EMSA Workshop on

Ballast Water Sampling for Enforcement

BW Sampling for Enforcement – Research Results

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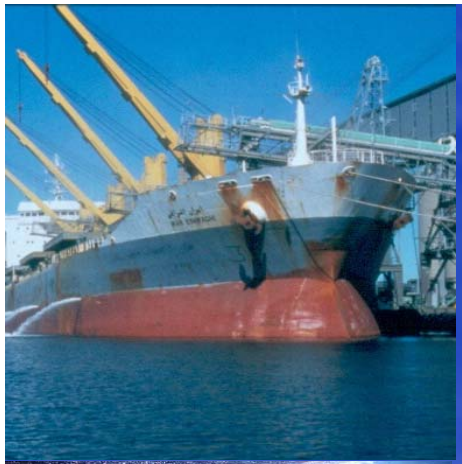


OUTLINE

- Study aims
- Materials and methods

Results

- Part 1 - Shipboard sampling test results
- Part 1 - Recommendations for representative sampling for D-2
- Part 2 - Indicative analyses methods
- Part 2 - Indicative sampling

Testing Sample Representativeness of a Ballast Water Discharge and developing methods for Indicative Analysis

Study aimed to:

1. develop a sampling protocol that obtains a representative sample of the whole discharged ballast water (representative sampling)

2. develop methods for indicative analysis/sampling that provides “clear grounds” for stopping a discharge and/or enforcement action (indicative analysis)

Materials & Methods

Part 1 – Representative sampling

- BW sampling onboard vessels
- 2 voyages, two different vessels
- Sampling of BW uptake and discharge
- Sampling of treated and untreated BW
- Sampling in ports and at sea (different concentrations and conditions)
- 44 samples taken and analysed

Voyage 1

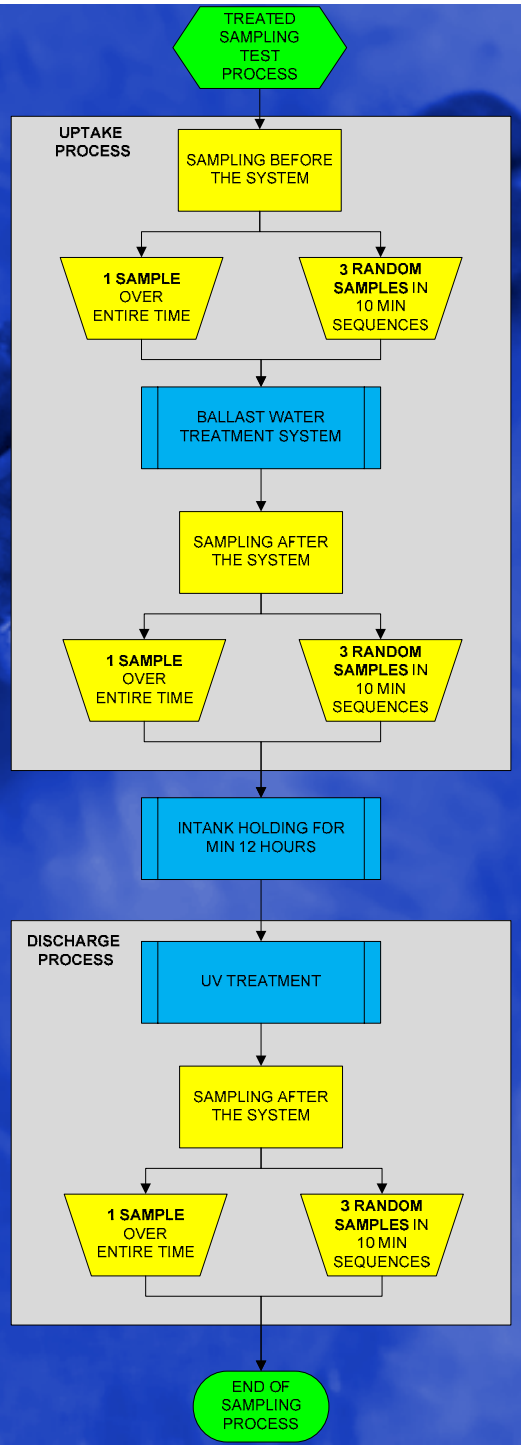
- Container vessel
- Mahle BWTS
  UV & filtration
- 24th to 29th July 2010
- Between the Ports of Leixoes (Portugal) and Algeciras (Spain)
- 1 treated test
- 2 untreated tests

Voyage 2

- General cargo
- No BWTS used
- 26th Sep to 1st Oct 2010
- Between the Ports of Terneuzen (The Netherlands), Karlshamn and Varberg (Sweden)
- 2 untreated tests

