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# Performance Evaluations of Instruments Designed for Rapid, Shipboard Detection of Living Microorganisms in Ballast Water

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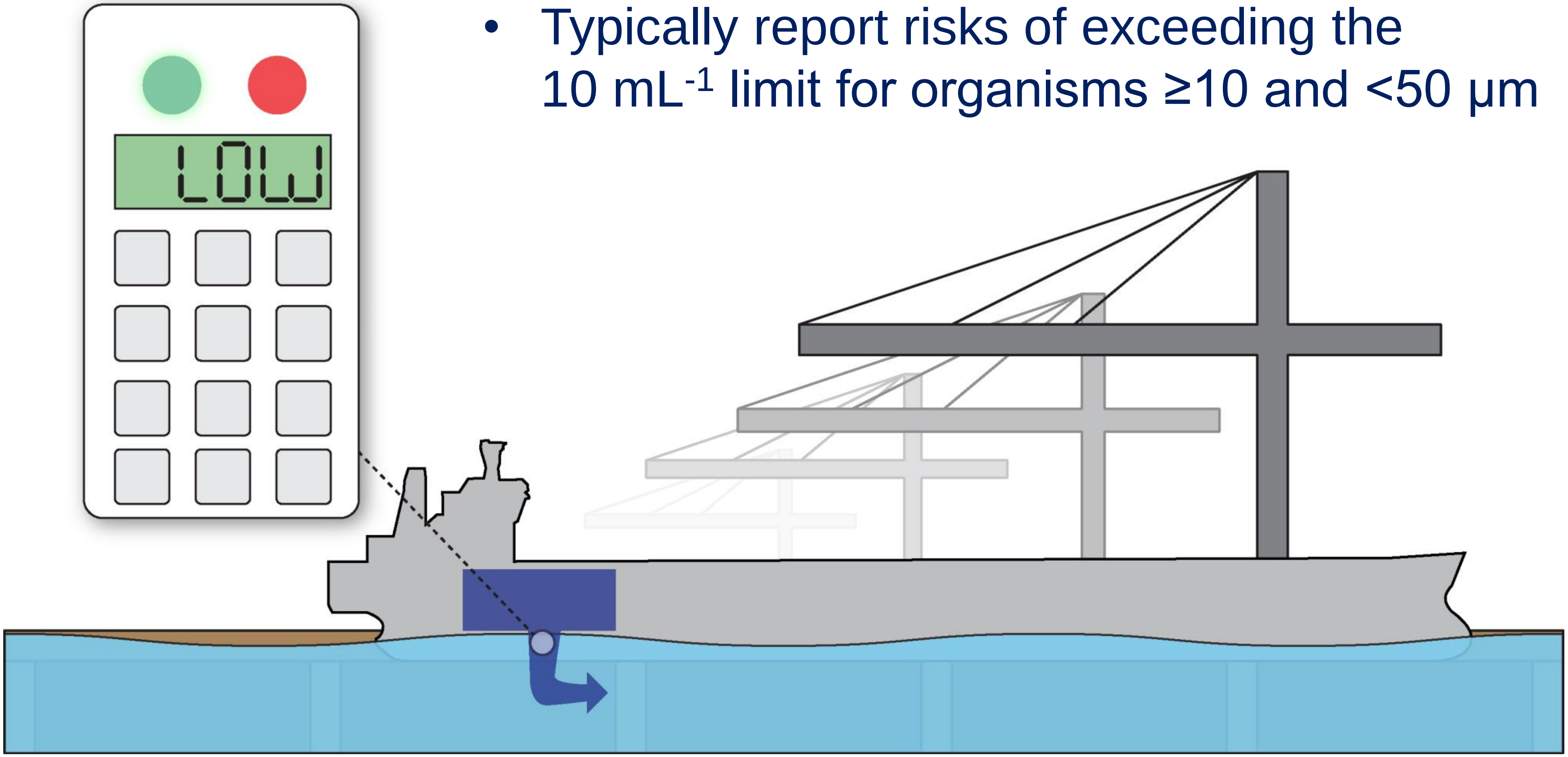
# Background on "Compliance Tools"

## Compliance Tools:

- Test ballast water as it is discharged
- Designed for rapid, shipboard analysis
- Typically report risks of exceeding the 10 $mL^{-1}$ limit for organisms ≥10 and <50 μm



# A Framework for Validation*

**2015 → 2016:**
Testing of compliance tools based upon variable fluorescence fluorometry

## Step 1: Proof-of-Concept

- Pilot study
- Subject matter workshops

## Step 2: Verification and Validation

- Rigorous, independent testing
- Tests with challenging conditions

## Step 3: Feasibility and Selection

Considerations include:

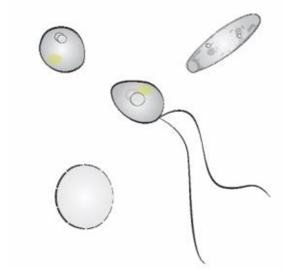
- Functional requirements
- Physical size and safety
- Cost and ease-of-use

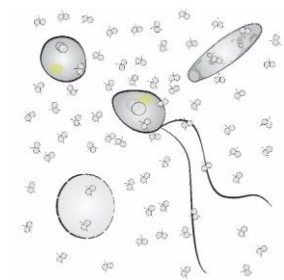
*Drake et al. (2014) Marine Pollution Bulletin 86: 122-128

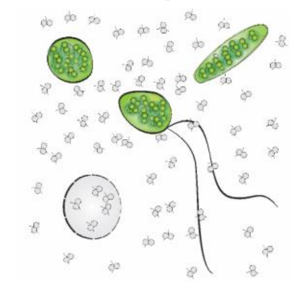
# Required Method for Organisms ≥10 µm and <50 µm

The **Environmental Technology Verification Protocol (ETV)*** stipulates an approach based upon epifluorescence microscopy

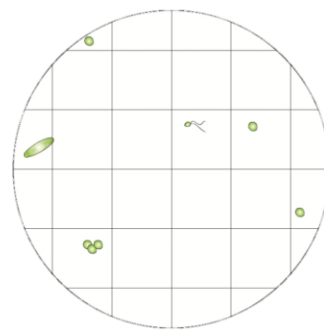
**Step 1: Labeling**
Two fluorescent probes are introduced into the sample

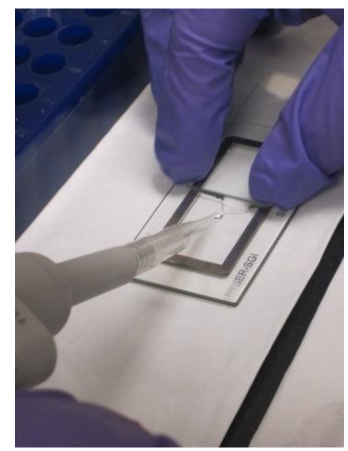






**Step 2: Manual microscopy**
Visual counts of fluorescing or moving (i.e., living) organisms





*U.S. Environmental Protection Agency, 2010; the ETV is the U.S. protocol for land-based verification testing of ballast water management systems

# Laboratory and Field Trials

**Laboratory trials:**
Tested a range of concentrations of one of two cultured microalgae


*Prorocentrum micans*


*Tetraselmis marina*

## Target concentrations:

| | |
|---|---|
| 0 $mL^{-1}$ | 20 $mL^{-1}$ |
| 5 $mL^{-1}$ | 50 $mL^{-1}$ |
| 10 $mL^{-1}$ | 100 $mL^{-1}$ |

**Field trials:**
Examined ambient samples at contrasting locations



# Fluorometry-based compliance tools

**YSI Ballast Monitor**
Xylem
86 x 103 x 30 cm
100 kg


**10Cells**
BBE Moldaenke
30 x 34 x 15 cm
5 kg


**BW680**
Hach
6 x 14 x 5 cm
0.3 kg

**Ballast-Check 2**
Turner Designs
9 x 18 x 5 cm
0.4 kg

**FastBallast**
Chelsea Tech. Group
20 x 24 x 5 cm
3 kg

# Testing and Analysis: 2015-2016

Round 1: June – September, 2015

Round 2: March – July, 2016

Sampling ambient organisms from seawater in Key West, FL

# Evaluation Criteria

## Linearity

- Do measurements of abundance change proportionately with cell concentrations?

## Precision

- Are repeated measurements of the same sample in agreement?

## Accuracy

- Does the instrument’s assessment (i.e., above or below the discharge standard) agree with microscope counts?

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# Results: Linearity (All trials)

## $R^2$ Values: Coefficient of Determination

### Microscope counts vs. compliance tool concentrations

| Tool | Laboratory Trials | | | Field Trials | | | |
|---|---|---|---|---|---|---|---|
| | *T. marina* | *P. micans* | Both | NRL | GSI | SERC | All Sites |
| **Ballast-Check 2 (2015)** | 0.46 | 0.98 | 0.90 | 0.63 | 0.64 | 0.12 | 0.10 |
| **10Cells** | 0.85 | 0.84 | 0.68 | 0.61 | 0.69 | 0.68 | 0.48 |
| **YSI Ballast Monitor** | 0.87 | 0.94 | 0.91 | 0.72 | 0.66 | 0.01 | 0.15 |
| **Ballast-Check 2 (2016)** | 0.33 | 0.90 | 0.82 | 0.73 | 0.46 | 0.39 | 0.36 |
| **FastBallast** | N/A | N/A | N/A | 0.13 | 0.75 | 0.71 | 0.37 |
| **BW680** | 0.57 | 0.92 | 0.86 | 0.66 | 0.61 | 0.82 | 0.66 |

