SURVIVAL OF BACTERIA AND PHYTOPLANKTON IN SHIP'S BALLASTS

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Billions of tons of ballast water are released world-wide every year. Living marine, brackish or freshwater organisms, including those noxious or unwanted, can travel by that way from a part of the world to another. A successful introduction needs: 1) survival during the pumping process. 2) survival in the ship's ballasts. 3) survival and reproduction in the new environment. To assess the survival conditions in ship's ballasts, a small scale pilot system has been built (MARTOB project), more realistic than laboratory vials and easier to handle than a real ballast. Bacteria and phytoflagellates coming from ship's sampling or from cultures were introduced in the system; their survival in several waters or sediments are studied, a useful method to assess the efficiency of further treatment processes at small scale and thus low cost.

1. Introduction

The most significant economic activities in coastal areas are the coastal fishing and aquaculture. Unwanted, noxious or pathogenic species introduced in these areas by ship's ballast waters and sediments release is a major threat for these activities: human or animal diseases, livestock and financial losses can affect entire regions.

A successful introduction of alien species supposes:
1) survival during pumping processes: this is the case for most of the small species, particularly phytoplankton and bacteria.
2) Survival in ship's ballast during travel or long storage of the ballast water (in the forepeak particularly): phytoplankton and bacteria producing cysts, are among the most fitted for survival in these apparently harsh conditions.
3) Successful setting in the new environment: again, phytoplankton and bacteria, due to mutation ability are generally the best fitted;

This is why it must be interesting to study their survival in the ballast environment. Working on this matter aboard a ship is difficult:

- Microbiologic experiments need a laboratory, hardly installed aboard a bulk or container or car carrier, as the samples taken regularly need to be cultivated at short notice.
- Phytoplankton introduced in ballast, generally tumble down unless some agitation by air lifting is organised.
- In real ballast, the huge size compels to put large volume of cultivated phytoplankton or bacteria to expect significant or at least usable samples.

This is why the work on medium or small scale pilot systems appears to be more convenient for the first trials.

2. Material and methods

2.1. The pilot scale system

Designed to carry studies on ballast environment conditions, the two tanks system has been built with naval grade steel, epoxy tar coated like a real ballast, and pivot mounted to simulate lurch if necessary. Internal framing provide areas for sedimentation (fig. 1).

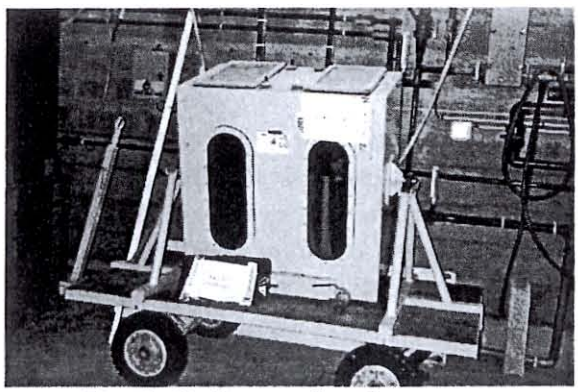

Fig. 1- Ballast scale pilot system.

2.2. Bacteria strain

To assess bacteria survival, a convenient cultivated strain is needed. Better than common E. coli, bacteria found in ship's ballasts seems more convenient. In this case, we used *Vibrio parahaemoliticus* discovered in water filling the hold (used as ballast tank) of a huge bulk carrier coming from South East Asia to La Rochelle (France).

The 1 litre (sterile bottle) sample was sent back to the lab at ambient temperature in thermal box (when looking for Vibrios, the cold storage must be avoided).

The typing has been done with selective medium (EPSA: Peptone Alkaline salted water), isolation on TCBS medium, subculturing on GNA and confirmation with Oxydase, salted Kigler media and API 20 E gallery.

Even the pathogenic characteristics (none here) have been checked by PCR. The isolated strain is since preserved in the lab on storage medium, sub cultured every 6 months. The culture for experiment is done on EPSA medium at 37 °c. The pilot scale tanks were filled with estuarine turbid water for the first experiment, then with bulk carrier ballast water for the second. The tanks were maintained in the dark. The water was previously analysed and if the ballast water contained some ubiquitous germs (as Pseudomonas), no *Vibrio parahaemoliticus* was found.

2.3. Phytoplankton

Waiting for a convenient natural phytoplankton bloom seeming quite difficult, we used a monospecific culture of Tetraselmis suecica (flagellate, Prasinophyceae).

This particularly resistant species was available in our research oyster hatchery, cultivated in 300 l Fiberglas culture vessels (fig. 2).

Fig. 2 - Phytoplankton culture vessels.

Ten litres were poured in each tank (already filled with 40µ filtered seawater) of the pilot scale system, and to avoid the sedimentation of the cells, stirring by air diffusion was maintained all the time.

One of the tanks was maintained in the dark, like a ship's ballast tank, the other one, acting as a control was well lit by neon light during the experiment, just like the culture vessels.

