Prediction of faecal contamination in shellfish production areas: Interest and limits of the salinity parameter

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Estuarine areas are sensitive to rapid and punctual contamination linked to heavy rainfall inducing rapid fresh water input. Thus, the increase of seawater contamination may be linked to a salinity decrease. The objective of this study was to measure salinity variation as a proxy to evaluate the risk of faecal contamination. Salinity sensors were set up on shellfish beds in Daoulas estuary (Bay of Brest) and monitored every 10 min over a four year period. Precipitation and river flows were also recorded. Moreover *E. coli* concentrations and salinity were measured in the estuarine surface water during different hydrodynamic conditions and seasons. The results indicated a significant relationship between *E. coli* concentration and salinity in the estuary. Mathematical models were then selected and validated to assess and predict salinity variations. In the upper estuary, statistical analysis shows that variation in salinity is a function of the rainfall of the previous day (first model with r^2= 0.66). The second model established a relationship between salinity and precipitation which reproduced 95% of the events, but failed to reproduce salinity variations during low rainfall (<1mm/day).

To conclude, the salinity parameter in Daoulas estuary was found to be relevant for assessing the sensitivity of the estuary to contamination. The prediction of salinity decrease was well modeled for the major conditions leading to faecal contamination. However, further investigations are necessary to directly relate shellfish contamination to rainfall events. This novel approach is promising and would help us to foresee the risk of microbiological contamination in shellfish harvesting areas and recreational sites located in estuaries. Nevertheless the relationship between rainfall and contamination is site dependant and application of the model to the other site would require a local database to validate the approach.

Keywords : Salinity, sensor, E. coli, shellfish, prediction, alert, Daoulas estuary.

Introduction

Estuaries in Brittany (France) are important areas for shellfish harvesting and thus play a key role in the local economy. Although much effort has been made during the last decade to protect and improve estuarine water quality, faecal water contamination remains a significant health concern. It is estimated that each year, about 10 % of Brittany shellfish production areas are closed (REMI, Ifremer) when rainfall occurs or/and when faecal indicators exceed shellfish water quality objectives established by the European Union (Directive EC/113/2006). Although it is well established that stormwater run-off carries significant levels of *Escherichia. coli* (Davies-Colley et al, 2008), it is not fully understood how river water flux impacts estuarine water quality, either temporally or spatially, due to the varying input rates, estuarine hydrodynamics and bacterial die-off rates (He et al, 2008). It is currently accepted that faecal contamination in estuaries is more or less related to salinity decrease (Pommepuy et al, 2006). The objectives of this study were first to assess the impact of rivers on estuarine water quality by measuring salinity, and second to link salinity variation to climatic events (rainfall amount and river flow). Thus, the final objective would be to set up an early warning system, based on the relationship between salinity variation and *E. coli* concentration in the estuarine water, and to assess salinity as a proxy for faecal contamination surveys.

