Rapid Compliance Monitoring using Indicative Tools

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May 25, 2016

Organism size

> ≥ 50 micrometers

10 – 50 micrometers

Toxicogenic Vibrio cholerae

Escherichia coli

Intestinal enterococci
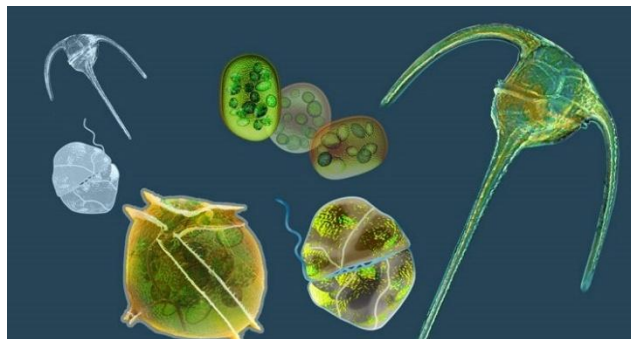
Amount allowable in discharge

<10 viable/living organisms per cubic meter

<10 viable/living organisms per milliliter

< 1 colony forming unit per 100 milliliters

< 250 colony forming unit per 100 milliliters

< 100 colony forming unit per 100 milliliters

➢ **Level 1 – Initial Inspection**

- Documentation, crew knowledge

➢ **Level 2 – More Detailed Inspection**

- Equipment operation, self-monitoring indicators

➢ **Level 3 – Indicative Measure of Gross Non-Compliance**

- Quickly, easily assess risk of non-compliance

➢ **Level 4 – Direct Measure of Compliance**

- Lowest risk, highest certainty, longest time

- ➤ A way to give port state officials and ship operators a quick and easy determination of whether treated ballast water is in risk of non-compliance with the standard
- ➤ Provides an indicative measure to avoid having to undertake a direct measure of compliance

➢ Sample volume ideal

➢ Algal abundance proportional to bulk fluorescence

➢ Cell viability calculated using variable fluorescence

➢ Methods are simple, quick, no reagents required

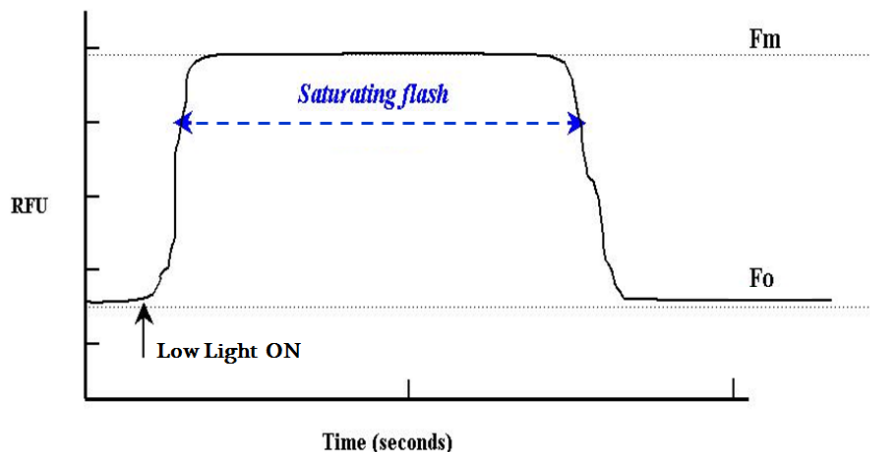➢ Respond to all treatment technologies

Indicative !

Algal abundance ~~ fluorescence detected from chlorophyll within algae

➤ Sample illuminated and light is absorbed by the cells

➤ Sample emits light (fluorescence)

➤ Fluorescence is quantified and displayed as a digital number estimating the algal abundance
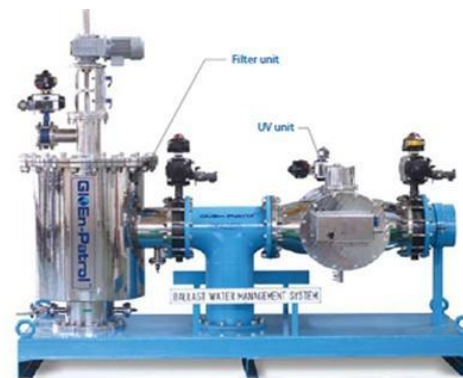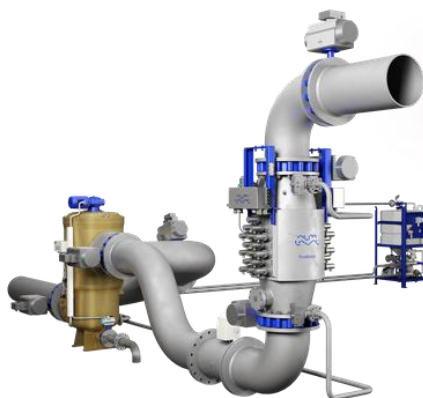
Chlorophyll Fluorescence

