UNIVERSITY OF PADUA

DEPARTMENT OF INDUSTRIAL ENGINEERING

Master Thesis in Environmental Engineering

DEVELOPMENT OF AN ECOSYSTEM MODEL OF THE LOWER PO RIVER FOR USE IN RISK ASSESSMENT OF XENOBIOTICS

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"Essentially, all models are wrong, but some are useful"

George E. P. Box

Abstract

Risk assessment methodology at the base of EU environmental directives (*Water Framework Directive* 2000/60/EC and *REACH* - Registration, Evaluation and Authorisation of CHemicals directive (EC) No 1907/2006) consists in the calculation of the Risk Quotient (RQ), that is the ratio between Predictive Environmental Concentration (PEC), calculated with fugacity and/or transport models, and Predictive Non Effect Concentration (PNEC) calculated on the basis of standardized eco-toxicological laboratory test, extrapolating individual effects to effects on the ecosystem by using assessment factors that can have a value between 5 to 1000 depending on the ecotoxicological knowledge. This approach is the one most favoured at present but, in spite of being developed in great detail by the European Commission, it is somewhat simplistic and further development to take better account of ecosystem complexity is to be expected [II]. The weakness of this index lies in the fact that PNEC refers to direct effects on a specific organism studied in laboratory and, even if it is a conservatory approach, information about indirect effects of a chemical along the trophic web are neglected.

According to these considerations, in this thesis work, a modelling approach to the aquatic risk assessment is considered, trying to mathematically simulate with AQUATOX (US-EPA) software the fate and effects of particular chemicals (Triclosan and LAS - Linear Alkylbenzene Sulfonate) contained in home and personal care products released in the aquatic environment of the largest Italian river, the Po river, with particular focus on the food web simulation.

A control ecosystem (with no chemicals) is developed and calibrated with observed data. The ecosystem control model is then used to test changes in organisms biomasses due to different input concentrations of the two chemicals LAS and Triclosan.

Problems encountered in the thesis work regard mainly the lack of observed data on Po river biota. To improve the model, a better quantitative knowledge of organisms biomass variation in time and of organisms diet is needed.

The results validate the thesis idea that chemical effects on organisms cannot be attributed only to individual toxicity effects (expressed with LC50 and EC50 toxicity parameters) but also to biota interactions with the entire ecosystem (indirect effects).

The ecotoxicological model of the Po river developed in this thesis can be considered as a draft useful for future development in order to reach the broader objective to built an ecotoxicological modelling of the Po river based on accurate observed data with the potential to became a tool for the achievement of protection aims and requirements of the chemical and environmental directives of the EU.

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1 Introduction

1.1 Overview

Over the past years, Europe environmental policy has been taking measures to organize and standardize methodologies to preserve our water bodies. Basically there are two types of regulation fields closely related to one another: regulations on environment management and monitoring status (that concern Member States responsibility on the water bodies status) and regulations on chemicals released in the environment (that concerns industry responsibility for the chemical produced). The key directive for environmental management and monitoring status is the *Water Framework Directive* (WFD; 2000/60/EC). With WFD a new view of water resources management in Europe has been developed. WFD requires Member States to evaluate the Ecological Quality Status (EQS) of water bodies and its final goal is to reach "good chemical and ecological status" for all waters by 2015 [I]. The word *ecological* represents the innovation aspect of the Directive: the water management has to be based mainly upon biological and ecological factors, with ecosystems being at the centre of the management decision (Borja, 2005). To reach WFD objectives, it is important to assess the actual and future ecosystem state and to find standardized method to "measure" it. Several indices have been tested and discussed during previous years and currently lots of methodologies are being developed for the evaluation of ecologic state. In this dynamic European contest, aquatic environment risk assessment plays an important role as it is a procedure that permits to evaluate or predict the effects of different inputs scenarios on the ecosystem and, thus, to evaluate the actual or future ecological status. The key directive for the control of generic chemicals (for non generic chemicals as biocides, pesticides and pharmaceutical there are other directives) is the so called *REACH* (Registration, Evaluation and Authorisation of CHemicals), proposed by European Commission (EC) and entered into force on 1st June 2007. It promotes a system in which industries that produce a certain generic chemical are responsible for the assessment and management of the human and environmental risk of that chemical [I].
Regarding aquatic risk assessment, both key directives refer to a specific methodology based on the calculation of the Risk Quotient (RQ), that is the ratio between Predictive Environmental Concentration (PEC) and Predictive Non Effect Concentration (PNEC). PEC is calculated with fugacity and/or transport models, PNEC is calculated on the basis of standardized eco-toxicological laboratory test, extrapolating individual effects to effects on the ecosystem by using assessment factors that can have a value between 5 to 1000 depending on the ecotoxicological knowledge.

This approach is the one most favoured at present but, in spite of being developed in great detail by the European Commission, it is somewhat simplistic and further development to take better account of ecosystem complexity is to be expected [II]. In fact, the weakness of this index lies in the fact that PNEC refers to direct effects on a specific organism studied in laboratory and, even if it is a conservatory approach, information about indirect effects of a chemical along the trophic web are neglected. Rossaro (1993), in his considerations on Po river management, says that real effects of chemicals on the environment are not easy to predict, and ecotoxicological studies in laboratory do not necessarily reflect the complexity of reality. Also Galic et al. (2010) underline the fact that current EU risk assessment schemes focus on individuals, but most of the protection goals focus on population, thus, there is the need for more "population oriented approaches". To assess the impact of a chemical taking in consideration the entire ecosystem requires the knowledge of several aspects of fundamental ecology such as species life-history traits, population structure, density-dependent regulation, species composition and interactions, landscape structure etc. Ecological and toxicological data and processes have to be integrated and in this context ecological models are a very powerful tool also because they allow extensive testing of various scenarios with low costs. Thus, the usage of ecological models in regulatory risk assessment should be promoted (Galic et al., 2010).

According to these considerations, in this thesis, a modelling approach to the aquatic risk assessment is considered, trying to mathematically simulate the fate and effects of particular chemicals (Triclosan and LAS - Linear Alkylbenzene Sulfonate) contained in home and personal care products released in the aquatic environment of the largest Italian river, the Po river, with particular focus on the food web simulation. Inspired by the paper of Galic et al. (2010), the challenge is to establish if ecotoxicological modelling of the Po river has the potential to became a tool for the achievement of protection aims and requirements of the chemical and environmental directives of the EU.

The first step will be to derive an ecological model of the river that is able to simulate the most important physico-chemical and biological processes without considering the effect of chemical release (control model), then, toxicological data on LAS and Triclosan will be integrated in the model that will be utilize to "test" the effects of changes in the concentration of the two chemicals (perturbed model).

In doing this, there is the complete awareness that the biological response of organisms inhabiting the ecosystem is influenced by a complex series of factors that act together, often synergistically, thus, it is difficult to understand what happens in the system in a mechanistic fashion. Moreover, the interactions between different factors make often difficult to interpret observed data. But a deterministic analysis and a simplified modelling approach are necessary when the complexity of the nature has to be reduced to numbers, and used to make decisions, for example for regulatory use.

In the following paragraphs the background and the objectives of the thesis are described in details. Chapter 2 is dedicated to materials and methods, it contains all the information about the data sets used for the calibration of the control model and physico-chemical and ecotoxicological parameters of LAS and Triclosan used in the perturbed model; also, all the methodologies applied in the study are described. In Chapter 3, the results of control and perturbed simulations are presented. In Chapter 4 results are discussed trying to assess the risk of chemicals in the modeled environment and to evaluate the validity of the use of a modelling approach in aquatic risk assessment.

1.2 Background

1.2.1 State of the art on risk assessment methodologies in Europe

With Directive 92/32EC and EC Council Regulation (EC) 793/93, the European Union requires risk assessment of new and existing substances (Vermeire, 1997). Then on June 2007 the European Union REACH regulation (EC) No 1907/2006 came into force, forcing companies to present reports about the features of chemicals produced and related risk assessment. The level of detail of the report depends on the quantity of substance produce. Risk assessment methodology and procedures are laid down in detailed Technical Guidance Documents (TGD) and the implementation is helped with the decision support tool EUSES (European Union System for the Evaluation of Substances). The general methodology at the base of TGD and EUSES software for a quantitative assessment of environmental risk is summarized in Figure 1.1 and is characterized by the following steps (Vermeire, 1997):

1) Exposure assessment: knowing emission rates of a chemical in the environment (observed or evaluated), with fugacity and dispersion models the local and regional distributions of the substance in the various environmental compartments and in the considered systems is evaluated. The result is the calculation of the PEC (Predicted Environmental Concentration) for each environmental compartment.

2) Effects assessment: it comprises hazard identification and dose-response assessment mainly derived from laboratory ecotoxicological studies on single species. Individual ecotoxicological parameters (LC50, NOEC, LOEC) found in laboratory are then extrapolated to the entire ecosystem through assessment factors in order to

calculate PNEC for each compartment (soil, sediment, water). PNEC is calculated by dividing the lowest toxicity value with the relevant assessment factor (Table 1.1). PNEC can be also calculated using statistical extrapolation techniques. The technical guidance documents provides information on the calculation of secondary poisoning through the bioconcentration factor (BCF) and concentration in food.

Table 1.1 - Assessment factor for the derivation of PNEC in water (from ECHA, 2008).

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels (fish, invertebrates (preferred Daphnia) and algae)	1000
One long-term EC10 or NOEC (either fish or Daphnia)	100
Two long-term results (e.g. EC10 or NOECs) from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term results (e.g. EC10 or NOECs) from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1
Field data or model ecosystems	Reviewed on a case by case basis

3) Risk characterization: for a quantitative evaluation of environmental risk the ratio between PEC and PNEC is calculated. If the PEC exceeds the PNEC, there is considered to be risk of environmental damage in proportion to the ratio of PEC to PNEC.

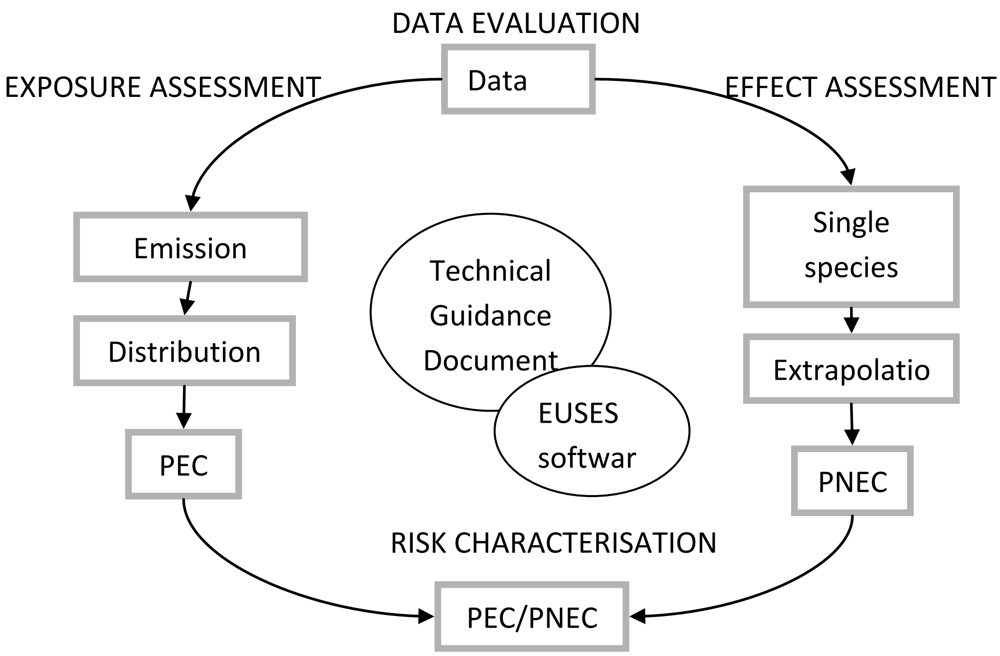


Figure 1.1 - Basic scheme of ecological risk assessment with TGD and EUSES (modified from Vermeire, 1997).

This risk assessment methodology is also mentioned in the WFD as a tool for the calculation of the so called Ecological Quality Ratio (EQR) for water bodies (see Paragraph 2.10).

As written in the overview, this scheme of evaluation does not take into account the numerous ecological processes that can act unpredictably or in a synergic way and influence the risk evaluation.

1.2.2 Case studies background

A similar thesis project was done by A. Lombardo (2013) on the river Thames. He used the US-EPA AQUATOX model to simulate a 4 km segment of river ecosystem, testing the effects of different input concentrations of Triclosan and LAS chemical compounds. Key challenges identified during the river Thames project include a) the limited bio-monitoring data available for a proper calibration/validation of the trophic web model and b) the limited relevance of standard ecotoxicological datasets in the context of the studied ecosystem.

In order to face all the other key challenges this thesis will further explore the potential of ecological models for use in chemicals risk assessment building upon the learnings from river Thames case study.

1.3 Objectives

1.3.1 Thesis objectives and work procedure

In this thesis work, an eco-toxicological model of the Po River is developed. This model has to be able to simulate the direct and indirect effects of home and personal care products on the ecosystem state and dynamics. The goal is to understand the relevance of ecological processes in the assessment of the risk produced by a chemical release in a riverine ecosystem, with particular attention to the indirect effects due to biota interaction. The case study will focus on a segment of the lower Po river in Italy. The presence of monitoring networks and several researches on this river should make it possible to create a satisfactory model and calibrate it against field observations. This addresses one of the key challenges identified in the river Thames project.

The thesis project will comprise the following steps:

- Review and collection of hydrological, climatic, environmental and bio-monitoring data from the Po river basin;
- Choice of the modelling tool to be used;
- Selection of the study area model boundaries and construction of an ecological model of the Po river ecosystem, with a focus on its food web. The temporal and spatial scale of the analysis will be chosen based on data availability;
- Model calibration against field observations such as biomass time series;
- Reconstruction of the concentration of selected ingredient(s) of home and personal care products in the Po river, e.g. by reconstructing inputs to the river system; the selected chemicals will be the same used in the river Thames project, in order to compare the effects of these compounds in different ecological systems.
- Simulation of the effect of changes in the concentration of selected ingredient(s) on the ecosystem dynamics, and assessment of the consequent ecological risk.

1.3.2 Broader modelling study objectives

Other broader objectives underlie the thesis work:

- To compare modelling and indices-based approaches in risk assessment;
- To understand if the risk assessment methodology used can be applicable as a decision support tool for real problems of river ecosystem management or chemicals testing;
- To highlight any shortcomings with regards to the knowledge and monitoring of Po River ecosystem.

2 Materials and methods

2.1 Modelling tool: AQUATOX program

The first important step of the thesis work is the choice of the modelling tool to use according to the goal to achieve. In this case the intent is to simulate the fate and effects of chemicals released in the aquatic environment of the Po River, with particular attention to the role of the trophic network, so that the final modelling tool will surely include the main features of fugacity models (e.g. modelling tool as RAIDAR, BETR, AquaWeb etc., see Mackay D. et al. 2009), risk models (e.g. TRIM modelling tool [III]) and trophic models (e.g. ECOPATH modelling tool [IV]). The modelling tool which incorporates satisfactorily the previous ones and that is applicable to aquatic environment is US EPA AQUATOX software (available on the web site <http://water.epa.gov/scitech/datait/models/aquatox/>). It is a simulation model that predicts the fate and effect of chemicals in aquatic environments. Through a graphical interface the user can specify the physico-chemical features of the study area, the parameters of the main organisms constituting the food web and their diets, the toxicological parameters referring to a particular chemical to be tested. On the basis of the input data, the program can simulate numerous processes that regard the partition of chemicals between different media and along the trophic web, and the direct and indirect effects of this chemical on the entire ecosystem in terms of variation in biota biomass during the time.

2.1.1 Model conceptualization and data needed

With AQUATOX it is possible to model a riverine ecosystem considering one or more connected segments of the stream. The segment can be view as a reach of the river that is homogeneous in space from the physico-chemical and ecological points of view. Each segment to be modeled requires the collection of site-specific data for the model construction and the calibration, thus, given the availability of data in the literature and the effort required by the time-consuming activity of data search, for this study only one segment is considered (see paragraph 2.2 for segment description).

The reach stream is modeled as a CSTR, in which the following main compartments (state variables) are simulated: organisms, nutrients, detritus (bacteria and non living organic matter), sediments (inorganic matter) and chemicals (Figure 2.1). State variables are quantified in terms of concentration or density variations in time (in mg/L or g/m^2) and depend on forcing or driving variables (water flow, temperature, light, nutrients loading), biotic/abiotic parameters values and inputs to the system (Park R.A. and Clough J.S., 2012.).

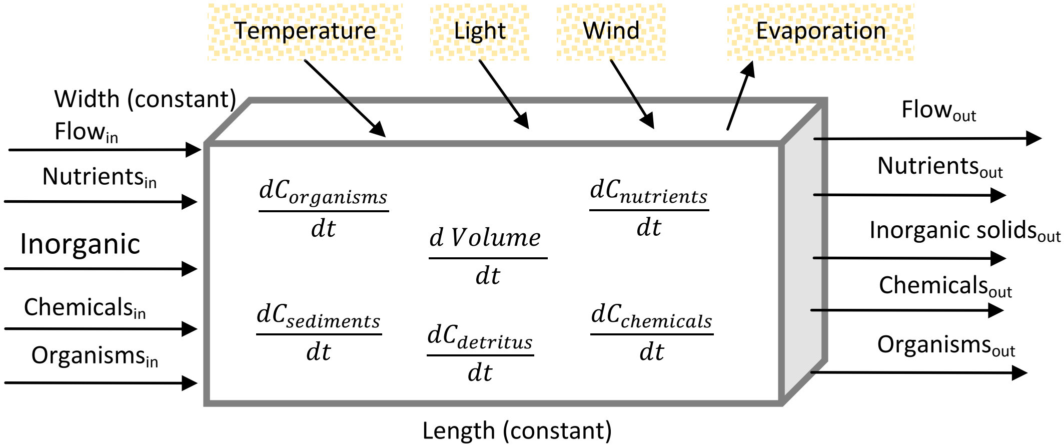


Figure 2.1 - Model conceptualization scheme.

AQUATOX has a user friendly interface and a wizard which may work as a checklist of data needed for simulation. Table 2.1 summarizes the main categories of parameters and data input used in the Po river simulation. Almost every input data can be chosen to be constant or variable (dynamic) in time. The following paragraphs describe in details the available starting data on biotic and abiotic factors referring to Po river segment under consideration and the initial conditions chosen for the state variables.

Table 2.1 - Summary of principal AQUATOX input data for the Po river model simulation.

Segment morphology	Length
	Average width
	Average surface area
	Average slope
	Altitude
	Bottom roughness
	Bottom surface composition
Segment hydrology	Flow rate
	Water depth and bathymetric approximations
	Water volume
Climate	Latitude
	Wind
	Light
Water physico-chemical properties	Water temperature
	Water pH
	Oxygen
	CO_2
	Nutrients (N & P)
	Detritus
	Inorganic solids
Biota	Time-varying biomass series for calibration
	Growth, respiration, excretion parameters
	Diets
Chemical compound	Physico-chemical parameters
	Ecotoxicological parameters

2.1.2 Temporal resolution and numerical stability

The Po river model is run for 3 years, from 1988 to 1990, because the observed available data for calibration refer to this period.

AQUATOX integrates differential equations over time to evaluate state variables changes. In order to avoid numerical instability and reduce truncation errors from the discretization, time steps should be small enough. In this case the model is too complex for a stability analysis to be done, and the smallest time step possible (0.01 d) is chosen to achieve a predetermined accuracy in the solution.

2.1.3 Calibration strategy

The objective of the calibration phase is to modify input parameters and constants until model outputs match in a satisfactory way the observed set of data. AQUATOX does not have an automatic calibration system that find the best set of parameters corresponding to the minimum error between the observed and simulated curves, but it provides graphical and tabular outputs with the possibility to import and plot external observed data for a graphical and mathematical comparison with the simulated ones. In addition it includes several tools for uncertainty and sensitivity analysis that can help during and after calibration step. Evaluation is limited by the quality and quantity of observed data, for this reason Park & Clough (2012) suggest to follow a weight-of-evidence approach with a sequence of tests, avoiding a stringent measure of goodness of fit.

The Po River model is iteratively calibrated on available observed biomass time series that refer to the years from 1988 to 1990, some time series relate only to a year, or are seasonal trends, for this reason the model results will be more accurate for some biota and less for others.

Calibration is done changing manually poorly defined parameters such as light saturation levels, maximum photosynthetic rates, and nutrient limitation parameters for plants and half saturation feeding, maximum consumption rate, minimum biomass for feeding, and carrying capacity for zooplankton, macroinvertebrates and fish.

For each calibration run, a series of tests suggested by Park & Clough (2012) are done:

- Is the model behaviour reasonable according to general experience?
- Do model curves fit in a reasonable way the observed ones?
- Are annual biomass averages respected by model curves?
- Do model curves fall within the errors band of observed data?

After the calibration with visual inspections, the goodness of the model has to be mathematically assessed doing a comparison between simulated and observed biomass time-varying series through four main calculations:

1) Comparison of the average annual biomass simulated and observed:

$$\varepsilon_i = \frac{\bar{B}_{obs\,i} - \bar{B}_{sim\,i}}{\bar{B}_{obs\,i}} \cdot 100 \qquad (33)$$

Where:

ε_i = percentage variation of the simulated average annual biomass with respect to the observed one;

$\bar{B}_{obs\,i}$ = average observed annual biomass of the organisms "i";

$\bar{B}_{sim\ i}$ = average simulated annual biomass of the organisms "i".

Values of ε_i lower that 1 are considered acceptable and it means that the model simulates in a good way the average annual biomass of the organism "i", values of ε_i larger than 1 should be commented taking in consideration also the quality of observed data set (i.e. the magnitude of uncertainties).

2) Comparison of the average annual biomass simulated in the 1 year model and the 3 years model:

$$\varepsilon_{i(1-3)} = \frac{\bar{B}_{i(1)} - \bar{B}_{i(3)}}{\bar{B}_{i(1)}} \cdot 100 \qquad (34)$$

Where:

$\varepsilon_{i(1-3)}$ = percentage variation of the simulated average annual biomass in 1 year model with respect to the average biomass in 3 years model;

$\bar{B}_{i(1)}$= average simulated biomass of the organisms "i" in 1 year model;

$\bar{B}_{i(3)}$= average simulated biomass of the organisms "i" in 3 years model.

The value of $\varepsilon_{i(1-3)}$ will necessarily be high, in fact the marked difference between the average biomass of 1 year and 3 years models is due to the fact that in 3 years model inputs change from one year to another and influence the simulation results. Moreover the average reference biomass is referred to a specific year (1989) and it is not an absolute mean done over many years, so that is not a valid reference value, but it is used, in this case, to verify that three years simulation average biomass is at least of the same order of magnitude of 1 year simulation ($\varepsilon_{i(1-3)}$<100%).

3) Comparison of observed and simulated biomass data points through Pearson linear correlation coefficient:

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}} \qquad (35)$$

Where:

r = Pearson coefficient;

n = number of observations;

x = observed data;

y = simulated data.

It measures the strength and sign of the linear relationship. If the value of r is positive and close to 1 it means that when simulated data values increase, observed data values increase too with the same proportion, and the model behaviour is correct.
This coefficient can assume values from -1 to 1. A values of -1 means that between observed and simulated data there is a perfect negative correlation, the perfect positive correlation is present when the value is equal to 1 and if the value is 0 it means that there is no correlation. In Table 3.1 the conventional interpretation of Pearson coefficient values is shown [XIV].

Table 3.1 - Conventional interpretation of Pearson coefficient values [XIV].

Pearson coefficient value	**Correlation**
0.00 - 0.20	Very low
0.20 - 0.40	Low
0.40 - 0.60	Regular
0.60 - 0.80	High
0.80 - 1.00	Very high

4) Comparison of observed and simulated biomass data points through the Nash–Sutcliffe efficiency (NSE) coefficient:

$$E = 1 - \frac{\Sigma\left(f_{obs}(t) - f_{pred}(t)\right)^2}{\Sigma\left(f_{obs}(t) - \langle f_{obs}\rangle\right)^2} \tag{36}$$

Where:

f_{obs} = observed value at time t;

f_{pred} = predicted value at time t;

$\langle f_{obs}\rangle$ = average of the observed data.

This index compares model prediction to the mean of observed values. While the square of Pearson coefficient is based on the dispersion of the variates around the regression line, Nash–Sutcliffe coefficient is based on the dispersion of variates around the line of equal values. It is sensitive to extreme values because of squared differences (Sexton, 2007). Efficiencies can range from $-\infty$ to 1. An efficiency of 1 ($E = 1$) corresponds to a perfect match of modeled output to the observed data and indicates that the model is a better predictor of observed data than the observed mean. An efficiency of 0 ($E = 0$) indicates that the model predictions are as accurate as the mean of the observed data, whereas an efficiency less than zero ($E < 0$) occurs when the observed mean is a better predictor than the model or, in other words, when the residual variance (described by the numerator in the expression above), is larger than the data variance (described by the denominator). Essentially, the closer the model efficiency is to 1, the more accurate the model is. If E is negative, it means that the model is performing badly (a straight line performs better) In Table 3.3 an interpretation of NSE coefficient values by Moriasi et al. (2007) is shown.

Table 3.3 - Genera performance ratings for recommended quantitative criteria from Moriasi et al. (2007) (see Sexton, 2007).

NSE coefficient value	Model performance
< 0.50	Unsatisfactory
0.50 - 0.65	Satisfactory
0.65 - 0.75	Good
0.75 - 1	Very good

It is important to underline that the calibration is done turning off the inputs of the chemicals to be tested (control model), and only when calibration is finished, the system is perturbed with chemical inputs. In this way two scenarios can be compared: the reference, unperturbed scenario and the one exposed to realistic and hypothetical concentrations of chemicals.

2.1.4 Sensitivity analysis

The sensitivity analysis gives a measure of the variation in model outputs with respect to changes in the values of model inputs. It can be carried out either before or after the calibration step. If before, it allows a preliminary assessment of parameters relevance, driving the choice of the proper range of value to be explored during the calibration; if after, it can be used as a check for the goodness of calibration results. A double-step sensitivity analysis can be also done in order to gain even higher precision.
In modelling the Po river, the sensitivity analysis is not done because with the help of AQUATOX technical documentation the user is able to understand what are the most important parameter to change and in what proportion they influence output.

2.1.5 Validation strategy

The calibration and sensitivity analysis should always be followed by a validation step, with the intent to get an outline of the reliability of the model by testing it against an independent set of data. Validation step is not possible in this case study because of the lack of independent set of data.

2.2 Study area

The river Po is located in Northern Italy and flows 642 km from West to East along the whole Pianura Padana before entering the Adriatic Sea with a delta of 380 km^2. It is the longest Italian river, the one with the largest catchment basin (approximately 74000 km^2) and also the one with the maximum annual average discharge (1450 m^3/s). Along its course are 141 tributaries, in its watershed inhabit approximately 16 million people and is concentrated more than a third of industries and of the Italian agricultural production. Water withdrawals amounted on average to 20.5 billion cubic meters per year, of which 12 % are for drinking use, 7 % for industrial uses, 81 % for irrigation use (Autorità di bacino del fiume Po, 2006). This makes the Po river and its basin a crucial area for the Italian economy and, at the same time, an important natural system to preserve.

The spatial heterogeneity of the Po river, in terms of hydro-climatic, morphological factors and ecosystem structure, must be reconciled with the requirement, dictated by AQUATOX program, to model the system as a sort of continuous stirred-tank reactor, in which variables change in time but not in space (an exception is made for stratified systems). This require a modelling approach that considers the river ideally divided into segments (reactors), homogeneous in space and possibly linked to one another.

After a careful analysis of the available data, in this study is considered only one segment approximately 41 km long, stretching from the closing section of the Po river catchment, Pontelagoscuro, up to Serravalle, immediately before the branching section of the delta, the average latitude is 44.9° in North hemisphere (Figure 2.2 and 2.3). The segment belongs to the final part of the river characterized by high flow, large bed with modest slope, fine bottom substrate subjected to high solid transport (Fenoglio & Bo, 2009).

This segment is considered to be homogeneous in space because the following elements are approximately constant lengthwise:

- The morphological features of the channel (slope, shape, riparian vegetation etc.)(see Par. 2.3.1);
- The discharge, because there are no tributaries or water withdrawals;
- The biotic factors, because the segment is quite far from abiotic factors that can alter the functional dynamics and composition of biological communities according to the River Continuum Concept of Vannote et al. (see Fenoglio & Bo,

2009, p.98). For example it is far enough (about 275 km) from Serafini island, that represents a sort of barrier for biota in the river. It is far enough also from the sea, so that saline wedge penetration does not affect the segment ecosystem.

