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Molluscan Shellfish Bacterial Contamination in Ria Formosa Coastal Lagoon: A Modelling Approach

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ABSTRACT

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Enteric bacteria such as *Escherichia coli* (*E. coli*) are commonly used as indicators of anthropogenic contamination in seawaters and in aquacultures. In Ria Formosa Both tourism and shellfish harvesting contribute to turn anthropogenic contamination into an important issue, bringing up a need to understand the sensibility of the shellfish growing-places to these pressures. In this study a modelling approach is used to simulate the accumulation of faecal coliforms in *Tapes decussatus* cultured in Ria Formosa. The objective is to determine the impact of treated urban sewages upon shellfish beds. A hydrodynamic model is used to compute the currents in Ria Formosa and the coliform concentration in the water column. This concentration is then used by a bottom model that simulates the accumulation/depuration processes in the shellfish. The model constants are obtained from laboratory and field experiments exposing clams to *E. coli* contaminated waters, and measuring periodically the faecal coliform concentration in the water and in sub-samples of the clam population. The experimental results show that the time response to variations in water concentration is small, of the order of minutes and there is a threshold of water concentrations over which the clam is unable to accumulate more bacteria. The application of the model shows that the maximum concentration in the clams presents approximately an exponential decay with the distance from the discharge. The decay constant is a function of advective transport and dry period of the clam bed.

ADDITIONAL INDEX WORDS: *Lagrangian model, shellfish model, water quality, tapes decussatus.*

INTRODUCTION

Legislation in several countries, including the United States, Brazil, and European Countries impose reference values for the concentrations of enteric bacteria concentrations in the water column and in shellfish. The coliform group of these bacteria is commonly used as indicator of anthropogenic contamination in seawaters and in aquacultures (LEE and YOUNGER, 2002). The European Union directive 91/492/CEE imposes three classes based on coliform or *E. coli* concentrations in bivalves.

In Ria Formosa both tourism and shellfish harvesting contribute to turn anthropogenic contamination into an important issue, bringing up a need to understand the sensibility of the shellfish growing-places to these pressures. Ria Formosa is a shallow coastal lagoon with more than 80 Km^2 and an average depth of less than two meters. It is located in the south of Portugal in a region where tourism is an important economic activity. The population increase due to tourism is reflected in higher discharges of treated urban waste waters into the lagoon. The traditional activity of shellfish farming has also grown in parallel with the population, using more areas in Ria Formosa.

In this study both experimental and modeling approaches are used to access the problem. A model was built to simulate the accumulation of faecal coliforms in Tapes decussatus cultured in Ria Formosa. This model is run coupled to a hydrodynamic and transport model. Laboratory and field work was performed to obtain the parameters for the accumulation model and to validate the results. The final objective is to determine the impact of treated urban sewages upon shellfish beds.

METHODS

Experimental Methods

Clams (*T. decussatus*) used in field and laboratory experiments where harvested from one of the least contaminated shellfish beds in Ria Formosa immediately before the onset of the experiments. This procedure was adopted since starvation during artificial depuration could negatively influence clam condition, and thus experimental results.

Laboratory

Three sets of aerated aquaria (6) each filled with 20L of seawater testing negatively for the presence of coliforms were contaminated with *E. coli* ATCC 25922, grown in A1 broth (Difco). MPN (Most Probable Number) of *E. coli* in the water was 3.0×10^8/100mL in the first experiment (exp 1), and 9.0×10^4/100mL in the second (exp.2). In each experiment, clams (22 Kg in exp. 1 and 8 Kg in exp.2) were randomly distributed in three batches and introduced in three sets of aquaria. In exp.1 samples of clams (30 ind.) and water were collected every 30 minutes during the first two hours, every 60 minutes for the next two hours, and every 120 minutes for the final 4 hours. In exp. 2 water and clam samples were taken at minute 0, 30, 60 and 120. *E. coli* concentrations in water and clams were determined through standard 5 tube MPN procedure, using A1 broth (Difco).