Light from Fluorometer

Chl

Chlorophyll Molecule
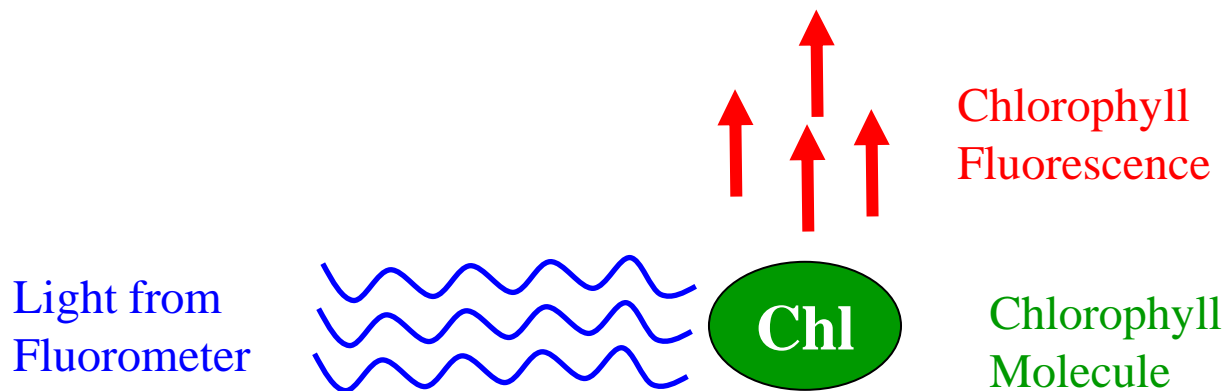
PAM measures photosynthetic efficiency (activity)

➤ Sample probed by low light level to determine background fluorescence (minimum fluorescence Fo)

➤ While monitoring background fluorescence, sample is blasted (saturated) with a high intensity light giving a maximum fluorescence value (Fm)

➤ These 2 values, Fo and Fm, are used to calculate an activity ratio that gives a good measure of how active algae are in the sample

• Typical values for active algal cultures range from 0.25 – 0.70

Water Type	Cell Count (10 – 50 μm)	Ballast-Check 2	
		Total Chl	Activity
Raw	163.7	2.20 $\pm$ 0.03	0.36 $\pm$ 0.02
Treated	NA	0.30 $\pm$ 0.06	ND
After 24 hour hold at 11 ppm Chlorine Concentration			
Treated (T_i)	0	0.30 $\pm$ 0.00	ND
Treated (T_m)	2.5	0.20 $\pm$ 0.05	ND
Treated (T_f)	7.6	0.80 $\pm$ 0.10	0.19 $\pm$ 0.04

Data taken Dec. 1st and 2nd, 2014 aboard the Golden Bear during a ballast water treatment test using chlorination method.

Sample numbers	Ballast tank #	Uptake Fv/Fm	Treated Discharge Fv/Fm
Untreated #1 (Treated #4)	1	0.65	0.10
Untreated #2 (Treated #5)	2P/2S	0.58	0.06
Untreated #3 (Treated #6)	4S/4P/5	0.64	0.12
Mean ± Standard Deviation		**0.62±0.04**	**0.09±0.03**
Untreated #7 (Treated #10)	1	0.52	0.15
Untreated # 8 (Treated #11)	2P	0.57	0.15
Untreated # 9 (Treated #12)	2S	0.57	0.13
Mean ± Standard Deviation		**0.55±0.03**	**0.14±0.01**
Untreated # 13 (Treated #16)	1	0.58	0.1
Untreated # 14 (Treated #17)	2P	0.57	0.13
Untreated # 15 (Treated #18)	2S	0.59	0.15
Mean ± Standard Deviation		**0.58±0.01**	**0.13±0.02**
Grand mean ± Standard Deviation		**0.59±0.04**	**0.12±0.03**

Data collected by David Wright, August 2014, for a Hyde Guardian treatment system in a fresh water test.

