MODELLING DIOXINS DETOXIFICATION IN MANILA CLAM (*Tapes philippinarum*)

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Abstract
Detoxification experiments on edible organisms contaminated by POPs can give direct practical advice for setting up management procedures aiming at decreasing risk to human health. However, ecotoxicological modelling permits a full exploitation of the empirical data and a better understanding of observed patterns and differences. Here an ecotoxicological modelling approach is set-up for Manila clam coupling a bioenergetic growth model with an accumulation model. The coupled ecotoxicological model allowed for a good representation of observed dynamics of concentration of each dioxin congener in clam flesh during detoxification experiments conducted in the Venice Lagoon in winter 2006. Moreover, it allowed for determination of rates and half-lives of clam metabolic degradation specific for each congener. These estimates are more reliable than estimates based on apparent detoxification rates. This preliminary ecotoxicological model provided evidence of its usefulness in representing dioxin dynamics in clam flash under different environmental conditions and thus it can be used for providing scenarios for risk management.

Introduction
Natural detoxification experiments conducted in Venice Lagoon showed the effectiveness of a culture-based fisheries strategy based on the removal of Manila clam (*Tapes philippinarum*) specimen from the contaminated Industrial Zone and their resettlement in cleaner areas of the lagoon[1-4]. Empirical data showed substantial decrease of toxicity to reference level[5] within 120 days from displacement[3]. However, summer and winter experiments showed differences in detoxification patterns that were connected with the sensitivity of the detoxification processes to temperature[3,4]. Although these experiments cover the extremes of natural environmental conditions in the Venice Lagoon in terms of both POPs concentration in sediments and water temperature, a modelling approach might allow to better understand, describe and forecast the bioaccumulation processes. A simple model describing POPs dynamics over time by means of two counteracting apparent processes was previously used for estimating apparent detoxification rates and half-lives of different congeners[4]. However, the ecotoxicological modelling approach allows for an explicit representation of both accumulation and detoxification processes, thus it permits for extending and broadening the findings of detoxification experiments, exploiting at best the information carried out by costly field measurements.
In the present work a preliminary ecotoxicological model is set up by coupling a bioenergetic model describing the growth of Manila clam with a model describing explicitly the main ecotoxicological processes. This coupled model is calibrated and validated with dioxins data collected during detoxification experiments previously carried out in the Venice Lagoon[3,4].

Material and Methods
Field experiments and data
Contaminated specimen of *Tapes philippinarum* at minimum marketable size (25mm of length) were collected in the area surrounding the Industrial Zone, transported in the southern part of Venice Lagoon and reared in two controlled areas that are characterized by sediments with low contamination by industrial sources[6,7]. These experiments were conducted both in summer[3] and in winter[4] natural conditions. Dioxins concentrations in clam flesh (both on wet weight and lipid basis) were determined at days 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 90 and 120 after resettlement. Moreover, superficial sediments (0-10 cm) were sampled in the site where clams are collected (Industrial Zone) and in the two sites (Site 1 and Site 2) chosen for detoxification and dioxin concentrations were measured on a dry weight basis. The quantitative determination of PCDDs/PCDFs was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections, as recommended by the US-EPA[8]. The reader is referred to previous works for a more detailed description of the analytical methods used[2] and for a full presentation of results of these experiments[3,4].

Ecotoxicological coupled model

The ecotoxicological model is set up for describing explicitly the influences of environmental factors (sediment contamination and temperature) and of phisiological processes on the dynamics of pollutants in clam flesh. This ecotoxicological model describes the uptake of pollutants through respiration and feeding processes, whereas POPs detoxification processes are explicitly accounted by representing clearence, excretion, biological metabolism and dilution due to growth. The POP concentration dynamics over time (t), therefore, follows the general equation[9,10]:

$$\frac{dC_B(t)}{dt} = k_1 \cdot C_S + k_D \cdot C_D - (k_2 + k_E + k_M + k_G) \cdot C_B(t) \quad (1)$$

