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Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll *a*

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Abstract:

Vibrio parahaemolyticus is one of the principal bacterial causes for seafood-borne gastroenteritis in the world. In the present study, three sites located on the French Atlantic coast were monitored monthly for environmental parameters over 1 year. The presence of total and pathogenic *V. parahaemolyticus* in sediment, water and mussel samples was detected following enrichment by culture and real-time PCR (toxR gene, tdh, trh1 and trh2 virulence genes). Using generalized linear models, we showed that the presence of *V. parahaemolyticus* in water could be explained by a combination of mean temperature over the 7 days before the day of sampling ($P < 0.001$) and turbidity ($P = 0.058$). In mussels, an effect of chlorophyll a ($P = 0.005$) was detected when an effect of the mean salinity over the 7 days before sampling was significant for the sediment ($P < 0.001$). We did not detect any significant effect of phytoplanktonic blooms or of the number of culturable bacteria on *V. parahaemolyticus* presence. No sample was revealed positive for tdh. The presence of trh1 and trh2 was positively influenced by the mean temperature during the 2 days before the day of sampling ($P < 0.001$ and $P = 0.032$). The importance of these ecological parameters is discussed in relation to the biology of *V. parahaemolyticus.*

INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative halophilic bacterium occurring naturally in aquatic environments worldwide. Its abundance in water is seasonally variable, reaching its maximum in summer when temperature is at its highest. The seasonal cycle of *V. parahaemolyticus* in the sediment, water and plankton of Chesapeake Bay (USA) was first reported by Kaneko and Colwell (1973; 1978). The bacterium might survive in sediments during the winter and be released into the water column in late spring or early summer when the temperature rises to 15 °C. Due to their filter-feeding activity, molluscs concentrate bacteria in their tissues. The consumption of raw or undercooked contaminated seafood (and, more rarely, the contact between a wound and contaminated water) may lead to the transmission of bacteria to humans. However, only bacteria producing virulence factors, i.e. thermostable direct haemolysin (TDH) and/or TDH-related haemolysin (TRH), are considered to be pathogenic and can cause acute gastroenteritis (or, more rarely, invasive septicaemia) (Nishibuchi *et al.*, 1995). Since 1996, the incidence of *V. parahaemolyticus* infections has increased dramatically from sporadic cases to large-scale outbreaks in America and epidemics in India and Asia (Matsumoto *et al.*, 2000). This increase in incidence seems to be related to the emergence of a new pandemic clone, belonging to the O3:K6 serovar. In Europe, the risk of *V. parahaemolyticus* infections is considered to be very low (European Commission, 2001; Su and Liu, 2007). Consequently, the monitoring of this microorganism has not been included in the most important European infectious disease surveillance networks. However, validation of the ISO standard (ISO/TS 21872-1 and -2, 2007) and the preparation of a draft code of hygienic practice for *Vibrio* spp in seafood (Codex Committee on Food Hygiene, *Codex Alimentarius* Commission 2008) could contribute to the monitoring of this bacterium in shellfish and water for risk assessment purposes (WHO-FAO, 2005). In France, the temperature of coastal water tends to increase (this is monitored by IFREMER; see

http://wwz.ifremer.fr/envlit/) and the O3:K6 serovar was shown to be present and persistent (Quilici *et al.*, 2005). However, apart from two outbreaks caused by imported shrimps (44 patients, Lemoine *et al.*, 1999) and mussels (100 patients, Hervio-Heath *et al.*, 2005), only sporadic cases have been reported (Quilici *et al.*, 2005) and there has only been one case of mortal septicaemia (Quilici *et al.*, 2004). A few environmental studies have shown the presence of pathogenic strains in French coastal areas; *tdh*+ samples were never detected but the prevalence of *trh*+ strains ranged between 3 and 10 % (Hervio-Heath *et al.*, 2002; Robert-Pillot *et al.*, 2004). With a national production of 60 000 t per year, France is the third largest mussel-producing country in Europe after Spain (250 000 t) and The Netherlands (100 000 t) (Ifremer, 2006). Pertuis Breton (Poitou-Charentes Region) is an important area for this activity economically, as it represents the second largest site for mussel culture in France (production ranging from 7 000 to 9 000 t). Moreover, it provides most of the young mussels to be grown on in Brittany and Normandy, two other French regions that do not produce spat (Dardignac-Corbeil, 2004). Poitou-Charentes is one of the two French regions where the highest number of vibrioses is reported (Quilici *et al.*, 2004).

Numerous authors worldwide have become interested in the identification of the causative or associative factors that affect *V. parahaemolyticus* incidence, but no such study had been made in France. The distribution of total coastal *V. parahaemolyticus* populations is influenced by environmental factors including temperature in temperate zones (Duan and Su, 2005; Parveen *et al.*, 2008), turbidity (Blackwell and Oliver, 2008; Parveen *et al.*, 2008), salinity (Martinez-Urtaza *et al.*, 2008) and factors related to plankton (planktonic species, plankton detritus, or other particulate matter trapped during plankton hauls) due to attachment and/or a chitinoclastic activity (Kaneko *et al.*, 1973). Similarly, climate, especially increasing temperature, has been shown to impact other pathogenic *Vibrionaceae* as *V. vulnificus* and *V. cholerae* diseases (Colwell, 1996; Lipp *et al.*, 2002; Paza *et al.*, 2007; de Magny *et al.*, 2008).

In particular, *Vibrio cholerae* has been extensively studied and associations with chlorophyll a, rainfall and temperature have been shown **(**de Magny *et al.*, 2008). In oysters, temperature, turbidity and dissolved oxygen were also positively correlated with *V. parahaemolyticus* densities (Parveen *et al.*, 2008). All of these studies evaluated the links between environmental factors and total *V. parahaemolyticus* but, as recently reported by Drake *et al.* (2007), less is known about pathogenic strains in shellfish or water.