A. Hypochlorite treatment
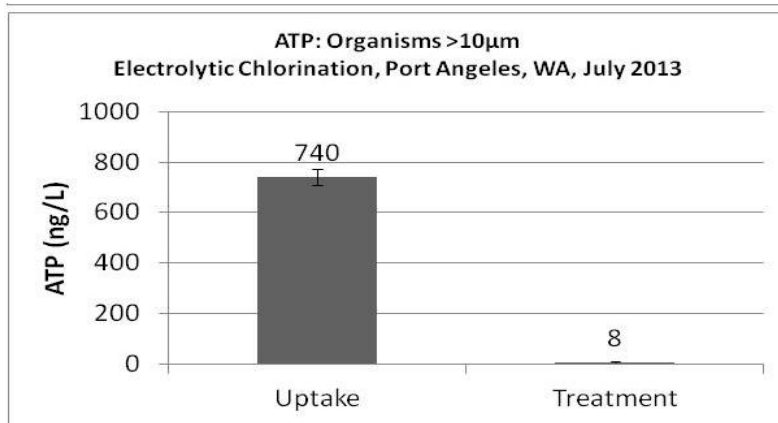
B. UV treatment
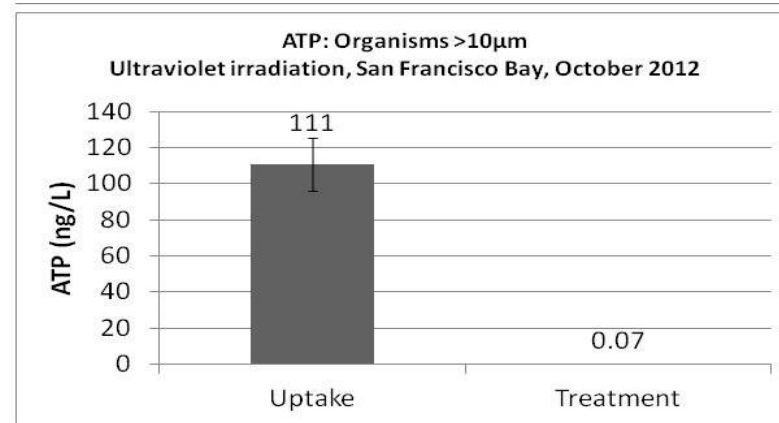
C. Chlorine dioxide treatment
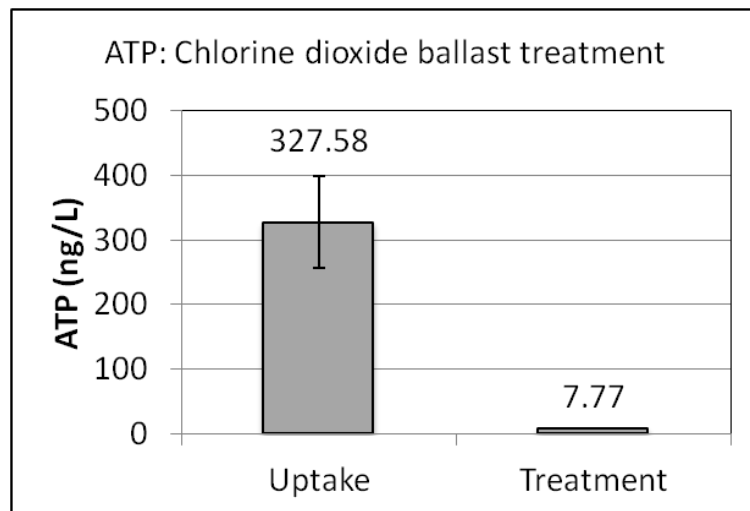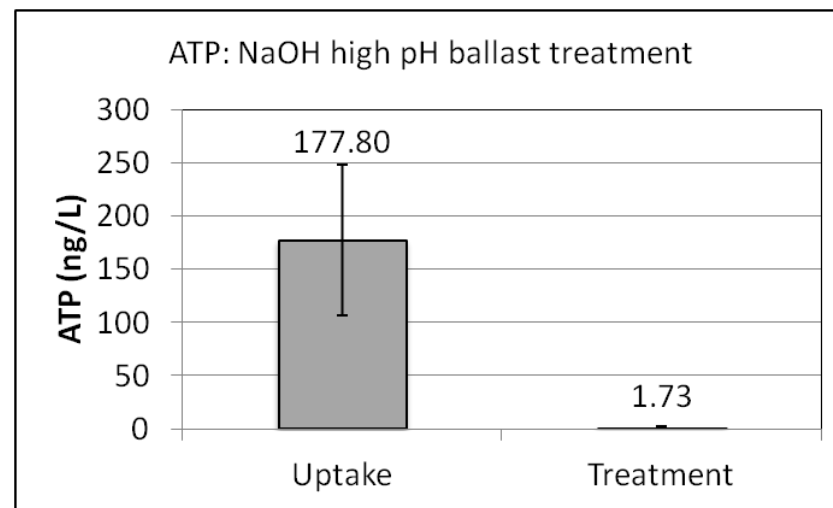
D. NaOH treatment (freshwater)

