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 \(\geq 10\) and <50 µm
A Framework for Validation*

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

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

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

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

*Image: Gert Hansen
SCCAP K-1137
http://media.nordicmicroalgae.org

**Target concentrations:**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>0 mL(^{-1})</th>
<th>5 mL(^{-1})</th>
<th>10 mL(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 mL(^{-1})</td>
<td>50 mL(^{-1})</td>
<td>100 mL(^{-1})</td>
</tr>
</tbody>
</table>

*Prorocentrum micans  Tetraselmis marina*
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?
### Results: Linearity (All trials)

**R² Values: Coefficient of Determination**
Microscope counts vs. compliance tool concentrations

<table>
<thead>
<tr>
<th>Tool</th>
<th>Laboratory Trials</th>
<th>Field Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T. marina</td>
<td>P. micans</td>
</tr>
<tr>
<td>Ballast-Check 2 (2015)</td>
<td>0.46</td>
<td>0.98</td>
</tr>
<tr>
<td>10Cells</td>
<td>0.85</td>
<td>0.84</td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td>0.87</td>
<td>0.94</td>
</tr>
<tr>
<td>Ballast-Check 2 (2016)</td>
<td>0.33</td>
<td>0.90</td>
</tr>
<tr>
<td>FastBallast</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>BW680</td>
<td>0.57</td>
<td>0.92</td>
</tr>
</tbody>
</table>

**R² Values: 0 to 1**

Legend:

- R² ≥0.90
- R² ≥0.75
- R² <0.50

Detailed reports available at: [www.act-us.info](http://www.act-us.info)
### Results: Precision (Laboratory trials)

**CV: Coefficient of Variation**

<table>
<thead>
<tr>
<th>Tool</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Median</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BallastCheck2 (2015)</strong></td>
<td>22%</td>
<td>230%</td>
<td>77%</td>
<td>59%</td>
<td>21</td>
</tr>
<tr>
<td><strong>10Cells</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>YSI Ballast Monitor</strong></td>
<td>0.2%</td>
<td>24%</td>
<td>4.7%</td>
<td>3.4%</td>
<td>36</td>
</tr>
<tr>
<td><strong>BallastCheck2 (2016)</strong></td>
<td>1%</td>
<td>99%</td>
<td>33%</td>
<td>29%</td>
<td>14</td>
</tr>
<tr>
<td><strong>FastBallast</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>BW680</strong></td>
<td>2%</td>
<td>105%</td>
<td>30%</td>
<td>16%</td>
<td>23</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>Tool</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Median</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BallastCheck2 (2015)</td>
<td>9%</td>
<td>61%</td>
<td>28%</td>
<td>26%</td>
<td>12</td>
</tr>
<tr>
<td>10Cells</td>
<td>6%</td>
<td>52%</td>
<td>24%</td>
<td>22%</td>
<td>20</td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td>0.1%</td>
<td>63%</td>
<td>13%</td>
<td>4.7%</td>
<td>36</td>
</tr>
<tr>
<td>BallastCheck2 (2016)</td>
<td>25%</td>
<td>113%</td>
<td>63%</td>
<td>53%</td>
<td>15</td>
</tr>
<tr>
<td>FastBallast</td>
<td>9%</td>
<td>42%</td>
<td>21%</td>
<td>18%</td>
<td>22</td>
</tr>
<tr>
<td>BW680</td>
<td>6%</td>
<td>101%</td>
<td>25%</td>
<td>17%</td>
<td>26</td>
</tr>
</tbody>
</table>

**CV(%)**: Standard deviation adjusted to the mean

*Only reported for mean values >10 units*

**Legend:**

- $CV < 25\%$
- $CV \geq 25\%$
### Results: Accuracy (Laboratory trials)

