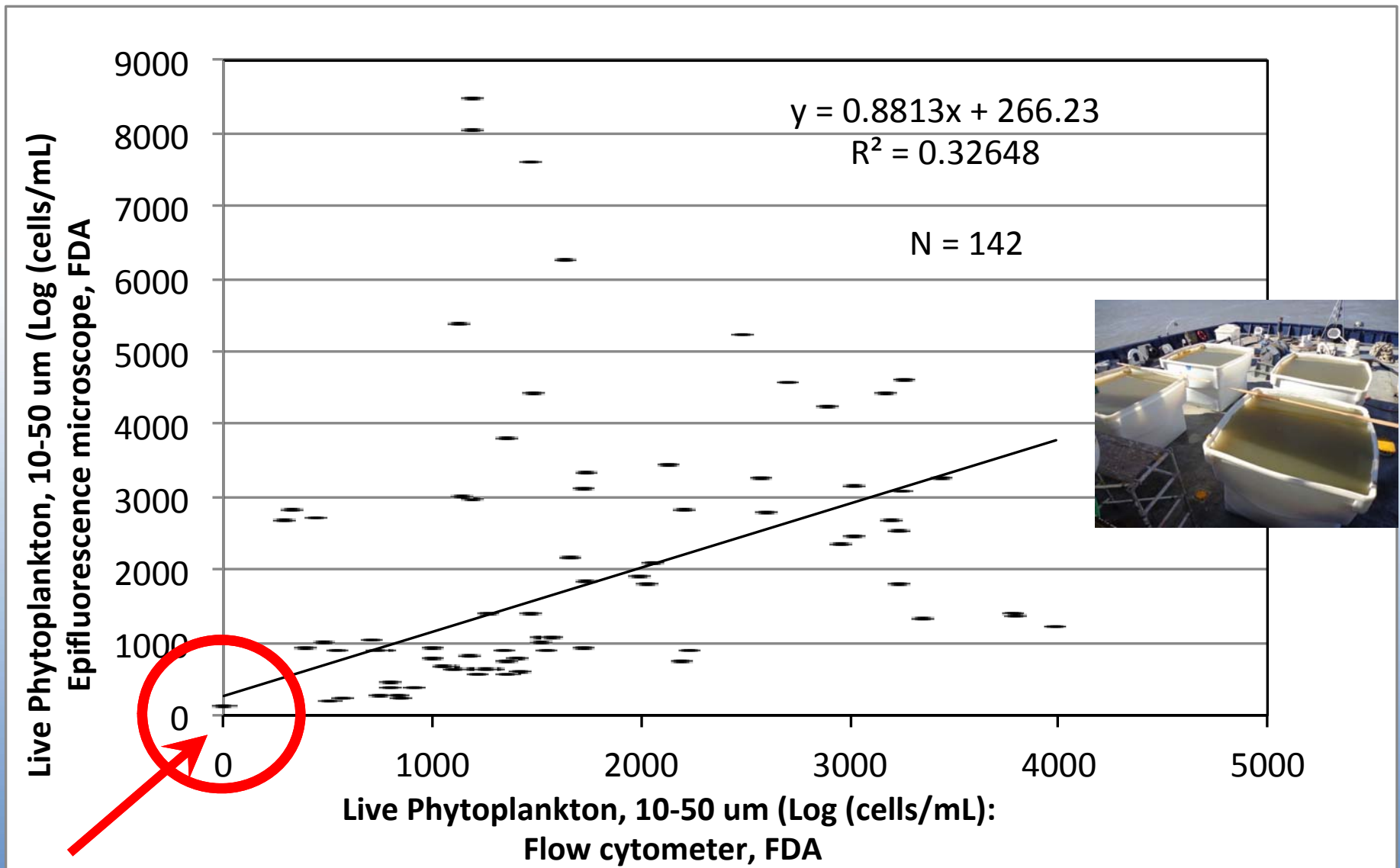


**Will it see 5 cells in a chain, or just one entity?**



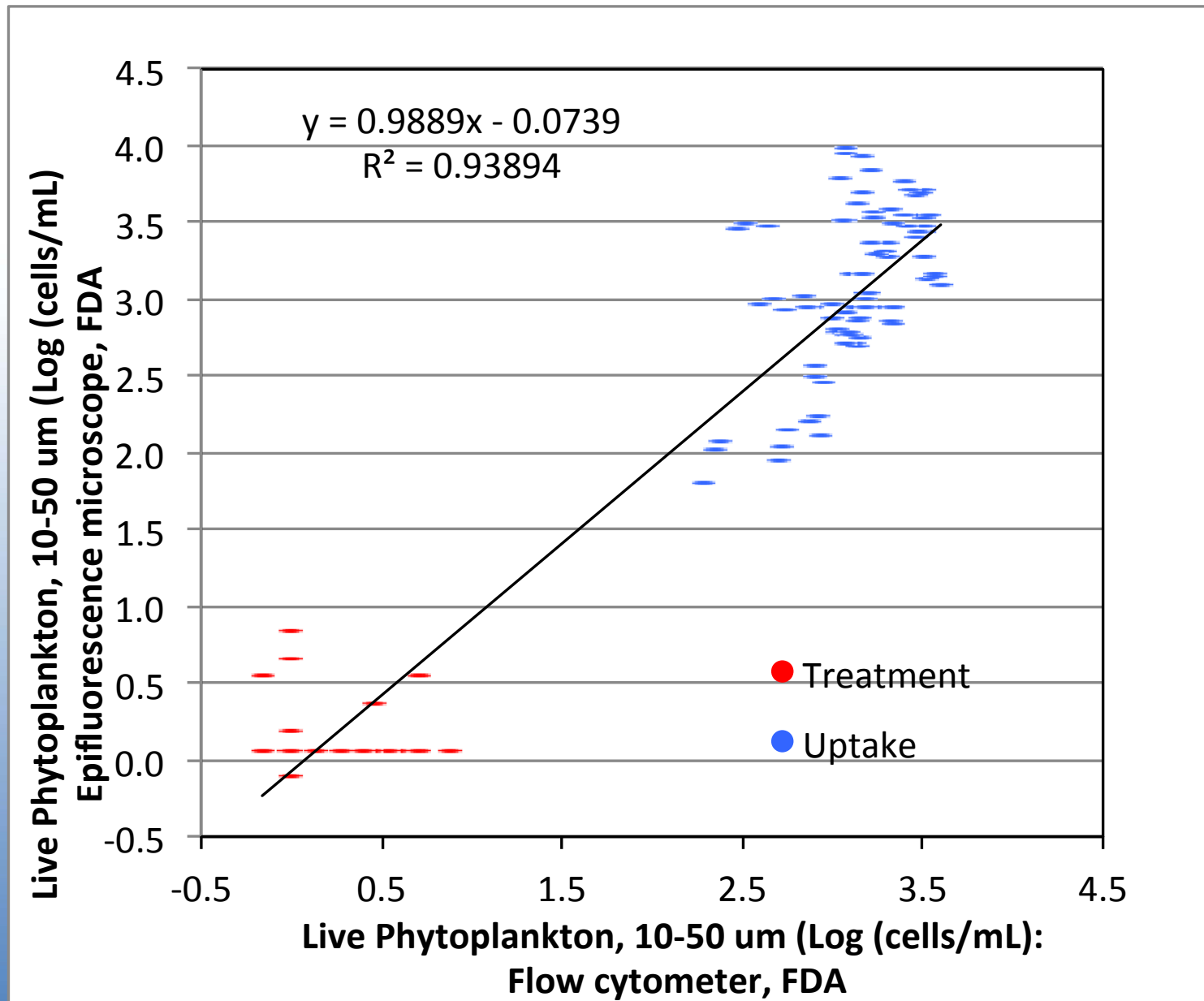


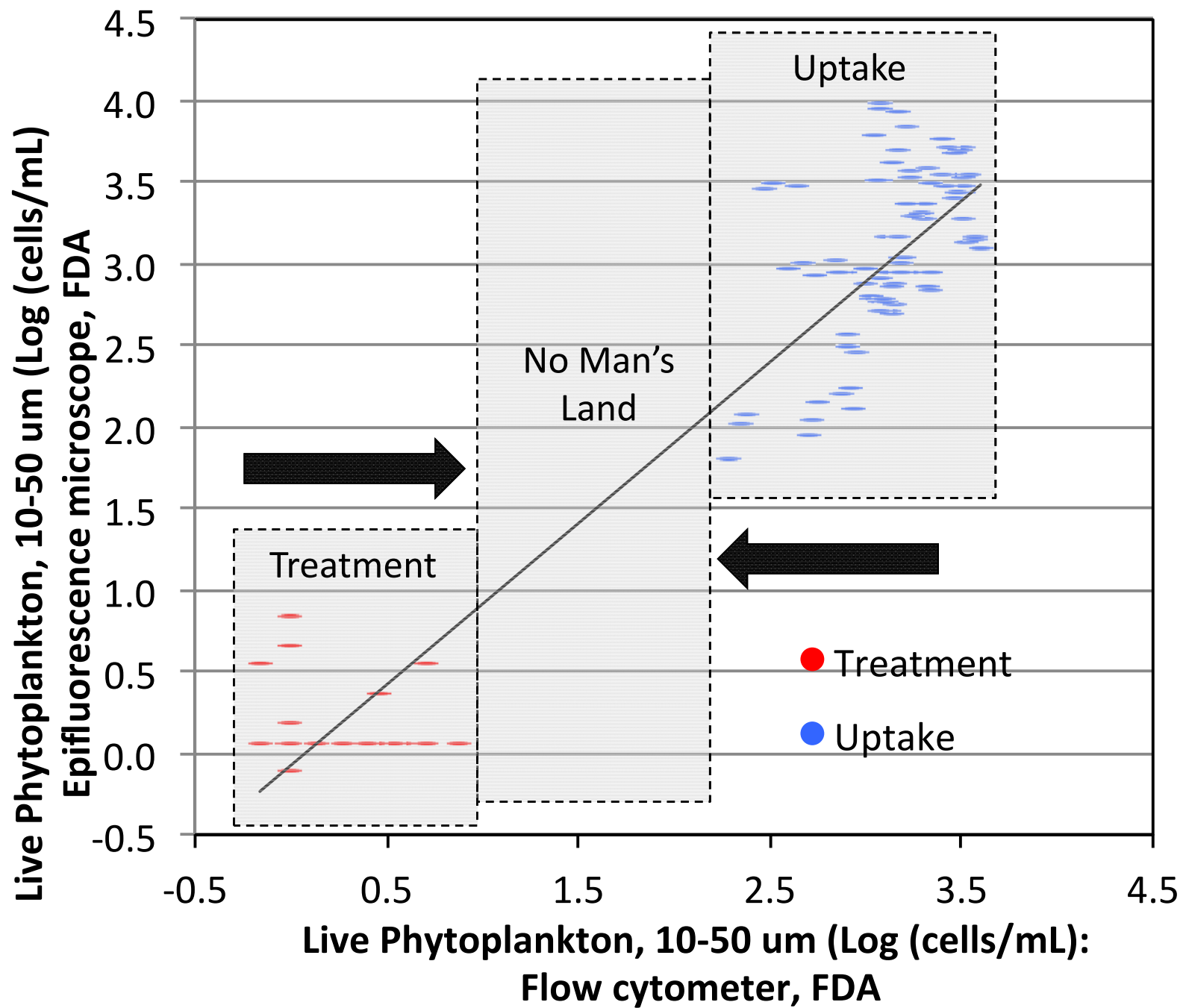