The objectives of the present study are first to determine the incidence of total and pathogenic *V. parahaemolyticus* in mussels, water and sediment in the Pertuis Breton, and second, to investigate the relationships with either abiotic (water temperature, salinity, turbidity, dissolved oxygen, chlorophyll *a*) or biotic parameters (phytoplankton blooms, number of culturable bacteria).

RESULTS AND DISCUSSION

Description of the data

All environmental (water temperature, salinity, turbidity and cholorophyll a) parameters showed temporal variation during this one-year study (see Fig. 1). All of these parameters are presented in Figure 1. Technical problems with sensors (battery or biofouling) led to a few gaps in water temperature and salinity data. Measurements of dissolved oxygen were insufficient in number (only 18 observations) for the variable to be included in the statistical analyses. The monitoring of phytoplankton showed blooms of *Pseudo-nitzschia* sp, *Chaetoceros* sp, *Skeletonema costatum* and *Leptocylindrus* (*danicus* and *curvatulus*), especially in May-June. All of these data were normalized (square root or log transformation) before statistical analysis.

Overall, total *V. parahaemolyticus* was present in mussels from May to October (present during winter at l'Eperon and in December-February and March in La Carrelère), in water

from June to October and in sediment from June to December (see Fig. 2 for details). No effect of sampling site was detected ($P = 0.381$) but the source effect was significant ($P = 0.035$). Data from the different sites were thus pooled but considered separately for mussels, water and sediment in the statistical analyses concerning the presence of total *V. parahaemolyticus*. When we considered the presence of pathogenic *V. parahaemolyticus*, *tdh* was not detected in any of the 483 *toxR* positive (*toxR+*) samples, which is in agreement with the other field studies conducted in France (Hervio-Heath *et al.*, 2002; Robert-Pillot *et al.*, 2004). *trh1* was detected in *toxR+* samples of mussels and sediment but never of water. The presence of the *trh2* gene was almost detected in all sites depending of the time. All of theses results are presented in Fig. 3. Based on the lack of substantial differences for the presence of *trh1+* or *trh2+* samples (presence of pathogenic *V. prahaemolyticus*) between the sources of samples and/or sampling sites (all $P > 0.400$), virulence data were pooled for the general linear models. Among the *toxR+* samples, 24.64 % (119/483) were *trh+*: 11/483 *trh1+* with 0/223 isolated strains and 113/483 *trh2+* with 34/223 isolated strains (15.25%), a few samples were simultaneously *trh1+* and *trh2+*. This prevalence is similar (considering isolated strains only) or a little greater (considering RT-PCR on DNA directly extracted from enriched samples and from strains) than those reported in previous short studies on the situation in France (3-15 %, see Introduction).

The influence of abiotic parameters on the presence of total V. parahaemolyticus *depends on the source (compartment)*

In water, the best model (minimal AIC = 31.851, $P < 0.001$, $R^2 = 0.506$) explaining the presence of total *V. parahaemolyticus* was composed of mean temperature over the seven days before the date of sampling, hereon referred to as *Temp-7* ($P < 0.001$), and turbidity ($P = 0.058$) (see Table 1). More than half of the variance ($R^2 = 0.506$) in presence-absence of total

V. parahaemolyticus in water could be attributed to differences in water turbidity and *Temp-7*. Increasing temperatures are linked to a higher risk of encountering *V. parahaemolyticus* in water (mean temperature when total *V. parahaemolyticus* were detected was 20 °C, compared with 13°C when they were not; Fig. 4). The same effect of temperature has been frequently observed in previous studies (Cook *et al.*, 2002; Blackwell and Oliver, 2008; Parveen *et al.*, 2008) and directly linked to the growing capacities of this bacterium. In the USA, the Gulf Coast has the warmest mean water temperature (22 °C, although it can reach 30 °C) and was seen to have the highest mean *V. parahaemolyticus* levels (11000 CFU/100 g oysters, 44/100 ml water), while the Pacific Coast presents the coldest water temperature and has lowest levels of *V. parahaemolyticus* (2100 CFU/100 g oysters, 2 CFU/100 ml water). The direct relationship between *V. parahaemolyticus* and water temperature seems to determine its geographical distribution in the USA, Europe and Asia (Igbinosa *et al.*, 2008). In the present study, we tested the effect of mean temperature calculated over a longer or shorter period (one, two and seven days before the sampling) and we found that a seven day-period allowed a better prediction of the presence of the bacteria. This makes sense, considering the time needed for the growth of *V. parahaemolyticus* once favourable temperature conditions arise. Turbidity, although clearly less important, was also often linked to the bacteria presence because nutrients levels were higher (when waters are more turbid and richer in organic matter) stimulating bacterial growth or attachment to sediment particles, or reducing the penetration of solar radiation (Jones and Summer-Brason, 1998; Blackwell and Oliver, 2008; Parveen *et al.*, 2008).