The first cell count was done in the bucket before pouring it in the tanks, using an image processing system (Samba technologies, France).

Every day, a one litre sample was taken in each tank, filled at different depth from bottom to top for a good mixing.

Probably due to water stirring, a lot of suspended matters and particles was soon observed, rending the processing system useless (unable to separate cells and particles).

So, a sub sampling was done, then: two 10 ml microscope observation chambers were filled from each sample and the counting was done manually (and painfully) on microscope (x100) after three hours for sedimentation (Fig. 3).

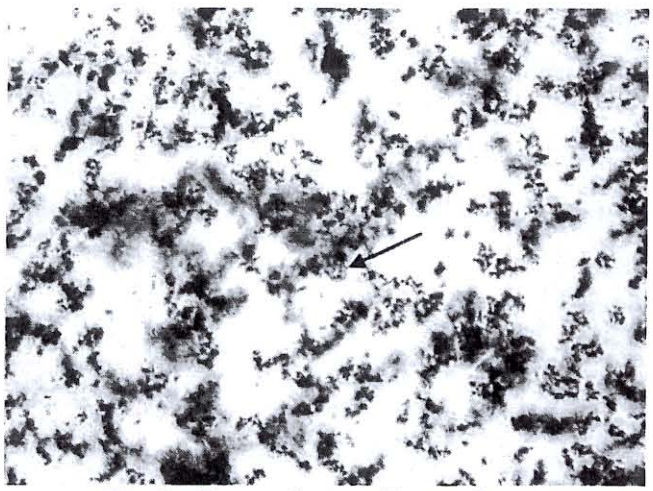

Fig 3 - Tetraselmis cell in sediment

The number of cells counted in the chambers was recalculated to a litre. This is the standard procedure used for the REPHY (french toxic phytoplankton watching network).

3. Results

3.1. Bacteria survival

The first experiment was done with water directly pumped from the Seudre estuary (25 km long) in close proximity of the station and used in the hatchery system (Fig. 3).

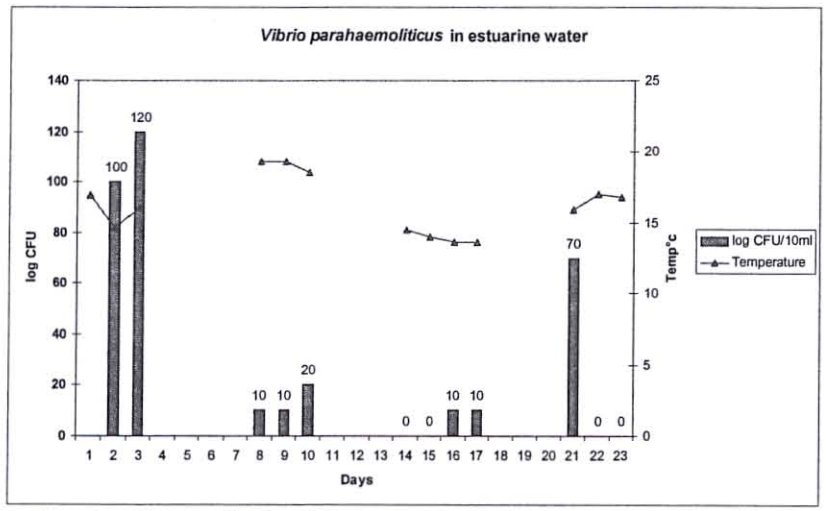

Fig. 4 - Survival of vibrio parahaemoliticus in estuarine water

After 24 days, V. parahaemoliticus has disappeared from the water. A month after, sampling in the sediment in the tank bottom let appear 1.3 CFU (colony forming unit)/ 100 ml: the germ is still present in the sediment.

For the second experiment, the pilot system was filled with real ballast water. This was done by taking double bottom ballast water aboard a bulk carrier coming from Pasajes (Spain), and containing a mixing of port and Gulf of Biscay water (Fig. 4).

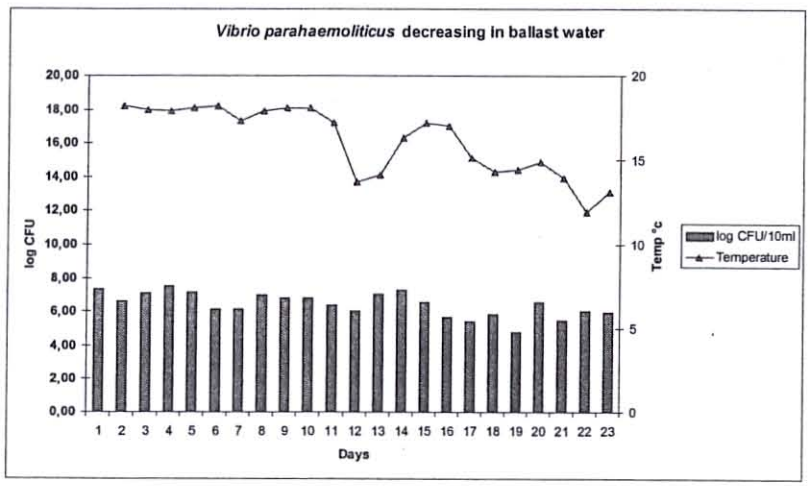

Fig.5 - Survival of Vibrio parahaemoliticus in ballast water

The decreasing is slow. After 45 days, the decreasing observed is only one log: 7.34 to 5.97 log CFU/100ml.