1. Methods

The Daoulas estuary is located on the Atlantic west coast of France (figure 1). Approximately 6 500 people live on the surrounding catchment, with a mean density close to 58 inhabitants/km^2 (188 inhabitants/km^2 near the sea). The total catchment area is 113 km^2 and the distance between the source of the rivers and the estuary mouth is about 8.7 km. The main river, La Mignonne, represents 60 % of the sub-catchment area with a mean flow of 1.45 m^3/s. Eight smaller rivers also flow into the estuary. Estuarine waters are partially mixed with riverine waters upstream of point A (figure 1), but are well mixed downstream of this point leading to homogeneous water quality in terms of salinity. The estuary experiences semi-diurnal tides, with a mean tidal range of 3.5 m and salinity varying from 12 to 35 ‰. Previous studies suggest that the estuary is significantly affected by La Mignonne's inputs, which contribute 85 % of the *E. coli* fluxes (Pommepuy et al, 2008). Within the estuary, about 200 t/year, mainly of oysters (*Crassostrea gigas*), are produced on 55 hectares used for shellfish farming. According to European regulation EC/854/2004 modified by regulation EC/113/2006), the level of classification of these shellfish harvesting areas is B for oysters. In situ investigations were carried out over a three year period (2005-2007). Discrete surface samples were collected from upstream and downstream sites to assess the salinity and *E. coli* concentrations for different hydrodynamic conditions and locations. Three sampling trials were, thus undertaken randomly across the estuary: the data set consisted of 145 measurements made on June 6th, July12th and August, 1st, 2005. Salinity was measured with a salinity probe (WTW Cond 340i) and *E coli* analysed in accordance with the technique NF EN-ISO 9308-3. A salinity probe (FSI's CT Sensor) was also positioned on a oyster rearing area and fixed to oyster racks at 50 cm above the bottom (figure 1, point A). Salinity and water depth were recorded every ten minutes. At point 2 (figure 1), a permanent gauge measured flow continuously on La Mignonne River (http://www.hydro.eaufrance.fr/). Rainfall information was obtained from three local Meteo-France stations (Guipavas, Lanveoc and Sizun). Precipitation and river flows were recorded every day. Mean deviations of daily salinity were then calculated for the whole period (Dec 2004 - January 2009), and Log $_{10}$ transformation applied to improve data normality. To construct the first multiple regression model, we used a time series of salinity at station A as the dependent variable. Independent variables included log-transformed same day and 1- to 3-days lagged river flow. The model was obtained from the databases for the period December 17, 2004 - October 30, 2005 and validated from May 17, 2006 to May 22, 2007. The flow of the river La Mignonne is hydrologically dependent on rainfall, thus the relationship between salinity and rainfall was investigated to developing a warning system (figure 2). The second model was established from May 17, 2006 to May 22, 2007 on the same period as for the first model, and validated from February 22, 2008 to January 11, 2009. The model based on the Thiessen' equation (Boots et al, 1980), uses the deviation of daily salinity as the dependant variable, and the independent variables are rainfall on the same day or rainfall with a lag of 1day before (t-1 local Meteo-France station data bases). Statistica software was used to test the normality of data distributions and then to perform statistical analyses. Normality and homoscedasticity of the residuals were verified. Unless otherwise stated, statistical significance for all tests was determined using a cutoff of $p< 0.05$.

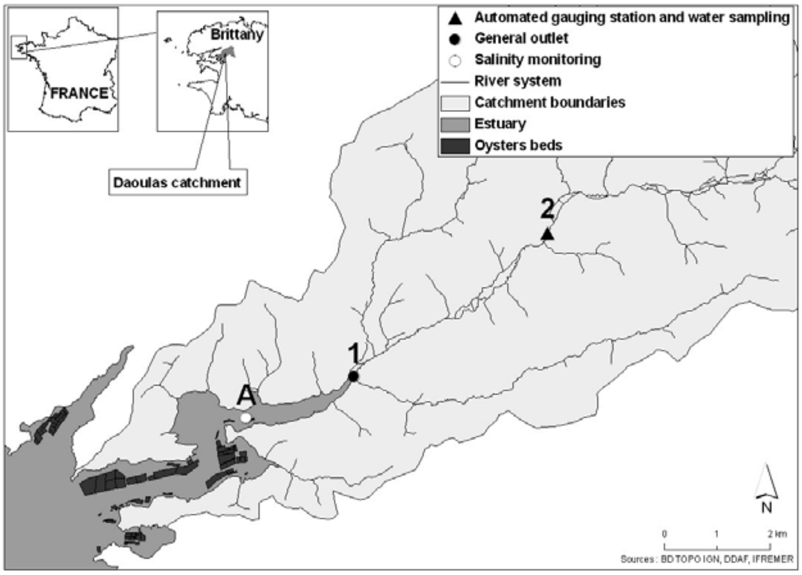

Figure 1. Presentation of the Daoulas estuary: location of the gauging station on La Mignonne river, point 1 and the sampling point A, in the estuary