where K_1 and K_D are the uptake rates from sediment (through respiration) and food, respectively. These uptakes are proportional to POPs concentration in sediment (C_S) and food (C_D). Detoxification processes, instead, depends on the concentration of POPs in clam flash (C_B) and are accounted by means of specific rates for clearence through respiration (K_2), excretion (K_E), metabolic degradation (K_M) and dilution due to growth (K_G). These ratios are generally assumed constant during time, whereas they are depending on phisiological activity of the animal. Therefore clam respiration (R), food intake (Q), food non-assimilated (UN) and growth (G), are influencing correspondent ratios as in the following:

$$\frac{dC_B(t)}{dt} = k_1(R) \cdot C_S + k_D(Q) \cdot C_D - [k_2(R) + k_E(UN) + k_M(R) + k_G(G)] \cdot C_B(t) \quad (2)$$

Moreover, phisiological factors (R, Q, UN, G) are varying over time, according to changes in individual size (weight, w of the organism), environmental temperature and food availability (F, chlorophyll). In order to have a model representing these factors changing dynamically, we coupled the bioaccumulation model presented in eq. (2) with a bioenergetic model of the clam growth. This bioenergetic model was previously calibrated and validated for *Tapes philipppinarum* in the Venice Lagoon[11] and it can provide estimates of phisiological factors (R(t), Q(t), UN(t), G(t)) for clam individuals. These estimates are used here in the ecotoxicological model for varying bioaccumulation/detoxification ratios (eq. 2). For relating phisiological factors to the ratios K_1, K_D, K_E and for accounting of the chemical property of the pollutant, namely the K_{ow}, we used empirical relationships presented in literature[9,10,12]. K_G does not need to be estimated, because the dilution due to growth is implicitly accounted since the POP concentration in clam flesh and its growth (wet weight) are both modelled. The coupled model is calibrated on dioxins data collected during 2006 experiment in Site 1 by estimating the parameter K_m, derived from grouping K_2, K_M. Validation is carried out by using experimental measures taken in Site 2.

Results and Discussion

The bioenergetic model is forced with water temperature and chlorophyll concentration specific for area and season (Fig. 1A), and represents their effects over time on physiological functions (respiration, R, food intake, Q, growth, G; represented in Fig. 1B) that are used as input for the bioaccumulation model.

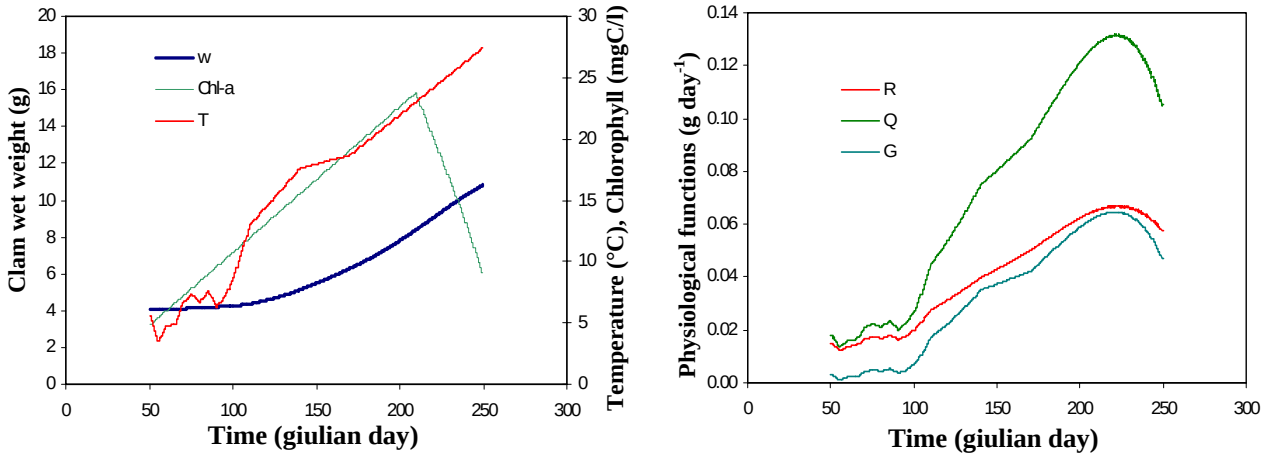

Figure 1. Forcings over time (left panel, A), i.e. water temperature and Chlorophyll concentration, affecting growth of Manila clam in the bioenergetic model, and thus influencing physiological factors (right panel, B) over time. Physiological factors are used in the bioaccumulation model.