More important, the sediment is contaminated at the same rate than previous experiment on estuarine water (1.4* 10^7 CFU/ 100ml).

3.2. Phytoplankton

From a 2 ml sample taken in the 10 l bucket, 0.1 ml was set on a haemocytometer slide and counted with the image processing. We had 174 million cells/l, giving 6.69 million/l in the 250 litre tanks (just like a real bloom at sea). The cell number decreasing was observed daily during 6 days (Fig.6).

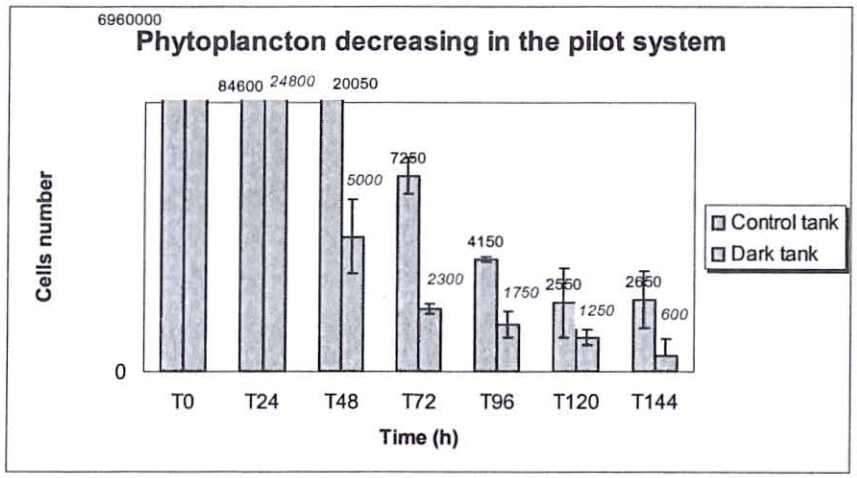

Fig. 6 - Tetraselmis suecica survival in pilot scale water

Even with air agitation, the number of cells decreases rapidly after 48 hours: 20 500 cell/l in control, 5000 cells/l in the dark. After 6 days, 2650cells/l remain in the control, versus 600 in the dark.

4. Discussion

4.1. Pilot scale

The use of this system as an alternate way for working aboard a ship presents some advantages:
- Manage the whole environmental conditions: temperature, salinity, dissolved oxygen, turbidity, light…,
- Start, stop and change the experiment conditions at any time,
- Handle reasonable volumes of water: from 250 l to several cubic metres (mesocosmic size), far from the thousand cubic metres of a real ballast;
- Spare money and time in prototype design and trials.

The main drawback is obviously the artificial situation, and especially for treatment assessment, further experiments must be carried aboard a ship.

4.2. Bacteria

The Vibrio parahaemoliticus survival has already been presented (see MARTOB subtask 4 report), but the comparison with phytoplankton is interesting: both species used are widely present in the coastal waters, and although non pathogenic, can be very similar in behaviour with pathogenic species or strains; The experiment with real pathogens (V. cholerae for example), far more complicated considering the danger, must be preceded by a work on non pathogenic similar species or strains.

4.3. Phytoplankton

The same remark applies to phytoplankton: we intend to carry a further work on some well known toxic dinoflagellates, as Alexandrium tamarense (this species being cultivated in some laboratories), including the cysts research in the sediments.

The Tetraselmis species used here is well known for its survival ability, even on the tiles of the culture room floor...The only difference between the pilot scale and the culture vessel was the lack of carbon dioxide bubbling, leading perhaps the cells to adhere on the walls of the pilot system. The experiment didn't last long due to technical considerations, but finding still 600 cells/l after six days in the dark suggest that the environmental conditions in the ballasts are not so harsh, after all...

5. Conclusion

The use of pilot scale system simulating ship's ballast conditions is a sort of technical challenge to work as close as possible to the real ballast environment. The size effect must be considered but other characteristics are very similar: dark, rusted (even muddy) environment, with steel walls and frames providing sediments traps.

It seems difficult to put contaminated water in the pilot system straight from the ship's ballasts, not knowing the nature of the contamination before; this is why the use of a cultivated bacteria strain is highly preferable. Its survival even after two months in the sediment is a technical challenge for treatment methods.

The same remark applies to phytoplankton flagellates, with their ability to make cysts, and a further work will study this possibility, with existing techniques.

6. Acknowledgements

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7. References

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