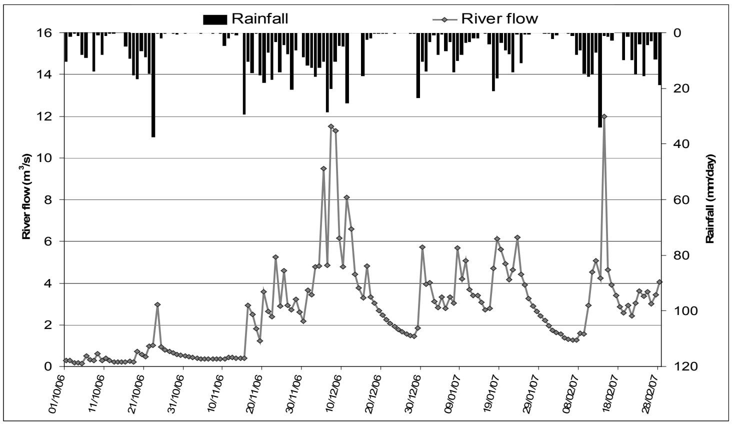

Figure 2: La Mignonne river flow and rainfall level (Daoulas, France)

2. Results

The Daoulas shellfish production areas are classified B for oysters according the EU regulation, this means that less than 90% of sanitary results are inferior to 4600 *E.coli*/100ml and 10% are superior to this limit. Considering the REMI survey from 2000 to 2009, the results indicate that the occurrence of shellfish contamination events and especially those superior to 4600 *E. coli* are dependant on rainfall events. For example, 63.33% of the cases when levels have exceeded the limit have been correlated with rainfall levels ≥ 10mm/48h. The source of this contamination is linked to different phenomena. Bougeard et al (in press) demonstrated that run-off plays a major role in this contamination, because the watershed size is very limited (110 km^2) and agriculture practices include manure spreading on fields. Pommepuy et al (2008) also underlined the effect of a wastewater treatment plant located upstream of the estuary where there was shellfish contamination. In this paper the role of the main river La Mignonne was investigated. Figure 2 presents the variation in rainfall and La Mignonne river flow from October 2006 to February 2007. After a dry weather period marked by flow inferior to $1m^3/s$, the river flow increased as a function of the rainfall level. During the wet period, due to the soil saturation, the base flow is higher than during the dry period (> $2m^3/s$). In all cases, watershed response to rainfall is very short (lag time of a day).

In the estuary, the investigation in the field allowed us to describe the spatio-temporal variation of salinity and *E. coli* values and to document how river flow and tidal currents determine the distribution of these parameters. Figure 3a illustrates a typical pattern of salinity and *E. coli* dynamics over several hours at the surface, during a spring tide. Each time the sampling was done in low-salinity water, the *E. coli* concentration was higher than in high-salinity water. Figure 3b shows how *E. coli* concentration decreases as salinity increases, moreover there is a strong relationship between the two parameters ($Y = - 0.0665 x + 3.26$, with $R^2 = 0.704$). This correlation has been found in other estuaries (Pommepuy et al, 1997) and means that the salinity parameter could be used as a surrogate for *E. coli* concentration for the purpose of estimating faecal contamination. This initial result was very encouraging and led us to make a more thorough investigation of the spatio-temporal evolution of salinity in the shellfish production areas. Figure 4 represents the variation in salinity and water depth, recorded on the shellfish bed at point A in the period January 14 - 25, 2005. This point, where shellfish are grown, is located in the middle of the estuary and is strongly influenced by river discharge. During the spring tide between January 14 - 16 and 24 - 25, characterized by a high variation in water depth, salinity ranged from 32 ‰ at high tide to more than 20‰ at low tide. During the neap tide, in contrast, salinity was less than 15‰ at the same point. This result clearly demonstrates the high variation of salinity, and suggests the neap tide could be a risk period for shellfish contamination in this part of the estuary

