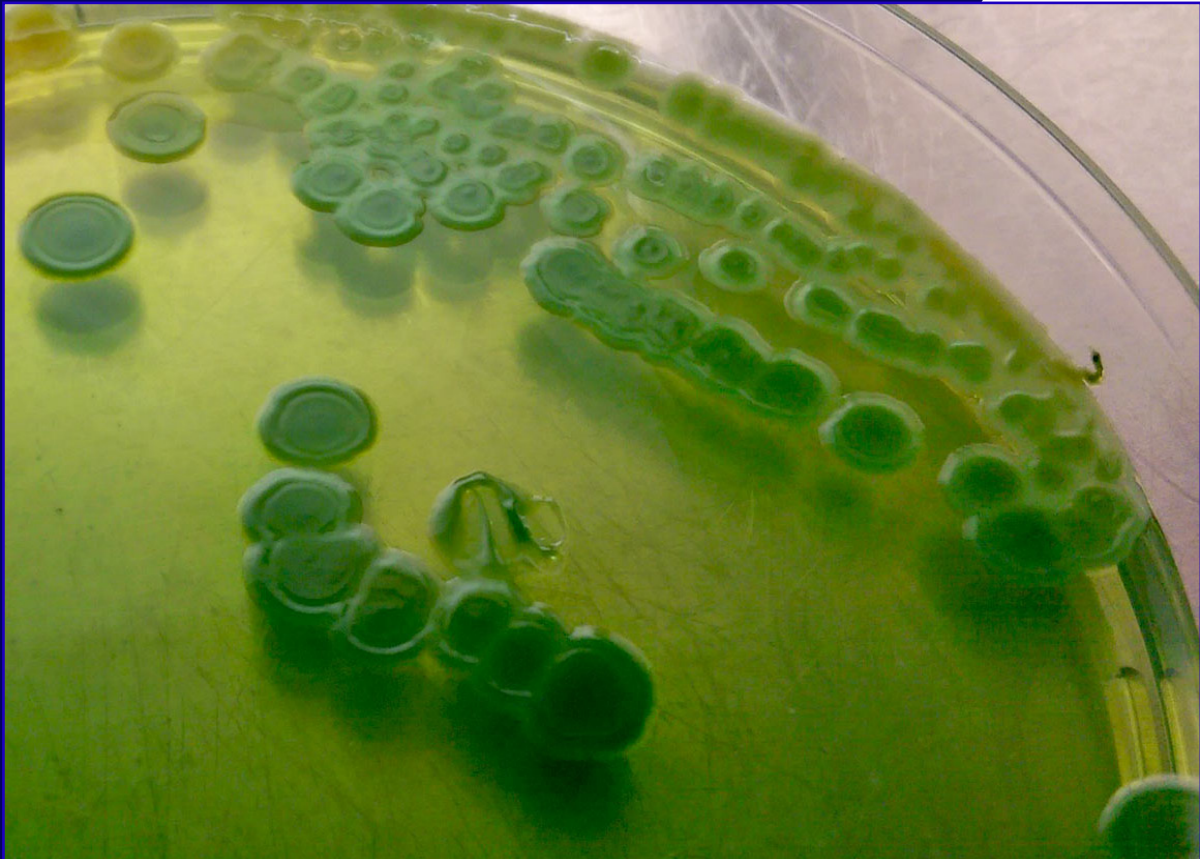


Vibrio spp. in the port of Rotterdam in the fall of 2014

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1. Summary

In the fall of 2014 five *Vibrio* species were recorded in the port of Rotterdam. Strains of two of these species are known human pathogens, i.e. *V. alginolyticus* and *V. parahaemolyticus*. Strains of the other three species can be pathogenic to shellfish, shrimp and/or fish species, i.e. *V. aestuarianus*, *V. anguillarum* and *V. campbellii/Vibrio harveyi*. In addition to the *Vibrio* species, two bacterium species were recorded that are also known to be potentially pathogenic to humans, i.e. *Proteus vulgaris* and *Shewanella putrefaciens*. These two species are known to cause respectively urinary tract infections and endocarditis.