$R^2$ Values: 0 to 1

Detailed reports available at: www.act-us.info

Legend:

$R^2 \geq 0.90$ $R^2 \geq 0.75$

$R^2 < 0.50$

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# Results: Precision (Laboratory trials)

## CV: Coefficient of Variation

| Tool | Minimum | Maximum | Mean | Median | n |
|---|---|---|---|---|---|
| **BallastCheck2 (2015)** | 22% | 230% | 77% | 59% | 21 |
| **10Cells** | N/A | N/A | N/A | N/A | N/A |
| **YSI Ballast Monitor** | 0.2% | 24% | 4.7% | 3.4% | 36 |
| **BallastCheck2 (2016)** | 1% | 99% | 33% | 29% | 14 |
| **FastBallast** | N/A | N/A | N/A | N/A | N/A |
| **BW680** | 2% | 105% | 30% | 16% | 23 |

CV (%): Standard deviation adjusted to the mean

Only reported for mean values >10 units

Legend:

CV <25%

CV ≥25%

# Results: Precision (Field trials)

## CV: Coefficient of Variation

| Tool | Minimum | Maximum | Mean | Median | n |
|---|---|---|---|---|---|
| **BallastCheck2 (2015)** | 9% | 61% | 28% | 26% | 12 |
| **10Cells** | 6% | 52% | 24% | 22% | 20 |
| **YSI Ballast Monitor** | 0.1% | 63% | 13% | 4.7% | 36 |
| **BallastCheck2 (2016)** | 25% | 113% | 63% | 53% | 15 |
| **FastBallast** | 9% | 42% | 21% | 18% | 22 |
| **BW680** | 6% | 101% | 25% | 17% | 26 |

CV(%): Standard deviation adjusted to the mean

Only reported for mean values >10 units

Legend:

CV <25%

CV ≥25%

# Results: Accuracy (Laboratory trials)

## Probability of measuring an exceedance at 30 $mL^{-1}$

| Compliance Tool | Laboratory Trials | | |
|---|---|---|---|
| | *T. marina* | *P. micans* | Both organisms |
| **Ballast-Check 2 (2015)** | 0.62 | 0.98 | 0.71 |
| **10Cells** | N/A: Insufficient readings exceeding 10 $mL^{-1}$ | | |
| **YSI Ballast Monitor** | N/A: Pass/Fail not reported | | |
| **Ballast-Check 2 (2016)** | N/A[2] | 0.99 | 0.64 |
| **FastBallast** | N/A: Instrument malfunction | | |
| **BW680** | 1.00 | 1.00 | 0.99 |

30 $mL^{-1}$: 3x the exceedance of the discharge standard

Legend:
Probability ≥0.90

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# Results: Accuracy (Field trials)

## Probability of measuring an exceedance at 30 $mL^{-1}$

| Compliance Tool | Field Trials | | | |
|---|---|---|---|---|
| | NRL | GSI | SERC | All Sites |
| **Ballast-Check 2 (2015)** | 0.97 | 0.26 | 0.07 | 0.28 |
| **10Cells** | 0.99 | 0.98 | 1.00 | 0.99 |
| **YSI Ballast Monitor** | N/A: Pass/Fail not reported | | | |
| **Ballast-Check 2 (2016)** | 1.00 | 0.22 | 0.05 | 0.25 |
| **FastBallast** | N/A: Insignificant regression | | | 0.70 |
| **BW680** | 1.00 | 1.00 | 0.99 | 0.97 |

30 $mL^{-1}$: 3x an exceedance of the discharge standard

Legend:
Probability ≥0.90

# Conclusions: Testing the Validation Framework

Tests provided challenging conditions, and in general, the compliance tools performed well for samples of:

- Monocultures of relatively “large” microalgae (i.e., *P. micans*)
- Oligotrophic waters (i.e., Florida Keys)

In field trials, compliance tools had a high probability (~99%) of detecting an exceedance when concentrations were ≥30 $mL^{-1}$:

- Therefore, probabilities of detecting gross exceedances (e.g., ≥100 $mL^{-1}$) would be very high (~100%)

Future rounds of testing may include technologies with other approaches, new instruments, or new models of these instruments

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# Acknowledgements: Participants

## Additional Test Participants

Dr. Katherine 'Jenny' Carney

Smithsonian Environmental Research Center (SERC)

Ms. Lisa Allinger and Dr. Euan Reavie

University of Minnesota-Duluth; Great Ships Initiative (GSI)

## Technical Advisory Committee (Fluorometry)

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U.S. Environmental Protection Agency

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U.S. Coast Guard

Dr. Carolyn Junemann

Maritime Administration

Dr. Sam Laney

Woods Hole Oceanographic Inst.