Data courtesy Nick Welschmeyer

➢ Continuous

- Installed & sampling all the time

- Check large sample of water

➢ Discrete

- Simple & quick

- Independent verification

- Low/no maintenance required
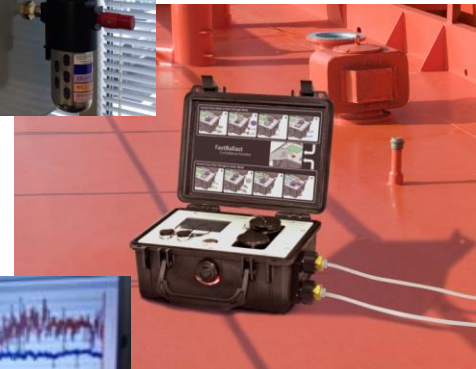
➢ Validation Round 1

- June – September 2015
- 2 discrete tools, 1 inline tool
- 1 set of laboratory-based experiments
- 3 sets of field-based experiments
- Side by side comparison with standard method

➢ Validation Round 2

- Started March 2016
- 3 discrete variable fluorescence tools
- 1 discrete ATP tool demonstrated
- Expected completion Summer 2016

Photo: M. First; NRL; June 2015

➢ Data show general agreement between indicative tools & microscope counts

➢ Results are preliminary & do not yet include all data

➢ Presentation given January 21, 2106 at ICMB-IX in Sydney

➢ Reports for individual tools will eventually be available through Alliance for Coastal Technologies (www.act-us.org)

NRL-KW
Subtropical, oligotrophic, marine

Photo: M. First; NRL, Key West, FL 2011

SERC
Temperate, eutrophic, brackish

Photo: M. First ; Edgewater, MD; August 2015

GSI
Temperate, mesotrophic, fresh

Photo: M. First; GSI, Superior, WI; May 2012

TURNER DESIGNS
Reliable Instruments for an Unreliable World
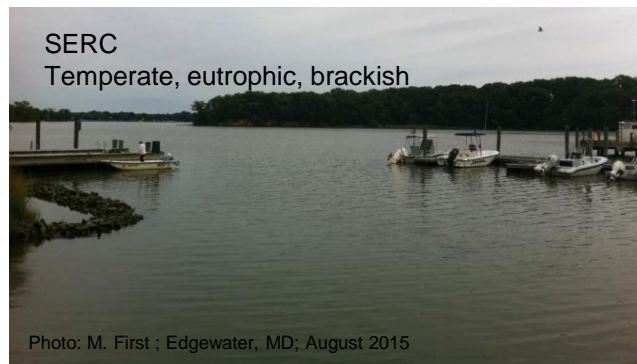
- Evaluate ballast sampling devices and analytic tools
- Mindelo, Cape Verde to Hamburg, Germany
- 2 weeks
- 19 researchers from 10 countries
- 28 experiments
- Several sampling devices
- Analytic devices: >=50 µm
- Analytic devices: 10 µm ≥ X < 50 µm
- Analytic devices: Indicator microbes

