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Modeling of *Escherichia coli* Fluxes on a Catchment and the Impact on Coastal Water and Shellfish Quality

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Abstract:

The simulation of the impact of *Escherichia coli* loads from watersheds is of great interest for assessing estuarine water quality, especially in areas with shellfish aquaculture or bathing activities. For this purpose, this study investigates a model association based on the Soil and Water Assessment Tool (SWAT) coupled with a hydrodynamic model (MARS 2D; IFREMER). Application was performed on the catchment and estuary of Daoulas area (France). The daily *E. coli* fluxes simulated by SWAT are taken as an input in the MARS 2D model to calculate *E. coli* concentrations in estuarine water and shellfish. Model validation is based on comparison of frequencies: a strong relationship was found between calculated and measured *E. coli* concentrations for river quality (r^2 = 0.99) and shellfish quality (r^2 = 0.89). The important influence of agricultural practices and rainfall events on the rapid and large fluctuations in *E. coli* fluxes from the watershed (reaching three orders of magnitude in <24 hours) is one main result of the study. Response time in terms of seawater quality degradation ranges from one to two days after any important rainfall event (greater than 10 mm/day) and the time for estuary to recover good water quality also mainly depends on the duration of the rainfall. In the estuary, three effects (rainfall, tidal dilution, and manure spreading) have been identified as important influences.

Keywords: SWAT; watershed; estuary modeling; *E. coli*; shellfish; water quality

Introduction

The microbiological quality of surface waters in rivers or estuaries determines their acceptability for shellfish culture and recreational use (Hooda, Edwards *et al.* 2000). Microbiological contamination often results from urban wastewater discharges or non-point source pollution, including land-applied animal manure, failure of septic systems and wildlife. On the coast, microbiological contamination can cause beach closures or prohibited shellfish sales, both of which have direct effects on the coastal economy. French shellfish growing zones were classified by a national commission according to European regulations that proposed new standards for the classification of shellfish waters (EC/113/2006), the shellfish grown in these areas (EC/854/2004 modified by regulation EC/1666/2006) and bathing activities (EC/7/2006) (Anonymous 2004, 2006a, c, b).

Moreover, this EU Directive is linked with the Water Framework Directive, which stipulates an integrated approach to river basin management. Modeling is frequently used in environmental sciences to analyze the impact of actual and alternate land management scenarios. Simulating and predicting *E. coli* fluxes and their impact on water and shellfish quality require a balance between accounting for all the phenomena and factors (e.g. rainfall, watershed physical parameters, sources, dilution in tidal areas and behavior of fecal bacteria) and the representation at different spatial and time scales. The existing models developed for coastal areas only consider physical factors (i.e. dilution and dispersion) and few references exist on the possible influence of fluxes variation on the variation of seawater quality (Kashefipour, Lin *et al.* 2006). The dilution and dispersion phenomena on Atlantic coasts are governed by tidal forcing, which play an important role especially in the present area, and possibly buoyancy effects wherever estuaries are significantly stratified. Thus, in coastal water subject to tidal currents, the physical dilution is more efficient than mortality to decrease *E. coli* concentrations (Salomon and Pommepuy 1990). Constant, day-/ night-time or variant decay rates have already been integrated into models to improve the results (Fiandrino, Martin *et al.* 2003), but this variation has been found of little consequence (Kashefipour, Lin *et al.* 2006) compared with the amplitude of other phenomena (e.g. dilution and fluxes).

The linkage of a coastal water model with a catchment model offers the possibility to integrate all the parameters that drive water and pollutant fluxes out of a watershed and into a coastal area. Coastal water models already set up at the regional scale allow to determine the movement of marine water, dissolved and particulate matter and to appreciate short- or long-term dispersion in the whole region (Bailly du Bois and Dumas 2005; Lazure and Dumas 2008). Catchment-scale water quality models have the capacity to simulate movements of pollutants from the land surface to receiving streams and to route the pollutants through the stream network towards the watershed outlet (Jamieson, Gordon *et al.* 2004). These watershed models can simulate the daily variations of river flow and contaminants fluxes. Considering this information, we decided to use two such models to simulate *E. coli* fluxes and estimate their impact on coastal water and shellfish quality.

The Soil and Water Assessment Tool (SWAT) (Arnold et al., 1998) was successfully applied to simulate river flow (Kannan, White *et al.* 2007), nutrient fluxes (Pohlert, Huisman *et al.* 2005) and *E. coli* fluxes (Sadeghi and Arnold 2002; Baffaut and Benson 2003; Guber, Pachepsky *et al.* 2007; Parajuli, Mankin *et al.* 2007). The version of the model incorporating both landscape and in-stream microbial processes (Jamieson, Gordon *et al.* 2004) was widely applied to simulate management scenarios for the reduction of river pollution (Arnold, Srinivasan *et al.* 1998; Pohlert, Huisman *et al.* 2005). SWAT allows modeling of bacteria fate and transport. Although the calibration of the model for faecal fluxes seems more complex than for flow, because of the paucity of data and insufficient understanding of *E. coli* biophysical process, this approach represents a major breakthrough to estimate *E. coli* fluxes (Baffaut and Benson 2003; Parajuli 2007).

Hydrodynamic modeling for applications at regional scale (MARS) has been practiced for a number of years (Lazure and Dumas 2008) to describe currents, dilution and transport of particles all along the French coast. Recently, applications on water quality variations have been realized, in particular faecal contamination in bathing and shellfish harvesting areas (Pommepuy, Hervio-Heath *et al.* 2005; Riou, Le Saux *et al.* 2007). These models are currently used to manage the impact of wastewater on the sea water (Fiandrino, Martin *et al.* 2003). However, to our knowledge, any study used daily flows simulated with a watershed model as an input into another hydrodynamic model to assess daily bacterial concentrations in estuaries. The coupling of these two types of model is of major importance in advancing our understanding and prediction of the microbiological contamination in an estuary, which is crucial for coastal management.

The goal of this study was to assess the effect of daily *E. coli* fluxes from a catchment on coastal water and shellfish quality by a modeling approach. The study was set up on a daily time step which was adapted to our objective and the catchment characteristics.

The first step of the work consisted in setting up the SWAT model on the 113 km² Daoulas catchment. After calibration and validation for river flow, *E. coli* daily concentrations were simulated from a scenario of contamination corresponding to local practices. Then, simulated *E. coli* fluxes were taken as an input in MARS 2D to assess their impact on shellfish quality. The models were calibrated and validated for *E. coli* concentrations in river water and in shellfish on several data sets obtained during the study and previous years. Frequency curves were plotted to compare simulated *vs.* observed *E. coli* concentrations. Once validated, the models were used to test the effect of agricultural practices on *E. coli* concentrations in river and coastal water as well.

Materials and Methods

- The study catchment and estuary

The 113-km² catchment of Daoulas estuary is located on the western French Atlantic coast in France (Figure 1).

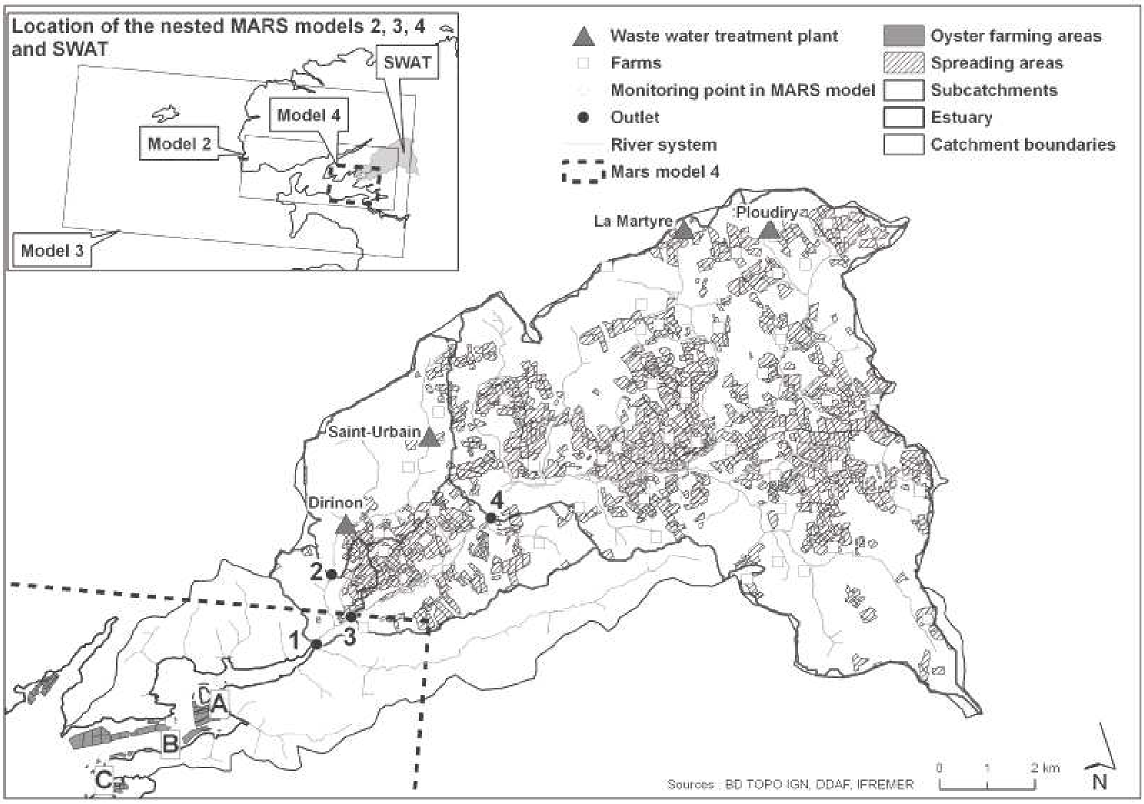

Figure 1. The Daoulas estuary and Mignonne River catchments: subcatchments, locations of farms and spreading areas, monitoring points in the estuary: A, B, C (MARS model) and B (REMI data), and the nested MARS models