Field Campaigns

Experiments 3, 4 and 5 involved placing clams loosely packed in ballasted nets, on the bottom of the salt marsh channel at 100m (St 1) and at 400m (St 2) of the outlet of the Faro Noroeste sewage treatment plant. The clams had just been harvested from a less contaminated area, with a concentration of fecal coliforms (FC) of 330 per 100 g of clam flesh and intervalval fluid. In order to ensure immediate submersion, in exp.3, clams were sunken 2 to 3 hours after spring low tide and water and clam samples were taken at time 0, 30, 90 and 180 (minutes). In exp. 4 the ballasted nets with the clams were sunken at spring high tide and sampled 30, 90 and 145 minutes after. In exp. 5 clams were submerged at spring high tide and were collected at neap high tide 7 days after. Fecal coliform concentrations in water and clam samples, were estimated using standard 5 tube MPN procedure, with A1 broth (Difco). Access

Table 1. *Concentration of E. coli in water and clams during laboratory experiments.*

Experiment	Time (min.)	*E.coli* MPN/100mL in water			*E.coli* MPN/100g in clams		
		Average	Min	Max.	Average	Min	Max.
Exp. 1	0	3.0×10^8	3.0×10^8	3.0×10^8	3.0×10^2	2.3×10^2	3.3×10^2
	30	2.8×10^8	2.4×10^8	3.0×10^8	1.0×10^8	7.0×10^7	1.3×10^8
	60	4.1×10^8	2.4×10^8	5.0×10^8	4.7×10^8	1.3×10^8	7.9×10^8
	90	8.0×10^8	3.0×10^8	1.6×10^9	3.7×10^8	1.7×10^8	4.9×10^8
	120	6.8×10^8	1.3×10^8	1.6×10^9	5.3×10^8	3.3×10^8	9.4×10^8
	180	6.6×10^8	8.0×10^7	1.6×10^9	4.3×10^8	1.8×10^8	7.9×10^8
	240	6.1×10^8	7.0×10^7	1.6×10^9	4.3×10^8	9.0×10^7	1.1×10^9
	360	5.8×10^8	3.0×10^7	1.6×10^9	7.0×10^8	3.3×10^8	1.3×10^9
	480	5.0×10^7	3.0×10^7	7.0×10^7	2.4×10^8	1.1×10^8	4.9×10^8
Exp. 2	0	9.0×10^4	8.0×10^4	1.1×10^5	1.9×10^3	7.9×10^2	2.4×10^3
	30	1.0×10^5	2.3×10^4	2.4×10^5	2.0×10^5	1.1×10^5	3.3×10^5
	60	8.0×10^4	5.0×10^4	1.1×10^5	4.6×10^5	2.3×10^5	7.0×10^5
	120	6.5×10^4	5.0×10^4	8.0×10^4	5.5×10^6	1.7×10^6	9.2×10^6

to St1 and St2 was only possible by boat, and thus limited by tide conditions. These limitations affected planed sampling time, imposing the 145 minutes after high tide as last sampling time.

Modelling Tools

The MOHID modeling system (http://www.mohid.com) was applied to Ria Formosa coastal lagoon to simulate the transport of fecal coliforms discharged from urban waste water treatment plants. An accumulation model was developed and coupled to this system to simulate the physiological behavior of the clams in respect to bacteria accumulation. The approach is here described.

Hydrodynamic and Transport Model

The MOHID system includes a 3D baroclinic hydrodynamic module for the water column and for the sediments and the correspondent eulerian transport and lagrangian transport modules. Parameters and processes involving non-conservative properties are object of specific modules (e.g. turbulence module, water quality, ecology and oil transformation). The hydrodynamic model solves the three-dimensional incompressible primitive equations (MARTINS *et al*., 2000). Hydrostatic equilibrium is assumed as well as Boussinesq approximation. The model uses a finite volume approach. This method makes the solution independent of the mesh geometry, allowing the use of a generic vertical mesh. The turbulence module uses the well known GOTM (General Ocean Turbulence Model http://www.gotm.net/). The model also solves a transport equation for salinity and temperature in order to compute the specific mass. The eulerian transport module used to transport these properties is based in the same finite volume method of the hydrodynamic model and is independent of the property transported. The lagrangian transport model tracks the trajectories of selected water masses using the transport fields from the hydrodynamic model in an explicit procedure. Dispersion is computed using the results from the turbulent model. For non conservative properties the decay is computed as a sink/source term. In this work a simple exponential law was used. This is a reliable approach in a large number of situations (BEAUEAU *et al*., 2001).

