NORTH SEA BALLAST WATER

# Experience from Facility & Research Centre with different rapid test methods

Louis Peperzak, NIOZ

Ballast Water Workshop 2014, Kiel-Schwentinental, Deutschland, June2.

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# North Sea Ballast Water Opportunity Project

Joint Land-based
Test facility
NIOZ-IMARES

One-stop-shop IMO
Type Approval with
Go-Consult+BSH

IMARES WAGENINGEN UR

><((((°> GoConsult

BSH
BUNDESAMT FÜR SEESCHIFFFAHRT UND HYDROGRAPHIE


RV Pelagia
Shipboard testing

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Scientific research

Development “compliance techniques” or “indicative rapid methods”

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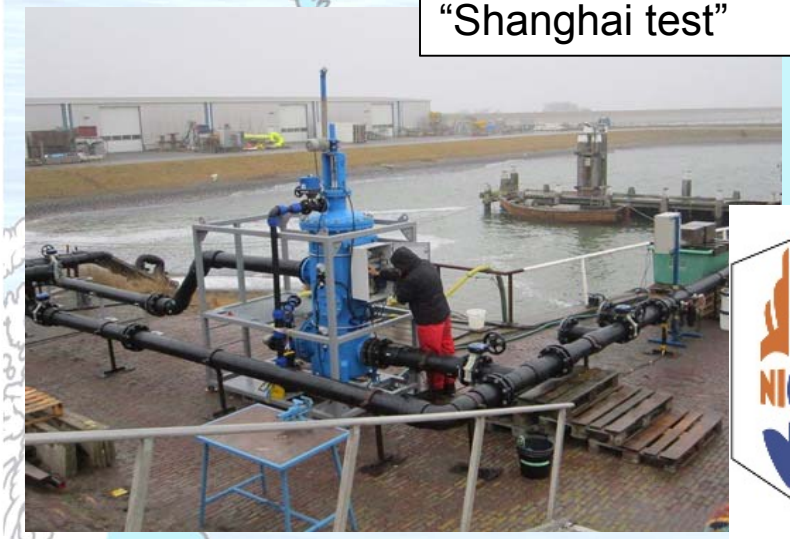
Component testing
Filter systems
“Shanghai test”



Ballast water box:
onboard
self-monitoring
ballast water*


Pilot
scale
tests


*www.ballastwaterbox.nl

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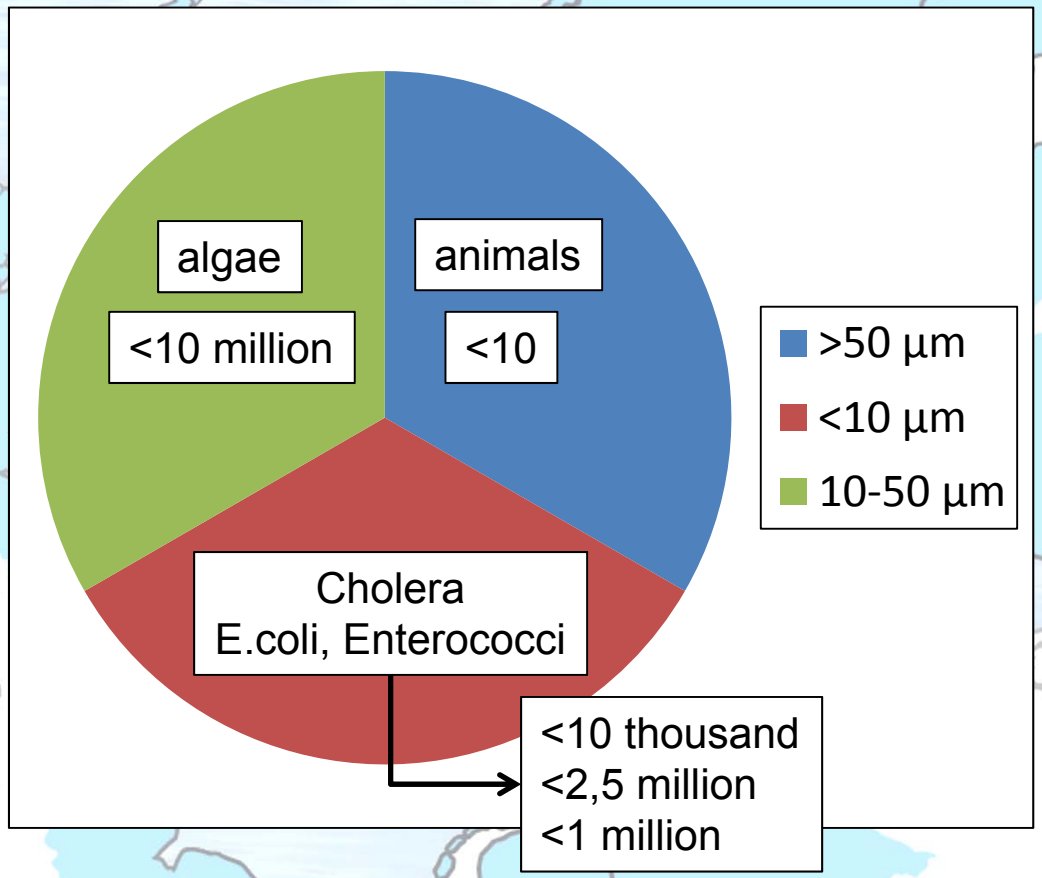
# The IMO D-2 standard: viable concentrations per cubic meter ($m^3$)




Even treated, ballast water may contain viable organisms, at different concentrations per size class

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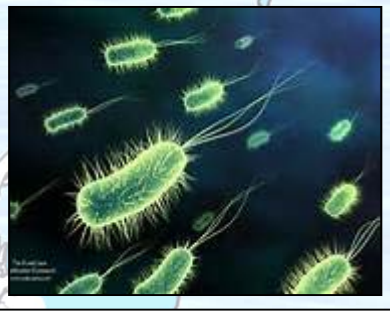
Size differences: bacteria + organisms 10-50 + >50 µm



1000x

Bacteria
(high/low concentrations)

Zooplankton
(low concentrations)

1000x

Many indicative methods focus on intermediate group: 10-50 µm
Highest D-2 standard concentration: 10 million per $m^3$ (algae)

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# The problem of viability:

viable

non-viable

zombie

viable ?