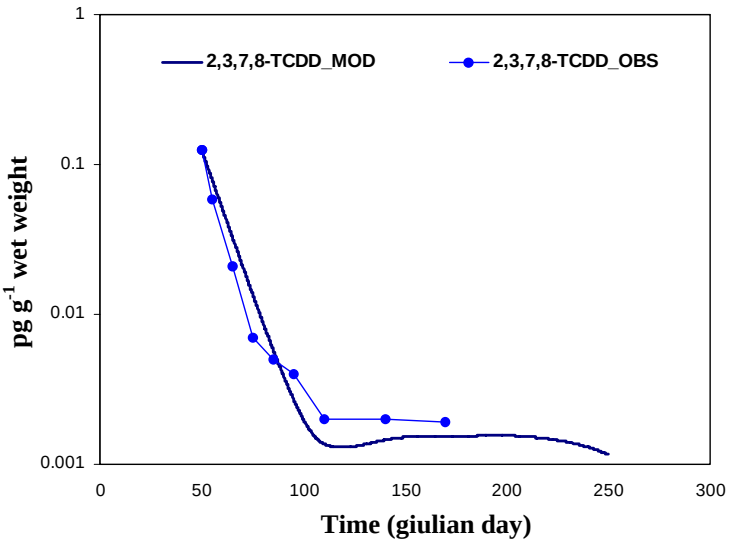

Figure 2.
Example of the goodness of fit between ecotoxicological model and field measurements. The growth model is forced with water temperature and food concentration (chlorophyll) typical of the southern part of the lagoon of Venice. Bioaccumulation model is calibrated for the metabolic detoxification rate (K_m). Resulting fitting (R^2= 97.1%) for the 2,3,7,8 TCDD exemplify the goodness of the model in representing detoxification pattern over time.

Calibration of the ecotoxicological model for TCDD congener is presented in Fig. 2 for exemplifying the goodness of the coupled ecotoxicological model in fitting the field measurements. Similarly, calibration was performed for all dioxin congeners (Table 1) by using specific ripartition constant (K_{ow}) and estimating with the model specific meabolic rate (K_m). The goodness of fit is quantified through the coefficient of determination (R^2) that shows very high values, suggesting the good capabilities of the ecotoxicological model in representing bioaccumulation/detoxification processes. Moreover, Table 1 reports also the results of the validation that is performed on data collected on Site 2 by using K_m estimated during calibration on Site 1 data. Validation for each congener shows high values for R^2 indicating the robustness of the model in representing different conditions: validation was performed changing background concentration of detoxification sediment. Estimated values of K_m permitted the estimation of metabolic half-lives of dioxin congeners specific for the congener-clam couple. These values, reported in Table 1, are independent from growth and excretion processes, which are processes not disentangled in the half-lives previously determined on the basis of apparent detoxification rates [2]. As can be seen from Table 1, true metabolic half-lives range from a minimum of 7 to a maximum of 28 days for 1,2,3,7,8,9 HCDF and 2,3,7,8 TCDF, respectively. These values, not surprisingly, are larger than those estimated from apparent detoxification rates[2]. Representing the dynamic of each dioxin congener by means of the ecotoxicological model, allowed for obtaining the dynamics of toxicity during detoxification experiments (Fig.3). As can be seen, the preliminary model can represent quite well the final toxicity level due to dioxins observed in the field experiment, although it represents roughly the first phase of sharp decreasing toxicity, evidencing the need for further analysis.

Table 1.
Results of the fitting of ecotoxicological model to field measurements for all dioxin congeners. Calibration against data from Site 1 allows estimating the clam metabolic detoxification rate (K_m) specific for each congener. This value is then used for representing detoxification in Site 2. R^2 as a measure of goodness of fit is presented both for calibration and validation procedures. Half-lives for each congener, estimated from K_m, are also reported.