The subcatchment of the Mignonne River represents 60% of the total catchment. The total stream length in this river system reaches 90 km of streams. Elevations range from 4 m near the coast to 293 m upstream, and 76% of the catchment is defined by a slope of less than 6%. Climate is of the oceanic temperate type and annual precipitation exhibits considerable spatial variation, from 700 mm on the coast to 1400 mm in the upstream part of the catchment. The geology of the catchment is mainly paleozoic shale and sandstone with holocene alluviums and colluviums appearing in river valleys. The predominant soil type is clay loam. Land-use include arable farming (44%), followed by pasture (25%), forest (20%), urban areas (10%), permanent crops (1%) and water (<1%). Livestock consists of cattle (3,364 heads), pigs (63,503) and poultry (383,400), on farms situated throughout the catchment. Approximately 6,600 people live in the Daoulas catchment, with a mean density of nearly 58 inhabitants/km² (188 inhabitants/km² on the coast).

Previous studies suggested that the estuary was significantly affected by loadings from the Mignonne River contributing 85% of the total *E. coli* flux (Pommepuy, Le Guyader *et al.* 2008). The pollution loads come from both point sources (i.e. discharge from four wastewater treatment plants or WWTPs) and non-point sources (especially spreading of livestock manure). Thus, this study considered only the discharges from the Mignonne River into the estuary. The Daoulas estuary is about 5 km long with a mean width of 500 m. On the Atlantic coast, the tidal currents are subject to lunar attraction that determines the periods of spring tides and neap tides. For example, each synodic month around the times of new and full moon, the tidal actions of the sun and moon are combined to amplify the tidal range (spring tide), and conversely for neap tide. The average range is 5.5 m for the spring tide and 3.0 m for the neap tide. At a low tide, the shores are broadly uncovered. The ratio between the volume of fresh water brought in by the river and the oscillating volume shows that the Daoulas estuary can be considered as an homogeneous estuary with limited salinity stratification near the mouth of the river (Allen 1972). Salinity profiles acquired in the estuary showed that the estuary downstream of location A (Figure 1) is homogeneous (Jean-Claude Le Saux, IFREMER, 2005, unpublished data).

The shellfish culture and harvesting activities in the Daoulas estuary involve oysters (*Crassostrea gigas*), mussels (*Mytilus edulis*), and clams (*Ruditapes decussatus*). The national shellfish monitoring network (REMI) surveys faecal contamination in shellfish harvesting areas, as required by the relevant European Union (EU) regulations (Anonymous 2009). REMI consists of shellfish quality monitoring, that has been conducted at monthly intervals for several decades by the French Research Institute for the Exploitation of the Sea (IFREMER) to assess quality of shellfish growing areas and to allow administrative services to classify areas according to regulation n°854/2004, modified by the regulation EC n°1666/2006 (classification A: 100% of results < 230 *E. coli*/100 g FIL (Flesh and Intravalvular Liquid) with a direct consumption of shellfish and classification B: 90% of results < 4 600 and 100% < 46 000 *E. coli*/100 g C.L.I. with purification of shellfish). The level of classification of shellfish harvesting areas in Daoulas estuary is B for oysters (at point B, Figure 1).

- **<u>Sampling investigations</u>**

A data set was created by monitoring subcatchments from January 2007 to January 2008 at outlets of subbasins 1, 2, 3 and 4 (Figure 1). Weekly sampling was performed and *E. coli* was analyzed with microplates NF EN-ISO 9308-3. Moreover, river discharges were systematically recorded to allow flux calculation. At points 1, 2 and 3, the flow was measured by a velocity sensor (A.OTT.GMBH Kempen) during field investigation each week (method with at least 4 verticals with 2 points in each vertical for a 2 meters large and 50 cm deep section). At point 4, a permanent gauge provided continuous flow in the Mignonne River (http://www.hydro.eaufrance.fr/).

For the monitoring period, we obtained 16, 39, 36 and 38 *E. coli* concentrations and associated flow values for points 1, 2, 3 and 4, respectively. These data correspond to different climatic conditions (e.g. rainfall events). The information was complemented by *E. coli* concentration values at location 1 for the period 2004 to 2006 (33 data points). During the study period, rainfall was monitored by the *Météo-France* stations located within a distance of about 10 km.

- The catchment-scale SWAT flow and water quality model

SWAT is a continuous time model that operates on a daily time step. It was developed by United States Department of Agriculture - Agricultural Research Service (USDA-ARS) (Arnold, Srinivasan *et al*. 1998; Arnold and Fohrer 2005). SWAT was chosen for this study because, first, it simulates the hydrological processes of a catchment and has been efficiently tested in another French catchment to simulate Phosphorus flows (Rollo and Robin 2010); second, submodels, including microbial survival and transport, have been added more recently; and third, it is an open source model run on a Geographical Information System (GIS) platform.

In the hydrologic component, runoff is estimated separately in each subbasin and routed to obtain the total runoff for the catchment. The runoff model uses a modified SCS (Soil Conservation Service) curve number method, and the peak flow is predicted from a modified rational formula. The estimation of potential evapotranspiration is calculated by the Penman-Monteith method (Neitsch, Arnold *et al.* 2005). Calculation methods or equations used in SWAT model are explained in Neitsch *et al.* (2005). AVSWAT (Di Luzio, Srinivasan *et al.* 2002) was developed as an interface between SWAT 2005 and Arcview 3.2 (ESRI, CA, USA).

The topography of the catchment was derived from a 15×15 m DEM (Digital Elevation Model). Land-use was digitized from the aerial orthophotos (BD ORTHO® RGE database from the French National Geographic Institute). Each parcel of the orthophotos was digitized and associated with six classes of pasture, forest, urban, permanent crops (orchards and coniferous trees), and water. For soil data, we used profiles made for soil permeability studies (personal communication with administrative services). The average depth of the soil profiles ranged from 1.0 to 1.9 meters and textures were generally clay loam or sandy loam (Bougeard, Le Saux *et al.* 2008). The majority profiles had a shale rock horizon. These characteristics were formatted to create a SWAT user's soil database for the modeling purposes.

SWAT's microbial sub-model

The SWAT's microbial sub-model considers the fate and transport of organisms and models faecal bacteria die-off and re-growth using a first order decay equation (1) (Moore, Smyth *et al.* 1989) expressed as

$$C_t = C_0 \times e^{-K_{20} t \theta^{(T-20)}} \qquad (1)$$

where C_t is the bacterial concentration at time t (count/100 ml); C_o is the initial bacterial concentration (count/100 ml); K_{20} is the first-order die-off rate at 20°C (day-1); t is the exposure time (days); θ is the temperature adjustment factor; T is the temperature (°C).

The fate and transport of bacteria is simulated as a function of bacterial populations resulting from manure applications, and the die-off and re-growth in soil and soil water, soil adsorption, and runoff partition (Sadeghi and Arnold 2002) . In addition, tillage incorporation affects how much bacteria is available for runoff transport, and filter strips can trap bacteria transported with runoff (Lim, Edwards *et al.* 1998; Sullivan, Moore *et al.* 2007). In streams, bacteria are subject to first order decay and point sources can be specified.

The current formulation of the SWAT bacteria transport model assumes bacteria are partitioned between the soil solution and the soil particles. The partition coefficient used in the model was 0.9, which means that 90% of the bacteria cells remain in the unattached state (i.e. in the soil solution). This value is in line with Soupir *et al*. (2008). The coefficient controls how much bacteria in the soil solution would be transported by runoff (Soupir, Mostaghimi *et al.* 2008).

Input parameters

The parameters used in SWAT to define *E. coli* behavior are presented in Table 1. Parameter values for die-off of less persistent bacteria were selected from Baffaut and Benson (2003) for bacteria in soil solution and adsorbed to soil particles, and from Benham *et al.* (2006) for bacteria in streams.

