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Merchant ships discharging unwanted marine species in close proximity of a French aquaculture area: Risks involved

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ABSTRACT

The most important oyster farming area in Europe is in a close proximity of two medium size merchant ports. Cargo ships deballast in this area before loading, releasing unwanted or noxious marine species. During a sampling campaign aboard these arriving ships, we found in some ballast water samples a huge number of potentially toxic dinoflagellates and some potentially pathogenic bacteria. A model was applied to find the potential geographical spread of the discharged ballast water. This model predicts the water to reach highly vulnerable shellfish farmed areas in six to eight days.

1. Introduction

Ocean ships introducing invasive or noxious organisms in new areas by the way of released ballast water can have fatal effects both on coastal environment and public health (Carlton and Geller, 1993; Gollasch, 1998).

On the Atlantic coast of France, the port of La Rochelle imports more than 3 million tons and exports 2.3 million tons of goods per year, mainly cereals. Estimating 40% of loaded cargo as discharged volume, the total volume of ballast water released reach 920,000 m^3 per year in or around the port itself (Fig. 1). The BWDA model (David et al., 2012) give an estimation of 33% of cargo loaded; 40% is the estimate given to us by numerous ship masters.

The most important oyster farming area in Europe is located several miles to the South of La Rochelle, between Oleron island and the coast: 25000 tons of oysters are produced here annually, employing circa 6000 people with approximately 270 million € turnover at maximum capacity. A large mussel cultivation area is situated to the North of the port. Cultivation practices for blue mussels comprise pole cultures ("bouchots") or long lines, producing circa 10,000 tons and employing around 500 people (approx. 30 million € turnover; all these economic data in: Anonymous, 2005). Therefore, these areas are particularly vulnerable to bacterial pollution or toxic phytoplankton blooms, which result in more and more, frequent closure of production areas (sales prohibitions), with bad economic consequences for this sector, not to mention public health problems when monitoring programs fail to detect them.

Two thirds of the ships arriving in La Rochelle for loading cargo are coming from Spanish and Portuguese coasts (Masson et al., 2000) where very frequent toxic algal blooms occur (Pazos et al., 1995), while others come from further away (South East Asia, America, Middle East), occasionally discharging ballast water containing exotic and noxious species (Masson et al., 2000, 2005).

To document this risk, several samples of ballast water were obtained in 2012 from ships arriving in the port for loading. As the main threats for aquaculture are pathogenic bacteria and toxin producing micro-algae, we focused on *Vibrio* genus for bacteria and potentially toxin producing phytoplankton species search.

2. Materials and methods

Sampling aboard arriving ships in France has become more and more difficult with the years, due to the reluctant and distant ship owners. The best way is to see the shipmaster at arrival and ask for permission. As the ship's name remains confidential, permission to sample is generally granted.

2.1. Ships

Access given to the ships arrivals through the port's database, the ships interesting to sample were chosen considering the last port (Iberian peninsula, North Africa, or more remote), the cargo (preferably huge quantity of cereals aboard bulk carriers) and the more convenient time for sampling (when the ship is loading). In 2010, 17 ships, in 2011, 12 ships and in 2012, only 7 ships were sampled.

2.2. Sampling

The sampling was generally carried in engine room by starting a ballast pump and opening the checking valve (David and Percovic,

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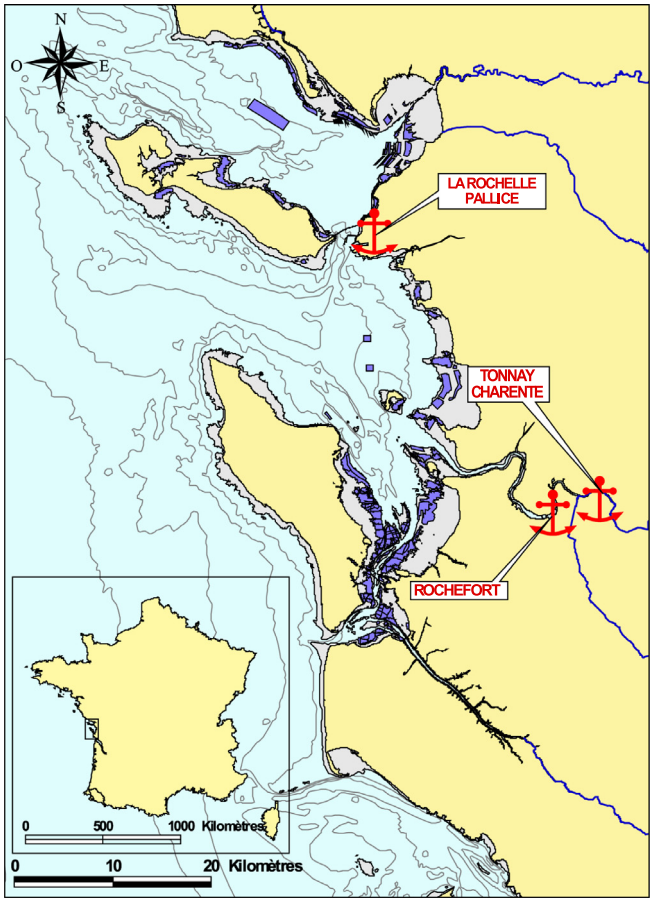