In sediment, the best model (AIC = 21.86, $P < 0.001$, $R^2 = 0.420$) explaining the presence of *V. parahemolyticus* included only mean salinity over the seven days before sampling, *Sal-7*, ($P < 0.001$; Table 1). The second best model included *Temp-2* ($P = 0.034$) and *Sal-2* ($P = 0.106$, AIC of the model = 23.536, $P = 0.001$, $R^2 = 0.430$). A few studies have reported the

influence of environmental parameters on the occurrence of vibrios in sediment. Kaneko and Colwell (1973; 1978) mention an effect of temperature on the *Vibrio* sp life cycle over-winter in sediment. Blackwell and Oliver (2008) only detected a positive influence of pH (no influence of water salinity or temperature) on the occurrence of *V. vulnificus* in sediment but an effect of water temperature and turbidity (no impact of salinity) on *V. parahaemolyticus*. In the present study, we showed that the bacteria are found during a longer period in sediment than in water, but they are not detected year-round in sediment. Moreover, salinity of water estimated over several days seemed to have a greater influence than temperature on the presence of *V. parahaemolyticus* in sediment. A positive influence of water salinity on the occurrence of vibrios in water has often been detected when the range of variation of salinity levels is broad enough and the sample size sufficient (Cook *et al.*, 2002; DePaola *et al.*, 2003; Zimmerman *et al.*, 2007; Martinez-Urtaza *et al.*, 2008; Parveen *et al.*, 2008). The particular link that we found between salinity and presence of total *V. parahaemolyticus* in sediment may also be due to a direct influence of salinity and/or of freshwater inputs and other water perturbations. The higher the salinity of the sea water, the less important freshwater inputs become and the less the sea water would be perturbed by them. High salinity would, therefore, allow a more important sedimentation of bacteria. Salinity has been shown to influence the deposition of coliform bacteria in bottom muds of an estuarine system, thus prolonging their survival (Roper and Marshall, 1979).

In mussels, only chlorophyll *a* was found to have a positive effect on the presence of the bacteria ($P = 0.005$, AIC of the model = 28.160, $P = 0.013$, $R^2 = 0.149$). However, biotic parameters (culturable bacteria or phytoplanktonic blooms) were never found to have a significant effect explaining the presence of total *V. parahaemolyticus*, even in mussels. This could be due to links between chlorophyll *a* – phytoplankton – zooplankton, but unfortunately, we were not able to monitor zooplankton during this study to confirm this.

Vibrio species have previously been shown to be influenced by the presence and abundance of zooplankton due to attachment and/or the chitinoclastic activity of the bacteria (see the introduction). The influence of chlorophyll *a* detected in mussel samples is not due to the bloom of any of the phytoplanktonic species reported in this study, but rather to an overall effect or to the influence of zooplankton in relation to phytoplankton, linked in turn to chlorophyll *a*. Chlorophyll *a* in water, assessed with remote sensing, is already used to predict cholera epidemics (de Magny *et al.*, 2008). It also seems to be able to explain some of the variation in *V. parahaemolyticus* levels in oysters (Phillips *et al.*, 2007).

Influence of mean temperature recorded prior to sampling on the presence of pathogenic V. parahaemolyticus *(detected in all* toxR+ *samples)*

The presence of pathogenic *V. parahaemolyticus* is mainly positively linked to past temperature *Temp-2* ($P < 0.001$, AIC for the model = 22.920, $P = 0.031$, $R^2 = 0.047$ for *toxR+ trh1+* samples and $P = 0.032$, AIC for the model = 38.635, $P = 0.011$, $R^2 = 0.017$ for *toxR+ trh2+* samples; Table 1 and Fig. 3). The models are highly significant but only explain a small part of the variance (<5%), suggesting that the major influencing factor was not found. Contrary to the model selected for the presence of total *V. parahaemolyticus* in water, *Temp-2* was a better explaining factor than *Temp-7* for pathogenic bacteria. This may be due to a faster growth of pathogenic strains than non-pathogenic ones or simply because the lowest variations in temperature occurred during summer (when pathogenic strains are more frequent) making it unnecessary to calculate the mean over as long a period as seven days. Moreover, even though several parameters influenced the presence of total *V. parahaemolyticus*, only one (*Temp-2)* had an impact on the presence of pathogenic strains. This raises two important points: although total *V. parahaemolyticus* data may be very informative and easier to predict (greater R^2), it is worth studying bacteria that are more

pathogenic as (1) according to Zimmerman *et al.* (2007) there are differences in predicting total and pathogenic *V. parahaemolyticus* and (2) there is a strong disadvantage of being a pathogenic strain during cold periods because pathogenic strains should have optimal growth at warm temperatures (at least 37°C for a human body) to be pathogenic for humans. Considering the three sites, *V. parahaemolyticus* was always detected in at least one of the samples even during cold months. This suggests that *V. parahaemolyticus* is always present in the environment and simply spatially redistributed between the different sources (shellfish, sediment and water). No difference was detected between sources either for the presence (see above) or the proportions of pathogenic strains (contingency table, all $P > 0.05$). This confirms the equal role of these different sources in the maintenance of pathogenic *V. parahaemolyticus*, as recently proposed by Johnson *et al.* (2009) on genetic grounds.

This study is based on qualitative data, but it would now be better to work with quantities of total and pathogenic bacteria measured directly in the samples (without any enrichment step) using quantitative PCR. Thus, methods for concentration (filtration) of materials and DNA extractions need to be further developed and validated. This would remove any potential culture bias, to enable us to detect more *V. parahaemolyticus* cells and to study how the bacteria are spatially and temporally structured using a genetic approach (multilocus sequence analysis; Johnson *et al.*, 2009). Further observations would contribute to the development of predictive models to evaluate proliferation of pathogenic species and thus, help to characterize risk periods for the benefit of public health.

EXPERIMENTAL PROCEDURES

Sampling sites and collection of samples

Pertuis Breton is located on the French Atlantic coast close to La Rochelle (Fig. 5). It represents an area of 350 km^2. The fresh water input from the Le Lay and La Sèvre Niortaise

rivers is relatively low (flow around 100m^3/s in winter) (Dardignac-Corbeil, 2004). Climate in the region is oceanic with mean annual temperature around 12 °C (from –6 to 36 °C, data from the Poitou-Charentes Regional environmental observatory http://www.observatoire-environnement.org/tbe/Climat.html).