Table 1. Parameters for river flow calibration and faecal bacteria simulations in SWAT

Variable name	**Description**	**Value**
Parameters for river flow calibration		
SURLAG	Surface runoff lag coefficient	0.208
ESCO	Soil evaporation compensation factor	0.9
EPCO	Plant uptake compensation factor	0.7
CANMX	Maximum canopy storage (mm H_2O)	0.011
SLSUBBSN	Average slope length (m)	10.7
SLOPE	Average slope steepness (m/m)	0.599
CH_N	Manning's "n" value for channel	0.025
CH_K2	Effective hydraulic conductivity in main channel alluvium (mm/hr)	68.3
ALPHA_BF	Baseflow alpha factor (days)	0.613
GW_DELAY	Groundwater delay times (days)	20
RCHRG_DP	Deep aquifer percolation fraction	0.132
REVAPMIN	Threshold depth of water in the shallow aquifer required for "revap" or percolation to the deep aquifer to occur (mm H_2O)	249.1
GW_REVAP	Groundwater "revap" coefficient	0.110
GWQMN	Threshold depth of water in the shallow aquifer required for return flow to occur (mm H_2O)	0.435
SOL_K	Saturated hydraulic conductivity (mm/hr)	+24.7%
SOL_AWC	Available water capacity of the soil layer (mm H_2O/mm soil)	+24.5%
CN2	Initial SCS runoff curve number for moisture condition II	-11.9%
Parameters for faecal bacteria simulations		
WDLPQ	Die-off factor for less persistent bacteria in soil solution at 20°C (1/day)	2.01
WGLPQ	Growth factor for less persistent bacteria in soil solution at 20°C (1/day)	0
WDLPS	Die-off factor for less persistent bacteria adsorbed to soil particles at 20°C (1/day)	0.023
WGLPS	Growth factor for less persistent bacteria to soil particles at 20°C (1/day)	0
WDLPRCH	Die-off factor for less persistent bacteria in streams (moving water) at 20°C (1/day)	0.35
WDLPRES	Die-off factor for less persistent bacteria in water bodies (still water) at 20°C (1/day)	1.030
BACTKDQ	Bacteria runoff extraction coefficient (m^3/Mg)	90
BACTKDDB	Bacteria partitioning coefficient	0.90
THBACT	Temperature adjustment factor for bacteria die-off/growth	1.070
WDLPF	Die-off for less persistent bacteria on foliage at 20°C (1/day)	0.016
BACT_SWF	Fraction of manure applied to land areas that has active colony forming units	1

The pollutant sources in the SWAT model were the discharges of the four WWTPs and the application of manure on pastures. The four WWTPs collect and treat the wastewater from the towns of Ploudiry, La Martyre, Saint-Urbain and Dirinon (Figure 1). The mean concentration of the discharges was 8.8×10^4 *E. coli*/100 ml (Pommepuy, Le Guyader *et al.* 2008). The discharges of each WWTP were determined in terms of equivalent inhabitant concept and ranged from 68 m^3/day (400 equivalent inhabitants) to 324 m^3/day (1900 equivalent inhabitants).

From literature (Geldreich 1966; Baffaut and Benson 2003; Machado, Maia *et al.* 2006) and French local administrative data, manure production was estimated from the number of livestock and the quantity of faeces produced by the different types of animals for each subcatchments. To reproduce the agricultural practices of the catchment, we used the available data on farm location and livestock numbers (Figure 1) (DDTM 2010). According to Aitken (2003), we assumed that the quantity of liquid manure spread corresponds to the quantity of faeces produced by the farms (Aitken 2003). From concentration in faeces (Geldreich 1966), a decrease of 3 log in *E. coli* concentration was applied to take into account the pathogen and *E. coli* decay during manure storage. Goss and Richards (2008) consider that 3 months is the time necessary for maturing of manure. Finally, fecal bacteria concentration in manure was set at 8.96×10^5 *E.* coli/g (dry weight).

From these data, we estimated a dummy spreading calendar according to four hypotheses. First, spreading is done at the rate of 30 t (wet weight) of liquid manure per hectare, *i.e* 1.5 t on dry weight, for one working day. Second, spreading is only realized in authorized areas shown in Figure 1. Third, spreading is realized during the authorized period (i.e. from January 15 to June 30). Fourth, spreading is only realized in dry weather conditions (i.e rainfall less than 5 mm/day).

Scenarios

The baseline simulation allowed us to calibrate and validate the SWAT model for *E. coli* concentrations in the river at point 1 and in shellfish at point B. This simulation included the WWTP discharges and spreading of manure on pastures (range grasses) where 10 m filter strips were set up to protect river quality. This scenario corresponds to the local practices observed during our study period and was backed up by information from the administration. Thus, the Water Framework Directive recommends to put filter strips in place between a potential pollutant-source area and a surface-water body that receives runoff, to reduce the amount of fecal bacteria in the runoff. Moreover, in the Daoulas area, spreading is done on range grasses and not on bare ground, limiting the risk of contaminated runoff. This scenario was used for the calibration and validation of the model.

Once calibrated and validated, the model was used to assess the effect of different inputs on river and estuarine quality. Two scenarios were chosen:

- Scenario 1 considered only the point source discharges (WWTP), without manure spreading, corresponding to an assessment of a banning of this practice on the watershed (or a dry-period, outside the spreading season). Overflow of raw wastewater was not simulated as no failure of the sewage network has been reported locally.

- Scenario 2 included the WWTP discharges and the same manure spreading calendar as for the baseline scenario, but without filter strips and on a bare ground. This scenario corresponds to bad spreading practices, such as what would occur after the corn harvest, for example. In this simulation, we considered that manure immediately entered adjacent watercourses, in contrast to the baseline scenario where recommended exclusion zones (10 m filter strip beside the river) were set up.

- The MARS-2D hydrodynamic model

MARS (Model for Applications at Regional Scale) is a 2D and 3D coastal model developed by the Coastal Oceanography Department of IFREMER (Lazure and Dumas 2008). It solves the so-called "primitives equations" of geophysical flows, which are basically the Navier-Stokes equations under the hydrostatic approximation. These equations are common to numerous oceanic and atmospheric models **(**Lazure and Dumas 2008); the primitive equations allow for a large spectrum of environmental flows, from deep ocean to coastal seas and estuaries or lakes. They are solved by MARS using finite differences on a 3D grid. The air-sea interface is a free surface and the external (surface gravity) mode is computed using an Alternate Direction Implicit (ADI) scheme, which allows (i) a much larger time step (shorter computations) than explicit schemes and (ii) an original external/internal mode coupling (Lazure and Dumas 2008). Furthermore a robust wetting-drying algorithm is implemented. The vertical structures are described using the so-called σ coordinate, enabling a simple formulation of the free surface and bottom boundary conditions. For this application however, the model was run in a 2D mode, meaning that the system considered is described by the Shallow Water (or Saint-Venant) equations. The choice of a 2D approach was justified by the very weakly stratified nature of the Daoulas River estuary (as discussed above) and allowed for a better horizontal resolution at the same computational cost. The 2D equations solved by the model can be found in Riou et al. (2006), for instance. A highly refined grid was thus used, with a horizontal step of 30 m in both directions, resulting in 475 x 345 grid points. Tidal forcing was propagated from a large-scale tidal atlas (FES99) (Lefevre, Lyard *et al.* 2002) into the high resolution grid, using a series of nested models of increasing resolution and decreasing spatial extent (Figure 1). The MARS model's hydrodynamic aspects (coastal currents) were already calibrated and validated (Lazure and Dumas 2008).

The calibration of hydrodynamics in the MARS model was performed in a previous study (P. Lazure and F. Dumas, IFREMER, 2009,private communication), which confirmed that the currents in the area of interest are strongly tidally driven. The calibration relies on comparisons of (i) the temporal evolution of the modeled sea level against independent tide predictions and (ii) the modeled currents *vs. in situ* measurements. The former is nowadays a routine check, while the latter does depend on the accuracy of the Digital Elevation Model. Actually Acoustic Doppler Current Profiler (ADCP) measurements on various ship tracks were performed in the Bay of Daoulas on March 2th, 2006 (IFREMER 2007) and made available for the afore-mentioned calibration study. The *rms* error on the currents was found to be in the 5-10 cm/s range, disregarding tide-reversal transects where model validation is poorer owing to small temporal offsets, which become relatively important when currents are reversing. Such phase lags (typically a quarter of an hour) do not affect the overall behavior of the advection-diffusion solution, however, and the model validation (especially when dealing with punctual *x,y,t* measurements such as those given by a ship-mounted ADCP) thus proved to be satisfactory for the present application. As far as the microbial submodel is concerned, a T90 value was selected as indicated earlier and *E. coli* concentrations in shellfish were calculated using the shellfish/water concentration ratio of 30.

MARS microbial sub-model

A microbiological model included in the MARS-2D allows faecal inputs and the fate of different microorganisms to be simulated (Riou, Le Saux *et al.* 2007). A constant bacterial decay rate (K) was used in MARS, which can be estimated as K = (log10/T90) where T90 is the time when 90% of a given initial population will disappear (Pommepuy, Hervio-Heath *et al.* 2005).

