Environmental Microbiology Reports
February 2010, Volume 2 Issue 1, Pages 185 - 191
http://dx.doi.org/10.1111/j.1758-2229.2010.00133.x

Chlorophyll a might structure a community of potentially pathogenic culturable *Vibrionaceae*. Insights from a one-year study of water and mussels surveyed on the French Atlantic coast

J. Deter[1], S. Lozach[1], A. Derrien[3], A. Véron[2], J. Chollet[1] and D. Hervio-Heath[1, *]

[1] Ifremer, centre de Brest, Département Environnement, Microbiologie et Phycotoxines (EMP), Laboratoire de Microbiologie, ZI de la pointe du diable, B.P. 70, 29280 Plouzané, France.
[2] Ifremer, centre de Nantes, EMP/Laboratoire National de Référence Microbiologie des Coquillages, BP 21105, 44311 Nantes Cedex 3, France.
[3] Ifremer, LERPC, centre de La Rochelle, place Gaby Coll, BP 7, 17137 L'Houmeau, France.

*: Corresponding author : D. Hervio-Heath, Tel. (+33) 2 98 22 43 49; Fax (+33) 2 98 22 45 94, email address : Dominique.Hervio.Heath@ifremer.fr

Abstract:

The present study focused on the isolation of culturable bacteria from mussels and sea water to identify *Vibrionaceae* potentially pathogenic for humans. Three sites located on the French Atlantic coast were monitored monthly (twice each month during summer) for 1 year. Environmental parameters were surveyed (water temperature, salinity, turbidity, chlorophyll *a*) and bacteria were detected by culture and identified by API 20E® systems (BioMérieux) and PCR. A total of seven species were detected (*Grimontia hollisae, Photobacterium damselae, Vibrio alginolyticus, V. cholerae, V. fluvialis, V. vulnificus and V. parahaemolyticus*) and species diversity was higher at the end of summer. Surprisingly, V. cholerae non-O1/non-O139 was detected in spring. No site effect was detected. Using Sørensen similarity indices and statistical analyses, we showed that chlorophyll a had a significant influence on the bacterial community detected in mussels and assemblages were more similar to one another when chlorophyll a values were above 20 µg l^{-1}. No significant effect of any parameter was found on the community detected in water samples. Such surveys are essential for the understanding of sanitary crises and detection of emerging pathogens.

INTRODUCTION

Many micro-organisms (microalgae, bacteria and heterotrophic protists) are naturally present in sea water. They are used as food resources (Langdon and Newell, 1990) by filter-feeding animals and may be accumulated in their tissues. Some of these bacteria, especially the *Vibrionaceae*, may be pathogenic and cause human diseases (gastro-enteritis, wound tissue infections or septicaemia) after seafood consumption or contact between a wound and water. *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus* are distributed worldwide and responsible for most food-borne infections caused by bacteria. Toxigenic strains of *V. cholerae* are the aetiological agents of cholera, causing more than 230 000 human cases, including over 6000 deaths, in 52 countries in 2006 (WHO, 2008). *V. parahaemolyticus* is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States of America (USA) and Japan, but the incidence is low in Europe (Su and Liu, 2007). Primary septicaemia due to *V. vulnificus* is the most deadly food-borne disease in the USA accounting for 95 % of all seafood-related deaths, with a mortality rate of approximately 50 % (Oliver, 2006). Other vibrios responsible for occasional severe infections are also found in the marine environment: *V. alginolyticus* (Rubin and Tilton, 1975), *Grimontia hollisae* (formerly *V. hollisae*) (Hinestrosa *et al.*, 2007), and *Photobacterium damselae damselae* (formerly *Listonella* or *V. damselae*) (Osorio *et al.*, 2005). Pathogenesis by these bacteria is often due to their ability to secrete extracellular virulence factors, whose nature may be more or less well known (Nishibuchi and Kaper, 1995; Oliver and Kaper, 2007).