Water (N = 16 x 3 sites = 48) and mussel *Mytilus edulis* (N = 48) samples were collected monthly (twice monthly between May and August, noted "Month-1" and "Month-2") from April 2008 to March 2009 from the three selected sites (L'Eperon, La Carrelère and Filière W, see Fig. 5) by the Laboratoire Environnement Ressources du Pertuis Charentais (LER/PC) IFREMER. Sediment samples (N = 12 x 2 sites = 24) were collected monthly during the same period in La Carrelère and L'Eperon (at low tide) but not in Filière W where the depth was too great and the mussels are never out of the water. Mussels were sampled from "bouchots" (wooden stakes) at La Carrelère and L'Eperon and on lines at Filière W. Water samples were collected just below the surface. All microbiological analyses were initiated within 20-24 h of sample collection following transportation to IFREMER Brest (transport in coolers at ~12 °C).

Environmental parameters were measured regularly by IFREMER LER/PC (between zero and two days before or after the sampling on average) for the REseau de surveillance du PHYtoplancton (REPHY) database (Ifremer phytoplankton monitoring network). Phytoplanktonic data concerned the monitoring of toxic species such as *Pseudo-Nitzschia*, *Alexandrium* and *Dinophysis*, but also the blooms of all other species (> 100 000 cells / l). Abiotic parameters were measured simultaneously in the upper 0.5 m of the surface water: chlorophyll *a* (µg / l), turbidity (in formazin nephtelometric units = FNU) and dissolved oxygen (mg / l, measured from June to September only) were measured. Temperature (in °C)

and salinity (no unit) were measured every 15 minutes by STPS sensors (NKE, France) placed in the proximity of the sampling points.

Preparation of samples

Mussels were washed, opened using a sterile scalpel and emptied of sea water. All of the following steps were done in sterilized conditions, with sterile instruments. Around 200 g of flesh were collected per site. Liquid (hemolymph) and solid (remaining flesh) phases were analysed separately for another experiment. As no difference was found between the two matrices, final results were pooled as "mussel" in the present study. The solid phase was then blended (3 x 20 sec. at high speed in a sterile Warring® Blender) and diluted 1:2 with sterile alkaline peptone water 1% NaCl (APW, 20g Bacto™ peptone Becton Dickinson and Co, 10 g NaCl per liter, pH 8.2). Eight hundred ml water from each site were filtered (nitrocellulose 0.22 µm, Millipore). Filter contents 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). For sediment samples, 20 g were 1:2 diluted with APW 1% NaCl.

Detection of V. parahaemolyticus

Direct culture and enrichment. Three filtrations of 10 ml, 50 ml and 100 ml water (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 (=MA) (Becton Dickinson and Co.), Difco™ Thiosulfate Citrate Bile Salts Sucrose agar (=TCBS) (Becton Dickinson and Co.), and CHROMagar™ Vibrio (=CA) (CHROMagar Paris France). One filter was deposited per plate (three plates / culture medium /site). For each site, 0.1 ml of hemolymph and 0.1 ml of the following two 10-fold serial dilutions with buffered physiological water (BPW, 4.52 g Na_2HPO^4, $12H_2O$, 0.4g KH_2PO_4, 7.2 g NaCl per liter) were

spread plated onto TCBS, MA and CA. We proceeded similarly for the 1:2 mussel and 1:2 sediment homogenates. All the plates were incubated 24 h at 37 °C.

Undiluted hemolymph, re-suspended filter contents, a 1:10 dilution (APW) of the 1:2 mussel homogenate and of the 1:2 sediment homogenate, were studied using the Most Probable Number method (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 (a negative control per series is placed at 4°C). The number of total halophilic bacteria culturable at 41 °C (selection of *Vibrionaceae*) was estimated using Mac Grady tables (Santé Canada, 1993). Bacteria were isolated from 10 µl of one positive tube per series on TCBS, MA and CA (incubation of one plate / medium during 24h at 37°C). No enumeration was done for sediment, but 6 ml of 1:2 diluted sediment were enriched in 24 ml of APW. After 20h at 41 °C, 100 µl of 10^{-4}, 10^{-5} and 10^{-6} dilution with BPW were spread-plated onto TCBS, MA and CA and incubated 24h at 37°C.

Biochemical identification. One colony of each morphotype per source/culture medium/site (up to three colonies) suspected to be *V. parahaemolyticus* (green on TCBS, purple on CA, white on MA) was isolated twice on Bacto™ Heart Infusion agar with 2 % NaCl (=HI) (Becton Dickinson and Company) and incubated at 37 °C. Oxidase disks and Api20E system (BioMerieux, France) were used for the biochemical identification of presumed *V. parahaemolyticus* strains.

DNA extraction and detection of total and pathogenic V. parahaemolyticus *by real-time PCR.* DNA from oxidase positive strains was extracted from a 10 µl inoculation loop washed twice with BPW (centrifugation 10 min at 5900 g). The pellet was suspended in 100 µl of DNase, RNase, protease-free water (5-Prime inc.), heated 15 min at 100 °C for cell lysis and

centrifuged 10 min at 5900 g at 4 °C. The lysate supernatant fluid was transferred to a new 0.5 ml tube.

Ten ml of enriched sediment were filtered (nitrocellulose 8µm, Millipore) and centrifuged 15 min at 2500 g. The pellet was suspended in 2 ml of BPW and centrifuged 5 min at 5900 g before the heating at 100 °C and performing the following steps.

Two ml of each MPN positive tube were centrifuged (10 min 5900 g). DNA was extracted as described above for strains. All DNA extractions were kept at -20 °C until real-time PCR (RT-PCR). DNA were tested for the presence of total and pathogenic *V. parahaemolyticus* using respectively *toxR* gene and *tdh*, *trh1* and *trh2* virulence gene detection (patent in process) by RT-PCR on a MX3000P™ (Stratagene). Each *toxR*+ sample was considered to be positive for the presence of *V. parahaemolyticus* and tested for the presence of the virulence genes.

