Impact of weather conditions on *Escherichia coli* accumulation in oysters of the Thau lagoon (the Mediterranean, France)

V. Derolez¹,*, D. Soudant², A. Fiandrino¹, L. Cesmat¹, O. Serais¹

¹ Ifremer, Laboratoire Environnement Ressources Languedoc-Roussillon, Sète Cedex, France
² Ifremer, Laboratoire DYNECO-VIGIES, Nantes Cedex 03, France

*: Corresponding author : Valérie Derolez, email address : vderolez@ifremer.fr

Abstract :

Aims : The Thau lagoon shellfish are regularly impacted by microbial pollution of faecal origin, which cause European health standards to be exceeded and closure of the shellfish harvest. The aims of this study were to investigate the impact of weather and hydrological conditions on *Escherichia coli* (*E. coli*) accumulation factor (AF) between water and oysters and to evaluate the relevance of the use of this ratio for the purpose of sanitary risk assessment.

Methods and results : Water and oysters (*Crassostrea gigas*) were sampled simultaneously in situ during 18 months in periods of dry weather and after rainfall events. Shellfish sanitary thresholds were exceeded in both periods. The *E. coli* AFs measured after rainfall (median = 6) were lower than in dry weather (median = 32), suggesting different shellfish faecal contaminations were operating in this system process.

Conclusion : The AFs we measured appeared to be relevant markers for generating sanitary risk assessments for Thau lagoon shellfish.

Significance and impact of the study : The results of the study address the need to assess the relationship between water quality measures and shellfish flesh quality. This study will contribute to the elaboration of a management tool to guide local authorities in prioritizing the sources of pollution and in optimizing public investment in the watershed.

Keywords : *E. coli* ; environmental health ; food safety ; shellfish ; water quality

1. Introduction

With up to 13 000 tons of oysters (*Crassostrea gigas*) and 2500 tons of mussels (*Mytilus galloprovincialis*) marketed every year, the Thau lagoon is the main shellfish harvesting area in the Mediterranean. Filter-feeding bivalves, such as oysters, filter large volumes of water from their environment and are able to concentrate large numbers of particles, which include micro-organisms pathogenic to human consumers (Bernard 1989; Plusquellec et al. 1990). According to European sanitary rules, the *Escherichia coli* concentrations found in Thau shellfish correspond to a Class B area. As a result, shellfish must be depurated before they can be marketed. Because Thau lagoon shellfish regularly exceed current health standards, authorities periodically restrict the shellfish harvesting area and, in some cases, suspend production. Moreover, several enteric virus outbreaks due to Thau lagoon oysters occurred during winter gastroenteritis epidemics in the local population (Le Saux et al. 2009).

Many studies have shown the impact of rainfall on microbial water quality in coastal and estuarine areas. Contamination due to rainfall most often results from urban wastewater discharges or from nonpoint pollution sources in the watershed (Lipp et al. 2001; Chigbu et al. 2005; Jeng et al. 2005; Coulliette and Noble 2008; Papastergiou et al. 2009; Chu et al. 2011; Conn et al. 2012). Microbial contamination can also occur in dry weather conditions, in which faecal material from seabirds resting on shellfish farming structures is the likely main
European regulations recommend monitoring coliform bacteria (e.g., *Escherichia coli*) in shellfish flesh to assess the extent to which shellfish growing areas are exposed to fecal pollution (Anonymous, 2004; Anonymous, 2006). USA or Australian regulations rely on the enumeration of indicator bacteria in water (Ogburn and White, 2009). The need to assess more thoroughly the relationship between water quality measures and shellfish flesh quality has been recently raised by international experts (Rees *et al*., 2010). To address this issue, several attempts have been made to model the ecophysiological accumulation of *E. coli* in shellfish growing areas (Fiandrino *et al*., 2003; Martins *et al*., 2006). However, most of the authors have assumed constant accumulation factors to evaluate shellfish microbial concentrations based on the microbial concentrations of the surrounding water (Pommepeuy *et al*., 2004; Riou *et al*., 2007; Bougeard *et al*., 2011) and on ratios between the concentrations of microorganisms in shellfish and in water, obtained from experiments performed under controlled conditions in laboratories (Cabelli and Heffernan, 1970; Lees *et al*., 1995; Burkhardt and Calci, 2000).