Accumulation Model

A new model was developed to simulate the accumulation of faecal coliforms in *Tapes decussatus* cultured in Ria Formosa. The faecal coliform concentrations in shellfish intervalvular flesh and intervalvular fluid depend basically on two factors: i) The concentration history of the water passing over the shellfish-growing place along time and ii) Shellfish physiologic behavior parameters such as filtration and retention rates (FIANDRINO *et al*., 2003). In the model developed the coliform retention is split into accumulation and elimination processes. The accumulation depends of the coliform concentration in the water column and is controlled by a accumulation constant k_a. The elimination depends of the coliform concentration in the clam mussel and is controlled by the elimination constant k_e. Using this approach the intervalvular concentration is governed by the differential equation 1.

$$\frac{dc}{dt} = k_a c_{H_2O} - k_e c \qquad (01)$$

Where c is the intervalvular concentration and c_{H2O} is the coliform concentration in the water close to the mussel. This equation is solved by the bottom module along time using the c_{H2O} values computed by the hydrodynamic and transport model. The k_a and k_e physiologic constants were determined from the laboratory and field experiments.

Table 2. *Concentrations of faecal coliforms in clams and water during the field experiments.*

Experiment	Time (min)	Station 1 - 100m		Station 2 - 400m	
		FC MPN/ 100mL in water	FC MPN/ 100g in clams	FC MPN/ 100mL in water	FC MPN/ 100g in clams
Exp. 3 half flood to flood	0	$2,4 \times 10^4$	$3,3 \times 10^2$	$1,7 \times 10^3$	$3,3 \times 10^2$
	30	$9,0 \times 10^2$	$2,4 \times 10^3$	$5,0 \times 10^2$	$2,4 \times 10^3$
	90	$1,1 \times 10^1$	$2,4 \times 10^3$	$2,3 \times 10^1$	$3,5 \times 10^3$
	180	$1,3 \times 10^1$	$5,4 \times 10^3$	<1	$2,4 \times 10^3$
Exp. 4 flood to half ebb	0	<1	$3,3 \times 10^2$	<1	$3,3 \times 10^2$
	30	$1,4 \times 10^1$	$2,4 \times 10^4$	8	$2,3 \times 10^3$
	90	$5,0 \times 10^2$	$2,4 \times 10^5$	$3,0 \times 10^2$	$2,4 \times 10^4$
	145	$2,4 \times 10^3$	$3,3 \times 10^5$	$5,0 \times 10^2$	$3,5 \times 10^4$
Exp. 5	0	<1	$3,3 \times 10^2$	<1	$3,3 \times 10^2$
	7 days	$1,6 \times 10^3$	$1,6 \times 10^5$	$2,2 \times 10^2$	$3,5 \times 10^4$

