Shellfish from Oosterschelde (North Sea) production areas contain pathogenic *Vibrio* species

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Introduction

Vibrio spp. are common inhabitants of various aquatic environments; some species are human pathogens that have been associated with wound and ear infections after exposure to contaminated waters, and gastroenteritis after consumption of contaminated seafood or water. Septicemia does occur, but is rare (Oliver and Kaper, 1997; Morris, 2003).
During the warm summer of 2006, *Vibrio* infections in bathers were reported from several countries in North-West Europe, including the Netherlands, where four people developed *V. alginolyticus* infections after swimming in the Oosterschelde (North Sea) that is also known for its production of bivalve mollusks (Schets *et al*., 2006). Bivalve mollusks concentrate pathogens such as *Vibrio* from contaminated water, and since they are generally consumed raw, they are potential vectors of disease. Outbreaks of shellfish related *Vibrio* infections have not been reported in the Netherlands to date, but such outbreaks are common in other parts of the world (Potasman *et al*., 2002; McLaughlin *et al*., 2007). *V. parahaemolyticus* is the leading cause of *Vibrio*-associated gastroenteritis in the United States (Potasman *et al*., 2002) and of food borne illness in Japan (Lee *et al*., 2001), whereas death associated with seafood consumption in the United States is mainly caused by *V. vulnificus* (Morris, 2003). Increased numbers of *V. parahaemolyticus* infections in the United States since the mid-1990s may be due to an increasing water temperature in the shellfish harvesting areas (Daniels *et al*., 2000).
The aim of this study was to detect and quantify *Vibrio* spp. in oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*) harvested from various Oosterschelde production sites, non-commercial *C. gigas* collected from the Oosterschelde, and *C. gigas* purchased in Dutch fish shops, and relate *Vibrio* numbers in Oosterschelde shellfish to the water temperature.

Materials and methods

Composite samples were prepared consisting of at least 25 gram of material obtained from at least six oysters or approximately ten mussels; one composite sample was analyzed per sampling day. Shellfish homogenates were incubated in alkaline buffered peptone water (ABPW) for 6-8 h at 36 $\pm$ 2 ºC and, in parallel, in ABPW without sodium chloride for 16-20 h at 36 $\pm$ 2 ºC. ABPW cultures were spread onto Thiosulphate Citrate Bile Sucrose agar plates which were incubated at 36 $\pm$ 2 ºC for 16-20 h. Presumptive *Vibrio* spp. were further tested and identified as described by Schets *et al*. (2010).
In all shellfish samples, *Escherichia coli* were enumerated using the most probable number (MPN) method according to ISO/TS 16649-3 (2005). The maximum likelihood method was used to estimate *Vibrio* and *E. coli* MPN in the shellfish by using Mathematica 5.1.

Results and Discussion

Different *Vibrio* spp. were detected in oysters and mussels destined for direct human consumption harvested from Oosterschelde production areas (Table 1), in oysters collected at non-commercial sites in the Oosterschelde (231->2398 MPN/g), and also in oysters purchased in Dutch fish shops (231->333 MPN/g). *V. alginolyticus* was the most frequently isolated species, followed by *V. parahaemolyticus*. One oyster sample from a non-commercial Oosterschelde site contained *V. cholerae* non-O1/O139.
Eighty percent of the total of 229 isolated presumptive *Vibrio* strains was *V. alginolyticus*; the species was found in 66% of the oyster and mussel samples. Although *V. alginolyticus* is a frequently reported cause of ear and wound infections, the species has not been associated with gastro-intestinal illness through consumption of contaminated seafood (Morris, 2003).

About 10% of the 229 isolated strains were identified as *V. parahaemolyticus*; 17% of the oyster and mussel samples contained this species. The 24 *V. parahaemolyticus* strains did neither have *tdh* nor *trh* genes.