Dr. Beth Stauffer

University of Louisiana Lafayette

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# Supplemental Slides

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# Testing and Analysis: 2015-2016

## Round 1: June – September, 2015
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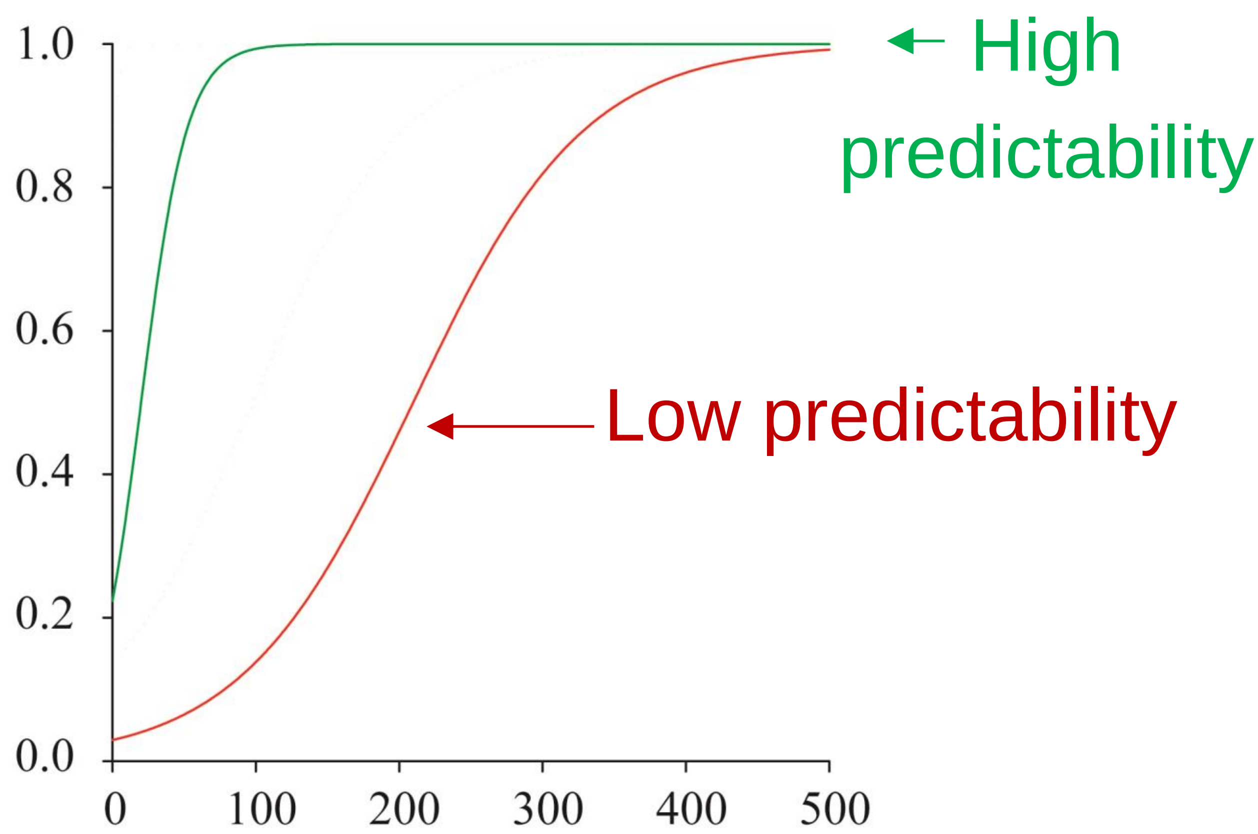
# Accuracy: Logistical Regression

Logistical Regression compares the relationship between:

- A continuous independent variable (cell concentration)
- A binary dependent variable (Pass/Fail)

Probability (of measuring an exceedance)




Cell concentration ($mL^{-1}$)

# Results: Linearity (Laboratory trials)

## $R^2$ Values: Coefficient of Determination

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| **FastBallast** | N/A | N/A | N/A |
| **BW680** | 0.57 | 0.92 | 0.86 |

Legend:
$R^2$ ≥0.90
$R^2$ ≥0.75
$R^2$ <0.50

$R^2$ Values:
0 (no linear relationship) to 1 (strong linear relationship)

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# Results: Linearity (Field trials)

## $R^2$ Values: Coefficient of Determination

### Microscope counts vs. compliance tool concentrations

| Tool | Field Trial Locations | | | |
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| BW680 | 0.66 | 0.61 | 0.82 | 0.66 |

Legend:

$R^2 \geq 0.90$

$R^2 \geq 0.75$

$R^2 < 0.50$

$R^2$ Values:

0 (no linear relationship) to 1 (strong linear relationship)