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.

MATERIALS AND METHODS

- 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.

MATERIALS AND METHODS

Sample staining

1 tonne sample filtered

HT system

250 µm
150 µm
50 µm

Zooplankton

90 or 60L Filtered 150 µm

5L Filtered 250 µm

Fire pump

Phytoplankton

100ml

Bacteria

MATERIALS AND METHODS

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 E 030 23	28/5 21:15	N 32 14 E 030 10
AP	29/5 00:00	N 32 32 E 029 09	29/5 00:30	N 32 34 E 028 59

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

MATERIALS AND METHODS

HIGH TEMPERATURE TREATMENT (HT)

- **Temperatures tested:**
  55, 60, 65 and 70^{o}C 75 and 80^{o}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.

MATERIALS AND METHODS

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 W 001 24	21/6 02:00	N 50 21 W 001 38
3U (S+P)	21/6 02:00	N 50 21 W 001 38	21/6 02:50	N 50 14 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:**
  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.

MATERIALS AND METHODS

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 –5ºC 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.

MATERIALS AND METHODS

ZOOPLANKTON SAMPLE PREPARATION

- 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

MATERIALS AND METHODS

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

MATERIALS AND METHODS

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)

RESULTS

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

RESULTS

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)

RESULTS

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

RESULTS

ZOOPLANKTON

Heat Treatment:

- No significant differences between tanks
- 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) (one-way ANOVA, $p<0.001$)
- No significant differences found between mortality of copepods and nauplii.
- No significant differences found in mortality between tanks.

RESULTS

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.