Zombieplankton after UV-treatment

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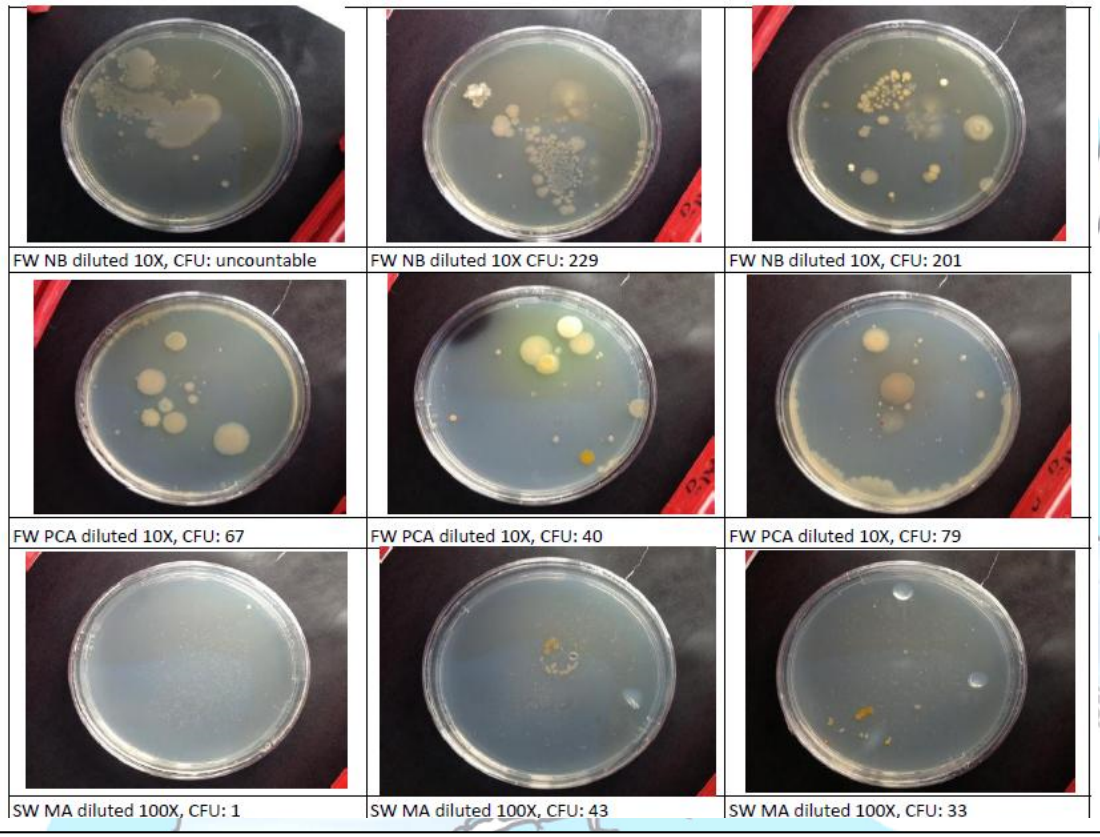
Viability (bacteria): viable cell grows out into a colony (≥ 24 hours)




A dead cell is a non-vital and non-viable cell.
A vital cell may be viable or non-viable: vitality can be measured in minutes