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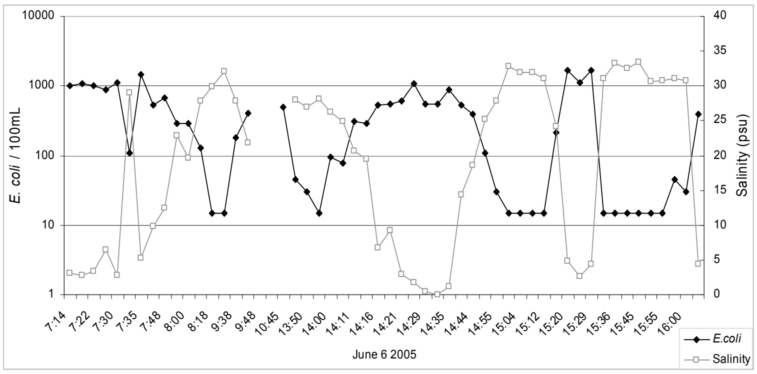
3a



3b

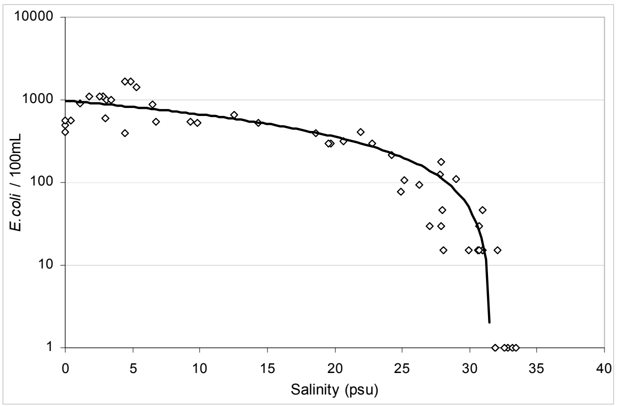


Figure 3. Relations between salinity (‰) and E. coli concentration (ufc/100ml) of surface water in Daoulas estuary on June 6, 2005. 3a) typical pattern of salinity and E. coli dynamics over several hours at the surface. 3b) Relationship between E. coli concentration and Salinity.

Statistical analyses were then performed as a first step towards analysing the very large recorded salinity database available at point A (December 17, 2004 - October 30, 2005). Statistical treatment demonstrated that the deviation of daily salinity (*DS*), expressed as its log, is directly correlated with the river flow (*RF*) occurring the same day and salinity one day earlier. The following multiple regression model was developed:

$$\log DS(t) = -0.736 + 0.414 \log RF(t) + 0.426 \log DS(t-1) \qquad \textbf{[eq. 1]}$$

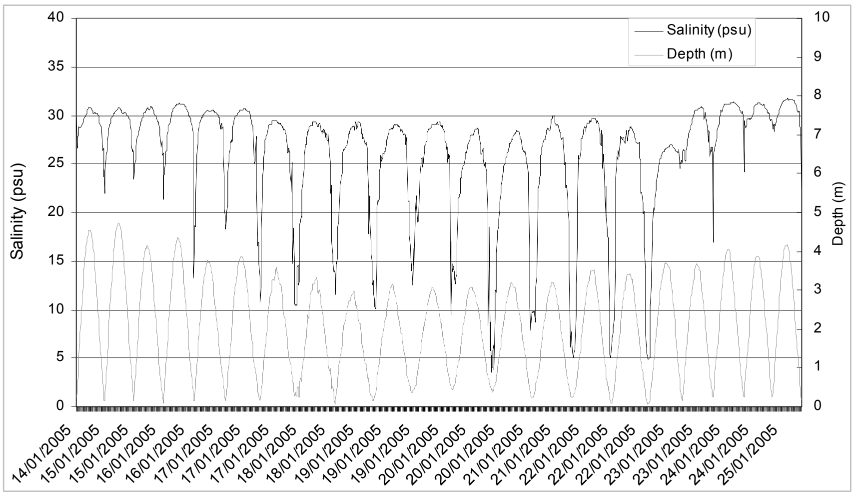


Figure 4. Salinity and water depth variations according to the tide, measured at point A in the Daoulas estuary.