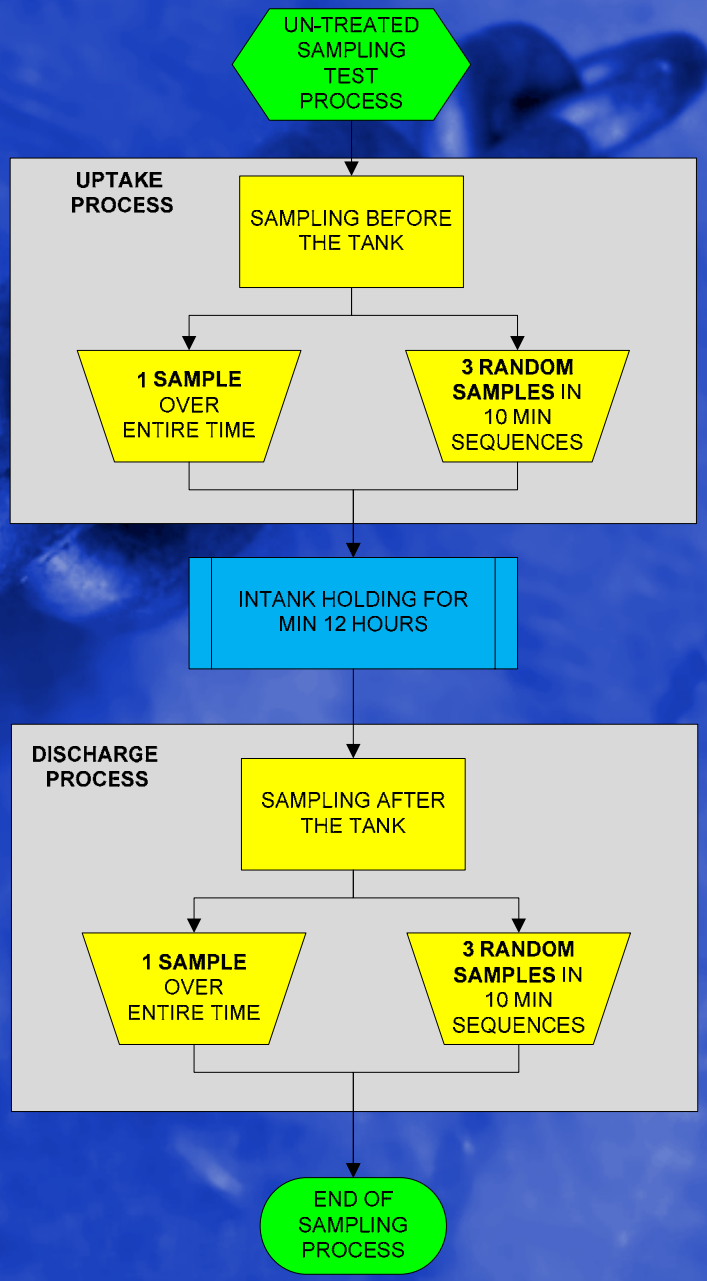
Treated test

- Uptake sampling before and after the system simultaneously
- In-tank holding >12 hours
- Discharge sampling after the system before discharge
- Sampling in parallel -1 sample over the entire time and 3 random samples in sequences
- 8 samples during uptake
- 4 samples at discharge



Untreated test

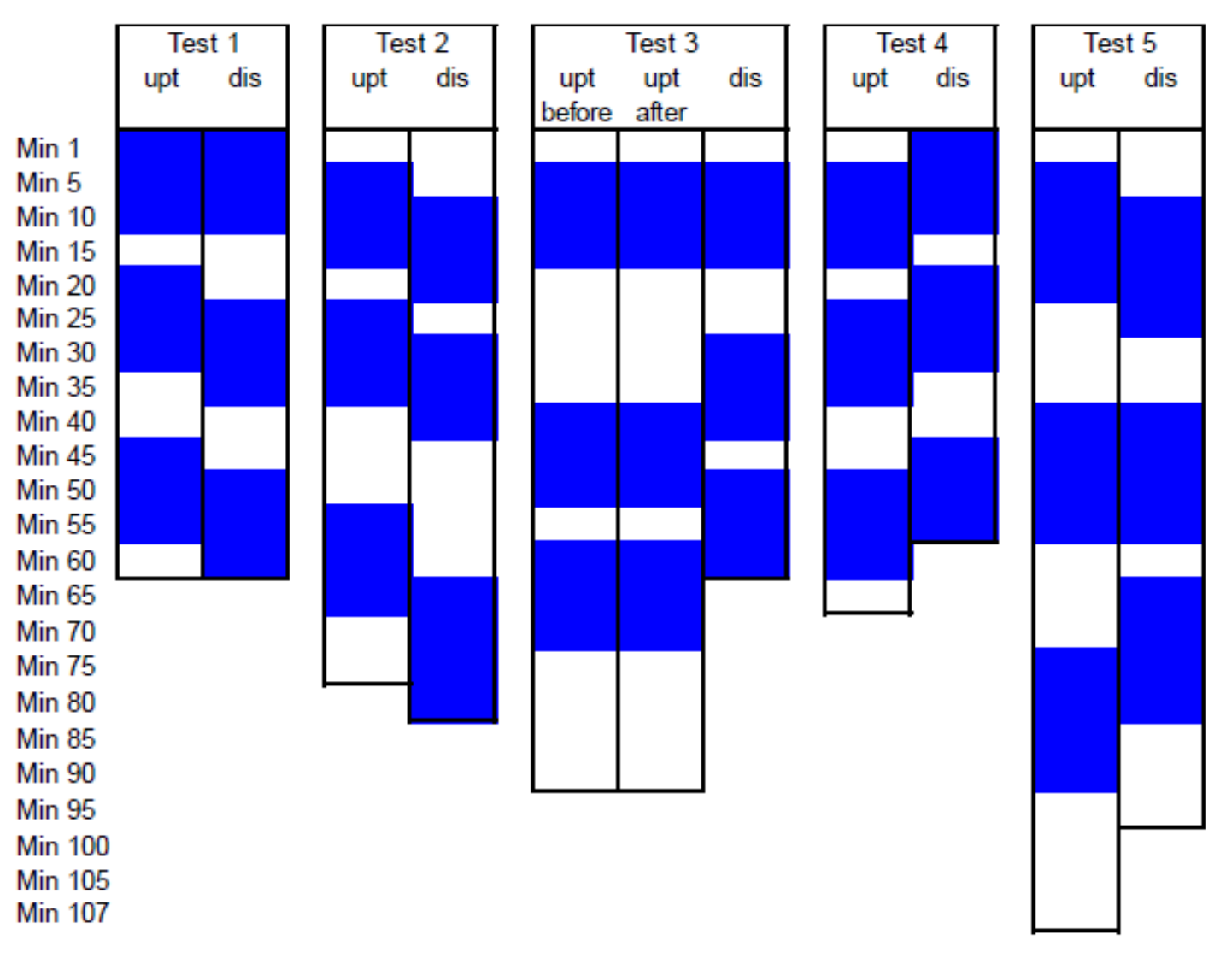
- Uptake sampling before tank
- In-tank holding >12 hours
- Discharge sampling after tank
- 4 samples during uptake
- 4 samples at discharge



Summary samples from onboard tests

- 5 uptake tests * (3 seqences + 1 over entire time (OET)) = 20 samples
- 1 uptake after the BWTS = 3 sequences + 1 OET = 4 samples
- 5 discharge tests * (3 sequences + 1 OET) = 20 samples
- All together 44 samples taken & analysed
- Treated 4 samples after BWTS + 4 at discharge = 8 samples
- All treated samples = no viable organisms detected