**N = 76**

Logarithmic plot of the same data shown previously





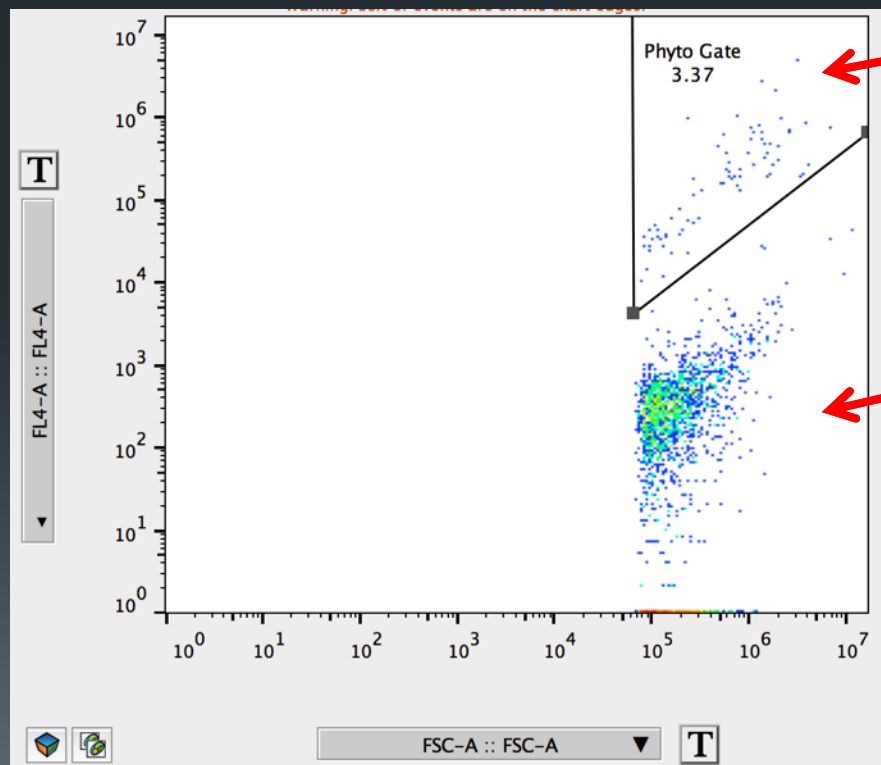
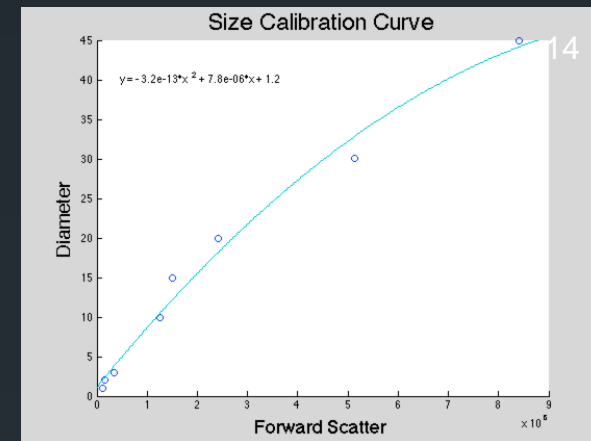
**Concept 2**: Size Fractionation      **Concept 3**: Challenge Concentration

