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

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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 source (Levesque *et al*., 2000; Wither *et al*., 2005; Ogburn and White, 2009; Derolez *et al*., 2010).

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 (Pommepuy *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 $\log_{10}$ *E. coli* 100 mL^{-1} for the water samples and 0.5 $\log_{10}$ *E. 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. 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. 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:

$$AF = C_o/C_w$$

where C_o = the *E. coli* concentration in oysters in cells per 100 g FIL and C_w = the *E. 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 90th 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 90th 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}, 90th 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.

198 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).

203 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 ($n = 46$).

207 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 ($n = 310$, $rho = 0.54$, $P < 0.0001$).

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

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

214 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* (Plusquellec *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 occured 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;

Coulliette and Noble, 2008). 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).

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

Rainfall sampling periods	Periods of sampling	Rainfall amount (mm)	Number of rainy days	Number of sampling sites	Sampling frequency
R1	3-7 October 2007	16	3	21	Daily
R2	2-7 January 2008	62	6	21	Daily and semi-daily (01/05)
R3	31st of October to 8th of November 2008	178	7	19	Daily and semi-daily (11/03 to 11/05)
R4	1-7 February 2009	78	8	6	Daily and semi-daily (02/02 to 02/04)

Table 2 Correlations between cumulative rainfall in the previous seven days and salinity, temperature, *Escherichia coli* concentrations in water (C_w) and oysters (C_o) 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.

	n	*rho*	*P*
Cumulative rainfall *vs*:			
salinity	312	-0.355	<0.0001
temperature	310	-0.147	0.01
C_w	318	0.323	<0.0001
C_o	318	-0.278	<0.0001
AF *vs*:			
cumulative rainfall	318	-0.452	<0.0001
salinity	312	0.239	<0.0001
temperature	310	0.030	0.593

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_w) and oysters (C_o) and accumulation factor ($\log_{10}$ 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_w) and oysters (C_o) during the dry weather periods (●) and the rainfall periods (●).

Figure 4 Salinity and *Escherichia coli* counts in water (△C_w) and oysters (● C_o) 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.