Random sequential tests per voyage



…every test also OET sample in parallel

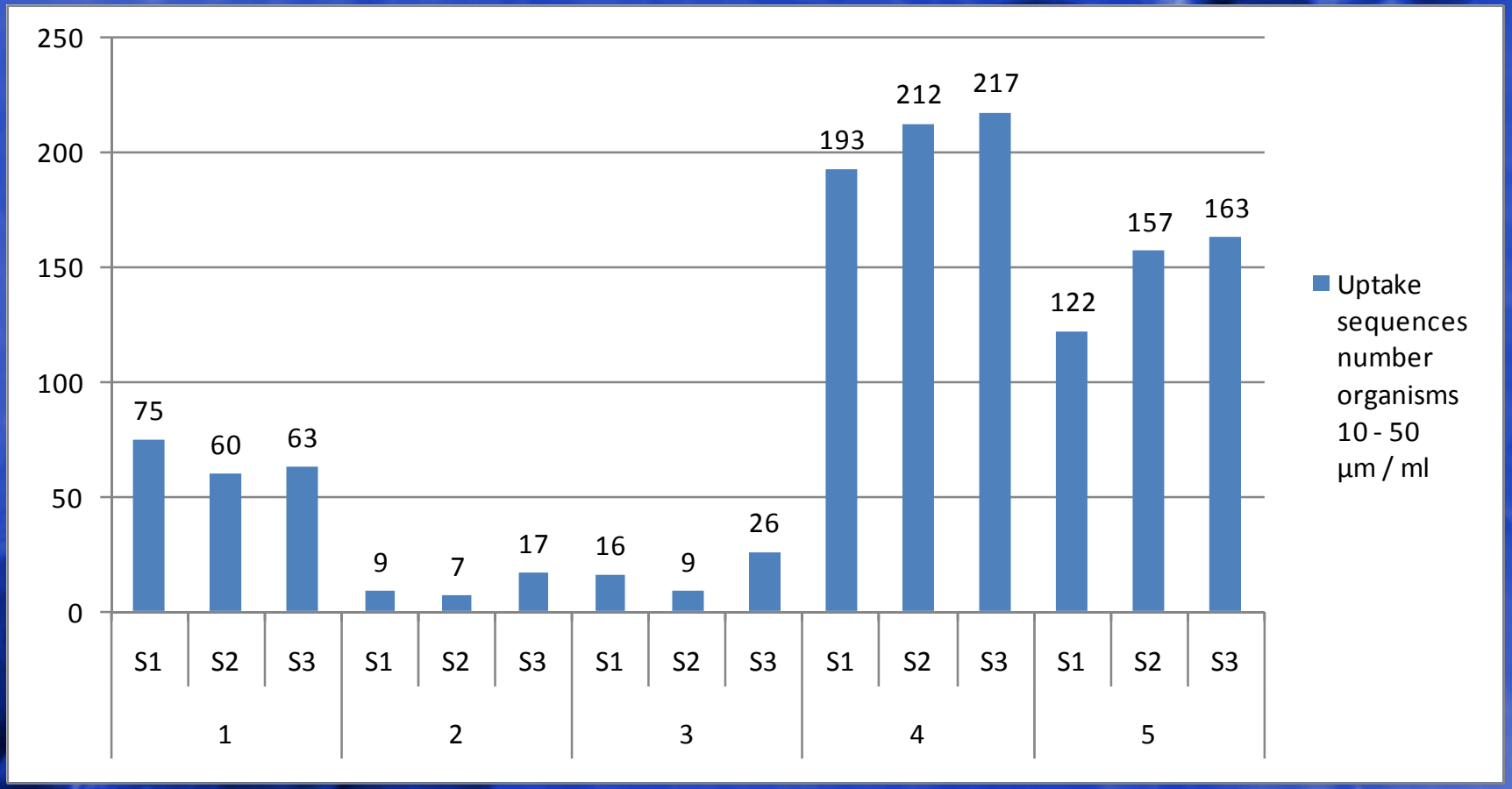
Results - REPRESENTATIVE SAMPLING

Sub-Questions to be answered:

- Is there a **homogeneity**, or lack of, **of organism concentrations in the uptake?**
- Is there a **homogeneity**, or lack of, **of organism concentrations in the discharge?**
- How **representative** are the **results observed during the entire discharge** of ballast water?, and
- Whether using an "**instantaneous**" or "**average**" testing sampling protocol will have an influence on the procedure used to obtain a representative sample?

Homogeneity of organism concentrations in the uptake

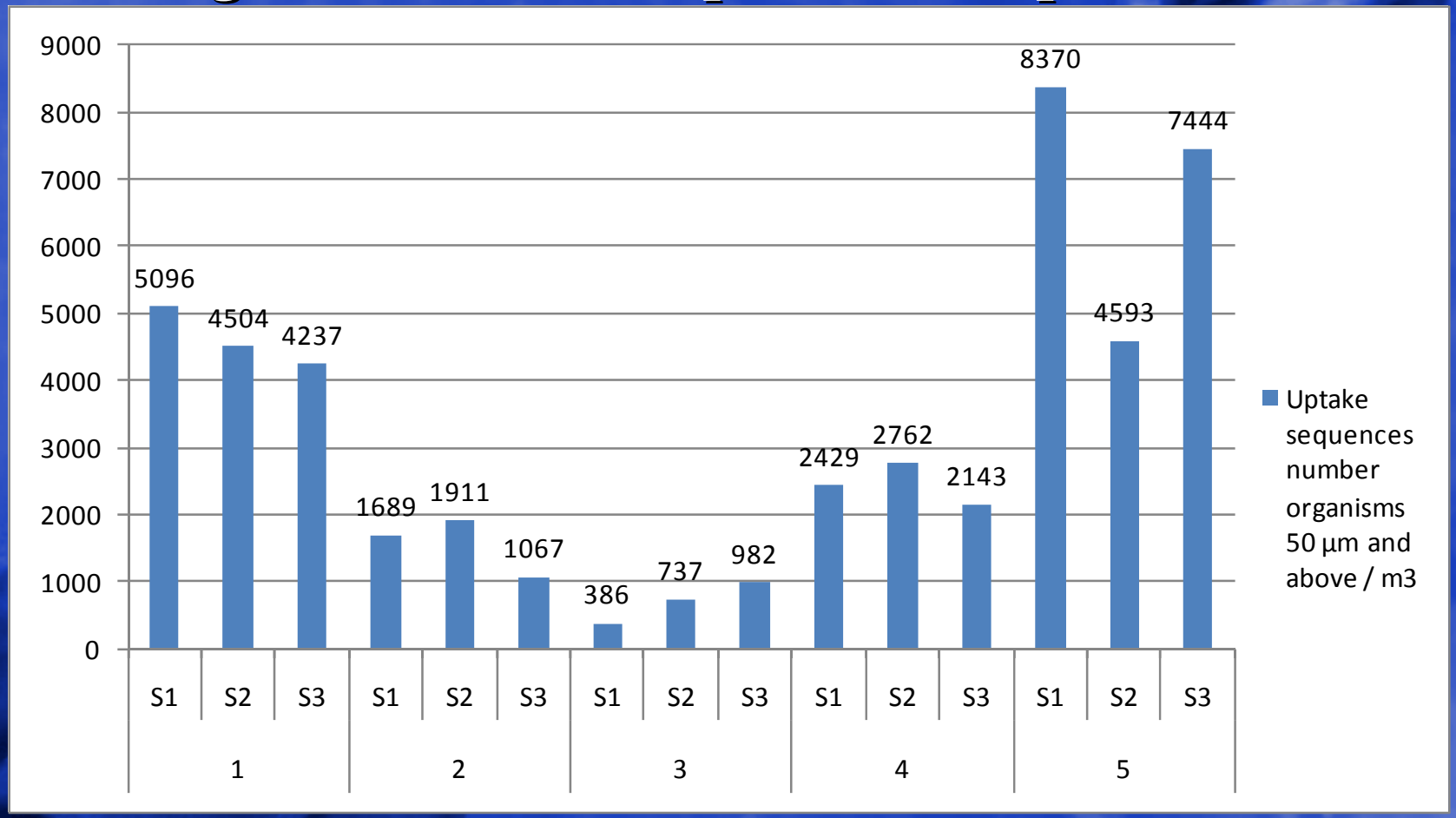
Organisms < 50 and > 10 – sequences compared