Project Leads: BSH and DFO

- ➢ PAM methods (4)
  - • bbe 10cells, Turner Designs Ballast-Check2, Hach BW680, Walz-Water PAM
- ➢ Microscopy (staining)
- ➢ ATP methods (2)
  - • SGS method (aqua-tools), Welschmeyer method
- ➢ Bulk FDA
- ➢ FDA pulse counting
  - ➢Satake Pulse Counter
- ➢ Flow cytometry
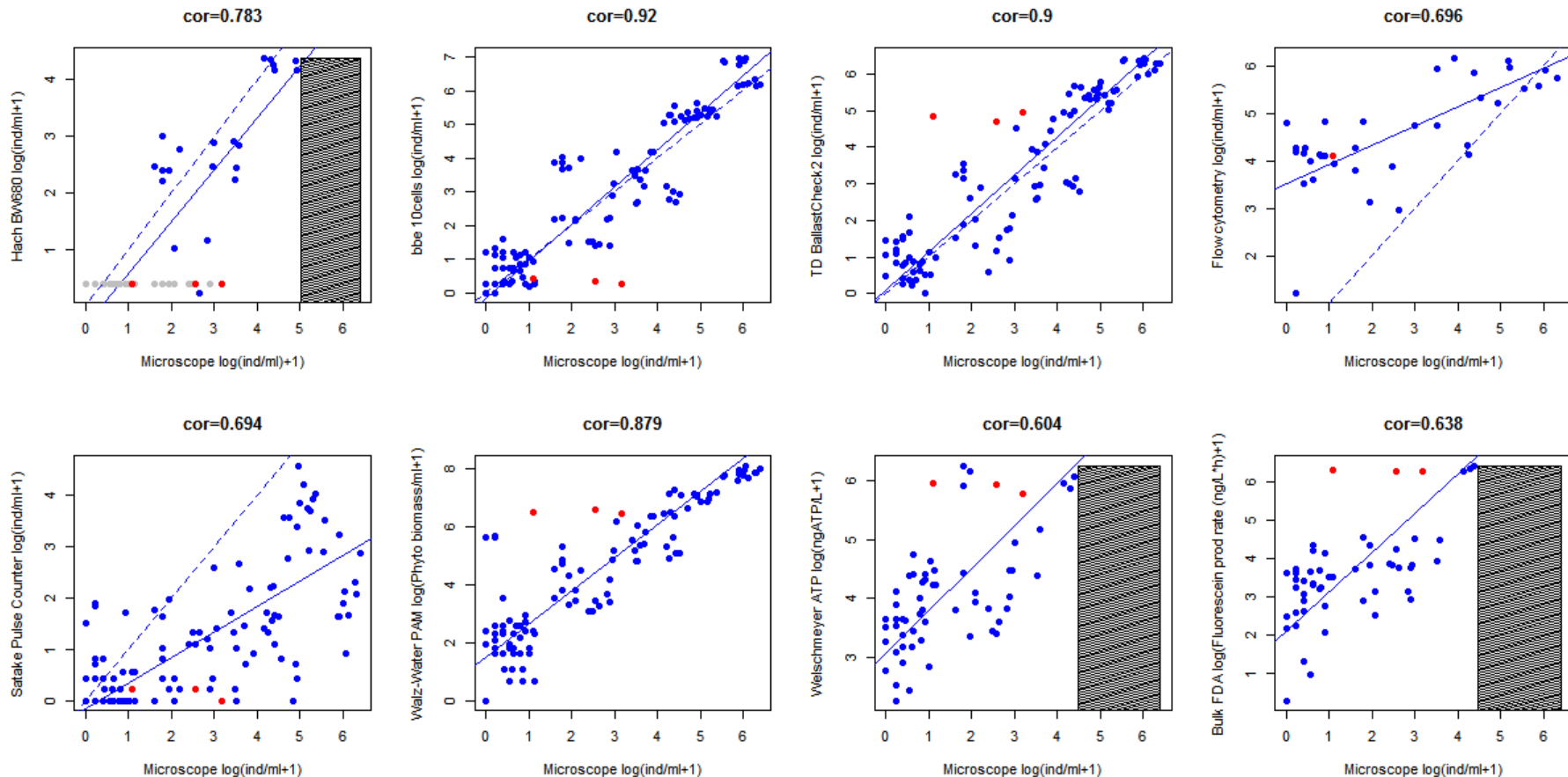
- Solid line is line of best fit based on Deming regression.
- Dashed line is 1:1 line for methods reporting results in individuals/ml.
- Red dots indicate data from experiments where BWMS was active. Grey dots indicate data below detection threshold.
- Shaded grey area shows region where data was not available.

