



Full Scale trials Biological assessment

Gemma Quílez-Badia

Tracy McCollin

Prof. C.L.J. Frid

Dr. M. Gill

School of Marine Science and Technology, University of Newcastle upon Tyne

INTRODUCTION

- As part of WP4, Onboard testing was required to assess effectiveness of different ballast water treatments.
- 3 treatments to be tested were:
 - High Temperature (UNEW)
 - Deoxygenation (SINTEF)
 - Oxidation method (BENRAD)
- Test carried out during 2 legs of the trip of the car carrier M/V Don Quijote:
 - High Temperature (HT): Suez (Egypt) to Zeebrugge (Belgium) from 27th May to 5th June 2003.
 - Deoxygenation (DEOX): Southampton (UK) to Manzanillo (Panamá) from 20th to 30th June 2003.

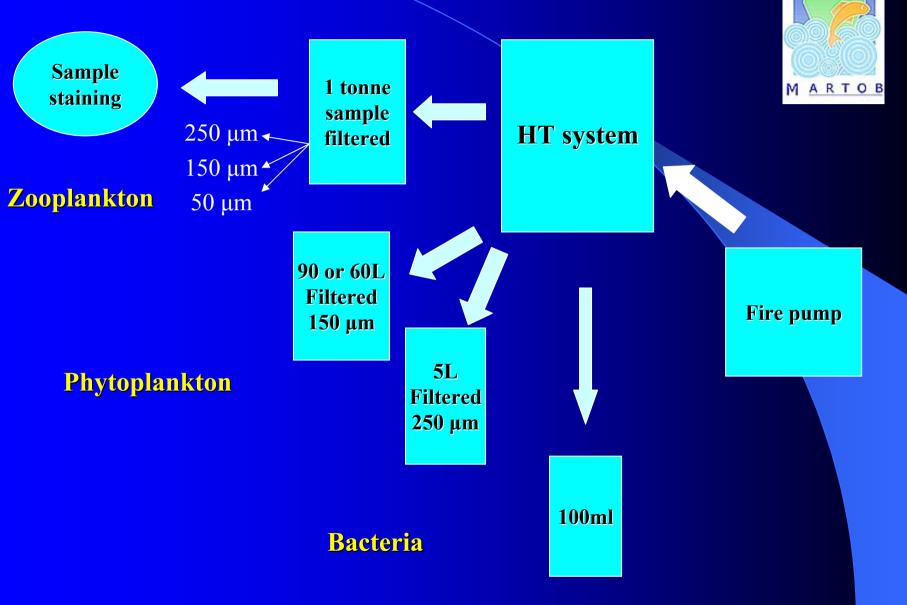


 Before both treatments: 5 samples of 1 tonne from Sea Chest (native organisms).

• At the end of both treatments: 3 samples of 1 tonne from the tank with oldest water (DB2S) (resting eggs).

 For both treatments: zooplankton, phytoplankton and bacterial samples taken using fire pump. Some of bacterial samples also taken from treated tanks via the sounding pipe.





HIGH TEMPERATURE TREATMENT (HT)



3 tanks: DB4P, DB4S and AP

Tank	Date/Time (UTC) Start	Start Position	Date/Time (UTC) Stop	Stop Position
DB4 (S+P)	28/5 20:40	N 32 11	28/5 21:15	N 32 14
		E 030 23		E 030 10
AP	29/5 00:00	N 32 32	29/5 00:30	N 32 34
		E 029 09		E 028 59

Table 1. Details of the ballasting of the tanks used in theHigh Temperature treatment.

HIGH TEMPERATURE TREATMENT (HT)



•Temperatures tested:

55, 60, 65 and 70°C 75 and 80°C were also applied to DB4S tank.

•Zooplankton:

3 replicates of 1 tonne from each tank and temperature tested. Before and after treatment.

•Phytoplankton:

samples of 5 and 90L, before and after treatment for each tank and temperature.

•Bacteria:

100ml samples from before and after treatment from each tank and temperature.

DEOXYGENATION TREATMENT (DEOX)



4 tanks: DB3 (S+P) and 3U (S+P)

Tank	Date/Time (UTC) Start	Start Position	Date/Time (UTC) Stop	Stop Position
DB3 (S+P)	21/6 01:00	N 50 26	21/6 02:00	N 50 21
		W 001 24		W 001 38
3U (S+P)	21/6 02:00	N 50 21	21/6 02:50	N 50 14
		W 001 38		W 002 01

Table 2. Details of the ballasting of the tanks used in the
Deoxygenation treatment.

