

Sampling and analysis during ship and landbased Certification

EMSA, Lisboa, February 2010

Dr. August Tobiesen

Norwegian Institute for Water Research
Gaustadalléen 21, NO-0349 Oslo, Norway

Shipboard testing

- Testing of BWMS under normal operation onboard
- Logistics



LNG tanker USA: Roar Lindefjeld/StatoilHydro



KCL Banshee



Wallenius Marine's Car Carrier M/V Aida

Shipboard testing

- Sampling and water management
- Several 1 m³ samples



Sampling on ship

- We used 3 in pipe valves to sample
 1. In pipe from seachest, influent sample during ballasting operation.
 2. In pipe from treated tank on deballasting
 3. In pipe from control tank on deballasting

Sampling on ship

- A calibrated flow meter was used to ensure correct volume of 1 m³ sample.

(flow should be regulated by pipe diameter and not by valve)

IMO guideline

- 3 samples for influent water both treated and control
- 3 samples deballasting control
- 9 samples deballasting treated

Sampling shipboard

For influent and Control:

Samples were spaced in time, in order to be representative of beginning, middle and end of ballasting operation.

Results from influent sampling

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	10260	69983	179133	55760	59800	5325
2	8550	30600	187000	68163	86200	8208
3	6618	59220	205567	82350	143588	17319
1	7473	54900	20733	63750	176400	11778
2	10735	65733	21033	59325	164000	13843
3	12730	59400	197100	49400	139650	19075
	passage	passage	harbour	harbour	harbour	harbour

Results from influent sampling

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	10260	69983	179133	55760	59800	5325
2	8550	30600	187000	68163	86200	8208
3	6618	59220	205567	82350	143588	17319
1	7473	54900	20733	63750	176400	11778
2	10735	65733	21033	59325	164000	13843
3	12730	59400	197100	49400	139650	19075
	passage	passage	harbour	harbour	harbour	harbour

Results from influent sampling

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	10260	69983	179133	55760	59800	5325
2	8550	30600	187000	68163	86200	8208
3	6618	59220	205567	82350	143588	17319
1	7473	54900	20733	63750	176400	11778
2	10735	65733	21033	59325	164000	13843
3	12730	59400	197100	49400	139650	19075
	passage	passage	harbour	harbour	harbour	harbour

Results from influent sampling

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	10260	69983	179133	55760	59800	5325
2	8550	30600	187000	68163	86200	8208
3	6618	59220	205567	82350	143588	17319
1	7473	54900	20733	63750	176400	11778
2	10735	65733	21033	59325	164000	13843
3	12730	59400	197100	49400	139650	19075
	passage	passage	harbour	harbour	harbour	harbour

Results from influent sampling

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	10260	69983	179133	55760	59800	5325
2	8550	30600	187000	68163	86200	8208
3	6618	59220	205567	82350	143588	17319
1	7473	54900	20733	63750	176400	11778
2	10735	65733	21033	59325	164000	13843
3	12730	59400	197100	49400	139650	19075
	passage	passage	harbour	harbour	harbour	harbour

Sampling $>50\text{ }\mu\text{m}$ Treated

$>50\text{ }\mu\text{m}$, Treated deballasting:

Sampling "continuously" $9\times 1\text{ m}^3$ samples while deballasting. Sample flow is regulated to fit deballasting operation

Results from Shipboard Treated sample deballast

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	14	0	0	10	1	9
2	11	0	8	4	1	1
3	4	1	2	3	3	1
4	8	0	3	2	1	0
5	6	1	2	10	1	2
6	5	4	2	11	3	2
7	3	4	0	12	17	3
8	0	6	3	20	14	0
9	0	4	0	10	15	1
average	5.7	2.2	2.2	9.1	6.2	2.1

Results from Shipboard Treated sample deballast

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	14	0	0	10	1	9
2	11	0	8	4	1	1
3	4	1	2	3	3	1
4	8	0	3	2	1	0
5	6	1	2	10	1	2
6	5	4	2	11	3	2
7	3	4	0	12	17	3
8	0	6	3	20	14	0
9	0	4	0	10	15	1
average	5.7	2.2	2.2	9.1	6.2	2.1

Results from Shipboard Treated sample deballast

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	14	0	0	10	1	9
2	11	0	8	4	1	1
3	4	1	2	3	3	1
4	8	0	3	2	1	0
5	6	1	2	10	1	2
6	5	4	2	11	3	2
7	3	4	0	12	17	3
8	0	6	3	20	14	0
9	0	4	0	10	15	1
average	5.7	2.2	2.2	9.1	6.2	2.1