Statistical analyses

We investigated the relationships between the environmental parameters measured and the presence of total (*toxR*+) and pathogenic (*tdh*+, *trh1*+ or *trh2*+) *V. parahaemolyticus* samples for each site and sampling session (binomial dependant variable: presence / absence). The dataset (overall pooled data) was tested for the effect of sampling site (Filière W, L'Eperon and La Carrelère) and source (mussel, water and sediment) using a logistic regression. We used generalized linear models with a logit function to test for the influence of (1) abiotic parameters (quantitative data): mean temperature (on the day of sampling itself, or estimated over the two preceding days = *Temp-2* or seven preceding days = *Temp-7*) × mean salinity (on the day of sampling, or estimated over two preceding days = *Sal-2* or seven preceding days = *Sal-7*) × turbidity × chlorophyll *a* × dissolved oxygen; and (2) biotic parameters: blooms of phytoplanktonic species (qualitative data) and number of culturable bacteria estimated by the

MPN method in mussels and water (quantitative data). The interactions between all of the variables in each model were investigated. We used an exhaustive approach, testing all the interactions between variables, and searched for the most parsimonious (the least number of variables but most explanation of results) using the Akaike Information Criterion (AIC) (Akaike, 1974; Burnham and Anderson, 1998). All analyses were based on Legendre and Legendre (1998) and performed with Statistica 6.1 (StatSoft, Inc.).

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TABLE AND FIGURE LEGENDS

Table 1. Description of the models selected to explain the presence / absence (P/A) of total and pathogenic (presence of *trh1* or *trh2* genes) *Vibrio parahaemolyticus* (=Vp). *Sal-y* or *Temp-y* = mean salinity or temperature estimated over *y* days before the sampling.

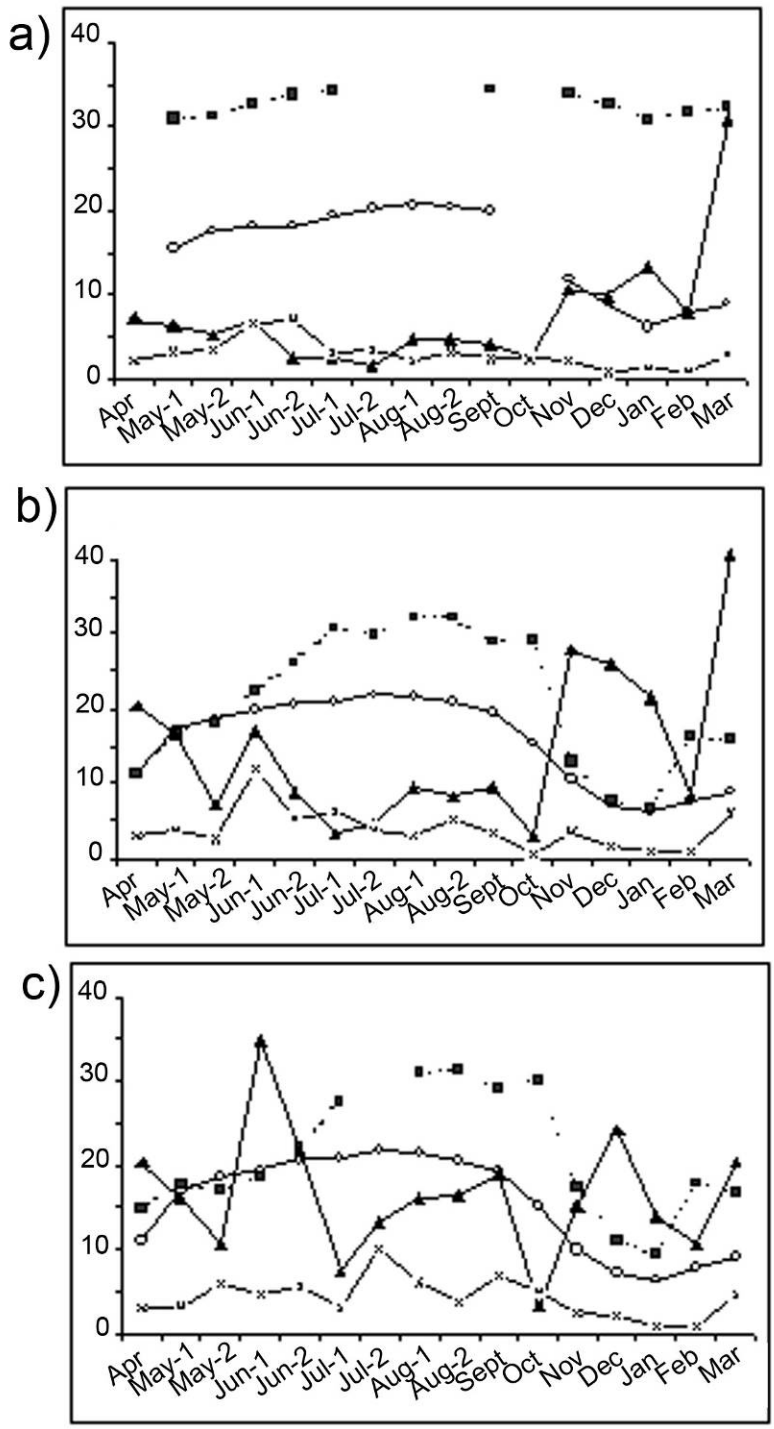
Variable to be explained	**Variables included in the selected model**	**Log likelihood**	χ^2	***P*-value**
P/A Vp in water	*Temp-7*	-41.155	40.309	< 0.001