MATERIALS AND METHODS DEOXYGENATION TREATMENT (DEOX) •Control tanks: MARTOB **DB3P and DB3S.** •Treated tanks (3US and 3UP): nutrient solution added to make water anoxic. From each tank on 21st, 24th, 26th and 28th June •Zooplankton: **3 replicates of 1 tonne** •Phytoplankton: samples of 5 and 90L (or 60L) •Bacteria: **100ml samples.** 1L samples taken on the other days via sounding pipe directly from treated tanks.

ZOOPLANKTON SAMPLE PREPARATION



After filtration through 50 µm sieve, the samples were:
Rinsed with filtered sea water
Stained with Neutral Red and fixed with 4% Formalin
Stored at –5°C overnight
Kept at room temperature until end of trials

At Dove Marine Laboratory (Newcastle):

 Counting analysis and taxonomic identification of samples or subsamples under stereomicroscope
 Preservation in 4% Formalin

Zooplankton identified to the level of subclass or class.

ZOOPLANKTON SAMPLE PREPARATION

MARTOB

•The following taxa were found:

 Copepods (adults) Copepods (nauplii) Cirriped larvae Cladocerans •Eggs (crustaceans) •Bivalve larvae •Echinoderm larvae Polychaete larvae Nematodes •Hydroids Gastropod larvae Chaetognath Appendicularia or Ascidian larvae

PHYTOPLANKTON SAMPLE PREPARATION



•Samples collected for 2 analyses: Chlorophyll *a* (Chl *a*) and direct cell counts.

•Chl *a* (5 litres, 250 µm):

•2L collected => divided into 3 replicates of 500ml => filtered using glass fibre filter

•Each filter folded in on itself once, wrapped with labelled foil square and frozen immediately

•Samples were stored at –20°C until end of trials, transported in dry ice to Marine Laboratory, Aberdeen, and transferred to a –20°C freezer immediately

PHYTOPLANKTON SAMPLE PREPARATION



•Direct cell count (90 or 60L, prefiltered 150 μm):

•Filtrate collected in buckets => divided into 3 replicates => filtered through 10 μm plankton net

Samples preserved with Lugols iodine

Samples stored in cool dark place until trials finished

•Cell counts using the Uthermöhl sedimentation method under an inverted microscope

•Phytoplankton counted to level of class Bacillariophyceae (diatoms) and Dinophyceae (dinoflagellates)



1) HIGH TEMPERATURE TREATMENT

ZOOPLANKTON

•Results from samples of 30th and 31st May
 •99.5% were adult copepods and nauplii =>
 Results and graphs based on them

•Results: AP tank DB4P tank DB4S tank

2) DEOXYGENATION TREATMENT



ZOOPLANKTON

•Results from samples of 21st and 28th June
•98.4% were adult copepods and nauplii => Results and graphs based on these groups
•Results: DB3P tank (control tank) DB3S tank (control tank) 3UP tank (treated tank) 3US tank (treated tank)



1) HIGH TEMPERATURE TREATMENT

PHYTOPLANKTON

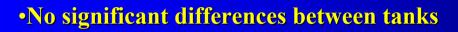
 Results based on analysis of all samples from HT treatment

•Very low levels of chl a

 Results from AP on 30th May and DB4S on 31st May

ZOOPLANKTON

Heat Treatment:



•No significant differences between before and after treatment

•In general, nauplii significantly more sensitive than adult copepods (Kruskal-Wallis, p<0.01)

Deoxygenation:

•Significantly higher naupli mortality over time in treated tanks (one-way ANOVA, p<0.001)

•Significantly higher copepod mortality over time in tank 3US (treated) (oneway ANOVA, p<0.001)

•No significant differences found between mortality of copepods and nauplii.

•No significant differences found in mortality between tanks.



MARTOB

PHYTOPLANKTON

Heat Treatment:

•Chl a:

Levels below detection limit.

Deoxygenation:

•No significant differences between tanks

•Significant differences over time

Cell count calculations currently being undertaken.