Vibrionaceae bacteria are particularly important for the seafood economy because pre-market depuration processes (before mollusc sale) generally fail to eliminate these microorganisms from shellfish, although they do eliminate conventional bacterial indicators (*Escherichia coli*) which are less strongly bound to mollusc tissues (Su and Liu, 2007). Consequently, the

improvement of our understanding of the ecological processes influencing *Vibrionaceae* populations is particularly relevant for the management of preventive actions. Such ecological studies are becoming increasingly numerous, especially in USA where the distribution of some coastal *Vibrio* populations is linked to environmental factors, including water temperature and salinity (Kaneko and Colwell, 1978; Mercedes *et al.*, 2000; Duan and Su, 2005; Oliver and Kaper, 2007; Blackwell and Oliver, 2008; Johnson *et al.* 2009). However, studies focusing on a global microbial community and its links with the environment remain scarce (Licciano *et al.*, 2007), even though methods for evaluating structure in bacterial community composition or the influence of environmental factors already exist (Clarke *et al.*, 2008).

In France (the third largest mussel-producing country in Europe (IFREMER, 2006)), only a few environmental studies have focused on potentially pathogenic vibrios (Hervio-Heath *et al.*, 2002; Robert-Pillot *et al.*, 2004). The presence of *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* non-O1/non-O139 has been observed in mussels (*Mytillus edulis*) and water at different sites (Hervio-Heath *et al.*, 2002; Robert-Pillot *et al.*, 2004). In the Pertuis Breton (Atlantic coast), a recent one-year study investigated the ecology of *V. parahaemolyticus* (Deter *et al.*, accepted). The results showed that water temperature and turbidity had an effect on the presence of total *V. parahaemolyticus* in water, but that chlorophyll *a* was the most important factor influencing the presence of the bacteria in mussels. In the present study, we monitored sea water and mussels (*Mytillus edulis*) at the same three sampling sites (Figure 1) for one year (one sampling per month, two per month from May to August). The purposes of the study were, first, to identify (presence/absence of species) the community of culturable potentially pathogenic *Vibrionaceae* and, then, to investigate the influence of environmental factors (water temperature, salinity, turbidity and chlorophyll *a*), the concentration of total potentially pathogenic culturable *Vibrionaceae*

species and the presence of total *V. parahaemolyticus* (results from Deter *et al.* accepted) on the structure of this entire community.

RESULTS AND DISCUSSION

Description of the data

All environmental parameters showed spatio-temporal variation during the study period (April 08-March 09): mean water temperature varied from 6.7 to 22.0 °C; salinity from 26.0 to 35.6 (no unit), with maximal values during summer; turbidity from 1.4 to 40.3 FNU (formazin nephtelometric units), with maxima in April and November-December; chlorophyll *a* from 0.6 to 22.0 µg / L, with maxima in June; and the number of culturable bacteria from 27 to 6400 bacteria / g mussel tissue (maxima in January) and from 0 to 24 bacteria / ml sea water (maxima during summer). Total culturable *Vibrionaceae* presented higher densities in mussels than in the surrounding water throughout the year (maximal MPN [most probable number of bacteria]) in mussels 6400 / g > 24 / ml sea water, Fig. 2). All of these parameters were adequately (log or square root) transformed to fit a normal distribution. Both MPN variables were highly correlated (r=0.400, P=0.005). A highly significant linear relationship was found between MPN of bacteria estimated in sea water and MPN of bacteria in mussels (Figure 2, linear regression, R^2=0.14, F=8.75, P=0.005). This meant that the concentration in the seawater could explain that in mussels and thus confirmed the ability of mussels to accumulate bacteria from surrounding environment, as already shown by Cavallo *et al.* (2009).