Do we provide an accurate assessment of  
Numerical “CHALLENGE” in 10-50  $\mu\text{m}$  counting?



## Conception 2. Natural organism concentrations are not challenging enough

- Analyze beads of known diameters to generate a calibration curve



Phytoplankton show red fluorescence due to chlorophyll content

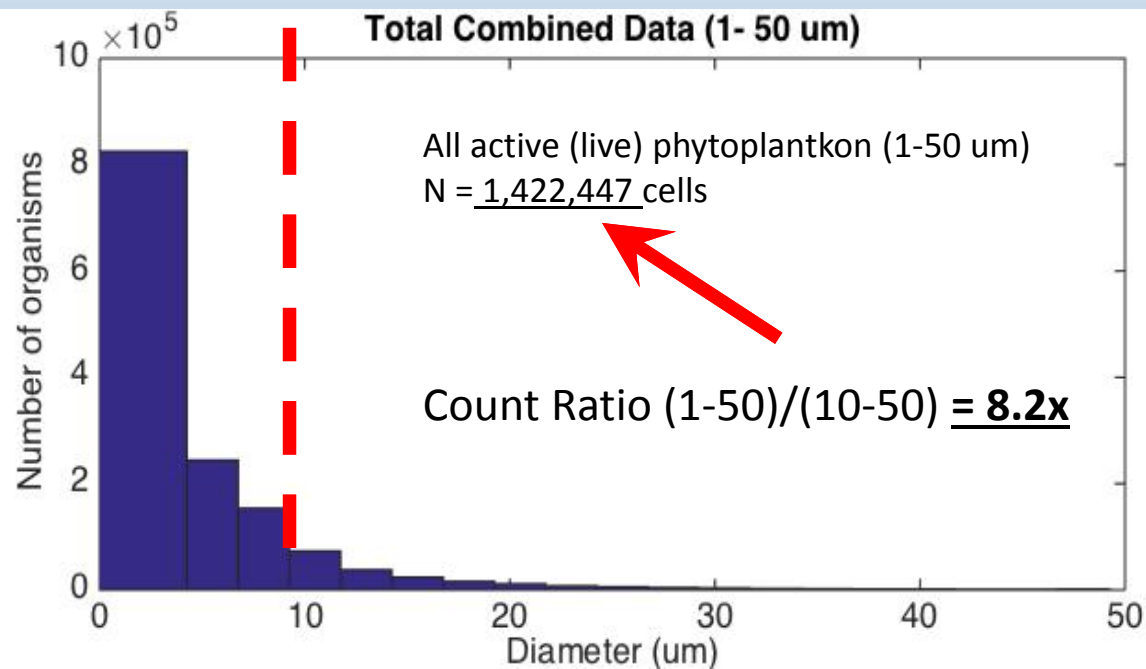
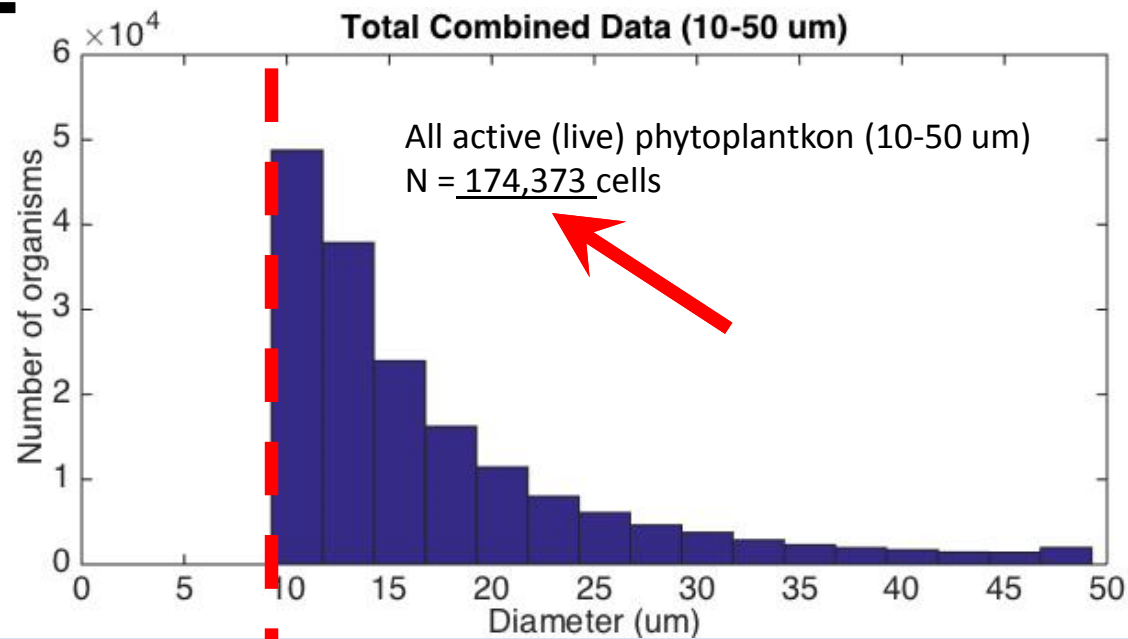
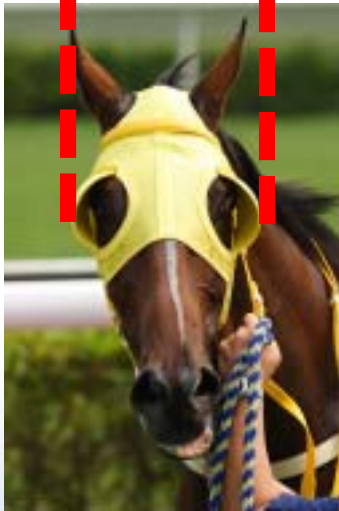
These particles exhibit low red fluorescence and are considered 'noise' (detritus, inorganic particles)