Results from Shipboard Treated sample deballast

	Boat A			Boat B		
Sample no.	cruise 1	cruise 2	cruise 3	cruise 1	cruise 2	cruise 3
1	14	0	0	10	1	9
2	11	0	8	4	1	1
3	4	1	2	3	3	1
4	8	0	3	2	1	0
5	6	1	2	10	1	2
6	5	4	2	11	3	2
7	3	4	0	12	17	3
8	0	6	3	20	14	0
9	0	4	0	10	15	1
average	5.7	2.2	2.2	9.1	6.2	2.1

Conclusion sampling

- Any one sample will not be representative for whole tank during deballasting
- Species diversity will not differ much in 1000m^3 or 10000 m^3
- Perhaps a standardised proportional sampler should be fitted together with the BWTS

Analysis of samples Shipboard

- *E. coli* and Intestinal *Enterococci* e were analysed according to ISO standards onboard.
- *Vibrio* were analysed according according to the method described by the American Public Health Association

Analysis of samples Shipboard

- >50 μm organisms were viewed on board using stereo microscope.
- >10-50 μm
 - Serial dilution cultures were started on board. Transported to lab in cooler.
 - Vital staining of sample prepared and frozen for later inspection in microscope

Testing while in harbour

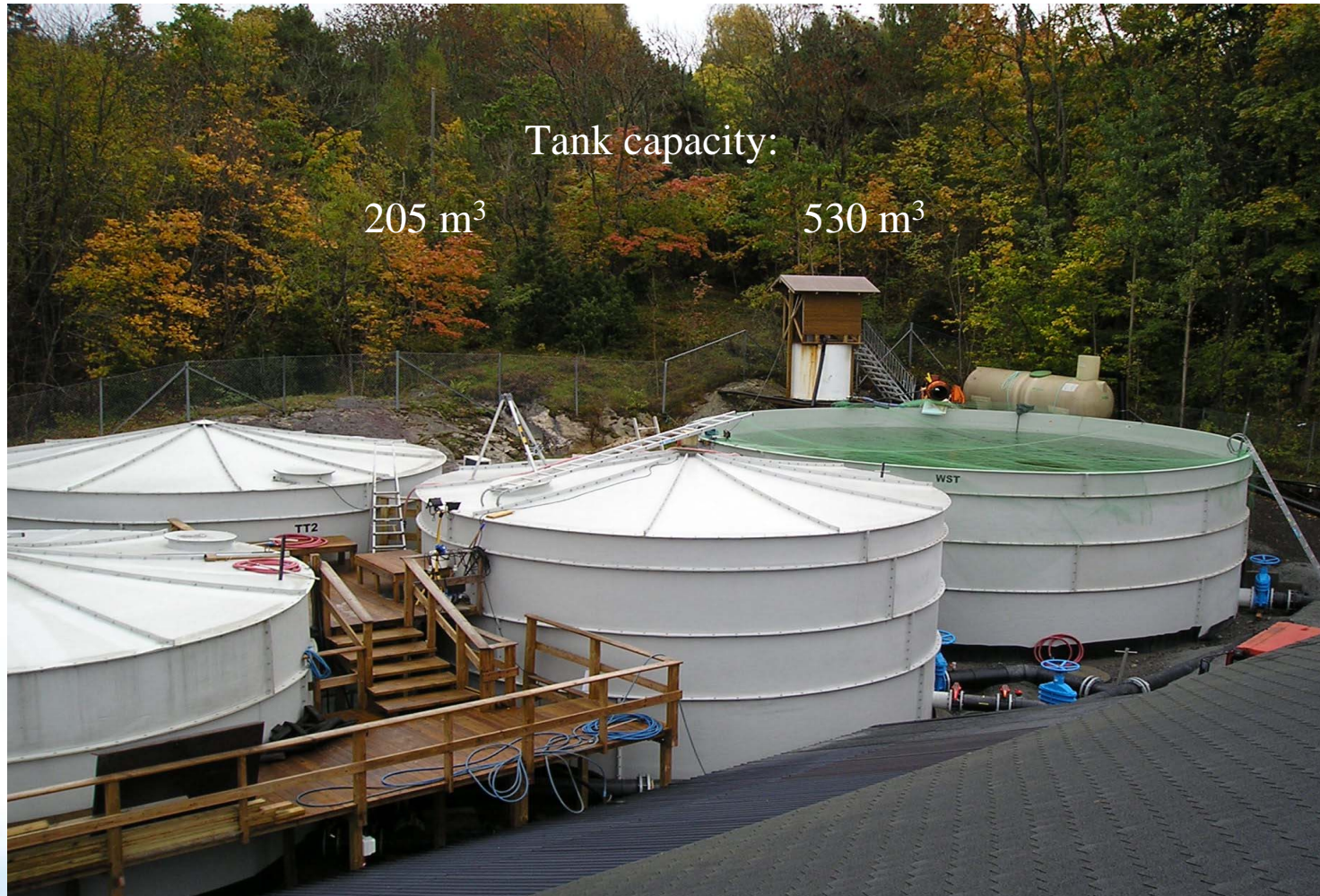
- Samples are send to laboratories for analysis.