Table 1: Total *Vibrio* levels (MPN/g) in commercial oysters and mussels from Oosterschelde production areas, and the *Vibrio* species isolated from the positive samples.

sampling date	oysters		mussels	
	MPN/g	isolated species (no.)	MPN/g	isolated species (no.)
14-06-2007	231	*V. alginolyticus* (6)	23	*V. alginolyticus* (7)
16-08-2007	23	*V. alginolyticus* (8) *V. parahaemolyticus* (1)	23	*V. alginolyticus* (4)
14-09-2007	622	*V. alginolyticus* (7) *V. parahaemolyticus* (3)	23	*V. alginolyticus* (5)
27-09-2007	62	*V. alginolyticus* (8)	62	*V. alginolyticus* (5) *V. parahaemolyticus* (7)
11-10-2007	62	V. alginolyticus (10)	0	none
25-10-2007	0	none	0	none
21-11-2007	0	none	0	none
19-12-2007	0	none	0	none
23-05-2008	0	none	0	*V. alginolyticus* (5)
17-07-2008	231	*V. alginolyticus* (5)	6	*V. alginolyticus* (5)
14-08-2008	231	*V. alginolyticus* (6) *V. parahaemolyticus* (1)	23	*V. alginolyticus* (5)
10-09-2008	231	*V. alginolyticus* (5)	231	*V. alginolyticus* (9)
08-10-2008	6	*V. alginolyticus* (8)	6	*V. alginolyticus* (8)
20-11-2008	0	none	0	none
18-12-2008	0	none	0	none
total		*V.alginolyticus* (102) *V.parahaemolyticus* (11)		*V.alginolyticus* (53) *V.parahaemolyticus* (8)

A temperature dependent occurrence of *Vibrio* spp. in the tested bivalve mollusks was observed (Figure 1), in agreement with findings published by others (Bauer *et al*., 2006; Lhafi and Kühne, 2007; McLaughlin *et al*., 2007). However, in contrast to what Bauer *et al*. (2006) and Lhafi and Kühne (2007) found, *Vibrio* spp. were not detected in shellfish when the Oosterschelde water temperature was below 13.5 °C. The suggested adaptation of *Vibrio* spp. to low water temperatures (Lhafi and Kühne, 2007) may not have occurred in the Oosterschelde, or the lack of detection in samples taken during colder months was due to low *Vibrio* levels and a limited amount of homogenate tested.

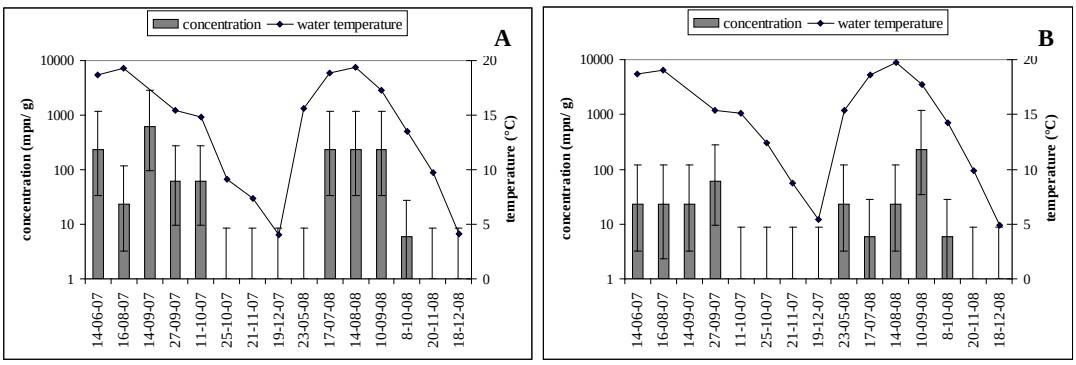

Figure 1: The concentration of *Vibrio* spp. in (A) oysters (*Crassostrea gigas*) and (B) mussels (*Mytilus edulis*) from Oosterschelde production areas. The bars display the 95%-confidence interval. The water temperature in the Oosterschelde was recorded on site at the time of shellfish sampling.