# Comparing 10-50um size class organisms across locations

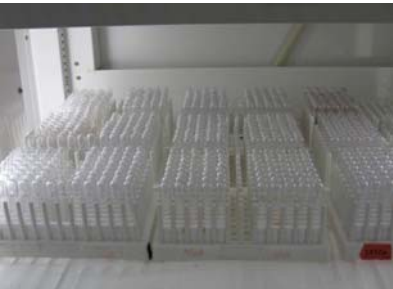
Location	Mean Volume ( $\mu\text{m}^3$ )	Equivalent Diameter ( $\mu\text{m}$ )	n
Seattle, WA	2,764	17.41	5485
Vallejo, CA	3,322	18.51	30325
San Francisco, CA	3,342	18.55	22694
Denmark	3,752	19.28	4229
Moss Landing, CA	7,204	23.96	50838
Port Angeles, WA	8,501	25.32	3425



→ 10-50  $\mu\text{m}$  ←

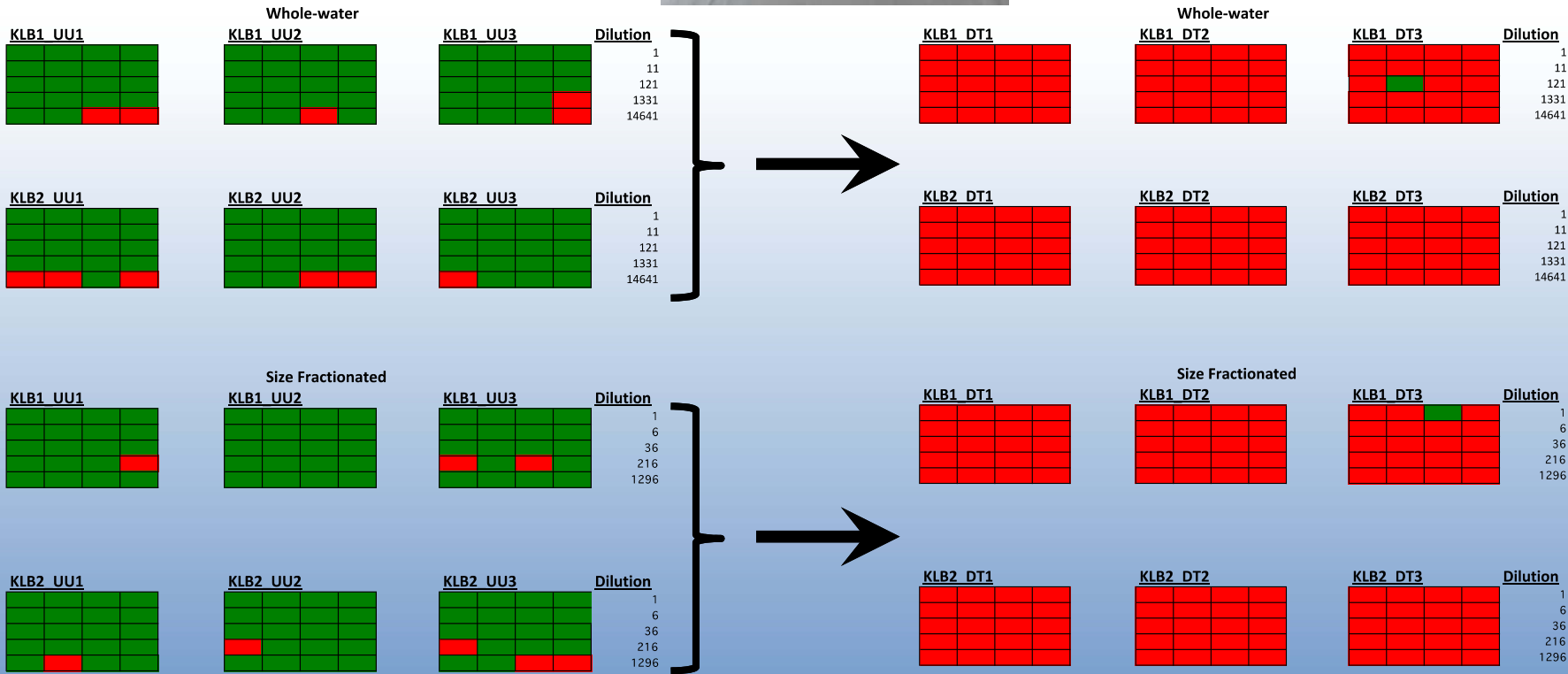


The outcome of 1) Whole-Water and 2) Size-fractionated (10-50 µm) MPN assays (UV)



Uptake

Treatment Discharge



The outcome of 1) Whole-Water and 2) Size-fractionated (10-50  $\mu\text{m}$ ) MPN assays (UV):  
**Numeric Challenge** and **Biological Efficacy** are significantly larger than we think

Uptake

Whole Water MPN  
concentrations

Sample Type	Live cells/mL
KLB1_UU1	3700
KLB1_UU2	7400
KLB1_UU3	1400
KLB2_UU1	2000
KLB2_UU2	3700
KLB2_UU3	7400

$X = 4267$

Biological Efficacy  
(Uptake/Discharge)



**46380x**

Treatment Discharge

Whole Water MPN  
concentrations

Sample Type	Live cells/mL
KLB1_DT1	0.094
KLB1_DT2	0.094
KLB1_DT3	0.083
KLB2-DT1	0.094
KLB2-DT2	0.094
KLB2-DT3	0.094

$X = 0.092$

Size-fractionated MPN  
concentrations

Sample Type	Live cells/mL
KLB1_UU1	240
KLB1_UU2	800
KLB1_UU3	140
KLB2_UU1	600
KLB2_UU2	240
KLB2_UU3	140

$X = 360$

**4615x**

Size-fractionated MPN  
concentrations

Sample Type	Live cells/mL
KLB1_DT1	0.078
KLB1_DT2	0.078
KLB1_DT3	0.078
KLB2-DT1	0.078
KLB2-DT2	0.078
KLB2-DT3	0.078