Structure of the *Vibrionaceae* community and influence of the environment

Bacterial isolates obtained from this study yielded very good to excellent identification using the API 20E® system, and *Vibrio* species were confirmed with conventional or Real-Time PCR (RT-PCR). Seven species were detected (Table 1). The presence of *G. holissae* is noteworthy as infection has never been reported on the European Atlantic coast except for one

human case 13 years ago in France (GrasRouzet *et al.*, 1996). All of the other species detected in this study had already been reported by Hervio-Heath *et al.* (2002) at Filière W after three sampling campaigns in summer 1999. As reported in other studies on samples from Europe and America, the most frequently isolated bacteria were *V. alginolyticus* (39 positive mussel samples out of 48 and 35/48 in water) and *V. parahaemolyticus* (17/48 in mussels and 16/48 in water) (Croci *et al.*, 2001; Hervio-Heath *et al.*, 2002; Blackwell and Oliver, 2008; Martinez-Urtaza *et al.*, 2008). The present one-year survey highlighted a higher diversity of bacterial species at the end of the summer (August-September, 4.5 species detected in mean) after maximal values of water temperature and salinity, two factors frequently reported as having an influence on these species (see Introduction). Surprisingly, *V. cholerae* non-O1/non-O139 was not detected during the summer but in spring (May in mussels at Filière W and February in sea water at L'Eperon). The detection of *V. cholerae* only in spring could be related to a lower competition of bacterial species on culture plates at this time of the year. The toxigenic form of this bacterium was reported in 36 imported cases of cholera in European Union in 2005 (ECDC, 2008). Contrary to toxigenic strains, which are rarely isolated, non toxigenic strains are normal inhabitants of aquatic ecosystems (Oliver and Kaper, 2007). The species had already been detected at the Filière W site ten years ago by Hervio-Heath *et al.* (2002). Knowing that *V. cholerae* non-O1/non-O139 may be associated with cases of gastro-enteritis and a small number of oyster-vectored deaths, notably in USA (Rippey, 1994), a more regular monitoring of this site should be considered.

Influence of total *V. parahaemolyticus* and of the environmental parameters on the *Vibrionaceae* community

The matrix of *Vibrionaceae* species detection (presence/absence per date at each site for mussels or sea water) was used to perform a Principal Component Analysis (PCA). All of the species were more or less correlated in their presence/absence (no antagonism between

species, see Fig.3 for water; similar results were obtained for mussels, data not shown). This suggested that similar parameters might influence each species and structure the entire community.

Analysis of similarities (ANOSIM), an approximate analogue of analysis of variance, was conducted to test the null hypothesis that there were no assemblage differences between groups of samples specified by a single qualitative factor (here sampling site or the presence/absence of total *V. parahaemolyticus*, as described in Deter *et al.* (accepted), with a RT-PCR detection after enrichment). The analyses were performed with Primer 6.1.11 software (Primer-E Ltd), according to Clarke and Gorley (2006), Clarke and Warwick (2001) and Clarke *et al.* (2008).

ANOSIM performed on the water similarity matrix did not show any significant effect of site (P=0.93) or of the presence of total *V. parahaemolyticus* (P=0.20). The BEST BIO-ENV procedure was then used to find the "best" match between the multivariate among-sample patterns of the assemblages (in water or mussels) and that from quantitative variables (water temperature, salinity, turbidity, chlorophyll *a*, MPN in water or in mussel) associated with those samples. This procedure was used to search (using all combinations of variables) for high rank correlations (called ρ) between the similarity matrix (species assemblage) and resemblance matrix (Euclidian distance), generated with environmental data. P-values were obtained with permutation tests (10000 permutations). In water samples, no parameters had a significant influence on the structure of the community (P>0.17). Consequently, other parameters that were not taken into account in the study (such as zooplankton or other bacteria, for example) should be considered as potentially more important.