Except $>50\text{ }\mu\text{m}$ is analysed on board

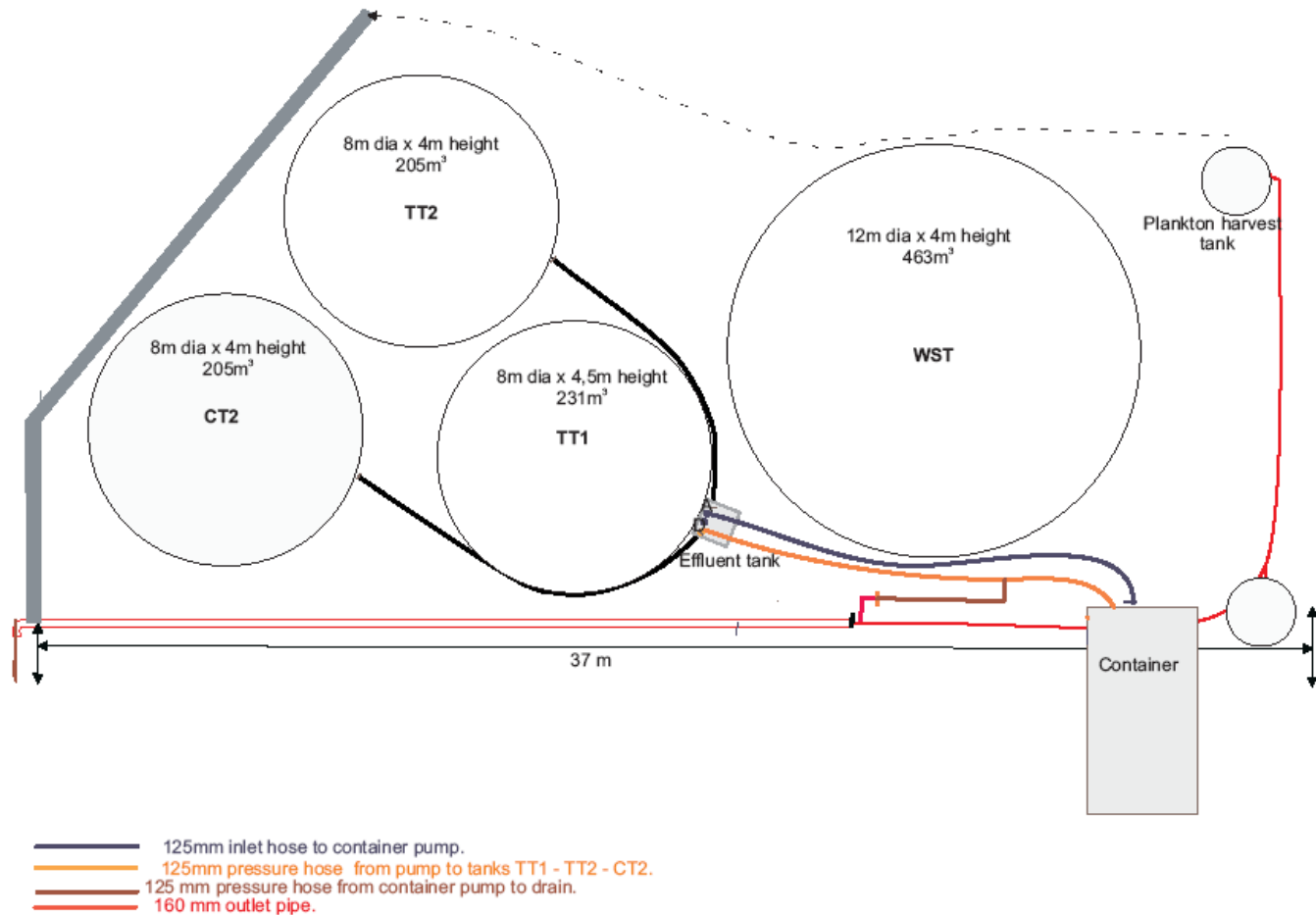
Landbased testing

At Solberstrand
Norway

Land based facility



Land based facility



Ballast Tech NIVA AS Testing facility.	
Overview ballast tanks. No. 15	
Oddbjørn Pettersen	3.10.2006

