Effect of Mid-Ocean Exchange of Ballast Water on Bacterial Community in Ballast Tanks

58,098 GT
Length(O.A.) 239.80m
Length(PP) 230.00m
Breadth 43.00m
Depth 20.50m

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Ballast Water Problems

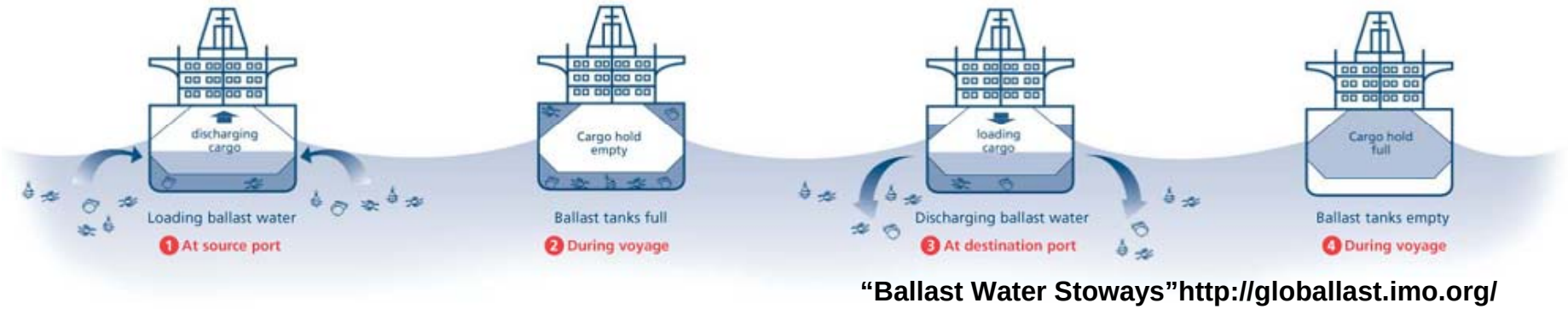
Cargo landing port = Uptaking Ballast water

Ballast water transfer alien species

→Natural ecosystem disturbance
→Economic loss
→Human illness ･･･ etc.

“Ballast Water Stoways”http://globallast.imo.org/

Ballast Water Problems



“Ballast Water Stoways”http://globallast.imo.org/

International maritime organization (IMO) adopted so-called

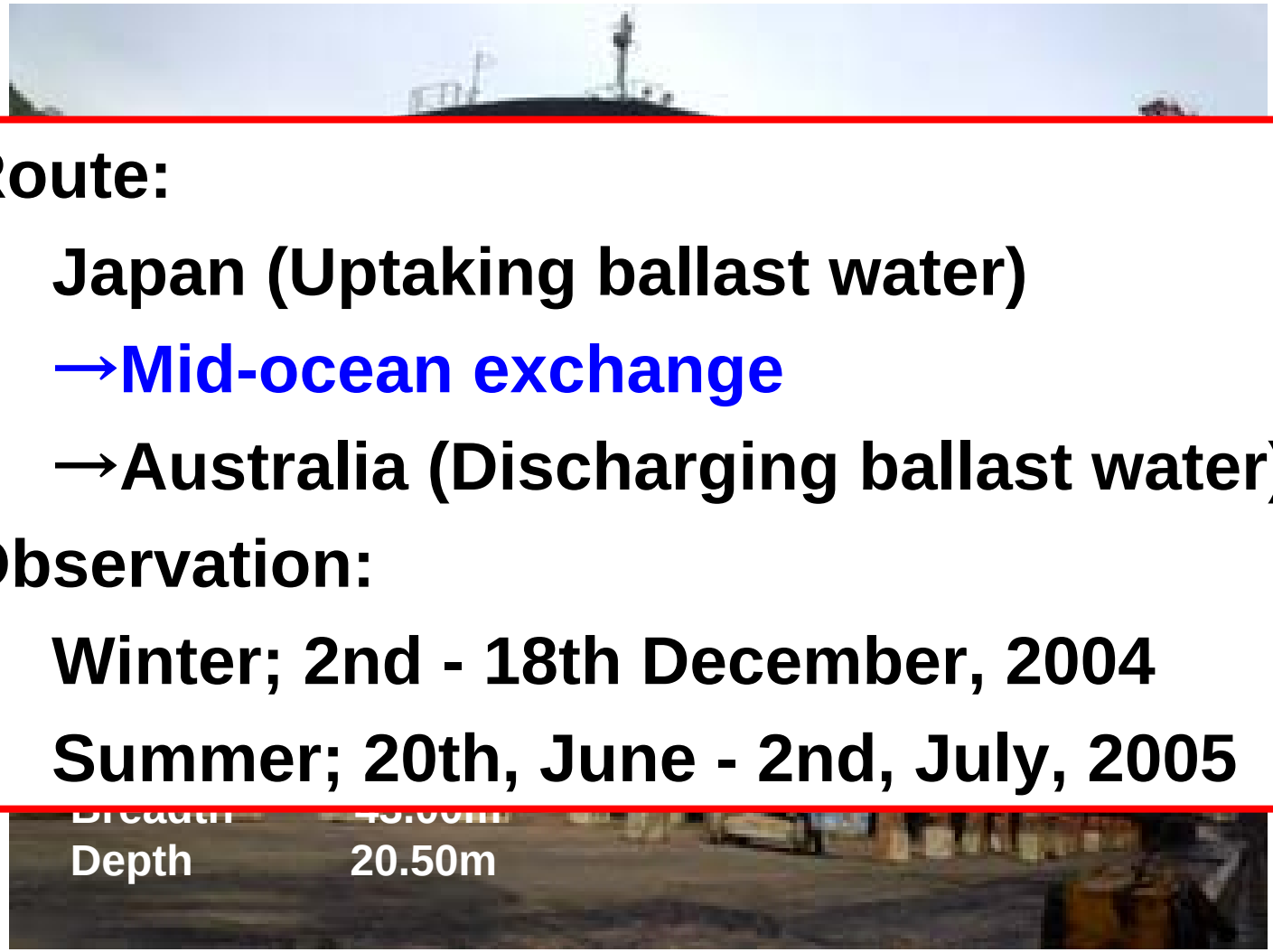
“Ballast water management convention”