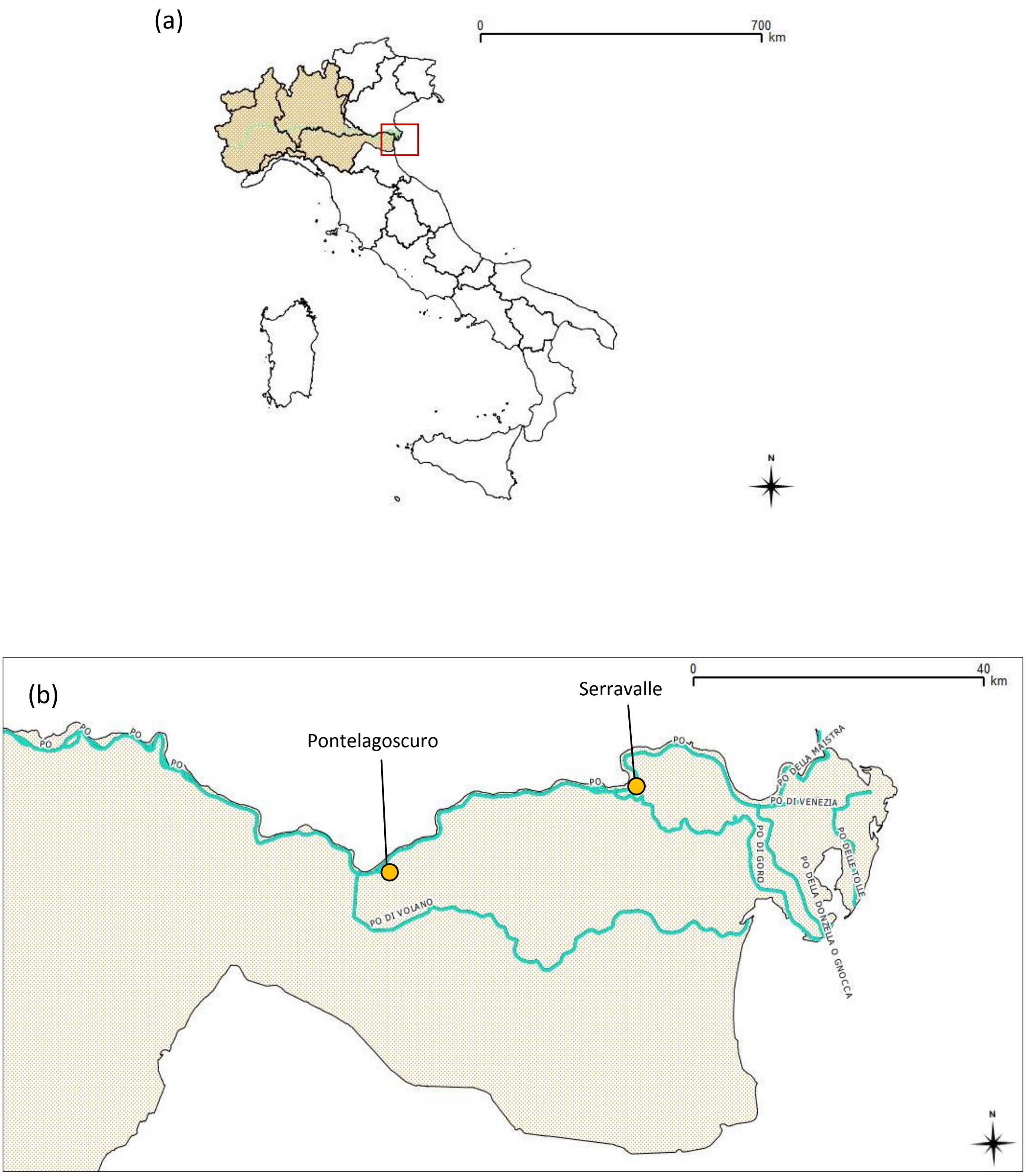


Figure 2.2 - Study area: (a) Po river basin (study area highlighted with the rectangle), (b) studied segment of the Po river from Pontelagoscuro to Serravalle (maps from SINANet web site [V] - download section, modified with Quantum Gis program).

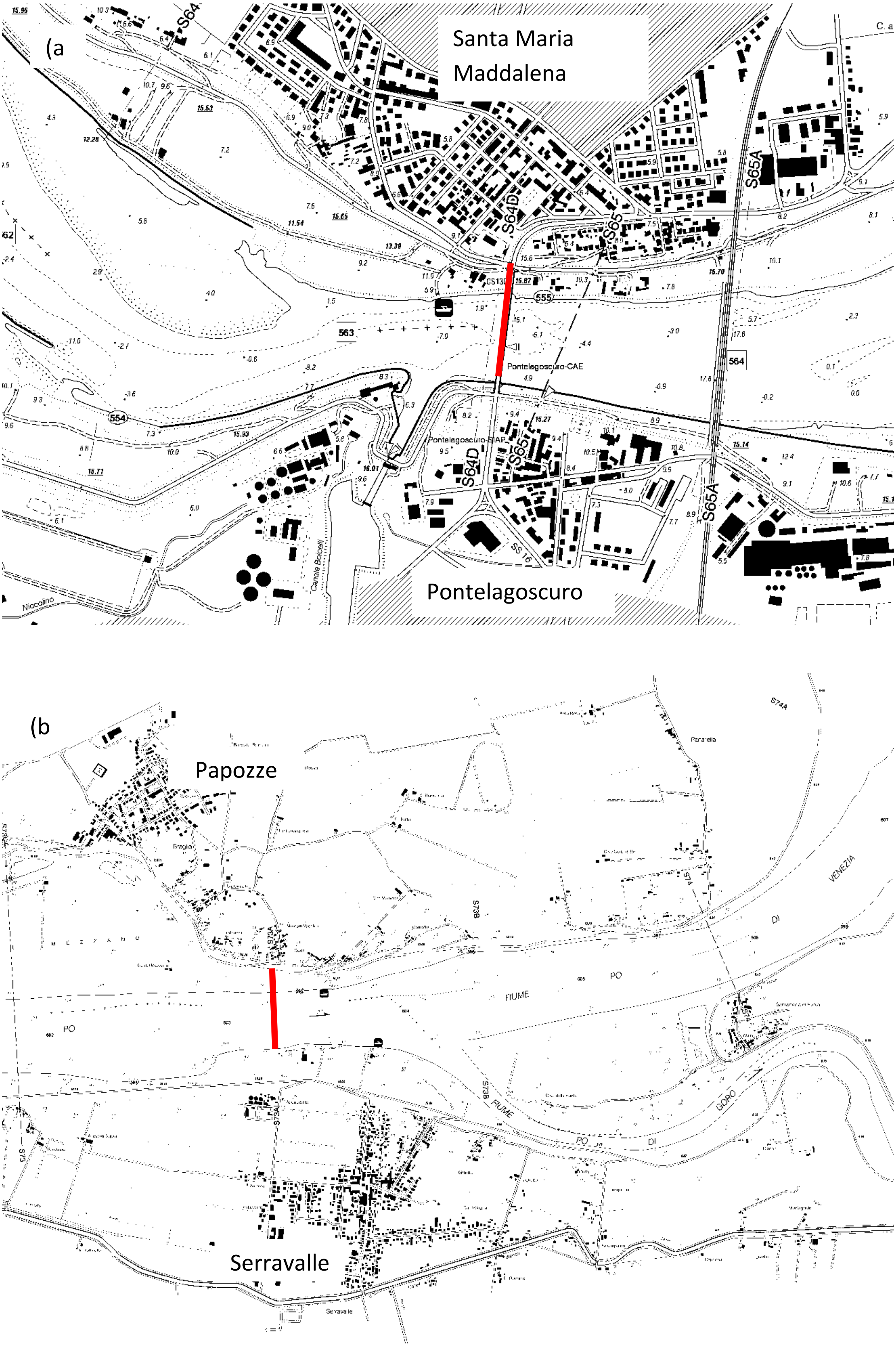


Figure 2.3 - Particulars of Pontelagoscuro (a) and Serravalle (b) sections (in red) (from ADBPO web site - http://www.adbpo.it/on-multi/ADBPO/Home/articolo952.html - Carta fiume Po in formato raster - Maps PO045 and PO048, scale 1:10,000).

2.3 River segment morphology

The knowledge of segment morphology is essential for modelling water depth distribution (5) and water volume (7), thus, indirectly, it affects water velocity, washout, residence time and light penetration, all factors determining the biomass trend of animal and plants. It is assumed, as a simplification, that the segment of the river Po going to be modelled represents the main channel (floodplain excluded) of the river and that it is characterized by homogeneous morphological features. Width between banks and section shape change (a little) along the river segment but it is not important in the modelling because the changes of state variables in space are not under consideration, moreover the river segment analyzed is part of a longer stretch considered in literature as the "lower part" of the Po river, that can be considered as homogeneous from the morphological point of view (Fenoglio & Bo, 2009). All the morphological features and data sources associated are here described in details.

2.3.1 Segment length

Segment length is measured with the "ruler tool" of Google Earth and it is about 41 km measuring the river path from Pontelagoscuro to Serravalle (Figure 2.4).

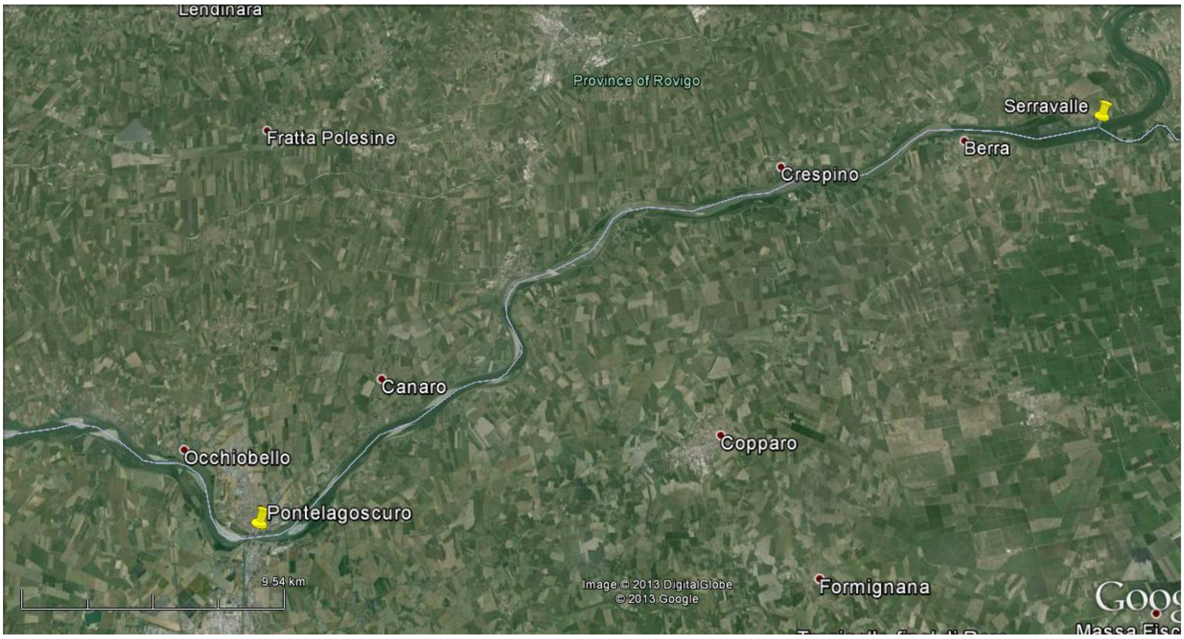


Figure 2.4 - Po river path (in blue) from Pontelagoscuro to Serravalle with Google Earth.

2.3.2 Segment width

Average segment width is calculated doing the mean of the average widths values of two river segment from Autorità di bacino del fiume Po (2010). The width measures refer to bankfull stage, as define by Leopold (1964) it is associated with the flow that just fills the channel to the top of its banks and at a point where the water begins to overflow onto a floodplain [III]. The analyzed segment are the following:

- The segment that goes from Road bridge A13 Occhiobello to Road bridge Polesella: average width between banks (bankfull) of 510 m;
- The segment that goes from Road bridge Polesella – tributary Po di Goro: average width between banks (bankfull) of 460.

Averaged width between banks is then calculated with the following formula:

$$Average\ Width = \frac{510 + 460}{2} = 485\ \text{m} \tag{3}$$

Measures refers to the bankfull, but river width is not constant vertically in the section, thus average width is probably overestimated. More accurate data can be obtained with an analysis of Po river sections drawings that are available, on request, from Autorità di bacino del fiume Po.

2.3.3 Surface area

Average segment surface area is calculated from average length and width:

$$Average\ Surface\ Area = 485 \cdot 41000 = 1988500\ \text{m}^2 \tag{4}$$

2.3.4 Channel slope

Po river slope has a constant value of about 0.03 ‰ from the section that corresponds to the entering of the Panaro river into Po river, to the beginning of the delta area (Colombo A. and Filippi F., 2008) (Figure 2.5).

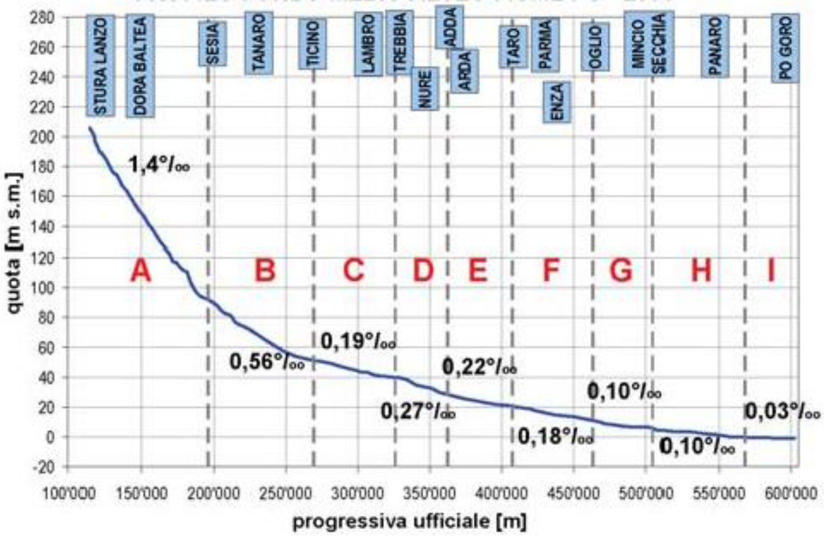


Figure 2.5 - Po River bed profile, from 2005 survey. From Colombo A. and Filippi F., 2008. Section "I" corresponded to the Po river segment studied.

2.3.5 Altitude

Site altitude is calculated considering the distance of hydrometric zero from the medium sea level. From Ufficio Idrografico e Mareografico di Parma - Bacino del Po (1988-1989) the altitude of the hydrometric zero is 8 m m.s.l..

2.3.6 Channel roughness

Channel roughness is modelled in AQUATOX with Manning's coefficient. Chow et al. (1988) suggest for this type of rivers, Manning coefficients around 0.03–0.04 s/m$^{1/3}$ for the main channel. Di Baldassarre and Montanari (2009) done an hydraulic model focused on a 330 km reach of the Po river from Sant'Antonio Island to Pontelagoscuro and found that the best performance was obtained by using Manning’s values equal to 0.03 s/m$^{1/3}$ for the main channel and 0.09 s/m$^{1/3}$ s for the floodplain. Thus, in this study Manning coefficient is set to 0.03 s/m$^{1/3}$.

2.3.7 Bottom surface composition

A mixture of flows and depths provides a variety of habitats to support fish and invertebrate life. In AQUATOX, there is the possibility to subdivide the environment modeled in different habitats by assigning a percentage of occurrence of run, riffle and pool. Pools are deep with slow water. Riffles are shallow with fast, turbulent water running over rocks. Runs are deep with fast water and little or no turbulence [III] (Figure 2.6).

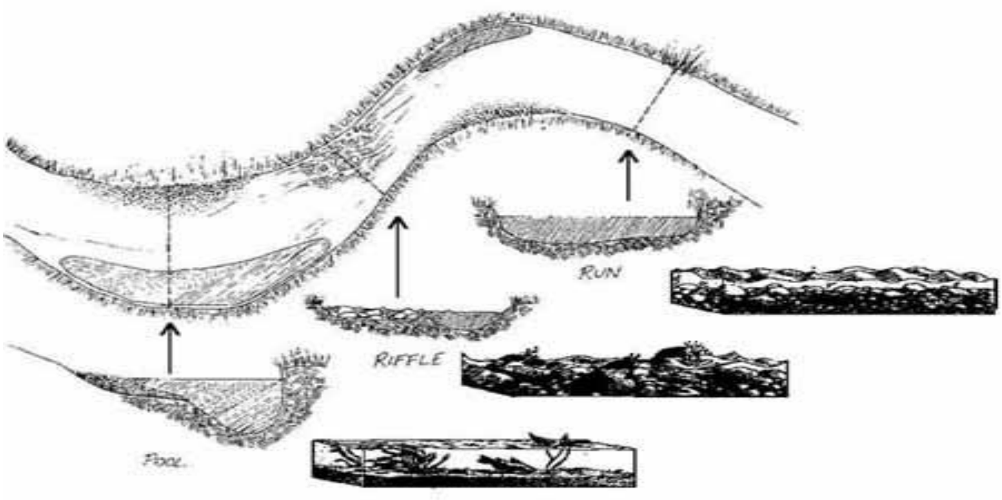


Figure 2.6 - River habitat types. From EPA web site [III]: http://water.epa.gov/type/rsl/monitoring/vms41.cfm.

Regarding the Po river, its habitat diversification has been greatly reduced after rectifications and canalization (Autorità di bacino del fiume Po, 2008). Bortoluzzi et al. (1998) classify the Po river morphology from Ostiglia to the Delta as *meandering* (Figure 2.7), with 53 % of straight section, 36 % of bends and 11% of meandriform stretches. Each type of river stretch is characterized by different bottom types and morphologies, reflecting a different erosion hydraulic regime, a different section of the channel and different relationships between suspended load and base load. In particular Leopold et al. (1964) defined a meander as a band with sinuosity greater or equal to 1.5 (see Bortoluzzi et al., 1998, cap.3, p. 39) composed by a deep and concave bank (pool) and a shallower zone between two pools called riffle. In the study of Bortoluzzi et al. is specified the position of this meanders in the study area (Table 2.2).

Table 2.2 - Meanders position, sinuosity index and radius of curvature from the study of Bortoluzzi et al., 1998.

Meanders	Sinuosity Index	Radius of Curvature
Ostiglia	2	5
Bergantino	2	7
Foce Torrente Panaro	1	6
Corbola	1	1
Bottrighe	1	1

As showed in Figure 2.8, in the Po river segment modeled (segment between the yellow points) there are not meanders (circled in red), for this reason pool and riffle percentages are set to 0 % and run to 100 %.

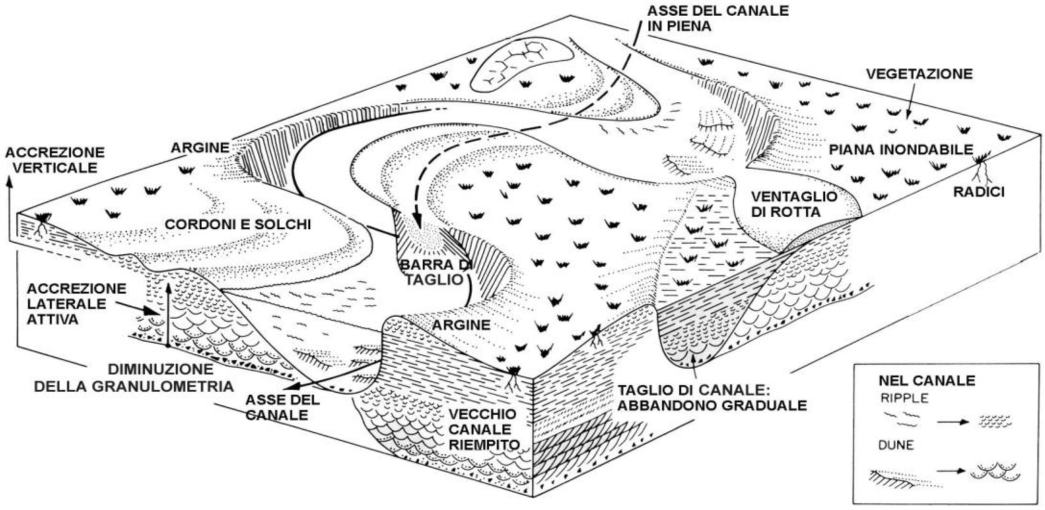


Figure 2.7 - Walker and Cant (1984) three-dimensional representation of morphological and stratigraphic elements of a meandering river system (see Bortoluzzi et al. (1998)).

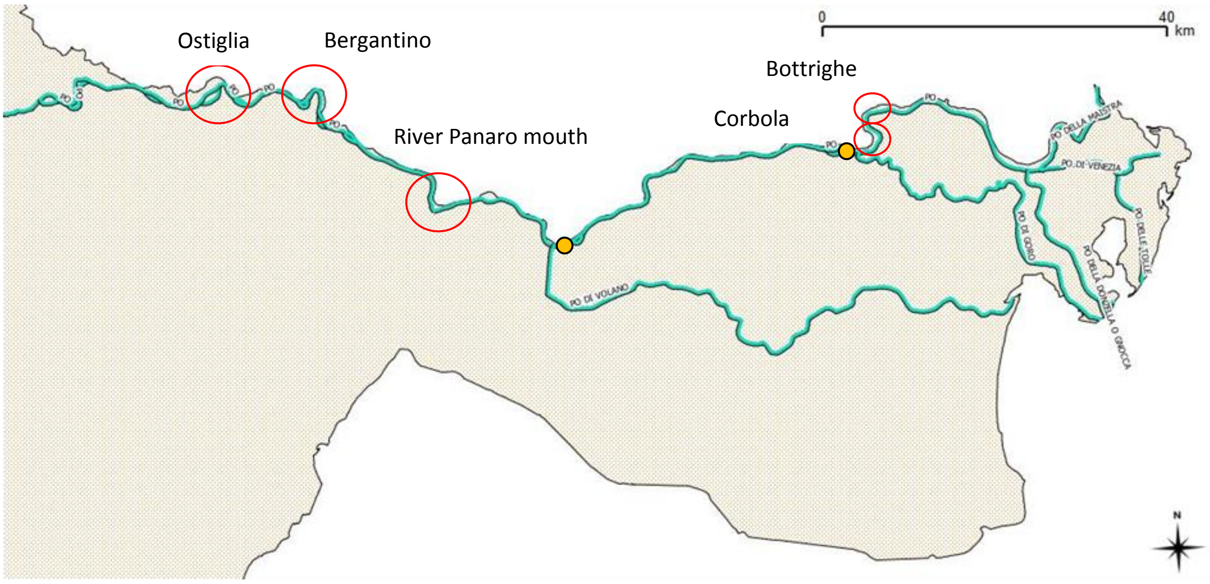


Figure 2.8 - Localisation of meanders (circled in red) in the Po river segment from Ostiglia to the beginning of the delta area. Yellow points show the borders of the Po river segment modelled. (Maps from SINANet web site [V] - download section, modified with Quantum Gis program).

In Table 2.3 the main morphological features of the river segment analysed are shown.

Table 2.3 - Main morphological features used in the model of Po River segment.

Description	**Value**	**Unit measure**
Length	41000	m
Width	485	m
Surface area	19885000	m^2
Slope	0.00003	unitless
Manning coefficient	0.03	$s/m^{1/3}$
Bottom surface composition	0 % pool 0 % riffle 100 % run	
Latitude	44.9	°

2.4 River segment hydrology

2.4.1 Flow rate

Po river flow rates are monitored since 1911 in various sections of the river. Flow data set used (Figure 2.9), is the one published in the hydrological annals and referring to the section of Pontelagoscuro from 1988 to 1990 (Appendix A, Table A.1).

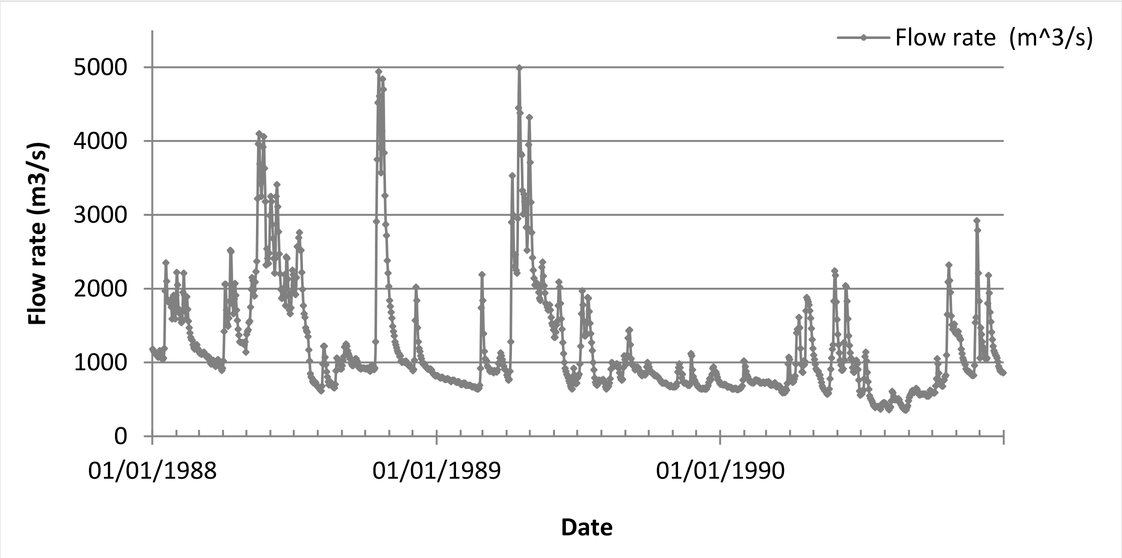


Figure 2.9 - Flow rate trend from January 1988 to Dicember 1990 in the section of Pontelagoscuro (Po river). From Ufficio Idrografico e Mareografico di Parma - Bacino del Po (1988-1989) and Agenzia Regionale Prevenzione e Ambiente - Regione Emilia Romagna - Servizio Idrometeorologico - Area idrologia (1990).

2.4.2 Water depth and bathymetric approximations

Water depth is very important when modelling light extinction into water, sedimentation, reaeration, photolysis and volatilization. Daily dynamic depth cannot be calculated directly from hydrometric measures because they are not available in the hydrological annals of 1988-1990 of the Po river. Daily depths are then calculated from Manning equation rearranged to yield:

$$Y = \left(\frac{Q \cdot Manning}{\sqrt{Slope} \cdot Width}\right)^{\frac{3}{5}} \quad (5)$$

Where:

Q = flow rate (m^3/s). From Ufficio Idrografico e Mareografico di Parma - Bacino del Po (1988-1989) and Agenzia Regionale Prevenzione e Ambiente - Regione Emilia Romagna - Servizio Idrometeorologico - Area idrologia (1990);

$Manning$ = Manning's roughness coefficient = 0.03 $s/m^{1/3}$;

$Slope$ = average segment slope = 0.03 ‰;

$Width$ = average segment width (1) = 485 m.

For the Po river segment analyzed average and maximum depth values resulted to be:

Mean depth: 5.15 m

Maximum depth: 12.32 m

Depth values found with Manning are calculated considering the river as a block with verticals walls and flat bottom. In reality river section is not rectangular, so that water depth changes according to the bottom and walls shape. Values calculated with equation (1) can be considering as approximate section-averaged daily depths, more precise values can be calculated using bathymetric approximations.

In AQUATOX, rivers section is approximated, according to Junge (1966), to extreme elliptic sinusoids (Figure 2.10), then the fraction of the total volume that is at a given depth is calculated (see Park & Clough, 2012, p.44).

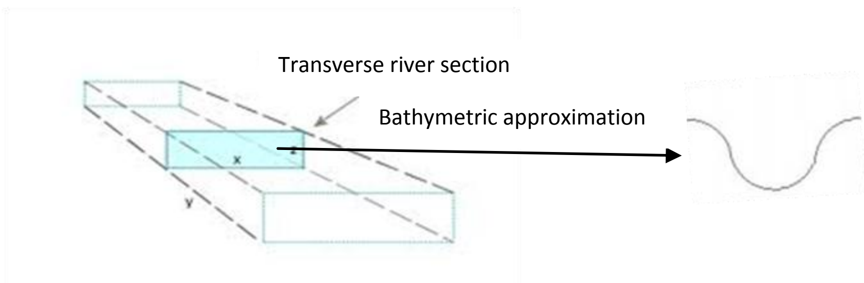
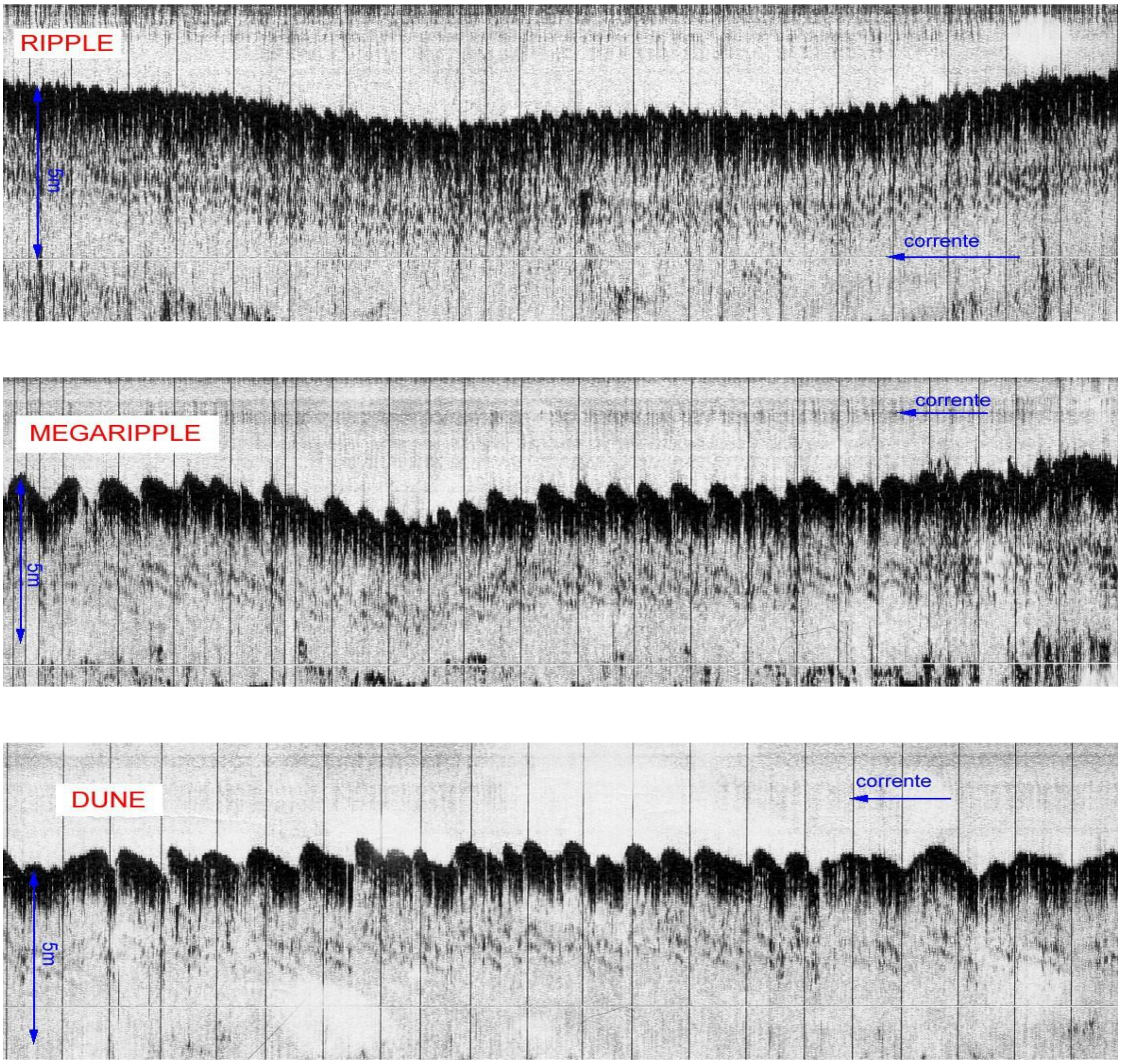


Figure 2.10 - Extreme elliptic sinusoid as bathymetric approximation of Po River section.