	Turbidity	-21.800	3.591	0.058
P/A Vp in sediment	*Sal-7*	-18.094	15.187	< 0.001
P/A Vp in mussels	Chlorophyll a	-27.017	8.034	0.005
P/A *trh1* gene in *toxR*+ samples	*Temp-2*	-23.102	13.205	< 0.001
P/A *trh2* gene in *toxR*+ samples	*Temp-2*	-18.811	4.623	0.032

1

2

Figure 1. Variation of the abiotic parameters: mean temperature (white circles) and salinity (black squares) of the day of sampling, turbidity (black triangles) and chlorophyll *a* (crosses). The study period (April 2008- March 2009) covered 16 sampling sessions (generally one sample per month year round and two per month between May and August) at a) Filière W, b) L'Eperon and c) La Carrelère. Gaps in temperature and salinity measurement are due to technical problems with sensors.


a)
b)
c)
40
30
20
10
0
Apr
May-1
May-2
Jun-1
Jun-2
Jul-1
Jul-2
Aug-1
Aug-2
Sept
Oct
Nov
Dec
Jan
Feb
Mar

1

2

Figure 2. Presence (P) / Absence (A) of total *Vibrio parahaemolyticus* in mussels (full black lines, squares), water (dotted grey lines, circles) and sediment (full grey lines, small triangles) per date of sampling at a) Filière W, b) L'Eperon and c) La Carrelère.

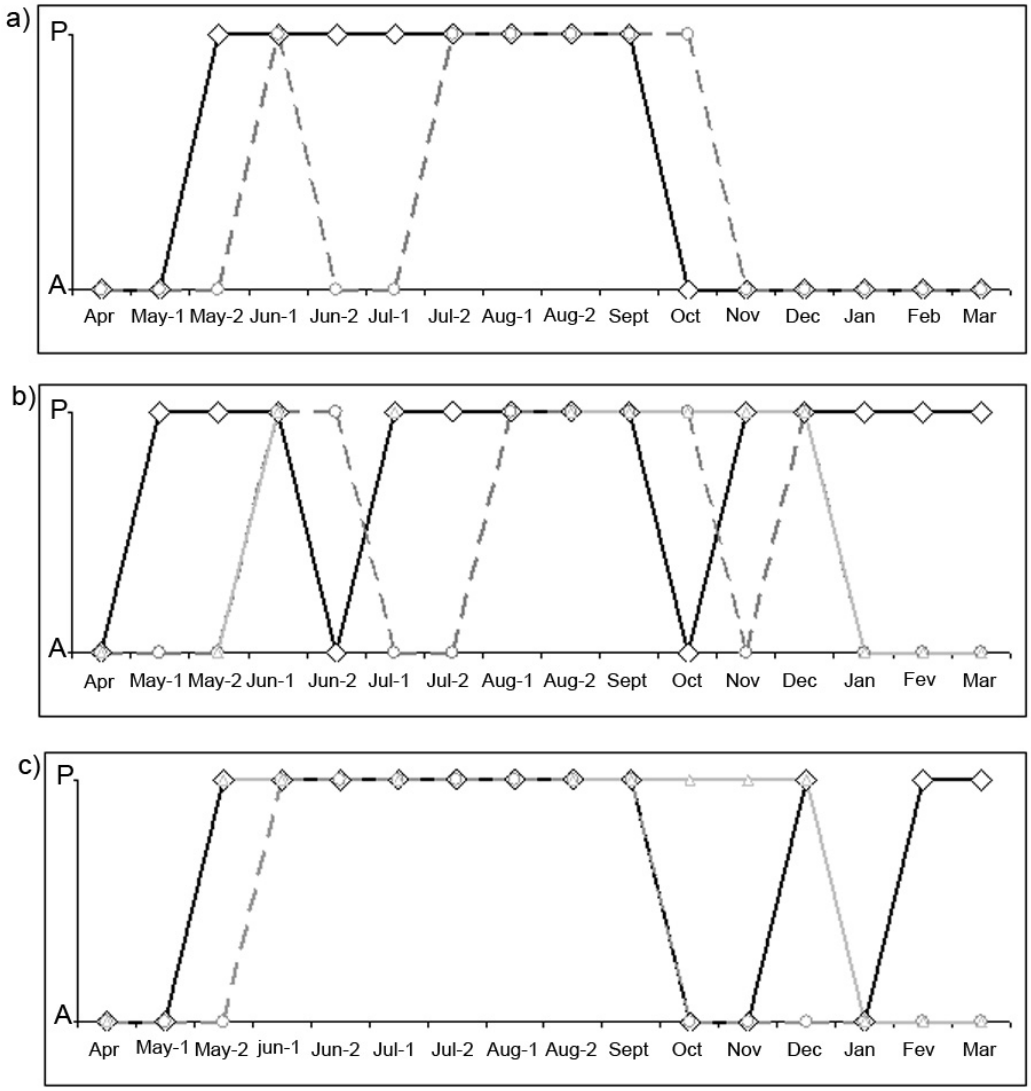
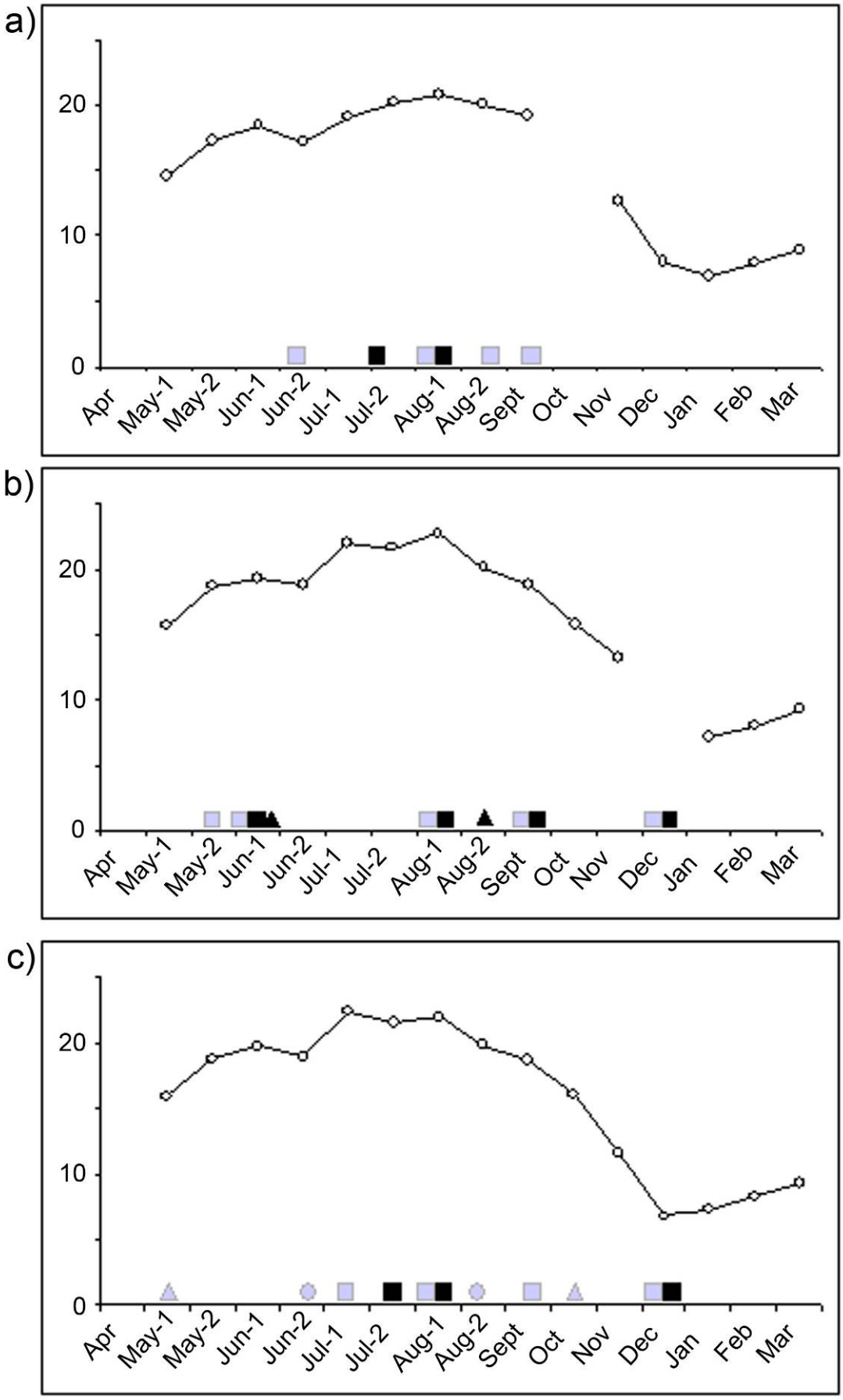