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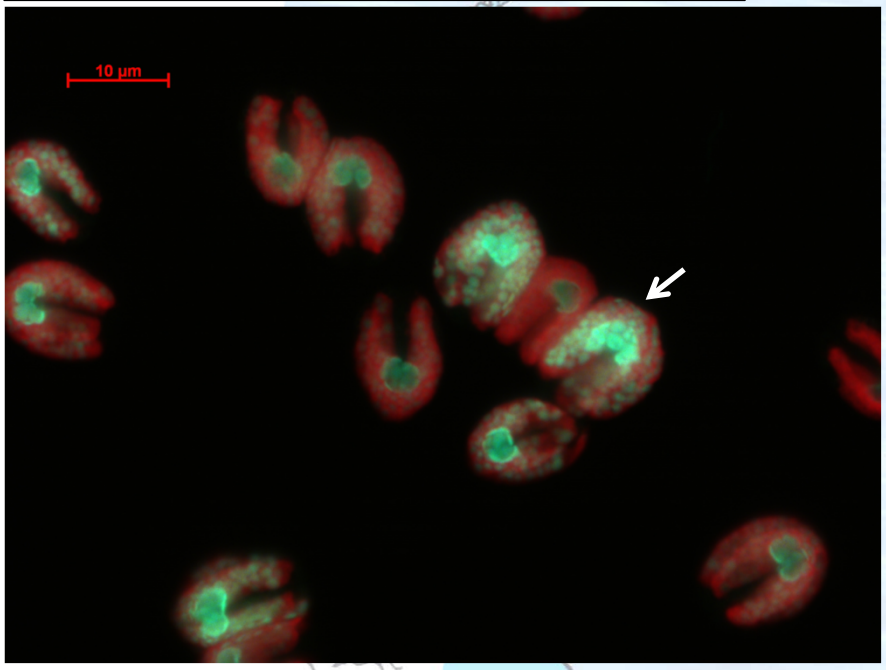
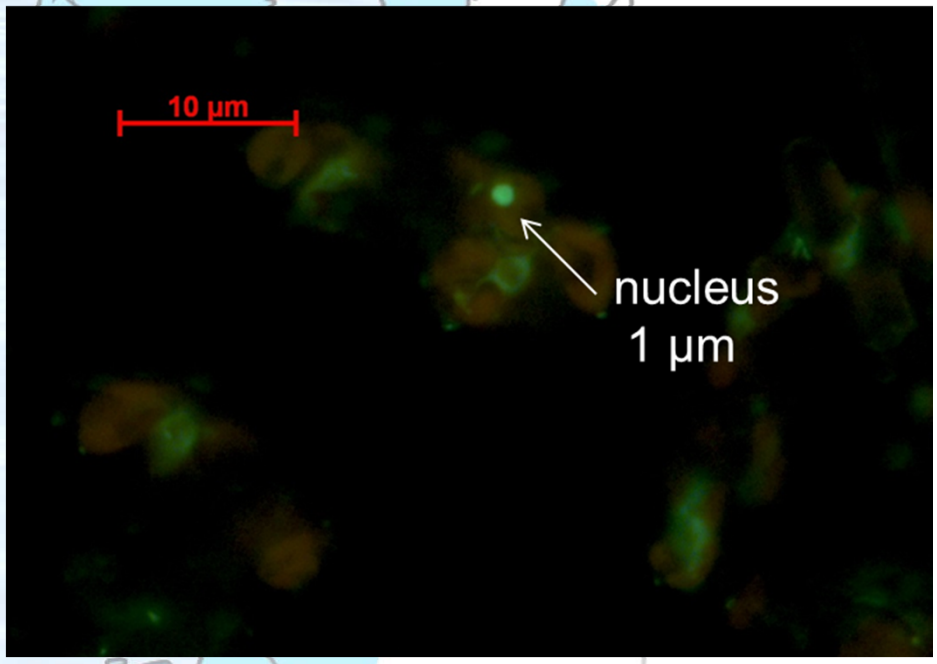
Viability (10-50 µm):

FDA/CMFDA stain: green = alive (enzyme acticity)

SYTOX stain: green = dead (membrane permeability)




These are vital stains: a proxy for live or dead
Should be measured in 6 hours
But: need for fluorescence microscope or flow cytometer (not practical on board)

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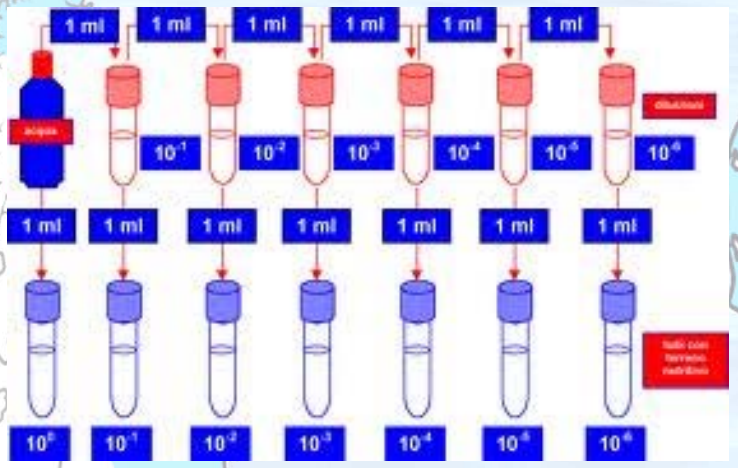
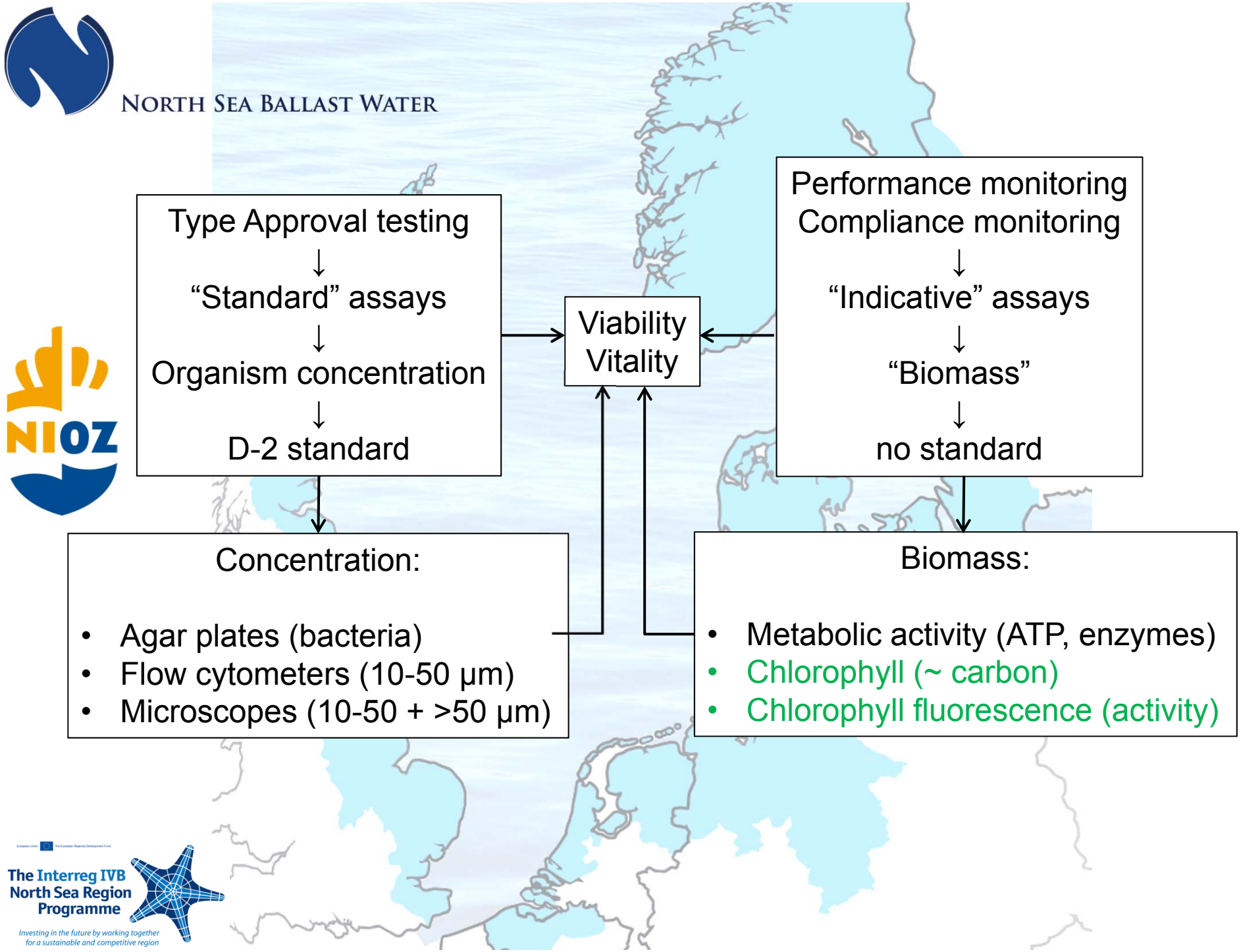
Alternative: Grow Out incubation experiments (true viability):
E.g. Most Probable Number (MPN) incubation:

- Dilute sample in steps until no viable cell is present
- Last tube with growth allows calculation of viable cell concentration in sample




Land-based tests:
Incubation experiments are feasible,
Ship-board: not practicle
Need for fast monitoring methods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Type Approval testing
↓
“Standard” assays
↓
Organism concentration
↓
D-2 standard
Viability
Vitality
Performance monitoring
Compliance monitoring
↓
“Indicative” assays
↓
“Biomass”
↓
no standard
NIOZ
Concentration:
• Agar plates (bacteria)
• Flow cytometers (10-50 µm)
• Microscopes (10-50 + >50 µm)
Biomass:
• Metabolic activity (ATP, enzymes)
• Chlorophyll (~ carbon)
• Chlorophyll fluorescence (activity)
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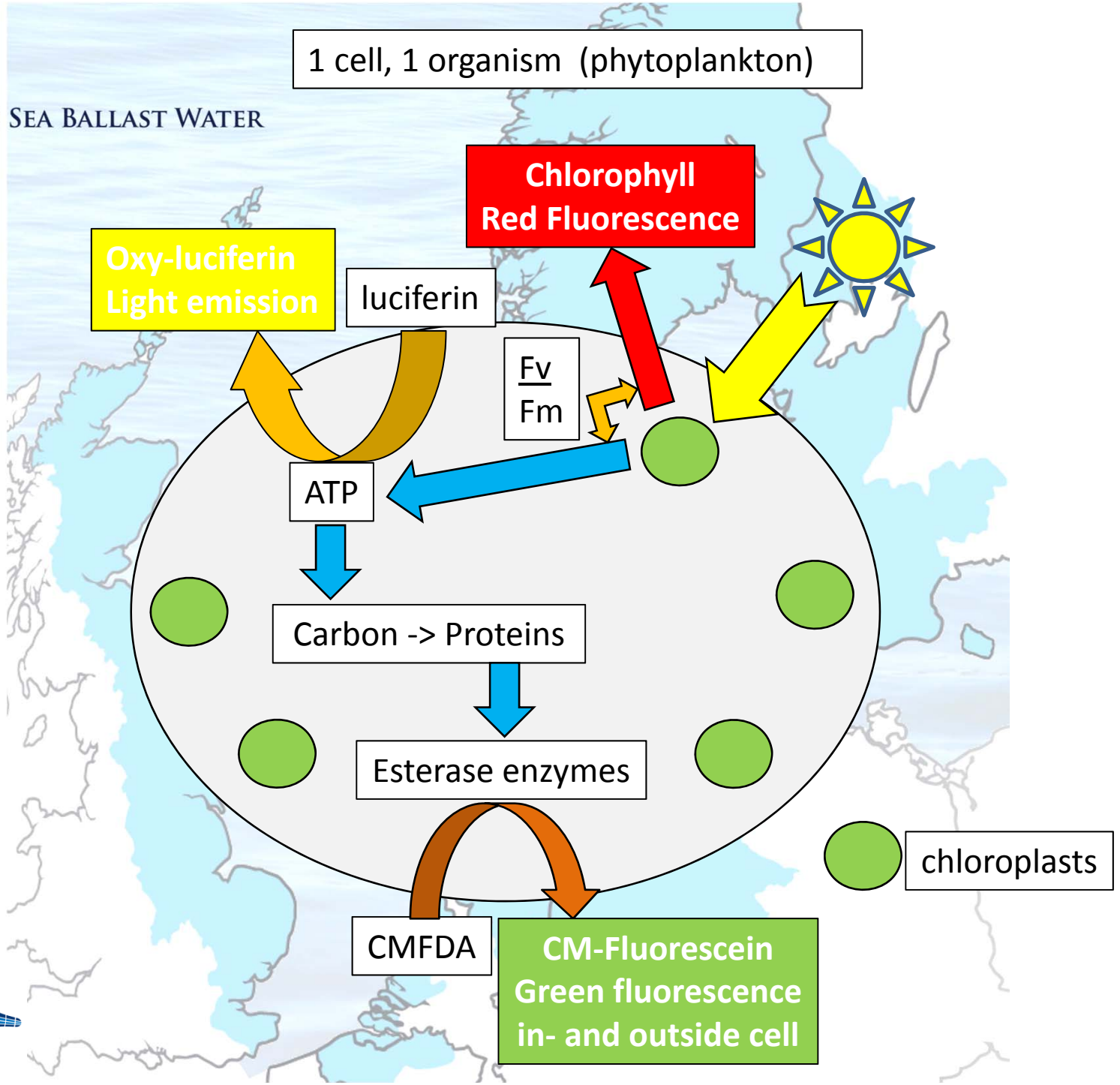


