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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.

### **Highlights**

► A sampling campaign has been carried on ship's ballast water in La Rochelle port ► The area is heavily shellfish farmed and thus highly vulnerable ► Some samples contained large number of potentially toxic dinoflagellates ► Mathematical model predicted the spreading of this released water to shellfish areas

**Keywords :** Ballast water ; Risk assessment ; Toxin producing dinoflagellates ; Shellfish farming ; La Rochelle ; Pathogenic bacteria

## 1. Introduction

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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 et al., 1993; Gollasch et al., 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<sup>3</sup> 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.

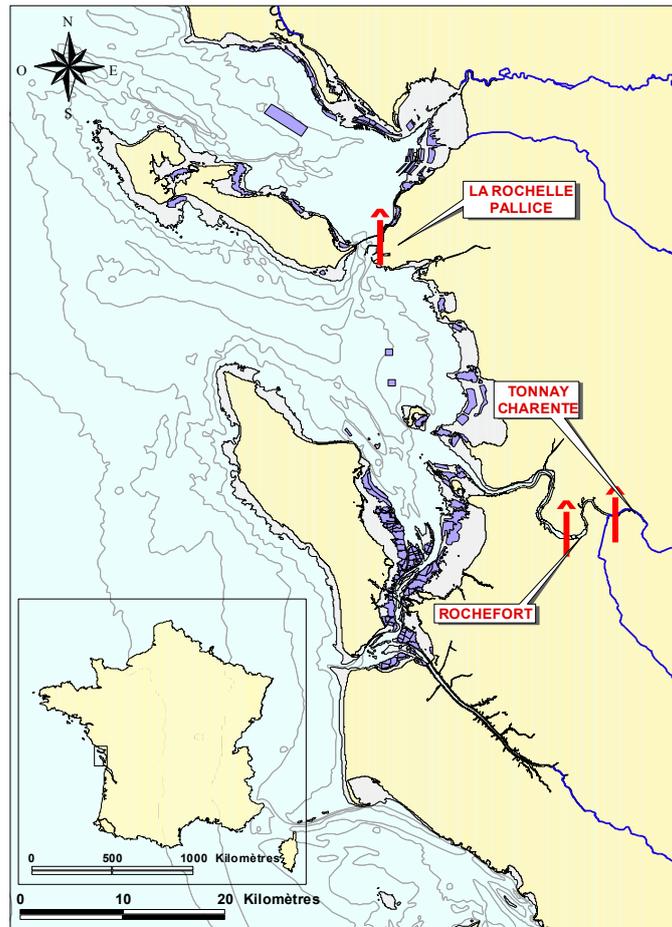


Fig 1: Mer des Pertuis area with Marennes –Oléron area (South) Baie de l'Aiguillon (North) aquaculture areas (grey) (In gray) and the merchant ports (î)

## 2. Material 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 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 minutes 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 hours 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 ed., 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

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### 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/litre) 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 (Table1)

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 one hour.

Table I: sampling campaign in 2012

Ship code, Length, Dead Weight Tonnage	Coming from:	Sampling date	T°C S‰	Phytoplankton observation after 10µm filtration and concentration in NaCl 35 ‰ solution	Vibrio research on TCBS, ChromAgar Vibrio media and API20E gallery identification
MA1 180m DWT: 33500t	<b>Bejaia (Algeria)</b>	28/03/2012	15.5°C 36.2 ‰	<b>Diatoms:</b> <i>Pseudo-nitzschia sp</i> larges. (seriata complex) <b>Dinoflagellates:</b> <i>Dinophysis</i> ( <i>sacculus</i> , <i>acuminata</i> ,) <i>Ceratium</i> , <i>Prorocentrum micans</i> , <i>Proto-peridinium spp</i>	<b><i>Chromobacterium violaceum</i></b> , pathogenic and opportunist germ in tropical et subtropical areas
SP1 99m DWT: 5750t	<b>Port of Pasajes (Spain)</b>	13/04/2012	15°C 34.6 ‰	<b>Diatoms:</b> <i>Pseudo-nitzschia sp</i> larges. (seriata complex): 618 cells / litre <b>Dinoflagellates:</b> numerous <i>Dinophysis</i> ( <i>sacculus</i> , <i>acuminata</i> , <i>caudata</i> , <i>acuta</i> ) and <b><i>Phalacroma</i>: 204 443 cells / litre</b> , numerous <i>Ceratium</i> ( <i>fuscus</i> , <i>furca</i> , <i>macroceros</i> , <i>lineatum</i> ), <i>Proto-peridinium spp</i>	No Vibrio researched (too late for beginning a culture)
MA2 125m DWT: 9822t	<b>La Coruña (Spain)</b>	16/04/2012	34.4 ‰	<b>Diatoms:</b> <i>Pseudo-nitzschia sp</i> larges. (seriata complex) in small quantity <b>Dinoflagellates:</b> numerous <i>Dinophysis</i> ( <i>sacculus</i> , <i>acuminata</i> ) and <b><i>Phalacroma</i>: 102 770 cells / litre</b> , numerous <i>Ceratium</i> ( <i>fuscus</i> , <i>furca</i> , <i>macroceros</i> , <i>tripos</i> ), <i>Proto-peridinium spp</i> , <i>Prorocentrum micans</i> .	<b><i>Vibrio vulnificus</i></b> <i>Shewanella putrefaciens</i>