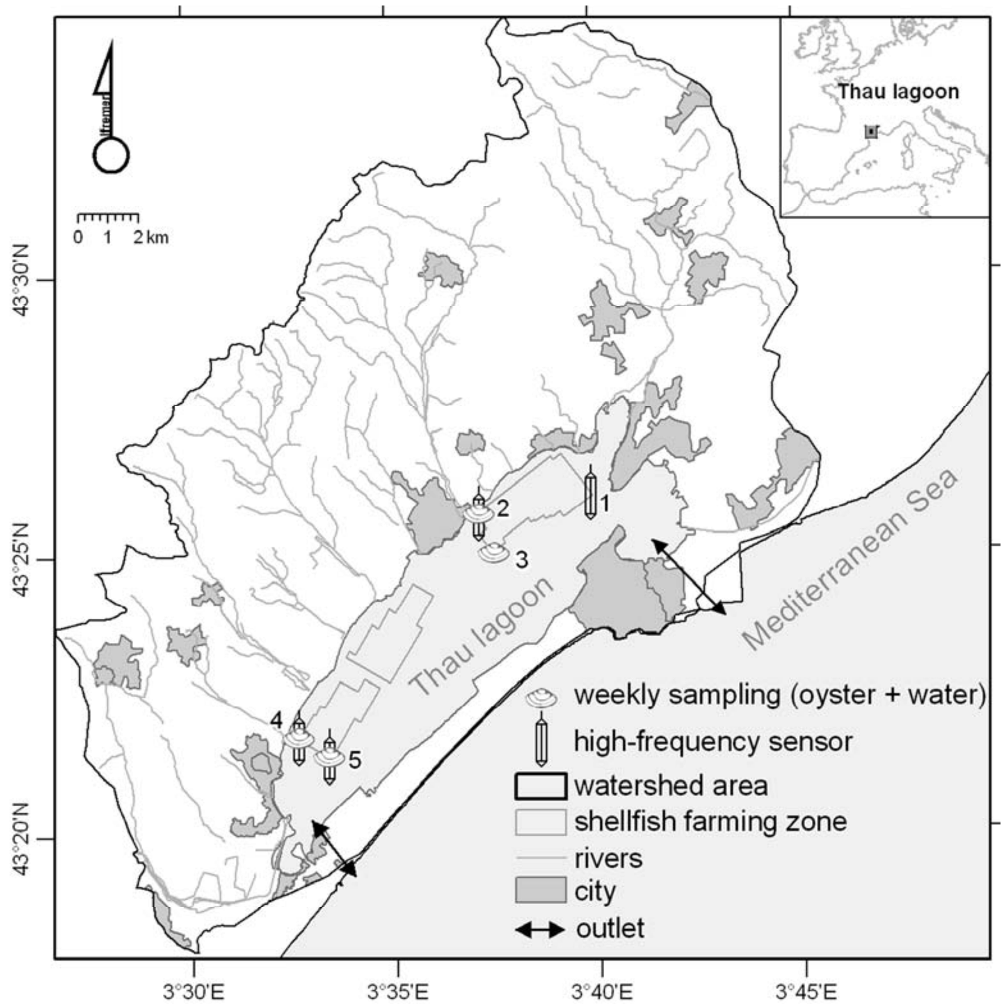

Figure 1 Location of Thau lagoon, its watershed and its shellfish farming areas. Location of high-frequency sensors (stations 1, 2, 4 and 5) and oyster and water weekly sampling stations (stations 2, 3, 4 and 5).
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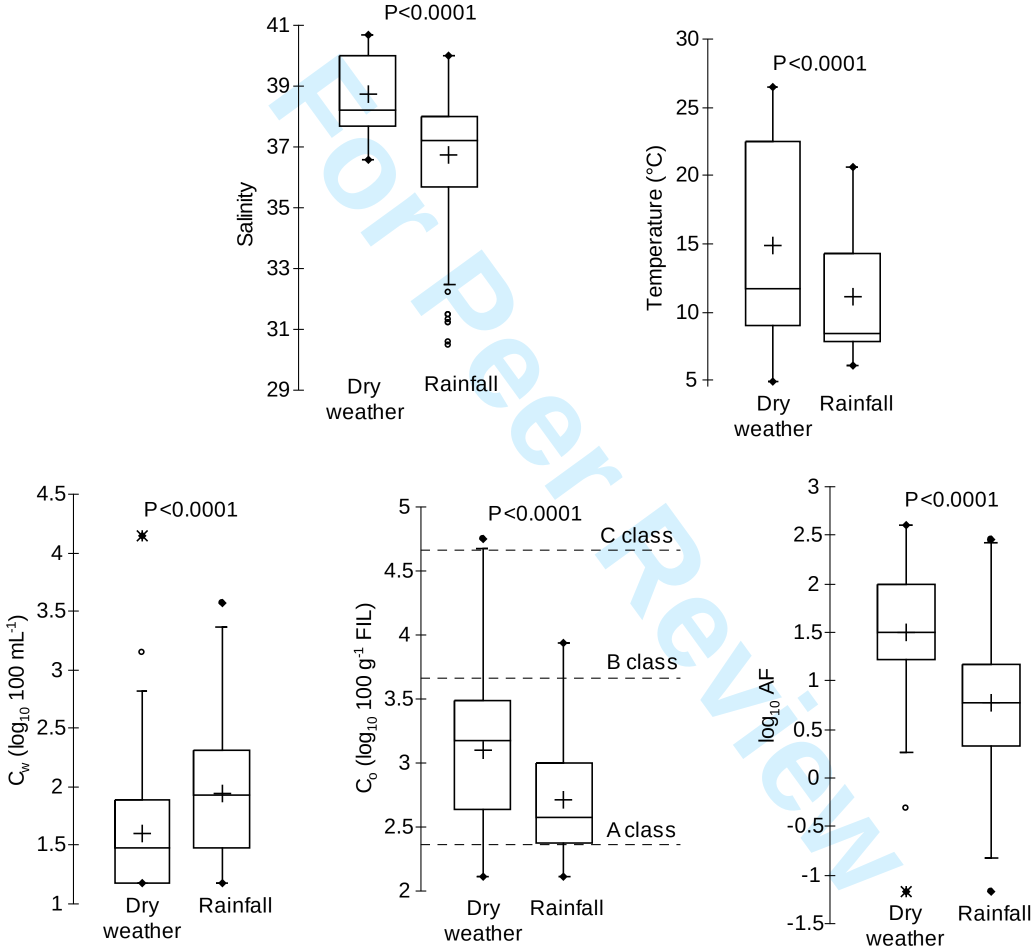
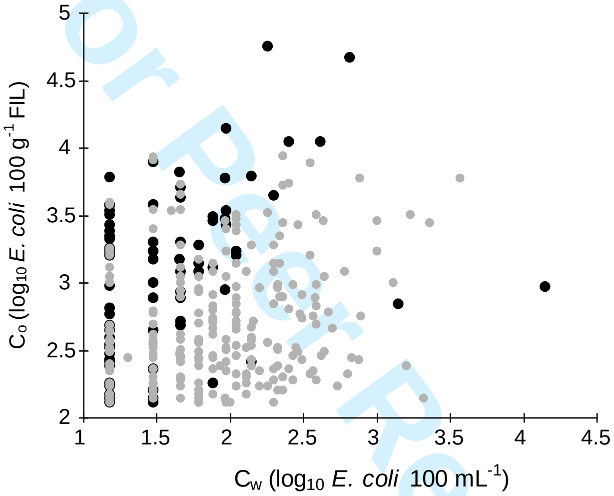
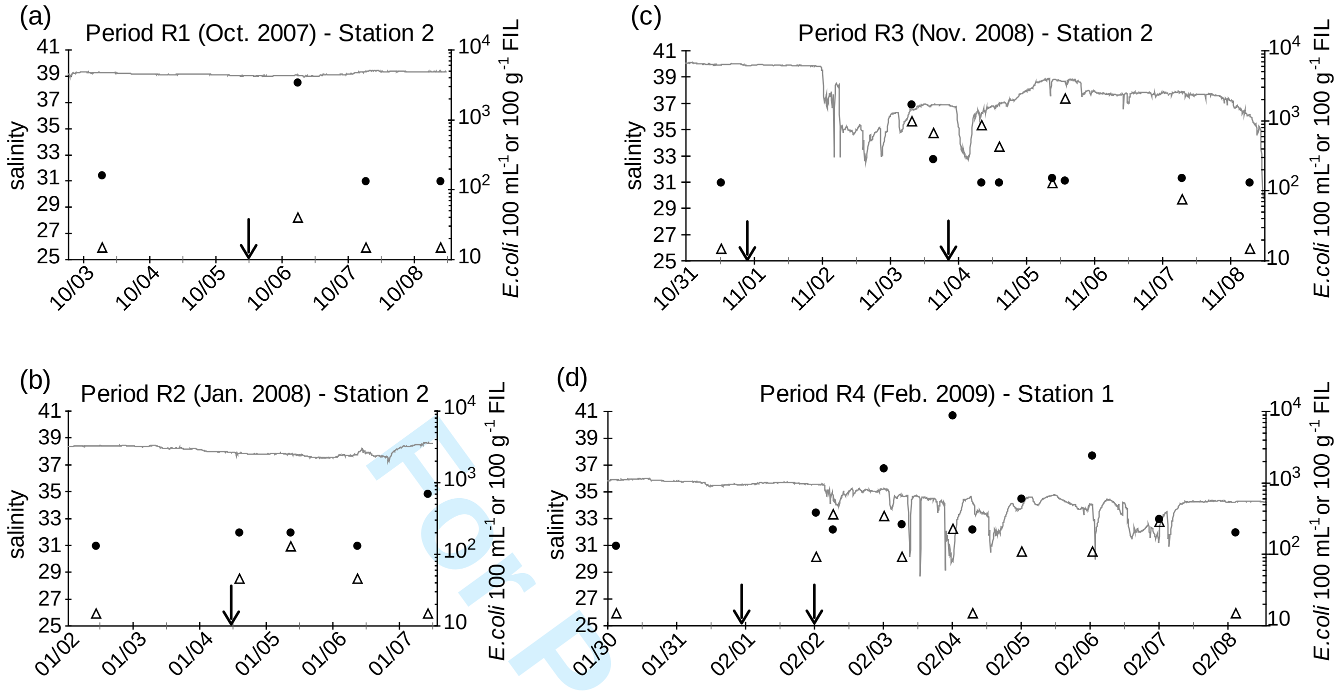