The model explains 66 % of the salinity variation (R^2 = *0.66*). Residuals follow a log normal law and their variances are constant over time. Then, the model was validated between May 17, 2006 - May 22, 2007.

The model accounts for a significant part of the variation in salinity and predicts salinity variations correctly in 94 % of cases. The importance of parameter *t-1* in the model corresponds to the capacity of the estuary to respond to river inputs.

As a second step, a regression model was established directly between the variation in salinity (*DS*) and the amount of rainfall (*R*)

log *DS (t)* = 0.279 + 0.725 log (*Ds t-1))* + 0.073 log *(R t-1))* ***[eq 2]***

Figure 5 shows the comparison between measured data and results predicted by the model. The model was established using the same periods as the previous model [eq. 1] and validated on the second period: R^2 was respectively *0.63* for May 2006 - May 2007, and *0.64* for February 2008 – January 2009 respectively. Rainfall lagged by day –1 was significantly related to salinity. The regression model was validated with independent data that were not used in model construction (May 2006 - May 2007). The estimated and observed salinity corresponds, and the model was run with another set of values to complete the validation exercise. Figure 6 represents the predicted versus observed salinity for the second dataset (February 2008 – January 2009). The model accounts for a significant part of the variation in salinity and predicts salinity variations correctly, the model includes 18 atypical data from the 315 values, representing standard error of 5% (Period 20008-2009). The model gives a good reproduction of the high values. However, the model failed to reproduce salinity variations during low rainfall periods (< 1 mm/day). The model failure has little consequence in terms of health hazards because summer is the lower risk period for shellfish contamination. Nevertheless, these results demonstrate the robustness of the relationship between salinity variation and rainfall at the monitoring point.

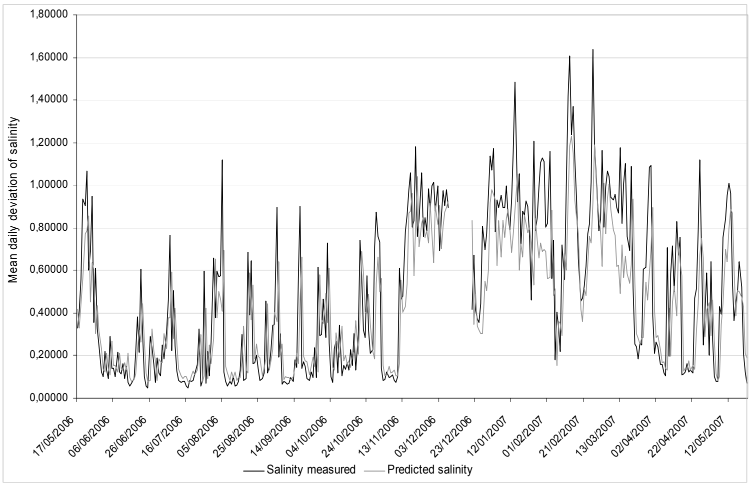


Figure 5. Comparison of the observed and the estimated salinity (mean daily deviation of salinity) from May 2006 to May 2007 Log DS (t) = 0.279 + 0.725 log (Ds t-1)) + 0.073 log (R t-1)) . Daoulas estuary, point A.