AN2 107m DWT: 6918t	<b>Las Palmas (Canarias)</b>	16/04/2012	36.6 ‰	<b>Diatoms:</b> <i>Pseudo-nitzschia</i> sp larges. (seriata complex), <i>Coscinodiscus</i> , <i>Paralia sulcata</i> , <i>Grammatophora</i> <b>Dinoflagellates:</b> <i>Dinophysis</i> ( <i>sacculus</i> ), <i>Phalacroma</i> , <i>Ceratium</i> ( <i>fusus</i> , <i>furca</i> , <i>macroceros</i> , <i>tripos</i> ), <i>Protopteridinium</i> spp., <i>Prorocentrum micans</i> .	Nothing found
MA3 125m DWT: 9561t	<b>Amsterdam</b>	23/05/2012	15.8 °C 8.2 ‰	Numerous skeleton shaped dinoflagellates (Ebriales) Not known as toxic. Can be present in Atlantic and Channel under 17-18°C	<i>Aeromonas hydrophila</i> <i>Klebsiella terrigena</i> (not surprising with 8.2‰ salinity)
GE1 89m DWT: 4270t	<b>Bilbao (Spain)</b>	14/06/2012	18.6 °C 33.3 ‰	<b>Diatoms:</b> <i>Pseudo-nitzschia</i> larges ( <i>seriata</i> complex) in huge quantity: <b>144 246 cells / litre</b> ballast water <b>Dinoflagellates:</b> numerous <i>Dinophysis</i> ( <i>sacculus</i> , <i>caudata</i> , <i>fortii</i> ) and <b>Phalacroma:</b> <b>5800 <i>Dinophysis</i> sp /litre</b> ballast water	No <i>Vibrio</i> researched (too late for beginning a culture)
MA4 142m DWT: 13971t	<b>Oran (Algeria)</b>	03/07/2012	20.4 °C 35.6 ‰	<b>Diatoms:</b> very few diatoms <b>Dinoflagellates:</b> numerous <i>Dinophysis</i> ( <i>sacculus</i> ): <b>1 000 <i>Dinophysis</i> sp / litre</b> ballast water	<b><i>Vibrio vulnificus</i></b> <b><i>Vibrio mimicus</i></b> (gastro entéritis) Possibly <b><i>Vibrio parahaemolyticus</i></b>

As we had generally a limited time aboard, only 10 litres for phytoplankton and 2 litres 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.

#### 4.2. Phytoplankton results

As a total flora determination and counting was time consuming and not relevant for risk assessment, we focused on known or potentially toxin producing species.

The huge quantities of *Dinophysis* sp found the April 13 and 16/ 2012 in ballast water taken in Pasajes and La Coruña respectively, is demonstrative of the ability to survive a short travel ( Zvyagintsev et al., 2009) the cells being still alive even after the passage in high pressure areas in the pumps. As we know Galicia, Cantabria (B.Reguera, pers. com.) and southwest French coasts (up to Arcachon oyster farming area, 200 Km north of Spanish border) were plagued by toxin producing dinoflagellate blooms at the same time, these ships pumped obviously their ballast water in blooms, in the last departure port or along the neighbouring coast. This is almost certain in the case of Passajes.