- Organisms patchiness present at open sea and in port
- BWTS exposed to organism density variations

Homogeneity of organism concentrations in the uptake

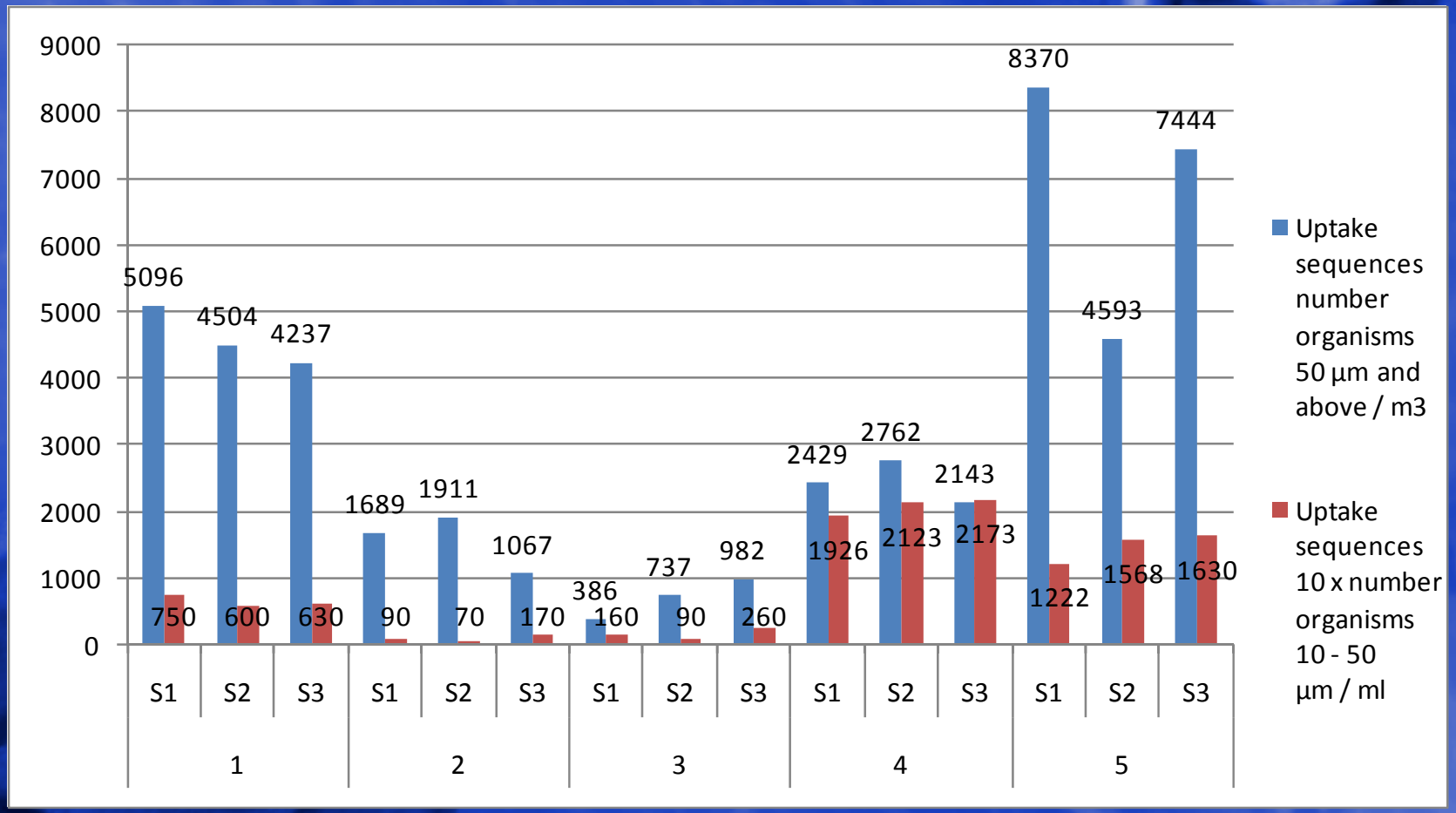
Organisms > 50 – sequences compared



- Organisms patchines present at open sea and in port
- BWTS exposed to organism density variations