We sampled water and oysters (*C. gigas*) simultaneously for 18 months during periods of dry weather and after rainfall events to evaluate accumulation factors between water and the oysters in the Thau lagoon, to investigate the impact of weather and hydrological conditions on this ratio and to evaluate whether the use of this ratio could be a relevant approach for assessing sanitary risk associated with shellfish consumption.

**Materials and methods**

The Thau lagoon has an area of 75 km² and is located in the South of France. It has a drainage area of 230 km² and it is connected to the Mediterranean Sea via two outlets (Fig. 1).
In three shellfish farming zones, oysters and mussels are fixed to ropes between three and ten meters in length, which are suspended under breeding structures made of wood or metal. Shellfish remain constantly immersed.

Sampling investigations

The lagoon and its watershed were monitored for 18 months, from September 2007 to February 2009. Five rain gauges, located in the watershed, collected rainfall data at intervals of 15 minutes. Rainfall is expressed in mm, as the depth of water that collects on a flat surface.

During this period, *C. gigas* oysters and water were sampled weekly at stations 2, 3, 4 and 5 (Fig. 1). During the four main rainfall events, hereafter referred as periods R1 to R4, six to 21 sites were sampled daily or semi-daily (Table 1) to monitor the impacts of these events on the lagoon microbial content. Additional samples were also collected in dry weather conditions on four occasions in 2008 to monitor the impact of seabirds roosting at night on the shellfish structures on the lagoon microbial content (see Derolez *et al.* (2010) for the detailed sampling strategy).

Oyster samples and water samples were each collected simultaneously, at one meter below the surface and at one meter off the bottom. Twelve oysters of a commercial size were collected in plastic bags for microbial analyses and were kept at temperatures between 2°C and 15°C before analyses. Water samples were collected in 500 mL sterile bottles and kept at temperatures between 1°C and 4°C before analyses. Oyster and water samples were analysed within 24 hours, according to NF V08-106 (Afnor, 2002), ISO 9308-3 (ISO, 1998) and ISO 7218 (ISO, 2007).

Hydrological measurements
Lagoon salinity and temperature were monitored at the surface and the bottom of the water column every 10 minutes by high-frequency sensors located at stations 2 and 5 during the entire sampling period (Fig. 1). During rainfall periods R3 and R4, sensors were located at an additional site (station 4 and station 1, respectively).

Point-in-time measurements of salinity and temperature were obtained systematically from WTW LF197S sensors when water and oysters were collected (surface and bottom sampling).

Before and after rainfall events, 27 water samples were analyzed for concentrations of suspended matter following a standardized method EN/872 (CEN, 2005).

**Microbial analyses**

E. coli concentrations in water samples were analyzed using the ISO 9308-3 standard method, i.e. the most probable number (MPN), scaled down for inoculation into liquid culture medium (ISO, 1998). Samples of oysters were analyzed using the NF V08-106 standard method (Afnor, 2002). This is an indirect method of estimating E. coli in live bivalves using a biosensor to measure impedance (Dupont et al., 2004). For each sample, about six oysters were washed, scrubbed under clean running water and opened with a sterile shucking knife.

Approximately 100 g of flesh and intravalvular liquid (FIL) were diluted 1:3 with tryptone salt water (SW) and were homogenized in a Waring blender. The samples were diluted 1:3, inoculated into selective media (Malthus coliform broth (Malthus Instruments) + tryptone 1 g L⁻¹ + NaCl 8.5 g L⁻¹) and incubated at 44°C in a Bac Trac 4300 (Sy-Lab, Neupurkersdorf, Austria).

Detection limits of the two methods were 15 E. coli 100 mL⁻¹ for the water samples and 130 E. coli 100 g⁻¹ FIL for the oyster samples. Uncertainty of the standard measurement methods, calculated according to ISO 5725-2 standard (ISO, 1994) (collaborative interlaboratory experiments, unpublished data), were 0.5 log₁₀ E. coli 100 mL⁻¹ for the water
samples and 0.5 $\log_{10}$ $E. \text{coli}$ 100 g$^{-1}$ FIL for the oyster samples and were determined using the following formula:

$$10^{\log_{10}(X) - 0.5} \leq X \leq 10^{\log_{10}(X) + 0.5}$$

where $X = E. \text{coli}$ 100 mL$^{-1}$ or 100 g$^{-1}$ FIL.