We were unable to measure the toxin level in these samples, but the toxicity of *Dinophysis* is highly probable, the Arcachon oyster producing area was closed for toxicity over regular threshold at that time, and also in most of the North West Spanish aquaculture areas too (Seoane et al., 2012).

### 4.3. Bacteria results

We choose to watch for the *Vibrio* genus only: although *Vibrio cholerae* (var O 1 and O 139) is specifically designated in the Ballast Water Management Convention, the interest in *V.parahaemolyticus*, *V.alginolyticus* and *V. vulnificus*, equally noxious for public health (sea food disease or skin illness) make sense (Hervio et al., 2002; Mc Carthy et al., 1994).

The results were not obvious, with the exception of *V.vulnificus* found in “la Coruña” sample, and *C. violaceum* in “Bejaïa” (Algeria) waters.

None of them seems pathogenic this time.

Further studies on more numerous samples should be done to have a good idea of the risk involved (in sea food contamination particularly), perhaps looking for heterotrophic and sanitary indicative bacteria, as in Zvyagintsev et al. (2010)

### 4.4. Risk assessment:

The ships coming to ports of La Rochelle or Rochefort (some 20 miles south, in the Charente river estuary) are waiting the appropriate tide level or a place along a quay, in an assigned area between the isles of Ré and Oleron. As the place is well protected, number of the moored ships uses this area to discharge their ballast.

The GPS coordinates of a ship moored in this area and which had been discharging were registered and used further in a MARS 2D mathematical model to assess the released water trajectory.

The ship coming from La Coruña loaded 5000 tons of wheat. The reasonable estimate for the subsequent discharged volume is 40% of the dry goods loaded (and 30% for liquids), then 2000 m<sup>3</sup> in this case.

The parameters used for a theoretical 2000 m<sup>3</sup> release permitted to describe the trajectory of diluted water and suspended matters too (Fig.2)

This model is more simple than 3D model used by Brickman et al. (2007) on Easter Canadian coast but in a far smaller (and a shallow) area.