(Feb. 2004)

Purpose of this study

To observe
the change of bacteria community
in ballast tanks before and after
the mid-ocean exchange,
and also during the voyage.

Voyages used for Observation



Route:

Japan (Uptaking ballast water)

→Mid-ocean exchange

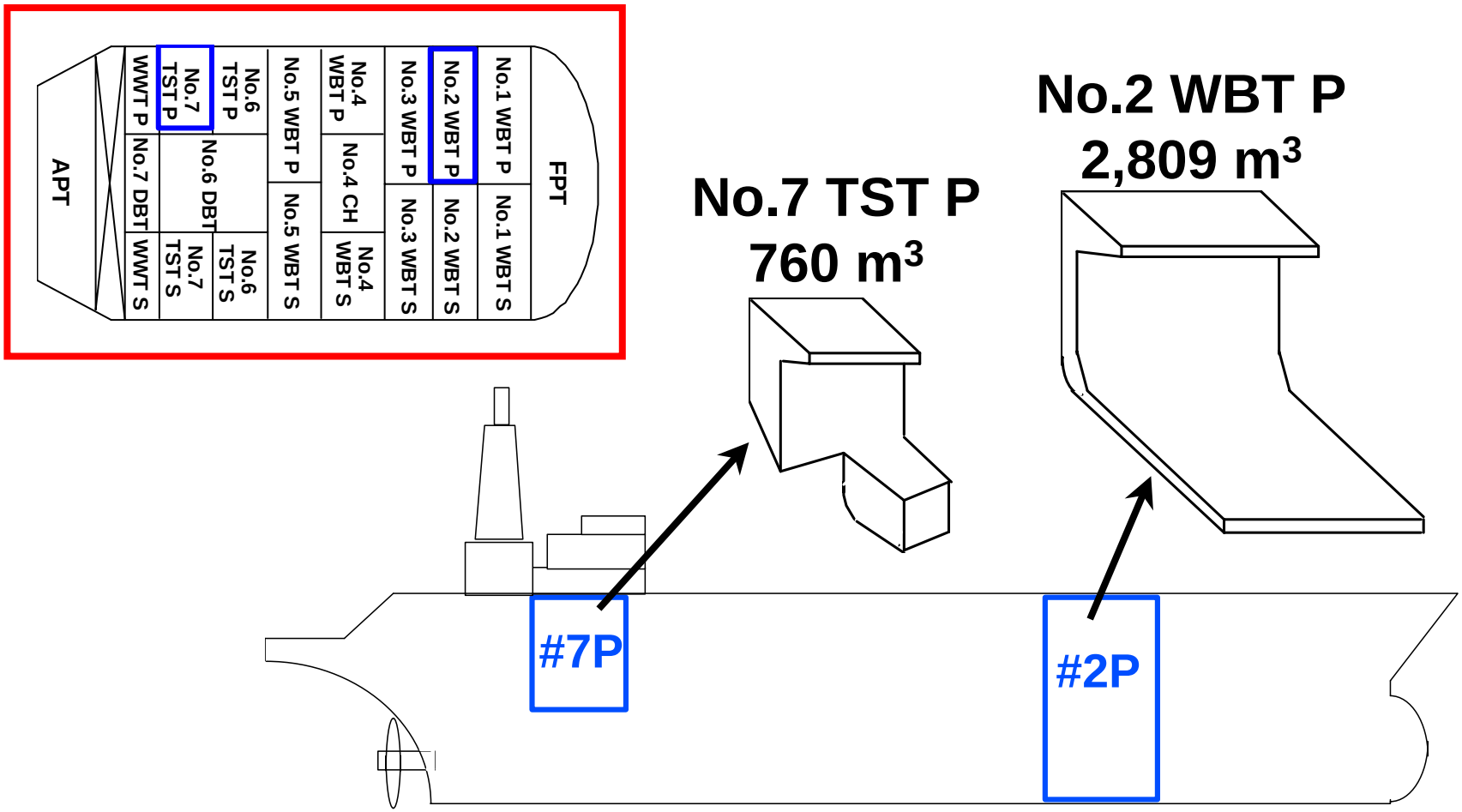
→Australia (Discharging ballast water)

Observation:

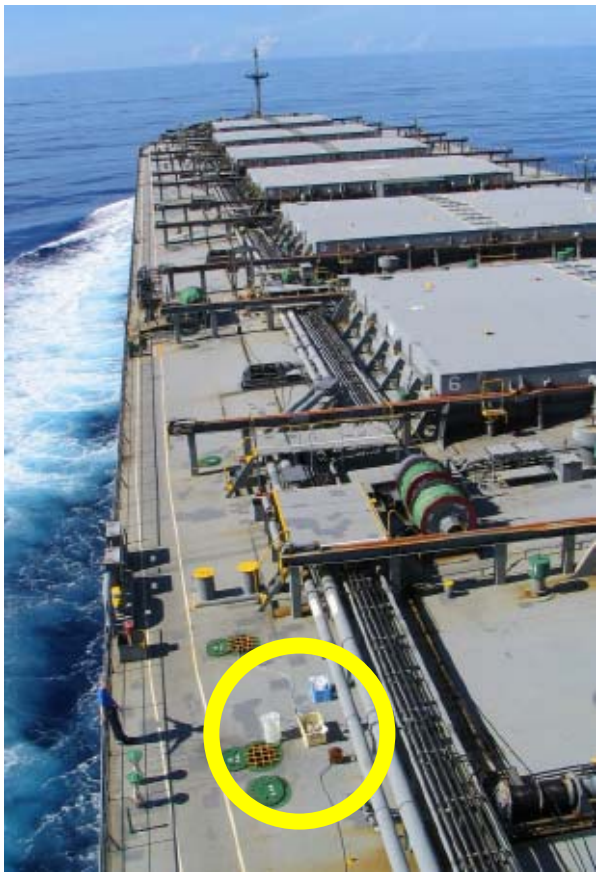
Winter; 2nd - 18th December, 2004

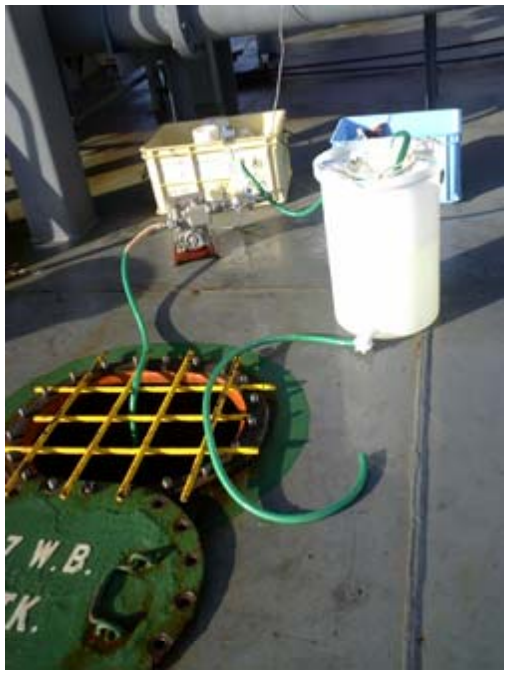
Summer; 20th, June - 2nd, July, 2005

Sampling from Ballast Tanks



Ballast Water Sampling





Surface
(Manhole)

Bottom
(Sounding pipe)

Quantitative

Direct count (Total count)
: No distinction between live and dead cells

Plate Count (Live count)
: Live cells

Qualitative

Denaturing Gradient Gel Electrophoresis (DGGE)
: All bacteria

Direct Count (Total count)

Preparation

Fixation, Stain

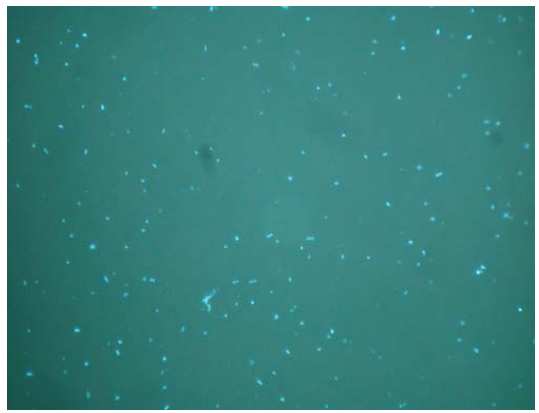
↓

Filtration

Keep on
-20° C

Count

Epifluorescence Microscopy



DAPI
x1000

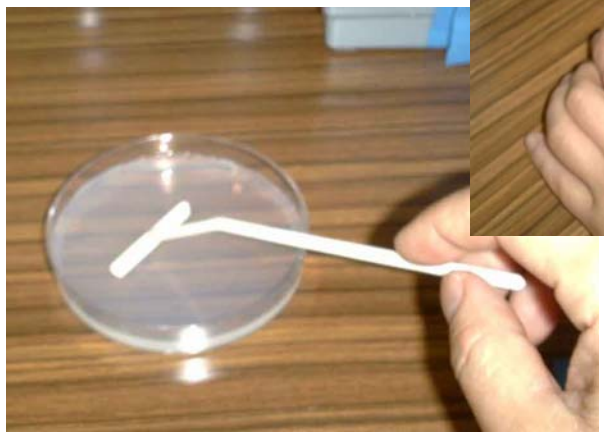
Bright blue-white cells

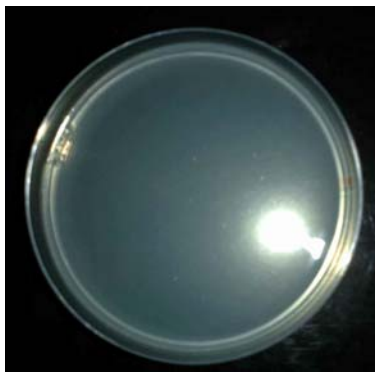
On board

Laboratory

Plate Count (Live count)