**Data analyses**

According to the high spatial variability of rainfall on the watershed, rainfall data collected at the western part or at the eastern part of the watershed were combined and averaged. Dry weather periods were defined as periods having a cumulative rainfall of less than 15 mm for the previous seven days. Rainfall weather periods corresponded to a cumulative rainfall amount of 15 mm or more for the preceding seven-day period equal to or higher. This threshold was consistent with those proposed by Australian and American authorities for authorizing shellfish to be marketed in estuarine areas (Lipp *et al.*, 2001; Chigbu *et al.*, 2005; Kirby-Smith and White, 2006; Coulliette and Noble, 2008; Ogburn and White, 2009).

When $E. \text{coli}$ concentrations exceeded detection limits of the methods for both water and oyster samples collected simultaneously, the accumulation factor (AF) was calculated following the Cabelli and Heffernan (1970) formula:

$$AF = \frac{C_o}{C_w}$$

where $C_o$ = the $E. \text{coli}$ concentration in oysters in cells per 100 g FIL and $C_w$ = the $E. \text{coli}$ concentration in water in cells per 100 mL.

Non-parametric statistics were used for data analyses. A Mann-Whitney U test, with normal approximation of the p-values for datasets with less than 50 samples, or a Wilcoxon signed rank test for paired samples, were performed to compare the two datasets (dry weather and rainfall). Spearman's rank correlation coefficients were computed and tested to assess the dependence between two parameters.
Results

Effects of rainfall amount on hydrology and fecal bacterial contamination

During rainfall periods, the maximum value of *E. coli* concentrations in oysters was 8,700 *E. coli* 100 g\(^{-1}\) FIL and less than 10% of samples exceeded 4,600 *E. coli* 100 g\(^{-1}\) FIL (n = 464), corresponding to Class B quality according to European sanitary standards (EC/854/2004) (Fig. 2). During dry weather periods, two samples were above 46,000 *E. coli* 100 g\(^{-1}\) FIL, with a maximum value of 56,000 *E. coli* 100 g\(^{-1}\) FIL (n = 396), which corresponded to a downgrade classification. In dry weather conditions, although the *E. coli* concentrations in water samples reached 14,000 *E. coli* 100 mL\(^{-1}\), the geometric mean was 19 *E. coli* 100 mL\(^{-1}\) and the 90\(^{th}\) percentile was 45 *E. coli* 100 mL\(^{-1}\). These two latter values corresponded to those of restricted shellfish growing areas according to US standards (geometric mean below 88 *E. coli* 100 mL\(^{-1}\) and 90\(^{th}\) percentile below 260 *E. coli* 100 mL\(^{-1}\)) (US FDA, 2009). During rainfall periods, these thresholds were exceeded and attained levels that corresponded to prohibited areas (geometric mean = 47 *E. coli* 100 mL\(^{-1}\), 90\(^{th}\) percentile = 270 *E. coli* 100 mL\(^{-1}\) and maximum = 3,700 *E. coli* 100 mL\(^{-1}\)). European and US monitoring systems (Anonymous, 2004; US FDA, 2009) would thus lead to different management options for Thau lagoon shellfish growing area.

Forty-nine percent of the water samples and 48% of the oyster samples collected during the sampling period (n = 860) had *E. coli* counts below the detection limits of the standard methods. These percentages were higher during dry weather periods (72% and 61%, respectively; n = 396) than during rainfall periods (28% and 38%, respectively; n = 464). Only samples with *E. coli* counts above the detection limits for both water and oysters were kept for the further analyses (n = 239 for rainfall periods and n = 79 for dry weather periods).
The *E. coli* concentrations found in oysters did not correlate with those found in water samples for all the periods ($n = 318$, $\rho = 0.089$, $P = 0.113$), but this relationship became significant if the samples collected during rainfall periods ($n = 239$, $\rho = 0.150$, $P = 0.019$) or the dry weather periods ($n = 79$, $\rho = 0.372$, $P = 0.001$) were considered separately (Fig. 3).