#### Probability of measuring an exceedance at 30 mL⁻¹

<table>
<thead>
<tr>
<th>Compliance Tool</th>
<th>Laboratory Trials</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T. marina</td>
</tr>
<tr>
<td>Ballast-Check 2 (2015)</td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>10Cells</td>
<td>N/A: Insufficient readings exceeding 10 mL⁻¹</td>
<td></td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td>N/A: Pass/Fail not reported</td>
<td></td>
</tr>
<tr>
<td>Ballast-Check 2 (2016)</td>
<td>N/A²</td>
<td>0.99</td>
</tr>
<tr>
<td>FastBallast</td>
<td>N/A: Instrument malfunction</td>
<td></td>
</tr>
<tr>
<td>BW680</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Legend:**
- Probability ≥0.90

**30 mL⁻¹:** 3x the exceedance of the discharge standard
## Results: Accuracy (Field trials)

### Probability of measuring an exceedance at 30 mL\(^{-1}\)

<table>
<thead>
<tr>
<th>Compliance Tool</th>
<th>Field Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRL</td>
</tr>
<tr>
<td>Ballast-Check 2 (2015)</td>
<td>0.97</td>
</tr>
<tr>
<td>10Cells</td>
<td>0.99</td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td>N/A: Pass/Fail not reported</td>
</tr>
<tr>
<td>Ballast-Check 2 (2016)</td>
<td>1.00</td>
</tr>
<tr>
<td>FastBallast</td>
<td>N/A: Insignificant regression</td>
</tr>
<tr>
<td>BW680</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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

Legend:
- Probability ≥0.90
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%)
Acknowledgements

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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
Supplemental Slides
Testing and Analysis: 2015-2016

Round 1: June – September, 2015
Round 2: March – July, 2016

Sampling ambient organisms from seawater in Key West, FL
Logistical Regression compares the relationship between:
- A continuous independent variable (cell concentration)
- A binary dependent variable (Pass/Fail)

Probability (of measuring an exceedance)

![Graph showing cell concentration vs. probability of exceedance with high and low predictability regions marked.](Image)

- High predictability
- Low predictability
## Results: Linearity (Laboratory trials)

### R² Values: Coefficient of Determination
Microscope counts vs. compliance tool concentrations

<table>
<thead>
<tr>
<th>Tool</th>
<th>Laboratory Trials</th>
<th>T. marina</th>
<th>P. micans</th>
<th>Both organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>BallastCheck2 (2015)</td>
<td></td>
<td>0.46</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td>10Cells</td>
<td></td>
<td>0.85</td>
<td>0.84</td>
<td>0.68</td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td></td>
<td>0.87</td>
<td>0.94</td>
<td>0.91</td>
</tr>
<tr>
<td>BallastCheck2 (2016)</td>
<td></td>
<td>0.33</td>
<td>0.90</td>
<td>0.82</td>
</tr>
<tr>
<td>FastBallast</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>BW680</td>
<td></td>
<td>0.57</td>
<td>0.92</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**Legend:**
- $R^2 \geq 0.90$
- $R^2 \geq 0.75$
- $R^2 < 0.50$

### R² Values:
0 (no linear relationship) to 1 (strong linear relationship)
## Results: Linearity (Field trials)

### R² Values: Coefficient of Determination

Microscope counts vs. compliance tool concentrations

<table>
<thead>
<tr>
<th>Tool</th>
<th>Field Trial Locations</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRL</td>
<td>GSI</td>
<td>SERC</td>
<td>All Sites</td>
</tr>
<tr>
<td>BallastCheck2 (2015)</td>
<td>0.63</td>
<td>0.64</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>10Cells</td>
<td>0.61</td>
<td>0.69</td>
<td>0.68</td>
<td>0.48</td>
</tr>
<tr>
<td>YSI Ballast Monitor</td>
<td>0.72</td>
<td>0.66</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>BallastCheck2 (2016)</td>
<td>0.73</td>
<td>0.46</td>
<td>0.39</td>
<td>0.36</td>
</tr>
<tr>
<td>FastBallast</td>
<td>0.13</td>
<td>0.75</td>
<td>0.71</td>
<td>0.37</td>
</tr>
<tr>
<td>BW680</td>
<td>0.66</td>
<td>0.61</td>
<td>0.82</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Legend:
- R² ≥0.90
- R² ≥0.75
- R² <0.50

R² Values:
0 (no linear relationship) to 1 (strong linear relationship)