Congeners	K_m (days^{-1})	Half life (days)	Calibration R^2	Validation R^2
2,3,7,8-TCDD	0.0867	**7.99**	97.1%	97.9%
1,2,3,7,8-PCDD	0.0654	**10.59**	93.0%	91.9%
1,2,3,4,7,8-HCDD	0.0542	**12.80**	83.2%	82.1%
1,2,3,6,7,8-HCDD	0.0514	**13.48**	85.9%	83.2%
1,2,3,7,8,9-HCDD	0.0533	**13.00**	84.2%	83.8%
1,2,3,4,6,7,8-HpCDD	0.0385	**17.98**	69.6%	69.5%
1,2,3,4,6,7,8,9-OCDD	0.0339	**20.47**	63.3%	62.5%
2,3,7,8-TCDF	0.0250	**27.72**	78.9%	73.6%
1,2,3,7,8-PCDF	0.0471	**14.73**	83.9%	83.7%
2,3,4,7,8-PCDF	0.0381	**18.19**	71.2%	72.6%
1,2,3,4,7,8-HCDF	0.0626	**11.07**	84.5%	86.1%
1,2,3,6,7,8-HCDF	0.0534	**12.97**	82.3%	82.6%
2,3,4,6,7,8-HCDF	0.0454	**15.26**	80.0%	78.7%
1,2,3,7,8,9-HCDF	0.0946	**7.33**	95.2%	98.6%
1,2,3,4,6,7,8-HpCDF	0.0573	**12.09**	75.2%	79.6%
1,2,3,4,7,8,9-HpCDF	0.0832	**8.33**	86.2%	91.1%
1,2,3,4,6,7,8,9-OCDF	0.0578	**12.00**	70.7%	77.8%

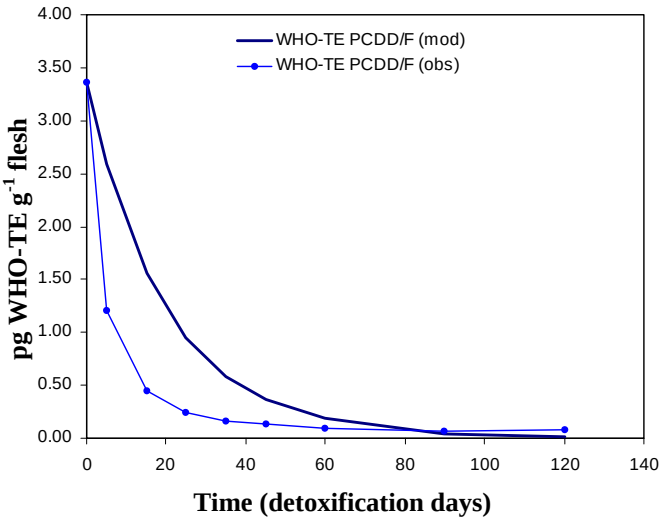

Figure 3.
Dioxin toxicity (WHO-TE) as measured in the 2006 winter detoxification experiment compared with the model outputs. Model toxicity is calculated on the basis of dynamics of all dioxin congeners simulated by the ecotoxicological model. The bioenergetic model for Manila clam uses water temperature and chlorophyll concentration as forcing function specific for the experimental area and season.

Conclusions

This work presents the development of an ecotoxicological model for Manila clam (*Tapes philippinarum*) obtained by coupling a bioenergetic growth model and a bioaccumulation model. The coupled model allows for a good representation of observed dynamics of concentration of each dioxin congener in clam flesh during detoxification experiments conducted in the Venice Lagoon in winter 2006[4]. It allows for determination of rates and half-lives of clam metabolic degradation specific for each congener that are more reliable than estimates based on apparent detoxification rates[2]. Although preliminar, the ecotoxicological model is a powerful tool for representing dioxin dynamics in clam flash under different environmental conditions (sediment contamination, water temperature, clam growth) and thus it can be used for providing scenarios for risk management.

Acknowledgments

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