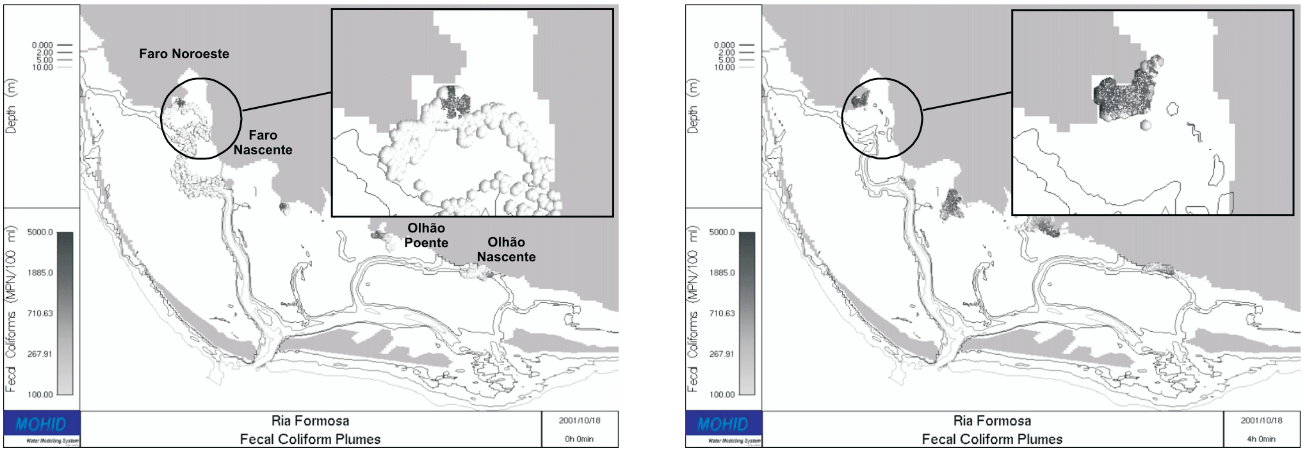

Figure 1. Coliform Plumes issuing from the four WWTP discharges in ebb (left) and flood (right). The zoom shows the sampled channel.

RESULTS

Experimental Results

Laboratory Results

Table 1 presents laboratory experimental results for the accumulation of *E. coli* in clams exposed to two different concentrations of this faecal coliform in water. Accumulation of *E. coli* in clams followed the same pattern in all replicate aquaria. but differed according to initial concentration in water. In fact maximum concentration detected in clams was 1.3 x 10^9 MPN/100mL during exp. 1, in which effective concentrations in clam flesh and intervalvar fluid did not exceed concentrations in water during the first six hours of this experiment. On the contrary, in exp. 2, in spite of the fact that accumulation follows the same type of curve, values in clams surpass those of the surrounding water right away, as determined from the first 30 minutes on. In both experiments the highest rate of *E. coli* uptake by clams occurred during these first 30 minutes of exposure.

Field Results

Data in Table 2 corresponds to concentrations of faecal coliforms (FC) detected in water and in clams introduced in the salt marsh channel at Station 1 (ST.1) and 2 (ST.2), located respectively at 100 m and 400 m from the outlet of the sewage treatment plant of Faro Noroeste. As in lab exp. 2, after only 30 minutes, FC concentrations in clams exceeded those of the surrounding water, both in exp.3 (while less contaminated water was flooding in from the sea) and in exp. 4 (when tidal conditions exposed the sampling locations to sewage contamination). During exp. 4 maximal faecal coliform contamination could not be assessed because of tidal limitations to boat access to St1 and St 2. Results of exp. 5 integrate the variation of FC concentration in clams during successive exposure to ebbs and floods, from a spring high tide to the neap high tide 7 days after, elucidating differences in water and clam contamination between spring and neap tides.

Modelling Results

Transport Simulations

For the hydrodynamic and transport simulations a grid with 183 x 151 cells is used. The spatial step is variable, ranging from 50 meters in the central part of the lagoon to more than 500 meters close to the open boundary. Only one layer is used in the vertical direction due to the intense vertical mixing present in the system. The tide elevation is imposed in the open boundary using tide gauge data with 22 harmonics. Wind and wind waves are not considered since its effect inside the lagoon is negligible. The hydrodynamic model was calibrated using water level and currents from several stations along the lagoon (MARTINS *et al.*, 2003).

The evolution of the plumes issuing from the four WWTP discharging into Ria Formosa is achieved using a lagrangian transport model. The faecal coliform decay in the water column was computed in the model using a exponential law with a T90 value (time elapsed until inactivation of 90% of initial load) previously calibrated for this system and conditions (DRAOT, 2003).

Figure 1 shows the coliform plumes in flood and ebb situations. The circle show the channel elected for the field campaign.

Accumulation in the Clams

The accumulation model is run to obtain the coliform concentration in the shellfish. The laboratory and field measurements are first used to calibrate the accumulation and elimination constants. After calibration the model is applied to the entire lagoon to understand the impact of discharges on shellfish concentrations.