Bottom river forms (ripple, magaripples, dunes) are not simulated because they do not influence significantly water depth, a reference can be made to understand the sediment transport in Po river bed. In this regard Bortoluzzi et al. (1998) did a bathymetric survey of the Po River from the Delta to Ostiglia and the examination of the river longitudinal profile highlights the great morphological variability of the bottom: about the 13 % of the studied segment area have a flat bottom, 32 % have ripple forms presenting a dominance of sand ripples of average height of less than 0.2 m, 35 % have megaripple forms characterized by a dominance of sand ripples with average height between 0.2 and 0.5 m, 19 % have dune forms characterized by sand ripples of average height between 0.5 and 1 m and 1% have sand ripples of average height of 1 m (Figure 2.11).



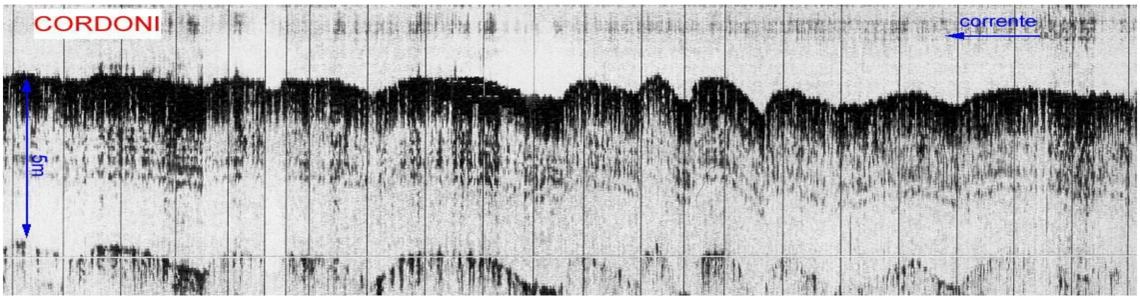


Figure 2.11 - Examples of Po River bottom profiles obtained with acoustic systems variable frequency between 2 and 16 khz ("Sub Bottom Profiler"SBP), from Bortoluzzi et al. (1998).

2.4.3 Water volume

Time-varying water volume is mathematically represented in AQUATOX by the following differential equation:

$$\frac{dV}{dt} = Inflow - Discharge - Evap \qquad (6)$$

Where:

$\frac{dV}{dt}$ = volume variation in time;

$Inflow$ = water entering water body (m^3/d);

$Discharge$ = water exiting from water body (m^3/d);

$Evap$ = water evaporated ((m^3/d).

Water evaporation (from AQUATOX available studies is about 15 in/year = 10^{-9} m/s) is negligible respecting the high flow modelled (on average 1540 m^3/s), so in this study mean and daily evaporation is set to 0.

The user is given several options for computing volume, in Po river simulation is chosen to calculate it for each time step with Manning's equation:

$$ManningVol = \left(\frac{Q \cdot Manning}{\sqrt{Slope} \cdot Width}\right)^{\frac{3}{5}} \cdot CLength \cdot Width \qquad (7)$$

Where the flow Q should be the sum of known discharge value at actual time step and daily evaporation, other parameters are the same described for equation (5). Average system volume results to be 1.02 * 10^8 m^3.

2.5 Study area climate

2.5.1 Latitude

Average latitude of the segment is computed by calculated the mean between Pontelagoscuro and Serravalle latitudes defined from Google Earth:

Pontelagoscuro: 44°53’18.39’’= 44.9°

Serravalle: 44°58’36.54’’= 44.98°

$$Average\ latitude = \frac{44.9+44.98}{2} = 44.9\ ° \tag{8}$$

2.5.2 Wind loadings

In AQUATOX, wind works as a drive variable for reaeration and volatilization. Time-varying wind data are not available from literature, then is used the default AQUATOX function that allows the assessment of variable wind speeds through a Fourier series of sine and cosine terms, the user must only specify wind annual mean (see Park and Clough, 2012, p. 59). Average annual wind can be derived from Figure 2.12 that represents the values for Emilia Romagna Region. Assuming that climate changes were negligible from 1988 to 2009, an average value of 3 m/s can be chosen for the studied area.

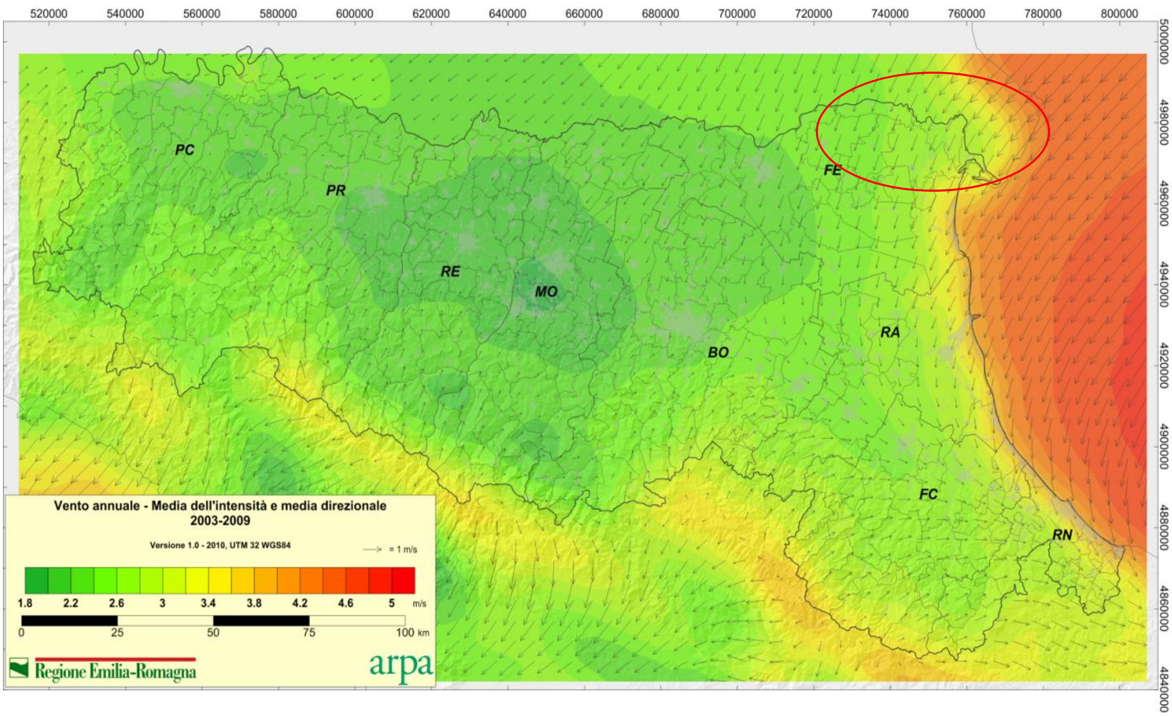


Figure 2.12 - Annual average wind intensity for Emilia Romagna Region, years 2003-2009. Study area circled in red. Map from ARPA web site [VI].

2.5.3 Light loadings

Light is the driving variable for photosynthesis and photolysis processes. Average daily incident light intensity values are calculated in river Po model with AQUATOX default function (9) derived from a variation of temperature function of Ward (1963) (see Park and Clough, 2012, p. 55-56):

$$Solar = LightMean + \frac{LightRange}{2} \cdot \sin\left(0.0174533 \cdot Day - 1.76\right) \cdot Frac_{Light} \qquad (9)$$

Where:

$Solar$ = average daily incident light intensity (ly/d);
$LightMean$ = user input mean annual light intensity (ly/d);
$LightRange$ = user input annual range in light intensity (ly/d);
Day = day according to Julian date (day numerated from 1 to 365,25);
$Frac_{Light}$ = fraction of site that is not shaded = $1 - 0.98 \cdot Canopy$;
$Canopy$ = user input fraction of site that is tree shaded = 0.08.

Monthly averaged daily light data are available for the years 1994-1999, for the Municipality of Ferrara, from Petrarca et al. (1999) (Annex A, Table A.3); thus mean, maximum, minimum values and annual range can be calculated:

Mean annual light intensity:	333.6 Ly/d
Maximum annual light intensity:	556.6 Ly/d
Minimum annual light intensity:	102.7 Ly/d
Annual range light intensity:	453.9 Ly/d

The solution of equation (9) for Po river segment is shown in Figure 2.13 (Annex A, Table A.2).

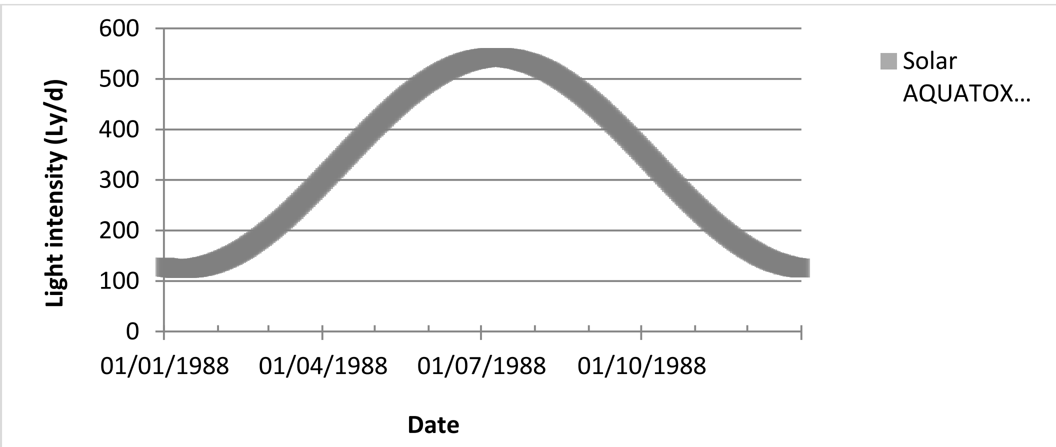


Figure 2.13 - Daily light intensity function, computed with AQUATOX.

2.6 Water physico-chemical properties

2.6.1 Water temperature

Temperature state variable is at the basis of all the modeled phenomena as it can influence process rates based on optimum temperature and biota mortality that depends in parts on minimum tolerated temperature. Monthly river water temperature in the section of Pontelagoscuro is available from Battegazzore, (1991) (Figure 2.14) (Appendix A, Table A.3), thus, these observed temperature loadings are entered into the model as a time-varying input. During the simulation, if the date that is being simulated does not appear on the input list date, AQUATOX uses interpolation to determine the correct loading value. Moreover AQUATOX assumes that the loadings "wrap around" with an annual cycle if the simulation date occurs before or after the first or last date of the loading time series.

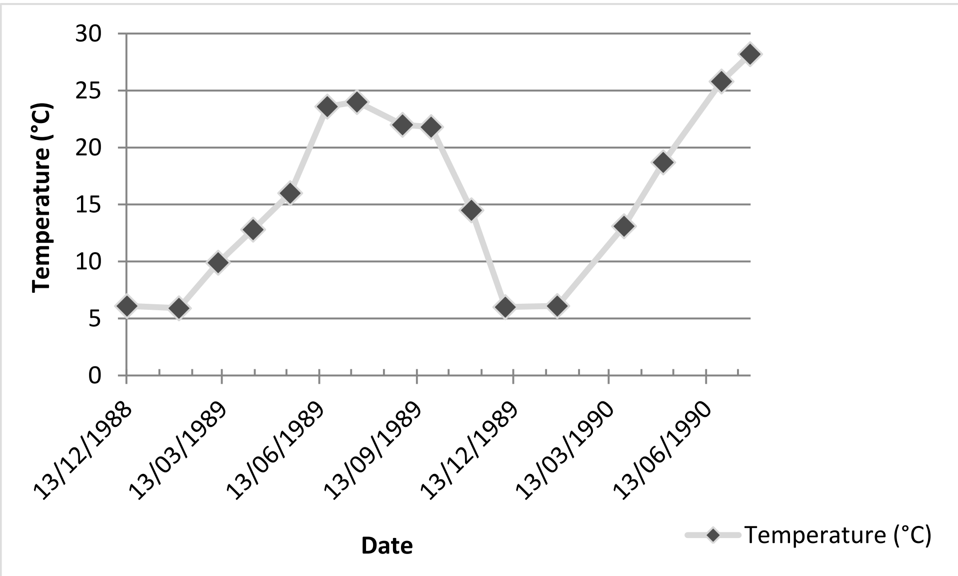


Figure 2.14 - Time-varying water temperature from 13 December 1988 to 24 July 1990 in Pontelagoscuro section. From Battegazzore, (1991).

2.6.2 Water pH

According to Park & Clough (2012), water pH state variable is important because it affects the following processes:

- ammonia ionization which results in a potential toxicity;
- organic chemicals hydrolysis and ionization which results in a potential toxicity;
- decay of organic matter and denitrification of nitrates;
- calcite precipitation (if pH > 7.5) which has a significant effect on the food web.

In AQUATOX, the user can use observed time-series of pH or can make the program calculate it. Monthly water pH values are available for the section of Pontelagoscuro from Battegazzore, (1991) (Figure 2.15) (Appendix A, Table A.4), thus, a time-varying pH input is chosen. As for temperature, if the date that is being simulated does not appear on the input list date, AQUATOX uses interpolation to determine the correct loading value. Moreover, the program assumes that the loadings "wrap around" with an annual cycle if the simulation date occurs before or after the first or last date of the loading time series. Average pH value is 8.1 (similar to river Thames pH found by Lombardo, 2013), this alkaline pH can be attributed to geologic features of the river that is in an area with presence of limestone rocks (source of calcium bicarbonate).

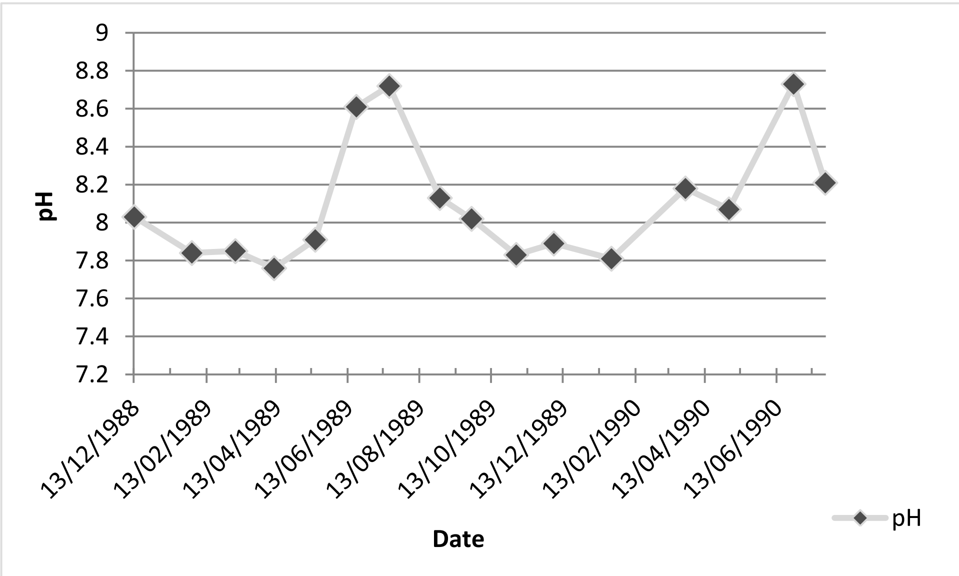


Figure 2.15 - Time-varying pH from 13 December 1988 to 24 July 1990 in Pontelagoscuro section. From Battegazzore, (1991).

2.6.3 Dissolved oxygen

Dissolved oxygen is a vital element for plants and animals, low oxygen concentrations can result in two main important phenomena (Park & Clough, 2012):

- fish and other organism mortality;
- decreased degradation of toxic organic chemicals.

AQUATOX simulates daily average dissolved oxygen by solving a differential equation (Park & Clough, 2012 p.169) that includes terms as reaeration, photosynthesis, respiration, decomposition and nitrification.

User can give a dynamic or constant input loading and initial condition for dissolved oxygen. In the Po river model, it is chosen to turn off the effect of loadings and washout (by selecting this option in oxygen check box), assuming that upstream processes governing oxygen are producing water concentrations identical to the current stream segment being modeled; in this way in stream processes can be analyzed without being dominated by upstream loadings.

Initial condition for dissolved oxygen concentration is set to 10 mg/L corresponding to the date 13/12/1988 of data set of Battegazzore (1991).

2.6.4 Carbon dioxide

Carbon dioxide can be a limiting nutrient for plants. It is simulated in AQUATOX similar to other nutrients with an equation that includes terms as carbon dioxide produced by respiration and decomposition, assimilation of carbon dioxide by plants, interchange of carbon dioxide with atmosphere (Park & Clough, 2012 p.169).

No observed time series are available for Po river, thus a constant input of CO_2 equal to 0.25 mg/L and an initial condition of 0.01 mg/L are chosen. These values are calibrated in order to guarantee a complete availability of CO_2 for plants, assuming that CO_2 is not the limitation factor for plants.

2.6.5 Nitrogen

According to Park & Clough (2012), in AQUATOX, two nitrogen compartments, ammonia and nitrate, are modeled with differential equations including processes as remineralisation, nitrification, assimilation by plants, denitrification.

Dynamic inflow loadings of NH_3, NH_4^+ and NO_3 are put in the model simulating nutrients coming from upstream. Monthly values are available for the section of Pontelagoscuro from C.N.R. Istituto di Ricerca Sulle Acque - Reparto Sperimentale di Idrobiologia Applicata - Archivio dati fiume Po (Figure 2.16) (Appendix A, Table A.5). Initial conditions are chosen on the basis of this observed data considering the values of 08/01/1988: NH_4-N = 0.29 mg/L, NO_3-N = 3.02 mg/L.

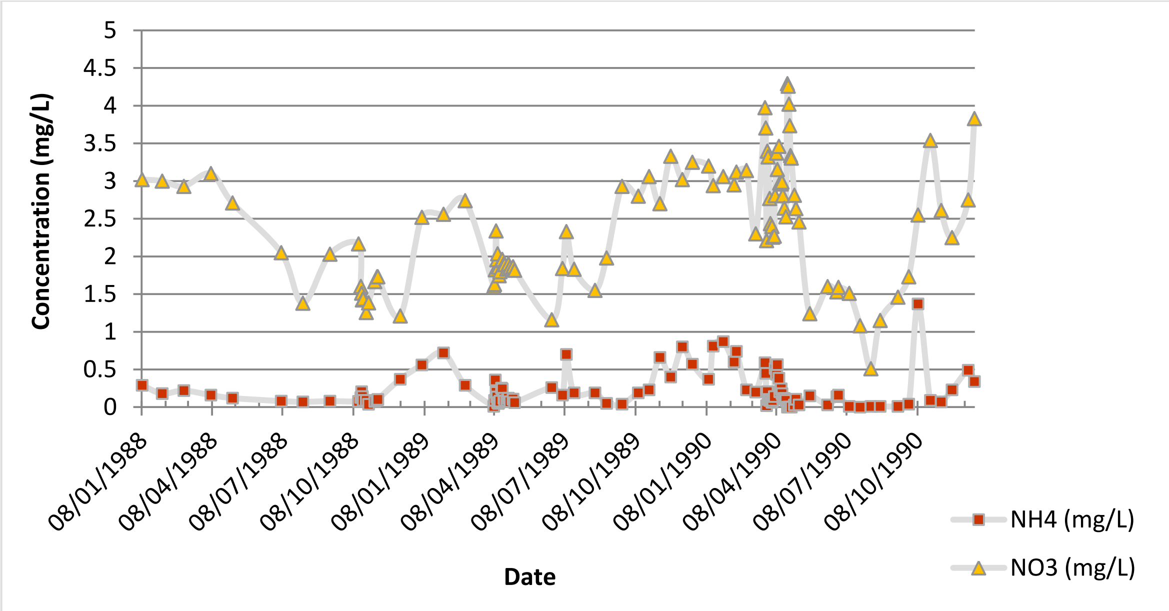


Figure 2.16 - Time-varying NH_4 and NO_3 input loading from 08 January 1988 to 20 December 1990 in Pontelagoscuro section. From C.N.R. Istituto di Ricerca Sulle Acque - Reparto Sperimentale di Idrobiologia Applicata - Archivio dati fiume Po.

2.6.6 Phosphorous

In AQUATOX, phosphate concentration is simulated taking into account various processes as decomposition, excretion and assimilation.

Dynamic inflow loadings of total soluble phosphorus are put in the model simulating nutrients coming from upstream. Monthly values are available for the section of Pontelagoscuro from Bertonati & Ioannilli (1991) (Figure 2.17) (Appendix A, Table A.6). Initial conditions are chosen on the basis of this observed data considering the value of 14/10/1988: TSP = 0.12 mg/L.

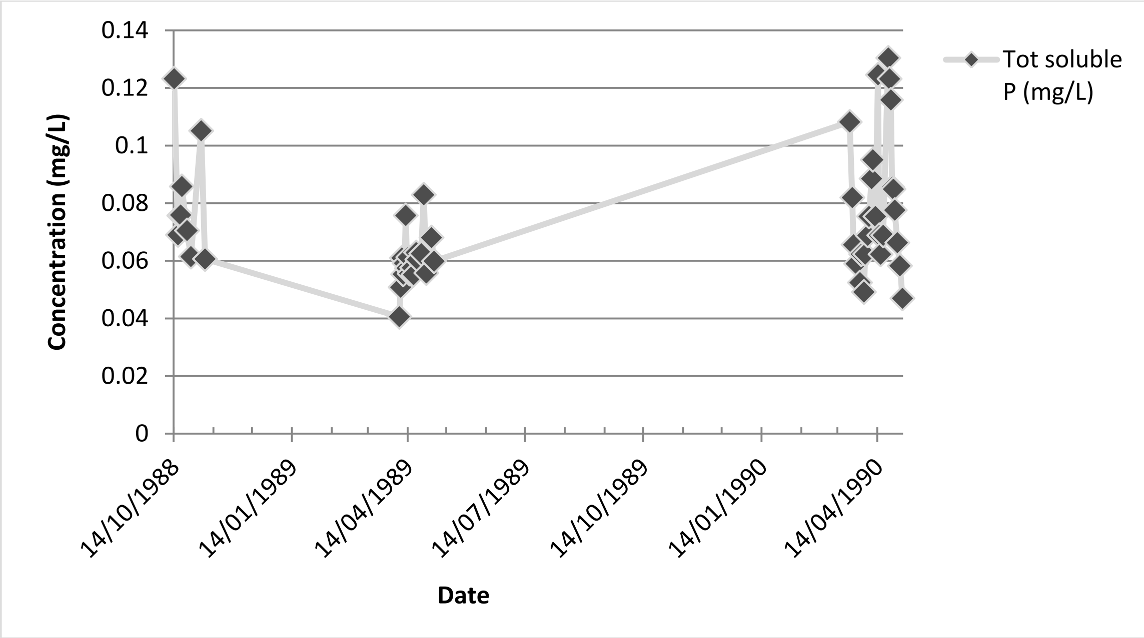


Figure 2.17 - Time-varying total soluble phosphorus input loading in the months of October 1988, April 1989 and April 1990 in Pontelagoscuro section. From Bertonati & Ioannilli (1991).

2.6.7 Detritus

In AQUATOX the term "detritus" refers to "non-living organic matter and associated decomposers (bacteria and fungi)" (Park & Clough, 2012). In fact, since the nonliving organic matter substrate and the metabolizing micro-organism are never isolated from one another in streams, consideration of these components as functionally separate compartments seems merely academic (Cummins, 1974). They are modeled as refractory and labile: refractory detritus does not decompose directly but it is converted to labile through colonisation, while, labile detritus is readily decomposed. Both refractory and labile detritus can be dissolved, suspended, sedimented or buried, so that eight detritus compartments are modeled.

Dynamic inflow loadings of suspended detritus (particulate and dissolved) are put in the model as TOC concentration, then AQUATOX will make the necessary conversions of TOC in OM, simulating organic matter coming from upstream. Monthly values are available for the section of Pontelagoscuro from Bertonati & Ioannilli (1991) (Figure 2.18) (Appendix A, Table A.7). Initial conditions are chosen on the basis of this observed data considering the value of 14/10/1988: TOC = 2.36 mg/L.

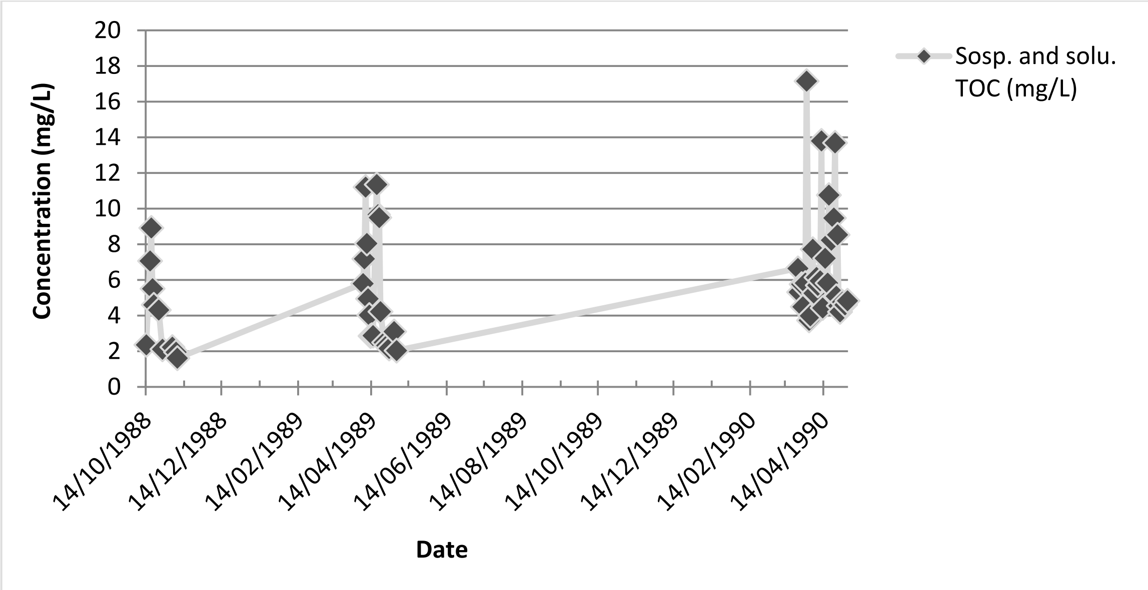


Figure 2.18 - Time-varying suspended and soluble TOC input loading in the months of October 1988, April 1989 and April 1990 in Pontelagoscuro section. From Bertonati & Ioannilli (1991).

For the calculation of the deposition of suspended detritus and resuspension of sedimented ones, the user has to specify initial conditions for total labile and refractory detritus in river bed and water column. Quantitative data on detritus in Po river sediment bed are not found in literature. Thus initial condition of detritus in sediment bed are set equal to the values of two AQUATOX studies Blue Earth River and Ohio stream (see AQUATOX software documentation). For detritus in water column literature data are found (Table 2.4).

Table 2.4 - Boundary conditions for detritus in stream bed and water column.

		Initial conditions
Detritus in sediment bed	Labile detritus (g dry /m^2)	0.3
	Refractory detritus (g dry /m^2)	1
Detritus in water column	Labile + refractory (mg /L)	0.01 [a]
	% of initial conc. that is particulate	30 [b]
	% of initial conc. that is refractory	60 [c]

[a] Initial condition from TOC data.

[b] percentage calculated from data on suspended and dissolved TOC in Bertonati & Ioannilli (1991).

[c] from suggested detrital boundary conditions based on literature in Park & Clough (2012), p. 140.

Burial detritus are not modelled because in this study it is considered only the part of detritus directly accessible by ecosystem organisms.

2.6.8 Inorganic solids

Inorganic sediments influence light penetration in water and can affect biota consumption and mortality. For these reasons, it is important to model in an appropriate way their formation and concentration in the water column.

Data on Total Suspended Solids are available from monthly surveys done by ARPA Emilia Romagna in the section of Pontelagoscuro for the years 2010-2011 [VI]. From these data, average monthly values are thus calculated (Figure 2.19) (Appendix A, Table A.8) and used in the Po river model as dynamic input loadings for TSS, assuming that the hydrological regime affecting the sedimentation and resuspention and detritus formation are not so different from conditions in 1988-1990. AQUATOX will calculate inorganic sediments concentration by subtracting to TSS input loadings the simulated phytoplankton and suspended detritus concentration. Initial conditions are chosen on the basis of TSS observed data considering the value of TSS = 36.5 mg/L.

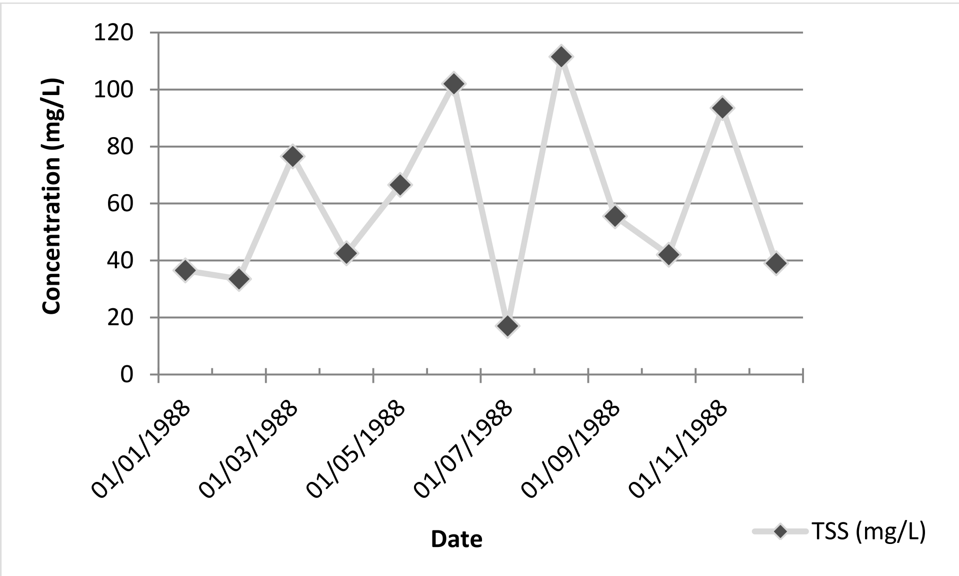


Figure 2.19 - Time varying TSS in Po river section of Pontelagoscuro. Monthly averages taken from data of 2010-2011 observed by ARPA Emilia Romagna [VI].

2.7 Biota

In AQUATOX biota are subdivided in plants and animals. Plants include macrophytes, periphyton and phytoplankton, animals include zooplankton, macro-invertebrates, insects and fish.

For each biota category five main steps are followed before starting the calibration phase:

1) Collection and analysis of observed data on biota of the Po river segment from literature;
2) Choice of the most representative group or species;
3) Derivation of time-varying series of biota biomass for the calibration;
4) Choice of default AQUATOX organisms to represent the ones of the Po river and possibly definition of biota parameters values from literature;
5) Choice of input loadings and initial conditions for biota.