Homogeneity of organism concentrations in the uptake

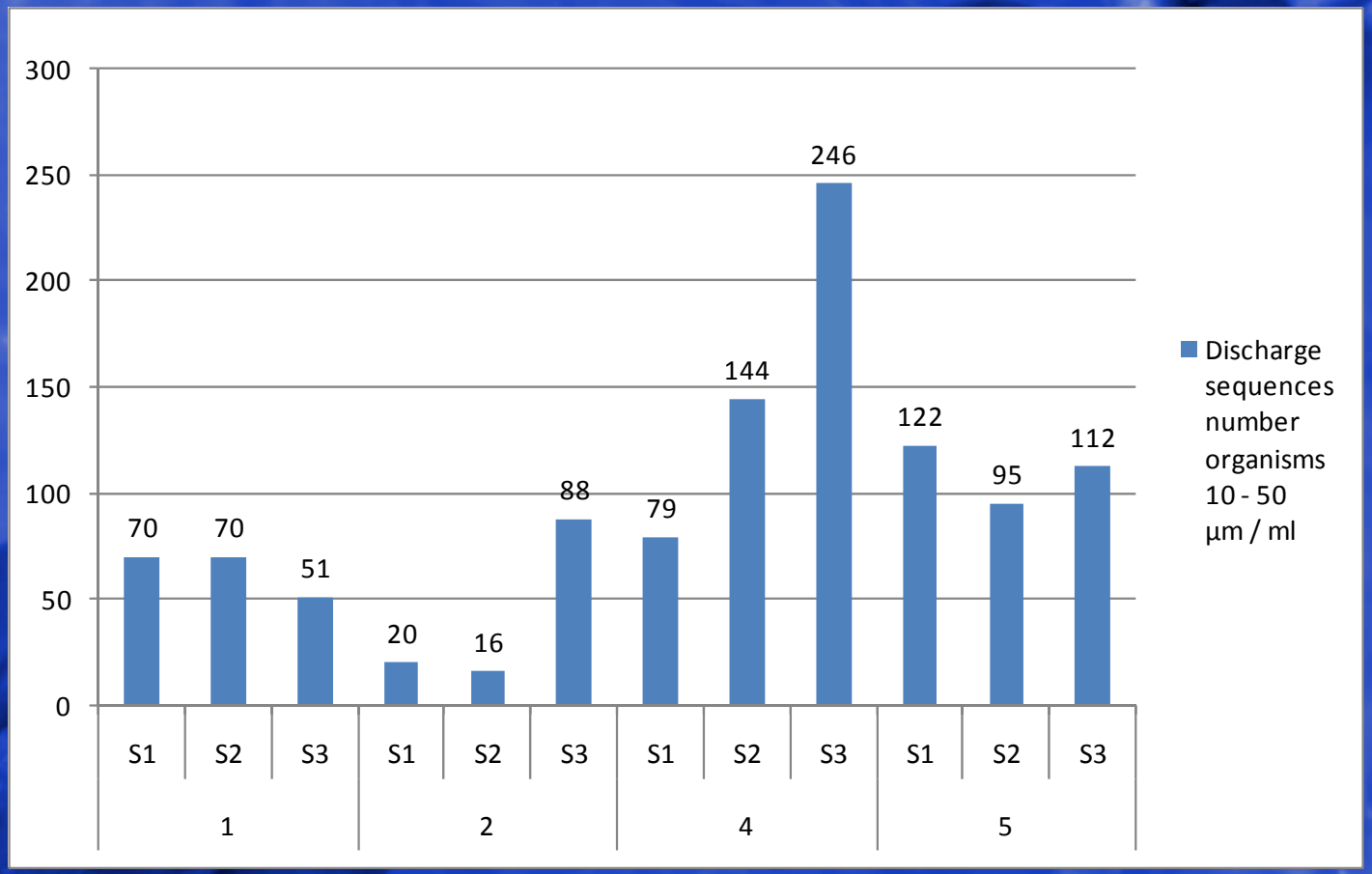
Organisms > 50 & < 50 and > 10 – sequences compared



- Frequently opposit concentrations, no pattern identified
- Nevertheless BWTS **performance seems not impacted**

Homogeneity of organism concentrations in the discharge

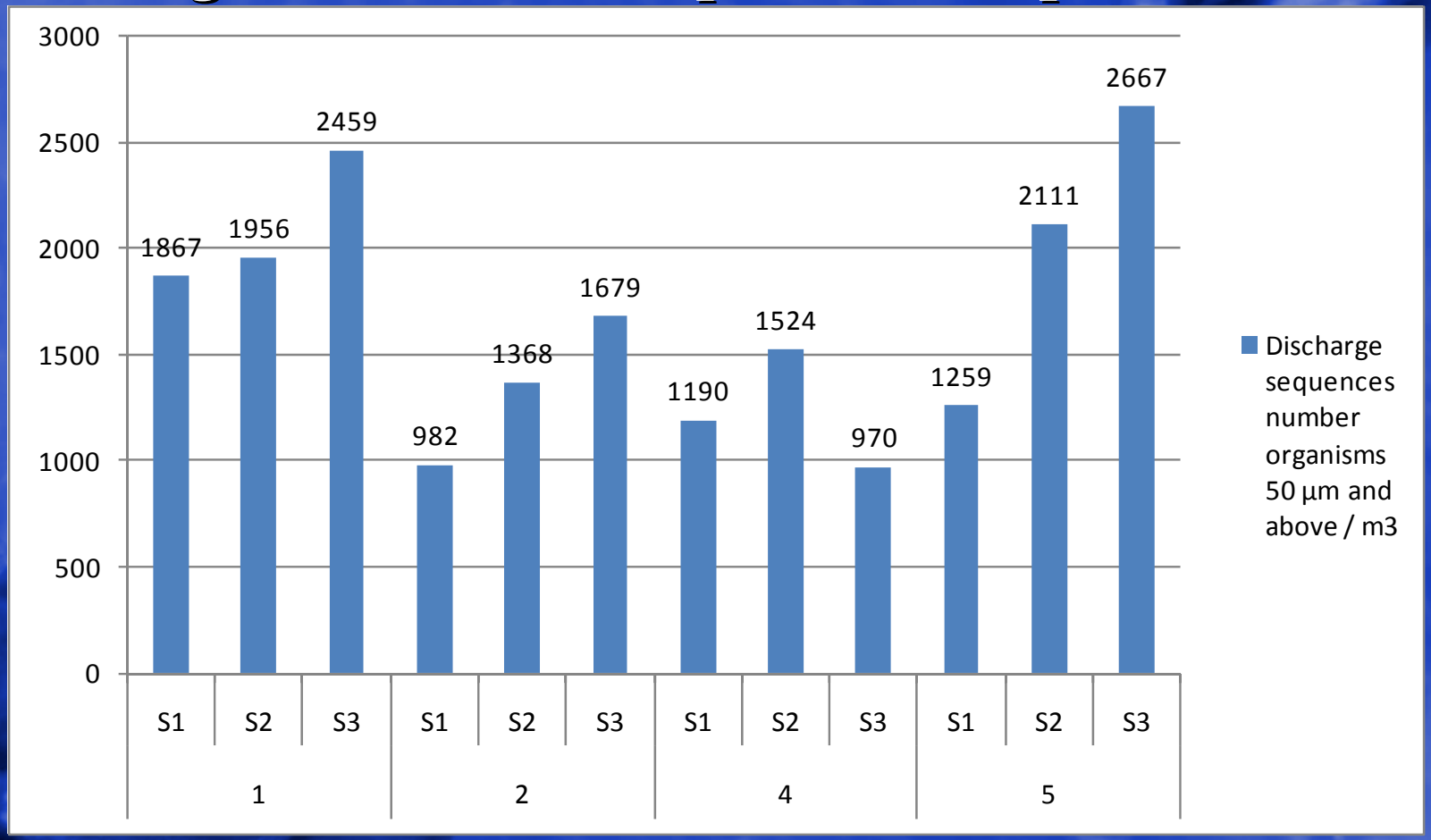
Organisms < 50 and > 10 – sequences compared