Model Calibration

For the elimination constant the concentration in the water column is considered to be null. With this assumption equation 1 simplifies to:

$$k_e = -\frac{\ln \frac{c(t)}{c(0)}}{t} \qquad (02)$$

The elimination constant is obtained adjusting equation 2 to experimental results obtained from (BENTO, 2001). The average elimination constant obtained was 5.5E-5 s^{-1}.

For the accumulation constant the elimination process can not be discarded since the clams are eliminating during the test. In the experiments performed the coliform concentration in the water remained constant. With this information and using the elimination constant obtained above, the accumulation constant can be computed from equation 3.

$$k_a = \frac{c(t) + c(0)\exp(-k_e t)}{c_{H_2O} t} \qquad (03)$$

The average accumulation constant obtained from the experiments is 1.1E-4 s^{-1}.

With this constants equation 1 is solved by the model using a simple Euler method as expressed in equation 4. (Runge-Kutta was also tested with minor improvements).

$$c(t+\Delta t) = c(t) + \Delta t\left(k_a c_{H_2O}(t) - k_e c(t)\right) \qquad (04)$$

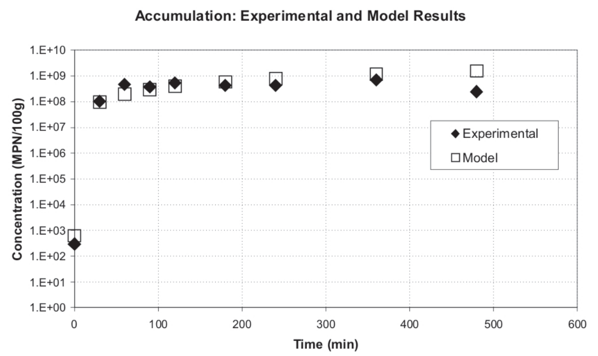

Figure 2. Faecal coliform concentrations in the shellfish. Calibration of the accumulation model with laboratory results.

In figure 2 the average accumulation results are compared with this equation for the accumulation experiment.

The model was then incorporated in the transport model and run in Ria Formosa using the actual discharges and tide conditions of the field experiments. In figure 3 the calibration procedure is illustrated. Coliform concentrations in the shellfish for the 400m station during the ebb experiment are compared with the *in situ* measurements.

From the field calibration a value of 4.5E-4 s^{-1} for the accumulation constant was derived. The elimination constant value of 1.1E-4 s^{-1} was left unchanged.

Model Results

The transport and accumulation models were applied in the entire Ria Formosa. The objective is to understand the impact of discharges on shellfish concentrations. An average tide condition and discharge loads are considered. The shellfish concentrations for several points along the discharge channels of the four treatment plants and over the intertidal areas were computed. In figure 4 the maximum modeled shellfish concentrations are plotted against the distance from the discharge. The three concentration levels imposed by European Union directive 91/492/CEE are also shown in the figure.

DISCUSSIONS

Laboratory and Field Results

Molluscan shellfish feed by concentrating particulate organic materials from the surrounding water. As pointed out by BURKHARDT III *et al.* (2000) the most important influence on the sanitary quality of harvest waters and shellfish areas is the discharge of wastewater from municipal treatment facilities and from combined sewage storm water overflows during rainfall events. Both type of discharges carry high proportions of adhered bacteria, amongst which are indicator bacteria of the coliform group, such as *E. coli.* In spite of disputes around the usefulness of the faecal coliform group as an indicator to ensure human safe consumption of shellfish, in the experimental

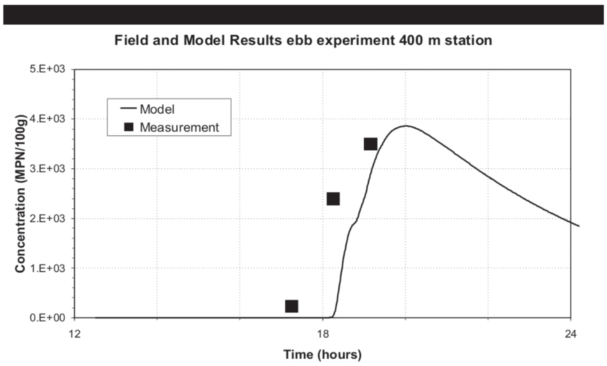

Figure 3. Faecal coliform concentrations in the shellfish. Calibration of the accumulation model with field results of exp. 4