- ➢ Collected treated water samples from 3 ships
  - Planning to do 2-3 more
- ➢ Comparing results from 3 indicative tools to microscopic cell counts
- ➢ Conclusions should be available early summer
- ➢ Still developing compliance assessment protocol regulations
  - Rulemaking expected later this year

TURNER DESIGNS
Reliable Instruments for an Unreliable World

➢ Port of Koper (Slovenia)

• Simulate PSC sampling process

• Test with several indicative tools onboard vessels

• Samples taken to laboratory, results compared with microscope counts of 10-50µm cells using microscopy & FDA stain

➢ 10 vessels sampled & tested

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David Consult

TURNER
DESIGNS
Reliable Instruments for an Unreliable World
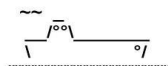
- ➢ Part of MEA-NL's ongoing intercalibration of instruments
- ➢ Comparing abundance with flow cytometry/ FDA staining
- ➢ Comparing viability with high end scientific instruments
- ➢ Performing land-based and shipboard tests of different type of BWMS

MEA-NL
MARINE ECO ANALYTICS

info@mea-nl.com

- How they work in real situations
  - During shipboard tests on ballast water management systems already installed /in use
- How their results correlate to laboratory-based tests
  - Flow cytometry and staining microscopy / cell counts;
- To what extent results are reliable and correspond to international standards
  - IMO & USCG
- A continuous series of measurements with the same indicative tools and laboratory-based techniques are being applied for L4 samples (Western English Channel).

- ➢ BWTS Testing Labs
- ➢ Treatment System manufacturers
- ➢ Sampling System manufacturers
- ➢ Port States

http://tritonmsc.de/

Ballast-Check 2 was used by the Glosten team to gain quick feedback on the efficacy of the USGS developed sodium hydroxide treatment system efficacy during 2015 shipboard trials, and now during 2016 trials. The table below aligns the obtained abundance and activity readings with those gained by CMFDA/FDA readings taken as per EPA ETV protocol.

FDA/cmFDA epiflourescent microscopy results (EPA ETV protocol) vs Turner Ballast-Check2

Test	Sample	Time (hrs)	cells/mL (#1)*	cells/mL (#2)*	Pass/Fail	Abundance	Activity	Risk
1	Uptake	0	21.0	-	Fail	1.7	0.21	**Low**
1	Control	48	7.6	30.3	Pass/Fail	2.9	0.18	Low
1	Low	48	0.9	0	Pass	0.7	0.14	Low
1	High	48	0.0	0	Pass	2.7	0.27	Low
2	Uptake	0	30.0	-	Fail	25.2	0.25	High
2	Control	48	6.3	26.3	Pass/Fail	5.6	0.23	Low
2	Low	48	0.0	0	Pass	2.3	0.09	Low
2	High	48	0.0	0	Pass	5.7	0.11	Low
3	Uptake	0	119.7	-	Fail	69.4	0.39	High
3	Control	48	52.2	242	Fail	13.0	0.37	High
3	Low	48	7.1	0.3	Pass	1.2	0.10	Low
3	High	48	5.1	0	Pass	8.2	0.14	Low
4	Uptake	0	211.7	-	Fail	59.9	0.47	High
4	Control	48	256.9	336.7	Fail	9.7	0.27	**Low**
4	Low	48	7.1	1.7	Pass	0.9	0.10	Low
4	High	48	7.2	0	Pass	7.0	0.09	Low

*#1 refers to results from the shipboard science team, #2 refers to results from a 2nd science team that analyzed samples sent by mail

High Risk:	High Algal Abundance	AND	High Algal Activity
Low Risk:	Low Algal Abundance	OR	Low Algal Activity

Abundance

# of live algal cells in 10-50 size fraction, <10 cells is a "PASS"

Measuring chlorophyll fluorescence from live algal cells expressed as (RFUBraw-RFUB10) * cal. Coeff.
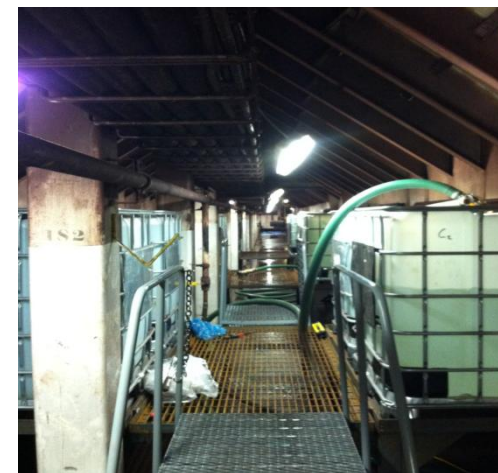
Activity

Range is 0.01-0.75 with >0.3 being normal for uptake

Measureing photosynthetic yield expressed as (Fm-F0)/Fm

American Spirit while underway with 80,000 tons of cargo.