Figure 3. Variation of mean water temperature (°C) estimated over the two days before the day of sampling (*Temp-2*) at a) Filière W, b) L'Eperon and c) La Carrelère. Presence of pathogenic (*trh1+* in black, *trh2+* in grey, no *tdh+*) *Vibrio parahaemolyticus* in mussels (squares), water (circles) and sediment (triangles) are indicated on the graphs. Gaps in temperature measurements are due to technical problems with sensors.


a)
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Aug-1
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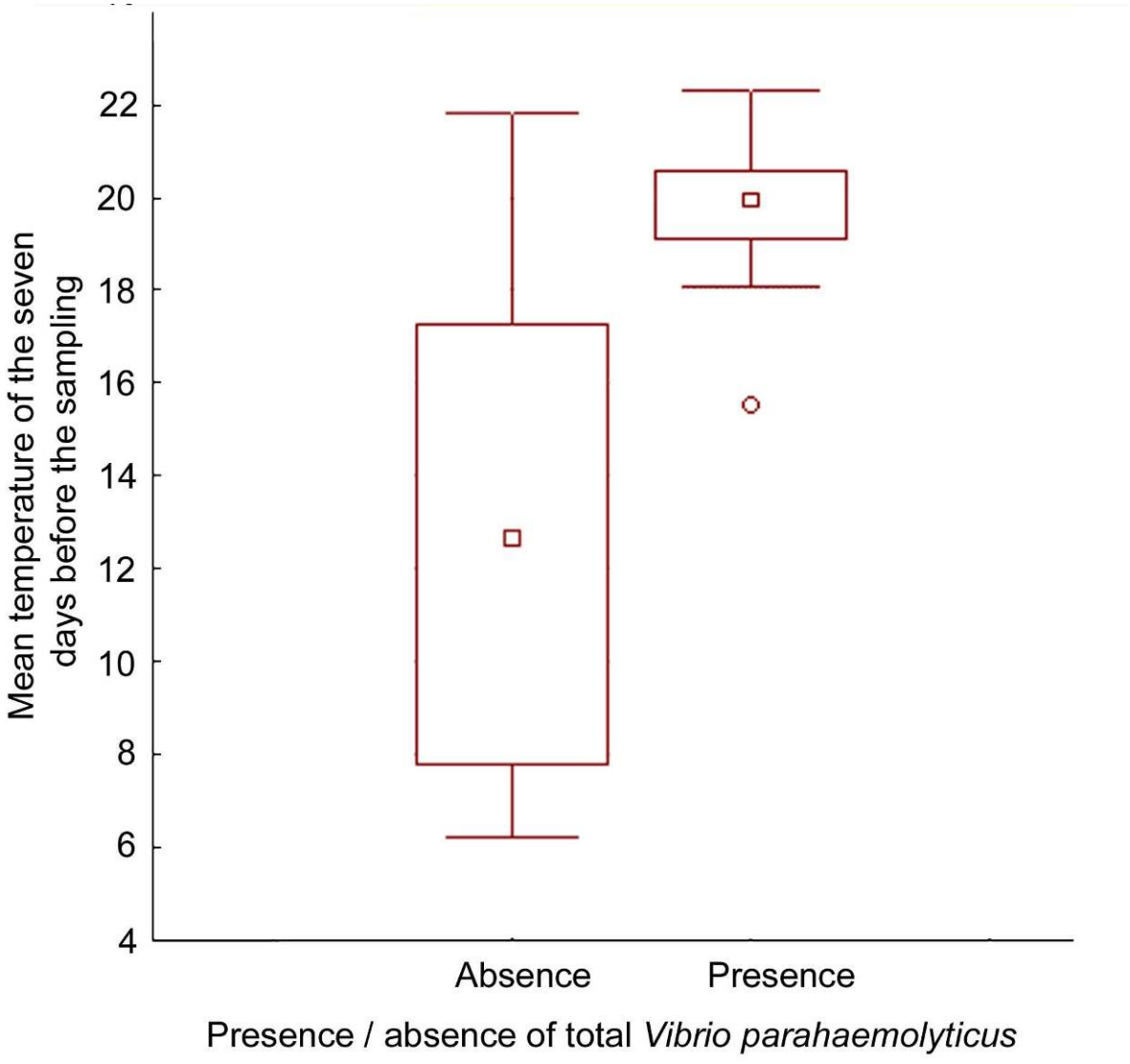
Figure 4. Positive relationship between the presence and absence of total *Vibrio parahaemolyticus* in water and mean water temperature over the seven days before sampling (*Temp-7* in °C). Squares represent the median of the data, box-plots show 25 % to 75 % of the data (boxes) and horizontal traits, how minimal and maximal values are distributed. Circles represent atypical points (the detection of total *V. parahaemolyticus* in October 2008 at L'Eperon).