Data collection and data analysis have been the longest procedures of this study. Po river does not have an organized biota monitoring network and the only data available in literature are from studies encouraged by ENEL company near Caorso and from CNR in 1991 to assess the quality of Po river water. To remedy this paucity of data, during the study, data from other periods or from other sections of the Po river are used, specifying and analyzing the feasibility of the assumptions done.

To choose the most representative species, the following considerations must be done:

- On the basis of the observed biomass from literature is the group/species significant in term of abundance and in terms of individual weight?
  The abundance discriminating value is chosen time to time for each biota category modelled. Individual weight is important too because AQUATOX works with biomass and if a species has few individuals with high weight, this must be taken in consideration.
- Among the non abundant species, is there some one that is important in the food web?

  If the trophic role of some rejected species is irreplaceable by others, this specie must be selected.
- Are there available data on time-varying biomass and parameters of the group/species?

If there are not sufficient observed biomass data, or values about the annual average density biomass on a group/species, this must be rejected.

On the basis of these consideration the algorithm in Figure 2.20 is used for the selection of most representative species to simulate.

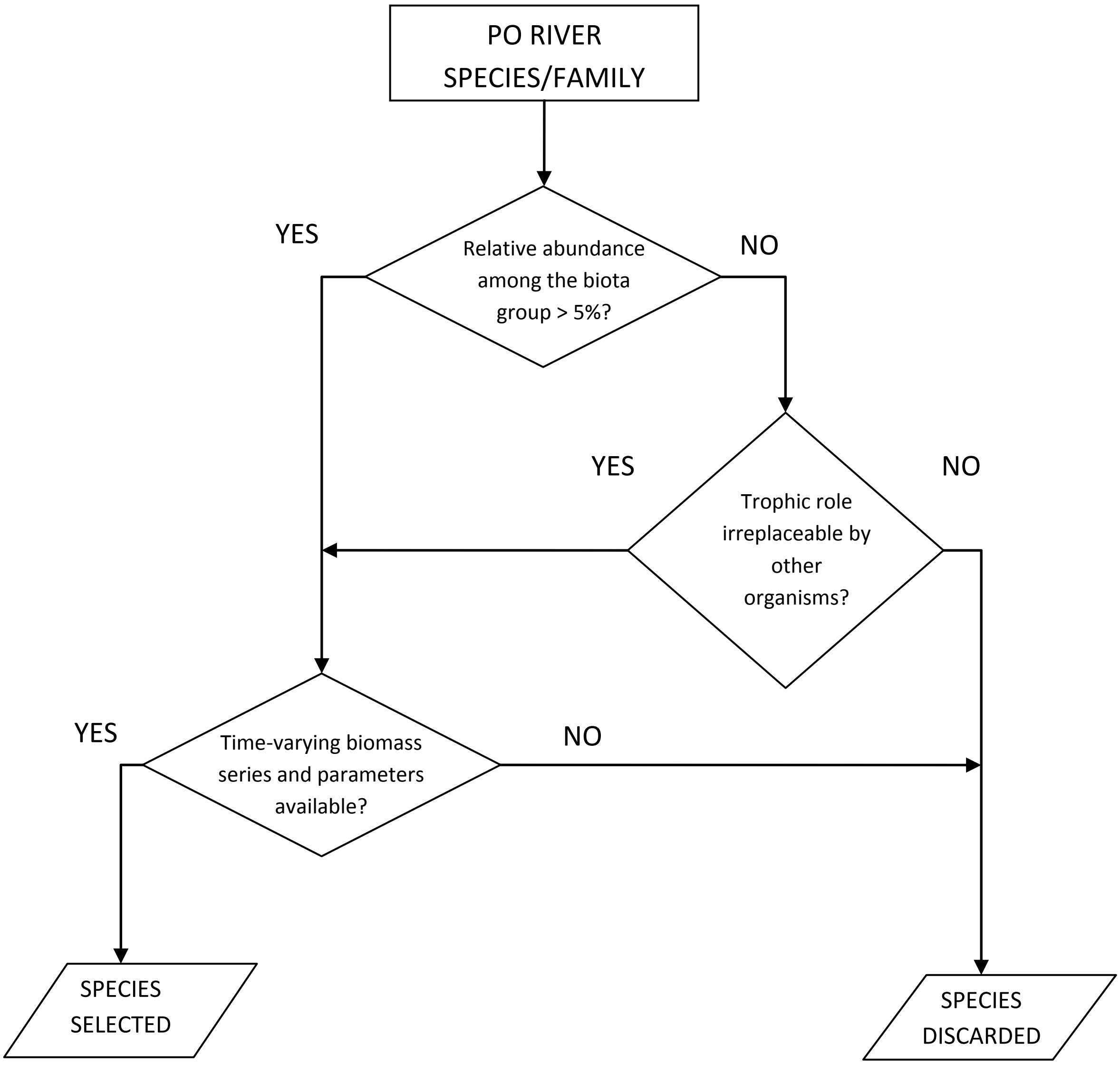


Figure 2.20 - Scheme of the algorithm used for the selection of the species to simulate.

Species chosen to be modeled are summarized in Table 2.5. All the principal and most representative functional groups of the Po river ecosystem are modelled.
To calibrate the model on biomass time series, it is fundamental to have observed data with the same unit of measurement of the simulated ones (mg/L or g/m^2), therefore data set found sin literature for Po River segment are modified in step 3 to be used for calibration.

Before calibrating it is appropriate to start from default parameters values of organisms similar to the ones present in Po river, or to find from literature parameters ranges. Default AQUATOX organisms chosen to represent Po river ones are summarized in Table 2.5.

Table 2.5 - Po river organisms modeled and default AQUATOX organisms chosen to represents the Po river ones.

Biota category	Po River	AQUATOX
Phytoplankton	Cyclotella	Cyclotella
	Chromulina	Isochryses
Zooplankton	Brachiuonus	Brachiuonus
Macroinvertebrates	Amphipoda	Ampelisca
	Young Chironomus	Chironomus
	Oligochaeta	Oligochaeta
	Trichoptera	Trichoptera
	Gastropod	Gastropod
	Odonata	Odonata
Fishes	Bleak	Bleak from River Thames
	Chub	Dace
	Young Wels catfish	Perca flavescens
	Adult Wels catfish	Perca flavescens

Regarding biota upstream loadings, of course, they could be significant inputs to the reach. Loading in "inflow water" are closely related to the volume of water entering the system (Park & Clough, 2012). In Po river the discharge is about 10^8 m^3/d and even a very low upstream loading concentration can result in a huge biomass input that can exaggeratedly influence the model outputs. For this reason it is chosen to set plants and animals loadings equal to zero in order to simulate only the initial biomass present in the system (specified with initial conditions) and analyse its behaviour that will not be influenced by upstream inputs but only by ecosystem intern processes.

In the following paragraphs, for each biota category, steps from 1 to 5 are described in details justifying the choices made.

2.7.1 Plants

Data analysis and plants species selection

In riverine ecosystems there could be three main categories of plants: macrophytes, periphyton and phytoplankton.

Aquatic macrophytes characterized by macroscopic dimension are found both near and within surface freshwater. They are very important in stream ecosystems for two main reasons: they provide shelter for invertebrates and food sources for many species. As analysed by Fenoglio e Bo (2009), in lotic systems the direct consume of macrophytes is not so important and this kind of plants enter the trophic web almost exclusively at the time of dieback, as detritus.
Regarding the Po river, information about macrophytes biomass are almost absent, a very useful study was done by Pellizzari (2009) which analyzed the vegetation of the Po River and its right bank, between Porporana and the Bianca Island (Province of Ferrara). Results emerging from the analysis can be summarized in the following points:

- Hydrophytic (that are free floating) and helophytic (perpetually submerged with only the root) communities are limited in the riverbed because of the current;
- The muddy and sandy banks host some different annual pioneer vegetation rich in alien species;
- Hygrophilous tall herb fringe communities colonize the river borders and dams with varying degrees of nutrient uptake; they are followed dynamically and structurally by bushy or woody riparial formations.

Thus, in Po River, only banks have a rich marginal vegetation while in the riverbed macrophytes have a limited and not constant presence, as documented by Pellizzari. Moreover, no data on macrophytes time-varying biomass are available. At the same time macrophytes represents an important source of food and shelter. The contribution of macrophytes to streams has been reviewed by Westlake and Fisher & Carpenter (see Anderson & Sedell, 1979) which concluded that the contribution of macrophytes to the productivity of stream ecosystems ranges from 1% in the River Thames, to 9-13% in intermediate-sized rivers, and up to 30% in springs, and it may be almost 100% of the primary production in polluted unshaded sections of some rivers. These plants are not usually grazed upon and thus represent a source of autochthonous detritus. The decomposition of macrophytes is quite rapid (ca 50% weight loss in a week) compared with terrestrial leaf material (ca 5-25% weight loss in a week) (Anderson & Sedell, 1979). For these reasons it is chosen to model macrophytes shelter function, considering a constant presence of 1 g dry weight/m^2 (modified on a qualitative and subjective basis from Anderson & Sedell, 1979 that considers 10 g dry /m^2 for deep waters) in order to simulate invertebrates protection from predation when Po river water reaches vegetated areas and avoiding an accurate macrophytes biomass assessment because of the lack of data for calibration. To simulate the trophic role of macrophytes, it is assumed that they are represented in the trophic web by labile and refractory detritus input (TOC).

Periphyton

Periphyton is a mixture of algae and cyanobacteria that are attached to submerged surfaces. Regarding the specific case of the Po river, in its lower sections there is a modest primary productivity because of the high depth and turbidity (Fenoglio & Bo, 2009), in addition no data on observed periphyton biomass are available from Po river, for this reasons periphyton is not modelled.

Phytoplankton

Phytoplankton is a mixture of algae and cyanobacteria that are floating in the water column. It is the main group of primary producer in Po river ecosystem (Fenoglio & Bo, 2009).

For the Po river segment analyzed, data on phytoplankton concentrations are available from Garibaldi (1991). He studied the phytoplankton community examining 30 samples collected monthly (from September 1988 to March 1990) from Po river at Pontelagoscuro. About one hundred species had been observed, but is not possible to model every one because of the increase in model complexity, then a selection is made based on the most abundant groups that result to be Diatoms (82%) and Chrysophytes (4.4 %) (Table 2.6 and Figure 2.21). For each modeled group, the most representative species, according to the abundance, is chosen: Cyclotella for the Diatoms group and Chromulina for the Chrysophytes group.

Table 2.6 - Total numeric density of phytoplanktonic species in Pontelagoscuro section of the Po river from 10/9/1988 to 27/03/1990 (modified from Garibaldi, 1991). (In orange the species chosen to simulate in Po river model).

Taxa	Numeric density ((ind/10^3)/L)	%
Diatoms	**321532**	**82.3**
Cyclotella	272377	69.7
Nitzschia	16271	4.2
Fragilaria	11899	3.0
Diatoma	8319	2.1

Asterionella	3966	1.0
Melosira	3101	0.8
Navicula	2866	0.7
Synedra	1151	0.3
Cymbella ventricosa	1102	0.3
Cocconeis	369	0.1
Surirella linearis	111	0.0
Chrysophytes (Other Algae)	**17143**	**4.4**
Chromulina globosa	11883	3.0
Chrysococcus sp.	3078	0.8
Oachromonas sp.	2182	0.6
Chlorophyceae (Greens)	**16210**	**4.1**
Scenedesmus	10094	2.6
Ankistrodesmus	1891	0.5
Coelastrum microporum	1495	0.4
Actinastrum hantzschii	1245	0.3
Oacystis lacustris	608	0.2
Chlamydomonas sp.	303	0.1
Tetraedron minimum	203	0.1
Logerheimia wralislaviensis	169	0.0
Euglena sp.	101	0.0
Schroederia setigera	101	0.0
Cryptophyceae (Other Algae)	**8923**	**2.3**
Rodomonas minuta	6018	1.5
Chryptomonas erosa	2905	0.7
Cyanobacteria (BlueGreens)	**7665**	**2.0**
Merismopedia tenuissima	5275	1.4
Oscillatoria sp.	1893	0.5
Lyngbia limnelica	295	0.1
Anabaena cilindrica	101	0.0
Coelosphaerium naegelianum	101	0.0

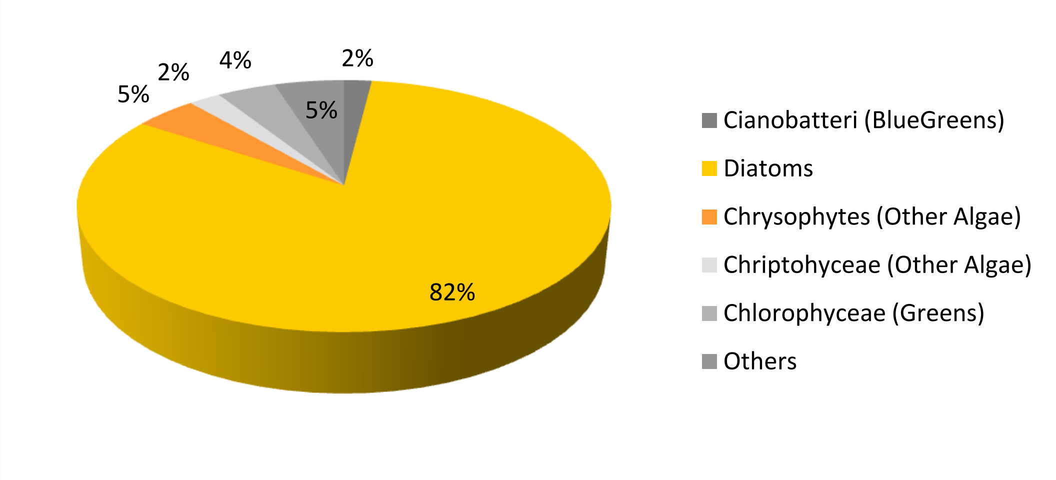


Figure 2.21 - Phytoplankton species percentages in Po river section of Pontelagoscuro in the period between 10/9/1988 and 27/03/1990 from Garibaldi L. surveys.

Time-varying biomass series elaboration

Time-varying biomass series are available from Garibaldi (1991) in (Ind /10^3)/L (Appendix B, Table B.1). To calibrate, phytoplankton biomass has to be converted in mg dry/L because this is the unit measure of AQUATOX output, so the dry weight of phytoplankton species cell has to be known. In Table 2.7 Cyclotella and Chromulina cell dry weights are shown, they are calculated from a regression equation (16) from Reynolds (1984). It is the equation of the line that best approximate data of cell dry weights against cell volumes of a selection of planktonic algae from literature or author's unpublished records. The equation of the regression is:

$$W_c = 0.47 \cdot V^{0.99} \quad (10)$$

According to the calculated weights, the time-varying biomass for the calibration is derived by multiplying the cell number by the cell weight (Figure 2.22) (Appendix B, Table B.2). The average daily biomass density over one year is assessed considering data of 1989, and it results to be 1.042 mg/L for Cyclotella and 0.042 mg/L for Chromulina.

Table 2.7 - Cell volumes and dry weights of Cyclotella (Diatom) and Chromulina (Chrysophyte).

Taxa	Cell Volume (micro m**3/cell)	Dry weight (pg/cell)
Cyclotella comensis	400[a]	177[c]
Chromulina (value refers to sp.)	440[b]	195[c]

[a] Jørgensen et al. (1991).
[b] Reynolds (1984).
[c] calculated from regression equation: $W_c = 0.47 \cdot V^{0.99}$ from Reynolds (1984).

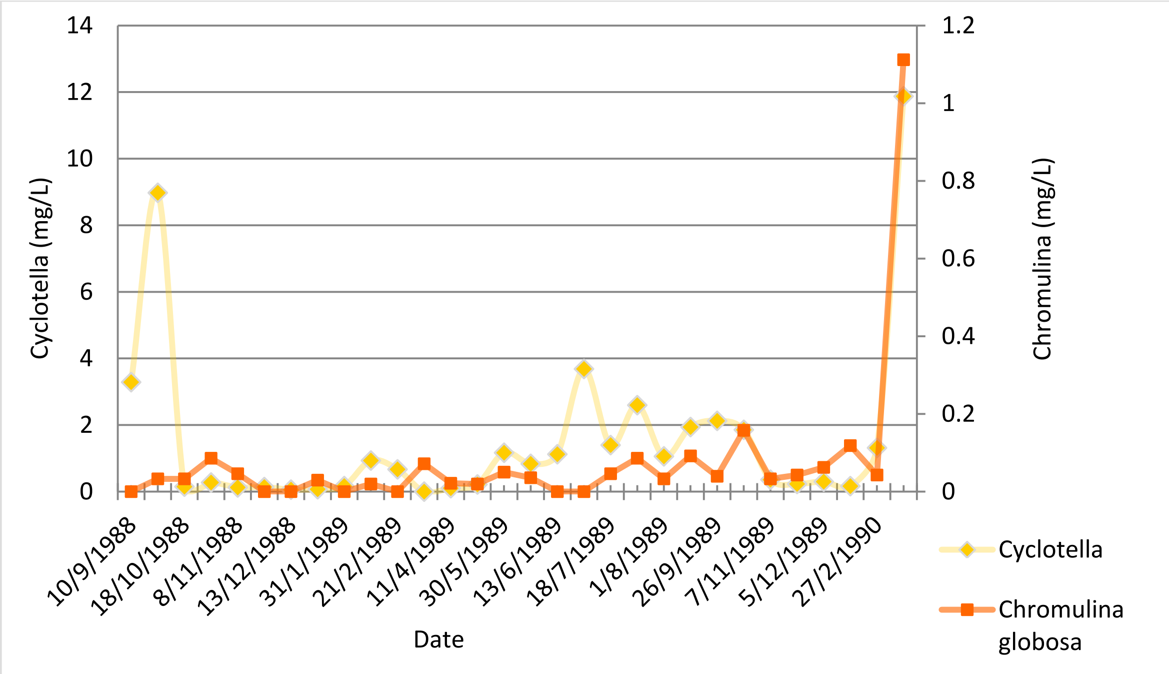


Figure 2.22 - Time-varying biomass density of Cyclotella (Diatom) and Chromulina (Chrysophyte) in the Po river section of Pontelagoscuro from 10/9/1988 to 27/03/1990.

Phytoplankton model assumptions

Residence time

An important aspect to be considered in modelling phytoplankton is the phytoplankton residence time. In fact, as the it moves with the water, phytoplankton residence time in the system is equal to water residence time (11):

$$t_{residence\ water} = t_{residence\ phytoplankton} = \frac{Volume}{Discharge} = 1.07\ d\ on\ average \tag{11}$$

Phytoplankton biomass remains one day in the river segment and then exit the system, thus if no input load is added, washout is much bigger than load, phytoplankton biomass goes to zero and primary productivity is not modeled.

As the intent is to model variations in phytoplankton biomass due to predation and water physico-chemical condition, the idea is that the initial phytoplankton biomass works as a seed for the all simulation period and that phytoplankton entering the system during the simulation period approximately equals phytoplankton exiting, in this way input and output flows do not affect variation in biomass in the system. To do this, AQUATOX tool called "Enhanced Phytoplankton Retention" is chosen.

This allows to calculated the load as a function of washout, with the following formula (12) (see Park and Clough 2012, p. 91):

$$Loading_{upstream} = Washout_{biota} - \left(\frac{Washout_{biota}}{\frac{TotLength}{SiteLength}}\right) \quad (12)$$

Where:

$Loading_{upstream}$ = loading of plankton due to upstream production (mg/L);
$Washout_{biota}$ = washout of plankton from the current reach (mg/L);
$TotLength$ = total river length (km);
$SiteLength$ = length of the modeled reach (km).

In order to make $Loading_{upstream} = Washout_{biota}$ the $TotLength$ should ideally tending to infinity. Thus the TotLength is chosen to be: 100000000 km. This is of course a modelling device essential to better simulate the segment processes and reach the objectives of this study. If the real TotLenght (of order of magnitude of 100 km) is used, the input load of phytoplankton is negligible and the washout is much bigger than load.

Light extinction

Analyzing data on Secchi depth and on phytoplankton mortality, it is visible that light penetration into the water column is widely reduced by turbidity of the water and therefore phytoplankton do not survive. This is due to the fact that, in AQUATOX, TSS and phytoplankton vertical distributions are constant, thus they are considered as well mixed variables, but in reality TSS concentration decreases in increasing water depth and phytoplankton concentration decreases in increasing water depth, but at the same time most of phytoplankton population avoid the surface layer, variously forming its peak concentration at depths between 1.5 and 2 times Secchi-disk extinction (Reynolds, 1984).
To reduce errors caused by this TSS vertical distribution simplification, the extinction coefficients modified as parameters to calibrate (Table 2.8).

Table 2.8 - Extinction coefficient modified for Po river model.

Parameter	AQUATOX default values	River Po
Extinction coefficient water (1/m)	0.02	0.02
Extinction coefficient sediment (1/m g/m^3)	0.17	0.1
Extinction coefficient DOM (1/m g/m^3)	0.03	0.03
Extinction coefficient POM (1/m g/m^3)	0.12	0.06

Moreover, in conditions with high TSS concentration, phytoplankton distribution over depth is strongly shifted towards the surface. For this reason it is absurd to assume that phytoplankton is equally distributed all over the water column depth. In AQUATOX there is the possibility to modeled phytoplankton not as mixed throughout all the water column, but as mixed in a 3 m surface layer. This option is chosen for the Po river simulation by using some modelling devices that consist in setting the wind value to 3 m/s (simulation of downward transport by Langmuir circulation) and in specifying phytoplankton as surface floating by checking the "surface floating" option.

Plants parameters

To compute calibration, it is appropriate to define in advance a range of variation of Cyclotella and Chromulina parameters or, in alternative, starting values from literature or from previous AQUATOX studies. This way of working ensures a proper calibration and avoids choosing parameters values that may satisfy calibration but have no real meaning.

Thus, for each modeled species, it is chosen the most similar one in AQUATOX plants library (Table 2.5), and the default values of the parameters are chosen as starting values for calibration (Table 2.9).

Table 2.9 - Cyclotella and Isochryses AQUATOX default parameters.

Parameter	Species	
	Cyclotella	**Isochryses**
Saturating light (Ly/d)	22.5	67
Max. saturating light (Ly/d)	300	
Min. saturating light (Ly/d)	22.5	
P half-saturation (mg/L)	0.055	0.046
N half-saturation (mg/L)	0.117	0.006
Inorg. C half-saturation (mg/L)	0.054	0.054
Temp. response slope	1.8	2
Optimum temperature (°C)	20	25
Maximum temperature (°C)	35	35
Min. adaptation temperature (°C)	2	2
Max. Photosynthetic rate (1/d)	1.87	2
Photorespiration coefficient (1/d)	0.026	0.026
Respiration rate at 20°C (g/g-d)	0.08	0.2
Mortality coefficient (g/g-d)	0.001	0.01
Exponential mortality coefficient (g/g-d)	0.05	0.04
P : Organics	0.007	0.007
N : Organics	0.059	0.059
Light Extinction	0.14	0.144
Wet to Dry	5	5
Sedimentation rate (KSed) (m/d)	0.005	0.31
Exp sedimentation coefficient	0.05	0.693

2.7.2 Zooplankton

Data analysis and species selection

The term "zooplankton" indicates a group of heterotrophic organisms of small size that can live in the water column for all their life (oloplanktonic) or that can also live for a period as benthos (meroplanktonic), the group main include protozoa, rotifers, micro-crustaceans, diptera Chaoboridae or Dreissena polymorpha larvae (Fenoglio & Bo 2009).

Rotifers are one of the principal components of the zooplankton in middle Po river (Rossetti et al. 2009), and the family Brachionidae results to be the most abundant, in particular the species *Brachionus calyciflorus*. This is demonstrated in particular in two studies: one from Antonietti et al. (1995) and the other from Ferrari et al. (1989). In the study of Antonietti et al., a series of water samples were taken in two sections of river Po, Torricella di Sissa (PR) and Casalmaggiore (CR) from February 20 to October 30 1990 with a 15 L Patalas trap and the rotifers species presented were analyzed (Figure 2.23) (Appendix B, Table B.3).

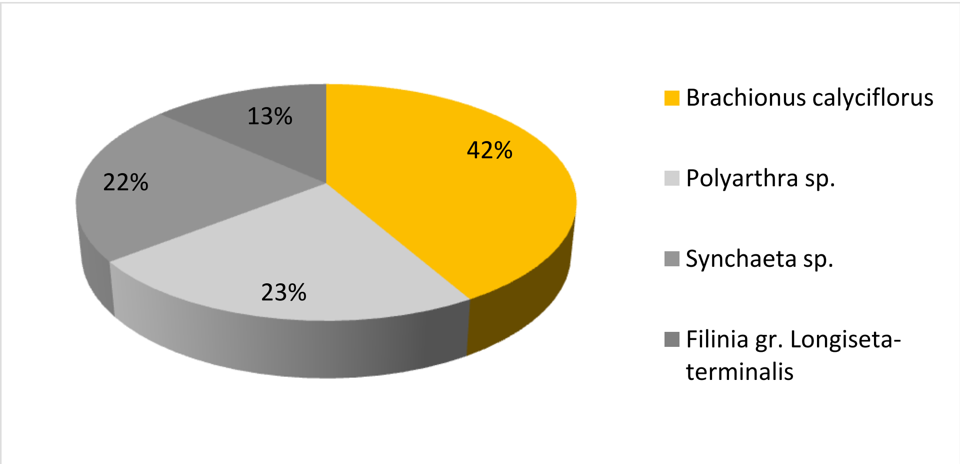


Figure 2.23 - Rotifers species percentages averaged between the two sample sections of Torricella di Sissa and Casalmaggiore from 20/02 to 30/10 1990. Data from Antonietti et al. (1995).

In the study of Ferrari et al., zooplankton samples from the middle reach of the Po River were collected daily from 27 July to 24 August 1988 from a station located near Viadana with a 15 L Patalas trap (Figure 2.24) (Appendix B, Table B.4).

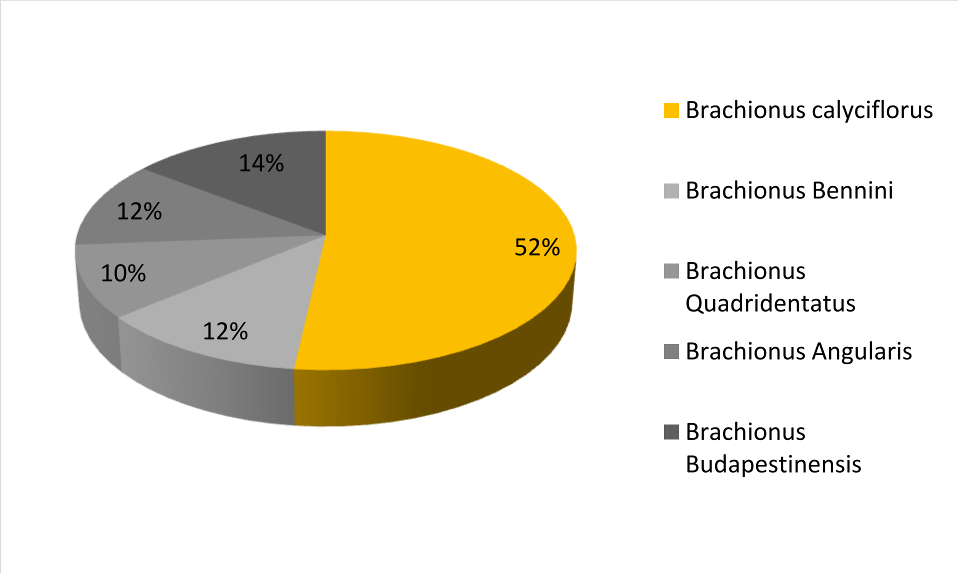


Figure 2.24 - Rotifers species density (ind/L) in the section of Viadana from 27/07 to 24/08 1988. Data from Ferrari et al. (1989).

On the basis of the available data set, it is chosen to model the species *Brachionus calyciflorus* because it is the most abundant in Po river.

The studies of Antonietti and Ferrari are chosen as the reference point for zooplankton simulation in Po river model, even if the samples are taken in sections about 125 km far from Pontelagoscuro. This is acceptable if it is demonstrable that factors affecting Rotifers biomass variation do not change very much from the Viadana section to the Pontelagoscuro section. A study from Battegazzorre et al. (1992) contains data on space variability of temperature, pH, DO, POC, DOC in ten sections along the River Po pathway in June 1990; from these data it is evident that abiotic variables do not have sensitive variations from Pontelagoscuro (600 km from source) to Casalmaggiore/Viadana (400 km from source) (Figure 2.25). Moreover the water velocity is high (about 1.5 m/s) and water takes about 3 days to reach Pontelagoscuro. The continuum of the river is not interrupted from Viadana to Pontelagoscuro (i.e. Serafini Island is upstream of Casalmaggiore).