Mesocosm tank set-up on ship during trials, checking high and low dose efficacy.

Data courtesy Kevin Reynolds, Glosten

➢ Verify presence of organisms when preparing to conduct shipboard tests

➢ During tests, immediately verify during discharge effectiveness of Guardian

➢ Found it to be "Easy to use, convenient, and very accurate at predicting the results of full-scale shipboard tests." Mark Riggio, Hyde Guardian Product Manager

➢ Verifying treatment system performance in R&D

- Lab & shipboard tests

➢ Samples from all regimes tested before and after treatment

- Marine, Fresh, Brackish water
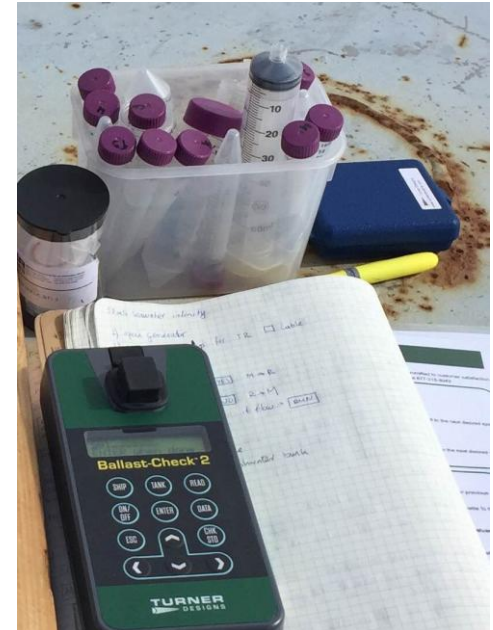
- ➢ Verify challenge water conditions before system tests
- ➢ Saves several hours vs cell counting

The expert in WATER ENVIRONMENTS

Hans Eikaas
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- ➢ Developed a small, closed system for BW sampling which can be permanently installed onboard ships
  - Footprint 30x50x40cm
- ➢ Using sampling system with indicative tools for
  - Rapid compliance testing onboard ships
  - Testing BW treatment system prior to their approval
- ➢ Together generates reliable, indicative results after less than 30 minutes

Triton MSC NP 6007 TG 18 system

TRITON MARINE SCIENCE & CONSULT

http://tritonmsc.de/

lothar.schillak@tritonmsc.de

➢ Online Compliance, Monitoring, Enforcement Training
- • Background – why is BWMC needed
- • Ballast water sampling
- • Measuring compliance with D-2 (detailed and indicative (CME) analysis)

➢ Joint program
- • World Maritime University (Josefin Madjidian)
- • NIOZ (Louis Peperzak)
- • GoConsult (Stephen Gollasch)
- • DavidConsult (Matej David)

➢ http://elearning.e-cmeballastwater.eu/

➢ Hands-on course at NIOZ Test Facility

- ➢ GEF-UNDP-IMO GloBallast Partnerships Programme created an online learning portal which includes the e-learning course Operational Aspects of BWM.
  - Introduction
  - Operational Aspects
  - Survey and Certification
  - Compliance Monitoring and Enforcement (CME).
- ➢ Free through registering at http://globallastlearning.com/login/index.php

➢ Ship Operators

- • Easy, inexpensive way to quickly verify treatment system performance and provide documentation

➢ Port State Control

- • Easy, portable way to quickly verify treatment system performance

➢ Treatment System Manufacturers

- • Inexpensive way to give ship owners independent verification of treatment system operation

- ➢ Established in 1972
- ➢ Focused on filter fluorometers for environmental & industrial markets
- ➢ Customers in >50 countries
  - University Researchers
    - Oceanographers, Limnologists, & Marine Biologists
  - Environmental Agencies & Consultants
  - System Integrators
  - Drinking Water & Waste Water Plants
  - Fisheries
  - Industries tracking Treatment Chemicals

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