- Organisms patchiness occurs
- No pattern identified

Homogeneity of organism concentrations in the discharge

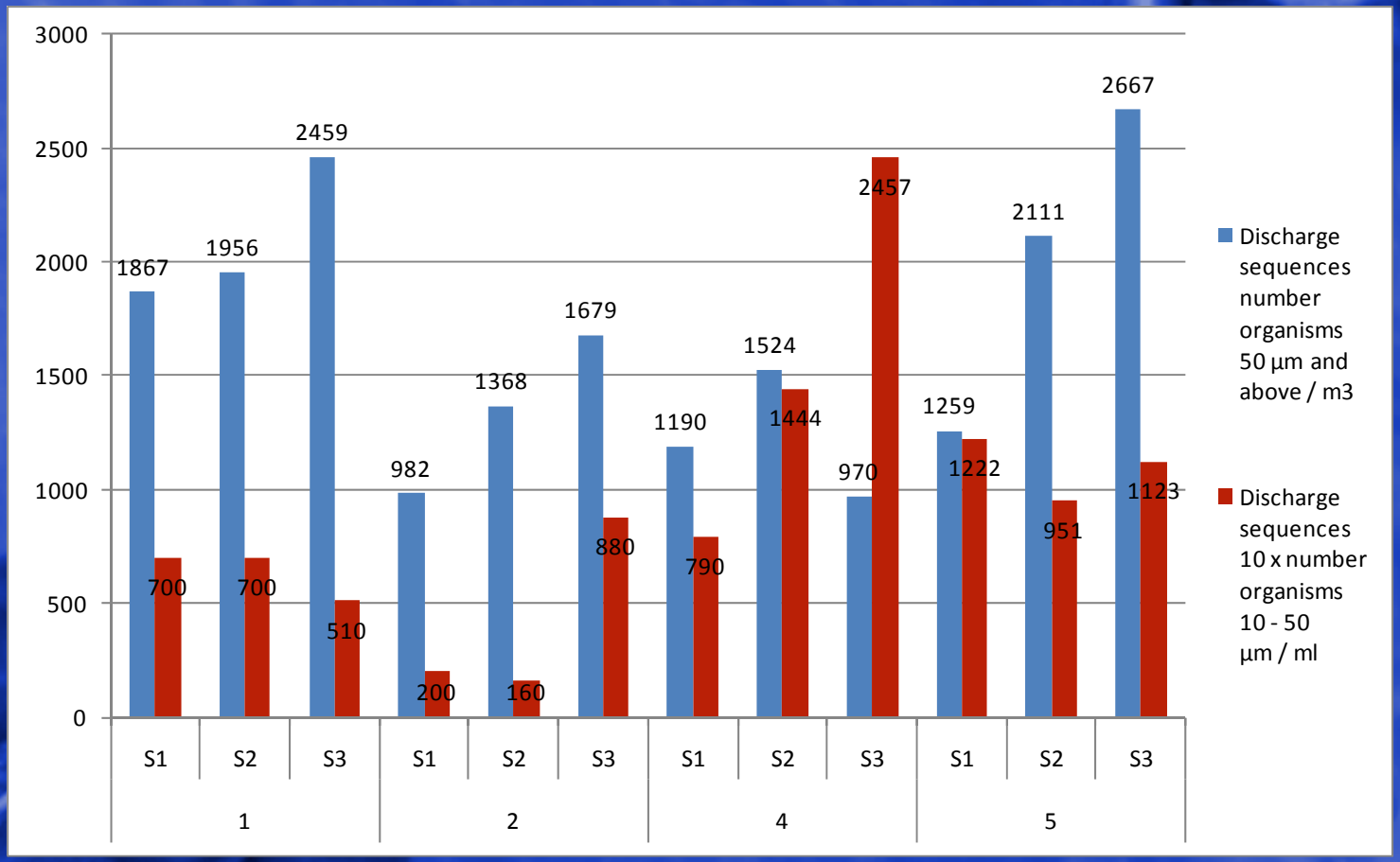
Organisms > 50 – sequences compared



- Organisms patchiness occurs
- Rise of organism concentration from beginning to the end

Homogeneity of organism concentrations in the discharge

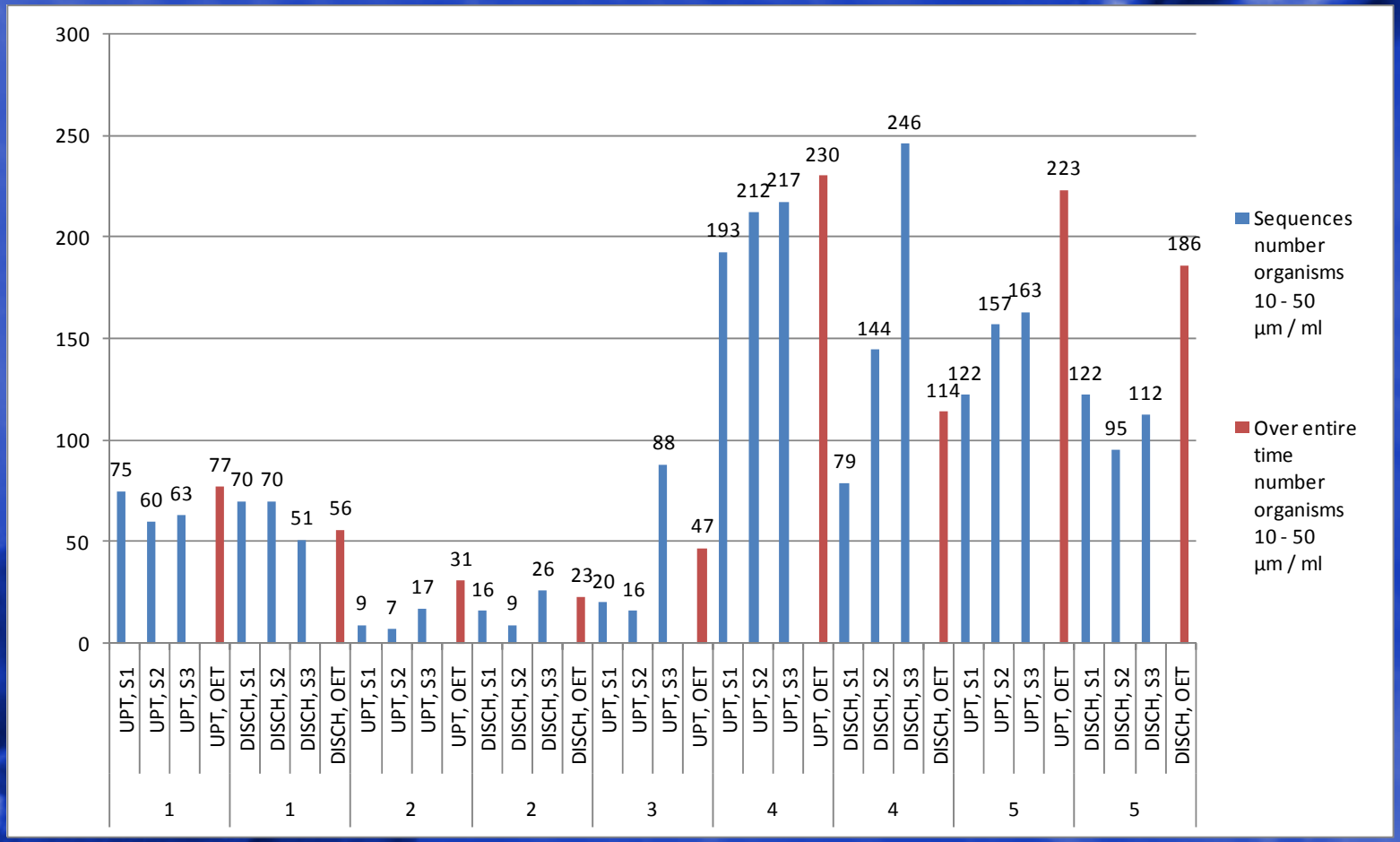
Organisms > 10 & < 50 and > 10 – sequences compared