In mussels, there was no significant difference between the groups of communities when sites were considered (ANOSIM, P=0.42), most probably because of the small spatial scale considered in this study (distance between sites ranged from 5 to 10 km). Presence of total *V.*

parahaemolyticus in mussels (Deter et al., accepted) had a significant influence on the similarities calculated between the assemblages observed in mussels (ANOSIM, P=0.03). ρ was optimised (0.325) with a model containing chlorophyll *a* and turbidity but the best one-variable solution produced a result that was almost as good with chlorophyll *a* alone (ρ=0.322; P=0.007) and which, on grounds of parsimony, might be preferable. Dissimilar samples were observed when chlorophyll *a* values were inferior to 18 µg/L. Samples presenting more similar assemblages were found when chlorophyll *a* presented values above 20 µg/L (Fig. 4). As an explanation, we suggest the influence of a link between chlorophyll *a* – phytoplankton – zooplankton but unfortunately, were not able to monitor zooplankton during this study. *V. parahaemolyticus* populations are known to be influenced by a factor related to plankton (planktonic species, plankton detritus, or other particulate matter trapped during plankton hauls) because of the attachment and/or a chitinoclastic activity (Kaneko *et al.*, 1973). In Deter *et al.* (accepted), the authors already linked the presence of total *V. parahaemolyticus* to chlorophyll *a*. This would not be due to the bloom of any of the phytoplanktonic species reported in Deter et al (accepted) but more to an overall effect or to the influence of zooplankton in relation with phytoplankton linked in turn to chlorophyll *a*. In the present study, chlorophyll *a* was also the parameter that most influenced the *Vibrionaceae* community found in mussels, indicating that attachment to plankton and chitinoclastic activity are characteristics shared by all *Vibrionaceae*. This would confirm the increasing role in ecology given to attachment and microbial biofilms for a majority of bacteria (Yildiz and Visick, 2008; Monds and O'Toole, 2009).

The present study showed a high diversity of potentially pathogenic *Vibrionaceae* on the French Atlantic coast. It is important to pursue such studies by including abundance values. Ecological studies on bacterial communities are still rare in Europe, but they could be very valuable in the event of environmental disorders (oyster summer mortalities (IFREMER,

2008)) or sporadic human cases (death after a *V. vulnificus* infection (DDASS Hérault, 2008)). Thus, such surveys would be important for the detection of emerging pathogens and/or to have knowledge of initial states in case of sanitary crises.

REFERENCES

Blackwell, K.D., and Oliver, J.D. (2008) The ecology of *Vibrio vulnificus*, *Vibrio cholerae* and *Vibrio parahaemolyticus* in North Carolina estuaries. *J Microbiol* **46**: 1-8.

Cavallo, R.A., Acquaviva, M.I., and Stabili, L. (2009) Culturable heterotrophic bacteria in seawater and *Mytillus galloprovincialis* from a Mediterranean area (Northern Ionian sea - Italy). *Environ Monit Assess* **14**: 465-475.

Chun, J., Huq, A., and Colwell, R.R. (1999) Analysis of 16S-23S r RNA Intergenic Spacer Regions of *Vibrio cholerae* and *Vibrio mimicus*. *Appl Environ Microbiol* **65**: 2202-2208.

Clarke, K.R., and Warwick, R.M. (2001) *Change in marine communities: an approach to statistical analysis and interpretation*, 2nd edition. In PRIMER-E. Plymouth.

Clarke, K.R., and Gorley, R.N. (2006) PRIMER v6: *User Manual/Tutorial*. In PRIMER-E. Plymouth.

Clarke, K.R., Somerfield, P.J., and Gorley, R.N. (2008) Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. *J ExpMar Biol Ecol* **366**: 56-69.

Croci, L., Serratore, P., Cozzi, L., Stacchini, A., Milandri, S., Suffredini, E., and Toti, L. (2001) Detection of Vibrionaceae in mussels and in their seawater growing area. *Lett Appl Microbiol* **32**: 57-61.

Deter, J., Lozach, S., Derrien, A., Véron, A., Chollet, J., and Hervio-Heath D. (accepted). Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effect of temperature, salinity, turbidity and chlorophyll a. *Env Microbiol*

DDASS Hérault (2008). Infection à *Vibrio vulnificus* avec décès suite à une séance de pêche dans l'étang de Vic (Hérault)

Duan, J., and Su, Y. (2005) Occurrence of *Vibrio parahaemolyticus* in two Oregon oyster-growing bays. *J Food Sci* **70**: M58-M63.