Compared to the US-FDA (2007) guideline value of 10,000 MPN/g *V. parahaemolyticus*, shellfish from Dutch production areas and oysters bought in Dutch fish shops contained low numbers of *V. parahaemolyticus*; *V. cholerae* non-O1/O139 was not found in these shellfish. Based on these results and the high *V. parahaemolyticus* dose required for infection (US-FDA, 2005), the risk of *V. parahaemolyticus* gastrointestinal infections through consumption of shellfish from Oosterschelde production sites is presumably low. Higher *Vibrio* levels were, however, found in samples of non-commercial oysters from the Oosterschelde, indicating that picking and consuming of these *C. gigas* may be a risk factor for gastrointestinal illness through infection with *V. parahaemolyticus*.
All commercial oysters and mussels tested contained low *E. coli* levels (oysters: 0-20 MPN/100 g; mussels: 0-128 MPN/100 g). As in other studies (Lhafi and Kühne, 2007), there was no correlation between *E. coli* counts and *Vibrio* presence.

Conclusions

Low numbers of pathogenic *Vibrio* species were detected in shellfish from the Oosterschelde production areas, indicating a low risk of gastrointestinal illness through consumption of these shellfish. However, the temperature dependent occurrence of *Vibrio* has been demonstrated and when global warming leads to elevated water temperatures in the moderate climate regions of north western Europe in future years, favorable growth opportunities for pathogenic marine *Vibrio* species may develop (Schijven and de Roda Husman, 2005). As a result human exposure to high numbers of these pathogens through consumption of contaminated seafood may increase.

References

Bauer A., Østensvik Ø., Florvåg M., Ørmen Ø., Rørvik L.M. (2006) Occurrence of *Vibrio parahaemolyticus*, *V. cholerae*, and *V. vulnificus* in Norwegian blue mussels (*Mytilus edulis*). *Applied and Environmental Microbiology* 72, 3058-3061.

Daniels N.A., MacKinnon L., Bishop R., Altekruse S., Ray B., Hammond R.M., Thompson S., Wilson S., Bean N.H., Griffin P.M., Slutsker L. (2000) *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *Journal of Infectious Diseases* 181, 1661-1666.

ISO/TS 16649-3. (2005) Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* -- Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide. International Organization for Standardization, Geneva, Switzerland.

Lee W., Lee M., Kim J., Park S. (2001) Foodborne illness outbreaks in Korea and Japan studied retrospectively. *Journal of Food Protection* 64, 899-902.

Lhafi K.L, Kühne M. (2007) Occurrence of *Vibrio* spp. in blue mussels (*Mytilus edulis*) from the German Wadden Sea. *International Journal of Food Microbiology* 116, 297-300.

McLaughlin J.B., DePaola A., Bopp C.A., Martinek K.A., Napolilli N.P., Allison C.G., Murray S.L., Thompson E.C., Bird M.M., Middaugh J.P. (2007) Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *New England Journal of Medicine* 353, 1463-1470.

Morris J.G. (2003) Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. *Clinical Infectious Diseases* 37, 272-280.

Oliver J.D., Kaper J.B. (1997) *Vibrio* species. In: M.P. Doyle, L.R. Beuchat and T.J. Montvilee (Eds.). *Food Microbiology – Fundamentals and Frontiers*. Chapter 13, 228-264, ASM Press, Washington DC, 768 pp. (ISBN 1-55581-117-5).

Potasman I., Paz A., Odeh M. (2002) Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. *Clinical Infectious Diseases* 35, 921-928.

Schets F.M., van den Berg H.H.J.L, Demeulemeester A.A., van Dijk E., Rutjes S.A., van Hooijdonk H.J.P., de Roda Husman A.M. (2006) *Vibrio alginolyticus* infections in the Netherlands after swimming in the North Sea. *Euro Surveilancel* 11(45).

Schets F.M., van den Berg H.H.J.L, Rutjes S.A., de Roda Husman A.M. (2010) Pathogenic *Vibrio* species in Dutch shellfish destined for direct human consumption. *Journal of Food Protection*, 73, 734–738.

Schijven J.F., de Roda Husman A.M. (2005) Effect of climate changes on waterborne disease in The Netherlands. *Water Science and Technology* 51, 79-87.

US-FDA. (2005) Pathogenic *Vibrio parahaemolyticus* in raw oysters – Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm050421.htm accessed 06052010.

US-FDA. (2007) National Shellfish Sanitation Program – Guide for the control of molluscan shellfish. http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/FederalStatePrograms/NationalShellfishSanitationProgram/ucm046353.htm accessed 06052010.