2. Introduction

In the spring of 2014 various *Vibrio* species were encountered during a species survey in the port of Rotterdam (Gittenberger *et al.*, 2014). Some of the *Vibrio* colonies that were identified concerned species of which certain strains are known as human pathogens. One of the species, i.e. *Vibrio cf brasiliensis*, may concern a non-native to Europe that was introduced by ballast water in the port of Rotterdam. The identifications were done by MALDI-TOF MS at the Erasmus Medical Center in Rotterdam. Although this method is commonly used for the identification of *Vibrio* species, not all species can be identified with certainty. The aim of the survey in the spring of 2014 was not to identify all *Vibrio* species however. It focused on recording the presence of *Vibrio cholerae*, a species that can be identified with certainty with a MALDI-TOF MS analysis. That species was not found.

The present report describes the results of an inventory that was done in the port of Rotterdam in the fall of 2014. This inventory did focus on recording all *Vibrio* species. To increase the certainty with which these species were identified in comparison to the spring survey, all *Vibrio* identifications were based on both a MALDI-TOF MS analysis and a DNA-analysis using the 16S marker. This marker is known for being diagnostic among bacterial species.

3. Materials & methods

The 7th of October 2014, a surface water sample and about 20 mussels were collected with a boat at three different locations in the port of Rotterdam, i.e. in the 8th Petroleumhaven, the Beneluxhaven and the Brittaniëhaven (Fig. 1). Each of these samples was used to assess the *Vibrio* species present. This was done by applying water samples and homogenised (with a stomacher) dissected intestinal organs of mussels onto *Vibrio* spp. specific growth media, after which the *Vibrio* species present were grown overnight. First undiluted samples were used, and if necessary, when relatively high numbers of colonies were present, dilutions were used of 1:1, 1:10 and 1:100. For each research area, a sample was taken from each colony that appeared different in its morphology, i.e. its colour and/or form. Each sample was applied to a separate petridish with *Vibrio* specific growth media and left overnight to grow into a larger colony. These colonies were analysed with MALDI-TOF MS both at the department of Medical Microbiology and Infectious Diseases of the Erasmus Medical Center in Rotterdam using the Bruker system and database, and at Baseclear, Leiden, using the

VITEK MS IVD Database as a reference. Additionally samples of the colonies were taken for DNA-extraction. For each of the *Vibrio* species that was identified with the MALDI-TOF MS analysis, the DNA of at least one colony was extracted to sequence the 16S marker. These 16S DNA-analyses were used in combination with the MALDI-TOF analyses to identify the colonies.