ECDC, The European Centre for Disease Prevention and Control (2008) Questionnaire for the first European communicable disease epidemiological. In: *European commission- Health and consumers directorate general DG SANCO / Eurostat*.

GrasRouzet, S., Donnio, P.Y., Juguet, F., Plessis, P., Minet, J., and Avril, J.L. (1996) First European case of gastroenteritis and bacteremia due to *Vibrio hollisae*. *Eur J Clin Microbiol* **15**: 864-866.

Hervio-Heath, D., Colwell, R.R., Derrien, A., Robert-Pillot, A., Fournier, J.-M., and Pommepuy, M. (2002) Occurence of pathogenic vibrios in coastal areas of France. *J Appl Microbiol* **92**: 1123-1135.

Hinestrosa, F., Madeira, R.G., and Bourbeau, P.P. (2007) Severe gastroenteritis and hypovolemic shock caused by *Grimontia* (*Vibrio*) *hollisae* infection. *J Clin Microbiol* **45**: 3462–3463.

IFREMER (2006). Aquaculture - Filière mollusques [www document]. URL http://wwz.ifremer.fr/aquaculture/filieres/filiere_mollusques/decouverte_mollusques

IFREMER (2008). Oyster (*Crassostrea gigas*) mortality events during summer 2008 in France [www document]. URL http://www.ifremer.fr/crlmollusc/page_labo/News.htm

Johnson C.N., Flowers, A.R., Young, VC, Gonzalez-Escalona, N., DePaola, A., Noriea, N.F., and Grimes, D.J. (2009) Genetic relatedness among *tdh+* and *trh+ Vibrio parahaemolyticus* cultured from Gulf of Mexico oysters (*Crassostrea virginica*) and surrounding water and sediment. *Microb Ecol* **57**: 437-43.

Kaneko, T., and Colwell, R.R. (1973) Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *J Bacteriol* **113**: 24-32.

Kaneko, T., and Colwell, R.R. (1978) Annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. *Microbiol Ecol* **4**: 135-155.

Langdon, C.J., and Newell, R.I.E. (1990) Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Mar Ecol: Progress Series* **58**: 299-310.

Lee, J.Y., Eun, J.b., and Choi, S.H. (1997) Improving detection of *Vibrio vulnificus* in *Octopus variabilis* by PCR. *J Food Sci* **62**: 179-182.

Licciano, M., Terlizzia, A., Giangrandea, A., Cavallob, R.A., and Stabilia, L. (2007) Filter-feeder macroinvertebrates as key players in culturable bacteria biodiversity control: a case of study with *Sabella spallanzanii* (Polychaeta: Sabellidae). *Mar Environ Res* **64**: 504-513.

Martinez-Urtaza, J., Lozano-Leon, A., Varela-Pet, J., Trinanes, J., Pazos, Y., and Garcia-Martin, O. (2008) Environmental determinants of the occurence and distribution of *Vibrio parahaemolyticus* in the rias of Galicia, Spain. *Appl Environ Microbiol* **74**: 265-274.

Mercedes, P., Rodo, X., Ellner, S.P., Colwell, R., and Bouma, M.J. (2000) Cholera Dynamics and El Nino-Southern Oscillation. *Science* **289**: 1766-1769.

Monds, R.D., and O'Toole, G.A. (2009) The developmental model of microbial biofilms: ten years of a paradigm up for review. *Trends Microbiol* **17**: 73-87.

Nishibuchi, M., and Kaper, J.B. (1995) Thermosatble direct hemolysin gene *of Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. *Infect Immun* **63**: 2093-2099.

Nhung, P.H., Ohkusua, K., Miyasakab, J., Suna, X. S., and Ezakia, T. (2007) Rapid and specific identification of 5 human pathogenic Vibrio species by multiplex polymerase chain reaction targeted to *dnaJ* gene. *Microbiol Infect Dis* **59**: 271-275.

Oliver, J.D. (2006) *Vibrio vulnificus*. In *Biology of* Vibrios. Thompson, F.L., Austin, B., and Swing, J. (eds). Washington, D.C., USA: American Society of Microbiology Press.