Cumulative rainfall amounts for the seven days prior to sampling correlated negatively with salinity, temperature and *E. coli* concentrations in oysters, and correlated positively with *E. coli* concentrations in water (Table 2). Similar results were obtained if data collected during rainfall and during dry weather periods were compared using Mann-Whitney U tests (Fig. 2).

For water samples collected after rainfall events, suspended matter concentrations were between 2 mg L$^{-1}$ and 44 mg L$^{-1}$ in the shellfish farming areas. The amount of suspended matter increased significantly with decreasing salinity ($\rho = -0.44$; $P = 0.019$; $n = 27$).

During rainfall periods, salinity and temperature were lower at the surface than at the bottom, according to the Wilcoxon signed rank test ($n = 43$ pairs, $P < 0.0001$ for both parameters). Conversely, *E. coli* concentrations in water and in oysters were higher ($P = 0.008$ and $P = 0.009$, respectively) at the surface than at the bottom. This pattern, which was due to the vertical stratification of the water column after rainfall, was not observed for samples collected in dry weather conditions.

**Escherichia coli** accumulation factors between water and oysters

Relationships between the accumulation factor and hydrological variables

*E. coli* accumulation factors (AFs) were calculated for water and oyster samples collected simultaneously. AFs covered a wide range of values [0.1 - 406] (Fig. 2) for the study period, and had an overall mean value of 30.5 and a median of 9.1.
AF correlated negatively with the amount of rainfall in the seven days that preceded sample collection and correlated positively with salinity (Table 2). The AFs calculated for samples collected during rainfall periods were also significantly lower than the AFs calculated for samples collected during dry weather periods, according to the Mann-Whitney U test ($P < 0.0001$) (Fig. 2).

AF did not correlate significantly with lagoon water temperature. However, during rainfall periods, the AF median was 8.9 for temperatures higher than 15°C ($n = 23$) and was 5.7 for lower temperatures ($n = 208$). Conversely, during dry periods, the AF median was 22 for temperatures higher than 15°C ($n = 33$) and 44.1 for lower temperatures ($n = 46$).

The three hydrological variables correlated with each other: rainfall amounts with salinity ($n = 312$, $\rho = -0.36$, $P < 0.0001$); rainfall with temperature ($n = 310$, $\rho = -0.15$, $P = 0.009$) and temperature with salinity ($n = 310$, $\rho = 0.54$, $P < 0.0001$).

There was no effect of water depth on AF for the entire period, for dry weather periods or for rainfall periods.

**Daily variations in salinity and in the E. coli concentrations in water and oysters after rainfall events at stations 1 and 2**

Results of high-frequency salinity measurements and E. coli counts in water and oysters performed at stations 1 and 2 during the four rainfall events monitored are illustrated in Figure 4 (a-d). After the rainfall events, whether salinity remained stable or decreased significantly (-7 units for periods R3 and R4) (Figs. 4a-d) depended on the amount of rainfall (Table 1). The E. coli concentrations in the water ($C_w$) and the oysters ($C_o$) varied significantly from day to day, and even during the day, especially during periods R3 and R4.

Although rainfall amount and E. coli concentrations in water (geomean $C_w = 120$ E. coli $100$ mL$^{-1}$) were at their highest during period R3, the geometric mean of the E. coli concentrations in oysters was only 390 E. coli $100$ g$^{-1}$ FIL. In contrast, despite the lowest
rainfall amount and $C_w$ in R1, the geometric mean of $C_o$ reached 610 $E. coli$ $100$ $g^{-1}$ FIL in this period. Intermediate situations were observed for the respective geometric means of these values for periods R2 ($C_w = 70$ $E. coli$ $100$ $mL^{-1}$ and $C_o = 380$ $E. coli$ $100$ $g^{-1}$ FIL) and R4 ($C_w = 100$ $E. coli$ $100$ $mL^{-1}$ and geomean $C_o = 650$ $E. coli$ $100$ $g^{-1}$ FIL). These results led to high AF values for the rainfall periods R1 (AF = 11.7; $n = 16$) and to lower values for the rainfall periods R4 (AF = 7; $n = 82$), R2 (AF = 5.9; $n = 50$) and R3 (AF = 3.5; $n = 78$).