Fig. 1. Mer des Pertuis area with Marennes–Oléron area (South) Baie de l'Aiguillon (North) aquaculture areas (grey) (In gray) and the merchant ports (î).

2004) after ten minutes. Ten litres for phytoplankton and two litres (in sterile bottles) for bacteria research were taken in this case. These volumes seemed the most appropriate, according to David et al. (2007) Samples were always filtered (outside ship or in laboratory) at 20 µm and lugol preserved when not immediately observed (see discussion below).

2.3. Conditioning

The samples (after recording temperature, salinity and water origin if known) were put in a black thermal box and driven to the laboratory 10 min away.

2.4. Bacteria

The genus *Vibrio* was specifically researched (after enrichment during 6 or 18 h at 41 °C) with TCBS and ChromAgar Vibrio media; suspicious colonies and some isolated strains were identified with API 20 E galleries. Then, nucleic acids have been extracted and genetic amplification has been applied to detect the potentially pathogenic strains.

Vibrio parahaemolyticus (*toxR* gene), *Vibrio vulnificus* (vvhA gene) and *Vibrio cholerae* (16S-23S RNA intergenic space) have been researched by PCR on nucleic acids in enrichment media and strains (Hervio-Heath et al., 2002; Deter et al., 2010).

2.5. Phytoplankton

Ten millilitre sub samples were put in observation chamber, let 3 h for sedimentation then observed by inverted microscope (10 ml sedimentation chamber, Utermöl method). The count led to the estimate number of cells per litre. This procedure is consistent with the French phytoplankton-monitoring programme REPHY (Sournia, 1978).

Only exotic (non indigenous) or known producing toxins species were determined and counted. Among them, special attention was given to dinoflagellates and Pseudonitzschia genera, the later in two groups: large (*seriata* complex) or thin (*delicatissima* complex).

3. Results

3.1. Phytoplankton (Table 1)

In 2010 no potential harmful species were detected in the 17 samples taken outside ships at openings in hull during ballast discharges.

In the 12 samples taken in 2011, 11 contained *Pseudonitzschia* sp. And only 4 contained *Dinophysis*, mainly *D. sacculus.*

In 2012, *Pseudonitzschia* was present in 4 of the 7 samples. On the other hand, the various *Dinophysis* species (particularly those known as toxin producing like *D. acuminata* and *D. sacculus*) were present in almost all the samples.

The most interesting results are the unusually high number (more than 200,000 cells/l) of *Dinophysis* (*sacculus, acuminata, caudata, acuta*) found in the ships coming from Pasajes and La Coruña on April 13 and 16 2012, respectively. Some sampling campaigns had been carried previously aboard ships arriving in French ports (Masson et al., 2000). We never observed before such a quantity of cells, coming undoubtedly by pumping in a bloom at (or close to) the previous port(s).

3.2. Bacteria (Table 1)

Some potentially pathogenic bacteria were also found: in the MA4 ship, coming from Algeria, *Vibrio parahaemolyticus* (in enrichment broth, *toxR* gene) and *V. vulnificus* (in enrichment broth and strains, *vvhA* gene) are present. On MA3 ship, (from Amsterdam), a 16S 23S RNA of *V. cholerae* was found by PCR in enrichment broth, without any certainty about pathogenic character, as non-O1 neither O 139. In MA2 ship from La Coruña, *V. parahaemolyticus* was detected (in enrichment broth, *toxR* gene).

4. Discussion

4.1. Sampling

The sampling was done through ballast pump checking valves, ten minutes after the start of the pump in order to avoid a possible bias (more concentrated sample at the beginning, as suggested by Gollasch and David, 2011), when the pumps had not already been on.

In most cases, though, the ship was loading cargo and releasing ballast water at the same time, the pumps running since at least 1 h.

As we had generally a limited time aboard, only 10 l for phytoplankton and 2 l for bacteria research were taken. These volumes had been found the most appropriate, as also retained by David et al. (2007) all the samples came from double bottom ballast tanks.

The close proximity of port and laboratory allowed to filter (phytoplankton) and to begin the cultures (bacteria) at short notice.