Oliver, J.D., and Kaper, J.B. (2007) *Vibrio* species. In *Food microbiology: fundamentals and frontiers*. Doyle, M.P., and Beuchat, L.R. (eds). Washington, D. C., USA: American Society of Microbiology.

Osorio, C.R., Collins, M.D., Romalde, J.L., and Toranzo, A.E. (2005) Variation in 16S-23S rRNA intergenic spacer regions in *Photobacterium damselae*: a mosaic-like structure. *Appl Environ Microbiol* **71**: 636-645.

Rippey, S.R. (1994) Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev* **4**: 419-425.

Robert-Pillot, A., Guénolé, A., Lesne, J., Delesmont, R., Fournier, J.-M., and Quilici, M.-L. (2004) Occurence of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates from waters and raw shellefish collected in two French coastal areas and from seafood imported into France. *Intern J Food Microbiol* **91**: 319-325.

Rubin, S.J., and Tilton, R.C. (1975) Isolation of *Vibrio alginolyticus* from Wound Infections. *J Clin Microbiol* **2**: 556-558.

Su, Y.-C., and Liu, C. (2007) *Vibrio parahaemolyticus*: A concern of seafood safety. *Food Microbiol* **24**: 549-558.

WHO, World Health Organization (2008) *Cholera*. Fact sheet N°107.

Yildiz, F.H., and Visick, K.L. (2008) *Vibrio* biofilms: so much the same yet so different. *Trends Microbiol* **17**: 109-118.

TABLES

Table 1. *Vibrionaceae* species detected during the one-year study with the number of occurrences (total number = 16 dates for each species) per site and origin of sample.

For sea water, three filtrations of 10 ml, 50 ml and 100 ml (5, 10 and 25 ml in summer) were realized for each site. Filters (nitrocellulose 0.22 µm Ø 47 mm, Millipore) were deposited onto three different culture media: Difco™ Marine Agar 2216 (Becton Dickinson and Company), Difco™ Thiosulfate Citrate Bile Salts Sucrose agar (Becton Dickinson and Company), and CHROMagar™ Vibrio (CHROMagar Paris France). One filter was deposited per plate (three plates / culture medium / site). Concerning the mussels, the 1:2 dilutions of mussel tissue (see Fig. 2) were made and spread onto the three media (1:2, 1:20 and 1/200 dilutions). All plates were incubated 24 h at 37 °C (temperature and media selecting for *Vibrionaceae* bacteria). Presumptive *Vibrionaceae* were isolated (one colony of each morphotype per source/culture medium/site/date) and identified as described hereafter. Biochemical analyses were performed from cultures of isolated colonies grown onto Bacto™ Heart Infusion medium with 2% NaCl (Becton Dickinson and Company) and incubated for 20-24 h at 37 °C. Biochemical tests (oxydase test, API 20E® system: BioMérieux, France) were performed following classical procedures. Real time-PCR on MX3000P™ (Stratagene) were performed to detect *V. parahaemolyticus* (*toxR* gene, patent pending). Conventional PCR PTC-200 (Mj Research) was used to confirm the identification of *Vibrio* sp bacteria: *V. alginolyticus* with primers VM-F and V.al2-MmR for the *dnaJ* gene (Nhung *et al*, 2007), *V. vulnificus* with VV-1 and VV-2R for the *vvh* hemolysin gene (Lee *et al.*, 1997), and *V. cholerae* with prVC-F and prVCM-R for the 16S-23S rRNA Intergenic Spacer Regions (Chun *et al.*, 1999) as already described by Hervio-Heath et al. (2002). Confirmed *V. cholerae* strains were sent to the Centre National de Référence des Vibrions et du Choléra (CNRVC,

Institut Pasteur, Paris France) to be tested with O1 and O139 antisera and for the presence of the *ctx* gene related to toxigenic strains.

DNA used for these molecular identifications were extracted from a 10 µl handle of isolated strain following Deter *et al.* (accepted). The DNA extracts were conserved at -20°C until PCR was performed.