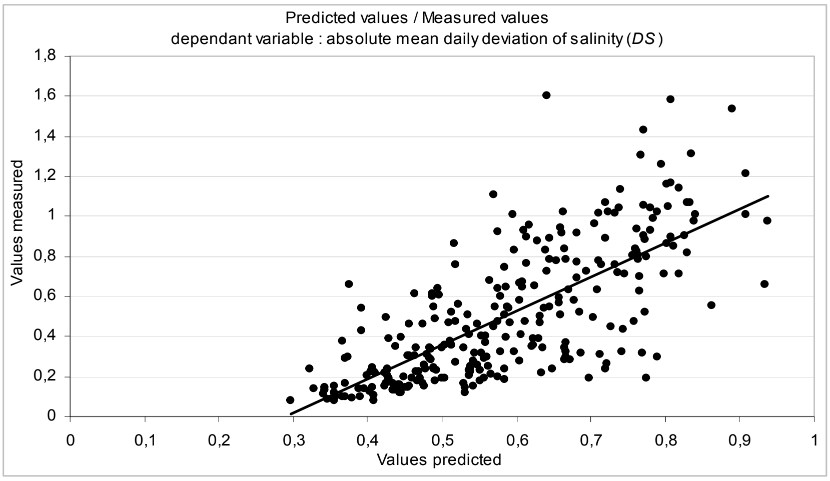


Figure 6. Predicted versus observed salinity using a multiple regression model. The observed values are those used to adapt the model. Log DS (t) = 0.279 + 0.725 log (Ds t-1)) + 0.073 log (R t-1)) (February 2008 – January 2009). Daoulas estuary, Point

3. Discussion and Conclusion

Shellfish harvesting areas are subject to closure when the amount of rainfall exceeds a given threshold, or when faecal indicator levels exceed established criteria. Considering that it takes at least 2 days to obtain bacterial analysis results for faecal indicators, making decisions on this basis is difficult. An alternative approach to providing more timely information is the use of predictive models based on forecasting and hydrological information (Clement et al, 2006; Le Saux et al, 2006). Multiple regression models are already used as tools for interpreting data collected in monitoring systems, or as alerts for health warnings (Boehm et al, 2007). Exploratory variables selected to establish the models can be readily measured *in situ* or in real time through online access to these data. In the present study, taking into account that the *E. coli* concentration is linked with salinity in Daoulas estuary, we have developed reliable salinity/river flow and salinity/rainfall models to predict the variations in salinity. We carried out regression analysis based and validated on field measurement, taking the specificities of Daoulas estuary into account. The multiple regression models make accurate simultaneous prediction of the variation of salinity. Thus, it allows the possibility to predict faecal contamination of the estuary with rainfall or river flow when salinity drops.

Nevertheless, there are limits to this approach. Firstly, salinity monitoring can also be applied in areas where the variations are significant or measurable (estuaries or areas submitted to large sewage outfall); secondly, the relationship between rainfall and salinity variation can vary from one site to another, and can be modified from season to season. For example, vertical stratification that occurs during winter could play a role in the relationship in a moderately stratified estuary. Soil saturation may also slightly modify the run-off and thus the parameters of the model. Finally, E. coli increases are usually related to salinity decreases, but other sources could be also cause contamination (sediment bed resuspension, unknown faecal sources, etc.). We found that this relation is site dependant and investigations should be conducted in situ before applying models. The site-specific character, often underlined and reported in previously studies (Boehm et al, 2007), means that models need to be validated by establishing model parameters for each site

To conclude, the alternative approach using statistical models to assess faecal contamination is promising for estuaries. Nevertheless further investigations still need to be done. For example the model could take into account the direct relationship between salinity in seawater and *E coli* in bivalve molluscan shellfish and be extended to predict *E. coli* concentration directly in bivalves. This approach, if successful, would offer the possibility of predicting faecal status of the water in which shellfish are grown from weather forecasts. This will help decision makers to take rapid and effective decisions about shellfish market closures or give management advice for Daoulas estuary.

Acknowledgment

This work was funded by IFREMER and Seafood Plus Program N° Food-CT-2004-506359 (Redrisk). The authors thank Ets Coïc (Logonna Daoulas), Robert Le Roux (Kristell Electronic), Morgane Bougeard and the team at the IFREMER microbiology laboratory.

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REMI The national shellfish monitoring network (REMI) surveys faecal contamination in shellfish harvesting areas (http://wwz.ifremer.fr/envlit/).