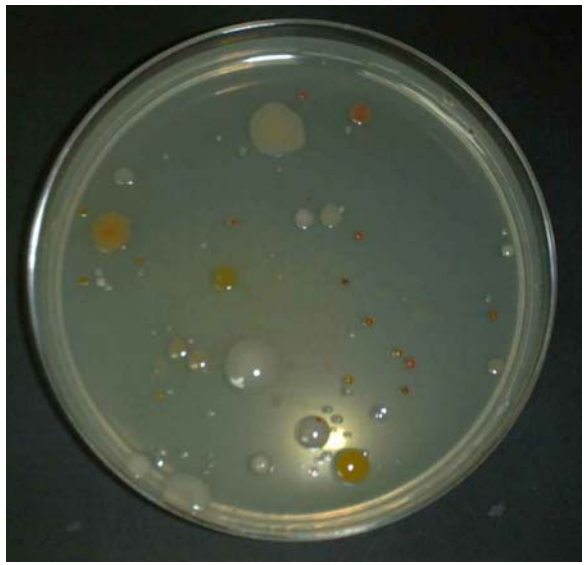
Inoculation





Culture:
Room temperature,
2days (non-selective)

Count

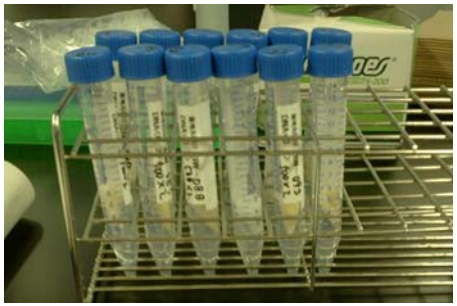


Marine Agar (Difco)

CFU = Colony forming unit

DGGE

(Denaturing Gradient Gel Electrophoresis)



Preparation (onboard)
5μm→0.2μm filtration
Keep under -20° C

DNA extraction

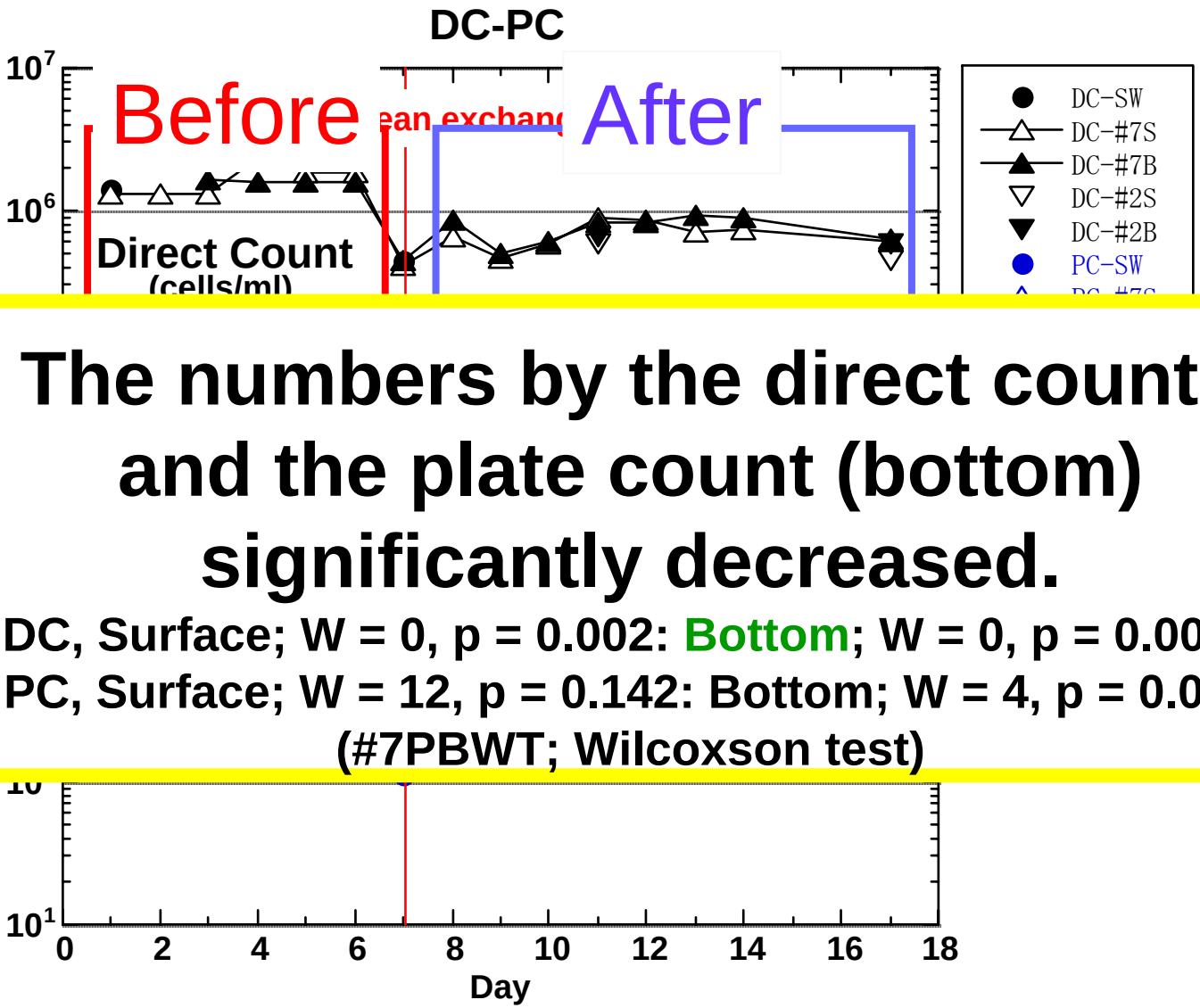
PCR amplify
Primer
with GC-richi sequenc
（F341GC,R907）