- Clear tank organisms patchiness
- BW sampling results clearly impacted by tank patchiness!

BW sampling Over Entire Time vs. Sequential

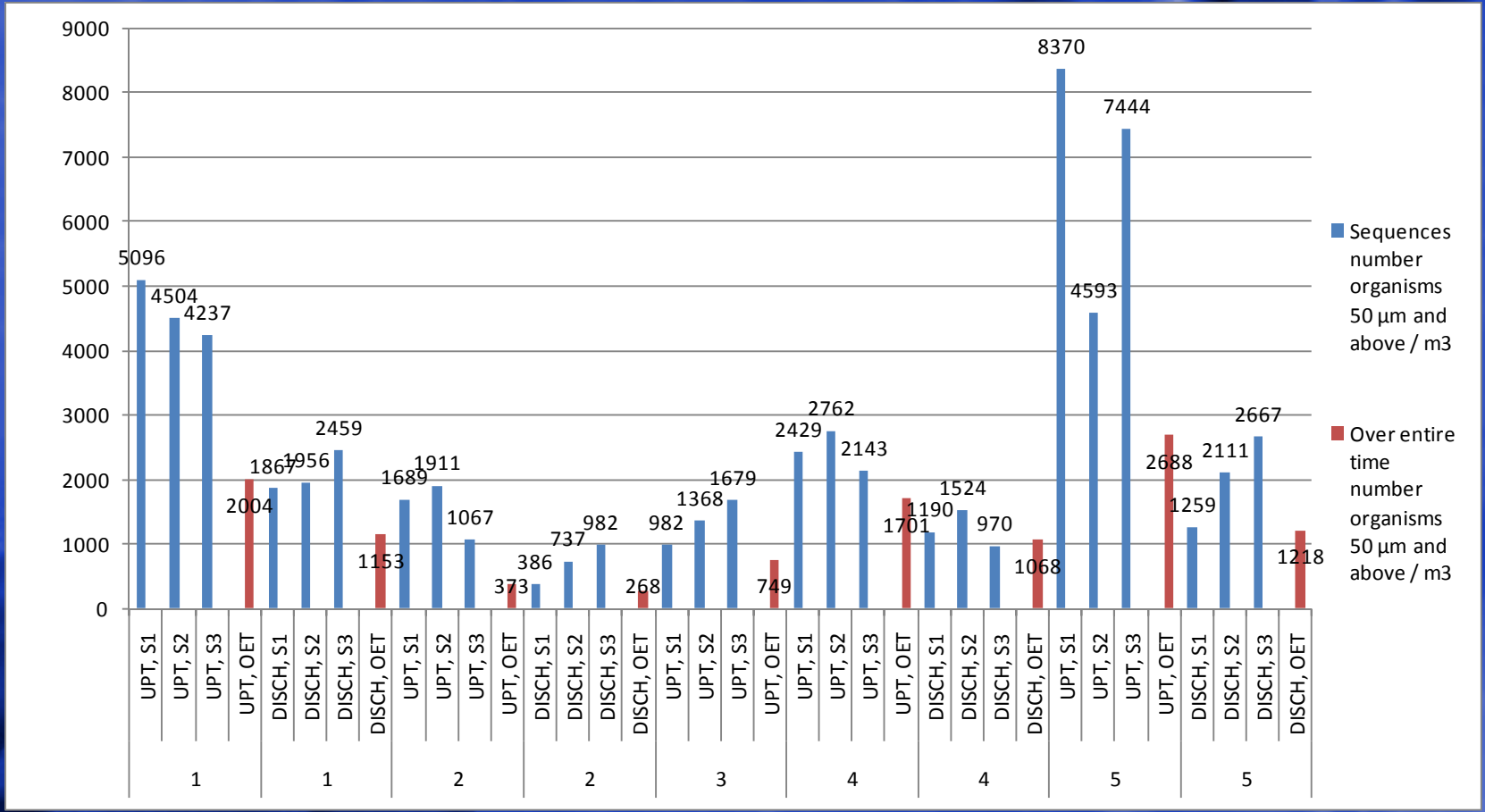
Organisms < 50 and > 10 – sequences compared



- Similar results OET and Sequences

BW sampling Over Entire Time vs. Sequential

Organisms > 50 – sequences compared



- Much higher concentrations in the sequence samples
- Longer time & higher volume sampling negatively impacts

“Instantaneous” sampling vs. “Average”

Definition of Instantaneous sampling:

- “one-point-in-time” sampling
- Shorter sampling time than OET

Definition of Average sampling:

- Average of two or more instantaneous samples, or
- OET sampling

"Instantaneous" sampling vs. "Average"

Organisms < 50 and > 10 – 3 sequences vs. OET compared

Test No.	Sample type	Total number organisms 10 - 50 µm / ml	Difference between sequence and OET (%)
1	DISCH, 3 SEQ AV	64	14
	DISCH, OET	56	
2	DISCH, 3 SEQ AV	41	-12
	DISCH, OET	47	
4	DISCH, 3 SEQ AV	156	38
	DISCH, OET	114	
5	DISCH, 3 SEQ AV	110	-41
	DISCH, OET	186	

Result:

- Similar probability to sample higher or lower concentration
- Similar difference in organism concentrations sampled

"Instantaneous" sampling vs. "Average"