NIOZ

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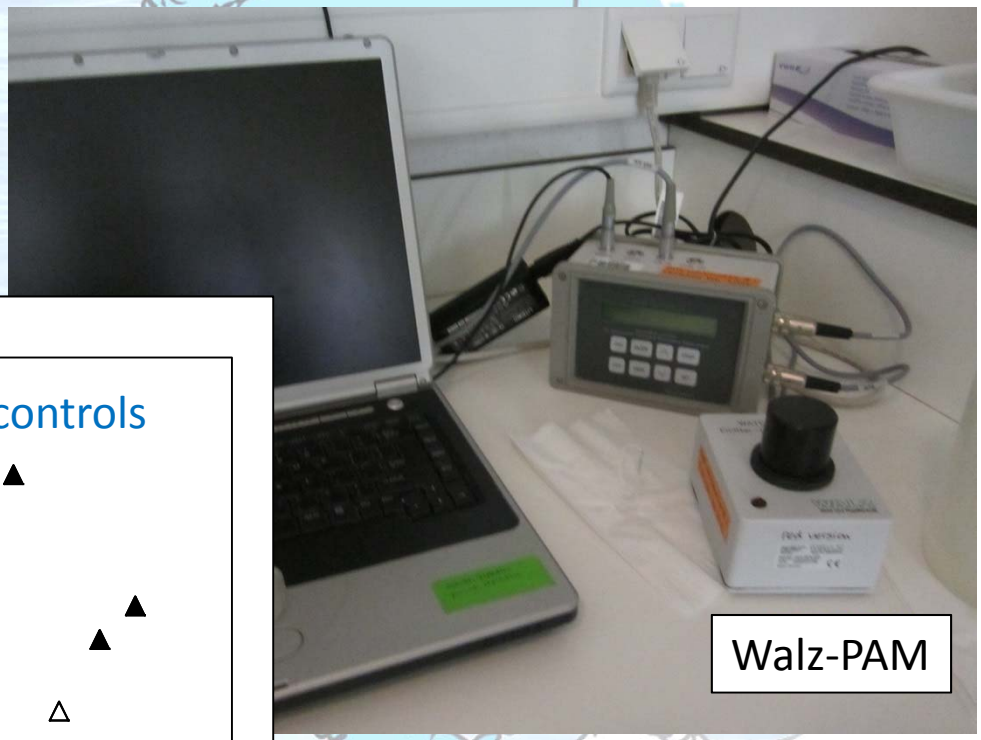
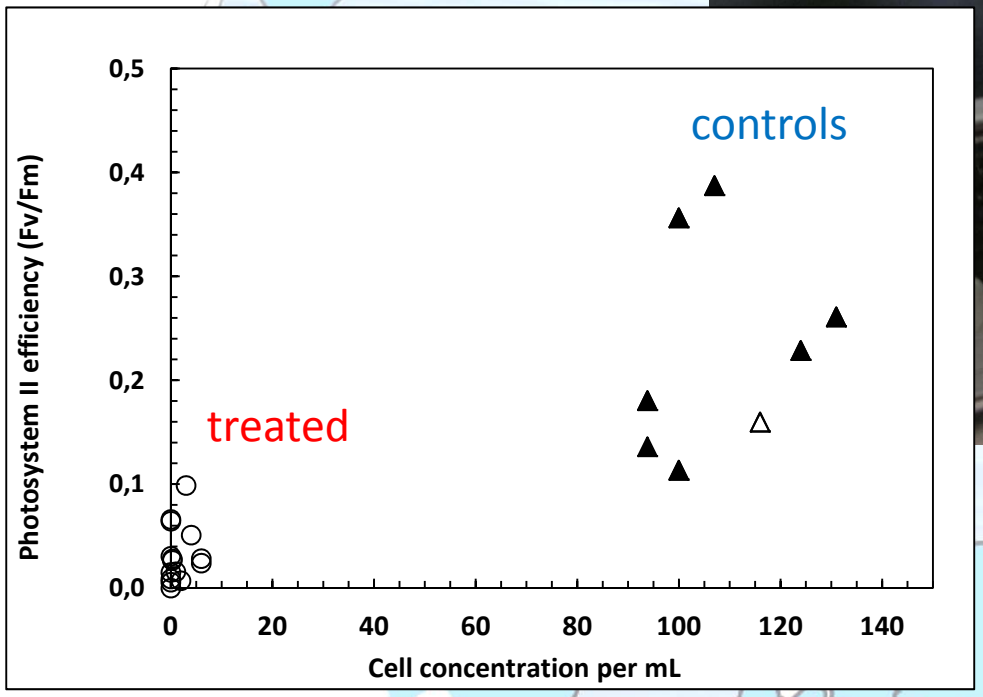
# Variable Fluorescence (algae): vitality (Fv/Fm) compared to vital cell concentration