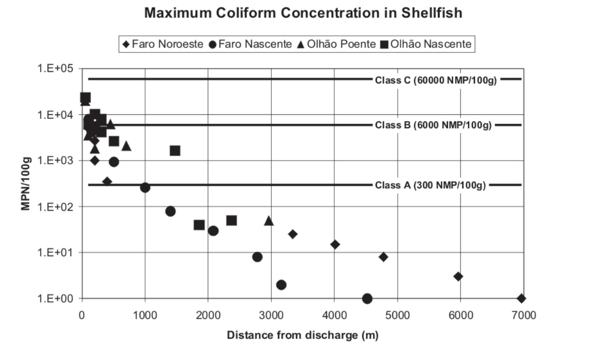

Figure 4. Faecal coliform concentrations as a function of the distance from the treatment plant discharge.

conditions used in this work they are a sure indicator of contamination of water and clams. Both laboratory and field experiments were designed in order to provide information on accumulation rates of bacterial indicators in clams. Results of exp. 1, exp. 2 , exp, 3 and exp.4 indicate that clams response to elevation of suspended matter is very rapid, confirming high rates of water filtration in the first 30 minutes of exposure. In fact the same type of results, with a rapid uptake of *E. coli* to a concentration of manifolds higher than that of the surrounding water, in the first half an hour of exposure, has been obtained in mussels (*Perna viridis*) by HO and TAM (2000). Results of exp.1 suggest a threshold of saturation limiting accumulation of *E. coli* to concentrations of a MPN of 10^9/100g, This threshold has a limited interest since even the discharge of untreated sewage would not produce such high concentrations of *E. coli* in waters. As expected, field experiment results revealed high influence of tidal conditions, but accumulation of FC in clams proceeded even during flood and high water, when FC concentration in water was minimal. Differences between spring and neap tides in clam and water contamination as illustrated by results of exp. 5 should be further investigated.

Model Results

The calibration procedure was divided in two parts: One using laboratory and other using field data. The laboratory data allowed the separation of elimination and accumulation processes, while in the field experiment the two processes were present simultaneously along with other factors not considered in the laboratory. In this way the accumulation and elimination constants obtained in the laboratory experiments were tested using the field experiments. The best agreement between model and field results was obtained using the accumulation constant derived in laboratory and a slightly higher elimination constant. The difference in elimination between the laboratory and the field experiments is probably due to temperature and average concentration levels in the water column. Nonetheless, the two elimination constants have the same order of magnitude which gives some confidence on the results.

The application in the entire systems shows that the maximum concentration in the clams reduces approximately with an exponential law. This average result is what should be expected from an exclusive diffusive transport in the water column and a constant concentration along the tidal cycle. The advective (tidal) transport and the number of dry hours are the processes responsible by the different behaviors from place to place. With this in mind it can be seen from figure 4 that Faro Nascente results decrease very fast with the distance. This WWTP is located in a shallow region so the bivalve beds are covered less hours than the other stations. Olhão Nascente, on the other hand, is located in a deep channel and the modeled points are immersed most of the tidal prism. The accumulation is thus higher. In the model it is considered that the bivalve eliminates during dry periods with the same rate. This behavior must be carefully investigated in the future.

The global model results show that Class A fields (clams can be consumed without depuration) can be expected for distances higher than 1500-2000 meters. Class B fields (clams must be depurated before consumption) can be expected for distances between 500 and 1500 meters and Class C fields are restricted to the first 500 meters which corresponds basically to the WWTP discharge channels.

CONCLUSIONS

The results show that the proposed model was able to simulate the accumulation processes of *Tapes decussatus* in Ria Formosa. The coliform accumulation in the clam was seen to be a function of the distance from the discharge and the number of hours the clam is in contact with the discharged plume in each tidal cycle. It was also shown that this modelling tool has a promising potential for the management of urban discharges.

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