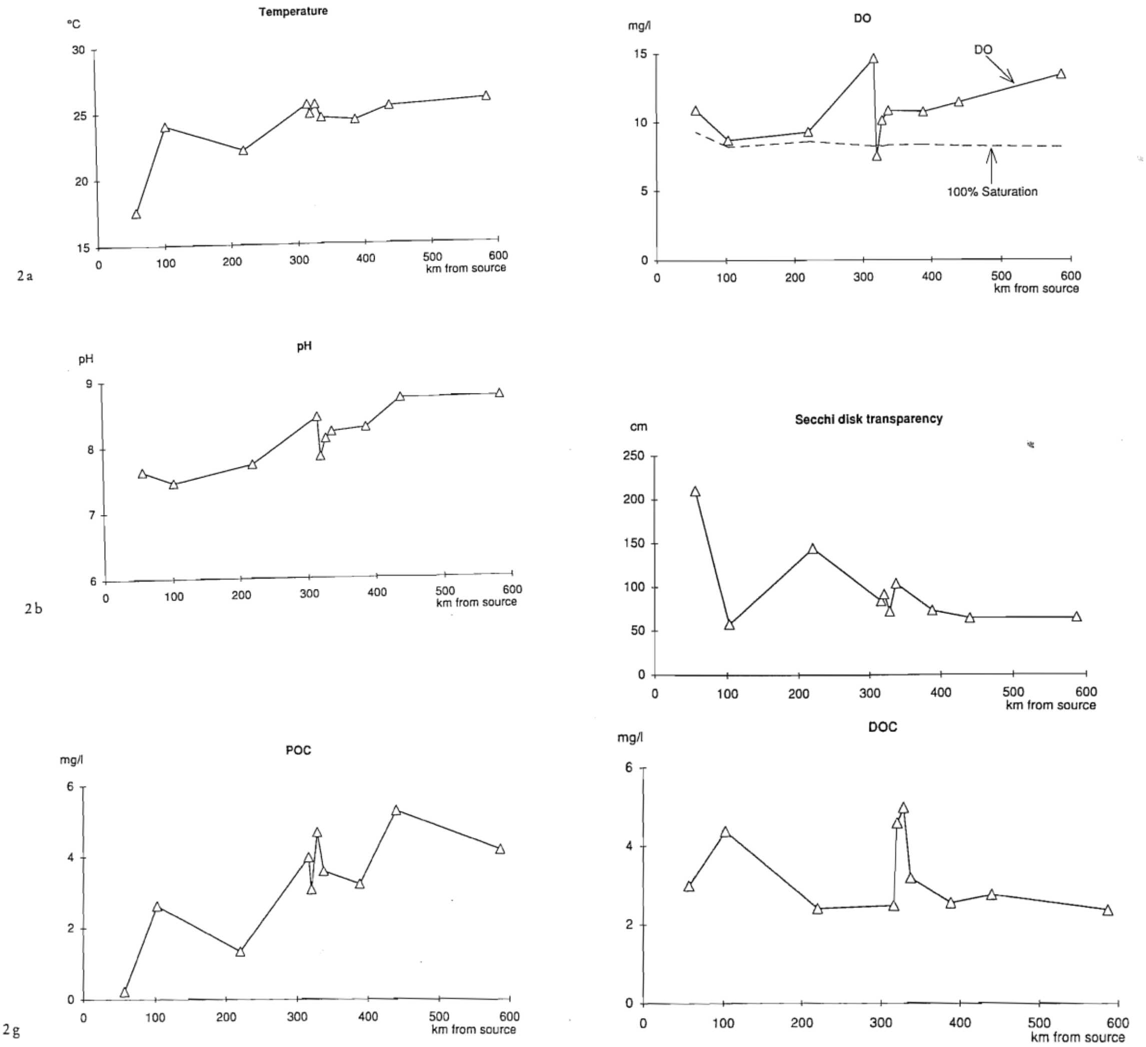


Figure 2.25 - Space-varying temperature, pH, DO, Secchi disk transparency, POC, DOC from Battegazzorre et al. (1992).

Time-varying biomass series elaboration

To derive *Brachionus calyciflorus* biomass series for calibration, data from Antonietti e Ferrari are used multiplying each numerical density (Ind/L) for the average dry weight of the species that is found to be 0.3 µg/ind (Palomares et al. 1993). Result is shown in Figure 2.26 (Appendix B, Table B.3). The average daily biomass density over one year is assessed considering data of 1990, and it results to be 0.0481 mg/L .

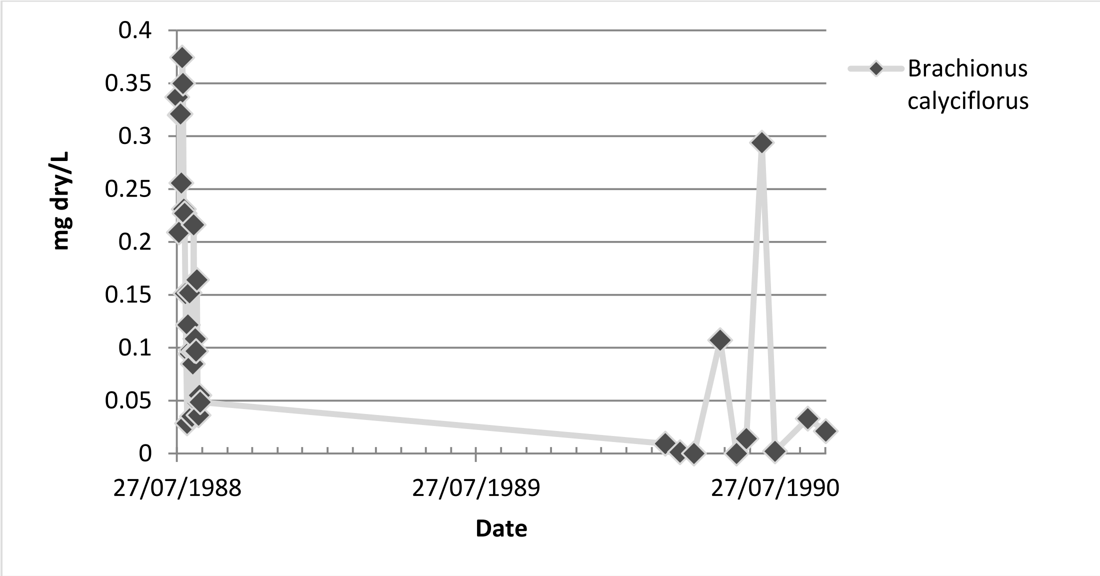


Figure 2.26 - Brachionus calyciflorus time-varying biomass from 27/07 to 24/08 1988 and from 20/02 to 30/10 1990. Data used for calibration.

Antonietti points out that the net decrease of Brachionus measured on 27/07/1990 is due to the increase of the density of its predator Asplanchna.

Zooplankton model assumptions

Residence time

As zooplankton moves with the water, in the same manner of phytoplankton, the same assumption done for phytoplankton in Paragraph 2.7.1 are used.

Parameters

Brachionus calyciflorus initial parameters values are taken from Brachionus data in the animal library of AQUATOX (Table 2.10).

Table 2.10 - *Brachionus calyciflorus* default parameter from animal AQUATOX library (search scientific name Brachionus).

Parameter	***Brachionus calyciflorus***	**Reference**
Half saturation feeding (mg/L)	1	Walz. 1995, p. 441
Maximum consumption (g/g*d)	3.4	From sev. papers, extrapolated from growth
Min. prey for feeding (g/m^2)	0.1	Walz. 1995, p. 441
Temp. response slope	2	Default
Optimum temperature (°C)	18	Walz. 1995, p. 443
Maximum temperature (°C)	25	Expert judgment
Min. adaptation temperature (°C)	5	cold-adapted (see Walz, 1995)
Mean wet weight (g wet)	1.2 * 10^(-7)	Walz. 1995, p. 441
Endogenous respiration (1/d)	0.34	Leidy & Ploskey, 1980, p. D20
Specific dynamic action (unitless)	0	Incl. in endogenous
Excretion : respiration	0.17	
N : Organics (frac dry)	0.09	Sterner & Elser 2002
P : Organics (frac dry)	0.014	Sterner & Elser 2002
Wet to dry	4.7	default
Gametes : biomass	0.18	Walz. 1995, p. 445
Gamete mortality (1/d)	0.06	Expert judgment
Mortality coefficient (1/d)	0.25	Walz. 1995, p. 443 (0.25)
Sensitivity to sediments	zero	Default -- no sediment effect
Carrying capacity (g/m^2)	4	LeCren & Lowe-McConnell, 1980, p. 260
Vel max. (cm/s)	400	Default
Mean lifespan (d)	4	Walz. 1995, p. 442
Fraction that is lipid (wet wt.)	0.012	

2.7.3 Macroinvertebrates

Data analysis and species selection

Macroinvertebrates of the Po river are a heterogenic group of organisms that constitute the zoobenthos, they are larger than a millimetre at the end of the larval development and they can be sampled with networks or sieves with a 500 micron mesh. In riverine ecosystems the macroinvertebrates communities include many phyla as: Porifera, Arthropods, Molluscs, Crustaceans, Anellids etc. (Fenoglio & Bo, 2009).

Regarding the river Po, a study on macroinvertebrates community was conducted by Battegazzore in 1991. He used artificial substrates Hester-Dendy to sample organisms in the section of Pontelagoscuro from December 1988 to July 1990 and he listed the time-varying numerical density of 131 species. Battegazzore data are referred to organisms that are found in the substrates after the average period of 1 month, data on the abundance are interpreted as a daily average value over a month, because organisms do not remain in the substrate for all the sampled period, in other words, the numbers found by Battegazzore are not cumulative biomasses but it is assumed that the number of organisms found in the substrate in the day of sample extraction from the river, is equal to the average daily number of individuals that have populated the substrate in the previous days of the month. This is the starting data set for the macroinvertebrates selection for Po river simulation.

The first step is the discarding of those groups that during the sample period (about 2 years) are found not to be significantly present in terms of individuals sampled (< 50 ind. sampled) but also in terms of biomass, because AQUATOX works with biomasses (if a species have few individuals but with high weight, this must be considered in the model). The following groups are rejected: Plecoptera, Coleoptera, Bivalvia, Hirudinea, Lepidoptera and Bryozoa. Of the remaining groups, only the most abundant (> 50 ind. sampled) and weight-significant species (> 50 ind. sampled) are selected (Table 2.11).

Table 2.11 - Selection of the groups with a significant number of individuals (group with n° of ind. > 50) and, for each group, selection of the most abundant species (species with n° of ind. > 50).

Group	Species	Total n° of ind. sampled	% over the total number of macroinvertebrates sampled	% over the total number of species belonging to that group
Amphipoda*	*Echinogammarus veneris* HELLER	3118	39.0	100
Diptera	*Rheocricotopus fuscipes* (K.)	321	4.0	19.7
	Rheopelopia ornata (Mc.)	286	3.6	17.6
	*Chironomus**	254	3.2	15.6
	*Polypedilum**	183	2.3	11.2
	*Cricotopus**	149	1.9	9.1
	Dictrotendipes sp.	81	1.0	4.97
	Orthocladius sp.	65	0.8	3.99
Trichoptera*	*Hydropsyche*	1334	16.7	98.96
Oligochaeta*	*Stylaria lacustris* (LINN.)	490	6.1	74.8
	Tubificidae gen. sp.	73	0.9	11.1
Gastropoda*	*Physa*	181	2.3	65.1
	Lymnea	63	0.8	22.7
Tricladida	*Dugesia* sp.	173	2.2	100
Hemiptera	*Aphelocheirus aestivalis* (F.)	153	1.9	100
Decapoda	*Atyaephyra desmaresti* (MILLET)	143	1.8	100
Ephemeroptera	*Caenis luctuosa* Burm.	108	1.3	56.25
Odonata*	*Pyrrhosoma nymphula* (SuLZER)	80	1.0	67.8
Isopoda*	*Asellus aquaticus* L.	55	0.7	100

* species or group present in AQUATOX animal library.

On the basis of this first data skimming, another selection has to be done, otherwise the model will become too complex. The second selection is based on the trophic function of organisms and their presence in AQUATOX library, in particular all the trophic functions should be included in the model. For each feeding behaviour it is chosen the most abundant organisms and the ones present in AQUATOX library. In particular the following groups/species are simulated: Amphipoda, Gastropoda, Diptera (Chironomus species), Oligochaeta, Trichoptera, Odonata (Table 2.12). Concerning the insects as Diptera, Trichoptera and Odonata, they are modelled as larvae, because in adult life they have wings and they are supposed not to be part of the aquatic system and also because the sample refers to the aquatic life stage. The adult insects are model all together as an external compartment.

Table 2.12 - Second selection based on feeding behaviour and presence in AQUATOX library.

Feeding behaviour	**Taxa**	**Selected organism**	**% over total macroinvertebrates**
Shredders	Amphipoda *(Echinogammarus)**	✓	39.0
	Decapoda *(Atyaephyra Desmaresti)*		1.8
	Isopoda *(Asellus acquaticus)*		0.7
Scrapers	Ephemeroptera *(Heptageniidae)*		0.2
	Gastropoda *(Physa, Limnea)**	✓	3.0
Collectors gatherers	Diptera *(Chironomus)**	✓	3.2
	Ephemeroptera *(Baetis, Caenis, Ephemeridae)*		2.2
	Oligochaeta*	✓	8.2
Filterers	Diptera *(Simuliidae)*		0.5
	Trichoptera *(Hydropsychidae)**	✓	16.7
Predators	Odonata *(Nymphs)**	✓	1.5

* species or group present in AQUATOX library.

----- organism rejected.

Time-varying biomass series elaboration

To compute calibration with AQUATOX is necessary to convert the numerical density (ind/sample) in biomass density (mg/m^2).

The first step is to derive the number of individuals over surface. The number of total individuals sampled (Table 2.11) is divided by 0.8 (5*0.16) considering that organisms are sampled with 5 substrates with a free surface of 0.16 m^2 each one (8 square plates 10 x 10 cm) (Table 2.13).

Table 2.13 - Individual density averaged over time calculated from Battegazzore dataset.

Taxa	Average density ($ind/m^2/d$)
Amphipoda *(Echinogammarus)* B	243.59
Ditteri *(Chironomus)*	19.84
Ephemeroptera *(Caenis)*	8.44
Oligocheti	43.98
Tricotteri *(Hydropsychidae)*	104.22
Odonata *(Nymphs)*	6.25
Gastropoda	19.06

The problem is that these density measures are very uncertain because Hester-Dandy is a selective sampler, so some organisms are attracted and others are not. To solve the problem it is necessary to found a reliable density reference, in order to compare it with the observed data from Battegazzore, in this regards the data from Cironi and Ruffo (1981) are analysed. In 1974-1876 they sampled every month macroinvertebrates of the Po river in 14 sections before and after Serafini island, in the river bottom, with a dredge and in the banks, by drying up an area of 907 m^2 and cutting macrophytes (Appendix B, Table B.4). Average density for the station of Monte isola de Pinedo (before the obstruction of Serafini Island) are listed in Table 2.14. For Trichoptera and Gastropoda no data are available so for these organisms data of Battegazzore are maintained.

Table 2.14 - Average density values of macroinvertebrates in Po river bottom and banks at station of Monte isola de Pinedo (modified from Cironi and Ruffo, 1981).

Taxa	Bottom density ($ind/m^2/d$)	Banks density ($ind/m^2/d$)
Amphipoda *(Echinogammarus)*	-	110.21
Diptera *(Chironomus)*	504.24	126.09
Oligochaeta	57110.91	-
Trichoptera *(Hydropsychidae)*	-	-
Odonata *(Nymphs)*	-	100.49
Gastropoda	-	-

Thus, individual time-varying densities of Amphipoda, Diptera, Oligocaeta and Odonata are calculated doing a simple proportion between averages from Battegazzore and from Cironi and Ruffo (Appendix B, Table B.5).

The second step is to transform the individuals densities in biomass densities. To do this dry weights in Table 2.15 are used.

Table 2.15 - Macroinvertebrates dry weights used for the time-varying biomass series calculation.

Taxa	Dry weight (mg/ind)	Min Dry weight (mg/ind)	Max Dry weight (mg/ind)	Mean (mg/ind)
Amphipoda (Echinogammarus)	0.037[a]			
Diptera (Young Chironomus)	0.14[a]			
	0.007 - 1.5[b]	0.007[b]	1.5[b]	0.754
Oligocheti	0.005[a]			
Trichoptera (Hydropsychidae)	0.026 - 0.768[b]	0.026[b]	0.768[b]	0.397
Odonata (Nymphs)	0.4[c]			
Gastropoda	0.05[b]			

[a] Palomares et al. 1993

[b] Jørgensen et al. 1991

[c] Smock 1980

Resulted time-varying biomass series for calibration are shown in Figure 2.27 (Appendix B, Table B.6). Data are average daily biomass density for every month. The average daily biomass density over one year is assessed considering data of 1989, and it results to be 0.0037 g dry/m^2 for Amphipoda, 0.1486 g dry/m^2 for Chironomids, 0.1467 g dry/m^2 for Oligochaeta, 0.0476 g dry/m^2 for Trichoptera, 0.00156 g dry/m^2 for Gastropoda, 0.0656 g dry/m^2 for Odonata.

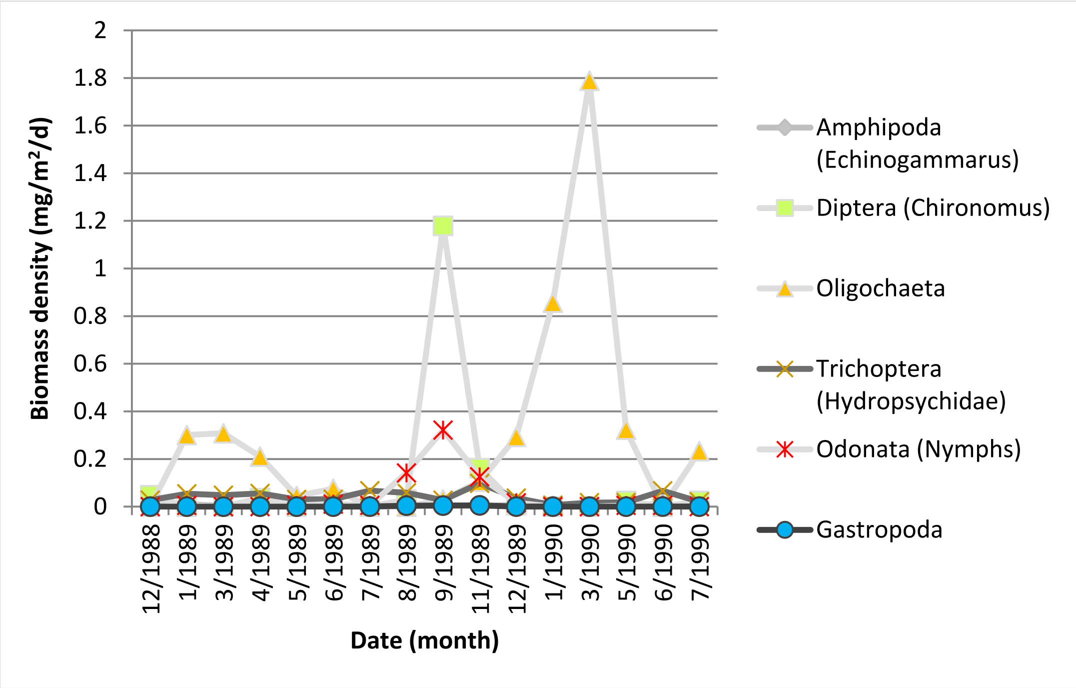


Figure 2.27 - Resulted average daily biomass density for every month from December 1988 to July 1990, data used for calibration.

Macroinvertebrates model assumptions

Drift

As the intent is to model variations in macroinvertebrates biomass due only to predation and water physico-chemical condition, the idea is that the initial animal biomass works as a seed for the all simulation period and that organisms entering the system during the simulation period equals organisms exiting, in this way input and output flows do not affect variation in biomass in the system. To do this the macroinvertebrates drift is set to zero for all simulated organisms.

Adult insects

Observed biomass series for calibration do not include adult insects because of the nature of the sample device (Hester-Dendy or dredge), thus in order to compare observe with simulated data, the macroinvertebrates have to be modeled excluding adult stages. At the same time adult insects are preyed by some fishes, so they have to be included in the model food web. To do this an adult insects compartment is added, so that it represents a constant source of food of 1 g dry/m^2 (a value obtained through calibration), but adult insects biomass variation is not modelled because of the lack of observed data for calibration.

Parameters

For each modeled species, it is chosen the most similar one in AQUATOX animal library (Table 2.5), and the default values of the parameters are chosen as starting values for calibration (Table 2.16).

Table 2.16 - Starting parameters values for macroinvertebrates.

Parameter	Species					
	Amphipoda	Chironomus (larvae)	Trichoptera (larvae)	Oligochaeta	Gastropoda	Odonata (larvae)
Half saturation feeding (mg/L)	0.25	0.25	0.25	0.25	0.01	0.5
Maximum consumption (g/g*d)	1.3	0.5	0.25	0.5	0.05	009
Min. prey for feeding (g/m^2)	0	0.2	0.1	0.1	0.7	0.1
Temp. response slope	2.4	1.62	2.4	2.4	1.4	2.4
Optimum temperature (°C)	20	25	20	20	20	30
Maximum temperature (°C)	35	37	35	28.7	38	40
Min. adaptation temperature (°C)	5	5	5	5	5	11
Mean wet weight (g wet)	0.005	0.0075	0.06	0.06	0.33	0.08
Endogenous respiration (1/d)	0.005	0.035	0.013	0.01	0.004	0.019
Specific dynamic action (unitless)	0.18	0.18	0	0.18	0.25	0.18
Excretion : respiration	0.17	0.17	0.17	0.17	0.17	0.17
N : Organics (frac dry)	0.09	0.014	0.09	0.014	0.09	0.09
P : Organics (frac dry)	0.014	0.014	0.01	0.014	0.01	0.014
Wet to dry	5	5	5	5	6	5
Gametes : biomass	0.01	0	0	0.09	0.1	0
Gamete mortality (1/d)	0.01	0	0	0.01	0.9	0
Mortality coefficient (1/d)	0.02	0.01	0.004	0.001	0.0038	0.002
Sensitivity to sediments	Zero	Zero	Zero	Zero	Zero	Zero
Carrying capacity (g/m^2)	10	25	1	10	30	5
Vel max. (cm/s)	400	125	250	200	400	400
Mean lifespan (d)	182	365	365	1000	1825	365
Fraction that is lipid (wet wt.)	0.05	0.05	0.05	0.05	0.05	0.05

2.7.4 Fishes

Data analysis and species selection

Fishes constitute an important trophic and ecological group in riverine ecosystems. Several qualitative and semi-quantitative studies on the fish community of the lower river Po has been conducted, merely quantitative studies are very difficult to find because there is not an active monitoring network for biota in river Po.

In order to have a first idea of what are the main species presented in the lower part o the Po river, the Fish Mapping ("Carta Ittica") of the Province of Rovigo (Maio et al., s.d.) is analyzed. The samples are taken with electric stunner in six sections (Figure 2.28) of the river Po in two days 6/7/1990 and 12/06/1991. Sampled points differ from one period to another: in 1990 the sampled stations are Crespino, Papozze, Polesella, Villanova Marchesana, in 1991 the sampled stations are Ficarolo and Melara. The Fish Mapping presents for each station the Moyle index resulted for the sampled species. For the use in Po river study, a space-average index is calculated for each period (Table 2.17). In Table 2.18 the space-averaged Moyle indices are shown.

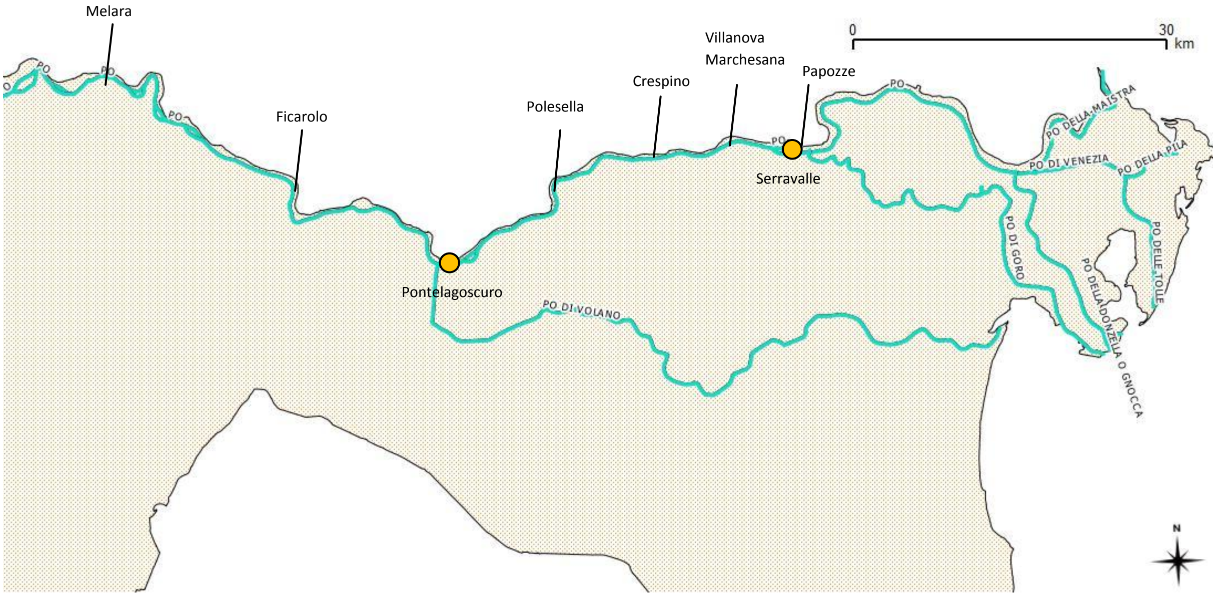


Figure 2.28 - Analysed sample stations from Fish Mapping ("Carta Ittica") of the Province of Rovigo (Maio et al., s.d.)

Table 2.17 - Moyle index meaning (Moyle e Nichols, 1973).

Abundance index of Moyle (1973)	
1 = scarce	1-2 individuals in 50 linear meters
2 = present	3-10 individuals in 50 linear meters
3 = frequent	11-20 individuals in 50 linear meters
4 = abundant	21-50 individuals in 50 linear meters
5 = dominant	>50 individuals in 50 linear meters

Table 2.18 - Moyle index of fishes species found in the 6 sections of Crespino, Ficarolo, Melara, Papozze, Polesella, Villanova Marchesana (from Turin et al., 1999).

Sampling Date	**06/07/1990 (4 sampled point)**		**12/06/1991 (2 sampled points)**	
Taxa	Samples (Moyle index)	Percentage of stations in which the specie appears	Samples (Moyle index)	Percentage of stations in which the specie appears
Ciprinids				
Alburnus alburnus alborella (Bleak)	2.75	100%	2.5	100%
Cyprinus carpio (Carp)	0.25	25%	0.5	50%
Gobio gobio (Gudgeon)	0.25	25%	1.5	100%
*Carassius carassius (Crucian)	0.75	25%	2.5	100%
Leuciscus cephalus (Chub)	2.75	75%	3	100%
Chondrostoma genei (Loose)	0.25	25%	1.5	50%
Chondrostoma soetta (Savetta)	0.25	25%	1.5	50%
Rutilus erythrophthalm (Roach)	0	0%	1	100%
Barbus plebejus (Barbel)	0.25	25%	0.5	50%
Scardinius erythrophthalmus (Rudd)	0	0%	1	50%
Tinca tinca (Tench)	0	0%	0.5	50%
Sunfishes				
*Micropterus salmoides (Largemouth bass)	0.75	75%	0.5	50%
*Lepomis gibbosus (Bluegill)	0.75	75%	1.5	100%
Blenniidae				
Salaria fluviatilis (Freshwater Blenny)	0.25	25%	0.5	50%
Pleuronectidae				
Platichthys flesus (European flounder)	0.5	25%	0	0%
Siluridae				
*Silurus glanis (Wels catfish)	0.5	25%	2	50%
Anguillidae				
Anguilla anguilla (Eel)	0.5	25%	1	100%
Mugilidae				
Liza ramada (Thinlip mullet)	0.5	25%	0	0%
Ictaluridae				
*Ictalurus melas (Catfish)	0.5	25%	0.5	50%
Gasterosteidae				
Gasterosteus aculeatus (Stickleback)	0	0%	0.5	50%

* allochthonous species

On the basis of these data, a first selection of organisms to be modelled is done, preferring animals with high Moyle index. The following specie are chosen: bleak, chub, wels catfish. In particular the latter is not so abundant but is chosen because of its importance in the trophic web (it is a tertiary predator) and for its high weight. Crucian is not selected even if it is present with a not negligible Moyle index because a value of average annual biomass density is not available for the Po river. This does not create a gap in the trophic web because Crucian has more or less the same trophic function of Chub, as both have the same omnivorous diet.

Time-varying biomass series elaboration

One of the main difficulties encountered during model elaboration is to find observed data on time-varying biomass of fishing in Po river. As already said, there is not a monitoring network of Po river biota and, unfortunately, several existing observed data have not been published. Literature data on Bleak are found in Vitali & Braghieri (1981), for Chub in Vitali & Braghieri, (1984), and for Wels catfish in Rossi et al., 1991. Data of Vitali & Braghieri refers to the zone of Caorso that is before Serafini Island, this is an area that can have different ecological features respecting to the Po river segment analysed. For this reason they are used only to assess relative seasonal variations of biomass, that are then compared with average annual biomass evaluated for Po river segment.

Bleak

Monthly surveys on Po river fishes were conducted by Vitali & Braghieri (1981) in the zone of Caorso from June 1974 to May 1977 in 18 stations with a fishing net. Bleak results to be 65 % of the total number of sampled fishes in a year and 4 % of the total weight of the sampled fishes in a year. Monthly number of organisms sampled and relative total weight are available, thus is possible to calculate an approximate estimate of the monthly bleak wet weight sampled for each section:

$$Monthly\ wet\ weight\ = \frac{Tot\ monthly\ wet\ weight * 0.04}{18} \tag{13}$$

Where:

$Monthly\ wet\ weight$ = wet weight of the bleak sampled in a month in a station (g);

$Tot\ monthly\ wet\ weight$ = wet weight of the total fishes sampled in a month in all the stations(g);

0.04 = fraction of total weight that is bleak;

18 = number of sampled stations.

To find a time varying biomass density in terms of g/m^2, a proportion is made using the annual average value of 2 g wet/m^2 of bleak in the Po river from Turin et al. (1999):

$$Wet\ density = \frac{2 \cdot Monthly\ \ wet\ weight}{Average\ weight\ in\ a\ section} \tag{14}$$

Where:

$Wet\ density$ = monthly bleak wet density (g/m^2);

2 = average annual wet density in Po river from Turin et al. (1999) (g/m^2);

$Average\ annual\ weight$ = calculated average annual weight in a section.

Then wet weight is transformed in dry weight according to Holmes and Donaldson (1969) that say: "The relation between wet and dry weight is fairly constant in healthy fish, since the relative amount of water in a fish is mostly around 72 % for both Osteichthyes and Chondrichthyes" (see Braunbeck et al., 1998, p. 259) (Table 2.19).
Time-varying biomass density use for calibration is shown in Figure 2.29. For every month it is calculated a daily average biomass. The average daily biomass density over one year is assessed considering data of Table 2.19, and it results to be 0.56 g dry/m^2.

Table 2.19 - Calculation results of bleak time varying dry density starting from total monthly weight sampled from Vitali & Braghieri (1981).