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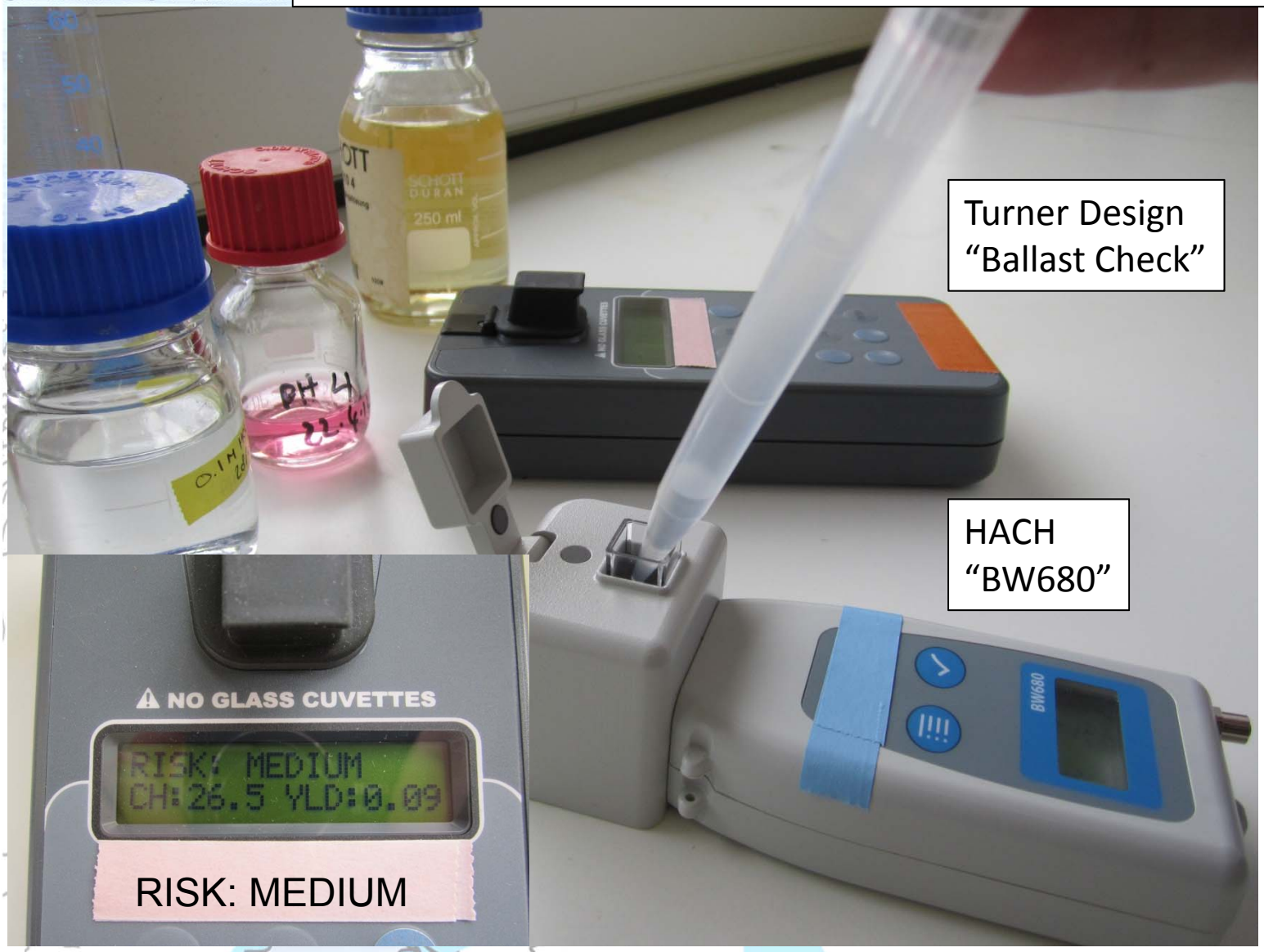
Variable Fluorescence (algae): biomass + vitality= risk

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Variable Fluorescence (algae):

biomass + vitality = vital cells/mL


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Validation studies rapid tools with: 1. Cultures in laboratory:


2. Test facility samples:

Shipboard samples: (e.g. S. Gollasch)

Goal: Compare indicative tools with IMO and ETV techniques for Type Approval.