Species	Mussels			Water		
	Filière W	**La Carrelère**	**L'Eperon**	**Filiere W**	**La Carrelère**	**L'Eperon**
Grimontia hollisae	2	3	0	2	1	0
Photobacterium damselae	2	2	3	2	3	4
Vibrio alginolyticus	13	12	14	11	12	12
Vibrio cholerae non-O1/non-O139	1	0	0	0	0	1
Vibrio fluvialis	2	1	4	5	6	4
Vibrio parahaemolyticus	7	6	4	4	7	5
Vibrio vulnificus	1	4	4	3	6	5

FIGURE LEGENDS

Figure 1. Map of the Pertuis Breton showing the locations of the sampling sites. These sites were monitored monthly (twice each month during summer) for one year for water and mussel samples. Environmental parameters (water temperature, salinity, turbidity chlorophyll *a*) and bacteria were surveyed at the same frequency.

Figure 2. Variation of the square-root transformed most probable number (MPN) of bacteria estimated in mussel (per g of mussel tissue) as a function of the square-root transformed number estimated in water (per ml of water). Regression equation: Y = 5.654 + 0.3369*X.
For the MPN method, around 200 g of mussel tissue per site were blended (3 x 20 sec. at high speed with a sterile Waring blender) and 1:2 diluted with sterile alkaline peptone water 1 % NaCl (APW, 20 g Bacto™ peptone Becton Dickinson and Company, 10 g NaCl per liter, pH 8.2). Water samples of 800 ml from each site were filtered (nitrocellulose 0.22 µm, Millipore). Deposits from filters were re-suspended in 25 ml (40 ml for l'Eperon and La Carrelère in summer) of sterile artificial sea water (water and Instant Ocean Sea Salt®, Instant Ocean). Re-suspended deposits from sea water and a 1:5 APW dilution of the 1:2 mussel homogenate were used for the MPN with three series of six tubes. Each series corresponded to a 1:10, 1:100 and 1:1000 dilution (with APW). Tubes were incubated 20 h at 41 °C (with a negative control per series at 4 °C). The number of total potentially pathogenic *Vibrionaceae* bacteria culturable at 41 °C (selection of *Vibrionaceae*~~s~~) was estimated using Mac Grady tables. A link between MPN in mussels and in water was sought by performing a linear regression using Statistica 6.1 (StatSoft, Inc.).

Figure 3. Principal Component Analysis (PCA) based on the presence/absence of the seven *Vibrionaceae* species in water samples. The purpose was to ordinate the samples and project

them onto a “best-fitting” plane (delimited by two or more principal components = axes) explaining as much of the variation in the data as possible. Species projected with the longest vectors (*V. parahaemolyticus*) are species that structure the data the most. Variables with vectors projected in the same plane may be considered as positively correlated. The analysis was performed with the Primer 6.1.11 software (Primer-E Ltd) according to Clarke and Gorley (2006) and Clarke and Warwick (2001).

Figure 4. Two-dimensional representation of the relative dissimilarities in *Vibrionaceae* species composition in the mussel samples. This graph is the result of a non-metric Multi-Dimensional Scaling (non-metric MDS using Primer 6.1.11 (Primer-E Ltd)) showing that the data are clearly structured (stress=0.01). Greater chlorophyll *a* (µg/l) values are represented on the figure by larger circles. Labels of non superposed circles corresponded to the date of the sampling (three first letters of the month) with a capital letter referring to the site (F=Filière W, C=La Carrelère and E=L’Eperon). Labels of the superposed circles are omitted for clarity; they correspond to all the remaining samples (11 samples for Filière W, 15 for l’Eperon and 11 for La Carrelère). For this analysis, the presence/absence matrix was transformed into a similarity matrix using Sørensen indices (Bray-Curtis index calculated on presence/absence data). Samples (in 2-D space) are represented as points in such a way that the relative distances separating all points are in the same rank order as relative dissimilarities of the samples as measured by the resemblance matrix. Points that are close together represent samples that are very similar in community composition (OctF and Jun-1C for example).