Electrophoresis
Denatured (urea and formamige)
polyacrylamide（acrylamide/bisacrlamide）gel

Visualization by Stain

Analysis of DGGE patterns

Bacteria Number - Winter



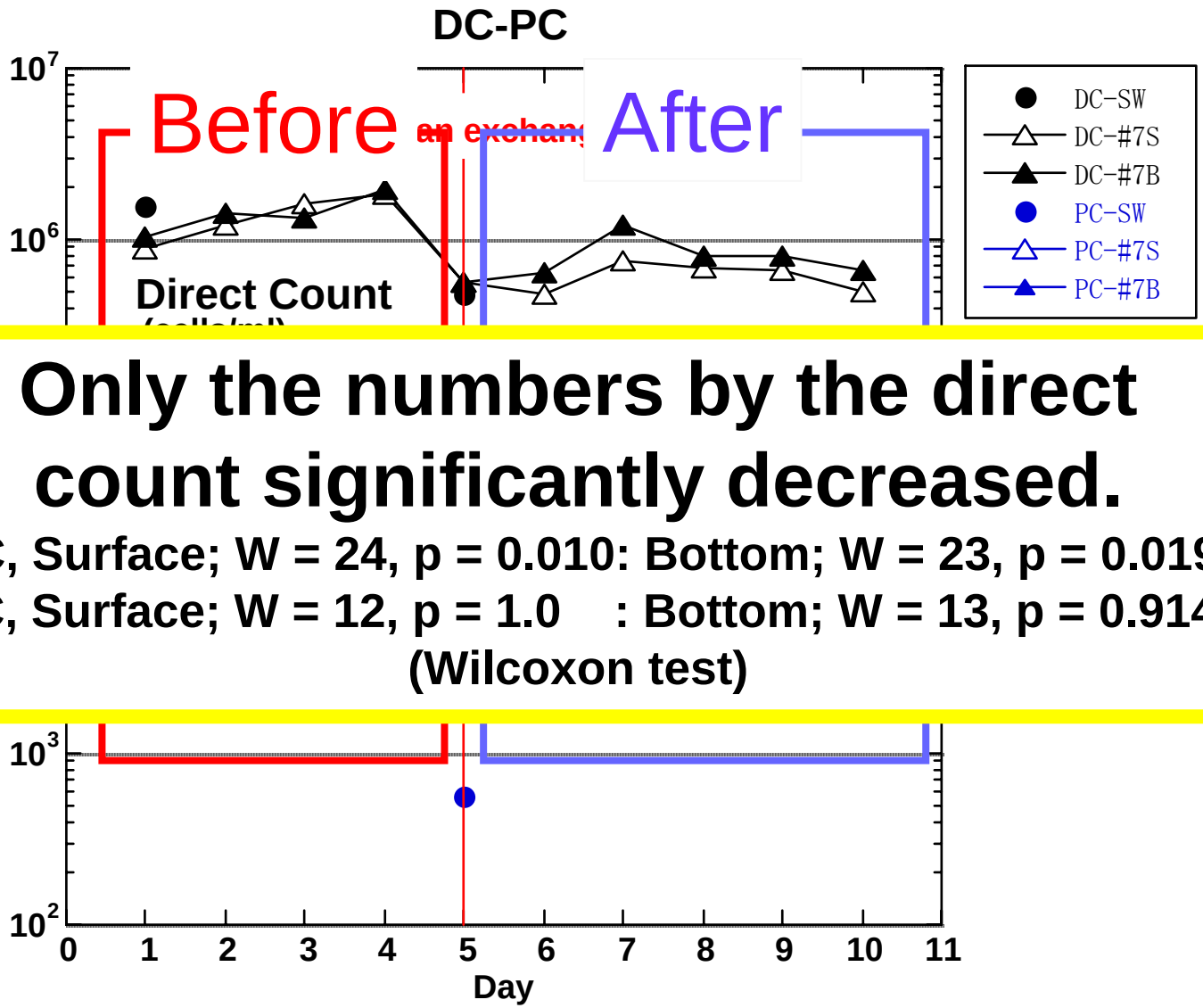
The numbers by the direct counts and the plate count (bottom) significantly decreased.

DC, Surface; W = 0, p = 0.002: Bottom; W = 0, p = 0.0007

PC, Surface; W = 12, p = 0.142: Bottom; W = 4, p = 0.048

(#7PBWT; Wilcoxson test)

Bacteria Number - Summer



Only the numbers by the direct count significantly decreased.