1 Figure 5. Map of the Pertuis Breton showing the locations of the sampling sites and sensors.

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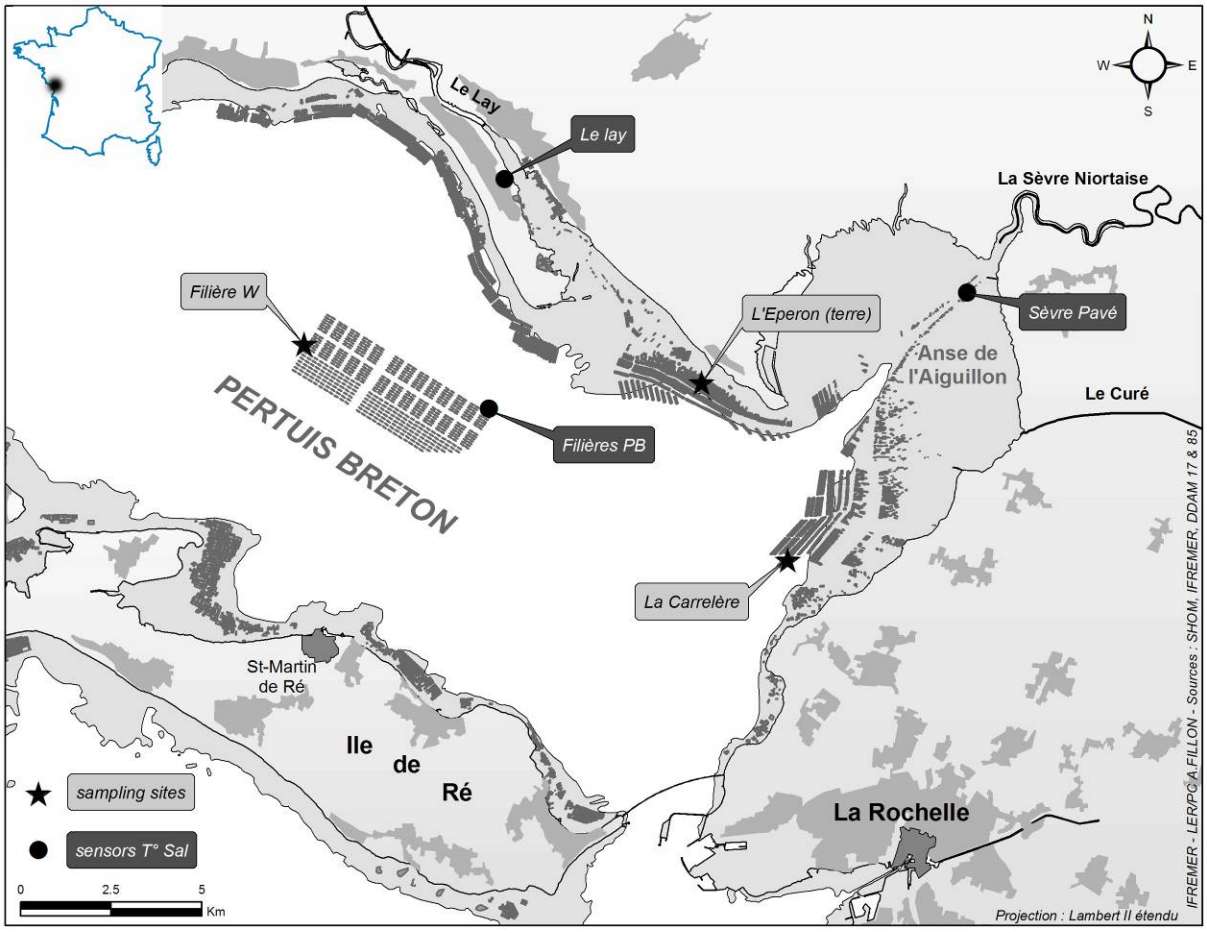

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