$X = 0.078$

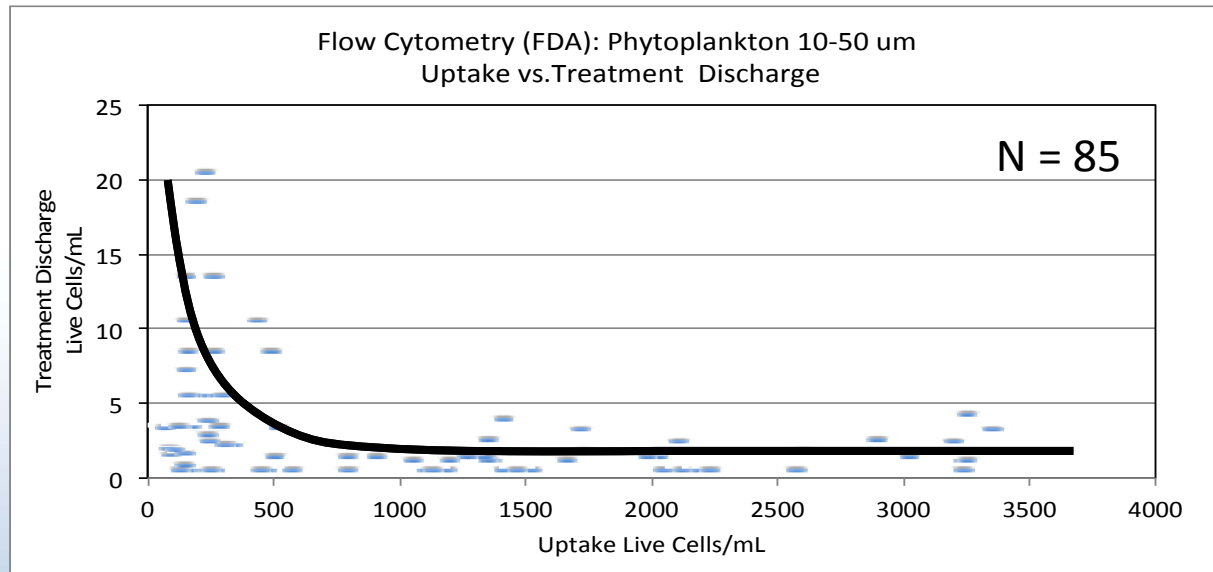
### **Conception 3 (again):**

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

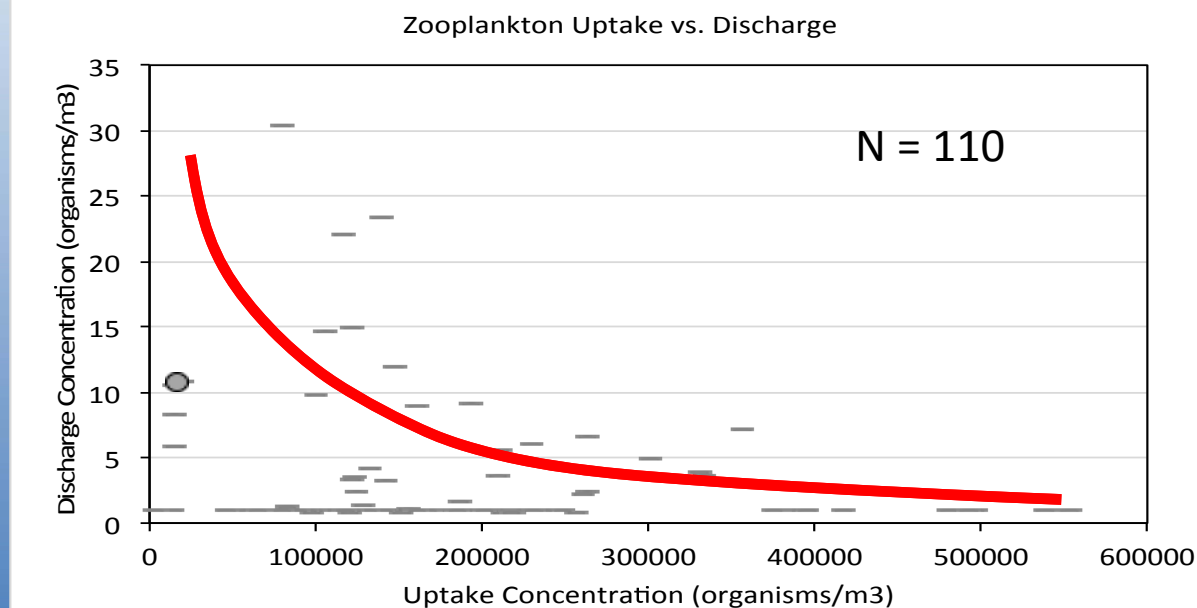
### **The CHALLENGE concept:**

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 ??



10-50  $\mu\text{m}$   
Live Phytoplankton (FDA)