Homogeneity in tanks

Particulate organic carbon										
Test cycle	1	2	3	4	5	6	7	8	9	10
WST	2	7.2	6.3	5.6	6.1	6	2.8	2.5	3	2.6
Control	2.1	6.8	6.3	5.8	5.9	5.6	2.8	2.2	2.7	2.7

Average ration wst/control is 0.97 for both

Total suspended solids										
Test cycle	1	2	3	4	5	6	7	8	9	10
WST	13.1	65.3	81.5	76.7	77.6	14.3	12.7	14.4	12.5	80
Control	13.1	63.6	75.7	64.8	70.4	15.8	14.2	14.2	11.8	73.3

Homogeneity in tanks

algal counts #/ml										
Test cycle	1	2	3	4	5	6	7	8	9	10
WST	3578	1876	1392	1564	2014	1651	1720	1409	1564	1219
Control	2679	1936	1504	1357	1737	1487	1513	1271	1513	1452
ratio wst/control	0.75	1.03	1.08	0.87	0.86	0.90	0.88	0.90	0.97	1.19

average ratio is 0.94 indicating a small reduction in algal numbers due to filters.

Analysis of samples

- Same methods as we use onboard ship.

Samples for 10-50 μm should not be concentrated prior to analysis.

Sample storage

- Bacteria (24h)
 - Most standards require analysis within 24 h.
- >50 μm (6 h)
 - A concentrated sample may become oxygen deficient because of degradation processes.

Sample storage

- 10-50 μm
 - A non concentrated sample may have good survival for 2-4 days when stored at 2-4 °C in the dark.

Analysis >50 μm

- Viability criteria = observed movement in a concentrated sample
- Advantages
 - Easily observed
 - Rapid (20 min per sample)

Analysis >50 µm

- Disadvantage
 - Not correct endpoint, should be reproductive ability
 - Some organisms may die during the concentration process

Analysis 10-50 μm

- Serial dilution culture (MPN)
 - 1 ml sample is added to 9 ml of medium in 5 test tubes. 1 ml from these dilutions is then further diluted giving a dilution series of 1/10, 1/100, 1/1000 etc.
 - Tubes are incubated in light for minimum 14 days before analysis of presence of species