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**Table 3. Photosynthetic Yield (Fv/Fm) determined at intervals following collection of challenge and treated water samples aboard M/V Coral Princess May 19th-21st 2013.** *Samples were transported to respective land-based laboratories by road (MLML) and air (NIOZ).*

| | Fv/Fm Challenge Water | Fv/Fm Treated Water |
|---|---|---|
| Day 0 (ERS) | 0.72 (Turner Ballast Check) | 0.34 (Turner Ballast Check) |
| Day 5 (MLML) | 0.61 (Walz) | 0.02 (Walz) |
| Day 7 (NIOZ) | 0.44 Turner Ballast Check) | 0.26 (Turner Ballast Check) |
| | 0.54 (Walz) | 0.01 (Walz ) |

Wright et al. A Case Study of Ballast Water Treatment Performance Assessment During a Shipboard Trial.

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# ATP: Energy of living organisms



More organisms = more ATP
Detection limit <10 per mL

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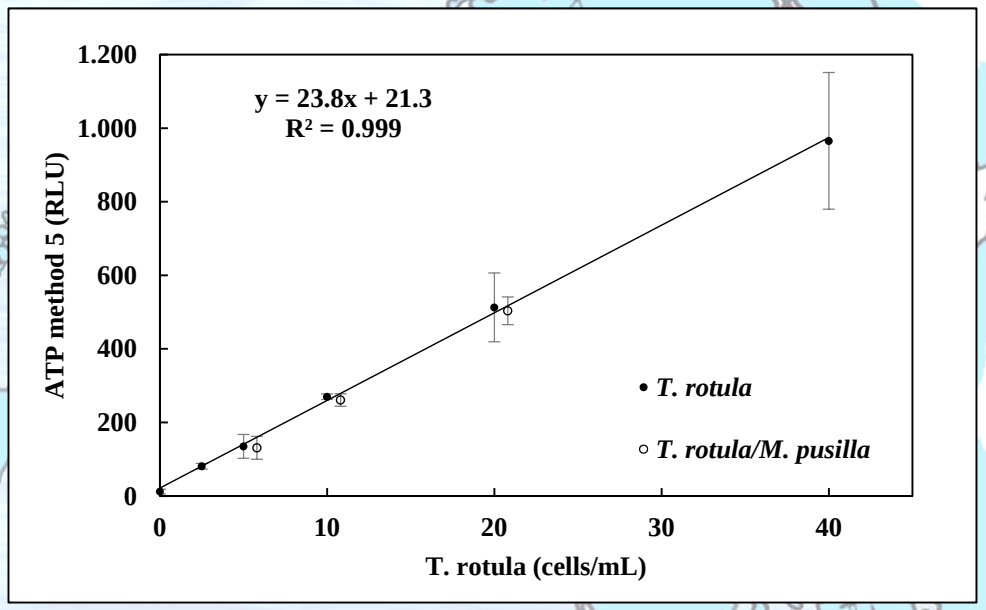


Figure 12. Detection limit test. Concentration method 5 using Total ATP swabs. Error bars represent the 95% c.i. of four or five measurements. Closed circles indicate results of only *T. rotula* cells. Open circles indicate test solutions containing *T. rotula* and *M. pusilla* in a 1:1,000 ratio. Open circles represent similar *T. rotula* cell concentrations as closed circles but were moved to the right to enhance visibility.

Van Slooten et al. Development of a sensitive and rapid ATP assay to measure living organisms in ships' ballast water.

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ATP:

FDA:

Fv

Fm

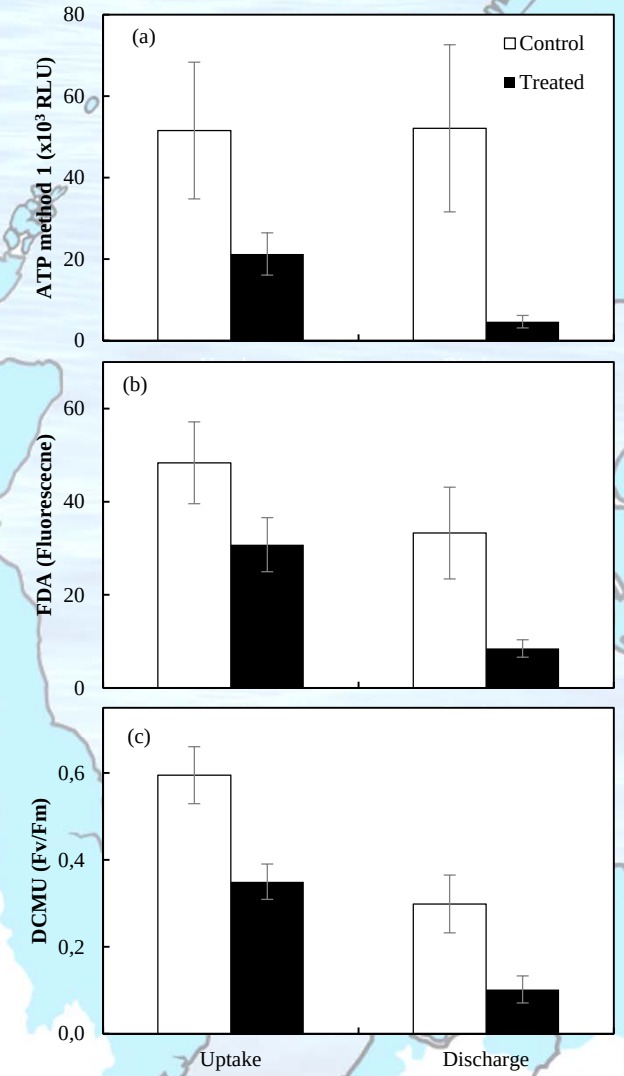


Figure 7. Full scale BWTS test. Three compliance tools used during the land based testing of an UV-based BWTS: (a) concentration method 1 using Total ATP swabs (b) FDA and (c) DCMU. Each bar represent the average of all tests carried out. Error bars represent the 95% c.i.