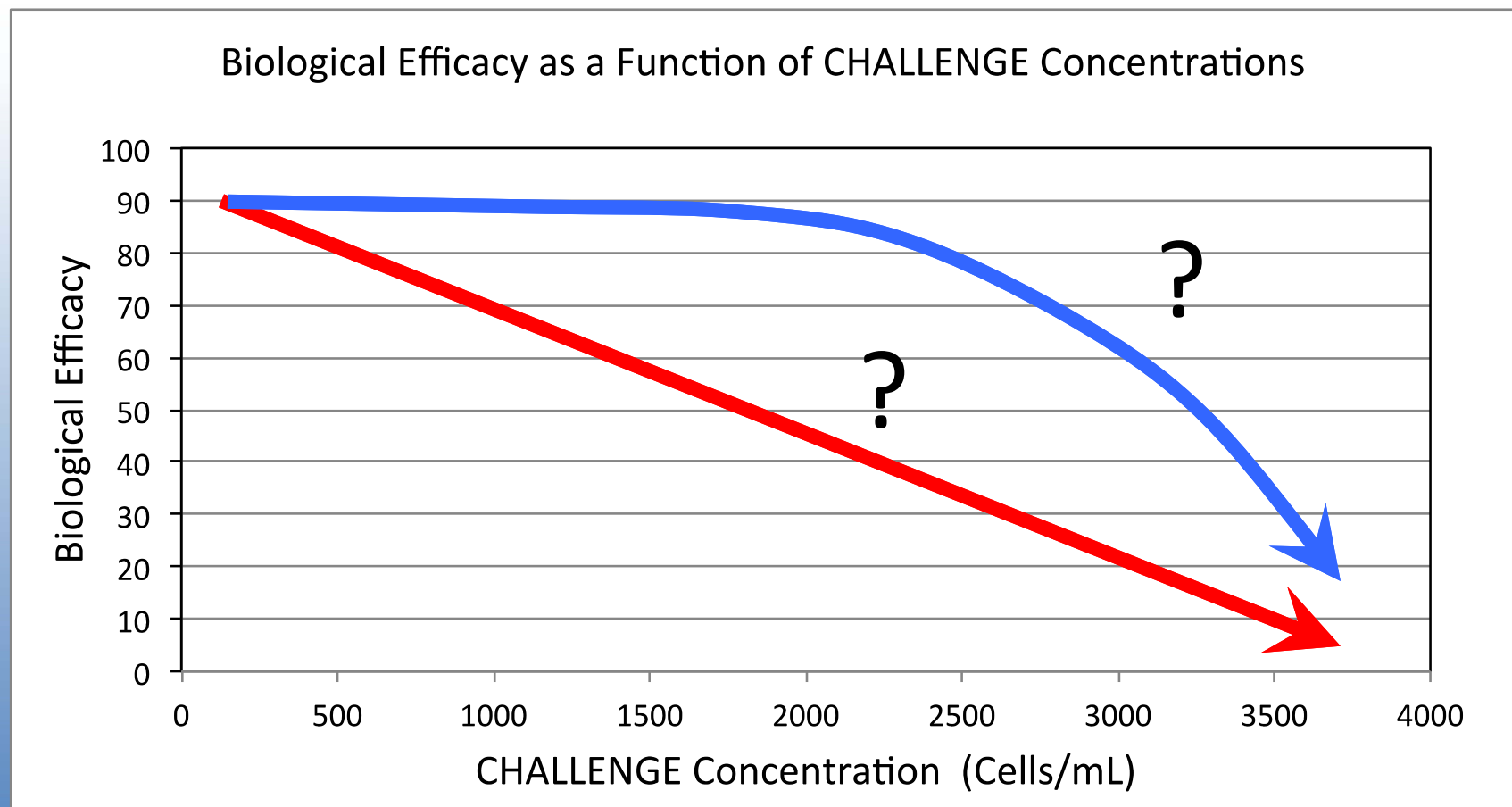


>50  $\mu\text{m}$   
Live Zooplankton

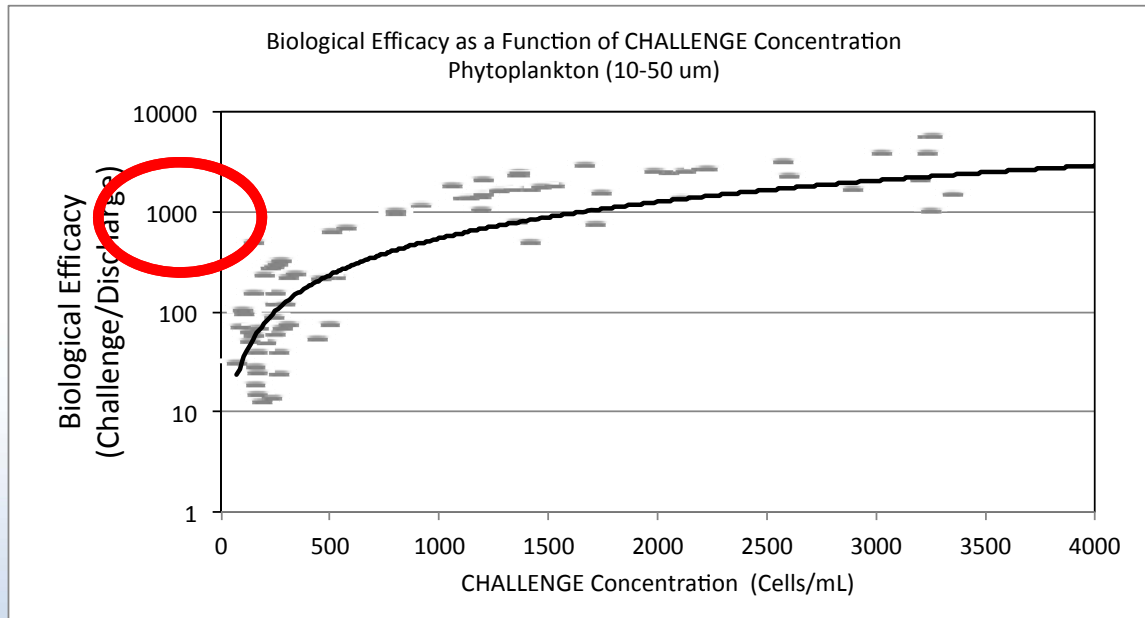
# “CHALLENGE” in Ballast Water Treatment Testing: Conceptions and Misconceptions

Nick Welschmeyer, Moss Landing Marine Laboratories, CA (CSU)

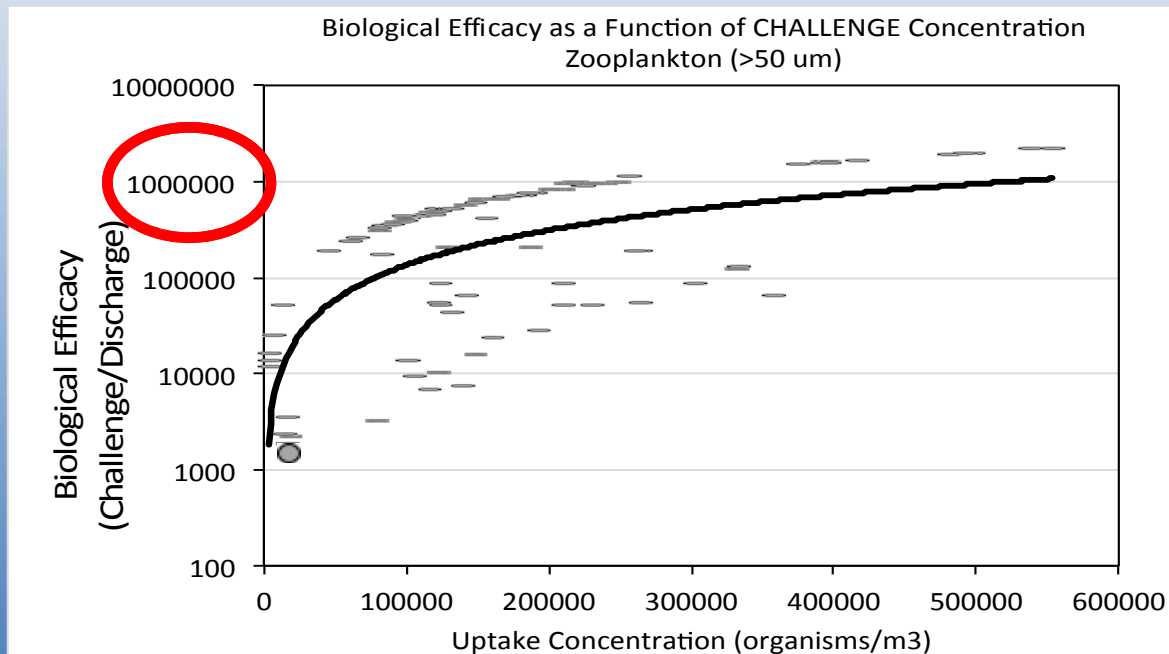
Presented: Feb 2, 2016; ETV Tech Panel, Baltimore MD



## Biological efficacy does not obey the CHALLENGE Concept in Ballast Water Testing



10-50  $\mu\text{m}$   
Live Phytoplankton (FDA)



>50  $\mu\text{m}$   
Live Zooplankton



# QUESTION:

## HOW ARE WE DOING IN BALLAST WATER TREATMENT?

### 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;

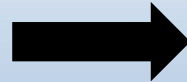
## Environmental Successes:

### 1. Visible reductions in Los Angeles smog



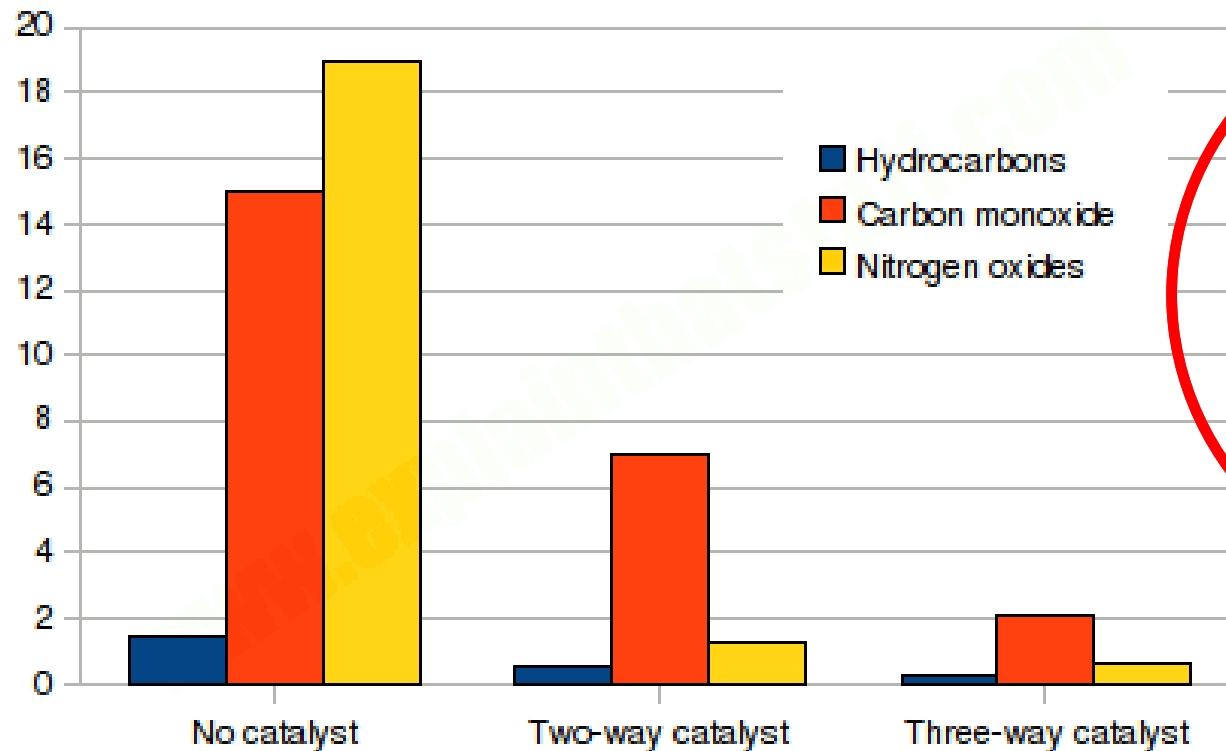
Then...

Now



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

## *Effectiveness of catalytic converters*

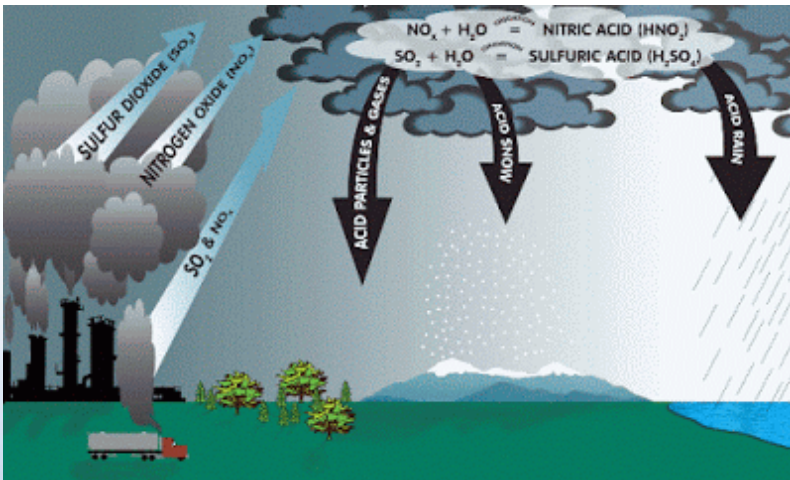


Roughly...  
**10x** reduction in  
pollution emissions,  
even with modern  
3-way converters