According to the AF formula, $C_o$ can be obtained by multiplying $C_w$ by AF. Considering rainfall and dry weather periods, median AF values (respectively 6 and 32) were used to evaluate $C_o$ and these were compared to measurements. Due to uncertainty of the standard measurement methods, the actual concentration in oyster is included in a computed interval. $C_o$ evaluations were included in these intervals for 89% of the samples collected during rainfall periods and for 94% of the samples collected during dry weather periods.

**Discussion**

Accumulation factors calculated according to Cabelli and Heffernan (1970) for the *Crassostrea gigas* oysters in our study (median = 9.1, $n = 318$) were in agreement with the values obtained from several experiments achieved under controlled conditions in laboratories: from 3.8 to 28 for *E. coli* in oysters (Burkhardt and Calci, 2000; Prieur et al., 1990; Shieh et al., 2003), from 6.5 to 12.5 for *E. coli* in *Mercenaria mercenaria* (Cabelli and Heffernan, 1970) and 9.8 for *E. coli* in *Mytilus edulis* (Plusquelllec et al., 1990). During our study, accumulation factor was shown to correlate with salinity, which is consistent with the observations of Hopkins (1936) and Prieur et al. (1990), who suggested that filtration efficiency is low when salinity is low, due to the closure of the bivalves. We obtained an insufficient number of values to test whether the accumulation factor related to the amount of suspended matter, but the latter increased significantly with decreasing salinity and occurred as
a consequence of turbid freshwater inputs to the lagoon. Suspended matter content in water is
known to affect the physiology of bivalves by reducing their filtration rates (Mane, 1975;
Plusquellec et al., 1990; Prieur et al., 1990). Whereas our dataset didn’t allow an effect of
temperature on accumulation factor to be clearly detected, Burkhardt et al. (1992) and
Campos and Cachola (2007) showed that bacterial accumulation by clams increased with
temperature. Other authors have reported that the optimal temperature range for the maximal
clearance and microbial accumulation rates of C. gigas (Bernard, 1989; Bougrier et al., 1995)
and Ostrea edulis (Solic et al., 1999) oysters is between 12°C and 19°C.

Our study suggests that the weather (rainfall or dry weather periods) was one of the main
factors influencing E. coli accumulation by C. gigas in the Thau lagoon. Rainfall led to higher
E. coli concentrations in lagoon water and was accompanied by decreasing salinity and
increasing turbidity. Low salinity and high turbidity lead to reduced clearance rates and to
consequently lower E. coli concentrations in oysters. These lower concentrations in oysters,
coupled with higher E. coli concentrations in the water lead logically to lower values of
accumulation factors. Temperature, season, food availability, reproduction cycles, size and
amount of light are known to impact the clearance rates of bivalves, corresponding to food
consumption and defined as the volume of water cleared of all 100% efficiently retained
particles per unit of time (Mane, 1975; Bougrier et al., 1995; Solic et al., 1999; Campos and
Cachola, 2007; Lopez-Joven et al., 2011). However, our study focused on meteorological and
hydrological parameters and didn’t address these factors.

The differences in the results obtained for the two types of weather conditions implied that
shellfish may have been impacted by different fecal sources (Ogburn and White, 2009).
Seabird fecal material is suspected as the main source of E. coli in dry weather conditions
(Derolez et al., 2010) whereas wastewaters inputs from the watershed are the main
contributors to microbial degradation of water quality after rainfall (Chigbu et al., 2005;
Although we did not measure the ratio between E. coli attached to suspended matter and E. coli remaining in the free-living state, this ratio could vary depending on weather conditions (Jeng et al., 2005) and the source of fecal material. Attached and free-living bacteria have different transport dynamics in the water column and have different shellfish retention efficiencies and digestion processes (Bernard, 1989; Prieur et al., 1990; Kach and Ward, 2008).