P<0.0001
Salinity
41
39
37
35
33
31
29
Dry weather
Rainfall
P<0.0001
Temperature (°C)
30
25
20
15
10
5
Dry weather
Rainfall
P<0.0001
C_w ($\log_{10}$ 100 mL^{-1})
4.5
4
3.5
3
2.5
2
1.5
1
Dry weather
Rainfall
P<0.0001
C_o ($\log_{10}$ 100 g^{-1} FIL)
C class
B class
A class
5
4.5
4
3.5
3
2.5
2
Dry weather
Rainfall
P<0.0001
$\log_{10}$ AF
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
Dry weather
Rainfall


5
4.5
4
3.5
3
2.5
2
1
1.5
2
2.5
3
3.5
4
4.5
Co (log10 E. coli 100 g-1 FIL)
Cw (log10 E. coli 100 mL-1)


(a)
Period R1 (Oct. 2007) - Station 2
salinity
E.coli 100 mL-1 or 100 g-1 FIL
10/03
10/04
10/05
10/06
10/07
10/08
(c)
Period R3 (Nov. 2008) - Station 2
10/31
11/01
11/02
11/03
11/04
11/05
11/06
11/07
11/08
(b)
Period R2 (Jan. 2008) - Station 2
01/02
01/03
01/04
01/05
01/06
01/07
(d)
Period R4 (Feb. 2009) - Station 1
01/30
01/31
02/01
02/02
02/03
02/04
02/05
02/06
02/07
02/08