## Environmental Successes:

### 2. Reduction in Acid Rain

Death to acid-intolerant forests



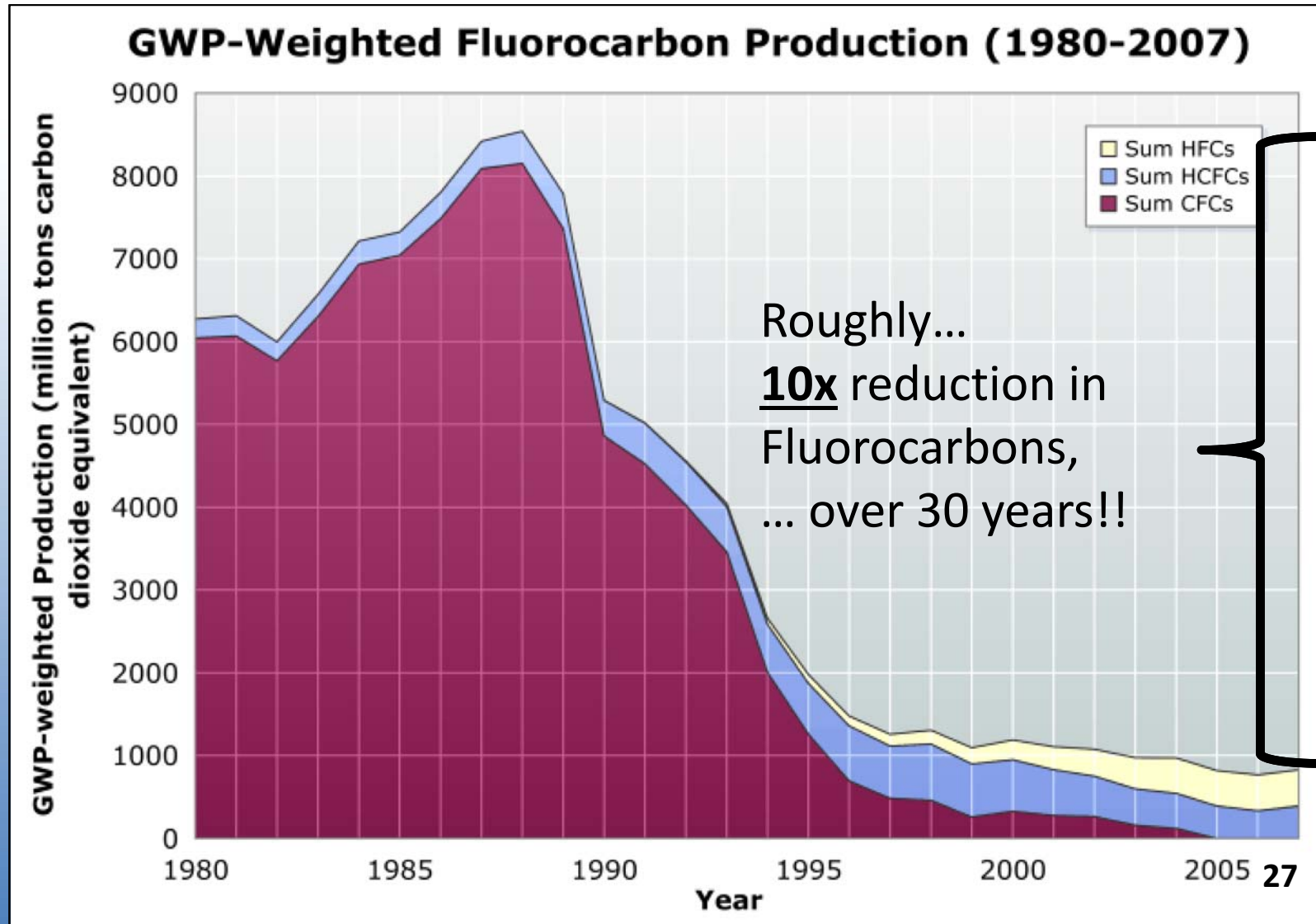
### The Clean Air Act 1970

Stack-gas scrubbers:  
Roughly...

**5x – 20x** reductions in  
 $\text{SO}_2$  and  $\text{NO}_x$

## Environmental Successes:

### 3. Reduction of the Antarctic Ozone 'Hole'



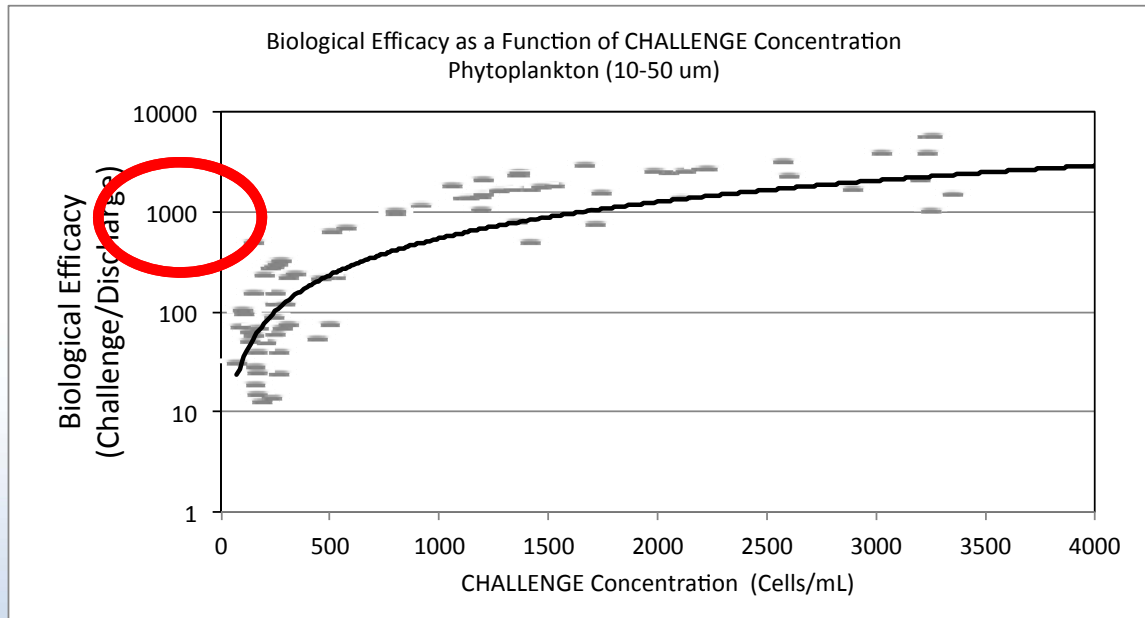
Three of the greatest environmental successes in modern history...

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

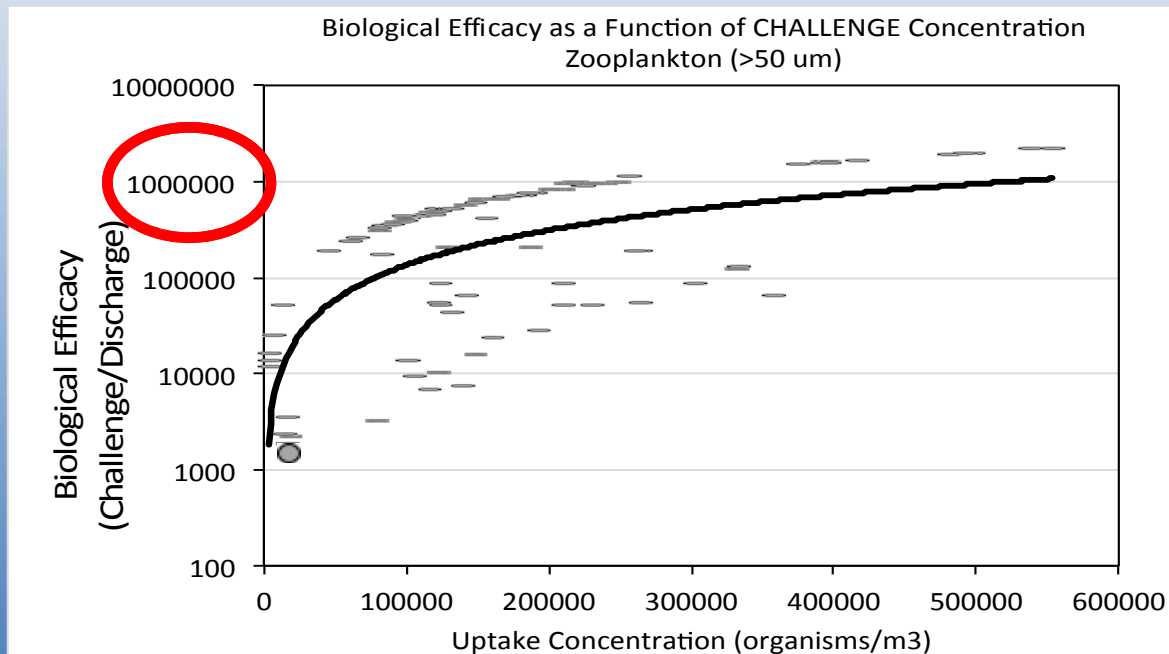
... were accomplished with reductions in the respective putative pollutants that were **approximately 10x**.

**HOW ARE WE DOING IN BALLAST WATER TREATMENT?**

## Biological efficacy does not obey the CHALLENGE Concept in Ballast Water Testing



10-50  $\mu\text{m}$   
Live Phytoplankton (FDA)



>50  $\mu\text{m}$   
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  $\mu\text{m}$  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!