DC, Surface; W = 24, p = 0.010: Bottom; W = 23, p = 0.019

PC, Surface; W = 12, p = 1.0 : Bottom; W = 13, p = 0.914

(Wilcoxon test)

Part of regulation D-2

Regulation D-2 Ballast Water Performance Standard

- Indicator microbes, as a human health standard, shall include:

  .1 Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony forming unit (cfu) per 100 millilitres or less than 1 cfu 1 gram (wet weight) zooplankton samples;

  .2 *Escherichia coli* less than 250 cfu per 100 millilitres;

  .3 Intestinal Enterococci less than 100 cfu per 100 millilitres.

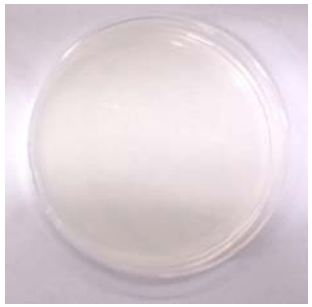
“Balast water management convention”

International Maritime Organization (IMO); February, 2004

Culture Media

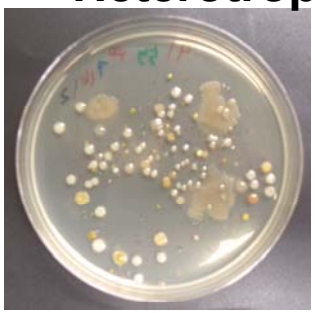
Non-selective Media

Marine Agar (Difco)



R2A (Difco)

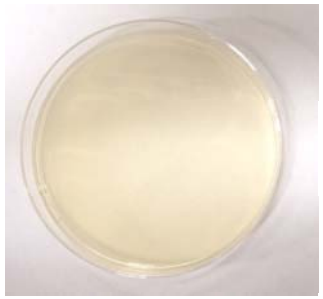
Heterotrophic bacteria



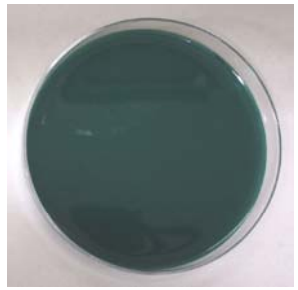
Marine bacteria

Fresh water bacteria

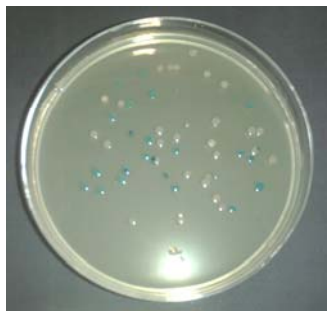
Selective Media



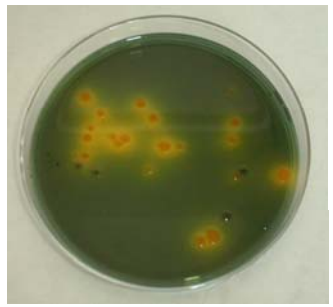
X-Gal Agar
(Nissui)



TCBS Agar
(Nissui)