Van Slooten et al. Development of a sensitive and rapid ATP assay to measure living organisms in ships' ballast water.

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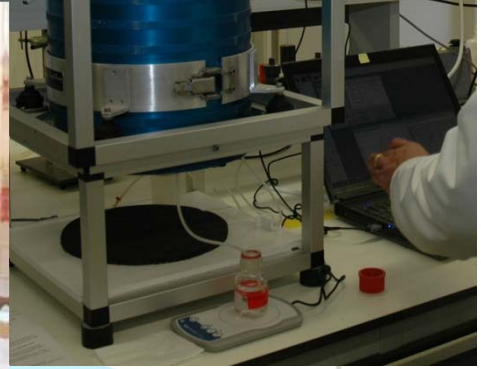
Sophisticated monitoring instruments

On-board flow cytometers:

- Cytobuoy
- BallastCam





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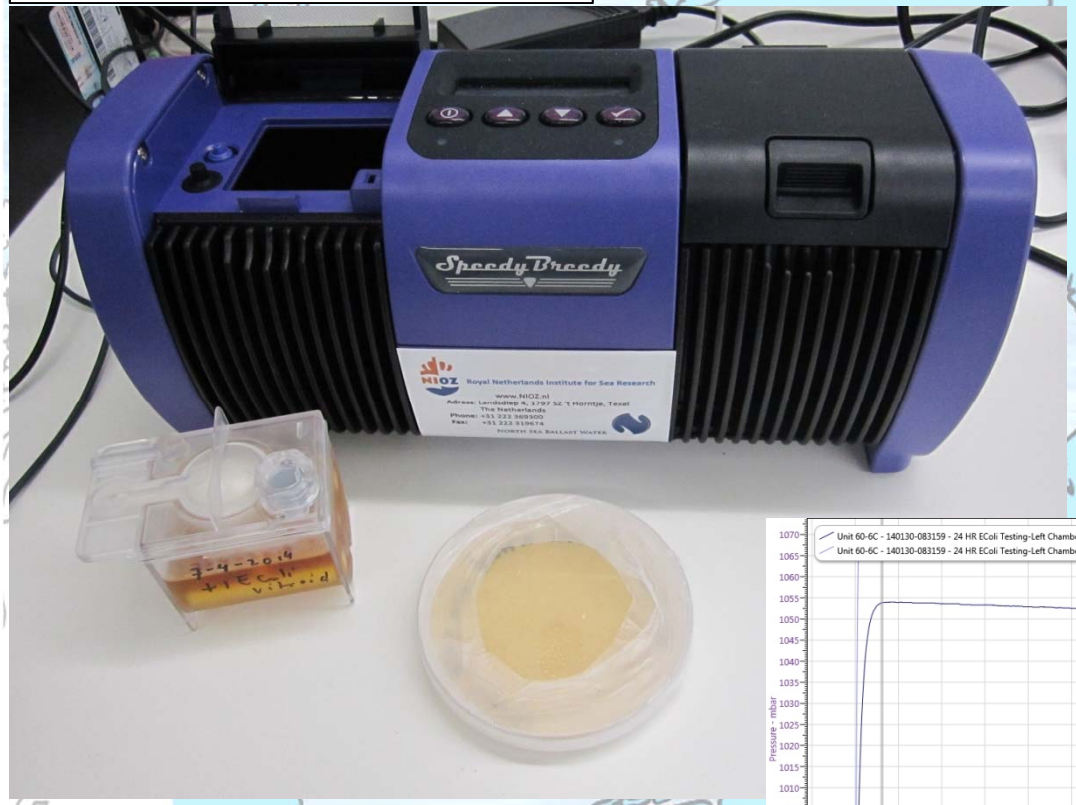
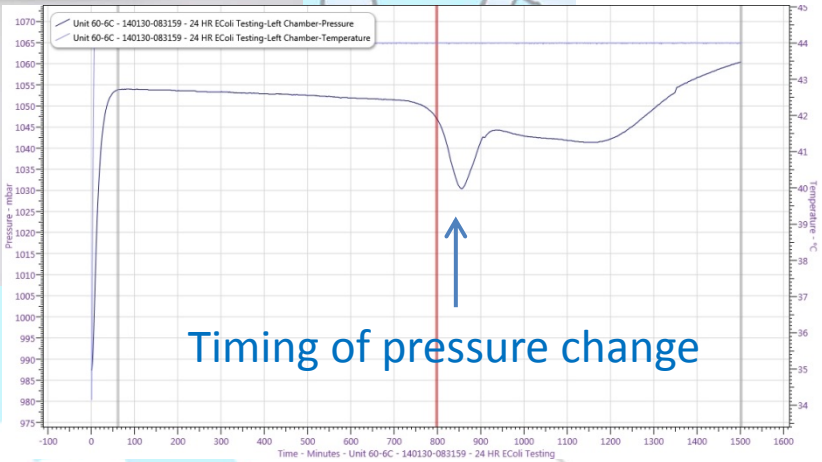
For bacteria (e.g. *E. coli*):
Speedy Breedy

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## Summary indicative techniques/methods:

- Faster than Type Approval Tests
- Robust and simple (only 10-50 µm organisms?)
- Cannot be more stringent than D-2

- Very fast (“biomass”): non-compliance measurement
- In near future: equivalent to a compliance measurement?
- How many samples? Statistical uncertainty remains

Conclusion:

- Several methods are already available
- More validation studies needed: laboratory and ballast water samples

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