Organisms < 50 and > 10 – 2 sequences vs. OET compared

Test No.	Sample type	Total number organisms 10 - 50 µm / ml	Difference between sequence and OET (%)
	DISCH, S1	70	25
	DISCH, S2	70	25
	DISCH, S3	51	-9
	DISCH, 2 SEQ AV	61	8
1	DISCH, OET	56	
	DISCH, S1	20	-57
	DISCH, S2	16	-66
	DISCH, S3	88	87
	DISCH, 2 SEQ AV	52	11
2	DISCH, OET	47	
	DISCH, S1	79	-30
	DISCH, S2	144	27
	DISCH, S3	246	116
	DISCH, 2 SEQ AV	162	43
4	DISCH, OET	114	
	DISCH, S1	122	-34
	DISCH, S2	95	-49
	DISCH, S3	112	-40
	DISCH, 2 SEQ AV	109	-42
5	DISCH, OET	186	

Method - Second highest sequence excluded

Result:

- Similar probability to sample higher or lower concentration
- Similar difference in concentrations sampled

"Instantaneous" sampling vs. "Average"

Organisms < 50 and > 10 – 2 sequences vs. OET compared

Test No.	Sample type	Total number organisms 10 - 50 µm / ml	Difference between sequence and OET (%)
	DISCH, S1	70	25
	DISCH, S2	70	25
	DISCH, S3	51	-9
	DISCH, 2 SEQ AV	61	8
1	DISCH, OET	56	
	DISCH, S1	20	-57
	DISCH, S2	16	-66
	DISCH, S3	88	87
	DISCH, 2 SEQ AV	18	-62
2	DISCH, OET	47	
	DISCH, S1	79	-30
	DISCH, S2	144	27
	DISCH, S3	246	116
	DISCH, 2 SEQ AV	195	72
4	DISCH, OET	114	
	DISCH, S1	122	-34
	DISCH, S2	95	-49
	DISCH, S3	112	-40
	DISCH, 2 SEQ AV	109	-42
5	DISCH, OET	186	

Method – beginning or end sequence excluded

Result:

- Similar probability to sample higher or lower concentration
- Similar difference in concentrations sampled

"Instantaneous" sampling vs. "Average"

Organisms > 50 – 3 sequences vs. OET compared

Test No.	Sample type	Discharge sequences number organisms 50 µm and above / m3	Difference between sequence and OET (%)
1	DISCH, 3 SEQ AV	2094	82
	DISCH, OET	1153	
2	DISCH, 3 SEQ AV	1343	79
	DISCH, OET	749	
4	DISCH, 3 SEQ AV	1228	15
	DISCH, OET	1068	
5	DISCH, 3 SEQ AV	2012	65
	DISCH, OET	1218	

Result:

- Always much higher organism concentration in sequences average

"Instantaneous" sampling vs. "Average"

Organisms > 50 – 2 sequences vs. OET compared

Test No.	Sample type	Total number organisms 10 - 50 µm / ml	Difference between sequence and OET (%)
	DISCH, S1	1867	62
	DISCH, S2	1956	70
	DISCH, S3	2459	113
	DISCH, 2 SEQ AV	2163	88
1	DISCH, OET	1153	
	DISCH, S1	982	31
	DISCH, S2	1368	83
	DISCH, S3	1679	124
	DISCH, 2 SEQ AV	1331	78
2	DISCH, OET	749	
	DISCH, S1	1190	11
	DISCH, S2	1524	43
	DISCH, S3	970	-9
	DISCH, 2 SEQ AV	1247	17
4	DISCH, OET	1068	
	DISCH, S1	1259	3
	DISCH, S2	2111	73
	DISCH, S3	2667	119
	DISCH, 2 SEQ AV	1685	38
5	DISCH, OET	1218	

Method - Second highest sequence excluded

Result:

- Always much higher organism concentration in sequences average

"Instantaneous" sampling vs. "Average"

Organisms > 50 – 2 sequences vs. OET compared

Test No.	Sample type	Total number organisms 10 - 50 µm / ml	Difference between sequence and OET (%)
	DISCH, S1	1867	62
	DISCH, S2	1956	70
	DISCH, S3	2459	113
	DISCH, 2 SEQ AV	2207	92
1	DISCH, OET	1153	
	DISCH, S1	982	31
	DISCH, S2	1368	83
	DISCH, S3	1679	124
	DISCH, 2 SEQ AV	1175	57
2	DISCH, OET	749	
	DISCH, S1	1190	11
	DISCH, S2	1524	43
	DISCH, S3	970	-9
	DISCH, 2 SEQ AV	1247	17
4	DISCH, OET	1068	
	DISCH, S1	1259	3
	DISCH, S2	2111	73
	DISCH, S3	2667	119
	DISCH, 2 SEQ AV	1685	38
5	DISCH, OET	1218	

Method – beginning or end sequence excluded

Result:

- Always much higher organism concentration in sequences average

Representativenes of OET sample

Organisms < 50 and > 10:

- OET sample is more representative (compared to average sequences)

Organisms > 50 :

- OET sample underestimates organism concentration

RECOMMENDATIONS FOR A BALLAST WATER SAMPLING PROTOCOL THAT IS REPRESENTATIVE OF THE WHOLE DISCHARGE

Sampling time duration - recommendations:

- **Recommended sampling time is ~10 minutes**

Reasoning:

- Longer sampling time negatively affects survival of organisms > 50, hence sample is underestimating “real” organisms concentration; and
- Shorter sampling times are still representative in organisms group < 50 and > 10.

Sampling timing - recommendations:

- **Do not start sampling during first 5 minutes after start of BW discharge**
- **Do not sample in the last 5 minutes before end of discharge**

Reasoning:

- High patchiness of organisms during that sampling times; and
- A lot of sediment present in that sampling periods what may negatively affect organisms survival in the sample and sample processing.

Number of samples - recommendations:

- **Two or more samples to be taken**
- **If more than one BW source, than at least one sample taken from each BW source**

Reasoning:

- Average of two random samples shown to be representative, but more samples may be taken; and
- As sample needs to be representative of whole discharge, if BW is loaded in different areas, BW water from each area needs to be sampled.

Sampled quantity - recomendations:

- **For organisms > 50; 350 – 500 litres of sample filtered and concentrated to ca. 100 ml**
- **For organisms < 50 and > 10; 5 litres of continuous drip sample during sampling, subsample of ca. 60 ml for transport**
- **For bacteria; 1 litre separated from the 5 litre continuous drip sample**

Reasoning:

- Suggested methods and quantities showed best results, concentrated sample easy to carry

THANK YOU
FOR ATTENTION!