MPN method

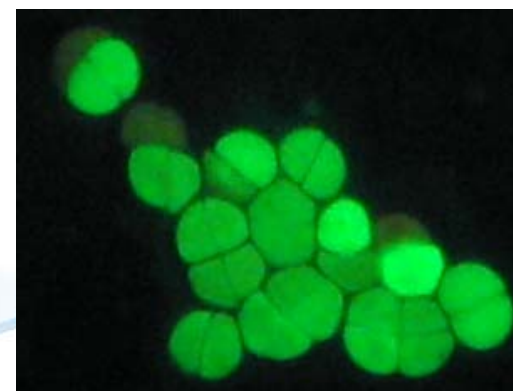
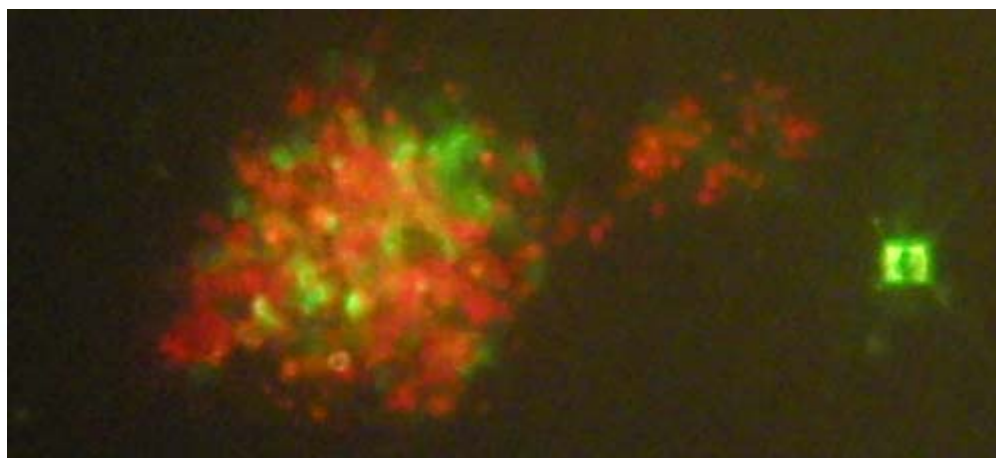
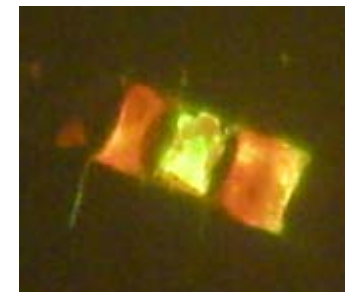
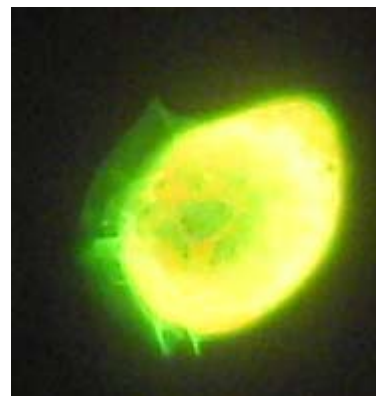
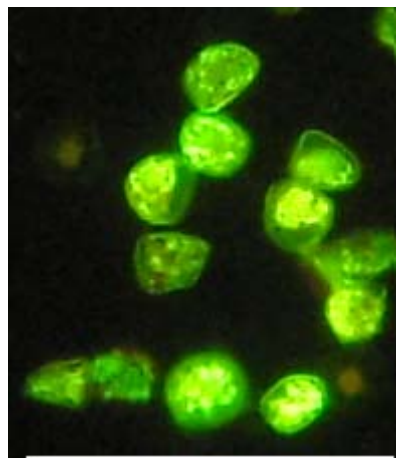
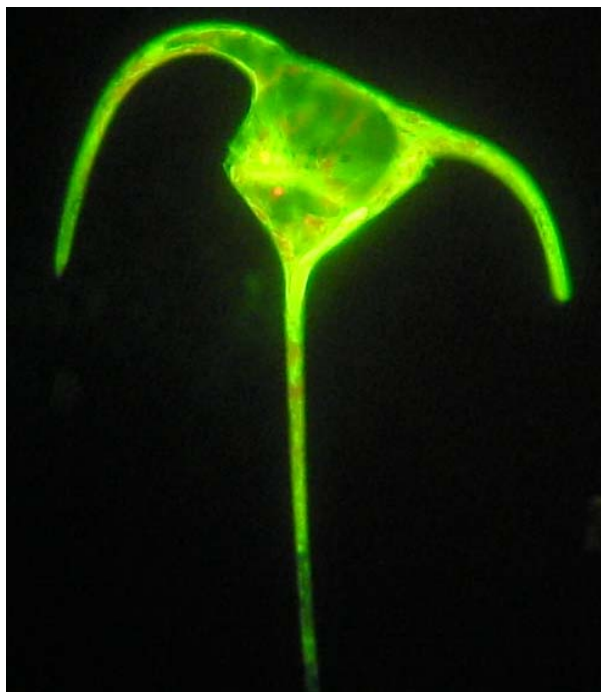
- Advantages
 - Only cells able to reproduce is detected
 - Several species can be enumerated in each dilution series

MPN method

- Disadvantages
 - 14 days before results
 - Need skilled personell
 - Some species do not grow in test tubes
(dinoflagellates, heterotropic cells,

Vital staining

- Unconcentrated sample is stained CFDA for 1 hour and then viewed in microscope



Vital staining

- Advantages
 - Include species that does not grow in test tubes.
 - Rapid one sample can be processed within 2 hours

Vital Stain

- Disadvantages:
 - Skilled personell
 - Some species do not stain (false negative)
 - Some species will stain even when not reproductive fit. (UV-treated)

UV treated samples

- May easily be categorized as alive relevant for both >50 and $10-50 \mu\text{m}$ group.
 - Subacute effect of UV is damaging DNA
 - UV treatment is energi consuming therefore there is a competition to reduce amount of UV.

Further development

- There is at present a gap between IMO definition of viability and what we are able to observe
- Image analysis (FlowCam, Cytobouy)
- Stains that target ability to reproduce.