Season	Date	Total weight of sampled bleak (g wet)	Wet Density (g wet/m^2)	Dry Density (g wet/m^2)
Summer 1976	01/06/1976	220.2	3.04	0.85
	01/07/1976	222.58	3.08	0.86
	01/08/1976	212.04	2.9	0.8
Autumn 1976	01/09/1976	209.04	2.9	0.8
	01/10/1976	11.45	0.16	0.04
	01/11/1976	156.8	2.17	0.6
Winter 1976 - 1977	01/12/1976	75.7	1.05	0.3
	01/01/1977	74.8	1.03	0.3
	01/02/1977	124.6	1.7	0.5
Spring 1977	01/03/1977	92.7	1.3	0.36
	01/04/1977	176.6	2.4	0.7
	01/05/1977	159.7	2.2	0.6
Average monthly value		144.7	2	

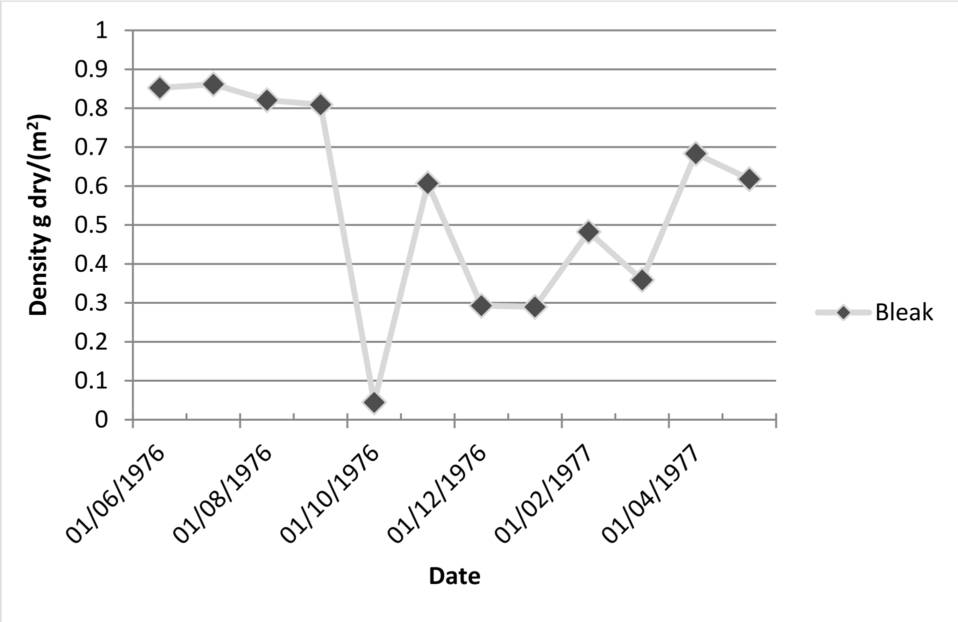


Figure 2.29 - Time varying biomass density of bleak, data use for calibration.

Chub

Monthly surveys of chub were done in 12 locations before Serafini Island (middle Po river) by Vitali & Braghieri from June 1974 to May 1977. Table 2.20 summarized the number of chubs captured for every season, and number of male and females.

Table 2.20 - Number of male, female and total chub sampled from June 1974 to May 1977 in 12 locations before Serafini Island (middle Po river) (from Vitali & Braghieri 1984).

Season	**N° of chubs sampled**	**N° Females**	**N° Males**
Summer 1976	83	57.436	25.564
Autumn 1976	132	83.688	48.312
Winter 1976-1977	83	53.95	29.05
Spring 1977	108	64.152	43.848

Male and female weights of different age classes are also reported in the study and from these data, male and female weight average over age are calculated: 236 g/ind for male, 394 g/ind for female (Annex B, Table B.7). Using these results, for every month is possible to calculate the total wet weight of chubs sampled for every station:

$$Total\ monthly\ wet\ weight = \frac{N°\ of\ female*394+N°\ of\ male*236}{12\cdot 3} \qquad (15)$$

Where:

$Total\ monthly\ wet\ weight$ = monthly wet weight of chub sampled in a station (g);

394 = average weight of chub female (g/ind);

236 = average weight of chub male (g/ind);

12 = number of stations;

3 = number of months in a season.

To find a time varying biomass density in terms of g/m^2, a proportion is made according to formula (14), using the average value of 9 g wet/m^2 of chub in the Po river from Turin et al. (1999).

Then wet weight is transformed in dry weight according to Holmes and Donaldson (1969) as done with bleak (Table 2.21). Time-varying biomass density use for calibration is shown in Figure 2.30. The average daily biomass density over one year is assessed considering data of Table 2.21, and it results to be 2.52 g dry/m^2.

Table 2.21 - Calculation results of chub time varying dry density, starting from total monthly weight sampled from Vitali & Braghieri (1984).

Season	**Total weight of sampled bleak (g wet)**	**Wet Density (g wet/m^2)**	**Dry Density (g wet/m^2)**
Summer 1976	796.4	7.5	2.1
Autumn 1976	1232.9	11.7	3.3
Winter 1976-1977	781.08	7.4	2.07
Spring 1977	989.8	9.4	2.6
Average monthly value	950.04	9	

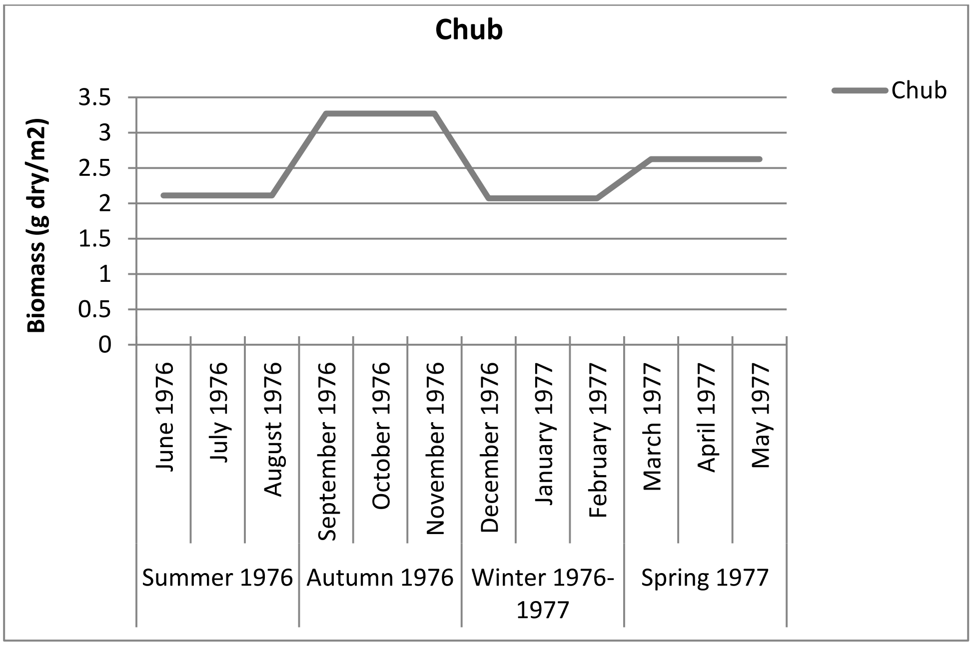


Figure 2.30 - Time varying biomass density of chub, data use for calibration.

Wels catfish

The only data available from literature are from a study of Rossi et al. (1991) in which the results of 23 samples collected in the lower Po river from March 1988 to October 1989 are presented, together with data on monthly sales of wels catfish in terms of weights collected in the fish market of Donata (RO) (Table 2.23).

Two age classes of wels catfish are modelled, on the basis of the fact that in gut analysis done by Rossi et al., organisms with a length less than 32 cm do not contain fish in their guts. Wels catfish are modelled as young (< 32 cm) and adult (> 32 cm). This are two distinct compartments in the model, so the growth of young fishes that became adults is not simulated and there are no biomass exchanges between the two age-classes.

The first step to do in order to derive time-varying biomass density series is to calculate the average individual weight of young and adult wels catfish. Thus, it is calculated the weight of wels catfish of different length, according to the following weight-length regression from Rossi et al. (1991):

$$Weight = 0.0096 \cdot Lenght^{2.9713} \quad (16)$$

Where:

$Weight$ = Wels catfish weight (g);

$Lenght$ = Wels catfish length (cm).

According to this calculations, the average wet weight of young and adult wels catfish on the basis of the percentage of young and adult sampled is derived (Table 2.22).

Table 2.22 - Length distribution of sampled wels catfish, weight calculated on the basis of regression (18) and average weights of young and adult organisms.

Individual length (cm)	Number of sampled organisms	% of total sampled	Weight from regression (g)	% Young < 32cm	% Adult >32 cm	Average weight < 32 cm (g)	Average weight > 32 cm (g)
5	19.00	9.45	1.145				
15	56.00	27.86	29.977	45.77		43.76	
25	17.00	8.46	136.763				
35	20.00	9.95	371.672				
45	18.00	8.96	784.263				
55	14.00	6.97	1423.675				
65	17.00	8.46	2338.733				
75	17.00	8.46	3578.004				
85	8.00	3.98	5189.836				
95	3.00	1.49	7222.397				
105	5.00	2.49	9723.688		44.28		3646.3
115	0.00	0.00	12741.563				
125	4.00	1.99	16323.751				
135	2.00	1.00	20517.855				
145	0.00	0.00	25371.373				
155	0.00	0.00	30931.701				
165	1.00	0.50	37246.140				
	201.00	100.00	1997.377				

The second step is to derive biomass density time-series. The only time-series available on Wels catfish presence in Po river is data collected in the fish market of Donata (RO) from Rossi et al. study (Table 2.23). This data set presents the monthly sales of Wels catfish from 1983 to 1987.

Table 2.23 - Monthly sales of wels catfish in terms of weights. Data collected in the fish market of Donata (RO) from 1983 to 1987 (Rossi et al. 1991).

Month	Monthly sales (kg)				
	1983	1984	1985	1986	1987
January	25	37	30	25	125
February	0	25	20	15	30
March	75	137	100	100	165
April	120	162	450	240	300
May	200	180	270	55	250
June	50	160	215	55	80
July	130	412	85	180	50
August	40	240	10	112	75
September	50	115	150	180	70
October	75	120	240	125	60
November	40	75	90	112	75
December	40	80	85	20	35

First of all an average monthly value is calculated from Table 2.23. Then, to find a time varying biomass density in terms of g/m^2, a proportion is made according to formula (14), using the average value of 45 g wet/m^2 of wels catfish in the Po river from Turin et al. (1999). Biomass of young and adult organisms are found multiplying the total monthly biomass density by the percentages of young and adults in Table 2.22. Then wet weight is transformed in dry weight according to Holmes and Donaldson (1969) as done with Bleak (Table 2.24). Time-varying biomass density use for calibration is shown in Figure 2.31. This monthly values are not referred to model years 1988-1990 and moreover they are derived from sales data, for these reasons during calibration they are used as a guide for the curves trend but are not considered as real observed data. The average daily biomass density over one year is assessed considering data of Table 2.19, and it results to be 5.767 g dry/m^2 for young wels catfish and 5.579 g dry/m^2 for adult wels catfish.

Table 2.24 - Calculation results of young and adult wels catfish time-varying dry density.

Date	Monthly average value (kg wet/month)	Monthly average density (g wet/m^2)	Young wels catfish (<32 cm) (g wet/m^2)	Adult wels catfish (>32cm) (g wet/m^2)	Young wels catfish (<32 cm) (g dry/m^2)	Adult wels catfish (>32cm) (g dry/m^2)
January	60.00	21.5	9.8	9.5	2.76	2.7
February	21.67	7.77	3.6	3.4	0.996	0.96
March	121.67	43.6	19.96	19.3	5.6	5.4
April	288.00	103.2	47.3	45.7	13.2	12.8
May	188.75	67.65	30.97	29.96	8.7	8.4
June	127.50	45.7	20.9	20.2	5.9	5.7
July	181.75	65.1	29.8	28.8	8.3	8.08
August	109.25	39.16	17.9	17.3	5.02	4.9
September	128.75	46.15	21.1	20.4	5.9	5.7
October	136.25	48.8	22.4	21.6	6.3	6.05
November	88.00	31.5	14.4	13.97	4.04	3.9
December	55.00	19.7	9.02	8.7	2.5	2.4

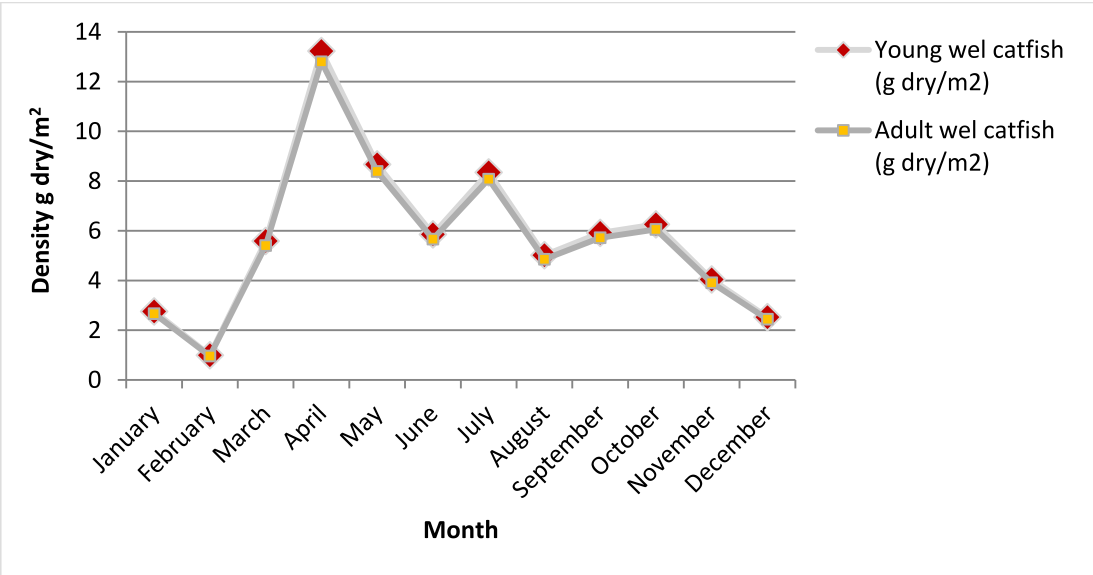


Figure 2.31 - Time varying biomass density of wels catfish, trends use for calibration.

Parameters

For each modeled species, it is chosen the most similar one in AQUATOX animal library (Table 2.5), default values of some parameters are chosen as starting values for calibration, other values are taken in literature (Table 2.25).

Table 2.25 - Starting parameters values for fishes.

Parameter	Species			
	Bleak	Chub	Young Wels catfish	Adult Wels catfish
Half saturation feeding (mg/L)	0.21	0.025	1	1
Maximum consumption (g/g*d)	0.11	0.29	0.07	0.07
Min. prey for feeding (g/m^2)	0.05	0.05	0.2	0.2
Temp. response slope	2.4	2.4	2.3	2.3
Optimum temperature (°C)	18	29	23	23
Maximum temperature (°C)	20	32	30.9	30.9
Min. adaptation temperature (°C)	10	10	1.1	1.1
Mean wet weight (g wet)	3.6	329	43.76	3150
Endogenous respiration (1/d)	0.025	0.026	0.0004	0.004
Specific dynamic action (unitless)	0.15	0.15	0.172	0.172
Excretion : respiration	0.05	0.05	0.05	0.05
N : Organics (frac dry)	0.01	0.097	0.1	0.1
P : Organics (frac dry)	0.025	0.0149	0.031	0.031
Wet to dry	3.7	3.7	4.5	3.7
Gametes : biomass	0.09	0.09	0.3	0.3
Gamete mortality (1/d)	0.9	0.9	0.01	0.01
Mortality coefficient (1/d)	0.006	0.01	0.0001	0.0001
Sensitivity to sediments	Zero	Zero	Zero	Zero
Carrying capacity (g/m^2)	12	0.8	0.9	0.9
Vel max. (cm/s)	400	400	400	400
Mean lifespan (d)	730	365	730	730
Fraction that is lipid (wet wt.)	0.02	0.02	0.03	0.03

2.7.5 Food web

Riverine ecosystems have a complex food web, that can be explained doing a functional categorization of stream biota on the basis of the way of feed and the character of food. According to Cummins K.W., (1974) riverine trophic web includes:

- *primary producers*, with two functionally distinguishable components, algae (or microproducers) and vascular plants (or macroproducers); they represent the internal energy supply for the system.
- *microconsumers*, in AQUATOX modeled as detritus together with the non-living organic matter. They constitute the food for the majority of invertebrates and they can be divided in two broad categories according to Cummins and Klug (1979): CPOM (coarse particular organic matter >1 mm) and FPOM (fine particular organic matter < 1 mm) that represents intermediates in the progression from CPOM to DOM (dissolved organic matter < 0.5 µm). In AQUATOX there is not a granulometric classification of detritus but only a classification in terms of particulate suspended, particulate sedimented and dissolved; the quantification of detritus in the three compartments is not done according to particle size but using mass balance.
- *macroconsumers*, in AQUATOX modeled as zooplankton, invertebrates and fishes and classified according to the way of feed in: detritivores (shredders, sediment feeders, suspended feeders, snails which feed mainly on detritus), grazers (which feed mainly on plants), primary predator (which feed mainly on other invertebrates), secondary predators (fishes that feed on invertebrates or plants), tertiary predators (fishes that feed mainly on other fishes).

In AQUATOX, the trophic web is modeled with a preference or diet matrix, in which for each predator the user must indicates the potential prey preferences in terms of percentages and define egestion coefficients. Egestion is the expulsion of that portion of ingested food not assimilated (feces) and should be distinguished from excretion, which is the elimination of nitrogenous compounds produced from assimilated material (Cummins, 1973).

Regarding the Po river ecosystem, it is very difficult to simulate the real trophic web, first of all it is impossible to simulate every animal really present in the segment because of the lack of observed data, especially on fish biomasses, and, thus, the impossibility to calibrate; second, information about diet habits are qualitative in most cases and not quantitative as required in AQUATOX. For these reasons, a selection of plants and animals is done according to the available data and animal importance in the food web, trying to reach an optimum level of complexity.

To built the preference matrix several qualitative and quantitative information about feeding habits of macroconsumers are collected for each biota. When information are few, initial value percentages are chosen and then changed during calibration in order to reach more precise values. In the following section the diets and egestion coefficients of each biota modeled are described in details, justifying the percentage chosen for the preference matrix.

In general, egestion coefficient assessment is based on Mathews (1993) that considers the food assimilation equal to 0,8 for each category of food except the detritus one.

Table 2.26 summarized biota modeled in Po River study and their role in the trophic web, Figure 2.32 is a schematic drawing of the Po river segment trophic web.

Table 2.26 - Biota simulates in Po river model and their trophic role.

Biota modeled	**Trophic role**
Cyclotella	Primary producer
Chromulina	Primary producer
Brachionus	Filter feeder of phytoplankton and fine particle of labile detritus
Amphipoda	Shredder of detritus
Oligochaeta (larvae)	Collectors gatherer of labile sedimented detritus
Young Chironomus (larvae)	Filter feeder of detritus
Trichoptera	Filter feeder of detritus, phytoplankton and invertebrates
Gastropod	Scraper of detritus
Odonata	Primary predator of macro-invertebrates
Bleak	Secondary predator: Planktivorous
Chub	Secondary predator: Omnivorous
Young Wels Catfish	Secondary predator: Carnivorous
Adult Wels Catfish	Tertiary predator: Carnivorous

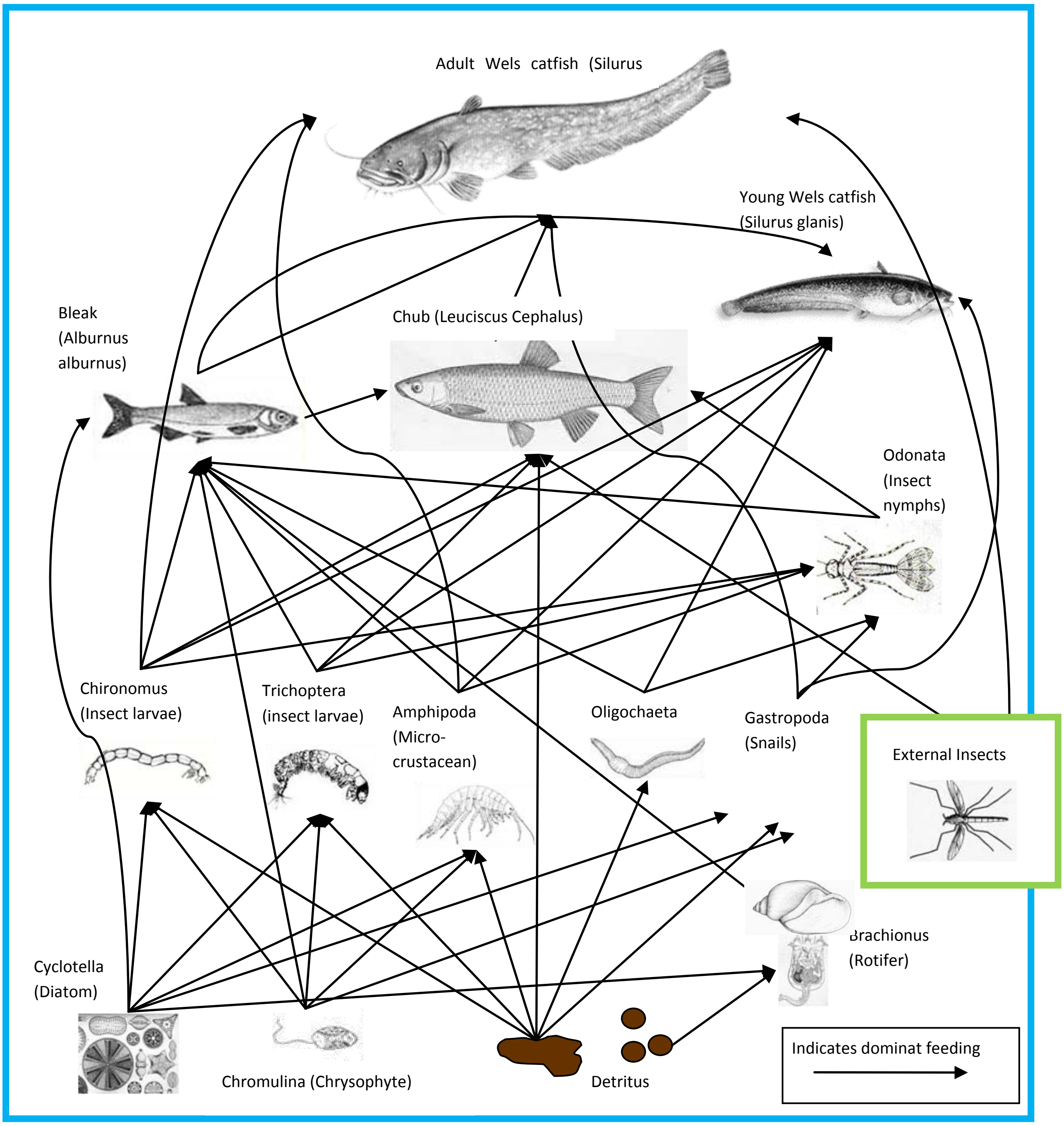


Figure 2.32 - Po river model food web scheme.

Rotifers (zooplankton)

No quantitative data on feeding preference of Po river zooplankton are found in literature, thus initial percentage values are chosen on the basis of qualitative data and then they are treated in the calibration phase as parameter to calibrate. According to Ricci and Balsamo (2000), because of their small dimensions, Rotifers facilitate energy transfer from bacteria and algae to higher trophic levels by feeding on particles of a size not efficiently grazed by larger invertebrates, thus size of food appears to be the most discriminating factor. Arndt (1993) says that filter feeding species (as Brachionus) feed on yeasts, bacteria and flagellates (see Ricci & Balsamo, 2000, p. 24). Also Obertegger et al. (2011) classify Brachionus as microphagous (see Bertani et al., 2012 p. 211). Thus, in deriving the preference matrix, labile detritus have a higher preference percentage respect to refractory detritus, because of the higher presence of bacteria in the first, and Chromulina have a higher preference percentage respect to Cyclotella because it is flagellate.

According to these qualitative information, Brachionus feeding behaviour is simulated in Po river model assuming an equal high preference for labile detritus and flagellates (Chromulina) and a little preference for diatoms and refractory detritus, simulating the accidental ingestion. In Table 2.27 the preference matrix for Rotifers is showed. According to Park and Clough (2012), because rotifers digest bacteria and defecate the remaining organic material, they have an assimilation efficiency different from zero only for labile detritus that are those detritus conditioned through microbial colonisation, while for refractory detritus the egestion efficiency is set to 1, because no bacteria are present on that type of detritus. The following egestion efficiencies are chosen: 1 for refractory suspended particulate detritus, 0.2 for labile suspended particulate detritus, 0.2 for Chromulina and 0.2 for of Cyclotella.

Table 2.27 - Preference matrix for Rotifers in the Po river. Percentage chosen on the bases of qualitative data analysis.

Prey	**Preference (%)**
Labile Suspended Detritus	40
Chromulina	40
Refractory Suspended Detritus	10
Cyclotella	10

Amphipoda (crustacean)

No quantitative data on feeding preference of Po river Amphipoda are found in literature, thus initial percentage values are chosen on the basis of qualitative data and then they are treated in the calibration phase as parameter to calibrate. In the segment of the Po River analysed, the most abundant species of Amphipoda is the Echinogammarus veneris, which belongs to the family Gammaridae (Battegazzore, 1991). Fenoglio and Bo (2009) classify Gammaridae Amphipods as shredders, these organisms feed on coarse particulate organic matter (CPOM) as leaves, wood, plant tissues in decomposition (conditioned through microbial colonisation). Several studies demonstrate that shredders prefer CPOM colonized and conditioned by bacteria because microbes make the detritus more digestible and increase bioavailable nutrients. Thus, in deriving the preference matrix, labile detritus will be selected over refractory detritus (Cummins & Klug, 1979).
According to these information, Echinogammarus veneris feeding behaviour is simulated in Po River model assuming the higher preference for labile suspended and sedimented detritus, and a low consumption of refractory detritus and phytoplankton simulating the accidental ingestion. In Table 2.28 the preference matrix for Amphipoda is showed. The following egestion efficiencies are chosen: 0.2 for labile suspended particulate detritus, 0.2 for labile sedimented particulate detritus, 1 for refractory suspended particulate detritus, 1 for refractory sedimented particulate detritus, 0.2 for Cyclotella and 0.2 for Chromulina.

Table 2.28 - Preference matrix for Amphipoda in Po river.

Prey	Preference (%)
Labile Suspended Detritus	47
Labile Sedimented Detritus	47
Refractory Suspended Detritus	2
Refractory Sedimented Detritus	2
Cyclotella	1
Chromulina	1

Oligochaeta

No quantitative data on feeding preference of Po river Oligochaeta are found in literature, thus initial percentage values are chosen on the basis of qualitative data and then they are treated in the calibration phase as parameter to calibrate. Oligochaeta are simulated in Po river model as a group and not as a single species. Fenoglio & Bo (2009) classify Oligochaeta as collectors and gatherers, organisms that feed on fine particulate organic matter (FPOM) and associated bacteria, collecting them directly from the substrate. Thus, in deriving preference matrix, labile sedimented detritus will be selected over other detritus types. In Table 2.29 the preference matrix for Oligochaeta is showed. The following egestion efficiencies are chosen: 0.2 for labile sedimented particulate detritus, 1 for refractory suspended particulate detritus.

Table 2.29 - Preference matrix for Oligochaeta in Po river.

Prey	**Preference (%)**
Labile Sedimented Detritus	99
Refractory Sedimented Detritus	1

Chironomidae (insects)

The most abundant Diptera family in the studied segment of the Po River is the Chironomidae (or Chironomids). Several species are present but for simplicity they are clustered in a unique modeled group called Chironomids.

Usually the Chironomids larvae and pupa are aquatic and spend from less that 2 to 7 weeks in water, depending on water temperature, before transforming in adults. Suspended organic matter in the water and in the mud is used as food by the developing larvae. Because they do not feed, adults live for only 3 to 5 days (Apperson et al., 2006). In the Po River model, only aquatic stages of Chironomids are simulated in order to be consistent with observed data.

According to Anderson & Sedell (1979), Chironomids in their larval stage are benthic organisms that feed by pumping suspended particulates. Berg (1995) says that, although the functional group categories are based partially on the morphology of the species, there is considerable flexibility in the mode of feeding among Chironomids and many factors, such as larval size, food quality and type of sediment might influence the larval feeding behaviour (see Henriques-Oliveira et al., 2003 p. 281). During a study on chironomid larvae feeding behaviour conducted in Rio da Fazenda, situated in the Parque Nacional da Tijuca, Rio de Janeiro, Brazil, the following percentage of ingested materials were found in Chironomus guts: 88 % of detritus, 12,6 % of leaf and wood fragments, 4.3 % of algae (Henriques-Oliveira et al., 2003). Considering detritus as labile organic matter and leaf and wood fragments as refractory organic matter, percentage

summarized in Table 2.30 are chosen. Almost the same percentages are found by Naser and Roy (2012) on muddy habitats of Curzon Hall campus of University of Dhaka.
Cummins (1973) points out that "very little is known about the digestive capabilities or efficiencies of aquatic insects. Although similarities might be found with terrestrial forms for aquatic representatives of orders that are predominantly terrestrial (Hemiptera, Lepidoptera, Coleoptera, Diptera), extension of such generalizations to the truly aquatic orders (Plecoptera, Ephemeroptera, Odonata, Trichoptera, Megaloptera) is not warranted". For refractory detritus an egestion efficiency equal to 1 is chosen, considering that they are not digested because of their refractory properties. For other items an egestion efficiency equal to 0.2 is chosen according to the default herbivores and detritivores values found in Ecopath user's guide (Christensen et al., 2000).

Table 2.30 - Preference matrix for Chironomids in Po river.

Prey	Preference (%)
Labile Suspended Detritus	44
Labile Sedimented Detritus	44
Refractory Suspended Detritus	6.3
Refractory Sedimented Detritus	6.3
Cyclotella	2.15
Chromulina	2.15

Trichoptera (insects)

According to Cianficconi & Moretti (1992) (see Fenoglio & Bo, 2009, p.73), Trichoptera is an order of insects with aquatic larvae and adults with wings. In the Po river segment analysed, the species Hydropsyche is the most abundant and, thus, the modeled one. Fenoglio & Bo (2009) classify Hydropsyche as filterers feeders, organisms that filter the suspended particulate detritus by building very fine-meshed nets, consisting of silky material secreted by themselves. Wallace & Webster (1996) describe as Hydropsychids feed on larger particles and select higher-quality food items such as diatoms and animal drift. This selectivity suggests that their major impact is on the quantity and type of particulate organic matter (POM) in suspension. Experimental studies of Georgian & Thorp (see Wallace and Webster, 1996, p. 125), estimated that two *Hydropsyche* species in riffles of a New York stream, removed 18% of drifting invertebrate prey per meter. Their results suggest that, when large net-spinning caddisfly populations are present in shallow streams, their predation may suppress stream drift. A study done by Coffman (1967) on macroinvertebrates of a woodland stream in Pennsylvania, gives the percentages of food preferences for three species of Hydropsyche (Table 2.31), the analysis are based on the percentages of each category in the gut (see Cummins, 1973, p. 201).