Daily values for river flow and *E. coli* concentration were taken as input into MARS, which was then run with realistic tide and windy conditions. The simulation period was from 01 February to 31 July 2007, because this is the sampling period. The die-off used in this study was 0.35 (at 20 °C). The selected die-off value is a mean for night and day conditions, which take the local hydrologic conditions and local oceanic temperate climate into account (Pommepuy, Hervio-Heath *et al.* 2005). MARS allows monitoring points to be set up in the estuary and *E. coli* concentrations to be traced during simulation (Figure 1). Monitoring point B corresponds to the REMI survey point.

From the simulated concentrations in the estuarine water, the *E. coli* concentrations in shellfish were calculated using a shellfish/water concentration ratio of 30. This ratio was estimated based on literature (Burkhardt, Calci *et al.* 2000) as well as the data on the Brittany climate and local oysters (*Crassostrea gigas*) (Anonymous 1996; Riou, Le Saux *et al.* 2007). Model results of shellfish contamination at point B were then compared with the shellfish monitoring database REMI.

- Model calibration procedures

For the calibration of river flow, autocalibrations were used with PARASOL (Parameter Solutions) method (van Griensven and Meixner 2007, Green and van Griensven 2008) and some parameters were adjusted from the initial SWAT values to match the simulated and observed daily flows. The coefficient of determination (r^2) and the Nash-Suttcliffe efficiency (Ens) were used to evaluate model prediction for flow. The r^2 value is an indicator of the strength of the relationship between the observed and simulated values (Cheng, Ouyang *et al.* 2007). Nash-Suttcliffe simulation efficiency indicates how well the plot of observed *vs.* simulated values fits a 1:1 line (Nash and Sutcliffe 1970). If the values of r^2 and Ens are equal to one, then the model prediction is perfect. According to literature, the model's efficiency is satisfactory if r^2 is greater than 0.6 and Ens greater than 0.5 (Santhi, Arnold *et al.* 2001; Gassman, Reyes *et al.* 2007; Moriasi, Arnold *et al.* 2007) .

For the calibration of *E. coli* concentration, as done in literature and due to uncertainty of contamination sources, a frequency curve analysis method was chosen to compare measured *vs.* predicted data for faecal coliform concentrations. This allows to appreciate the quality of the simulation and to compare impacts of different management scenarios (Baffaut and Benson 2003; Pachepsky, Sadeghi *et al.* 2006; Guber, Pachepsky *et al.* 2007; McGechan, Lewis *et al.* 2008; Parajuli, Mankin *et al.* 2009). Nevertheless, r^2, Ens, and frequency curve analysis for *E. coli* concentrations in the river were computed for the purpose of calibrating the SWAT model, and for *E. coli* concentrations in shellfish for the purpose of calibrating the MARS model. Because few data on coastal water quality were available, these statistics were not computed for the coastal MARS model.

Data used for calibration and validation

For calibration and validation of daily river flow, a 7-year period of gauging values at point 4, and weekly measures from February to July 2007 at points 2 and 3 were used.

For *E. coli* concentration frequencies in the river, 49, 39, 36 and 38 data points measured from 2004 to 2007 at points 1, 2, 3 and 4 were used, respectively. Average concentrations were very close for the four points, with the maximal value at point 2 ($5.11 \times 10^3 \pm 1.51 \times 10^4$ *E. coli*/100 ml) and the minimal value observed at point 3 ($1.47 \times 10^3 \pm 2.62 \times 10^3$ *E. coli*/100 ml). This limited dataset includes only isolated measurements during one year of study and a future larger database would be very helpful to improve the robustness of the model.

For shellfish quality, the calibration for *E. coli* concentration frequencies was realized at point B with a large database available on shellfish (REMI survey, N=162) from 1991 to 2007. The dataset on shellfish indicates an average *E. coli* concentration of $8.47 \times 10^2 \pm 3.43 \times 10^3$ *E. coli*/100 g. FIL.

Results

<u>- Calibration and validation for river flow</u>

The hydrological calibration was made in subbasin 4 over a 4-year period (2000 to 2003) using the embedded autocalibration function of SWAT. The parameters used to calibrate river flow and final values chosen for each one were presented in table I. The calibration results indicated a good reproduction of river flows (Figure 2); $r^2 = 0.84$, Ens = 0.84.

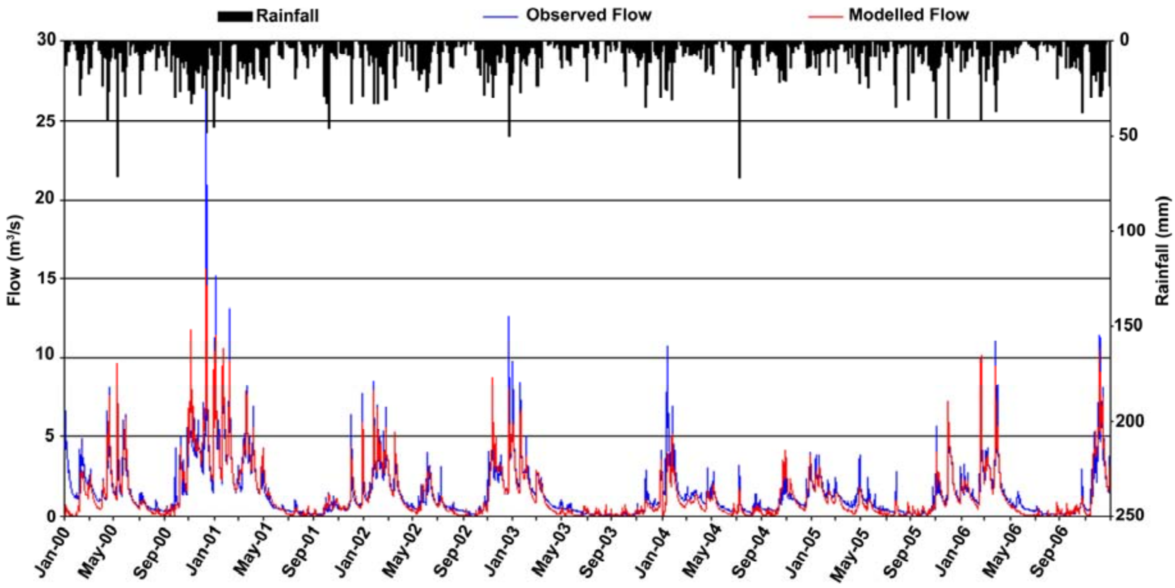

Figure 2. Simulated and measured flows from January 2000 to December 2006 for subcatchment 4 at the gauging station (upstream in the Daoulas catchment)

The validation of the model over the 3-year period (2004-2006, Figure 2), indicated a good simulation performance ($r^2 = 0.84$, Ens = 0.82). The model performance was judged to be compatible with studies published in the literature (Pohlert, Huisman *et al.* 2005; Michaud, Deslandes *et al.* 2006).

In addition, the calibration for subbasins 2 and 3 was done from February to July 2007 using weekly measurements of streamflows and the results were judged to be satisfactory for subbasin 2 (r^2=0.73 and Ens=0.80) and acceptable for subbasin 3 (r^2=0.59 and Ens=0.46), which illustrates the robustness of the model simulation. Results on subbasin 3 were less satisfactory because the calibration was done on a limited number of values corresponding to punctual measurement and not data from a gauging station.

- **<u>Calibration and validation for *E. coli* concentrations in river</u>**

Figure 3 presents *E. coli* daily simulated concentrations for baseline scenario and observed concentrations at the outlet of the subcatchment 4 (point 4).

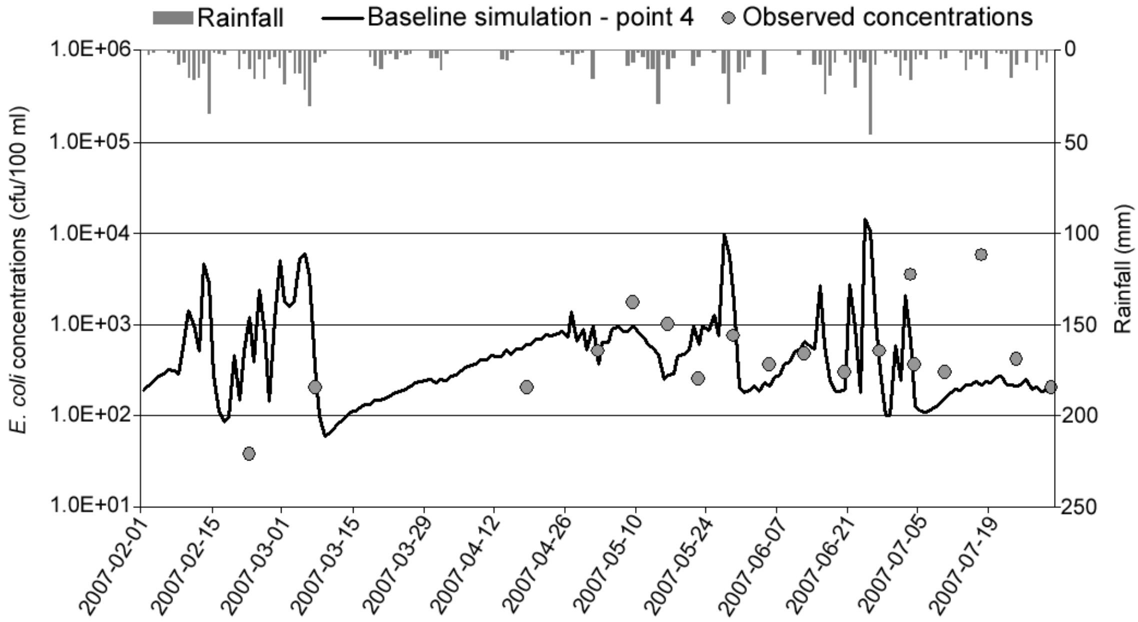

Figure 3. Simulated and observed *E. coli* concentrations in the Mignonne River (Daoulas catchment) at point 4 according to baseline scenario from February to July 2007