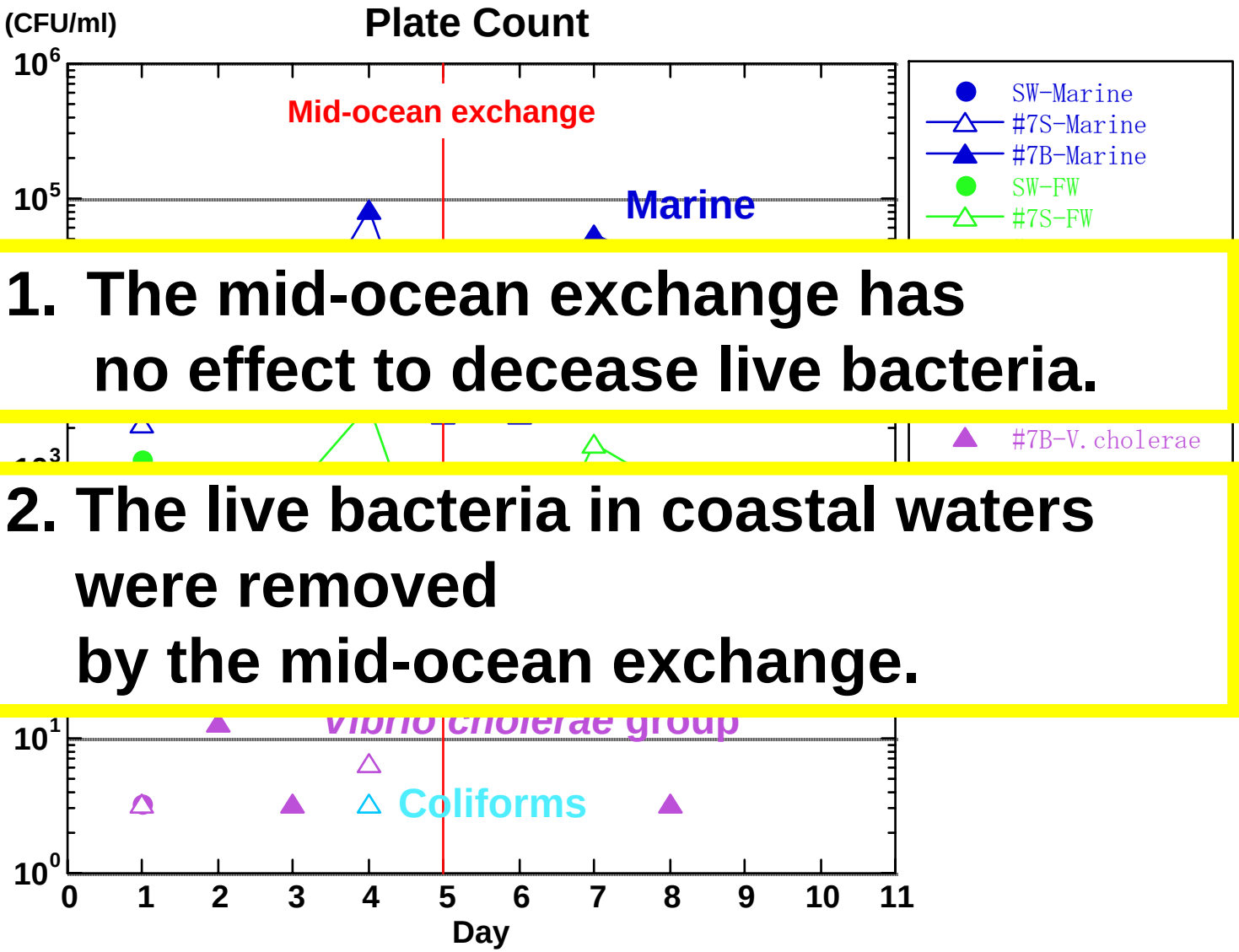


Coliforms
(Blue color colonies)



Group of
Vibrio cholerae
(Yellow color colonies)

Plate count (Live bacteria)
- Summer



1. The mid-ocean exchange has no effect to decease live bacteria.

2. The live bacteria in coastal waters were removed by the mid-ocean exchange.

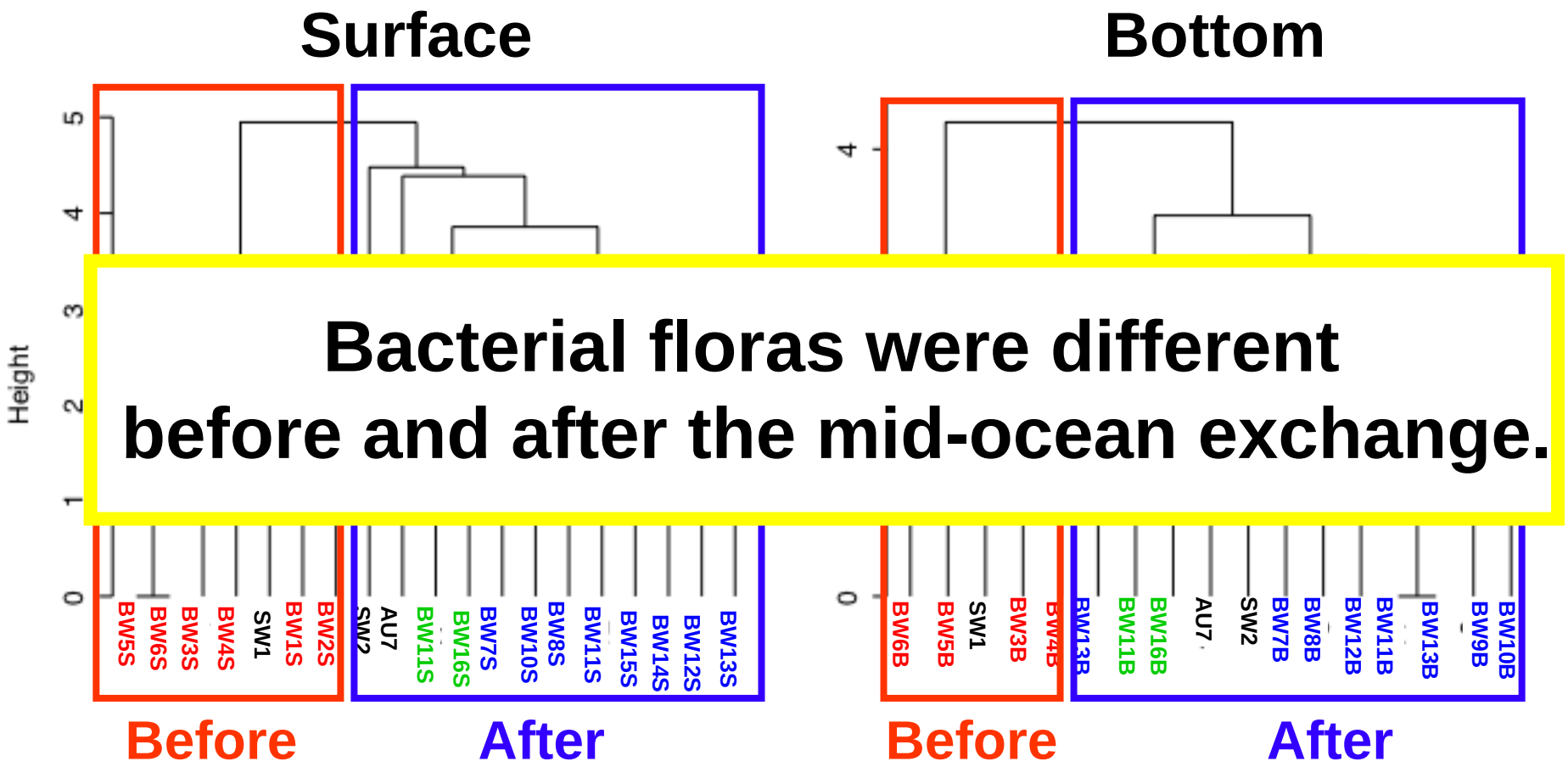
DGGE-Winter

- **SW (Black): Uptaking sea water sample;**
  #1, Japan port area; #2, Mid-ocean water.
- **BW: Ballast water; S, Surface; B, bottom;**
  #, days after charge;
  Red, Before the mid-ocean exchange (#7tank);
  Blue, After the mid-ocean exchange (#7tank);
  Green, After the mid-ocean exchange (#2tank).