Table 2.31 - Hydropsychidae food habits from a study of Coffman (1967) on a woodland stream in Pennsylvania , basing on the % of each category in the gut (see Cummins, 1973, p. 201).

Taxon	Food habits (%)			
Hydropsychidae	Algae	Live vascular plant tissue	Detritus	Animals
H. bettenl	2	0	1	97
H. bronta	39	0	6	55
H. slossonae	18	0	3	79

Animals percentages in guts are high because probably prey drift is high and Hydropsyche select higher-quality food items. In Po river model animal drift is not simulated and as Hydropsyche feed on animal drift, animal predation by Trichoptera is not modelled. For this reason Hydropsyche preferences in Po river model are subdivided only between algae and labile suspended detritus (Table 2.32). The following egestion efficiencies are chosen: 1 for refractory detritus, 0.2 for other items.

Table 2.32 - Preference matrix for Trichoptera in Po river.

Prey	**Preference (%)**
Labile Suspended Detritus	32
Cyclotella	32
Chromulina	32
Refractory Suspended Detritus	4

Gastropoda (mollusca)

No quantitative data on feeding preference of Po river Gastropoda are found in literature, thus initial percentage values are chosen on the basis of qualitative data and then they are treated in the calibration phase as parameter to calibrate. According to Fenoglio & Bo (2009), Gastropoda feed mainly on plants and fouling organisms. In most gastropods, food is eventually engaged by the radula, which is controlled by the buccal muscles. These muscles cause the radula to protract out of the mouth towards the food and then to pull the food into the buccal cavity, or to rasp the food, with a retraction movement, thus they can be considered scrapers (Elliott and Susswein, 2002). On the basis of these considerations, high percentages are chosen for labile sedimented detritus as they represent macrophytes and dead organisms in the model. Percentage summarized in Table 2.33 are used in the preference matrix. The following egestion efficiencies are chosen: 1 for refractory detritus and 0.2 for other items.

Table 2.33 - Preference matrix for Gastropoda in Po river.

Prey	**Preference (%)**
Labile Suspended Detritus	5
Refractory Suspended Detritus	3
Labile sedimented detritus	79
Refractory sedimented detritus	3
Cyclotella	5
Chromulina	5

Odonata (insects)

Odonata is an order of insects belonging to the group of Paleoptera. They are aquatic or semi-aquatic when juveniles, and terrestrial when adults. According to Cummins (1973), the presence of a modified labium in all nymphal Odonata is considered sufficient evidence to conclude that all species are predaceous, even though the food habits of only an insignificant number of species have actually been studied. Odonata larvae extend their unique large lower lip in front of the body to catch prey which may include, in the early stages, very small Crustaceans, Copepods and Cladoceri. In more advanced stages, they hunt any prey that is suitable in size. Some large larvae of Aeshna can catch even small vertebrates such as tadpoles and juveniles ([VII] section "sviluppo e maturazione").

In Po river model, only Odonata aquatic stage is simulated and, for simplicity, no distinction on age basis is done. It is assumed an equal preference for all the possible animal preys (Table 2.34). It is chosen an egestion efficiency equal to 0.2 for all items.

Table 2.34 - Preference matrix for Odonata in Po river.

Prey	Preference (%)
Amphipoda	20
Young Chironomids	20
Oligochaeta	20
Trichoptera	20
Gastropods	20

Bleak (fish)

Bleak belongs to the family of Cyprinids, a wide variety of specialists and generalists fish feeding on all trophic levels (Lammens & Hoogenboezem, 1991). According to several authors as Politou (1993), Herzig (1994) and Vinniet (2000), bleak is a specialised open water feeder, foraging primarily on zooplankton throughout its entire life. No evidence for filter feeding ability in bleak has been found in literature (see Vašek and Kubečka, 2004). Lammens and Hoogenboezem studied the diets of the most common European cyprinids and concluded that micro-crustaceans and adult dipteran are the preferred or very highly consumed prey for the bleak.

Turin et al. (1999) studied the fish population of the inland freshwater of the province of Rovigo, Po river included, and wrote that bleak diet is various: very important is the phytoplanktonic component, although the vegetable diet is supplemented by insect larvae (Odonata, Trichoptera, Chironomids), Oligochaeta and Crustaceans.

According to these information, giving more weight to data referring to Po river, percentages in Table 2.35 are chosen. It is chosen an egestion efficiency equal to 0.2 for all items.

Table 2.35 - Preference matrix for bleak in Po river.

Prey	Preference (%)
Cyclotella	32
Chromulina	32
Amphipoda	6
Young Chironomids	6
Oligochaeta	6
Trichoptera	6
Odonata	6
Rotifer	6

Chub (fish)

Chub belongs to the family of Cyprinids as bleak. Mann (1976) studied feeding behaviour of chub in the river Stour and analysed the contents of the anterior one-third of the alimentary canal of three size-groups of chub (Table 2.36). Chironomids larvae appear to be the most important constitute of the diet.

Table 2.36 - Contents of fore-gut of chub from River Stour, averaged from three size-groups.

Prey	Preference (%)
Ephemeroptera nymphs	5.13
Ephemeroptera adults	1.63
Trichoptera larvae	6.47
Coleoptera	4.43
Simulium larvae	6.73
Choronomidae larvae	17.23
Tipulidae larvae	1.5
Hemiptera	12
Astacus pallipes	2.9
Gammaris	2.07
Cladocera	9.7
Mollusca	0.73
Pisces	12.13
Anas platyrhynchos	0.73
Other aquatic organisms	10.3
Other terrestrial organisms	6.2
	Occurrence (%)
Macrophytes	15.23
Algae	8.5
Empty stomachs	29

Turin et al. (1999) studied the fish population of the inland freshwater of the province of Rovigo, Po river included, and wrote that chub is an omnivorous fish with a diet that includes: insect larvae, insect with wings, macrophytes, fish eggs and, for larger organisms, other fishes.

According to these information, giving more weight to data referring to Po river, percentages in Table 2.37 are chosen.

Table 2.37 - Preference matrix for chub in Po river.

Prey	Preference (%)
Chironomids larvae	29
External insects	14
Trichopter (larvae)	14
Odonata (larvae)	14
Retractable suspended detritus (macrophytes)	10
Labile suspended detritus (macrophytes)	10
Bleak	9

It is chosen an egestion efficiency equal to 0.2 for all items.

Wels catfish (fish)

Wels catfish is modelled as young (<32 mm) and adult (> 32 mm) according to the different diets. For Turin et al. (1999) it is a ravenous predator, particularly active at night during which moves from the riverbed to rise to the surface where it hunts for fish of all kinds, but also for other vertebrates as mice, amphibians and aquatic birds. Young organisms hunt only tadpoles and small fish and complement the diet with benthic macro-invertebrates.

Rossi et al. (1991) studied Wels catfish population in the end part of the Po river and determinate young and adult diets on the basis of guts analysis (Table 2.38).

Table 2.38 - Preference matrix for Wels catfish in Po river, from guts analysis of Rossi et. al. (1991).

Prey	**Spring-Summer**		**Autumn-winter**		**Average**	
	Young (<32 mm)	Adult (>32 mm)	Young (<32 mm)	Adult (>32 mm)	Young (<32 mm)	Adult (>32 mm)
Amphipoda	61.0	7.1	78.4		69.7	3.55
Isopoda	2.4		7.8	10.0	5.1	5
Decapoda			2.0	10.0	1	5
Trichoptera (larvae)	13.8		0.7		7.25	
Ephemeroptera (larvae)	4.9				2.45	
Etheroptera			5.2		2.6	
Diptera (larvae)	5.7	7.1	1.3		3.5	3.55
Gasteropoda	8.9			10.0	4.45	5
Nematoda	2.4		3.3		2.85	
Others macro-inv.	0.8		1.3		1.05	
Bleak		21.4		10.0		15.7
Chub		32.1		10.0		21.05
Crucian		3.6		20.0		11.8
Chondrostoma soetta		3.6				1.8
Roach		14.3				7.15
Flounder				10.0		5
Others fishes		10.7		20.0		15.35

Average percentage are chosen for the preference matrix of Po river model (Table 2.39). In particular the Isopoda and Decapoda percentages are clustered in Amphipoda group, Ephemeroptera and Eteroptera percentages are divided between Trichoptera and Diptera Chironomidae, Nematoda percentages are considered as Oligochaeta and percentages of fish that are not modelled are clustered in Bleak and Chub group keeping constant the relative percentages between the two species. Moreover, a small percentage of bleak is added in young organisms according to the professional judgment of AQUAPROGRAM (company of Vicenza expert in fishes of North-East Italy) and a percentage of external insect is added for adult organisms according to Turin et al. (1991).

Table 2.39 - Preference matrix for Wels catfish in Po river model.

Prey	**Young (<32 mm)**	**Adult (>32 mm)**
Amphipoda	75.8	13.5
Trichoptera (larvae)	10.7	
Chironomus (larvae)	5.1	3.6
Gasteropoda	4.4	5
Oligochaeta	2.8	
External Insects		5
Bleak	1.2	30.5
Chub		42.4

The complete preference matrix and the egestion coefficients are shown respectively in Table 2.40 and Table 2.41.

Table 2.40- Initial preference matrix for Po river model (numbers are %).

Prey	**Predator**											
	Amph.	**Esternal insects**	**Chiron. (larvae)**	**Oligoch.**	**Trich.**	**Rotifer**	**Gastrop.**	**Odon.**	**Bleak**	**Chub**	**Yonug wels catfish**	**Adult wels catfish**
R detr sed	2.0		6.0	1.0			3.0					
L detr sed	47.0		42.0	99.0			79.0					
R detr part	2.0		6.0		4.0	10.0	3.0			10.0		
L detr part	47.0		42.0		32.0	50.0	5.0			10.0		
Cyclotella	1.0		2.1		32.0	10.0	5.0		32.0			
Chromulina	1.0		2.0		32.0	30.0	5.0		32.0			
Macrophytes												
Amphipoda								20.0	6.0		75.8	13.5
Esternal insects										14.0		5.0
Chironomid								20.0	6.0	29.0	5.1	3.6
Oligochaeta								20.0	6.0		2.8	
Trichopter								20.0	6.0	14.0	10.7	
Rotifer (Brachionus)									6.0			
Gastropod								20.0			4.4	5.0
Odonata									6.0	14.0		
Bleak										9.0	1.2	30.5
Chub												42.4
Young wels catfish												
Adult wels catfish												

Table 2.41 - Initial egestion coefficient for Po river model.

Prey	Predator											
	Amph.	**Esternal insects**	**Chiron. (larvae)**	**Oligoch.**	**Trich.**	**Rotifer**	**Gastrop.**	**Odon.**	**Bleak**	**Chub**	**Yonug wels catfish**	**Adult wels catfish**
R detr sed	1		1	1			1					
L detr sed	0.1		0.2	0.3			0.2					
R detr part	1		1		1	1	1			1		
L detr part	0.1		0.2		0.2	0.2	0.2			0.2		
Cyclotella	0.8		0.2		0.2	0.2	0.2		0.2			
Chromulina	0.8		0.2		0.2	0.2	0.2		0.2			
Macrophytes												
Amphipoda								0.2	0.2		0.2	0.2
Esternal insects										0.2		0.2
Chironomid (larvae)								0.2	0.2	0.2	0.2	0.2
Oligochaeta								0.2	0.2		0.2	
Trichopter (larvae)								0.2	0.2	0.2	0.2	
Rotifer (Brachionus)												
Gastropod								0.2	0.2		0.2	0.2
Odonata (larvae)									0.2	0.2		
Bleak										0.2	0.2	0.2
Chub												0.2
Young wels catfish												
Adult wels catfish												

2.8 Organic chemicals properties

Since the objective of this thesis is to assess fate and effects in Po river ecosystem of Linear Alkylbenzene Sulfonate (LAS) and Triclosan (TCS), it is important to define and analyzed all the compounds properties involved in the physico-chemicals reactions that take place in water, detritus and biota.

Regarding the fate aspect, AQUATOX calculates time-varying non-equilibrium concentration of the compound in different means (water, detritus, biota); thus, for every mean, several processes as microbial degradation, biotransformation, photolysis, hydrolysis, volatilisation are simulated with kinetic equations and for every time step a balance between chemical mass entering and exiting the mean is done.

Regarding quantification of chemical mass that goes to detritus (non-dissolved), AQUATOX distinguishes partition between refractory detritus (relatively non-polar, used as a surrogate for sediments in general) and labile detritus. Both calculations require the knowledge of octanol water partition coefficient (k_{OW}), sorption rate constant ($k_{1\ Detr}$) and desorption rate constant ($k_{2\ Detr}$) of the compound (see Park & Clough, 2012).

The assessment of chemical mass that goes to biota involves three main parameters: BCF (bioconcentration factor), k_1 (uptake rate constant) and k_2 (desorption rate constant). This parameters are related (in steady state conditions) with the following formula:

$$BCF = \frac{C_b}{C_w} = \frac{k1}{k2} \tag{17}$$

Where:

BCF = bioconcentration factor (L/g);

C_w = concentration of the chemical in water (mg/L);

C_b = concentration of the chemical in the biota (mg/g);

k_1 = uptake rate constant (L/g d);

k_2 = desorption rate constant (1/d).

Involved parameters as BCF, k_1 and k_2 can be recovered on the basis of K_{ow}. According to Lombardo (2013), compounds behaviour in the environment is highly influenced by other factors and an assessment based on K_{ow} value can be meaningless (especially for LAS that is a surfactant), thus data used in this study are recovered as much as possible from literature.

Regarding effects, AQUATOX requires, for each modelled organisms, LC50 and EC50, with the relative exposure time, for each tested compound. As ecotoxicological data of LAS and TCS are not available for some modelled organisms, it is necessary to take parameters from similar biota (read-across procedure). If only chronic toxicity parameters as LOEC and NOEC are available for an organism, these are converted in LC50 or EC50 using the same relationships used by Lombardo (2013) in this study on river Thames:

- The average acute/chronic ratio (ACR) for LAS expressed as the ratio between LC50 value and chronic toxicity values (LOEC and NOEC) found for several tests on different animals. This value is about 6. (See Table C1 p. 104 of the ECETOC technical report 91) (ECETOC, 2003), ACR for plants is not available;
- The average ratio between lethal acute toxicity and effect acute toxicity ratio for animals or plants (LC50/EC50). For plants this ratio value chosen is about 10 (AQUATOX default studies) for both the pollutants. It was used to estimate LC50 because only data of EC50 were found in literature for both the toxicants. Animal average LC50/EC50 change from Linear alkilbenzene sulfonate (LAS) and Triclosan (TCS). For LAS is equal to 1.67 and for TCS is equal to 3.86;
- The ratio between acute effect toxicity (EC50) and chronic toxicity (CT) (LOEC or NOEC). This value is unknown. A single constant value equal to 2 has been chosen for both the pollutants to simplify the assumptions. This value was chosen by expert judgment (Marshal, 2013) to guarantee that the ACR founds for the two Chemicals were as close as possible to the median value of 6.

The following equation shows the relation between the three ratio:

$$ACR = \frac{LC_{50}}{EC_{50}} \cdot \frac{EC_{50}}{CT} \qquad (18)$$

LC50 and EC50 values are the basis for several calculations that lead to assess internal concentration causing 50% mortality for a given period of exposure by knowing BCF (bioconcentration factor), k_1 (uptake rate constant) and k_2 (desorption rate constant).

In this paragraph LAS and Triclosan physico-chemicals, ecotoxicological and bioaccumulation properties are going to be described in detail.

2.8.1 LAS

The acronym LAS (CAS No. 68411-30-3) stands for Linear Alkylbenzene Sulfonate, it is a synthetic surfactant and is the primary cleaning agent used in laundry detergents and cleaners at concentrations up to 25 percent in consumer products and at higher concentrations in industrial/commercial products (UNEP Chemicals, 2007). The LAS molecule (Figure 2.33) contains an aromatic ring sulfonated at the para position and attached to a linear alkyl chain at any position except the terminal carbons, the linear alkyl chain has typically 10 to 13 carbon units (HERA, 2013). LAS is manufactured by reaction between linear alkyl benzene (LAB) (proceeds by reacting paraffins with benzene) and sulphuric acid. This reaction produces sulphonic acid that is neutralized with sodium hydroxide (NaOH) to give the final molecule: sodium salt of LAS.

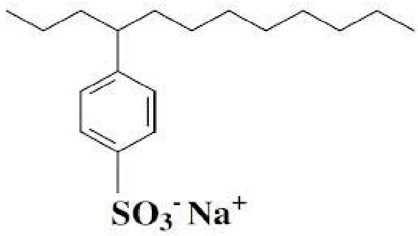


Figure 2.33 - LAS representative molecular structure (alkyl chain: C_{10}-C_{13}). From www.scienceinthebox.com.

It is an anionic surfactant, thus in water solution its molecule dissociates to give an amphiphilic organic anion (negatively charged ion) and a small inorganic cation (positively charged ion: Na^+). Amphiphilic anion consists of two different parts: a hydrophilic head (given by the presence of SO_3^-) and a hydrophobic tail (given by the presence of linear alkyl chain). This combined structure gives them a tendency to collect at aqueous/organic-phase boundaries (EOSCA, 2000) and make it difficult to give a standard interpretation of K_{ow} laboratory values.

2.8.1.1 Physico-chemical properties

Physico-chemical properties of a substance, such as solubility, vapour pressure and sorption properties, are parameters that can be used early in an evaluation process to assess its likely fate and to determine the environmental compartments into which it will partition.

Physico-chemical parameters of commercial $C_{11.6}$ LAS are summarized in Table 2.42 (HERA, 2013).

Table 2.42 - Commercial $C_{11.6}$ LAS physico-chemicals parameters (HERA, 2013).

LAS	Value/Range	Notes
Molecular weight (g/M)	342.4	$(C_{11.6}H_{24.2})C_6H_4SO_3Na$
Vapour pressure at 25°C (Pa)	$(3\text{-}17) \cdot 10^{-13}$	Calculated as C_{12}
Boiling point (°C)	637	Calculated as C_{12}
Melting point (°C)	277	Calculated as C_{12}
Octanol-water partition coefficient (log K_{OW}) (L/kg)	3.32	Calculated as $C_{11.6}$
Organic carbon-water partition coefficient K_{OC} (L/kg)	2500	Calculated as $C_{11.6}$
Water solubility (g/L)	250	Experimental
Sorption coefficient between soil/sediment and water, K_d (L/kg)	2-300	Experimental
Density (kg/L)	1.06 (relative) 0.55 (bulk)	Experimental
Henry's constant (Pa * m^3/mole)	$6.35 \cdot 10^{-3}$	Calculated as C_{12}
Dissociation constant		Not present. It is a salt.

2.8.1.2 Degradation properties

LAS does not undergo significant degradation by abiotic mechanisms under environmentally relevant conditions because photolyzable and hydrolyzable groups are absent from the chemical structure (UNEP Chemicals, 2007).

Regarding LAS degradation operated by biotic factors, an extensive database of studies demonstrates rapid and complete (ultimate) biodegradation of LAS in many of the available aerobic biodegradation tests, including soil and the aqueous environment (UNEP Chemicals, 2007). While LAS degrades rapidly under aerobic conditions, it does not degrade under anaerobic conditions, except under special conditions. LAS "Primary Biodegradation" is the transformation induced by microorganisms with formation of sulphophenyl carboxylates (SPCs) (Figure 2.35) as biodegradation intermediates. This biodegradation stage corresponds to the disappearance of the parent molecule and the loss of interfacial activity as well as the toxicity to aquatic organisms. Biodegradation proceeds further with the cleavage of the aromatic ring and the complete conversion of LAS and SPCs into water, CO_2, inorganic sulphates and biomass. This step is also known as "Ultimate Biodegradation" or mineralization [VIII].

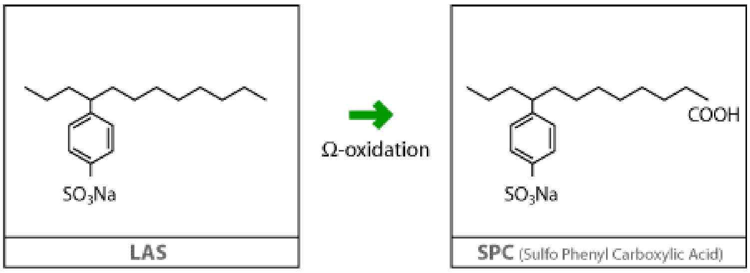


Figure 2.35 - Molecular structure of Sulphophenyl Carboxylates (SPCs), LAS primary biodegradation product [VIII].

Most relevant data on LAS biodegradability are summarized in Table 2.43 (HERA, 2013).

Table 2.43 - Most relevant data on LAS biodegradability (HERA, 2013).

Biodegradation properties		**Half-life time**	**Degradation rate**	**Notes**
Biodegradation in river water	Die-away	12 h	0.06 1/h	(prim. biod.)
	Die-away	18 h	0.04 1/h	(ultim. biod.)
	River monitoring	1-3 h	0.69 - 0.23 1/h	(prim. biod.)
Biodegradation in soil	Field study	1-7 d	0.69 - 0.10 1/d	(prim. biod.)
	Laboratory study	2-26 d	0.35 - 0.03 1/d	(ultim. biod.)
Biodegradation in oxic sediments		7 d	0.1 1/d	
Biodegradation in bulky sediments		70 d	0.01 1/d	

In the present risk assessment study, protective primary biodegradation values are considered by choosing the highest values for half-life and the lowest values for degradation rates. Ultimate biodegradation values, that are inherent to metabolite (SPCs) degradation, are not considered because, according to Kimerle et al. (1977), SPCs are not persistent and their toxicities are several orders of magnitude lower than that of the parent molecule.

2.8.1.3 Bioconcentration

LAS bioaccumulation factor should not be predicted on the basis of its octanol/water partition coefficient (K_{ow}), in fact, such predictions are not applicable to surfactants because of their surface active properties (HERA, 2013). It is difficult to obtain reliable partitioning (log K_{ow}) or bioconcentration factor (BCF) data for inclusion in current models used in performing environmental risk assessments. The difficulties revolve largely around the intrinsic property of surface-active substances to adsorb to surfaces and to accumulate at phase interfaces, so that bulk concentration of a surfactant would not be in equilibrium between the water and octanol phases but in equilibrium with octanol-water interface concentration (EOSCA, 2000) (Figure 2.34). For this reason K_{ow} is not used in assessing BCF.

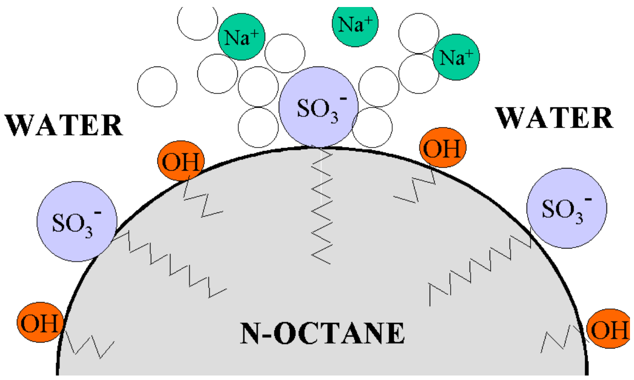


Figure 2.34 - Representation of LAS micelle structure from EOSCA (2000).

Thus, experimental literature values are used as much as possible, even if, as indicated by Tolls et. al. (1995) very few studies can differentiate between parent compounds and metabolites or other breakdown products. Because of this limitation, many reported concentration factors are probably significant overestimates.

Moreover studies of Comotto et al. (1979) and Kimerle et al. (1975) suggest that a slight increase in the length of the alkyl chain (from C12 to C13) significantly increases the bioaccumulation potential of the compound (EOSCA, 2000). In this study BCF referring to commercial $C_{11.6}$ LAS is chosen.

Plants

For algae, BCF_{dry} and k_2 Renauld literature values from Lombardo (2013) are considered (Table 2.44). k_1 is calculated by AQUATOX with equation (20) (the option "enter k_2 and BCF, calculate k_1" is chosen in the Chemical toxicity parameter screen).

Table 2.44 - BCF_{dry}, uptake rate (k_1) and desorption rate (k_2) of LAS for phytoplankton (Lombardo, 2013).

Taxa	BCF_{dry} (L/kg)	k_1 (L/kg d)	k_2 (1/d)	Lipid fraction
Cyclotella	5450	52320	9,6	0.005 [b]
Chromulina	5450	52320	9,6	0.005 [b]

[b] from Lyndall et al. (2010).

Animals

For animals, BCFs values are adapted from the river Thames model of Lombardo (2013), k_2 is calculated by AQUATOX from Barber equation, while k_1 is calculated from equation (20). An exception is made for Rotifer and Amphipoda: in fact, as Barber equation is based on individual weight, the very low Rotifer and Amphipoda weights (smaller respectively than 1 mg/L and 1 g dry/m^2) result in high k_2 estimated values that cause an overestimation of toxicant effects and are unreal. It is chosen to set Rotifer and Amhipoda k_2 equal to the highest one calculated with Barber equation (it results equal to 77,8 1/h, the same of Chironomids).

In fish, most surfactants are rapidly taken up and distributed within the body, moreover, as demonstrated by Tolls et al. (1994) (EOSCA, 2000), gills are an important uptake site for dissolved surfactants from the aqueous phase. Data from a study of Versteeg & Rowlings are used by Lombardo, in particular BCF_{wet} for all fishes is set equal to 80 L/kg that is an average value of a study carried out on minnow (*Phimepales Promelas).*

Regarding invertebrates, data from Versteeg & Rowlings (2003) study are considered by Lombardo, on the basis of these data in Po river model the following choices are taken: BCF_{wet} of *Hyallella* equal to 73.7 L/kg is used for the Amphipoda, BCF_{wet} of *Corbicula* equal to 21.25 L/kg is used for Gastropoda and feeders (Trichoptera, Chironomids and Oligochaeta), while BCF_{wet} of *Elimia* equal to 27 L/kg is used for invertebrate predators (Odonata). For Zooplankton BCF_{wet} is expressed as the average value of the BCF found in this study: 37.6 L/kg.

The BCF_{dry} are then found multiplying the BCF_{wet} from literature for the wet/dry weight ratio characteristic of each species (Table 2.45). Wet/dry ratio for Odonata and Chironomus are set equal to Trichoptera ratio because they belong to the same group of the aquatic insects. For Chub and Wels catfish only average wet weights are available from Rossi et al. (1991) and Vitali & Braghieri (1994). Knowing from Lyndall et al. (2010) that the average water percentage in young fish is 77.8 and 73 % for adult fishes, the wet/dry ratio can be calculated.

Table 2.45 - BCF_{dry} for LAS calculated on the basis of literature data on BCF and wet/dry weight ratio.

Taxa	**BCF_{wet} (L/kg)**	**Wet/dry ratio**	**BCF_{dry} (L/kg)**
Brachionus	37.6	4.7 [b]	176.7
Amphipoda	73.7	3.64 [a]	268.3
Chironomus	21.25	4.7	99.9
Oligochaeta	21.25	4.83 [b]	102.6
Trichoptera	21.25	4.7 [b]	99.9
Gastropoda	21.25	4.7 [b]	99.9
Odonata	27	4.7	126.9
Bleak	80	3.7 [d]	296
Chub	80	3.7 [f]	296
Wels catfish (young)	80	4.5 [c]	360
Wels catfish (adult)	80	3.7 [c]	296

[a] Jørgensen (1991)

[b] from Lyndall et al. (2010)

[d] [XIII]

[e] calculated from data of Rossi et al. (1991)

[f] calculated from data of Vitali & Braghieri (1994)

Table 2.46 shows k_2 values calculated by AQUATOX with Barber equation, and the calculated values of k_1 from equation (20).

Table 2.46 - LAS k_1 (uptake rate constant) and k_2 (desorption rate constant) for animals in Po river model.

Taxa	**k_1 (L/kg d)**	**k_2 (1/d)**	**Average wet weight (g)**	**Lipid fraction**
Brachionus	208182.3	77.8	$1.2 * 10^{-7}$ [c]	0.012 [a]
Amphipoda	71103.9	77.8	$3.7 * 10^{-5}$ [c]	0.012 [a] (same of zooplankton)
Chironomus	15206.6	85.91	0.0075 [c]	0.013 [a] (same of Trichoptera)
Oligochaeta	10143.3	98.86	0.06 [c]	0.0075[a]
Trichoptera	15206.6	85.91	0.06 [c]	0.013 [a]
Gastropoda	4072.5	40.77	0.33 [c]	0.013 [a]
Odonata	6839.1	53.89	0.08 [c]	0.013 [a] (same of Trichoptera)
Bleak	4898.5	16.55	3.6 [d]	0.02 [b]
Chub	1006.3	3.40	329 [e]	0.02 [b] (from Dace)
Wels catfish (young)	1821.1	5.06	43.76 [f]	0.03 [b] (from Perch)
Wels catfish (adult)	429.9	1.45	3150 [f]	0.03 [b] (from Perch)

[a] Lyndall et al., 2010.
[b] Lombardo, 2013.
[c] AQUATOX default value.
[d] Vitali & Braghieri, 1981.
[e] Vitali & Braghieri, 1984.
[f] Rossi et al., 1991.

2.8.1.4 Ecotoxicological data

According to the review of EOSCA (2000), surfactants generally seem to impact on higher aquatic organisms via their respiratory structure by changing the epithelial membrane permeability, with the result of cellular lysis and impairment of cellular respiration. Also in lower organisms surfactant toxicity appears to result from an initial disruption of normal membrane function followed by physical disruption of the cellular membrane.

In the aquatic environment, different LAS homologues and isomers are present. Each of these components has a different degree of ecotoxicity, with the shorter chain lengths being less toxic than the longer ones (HERA, 2013). If ecotoxicological data on different chain lengths are available, the higher value is taken in order to maximize the assessed risk.

In Table 2.47 the organisms associations for the ecotoxicological parameters assessment are summarized. Associations are made comparing organisms used in LAS ecotoxicological tests from the web site [XI] and Po river modeled organisms. Information for fish comparisons are taken from web sites [IX] and [X]. Fathead minnow (*Phimphales Promelas*) toxicity records are used for Bleak (*Alburnus Alburnus*) because they have similar size (5-8 cm), weight (2-5 g) and alimentary behaviour (omnivorous). For the same reason Tilapia (*Oreochromis niloticus*) is associated to Chub (Squalius cephalus), both have same size (on average 30 cm) and almost same diet (omnivorous), Bluegill (*Lepomis macrochirus*) is associated to young Wels catfish (*Silurus glanis*), in fact both are carnivorous and of small dimensions (max 40 cm). Rainbow trout (*Oncorhynchus mykiss)* is associated to adult Wels catfish because both are carnivorous and have large size (max 120 cm). Regarding macroinvertebrates, all groups or species modeled are found in available ecotoxicological tests except for Trichoptera and Odonata.