E. coli concentrations ranged from 6.0 x 10^1 to 1.4 x 10^4 cfu/100 ml for simulated values and from 3.8 x 10^1 to 5.8 x 10^3 cfu/100 ml for observed values in the river. This graph reveals several characteristics of bacteria transport in this watershed

The graph demonstrated the rapid response of the watershed to rainfall in terms of *E. coli* concentrations, as concentrations increased by a factor 100 or 1000 over a short time. This result was mainly due to the characteristics of the watershed including size (113 km²) and short time of concentration (less than a day), which provide little time for *E. coli* degradation during transport. Additionally, despite the fact that in our simulation, manure was not spread when the precipitation was more than 5 mm/day, agricultural practices had a strong influence on water quality due to the persistence of *E. coli* in soil and its rapid change in rainfall conditions. Moreover, the large percentage of agricultural land (44% of arable land and 25% of pastures) accentuates the impact of agricultural practices on water quality. On the other hand, it is clear that during dry weather, the WWTP discharges were major sources of contamination.

Regarding observed concentrations, most were within the same range of simulated concentrations. For example, on 6/20/2007: observed *E. coli* concentration was 200 cfu/100 ml and the model simulated 299 cfu/100 ml in the river. Nevertheless, there are some differences between the model results and the measured values. They could be due to the analytical uncertainty introduced by the laboratory method employed to measure the sample contaminant level (McCarthy 2008) or the type of samples collected. The model simulated a daily average concentration in the river, while observed concentration come from a grab sample realized during the day. Most of the results were rather acceptable excepted for the observed concentration on 7/17/2007 equal to 5840 cfu/100 ml whereas the model simulated 216 cfu/100 ml. Without exact knowledge of the spreading dates, it is difficult to determine if the poor model's performance on that date is caused by a deficiency of the model or lack of detailed input data. Additionally, other possibilities of unknown inputs include punctual events like a failure of sewer system or outflow from a manure pit, not taken into account in our scenario.

From February to July 2007, the simulated values were compared with *E. coli* concentrations observed on river samples to calibrate the *E. coli* concentrations in the river. This analysis demonstrated a poor model efficiency: $r^2 = 0.0007$ and Ens = -0.21 at point 4. These values were comparable to those found by Baffaut and Benson (2009) and Parajuli (2007). In order to overcome the problem due to uncertainty of factors and mechanisms implicated in biological models, these authors recommend analyzing and comparing the frequency curves of simulated *vs.* observed concentrations.

The calibration of *E. coli* simulation was realized at point 1 with measured data (2004-2007, N=49). Figure 4 presents frequency curves and shows a good correlation between simulated and observed concentrations frequency (r^2=0.99).

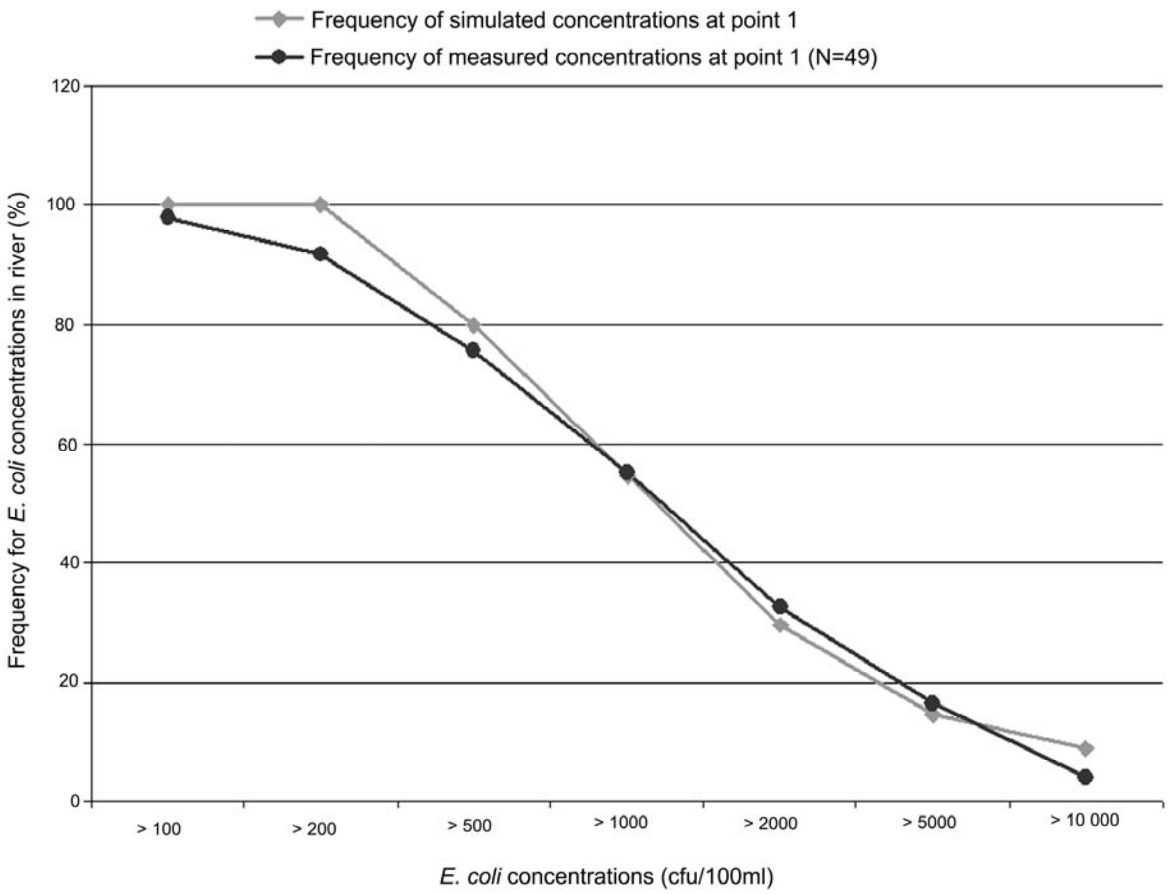

Figure 4. Cumulative frequency curves (%) for *E. coli* concentrations in the river at point 1 for observed data and simulated data from February to July 2007

For example, 76% and 80% of measured and simulated *E. coli* concentrations are superior to 500 cfu/100 ml, respectively.

To validate the model, a similar comparison between simulated and observed frequencies was made at points 2, 3, and 4 (Figure 5).

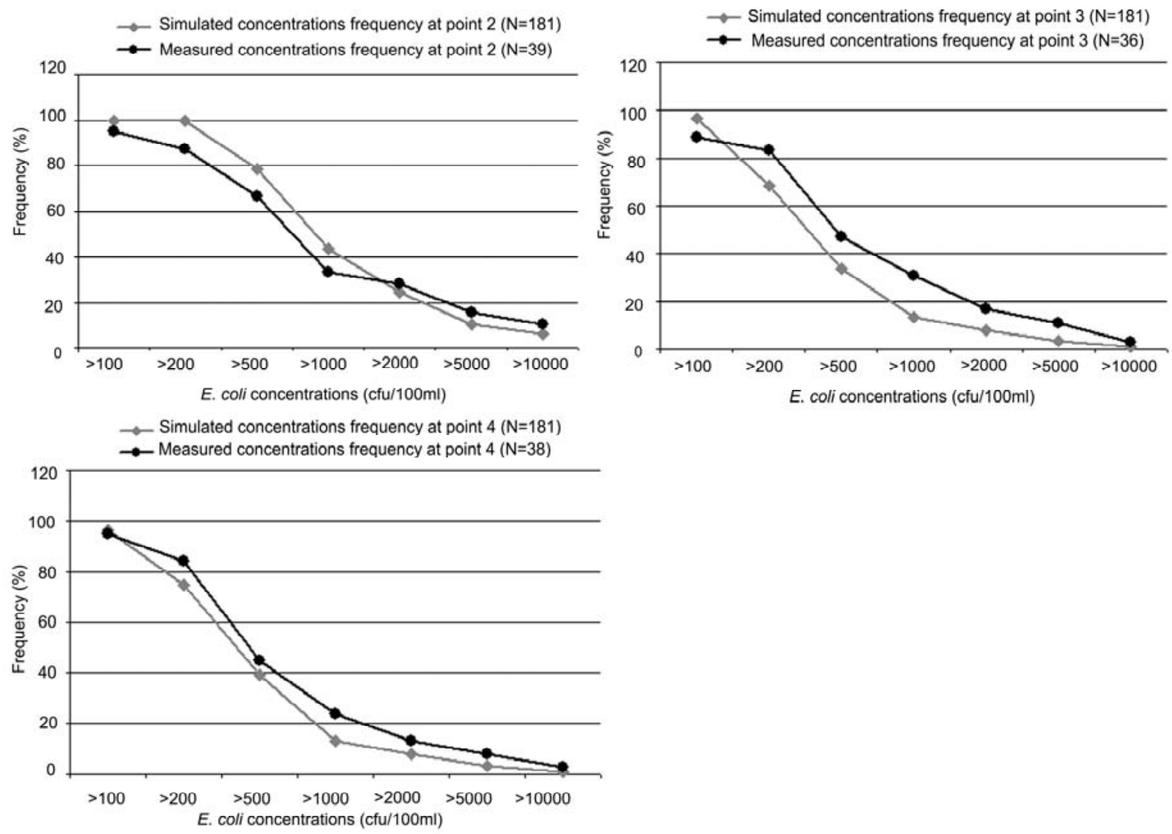

Figure 5. Cumulative frequency curves (%) for *E. coli* concentrations in the river at points 2, 3 and 4 for observed data and simulated data from February to July 2007