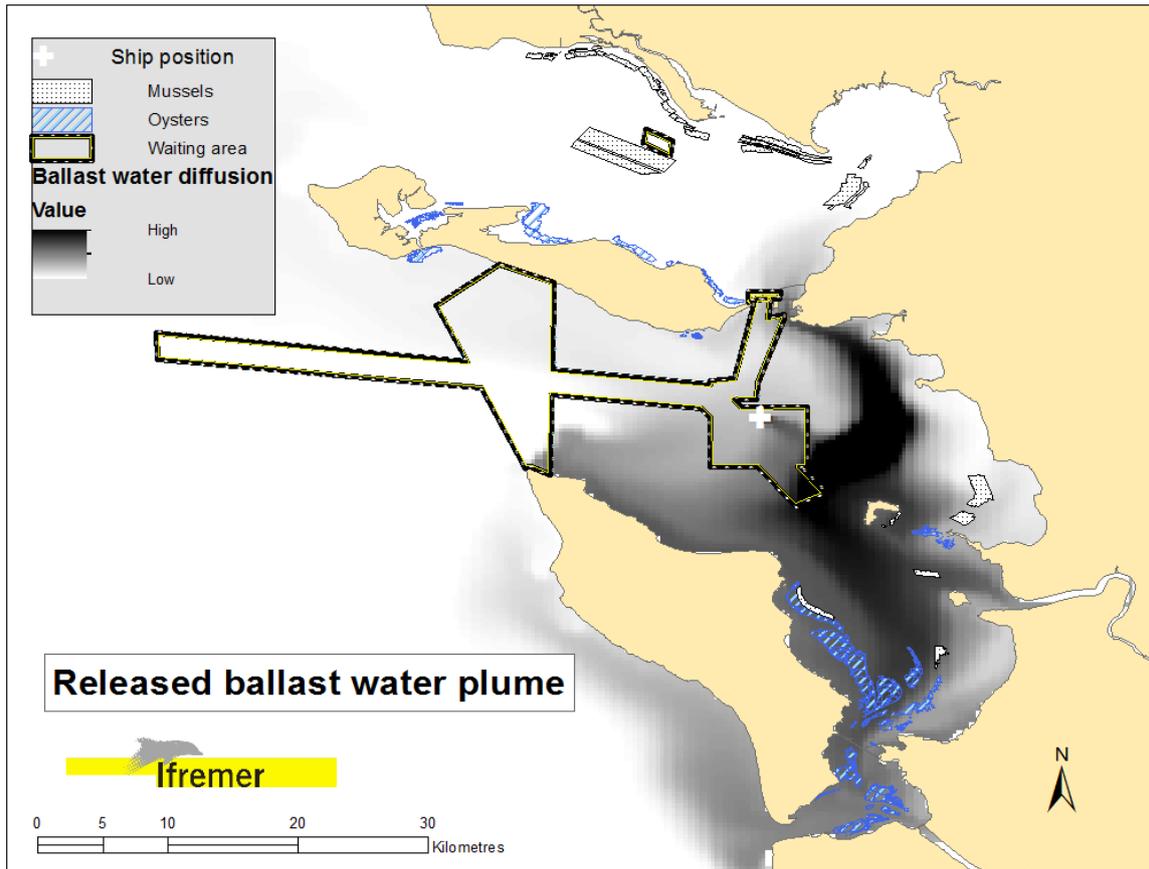


Fig 2: Spreading of ballast water from waiting area

The results are particularly worrying: in an 8 days time, the released waters reach the oyster beds and mussel longlines or mussel poles of Marennes-Oléron area. Any unwanted organism carried in these waters is spread away in this area.

In the April 13. 2012 example, we can estimate the number of *Dinophysis* cells spread in this area up to several billions, even if a sample bias is taken into account. As the statistical organisms distribution can most probably be described by a negative binomial function (organisms not dispersed at random but in aggregates, as suggested in Wright, 2012), the estimated number seems reasonable enough.

Some ships are discharging at the port entry (Fig.3). In this case, we can observe a different spreading: the released waters go North, and in the same amount of time spread on the mussel poles and mussel longlines in the Baie de l'Aiguillon and in the straight of Pertuis Breton respectively, very important mussel production areas.