In our study, the E. coli concentration in oysters correlated poorly with those in water (Fig. 3), which is consistent with other field surveys performed on oysters (Bernard, 1989; Ogburn and White, 2009) or clams (Campos and Cachola, 2007). On the contrary, some studies of bacterial accumulation by Mytilus galloprovincialis and M. edulis showed good correlations between fecal coliform concentrations in shellfish and in water (Solic et al., 1999; Plusquellec et al., 1990). In laboratory studies, maximum E. coli concentrations in bivalve are obtained after short delays: 30 minutes for M. edulis or Tapes decussatus (Plusquellec et al., 1990; Martins et al., 2006) and three to four hours for C. gigas or C. virginica (Bernard, 1989; McGhee et al., 2008). Microbial depuration processes are slower: C. virginica took five to ten days to achieve EU regulatory standards (< 230 E. coli 100 g⁻¹ FIL) from initial concentrations of 1,000 E. coli 100 g⁻¹ FIL (temperature: 24-25°C, salinity: 24-28 units) (McGhee et al., 2008; Love et al., 2010). These delays, coupled with the high and rapid variability in the hydrology of the Thau lagoon (Fig. 4), raise doubt as to whether “steady state” conditions, in which E. coli intake and removal are equal, were achieved by oysters monitored in situ (Bernard, 1989; Ogburn and White, 2009). Accumulation factor is a ratio between bacterial concentrations (Cabelli and Heffernan, 1970) that arise from many complex environmental and ecophysiological processes. Several attempts have been made to adapt ecophysiological models to evaluate E. coli concentrations in shellfish (Fiandrino et al., 2003; Martins et al., 2006). Such models are calibrated with laboratory experiments and take into...
account filtration, retention and depuration processes depending on hydrological variables (salinity, temperature, suspended matter). The application and calibration of such controlled models to data collected in situ is questionable, due to the major and rapid changes that take place in the water surrounding the shellfish, especially after rainfall events.

The simultaneous measurements made in the watershed and the lagoon during the OMEGA-Thau Project, of which this study is a part, will enable calibration and validation of hydrodynamic models, coupled with a model simulating *E. coli* fate in Thau lagoon water (Fiandrino et al., 2003). These models will allow the simulation of fecal pollution transfer from the watershed to the shellfish harvest areas. As no calibrated ecophysiological model was available to simulate the accumulation of *E. coli* in oysters in the Thau lagoon, the use of median values of accumulation factors could lead to a sufficiently accurate estimation of *E. coli* concentrations in oysters from *E. coli* levels simulated in water, for the purpose of sanitary risk assessment. This approach will allow maximum allowable daily loads of *E. coli* to be determined, which are similar to the Total Maximum Daily Load (TMDL) program, recommended by US EPA regulation (EPA, 1997), and above which shellfish microbial quality falls below public health safety thresholds (Loubersac et al., 2007). These *E. coli* loads will be used as guideline values by local authorities to determine priority interventions concerning the watershed to maintain shellfish quality at or above the current Class B standard, to limit closures of the shellfish harvest and to improve consumer health protection (Pommepuy et al., 2005; Le Saux et al., 2006; Gourmelon et al., 2010).

**Acknowledgements**

We would like to thank the following organizations: Ifremer LER/LR in Sète for sampling and analyses and Grégory Messiaen for the map of Thau lagoon. The OMEGA-Thau Project: financial support (AERM&C, Région Languedoc-Roussillon, Conseil Général de l'Hérault,
FEDER), technical support (SMBT, EGIS-eau, BRLi, DRE-CQEL/LR, CRCM, LDV34, Thau-Agglo and CCNBT). The authors are grateful to Jean-Claude Le Saux for helpful advice during OMEGA-Thau Project, and to Soizig Le Guyader for reviewing this manuscript. Thanks to Cédric Duvail for layout and design of the figures.

References


Table 1  Periods of sampling and sampling strategy for the four rainfall periods monitored.