There is a good correlation between simulated and measured frequency curves and the coefficients of determination between frequencies are equal to 0.98, 0.95 and 0.99 for points 2, 3, and 4, respectively.

Thus, we estimated that the SWAT calibration and validation was sufficient because the model simulation performance was in agreement with those documented in literature (Parajuli 2007; Baffaut and Benson 2009).

- Impact of *E. coli* fluxes on coastal water quality

Baseline scenario results at the main outlet of the catchment were taken as inputs into the MARS-2D model and the figure 6 presents daily mean *E. coli* concentrations simulated by the MARS model in estuary water for baseline scenario.

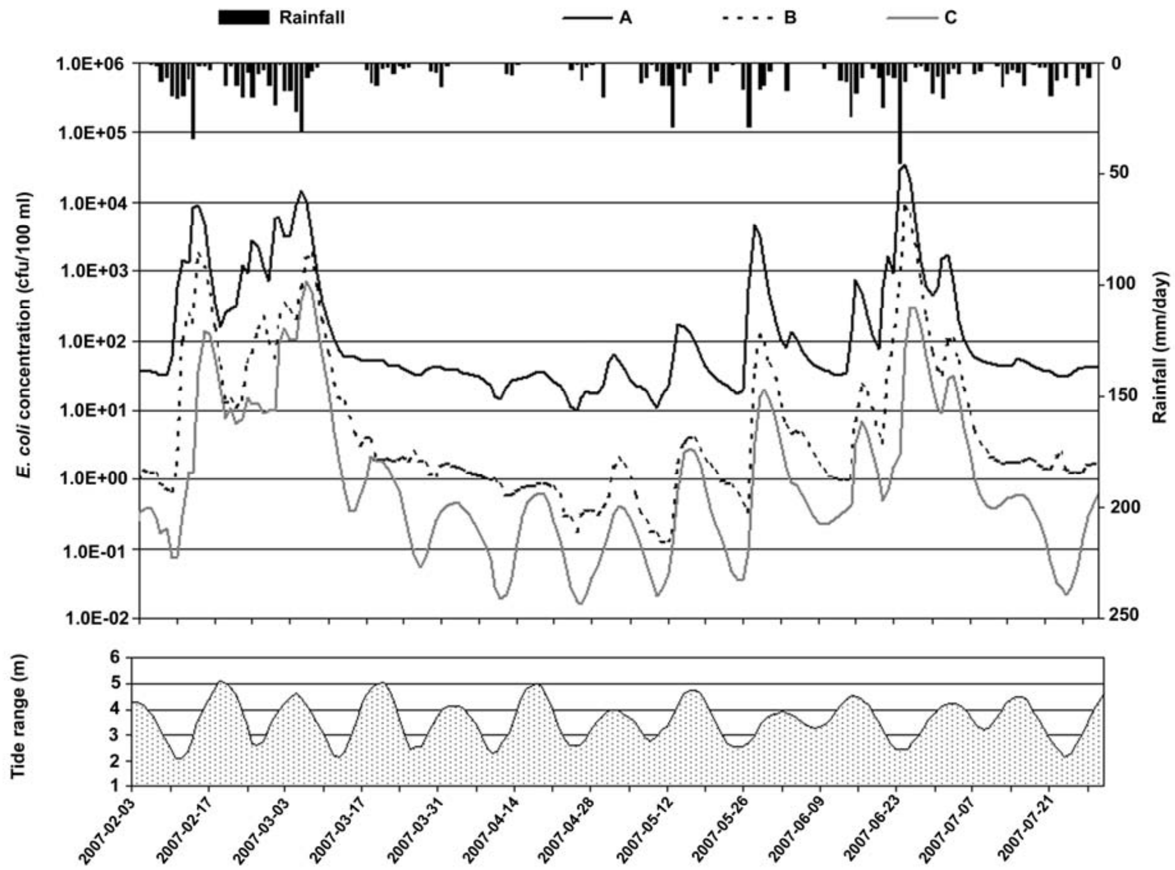

Figure 6. Baseline scenario : *E. coli* concentrations in estuarine waters at the 3 monitoring points (A, B and C) with rainfall data and tidal range from February to July 2007

In MARS, three points were used to estimate concentration variation in the estuary during the simulation period: A (upstream), B (in the middle estuary), and C (downstream). The succession of spring and neap tides results in variation of tidal range and induces an effect on water amplitude.

Figure 6 suggests that point A was highly impacted by river discharges throughout the simulation period. Indeed, marked peaks of simulated faecal contamination in the estuary follow *E. coli* peaks in the river, with a maximal concentration of 3.4 x 10^4 *E. coli*/100 ml one day after the rainfall event of 45.4 mm occurred on 24 June 2007. Point A is located in the upstream of the estuary, where there was almost no impact of the fortnightly tidal cycle. The *E. coli* concentrations at this point were only affected by the catchment discharges, even for March and April when the precipitation was relatively low. Two phases were observed in the simulated results regarding the recovery of estuarine water quality after a rainfall event. In the first two days after the rainfall event, concentrations decreased by a factor of 100. It then took several tidal periods from February to April before a total recovery of water quality was observed due to new inputs from river and the dilution capacity of the estuary water (Figure 6: February event and period until end of April).

At point C, located close to the open sea (Bay of Brest), the effect of tidal range variation was particularly well marked during March and April because river input was low. A correspondence between water quality fluctuation and tidal range variation could be observed during this period. The fortnightly tidal cycle induced these variations in concentration, possibly because of the shape of the estuary. This closed estuary drains out more than 50% of its water volume during low tide. During spring tides, the foreshore was exposed for a long time to larger variations of *E. coli* concentration than during neap tide (i.e. in April). Daily mean *E. coli* concentration was therefore higher during spring tide at point C.

Nevertheless, it is important to note that, from March to April, simulated concentrations at points B and C fluctuated between 10^{-2} and 10^{0} *E. coli*/100 ml, *i.e.* very low concentrations that remained within the European restrictions for excellent bathing conditions (i.e. 250 cfu/100 ml, European directive 2006/7/EC) and shellfish consumption (14 cfu/100 ml, USEPA criteria) (USEPA 1986) .

However, during contamination (i.e. rainfall) events, point C was impacted by river discharges, as indicated by simulated concentrations as high as 3.0 x 10^{2} *E. coli*/ 100 ml on 24 June 2007. After April, the *E. coli* fluxes were increasing because the rainfall events resulted in additional loads. Thus, the estuary response was very rapid with a coincident variation with the streams. When concentrations were increased by two orders of magnitude in the river (Figure 6), there was similar increase at the other monitoring points as well. The dilution effect and bacterial die-off consistently caused a concentration decrease from A to C. This effect was greater during dry periods (March to April) than during wet periods when discharges were higher. Moreover, a one-day time lag was observed between river discharges and contamination peaks at points A and B, and a two-day lag was observed at point C. Therefore, according to the model, the estuary reacted to the river inputs within a short time. Furthermore, water quality recovery was also very fast over the first days, but may take several weeks to return to be fully recovered, indicating a high sensitivity of the estuary to the rainfall events and/or the watershed fecal inputs.

- *E. coli* concentrations in shellfish

The validation method for shellfish quality was similar to that applied for the SWAT *E. coli* validation. That is, the validation was assessed by comparing frequency curves. The results at point B (Figure 7) indicated that for simulated and measured data, up to 32% of the *E. coli* concentrations exceeded 230 *E. coli*/100g FIL.