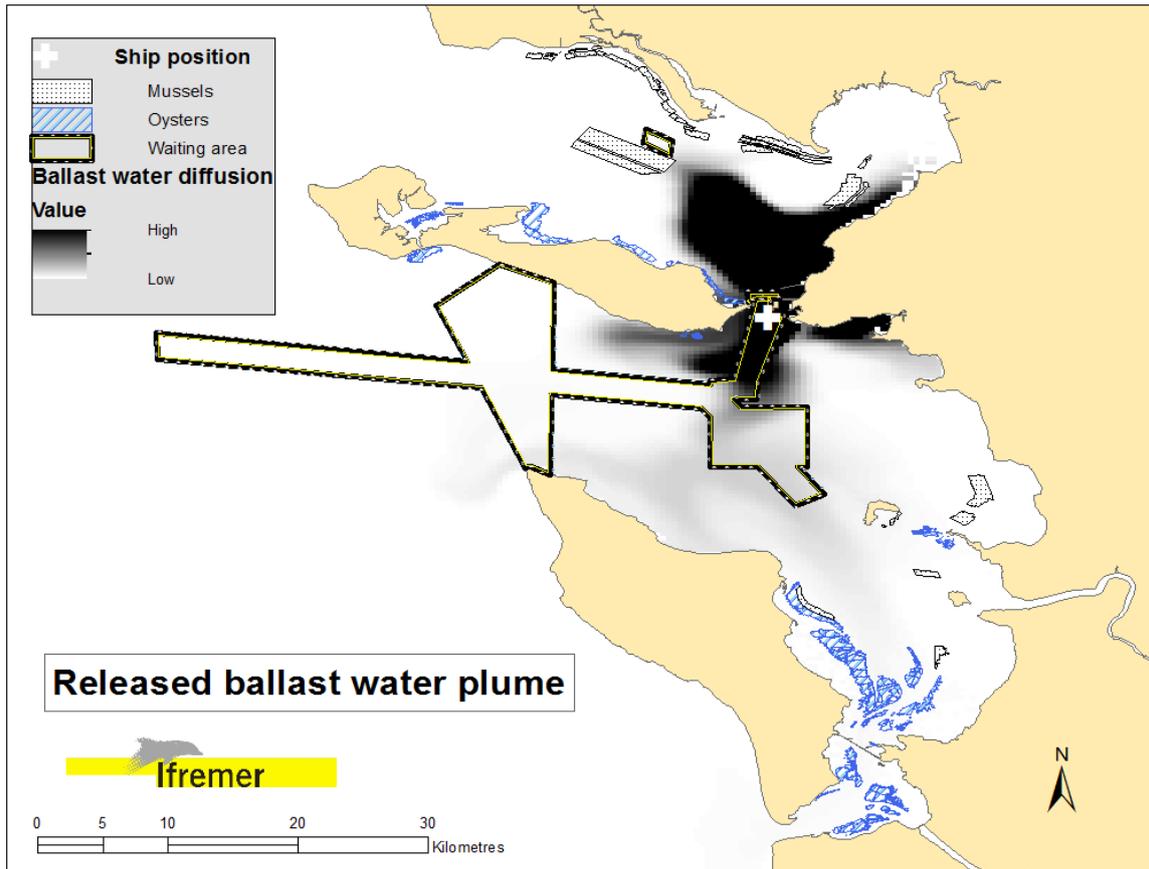


Fig.3: Spreading of ballast water from port entrance

Although we do not have definite and documented proof of noxious organisms introductions by ship's ballast waters in France, some cases are questioning:

- Toxin producing *Alexandrium catenella* appeared for the first time in the Thau lagoon area on Mediterranean coast, in 1993(Lilly et al., 2002): a bloom in the eastern part of the lagoon led to the temporary prohibition of oyster's sales in the area. Since then, several blooms have occurred with the same consequences. Genetic sequencing (ibid) gave an Asiatic origin of this strain; as the first blooming area in the lagoon was in close proximity of the channel connected with the merchant port, the introduction by ship's ballast water is highly probable.
- After the Xynthia storm (2010), a *Pseudo-nitzschia australis* toxin producing bloom occurred in the Baie de l'Aiguillon area (Nezan et al., 2010), probably induced by the post-storm environmental conditions (waters returning from flooded agricultural land) This species which have never been observed here before, occurred for the first time in Brest (Nezan et al. 2010) The same little bulk carriers coming from Spain come regularly in Brest and La Rochelle areas, probably introducing (and spreading) this species.

On April 2012, this release of several billions of *Dinophysis* cells in Marennes Oléron area (maximum estimate: 200 million cells/m<sup>3</sup>, 2000 m<sup>3</sup> release in 1 billion m<sup>3</sup>, high tide, 120 coefficient) didn't start a bloom. But we can foresee occurrence of some other noxious species like *Alexandrium* or *Heterosigma*, already found in ship's ballasts in La Rochelle (Masson et al., 2000) Cysts production could lead to subsequent blooms there in the next years, may the environmental conditions become favourable. This way of introduction has been well described by Hallegraeff et al. (1991)

## 5. General conclusion

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These few samplings contain numerous potentially toxin producing phytoplankton species. For the first time in France, we found samples of ballast water obviously pumped in a potentially toxic bloom. A simulation of this discharge trajectory predicts a spreading on oyster and mussel farming areas and can be seen as an unacceptable risk by the shellfish farmers.

This far from exhaustive study was carried to document the risk involved by ship's ballast water discharging in this specific area. The most important oyster producing area in Europe, close to an important mussel farming activity, all highly vulnerable to toxin producing blooms and pathogenic bacteria contamination, deserved a particular assessment, intended first for the port authorities awareness.

Other aquaculture areas in France are in close proximity of merchant ports (around Loire estuary or in the bay of Seine for example), with the same risk. More studies should be necessary for the authorities general awareness, before entry in force of the Ballast Water Management Convention and its subsequent regulation constraints (risk management, training for enforcement procedures)

The French REPHY monitoring programme running since 1983 is particularly efficient to detect toxin-producing phytoplankton, non-indigenous species included. But this new parameter (ballast water discharge) deserves to be taken in account to improve the system.

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