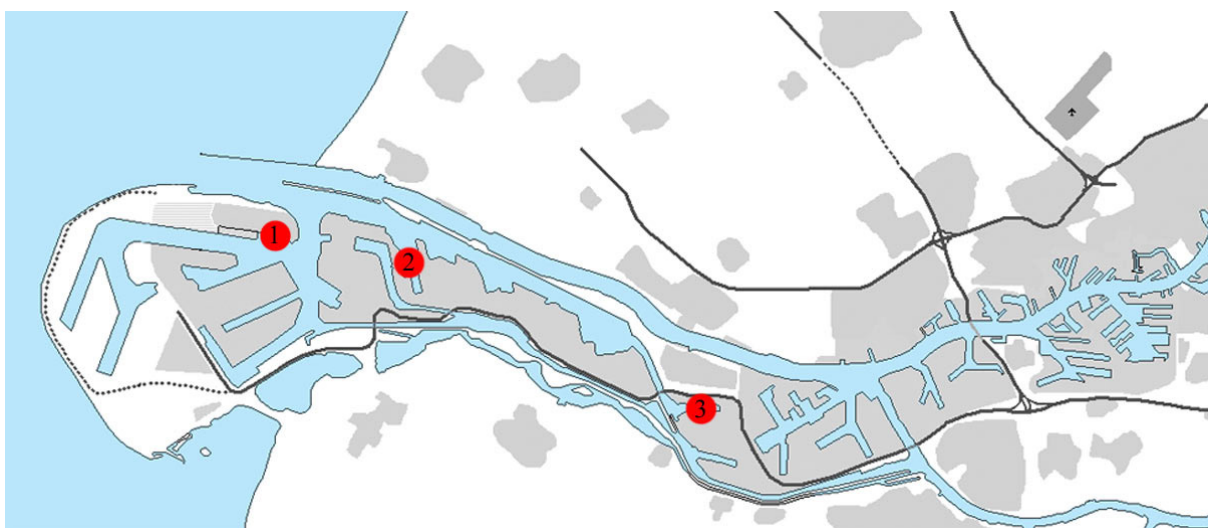


Fig. 1. The locations in the port of Rotterdam where the water samples were taken: [1] the 8th Petroleumhaven; [2] the Beneluxhaven; [3] the Brittaniëhaven.

4. Results

In total 29 bacterial colonies that grew on the *Vibrio* specific growth media, were selected to be analysed. Most bacterial colonies and species were found in the samples taken from the mussel intestines. Therefore 28 of the 29 colonies that were used for the analyses originated from mussel intestines and only one colony originated from a surface water sample. The MALDI-TOF MS analysis was successful for 25 of these samples. In combination with 23 additional 16S sequences, in total six bacterial species could be identified to the species level (Table 1). One species was recorded that could not be identified to the species level. It concerns either *Vibrio campbellii* or *V. harveyi*. These two *Vibrio* species are closely related sister species (Orata & Hedreyda, 2011) and could therefore not be distinguished from each other on the basis of the MALDI-TOF MS and the 16S analyses. The largest diversity of species was found in the 8th Petroleumhaven (Fig. 1).

Species	Research areas
<i>Vibrio aestuarianus</i>	8th Petroleumhaven Beneluxhaven Brittaniëhaven
<i>Vibrio alginolyticus</i>	Beneluxhaven Brittaniëhaven
<i>Vibrio anguillarum</i>	8th Petroleumhaven
<i>Vibrio campbellii</i> / <i>Vibrio harveyi</i>	8th Petroleumhaven
<i>Vibrio parahaemolyticus</i>	8th Petroleumhaven
<i>Proteus vulgaris</i>	8th Petroleumhaven
<i>Shewanella putrefaciens</i>	8th Petroleumhaven

Table 1. Bacterial species identified in the 8th Petroleumhaven, the Beneluxhaven and the Brittaniëhaven (Fig. 1). All species were identified on the basis of both MALDI-TOF MS and 16S analyses, with the exception of *Shewanella putrefaciens*, which was only identified with MALDI-TOF MS.

4.1 Species

All bacterium species that were recorded are known to occur in northern European waters and to have circumglobal distribution. Two of the recorded *Vibrio* species, i.e. *V. parahaemolyticus* and *V. alginolyticus*, are known human pathogens occurring in Dutch recreational waters (Schets et al., 2012). Wound infections and ear infections caused by *V. alginolyticus* were for example recorded in several persons that had been swimming in the Oosterschelde, a water inlet in the south of the Netherlands (Schets et al., 2006, 2011). Pathogenic strains of *Vibrio anguillarum* are known to cause large mortalities among fish and shellfish populations (Frans et al., 2011). *Vibrio aestuarianus* is especially known as an oyster pathogen that has been associated to summer mortalities in Pacific oyster (*Crassostrea gigas*) populations worldwide (Garnier et al., 2008; Vezzulli et al., 2014). *Vibrio campbellii* and *V. harveyi* are closely related species, which are hard to distinguish from each other. They are known as important pathogens for reared aquatic organisms. They have been associated with for example tail rot disease of sea breams (Haldar et al., 2010) and “shrimp disease” (Orata & Hedreyda, 2011). *Proteus vulgaris* concerns a bacterium that is known to cause urinary tract infections (Almeida et al. 2013) in humans and animals. It is found in soil, water and faecal matter. *Shewanella putrefaciens* is a marine bacterium species that in rare cases can cause endocarditis infections in humans (Constant et al., 2014).

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