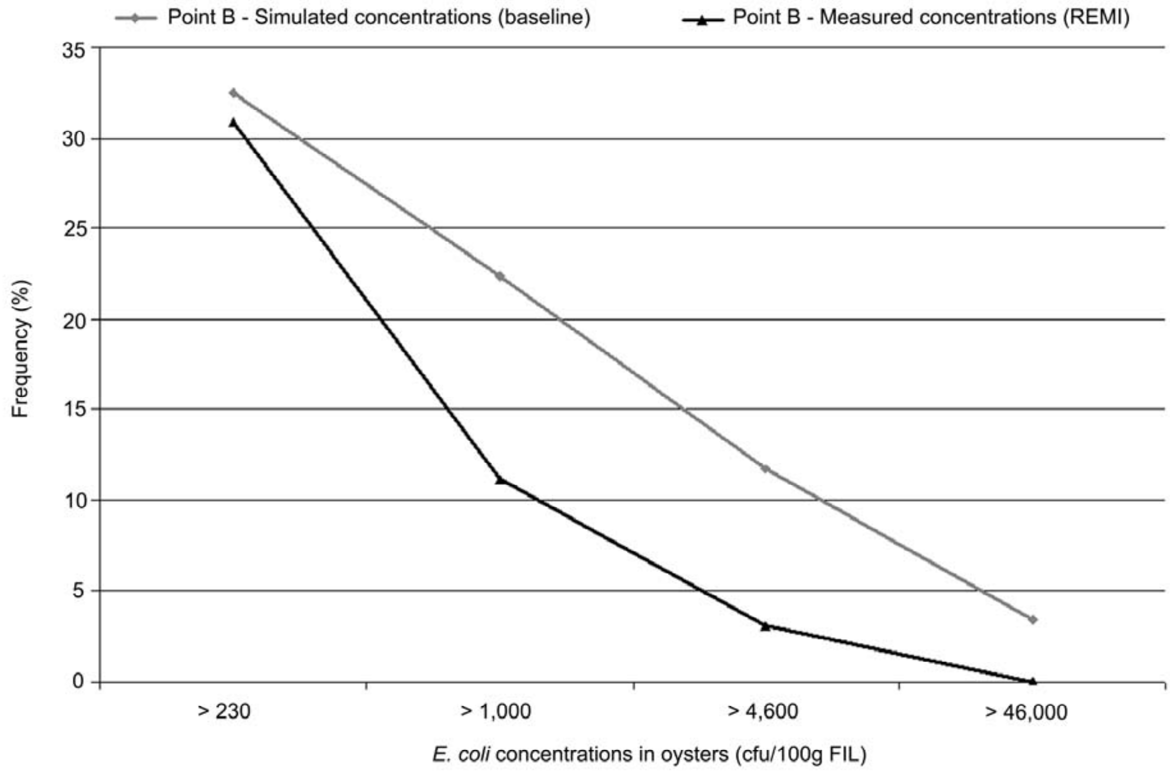

Figure 7. Cumulative frequency curves (%) of different *E. coli* contamination levels in oyster flesh and intravalvular liquid (FIL) in point B for baseline scenario and measured data (REMI) for February to July 2007

Approximately 11% of measured concentrations exceeded 1000 *E. coli*/100 ml in REMI data, while 22 % of simulated concentrations exceeded. No measured value within the REMI database exceeded 46 000 *E. coli*/100g FIL, and only 3 % of the simulated values exceeded. These results showed that the models could reproduce shellfish quality frequency with an acceptable reliability.

<u>- Effect of agricultural practices on water and shellfish quality</u>

Once calibrated for flow and *E. coli* concentrations both in the river and shellfish, the models were used to simulate two other input conditions: first, the WWTP discharges only and, second, the WWTP discharges and spreading on bare ground.

The *E. coli* concentrations in the river and coastal water, as well as in shellfish, under these scenarios were compared to those of the baseline scenario. We compared the results obtained in the scenarios with the requirements of bathing and shellfish Directives (2006/7/EC and 2006/1666/EC). According to Directive 2006/7/EC, the bathing water quality requirement in rivers (inland waters) is not met if the *E. coli* concentration is higher than 900 cfu/100 ml (based upon a 90-percentile evaluation).

Figure 8 presents *E. coli* concentrations in the river at point 1 for scenarios 1 and 2 and baseline simulation.

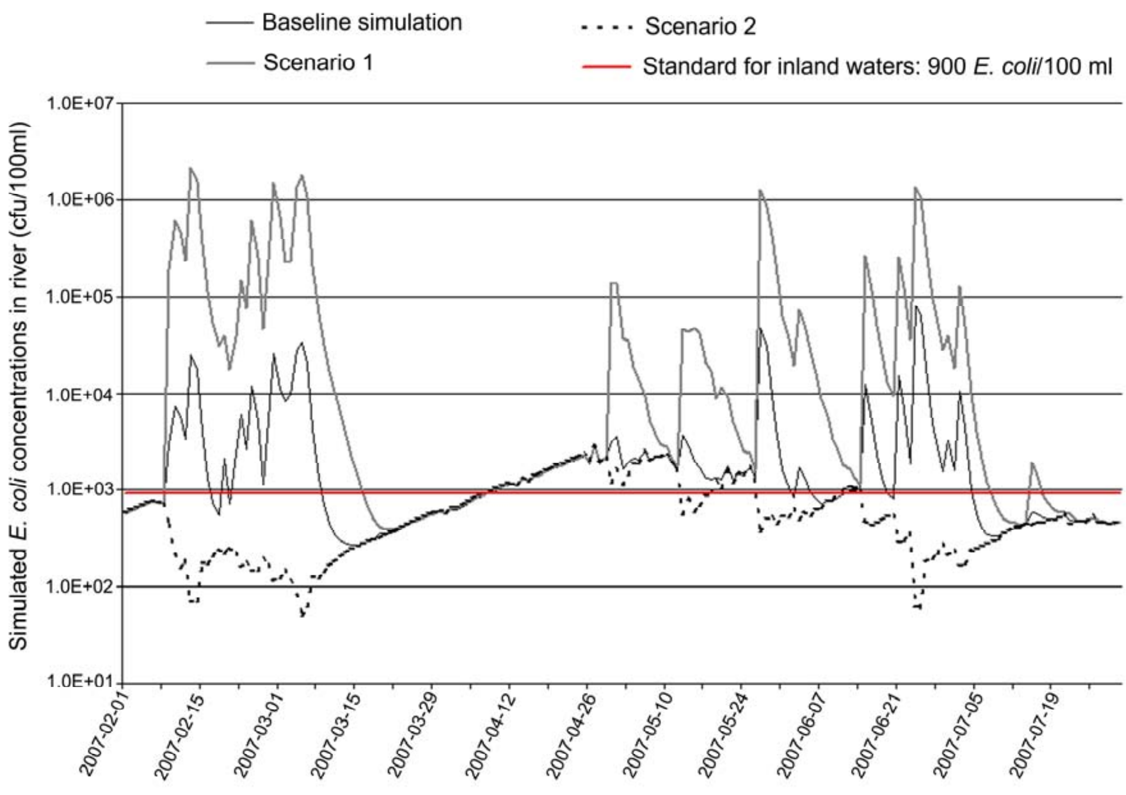

Figure 8. *E. coli* concentrations in the river (point 1) for baseline scenario and scenarios 1 and 2

Overall, *E. coli* concentrations were 1.5 times lower when there was no spreading activity (scenario 1), but could be 15.5 times higher when spreading was conducted without respecting the regulations (scenario 2).

The effect of these different scenarios are discussed here with regard to the EU bathing Directive 2006/7/EC, which stipulates *E. coli* concentrations should be lower than 500 cfu/100 ml in water for bathing uses. Water quality was considered as excellent and good quality if the 95-percentile value was not higher than 250 and 500 cfu/100 ml, respectively. Figure 9 presents the *E. coli* concentrations for the scenarios, and the baseline simulation at point B; and we calculated the 95- and 90-percentile for each scenario at each point (A, B and C).