- **Classification methods→Cluster Analysis**
- **Ordimation methods**
  →Nonmetric Multidimentional Scaliing（MDS）

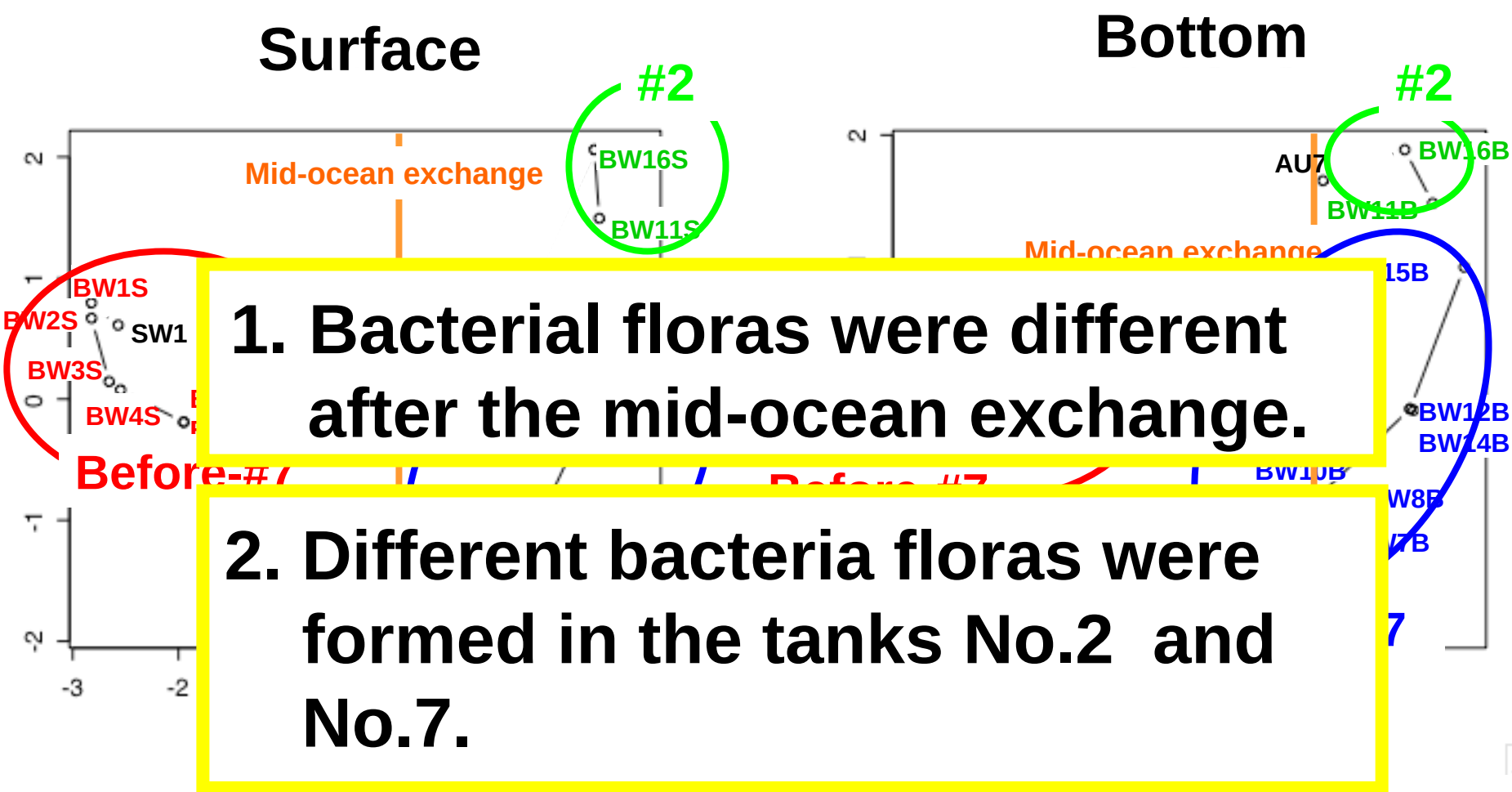
BW(surface&bottom) 050126: SW01, soma; SW02, ocean; S, surface; B, bottom; BW01-05, before exchanged ballast water; BW07-16, after exchanged ballast water; BW01-15, #7P BWT; BW16, #2P BWT; AU: Australia

Cluster Analysis - Winter



Bacterial floras were different before and after the mid-ocean exchange.

MDS - Winter



1. Bacterial floras were different after the mid-ocean exchange.

2. Different bacteria floras were formed in the tanks No.2 and No.7.

Summary

1. **After the mid-ocean exchange, total bacteria numbers significantly decreased.**

2. **Live bacteria number did not change significantly after the exchange, but live bacteria in the coastal waters were removed by the exchange.**

3. **Bacteria flora changed gradually in ballast tanks day by day, and became drastically different by the exchange.**