Materials & Methods

Part 2 – Indicative analysis

- Definition of method selection criteria
- Internet search for suitable methods
- Selected methods tested on 2 voyages
- BW uptake and discharge samples processed
- Treated and untreated BW samples processed
- Samples with different organism concentrations and water conditions

Indicative Analysis

Method selection criteria

- Reliable results to proof (non-)compliance
- Deliver prompt results (at best less than 30 minutes)
- Address all D-2 organism groups
- Simple to use
- Portable
- Cost-competitive (both, capital and running costs)
- Expertise needed to apply method

Results - INDICATIVE ANALYSIS

D-2 organism groups

- Organisms less than 50 and greater than or equal to 10 micrometres in minimum dimension
  - 8 methods considered
- Organisms greater than or equal to 50 micrometres in minimum dimension
  - 6 methods considered
- Methods for bacteria analysis
  - 11 methods considered

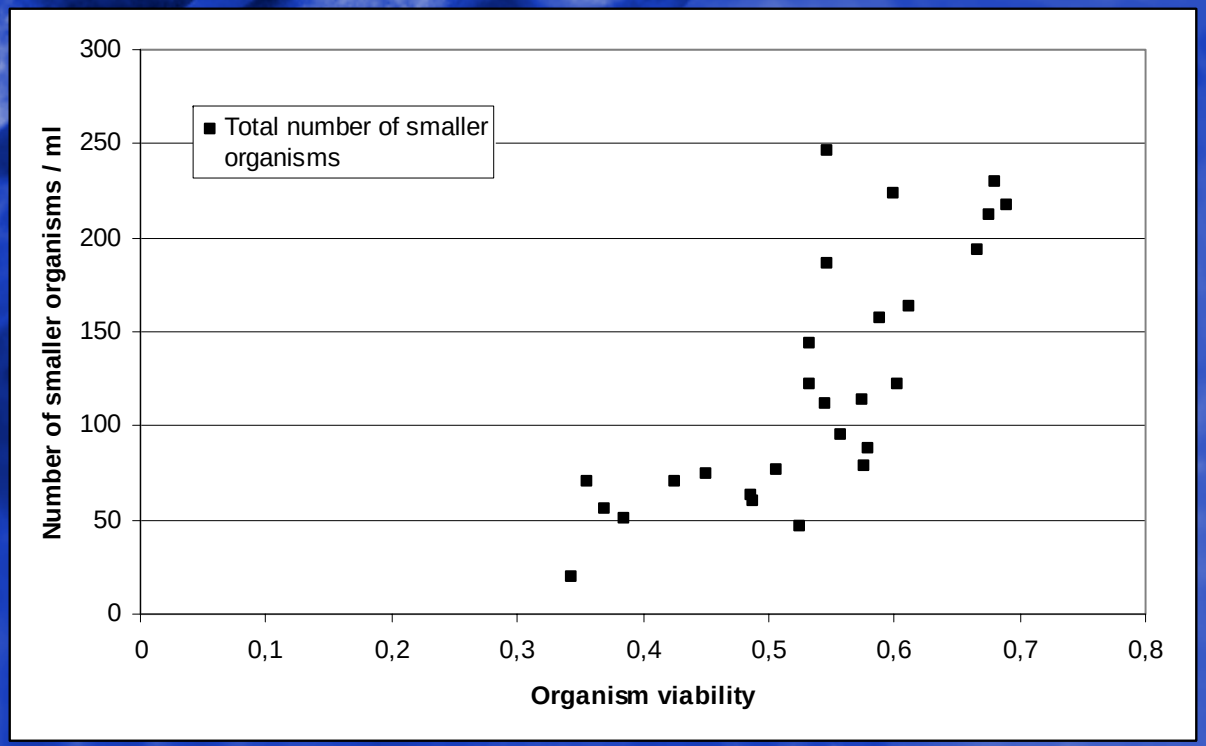
Indicative Analysis Methods

Organisms less than 50 and greater than or equal to 10 µm in minimum dimension

- Presence/absence methods (no viability, no counts)
  - e.g. DNA, ATP, “traditional” Chl *a* methods deliver results in less than 60 minutes
- Viability and counts
  - Flow cameras (less than 60 minutes, not portable, viability stain needed)
- Best compromise: PAM
    - portable, easy to use, low expertise needed
    - Viability in less than 10 minutes
    - No counts, but biomass and Chl *a* indication

Pulse-Amplitude Modulated fluorometry (PAM)

- PAM measures phytoplankton biomass and viability
- No direct counts
- Our results show a clear relation of biomass and viability measurements with organism numbers
- Suitable tool to show clear grounds that D-2 was not met
- Detection limit is 1 org / ml (calculated)



Indicative Analysis Methods

Organisms greater than or equal to 50 µm in minimum dimension

- Presence/absence methods (no viability, no counts)
  - e.g. DNA, ATP methods deliver results in less than 60 minutes
- Counts (no viabilty)
  - Hand-held flow cameras (less than 30 minutes)
- Best compromise: Stereomicroscope (counts & viability)
  - portable, easy to use, high expertise needed
  - results in less than 40 minutes

Indicative Analysis Methods

D-2 Bacteria

- Presence/absence methods (no cfu and/or counts)
  - e.g. DNA, ATP methods deliver results in less than 60 minutes
- All methods to determine cfu require incubation time of 24 - 72 hours
- Best compromise: Hand-held fluorometer
  - portable, easy to use, low expertise needed
  - presence/absence in < 10 mins to 4 hours
  - semiquantitative, i.e. high reading = high bacteria numbers

Indicative Analysis Methods, Summary

- No single method to address all D-2 organism groups
- Presence/absence methods
  - Document presence of organisms, but no counts, neither viability analysis
  - Suitable as first indication
- Count and viability methods
  - Document number of viable organisms
  - Suitable for D-2 compliance control
- Compromise needed

Indicative Analysis Methods, Suggestions 1

- Start with one method to evaluate one organism group in D-2
- Should this show presence or high numbers, take result as indication of a failed treatment system
- Should this show absence or low numbers, continue with second (and third) D-2 organism group to confirm results
- The easiest to start with may be the analysis for phytoplankton (PAM), followed by bacteriae (hand-held fluorometer) and zooplankton (stereomicroscope)

Indicative Analysis Methods, Suggestions 2

- Consider to equip a van with organism detection technology
- Drive from vessel to vessel in a port
- Send sampling team onboard and deliver the samples as soon as possible to van for analysis
- In this scenario the organism detection tools would not need to be carried onboard
- Sampling team “only” to board the vessel, no need to bring organism detection team onboard as well

Indicative Analysis and Indicative Sampling

- Different organism groups require different sampling strategies (e.g. gear, water volumes, number of samples)
- We recommended that for indicative ballast water sampling, one sequential sample is taken using the same sampling methodology as for a full D-2 compliance test (i.e. short sampling time)
- If high risk ballast water onboard, no samplings from discharge line during discharge, but from tank (manhole, sounding pipe)
- This may not be representative of the whole discharge but an indicative compliance control analysis is enabled without discharging ballast water with critical organisms

Indicative Analysis Approach