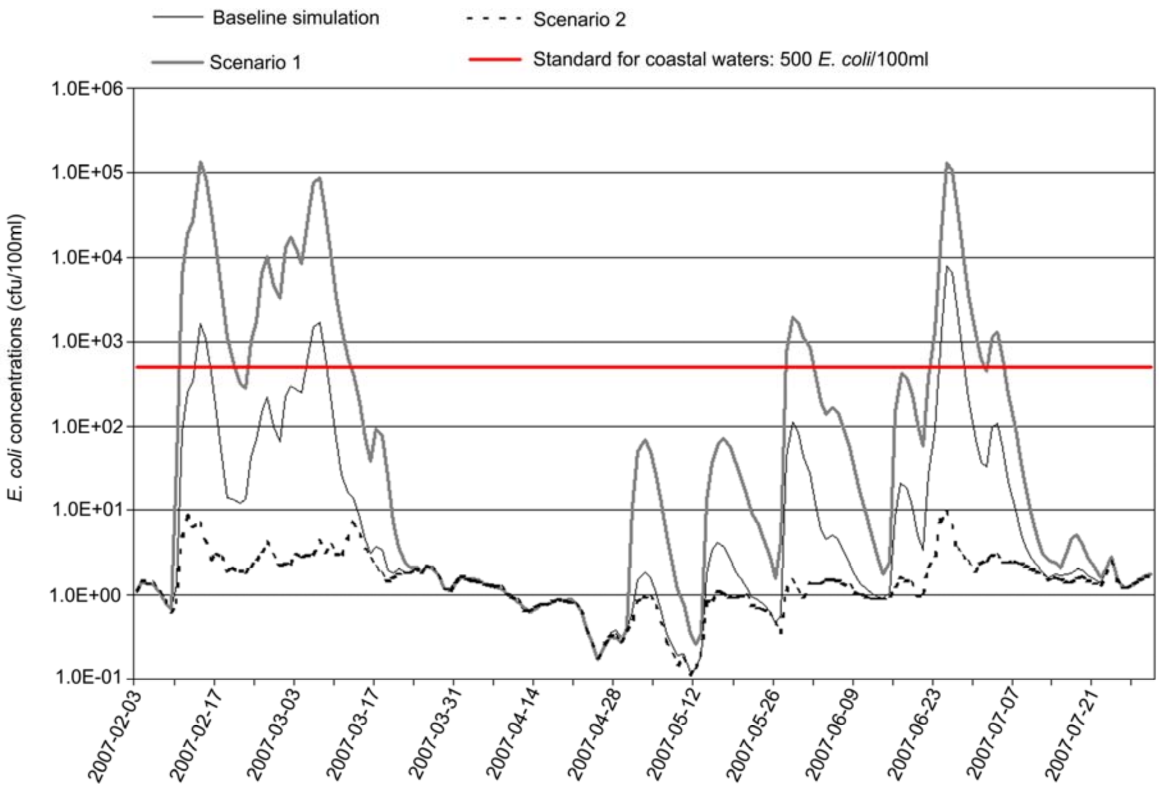

Figure 9. *E. coli* concentrations in coastal waters (point B) for baseline scenario and scenarios 1 and 2

For baseline simulation, the water quality would be non-sufficient at point A, sufficient at point B, and excellent at point C; whereas, for scenario 1, water quality would be excellent at the three points. The water quality for scenario 2 would be non-sufficient at points A, B and C.

Discussion

The integrated SWAT and MARS models were used to estimate the impact of catchments' agricultural practices and wastewater discharges on the quality of the aquatic environment in the estuary. This association of models has the ability to simulate *E. coli* concentrations from the Daoulas catchment, *via* streams and watershed outlet, and into the estuary. These models, set up for the Daoulas watershed and estuary, consider the dynamic processes, and incorporate daily and variable *E. coli* fluxes into the hydrodynamic model. This improved the prediction accuracy compared with the previous applications using only discrete inputs values (Kashefipour, Lin *et al.* 2006; Riou, Le Saux *et al.* 2007).

The MARS model in the Bay of Brest, at which the Daoulas River discharges were integrated, enables the consideration both of the riverine and marine processes (Bailly du Bois and Dumas 2005). The water quality, as in classic estuaries, is submitted to different factors including neap and spring cycles, and seasonal variations of river flow or wind that create variable conditions for the dilution of faecal contamination. The SWAT model was implemented, information on *E. coli* sources introduced, and a scenario close to local activities runs in the model. The validation, using available databases, showed that the simulation reproduced frequency of water or shellfish contamination close to those measured in the field. Adding continuous and variable river flow and *E. coli* fluxes as inputs to the coastal water model provided improvement in the description of changes in coastal water quality.

Indeed, the information obtained in the study is critical for the understanding of coastal contamination. The first result concerns the rapid response of the full watershed/estuary system to faecal contamination. One day was sufficient for *E. coli* spread on a field to be introduced in the estuary, and two days were needed to reach the mouth of the estuary. This result further confirms the need to determine the scale and characteristics of the watershed, which have to be taken in consideration to manage coastal areas. Other studies have showed the major impacts that small watersheds close to the sea can have for fecal contamination (Crowther, Wyer *et al.* 2003; Kay, Wyer *et al.* 2005). Crowther *et al*. (2003) have indicated that *E. coli* concentrations were strongly correlated with land use in close proximity to the subcatchment outlet. Furthermore, the model provided information concerning the time needed for water quality recovery: several days were needed, the exact duration depending on the duration of rainfall events: about 10 days for 15.8 mm of rainfall/day, and about 14 days for 30.8 mm/day. But, if the rainfall event is continuous during several days, the time for water quality recovery is longer because of the important discharge of the river and the impossibility to the estuary to dilute contamination. In a previous study, Riou *et al*. (2007) demonstrated that the seawater removal was a function of tidal and windy conditions. This study shows that rainfall and river associated *E. coli* discharges, and not only tidal dilution conditions, need to be taken into account to explain the time for seawater to return to a good quality. In relation to these phenomena, the daily time step used in the model seemed to be well adapted to the watershed size (113 km^2, 90 km river long). The second result indicates that the flux variation is of the same order of magnitude that those due to tidal variation (*i.e.* dilution and dispersion phenomena). This point underlines the need to introduce continuous and variable fluxes in MARS model.

Moreover, the results of the models were also used in the management of coastal areas, especially bathing areas. For baseline simulation and scenarios 1 and 2, 90-percentile evaluation on *E. coli* concentrations in the river were all above 900 *E. coli*/100 ml corresponding to non-sufficient water quality. Thus, the stopping of spreading would not lead to an improvement in classification for bathing water in this river. Nevertheless, in the estuary, if there is no spreading on the catchment, the quality of coastal water would be excellent at point A, even though it is close to the river outlet (scenario 1). In contrast, the scenario 2 with poor spreading practices would create non-sufficient water quality for bathing activities in the entire estuary.

This study was a first approach of coupling a watershed and an estuarine model to assess *E. coli* contamination in an estuary. The simulation of contamination frequencies is of a great interest to assess the contamination risk and test best management options. The model simulated the bacterial runoff due to rainfall events and agricultural practices, and allowed the incorporation of realistic sources of contamination in the catchment. A better knowledge of agricultural practices (dates of spreading, concerned parcels…) would probably improve the goodness of fit between simulated and measured concentrations. However, even if the modeling was based on known fecal contamination sources, the model would fail to reproduce punctual events for which no information was available and, thus, cannot be introduced in the scenario.

It would also be interesting to study the fate and the transport of fecal indicator bacteria in the river at a sub-daily time step since several authors have shown that rainfall-runoff substantially increased *E. coli* concentrations (one to two orders of magnitude) in a very short time (2 to 3 hours) (Jamieson, Joy *et al*. 2005; Davies-Colley, Lydiard *et al*. 2008).

Moreover, the validity of the model's applications should be tested further by comparing simulations with other field data on a longer period. In this study we also showed that, as in other coastal areas, agriculture is one of the major sources of *E. coli* in rivers (Hooda, Edwards *et al.* 2000; Avery, Moore *et al.* 2004). Nevertheless, as previously described, even though good manure management practices are currently implemented in this area, current investigations suggest that faecal pollution from cattle in field or tank failures in some farms could cause water pollution (Aitken 2003). More precise information on slurry condition and manure storage could also be relevant to improve the simulations.

Further confirmation of parameter values should be made for some parameters of the models, such as die-off rate. In our application we used a die-off equal to 0.35, which is a good compromise with local turbidity and salinity conditions in the estuary (Pommepuy, Hervio-Heath *et al.* 2005), but day and night decay rates could also be introduced to better reproduce bacterial behavior. Introduction of sediment resuspension phenomena would also be interesting to improve the model approach. Indeed, the influence of bacteria stored in bed sediment is important on water contamination during high flow (Crowther, Kay *et al.* 2002; Steets and Holden 2003). Low quality waters were found to be related with *E. coli* transported through the soil during erosion events (Vinten, Lewis *et al.* 2004). This was also confirmed by Jamieson, Gordon *et al.* (2004) indicating that stream sediment could be the primary source of contamination during summer months in Canada.

Conclusion

Current microbial water quality models are lacking in their ability to handle continuous fluxes arriving in coastal water. In this study, the simulation of continuous fluxes from a catchment, linked with climatic conditions and watershed practices, constitutes a better approach to observing the capacity for an estuary to dilute contamination. Moreover, it allowed the assessment of response time and the time for water quality recovery for an estuary in realistic conditions. In order to assess water quality for bathing and shellfish activities, the two models used allowed the testing of several solutions to improve quality, like best management practices and observation of their impact on estuarine water contamination. To prevent microbial contamination of river and estuarine water, it is essential to evaluate the effect of best and worst agricultural practices on contamination and to understand the complexity of hydrological and hydrodynamic properties of the unity “catchment-estuary”. This association is important to set up a coherent management strategy for coastal areas, including shellfish growing areas. Furthermore, these simulations permitted the evaluation of the non compliance of bathing areas with regulation associated with a risk to humans exposed to contaminated water (Bougeard, Le Saux *et al.* 2010). Finally, further progress will allow us to improve knowledge about contamination sources like septic systems and bovine pasture, and the implementation of small coastal subcatchments corresponding to local sources close to estuarine activities. Moreover, the appreciation of the sub-daily variations of the river contamination would allow improving the capacity of the model to reproduce *E. coli* concentrations.

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