<table>
<thead>
<tr>
<th>Rainfall sampling periods</th>
<th>Periods of sampling</th>
<th>Rainfall amount (mm)</th>
<th>Number of rainy days</th>
<th>Number of sampling sites</th>
<th>Sampling frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 3-7 October 2007</td>
<td>16</td>
<td>3</td>
<td>21</td>
<td>Daily</td>
<td></td>
</tr>
<tr>
<td>R2 2-7 January 2008</td>
<td>62</td>
<td>6</td>
<td>21</td>
<td>Daily and semi-daily (01/05)</td>
<td></td>
</tr>
<tr>
<td>R3 31st of October to 8th of November 2008</td>
<td>178</td>
<td>7</td>
<td>19</td>
<td>Daily and semi-daily (11/03 to 11/05)</td>
<td></td>
</tr>
<tr>
<td>R4 1-7 February 2009</td>
<td>78</td>
<td>8</td>
<td>6</td>
<td>Daily and semi-daily (02/02 to 02/04)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Correlations between cumulative rainfall in the previous seven days and salinity, temperature,
Escherichia coli concentrations in water (C<sub>w</sub>) and oysters (C<sub>o</sub>) and between the Escherichia coli accumulation
factor (AF) and cumulative rainfall in the previous seven days, salinity and temperature. Number of samples (n),
Spearman's rank coefficient (rho) and p-values (P) are given.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>rho</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative rainfall vs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>salinity</td>
<td>312</td>
<td>-0.355</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>temperature</td>
<td>310</td>
<td>-0.147</td>
<td>0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;w&lt;/sub&gt;</td>
<td>318</td>
<td>0.323</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C&lt;sub&gt;o&lt;/sub&gt;</td>
<td>318</td>
<td>-0.278</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AF vs: cumulative rainfall</td>
<td>318</td>
<td>-0.452</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>salinity</td>
<td>312</td>
<td>0.239</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>temperature</td>
<td>310</td>
<td>0.030</td>
<td>0.593</td>
</tr>
</tbody>
</table>

Figure 1 Location of Thau lagoon, its watershed and its shellfish farming areas. Location of high-frequency
sensors (stations 1, 2, 4 and 5) and oyster and water weekly sampling stations (stations 2, 3, 4 and 5).

Figure 2 Distribution of water temperature, salinity, Escherichia coli concentrations in water (C<sub>w</sub>) and oysters
(C<sub>o</sub>) and accumulation factor (log<sub>10</sub> AF) for all the sampling period (n = 318), the dry weather periods (n = 79)
and the rainfall periods (n = 239). P-values (P) of Mann-Whitney tests performed between samples collected
during rainfall and dry weather periods are given. European sanitary thresholds for oysters (Regulation
EC/854/2004) are indicated with dotted lines.

Figure 3 Distribution of Escherichia coli concentrations in water (C<sub>w</sub>) and oysters (C<sub>o</sub>) during the dry weather
periods (●) and the rainfall periods (★).

Figure 4 Salinity and Escherichia coli counts in water (△C<sub>w</sub>) and oysters (● C<sub>o</sub>) monitored at the surface
during: rainfall periods R1 to R3 on station 2 (a-c), and during period R4 on station 1 (d). Arrows (↓) represent
the peak of rainfall intensity and rainfall daily amounts.
Figure 1 Location of Thau lagoon, its watershed and its shellfish farming areas. Location of high-frequency sensors (stations 1, 2, 4 and 5) and oyster and water weekly sampling stations (stations 2, 3, 4 and 5).
For Peer Review

Paired F-tests were conducted to evaluate the significance of differences between samples that were obtained under dry weather and rainfall conditions. The following box plots illustrate the comparative distributions:

- **Safety**: Comparison between dry weather and rainfall with a p-value of <0.0001 for the paired t-test.
- **Temperature**: Comparison with a p-value of <0.0001 for paired t-test.
- **C\(_{	ext{w}}\) (log\(_{10}\) 100 g FFL)**:
  - Dry weather: C\(_{	ext{w}}\) values ranging from 1.5 to 4.5 with a p-value of <0.0001.
  - Rainfall: C\(_{	ext{w}}\) values ranging from 1.5 to 4.5 with a p-value of <0.0001.
- **C\(_{o}\) (log\(_{10}\) 100 g FFL)**:
  - Dry weather: C\(_{o}\) values ranging from 2 to 4.5 with a p-value of <0.0001.
  - Rainfall: C\(_{o}\) values ranging from 2 to 4.5 with a p-value of <0.0001.
- **log F**:
  - Dry weather: log F values ranging from 0 to 3 with a p-value of <0.0001.
  - Rainfall: log F values ranging from 0 to 3 with a p-value of <0.0001.