The invertebrate predator *Limnodrilus Hoffmeisteri* is associated to Odonata, while ecotoxicological data of Chironomus are used for Trichoptera because it is an aquatic insect too. Tests on Corbicula are associated to Gastropoda and Oligochaeta because they are filter feeders. For phytoplankton, association from Lombardo (2013) are considered.

Table 2.47 - Organisms associations for the ecotoxicological parameters assessment.

AQUATOX state variable	**Group/family**	**Species**	**Taxonomic type**	**Toxicity record**	**Toxicity parameter available from ECHA**
Diatoms1	Diatom	Cyclotella	Phytoplankton	*Selenastrum capricornutum*	$EC50_{photo}$
Other Algae1	Chrysophyte	Chromulina	Phytoplankton	*Microcystis aeruginosa*	$EC50_{photo}$ and NOEC
Grazer1	Rotifer	Brachionus	Pelagic Invertebrate	*Brachionus calyciflorus*	$EC50_{growth}$
Shredder1	Amphipoda		Crustacean	*Hyalella azteca*	LC50
SedFeeder1	Chironomids	Chironomus	Benthic insect	*Chironomus riparius*	LC50
SedFeeder2	Oligochaeta		Aquatic worm	*Corbicula*	LC50
SuspFeeder1	Trichoptera		Benthic insect	*Chironomus riparius*	LC50
Snail1	Gastropoda		Benthic invertebrate	*Corbicula*	$EC50_{growth}$
PredInvt1	Odonata		Benthic insect	*Limnodrilus Hoffmeisteri*	LC50
SmForageFish1	Cyprinids	Bleak	Fish	*Pimephales promelas*	LC50 and NOEC, LOEC
LgBottomFish1	Cyprinids	Chub	Fish	*Oreochromis niloticus*	NOEC
SmGameFish1	Siluridae	Wels catfish (young)	Fish	*Lepomis macrochirus*	LC50 and $EC50_{growth}$
LgGameFish1	Siluridae	Wels catfish (adult)	Fish	*Oncorhynchus mykiss*	NOEC

Data expressed as NOEC or LOEC have to be converted in LC50 and EC50 with the relation described before. For plants the only data available are EC50, according to many AQUATOX studies (Ohio stream in the U.S. and Skensved stream in Denmark) (Park & Clough, 2012) LC50 is estimated as ten times EC50 for plants. Regarding animals, for chub and adult wels catfish only NOEC values are available, thus the conversion in $EC50_{growth}$ is made by using the relation NOEC x 2, and LC50 is then calculated by using the relation $EC50_{growth}$ x 1.67. When only LC50 is known for an animal the $EC50_{growth}$ is calculated with the relation $EC50_{growth}$ x 1.67.

Results for LC50, $EC50_{growth}$ and $EC50_{reproduction}$ are summarized respectively in Tables 2.48, 2.49, 2.50.

Table 2.48 - LC50 values for LAS used for organisms in Po river model.

Organism	LC50 (mg/L)	Exposure time (h)	Reference/calculation
Cyclotella	290	96	$EC50_{photo}$ x 10
Chromulina	9.1	96	$EC50_{photo}$ x 10
Brachionus	3.34	48	$EC50_{growth}$ x 1.67
Amphipoda	7.6	48	Test on *Hyalella azteca* (ECHA)
Chironomus	8.6	48	Test on *Chironomus riparius* (ECHA)
Oligochaeta	1.02	768	$EC50_{growth}$ x 1.67
Trichoptera	8.6	48	Test on *Chironomus riparius* (ECHA)
Gastropoda	1.02	768	$EC50_{growth}$ x 1.67
Odonata	2.4	48	Test on *Limnodrilus Hoffmeisteri* (ECHA)
Bleak	3.2	48	Test on *Pimephales promelas* (ECHA)
Chub	0.835	2160	$EC50_{growth}$ x 1.67
Wels catfish (young)	1.67	96	Test on *Lepomis macrochirus* (ECHA)
Wels catfish (adult)	0.77	1728	$EC50_{growth}$ x 1.67

Table 2.49 - $EC50_{growth}$ values for LAS used for organisms in Po river model.

Organism	$EC50_{growth/photo}$ (mg/L)	Exposure time (h)	Reference/calculation
Cyclotella	29	96	Test on *Selenastrum capricornutum* (ECHA)
Chromulina	0.91	96	Test on *Microcystis aeruginosa* (ECHA)
Brachionus	2	48	Test on *Brachionus calyciflorus* (ECHA)
Amphipoda	1.7	576	Test on *Hyalella azteca* (ECHA)
Chironomus	8.0	672	Test on *Chironomus riparius* (ECHA)
Oligochaeta	0.61	768	Test on *Corbicula* (ECHA)
Trichoptera	5.15	48	LC50/1.67
Gastropoda	0.61	768	Test on *Corbicula* (ECHA)
Odonata	1.44	48	LC50/1.67
Bleak	2.4	4704	Test on *Pimephales promelas* (ECHA): LOECx2
Chub	0.5	2160	Test on *Oreochromis niloticus* (ECHA): NOECx2
Wels catfish (young)	2	672	Test on *Lepomis macrochirus* (ECHA):NOECx2
Wels catfish (adult)	0.46	1728	Test on *Oncorhynchus mykiss* (ECHA): NOECx2

Table 2.50 - $EC50_{repr}$ values for LAS used for organisms in Po river model.

Organism	$EC50_{repr}$ (mg/L)	Exposure time (h)	Reference/calculation
Brachionus	2	48	Set equal to $EC50_{growth}$
Amphipoda	1.7	576	Set equal to $EC50_{growth}$
Chironomus	8.0	672	Set equal to $EC50_{growth}$
Oligochaeta	0.61	768	Set equal to $EC50_{growth}$
Trichoptera	5.15	48	Set equal to $EC50_{growth}$
Gastropoda	0.61	768	Set equal to $EC50_{growth}$
Odonata	1.44	48	Set equal to $EC50_{growth}$
Bleak	2.4	4704	Set equal to $EC50_{growth}$
Chub	0.5	2160	Set equal to $EC50_{growth}$
Wels catfish (young)	2	672	Set equal to $EC50_{growth}$
Wels catfish (adult)	0.46	1728	Set equal to $EC50_{growth}$

2.8.1.5 Loads and concentration

LAS loads (tonnes/d) in river Po catchment and LAS concentration (mg/L) in Pontelagoscuro section have to be estimated in order to evaluate the perturbed model behaviour under realistic chemicals loads and to have starting point values to increase or reduce analyzing model response.

Data on LAS loads referring to the period 1988-1990 are few, a study conducted by Bruna & Divo (1988) documents that the LAS production in 1987 in Europe amounts to 485000 tons and in Italy to 94000 tons (see Capri et al., 1991), but no other information are given, for this reason LAS loads will be calculated on the basis of actual consumption values and sewage treatment plant removal efficiencies (Figure 2.36).

LAS entering the treatment plant is estimated considering the total laundry products consumption in a year divided by 365 days/year and assuming an average inclusion level of 15% in laundry formulations (19).

$$LAS_{down\ the\ drain} = \frac{Laundry\ use\ (g/cap/y)}{365\ (d/y)} \cdot 0.15 \qquad (19)$$

Where:

$LAS_{down\ the\ drain}$ = per capita daily LAS mass down the drain entering in the treatment plant (g/cap/d);

$Laundry\ use$ = per capita annual Italian consumption of products containing LAS (g/cap/year).

The quantity of LAS exits the sewage treatment plant is then calculated by multiplying the quantity of LAS exiting the treatment plant by the fraction of total sewage that is not treated (6%) and the fraction of LAS that remains in the treated effluent assessing from Franco et al. (2013) (20).

$$LAS_{into\ po\ catchment} = LAS_{down\ the\ drain} \cdot (1 - Frac\ treated\ in\ STP) + LAS_{down\ the\ drain} \cdot (Frac\ treated\ in\ STP \cdot Frac\ to\ effluent) \qquad (20)$$

Where:

$LAS_{into\ po\ catchment}$ = per capita daily LAS mass exiting the treatment plant and entering Po river catchment (g/cap/d);

$Frac\ treated$ = LAS fraction treated in Sewage treatment plant (STP);

$Frac\ to\ effluent$ = LAS fraction remains after treatment.

Overall LAS load into Po river catchment is then calculated multiplying the per capita Load by the total basin population (21).

$$Total\ LAS\ load = LAS_{into\ po\ catchment} \cdot Basin\ population \tag{21}$$

Where:

$Total\ LAS\ load$ = daily LAS mass entering Po river catchment (tonnes/d);

$Basin\ population$ = Po river catchment population.

Resulting LAS load (Table 2.51) is of the same order of magnitude of the values found from Price et al. (2009) of 2.16 g/cap/d for the UK.

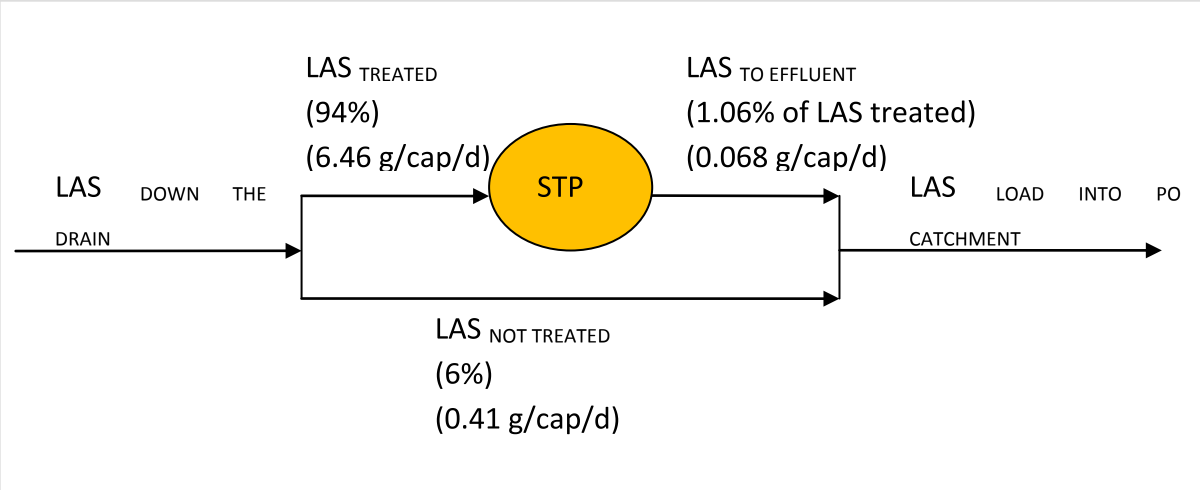


Figure 2.36 - Scheme of the LAS load assessment model.

Table 2.51 - Data and calculation of LAS loads in Po river catchment.

	Value	Unit	Reference	Comments
Po basin population	15,916,707	cap	Autorità di bacino del fiume Po (2006).	Referred to 2001
Po flow at Pontelagoscuro	1,450	m^3/s	[XII]	
Frac. treated in STP	0.94		[XIII]	Referred to 2005
% LAS in laundry	15		Unilever estimate	
Laundry use	16714	g/cap/y	Price et al., 2010	Emission in EU (value for Italy) referred to 2008
LAS down the drain	6.87	g/cap/d	Calculated	
Fraction to effluent STP	0.0106		Franco et al., 2013	
LAS into Po catchment	0.48	g/cap/d	Calculated	
LAS load into Po catchment	7.65	tonnes/d	Calculated	

Data on LAS concentrations in the section of Pontelagoscuro in the period September 1988 - September 1989 are available from a study of Galassi et al. (1991). Samples of water were taken near the water intake of the aqueduct and LAS molecules were extracted by means of XAD-2 resins. Results are summarized in Table 2.52.

Table 2.52 - LAS concentration in the section of Pontelagoscuro from September 1988 to September 1989 (Galassi et al., 1991).

Date	LAS C_{11} (ng/L)	LAS C_{12} (ng/L)	LAS C_{13} (ng/L)
09/1988	174	164	104
11/1988	118	16	0
01/1989	266	48	0
02/1989	98	0	0
04/1989	246	132	74
05/1989	500	228	108
06/1989	138	0	0
07/1989	126	0	0
08/1989	150	0	0
09/1989	126	0	0

Actual LAS concentration in Pontelagoscuro (final catchment section) can be calculated knowing the average in-river removal rate and the retention time of the chemical into Po river before reaching Pontelagoscuro.

Average in-river removal rate is chosen to be k = 0.058 1/h (the lowest value from those proposed by Price et al., 2009), that correspond to an half-time of 11.95 h. This value is very closed to biodegradation constant (0.06 1/h), in fact biodegradation should be the principal LAS elimination process in water.

LAS retention time in an ideal system that goes from STPs to Pontelagoscuro section can be assessed with the following formula:

$$RT = \frac{Length}{Velocity} \tag{22}$$

Where:

RT = retention time (d);

$Length$ = average length of route (m);

$Velocity$ = average water velocity in Po river (m/s) = 1 m/s ([XII]).

The retention time varies on the basis of the distance of the loading point from Pontelagoscuro. In this case it is considered a subdivision of the river basin in 12 sub-basins, the LAS load from each sub-region is calculated on the basis of the population distribution data (Marchetti, 1991). Residence time is then evaluated with (22), where length is the distance sub-basin/Pontelagoscuro. LAS that arrive at the closure section can thus be calculated as follow:

$$LAS\ (RTi) = LASi \cdot e^{-k \cdot RT_i} \tag{23}$$

Where:

$LAS\ (RTi)$ = LAS load that arrives in the closure section after RTi (g/d);

RTi = retention time of the LAS load coming from sub-basin i (h);

$LASi$ = LAS load from sub-basin i (g/d);

k = dissipation rate (1/h).

LAS concentration in Pontelagoscuro section is calculated with the following formula:

$$C_{LAS} = \frac{\sum_{i=1}^{12} LAS\ (RTi)}{Flow\ at\ Pontelagoscuro} \tag{24}$$

Results are summarized in Table 2.53.

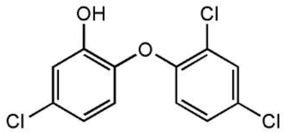
Table 2.53 - Calculation for the LAS loads and concentration in Pontelagoscuro section.

Sub-basin number	Basin Name	Basin distance from Pontelagoscuro (km)	Sub-basin population	LAS Load (g/d)	Retention time (h)	LAS load remained (g/d)
1	Alto Po	608.6035	477268.30	229359.12	166.29	14.86
2	Dora Riparia	546.733	1728104.88	830469.18	149.38	143.40
3	Dora Baltea	496.125	495058.15	237908.33	135.55	91.61
4	Sesia	445.75	542294.14	260608.36	121.79	222.96
5	Tanaro	411.241	881536.00	423636.60	112.36	626.22
6	Ticino	368.268	1714603.92	823981.07	100.62	2406.58
7	Lambro	315.348	3965376.23	1905626.7	86.16	12874.49
8	Adda	290.978	1962445.36	943085.35	79.50	9374.84
9	Taro	203.973	569899.95	273874.78	55.73	10808.27
10	Oglio	166.176	1405426.85	675400.95	45.40	48517.10
11	Mincio	135.739	822029.42	395039.74	37.09	45967.34
12	Panaro	54.906	1352663.80	650044.80	15.00	272311.38
Total river length		**652**	15916707	7649034.95		403359.07

Remained load is about 5 % of the initial total load. Actual concentration in Pontelagoscuro section results 3.22 µg/L, that is slightly higher respecting the concentration measured in the section in 1988 (Table 2.52) and slightly lower respecting the concentration in UK rivers (29-48 µg/L, from Price et al., 2009).

2.8.2 Triclosan

Triclosan (TCS) is the INCI (International Nomenclature of Cosmetic Ingredients) name of the molecule 5-chloro-2-(2,4-dichlorophenoxy)-phenol (CAS number 3380-34-5). It is a synthetic antimicrobial that is used in personal care products as soaps, deodorants, toothpastes, cosmetics since the 1970s, when it began to be use in US in soaps. TCS molecule (Figure 2.36) can be classify as phenylether, or chlorinated bisphenol (APUA, 2011) and it is produced by treatment of 2,4,4'-trichloro-2'-methoxydiphenyl ether with aluminum chloride in benzene under reflux (NICNAS, 2009). According to Levy et al. (1999) Triclosan blocks the active site of the ENR enzyme which is essential in the synthesis of fatty acids in bacteria (sees APUA p. 4).



Formula: $C_{12}H_7Cl_3O_2$

Figure 2.37 - TCS molecule structural and empirical formula (from web site [XI]).

Typically it occurs in aquatic ecosystem because a percentage variable between 30% and 2% remains in the effluent of waste water treatment plants (Lyndall et al., 2010). There is a current debate on the safety, and regulation of use of Triclosan.

2.8.2.1 Physico-chemical properties

Triclosan appears as a white to off-white crystalline powder with a faint aromatic odour (NICNAS, 2009), it is hydrophobic with an high octanol water partition coefficient (K_{ow}), it is ionisable because it contain the phenolic group (OH) (Lyndall et al., 2010). In particular in water with pH 7, TCS is present mainly in its neutral form, while in water with pH 8 about 55% of TCS will be in its neutral form and 45% in its ionized (anionic) form (values based on Multispecies Model calculation) (Environment Canada, 2012). In Table 2.54 the main physico-chemical parameters are summarized.

Table 2.54 - Triclosan physico-chemicals parameters (HERA, 2013).

TRICLOSAN	Value/Range	Notes
Molecular weight (g/M)	289.54[a]	C12H7Cl3O2
Vapour pressure at 25°C (Pa)	$1.8 \cdot 10^{-4}$ [b]	Experimental data
Boiling point (°C)	-	Decomposes before boiling
Melting point (°C)	56.4 [c]	Experimental data
Octanol-water partition coefficient (log K_{OW}) (L/kg)	4.76 [b]	Experimental data
Organic carbon-water partition coefficient K_{OC} (L/kg)	4.28 [b]	Experimental data
Water solubility (mg/L)	12 [b]	In neutral form
Sorption coefficient between soil/sediment and water, K_d (L/kg)	1.73 [d]	Experimental data
Density (kg/m^3)	1550 [c]	At 22 °C
Henry's constant (Pa * m^3/mole)	$2.3 \cdot 10^{-3}$ [b]	Low
Dissociation constant pka = -Log Ka	8.14 [b]	Ionisable

[a] NICNAS, 2009.

[b] Lyndall et al., 2010.

[c] [XI].

[d] Environment Canada, 2012.

2.8.2.2 Degradation properties

Regarding abiotic degradation processes in water, it is demonstrate by several studies that TCS is susceptible to phototransformation (primarily half life 3 hours, in laboratory conditions, summer sunlight, 46 °N); degradation products are 2,4-DCP and 2,7/2,8-DCDD, given their probable transient state in the environment and low toxicity, these DCDDs are not likely to be of environmental concern (Environment Canada, 2012). Thus, as a conservative assumption, photo transformation is not considered in river Po simulation, moreover considering the turbidity of the river photodegradation process can be neglected.

Considering biotic degradation processes, as written by Lyndall et al. (2010), TCS can be biodegraded under aerobic conditions. Products of degradation are 2,4-dichlorophenol and 2,8-dichlorodibenzo-p-dioxin, they are not considering in this risk assessment model because it is demonstrated by several studies that they do not accumulate in fishes and are less toxic than TCS, moreover they occurs at lower concentrations (see Lyndall et al., 2010). Table 2.55 summarizes the main biodegradation properties useful for the model, they are the same of the study of Lombardo (2013), half time is taken from Lyndall et al. (2010) while degradation rate (k) is calculated with the following formula:

$$k = \frac{ln2}{t_{1/2}} \qquad (25)$$

Table 2.55 - Most relevant data on TCS biodegradability.

Biodegradation properties	**Half-life time [a]**	**Degradation rate (1/d)**
Biodegradation in water	60 d	0.012
Biodegradation in soil	120	0.006
Biodegradation in sediments	540 d	0.001

[a] Lyndall et al. (2010).

2.8.2.3 Bioconcentration

Bioconcentration factor (BCF) and lipid fraction for phytoplankton, zooplankton, invertebrates are adapted from the study of Lombardo (2013). Fish BCF are calculated from BCF_{lipid} for fish equal to 165000 L/kg (Rüdel et al., 2012) and applying the same formula used by Lombardo:

$$BCF_{dry} = BCF_{lipid} \cdot f \cdot \frac{Wet_{weight}}{Dry_{weight}} \quad (26)$$

Where:

BCF_{dry} = bioconcentration factor on dry weight basis (L/kg_{dry});

BCF_{lipid} = bioconcentration factor on lipid weight basis (L/kg_{lipid});

f = lipid weight fraction ($kg_{lipid}/kg_{wet\ weight}$);

$\frac{Wet_{weight}}{Dry_{weight}}$ = biota wet to dry weight ratio (kg_{wet}/kg_{dry}).

Values for k_2 are calculated by AQUATOX with Barber equation, and k_1 values from equation (20) (Table 2.56).

Table 2.56 - BCF_{dry}, uptake rate (k_1), desorption rate (k_2), average wet weight, lipid fraction and wet/dry weight fraction of TCS for simulated organism.

Taxa	**BCF_{dry} (L/kg)**	**k_1 (L/kg d)**	**k_2 (1/d)**	**Average wet weight (g)[a]**	**Lipid fraction[a]**	**Wet/dry [a]**
Cyclotella	36332	563289	15.5		0.005	-
Chromulina	36332	563289	15.5		0.005	-
Brachionus	1700	50589.34	29.76	$1.2 * 10^{-7}$	0.012	4.7
Amphipoda	1700	48110.85	9.62	$3.7 * 10^{-5}$	0.012 (same of zooplankton)	3.64
Chironomus	1700	5302.84	3.12	0.0075	0.013 (same of Trichoptera)	4.7
Oligochaeta	1700	17947.46	3.59	0.06	0.0075	4.83
Trichoptera	1700	3520.5	2.07	0.06	0.013	4.7
Gastropoda	1700	7540.91	1.51	0.3	0.013	4.7
Odonata	1700	3326.50	1.96	0.08	0.013 (same of Trichoptera)	4.7
Bleak	12210	7336.42	0.60	3.6	0.02	3.7
Chub	12210	1507.16	0.12	329	0.02 (from Dace)	3.7
Wels catfish (young)	22275	612.23	0.12	43.76	0.03 (from Perch)	4.5
Wels catfish (adult)	18315	263.66	0.05	3150	0.03 (from Perch)	3.7

[a] See Table 2.46.

2.8.2.4 Ecotoxicological data

Data on TCS ecotoxicology are available only for some biota, thus, values from similar organisms are considered (Table 2.57). The starting point are data from Lombardo (2013). Cyclotella and Chromulina are associated to *Desmodeus Subspica*. Regarding zooplankton, data on *Paramecium Caudatuma* are used. Amphipoda is associated to *Hyalella azteca* because are both micro-crustaceans, Gastropoda to *Perna Perna* because they are molluscs and aquatic insects as chironomids, trichoptera, odonata are associated to *Chironomus Tentants*. Fathead minnow (*Phimphales Promelas*) toxicity records are used for Bleak (*Alburnus Alburnus*) because they have similar size (5-8 cm), weight (2-5 g) and alimentary behavior (omnivorous). For the same reason Tilapia (*Oreochromis niloticus*) is associated to Chub (Squalius cephalus), both have same size (on average 30 cm) and almost same diet (omnivorous), Bluegill (*Lepomis macrochirus*) is associated to young and adult Wels catfish (*Silurus glanis*), in fact both are carnivorous. No data on Oligochaeta is available, it is associated with *Perna Perna* because they are both filter feeders.

Table 2.57 - Organisms associations for the ecotoxicological parameters assessment.

AQUATOX state variable	**Group/family**	**Species**	**Taxonomic type**	**Toxicity record**
Diatoms1	Diatom	Cyclotella	Phytoplankton	*Desmodeus Subspica*
Other Algae1	Chrysophyte	Chromulina	Phytoplankton	*Desmodeus Subspica*
Grazer1	Rotifer	Brachionus	Pelagic Invertebrate	*Paramecium Caudatuma*
Shredder1	Amphipoda		Crustacean	*Daphnia magna*
SedFeeder1	Chironomids	Chironomus	Benthic insect	*Chironomus Tentants*
SedFeeder2	Oligochaeta		Aquatic worm	*Perna Perna*
SuspFeeder1	Trichoptera		Benthic insect	*Chironomus Tentants*
Snail1	Gastropoda		Benthic invertebrate	*Perna Perna*
PredInvt1	Odonata		Benthic insect	*Chironomus Tentants*
SmForageFish1	Cyprinids	Bleak	Fish	*Pimephales promelas*
LgBottomFish1	Cyprinids	Chub	Fish	*Oreochromis niloticus*
SmGameFish1	Siluridae	Wels catfish (young)	Fish	*Lepomis macrochirus*
LgGameFish1	Siluridae	Wels catfish (adult)	Fish	*Lepomis macrochirus*

All ecotoxicological values are taken from Lombardo (2013) and summarized in Table 2.58, 2.59, 2.60.

Table 2.58 - LC50 values for TCS used for organisms in Po river model.

Organism	LC50 (µg/L)	Exposure time (h)	Reference/calculation
Cyclotella	16.1	72	Test on *Desmodeus Subspica*
Chromulina	16.1	72	Test on *Desmodeus Subspica*
Brachionus	1544	48	Test on *Paramecium Caudatuma*
Amphipoda	200	240	Test on *Hyalella azteca* (ECHA)
Chironomus	400	240	Test on *Chironomus Tentants*
Oligochaeta	1260	48	Test on *Perna Perna*
Trichoptera	400	240	Test on *Chironomus Tentants*
Gastropoda	1260	48	Test on *Perna Perna*
Odonata	400	240	Test on *Chironomus Tentants*
Bleak	260	96	Test on *Phimepales Promelas*
Chub	260	96	Test on *Pimephales promelas*
Wels catfish (young)	370	96	Test on *Lepomis macrochirus*
Wels catfish (adult)	370	96	Test on *Lepomis macrochirus*

Table 2.59 - $EC50_{growth}$ values for TCS used for organisms in Po river model.

Organism	$EC50_{growth/photo}$ (µg/L)	Exposure time (h)	Reference/calculation
Cyclotella	1.61	72	Test on *Desmodeus Subspica*
Chromulina	1.61	72	Test on *Desmodeus Subspica*
Brachionus	400	120	Test on *Paramecium Caudatuma*
Amphipoda	250	240	Test on *Hyalella azteca* (ECHA)
Chironomus	280	240	Test on *Chironomus Tentants*
Oligochaeta	135	48	Test on *Perna Perna*
Trichoptera	280	240	Test on *Chironomus Tentants*
Gastropoda	135	48	Test on *Perna Perna*
Odonata	280	240	Test on *Chironomus Tentants*
Bleak	67	96	Test on *Phimepales Promelas*
Chub	67	96	Test on *Pimephales promelas*
Wels catfish (young)	96	96	Test on *Lepomis macrochirus*
Wels catfish (adult)	96	96	Test on *Lepomis macrochirus*

Table 2.60 - $EC50_{repr}$ values for TCS used for organisms in Po river model.

Organism	$EC50_{repr}$ (μg/L)	Exposure time (h)	Reference/calculation
Brachionus	400	120	Set equal to $EC50_{growth}$
Amphipoda	250	240	Set equal to $EC50_{growth}$
Chironomus	280	240	Set equal to $EC50_{growth}$
Oligochaeta	135	48	Set equal to $EC50_{growth}$
Trichoptera	280	240	Set equal to $EC50_{growth}$
Gastropoda	135	48	Set equal to $EC50_{growth}$
Odonata	280	240	Set equal to $EC50_{growth}$
Bleak	67	96	Set equal to $EC50_{growth}$
Chub	67	96	Set equal to $EC50_{growth}$
Wels catfish (young)	96	96	Set equal to $EC50_{growth}$
Wels catfish (adult)	96	96	Set equal to $EC50_{growth}$

2.8.2.5 Loads and concentration

Data on TCS loads referring to the period 1988-1990 are absent, for this reason TCS loads will be calculated on the basis of actual consumption values in the same manner done for LAS. TCS use per capita in a year is estimated considering a consumption equal to 350 tonnes/y in Europe (Von der Ohe et al., 2011). The quantity of TCS exits the sewage treatment plant is then calculated by adding the fraction of total sewage that is not treated (6%) and the fraction of TCS that remains in the treated effluent assessing from Franco et al. (2013) (Table 2.61). Resulting TCS load is comparable to values estimate for UK 0.63 - 2.74 mg/cap/d by Price et al. (2009).

Table 2.61 - Data and calculation of LAS loads in Po river catchment.

	Value	Unit	Reference	Comments
Po basin population	15,916,707	cap	Autorità di bacino del fiume Po (2006).	Referred to 2001
Po flow Pontelagoscuro	1,450	m^3/s	[XII]	
Frac treated in STP	0.94		[XIII]	Referred to 2005
TCS use	350	t/y	Von der Ohe et al., 2011	In Europe
TCS down the drain	1.2958	mg/cap/d	TCS use/365/Europe cap	
Fraction to effluent STP	0.055		Franco et al., 2013	
TCS into Po catchment	0.145	mg/cap/d	TCS down the drain*(Fraction treated in STP*fraction to effluent + (1-frac treated)	
TCS load into Po catchment	0.0023	tonnes/d	LAS into Po catchment *Po basin population	

Actual TCS concentration in Pontelagoscuro (final catchment section) can be calculated in the same manner used with LAS. Average in-river removal rate is chosen to be 0.06 1/h (the lowest value from those proposed by Price et al., 2010). The average residential time is the same as the value used for LAS: 99 h (Table 2.62).

Table 2.62 - Calculation for the LAS loads and concentration in Pontelagoscuro section.

Sub-basin number	Basin Name	Basin distance from Pontelagoscuro (km)	Sub-basin population	TCS Load (g/d)	Retention time (h)	TCS load remained (g/d)
1	Alto Po	608.6035	477268.30	69081.1	166.29	3.342854
2	Dora Riparia	546.733	1728104.88	250130.5	149.38	33.2362
3	Dora Baltea	496.125	495058.15	71656.05	135.55	21.75347
4	Sesia	445.75	542294.14	78493.11	121.79	54.2358
5	Tanaro	411.241	881536.00	127595.9	112.36	154.8723
6	Ticino	368.268	1714603.92	248176.4	100.62	607.5657
7	Lambro	315.348	3965376.23	573959.2	86.16	3333.786
8	Adda	290.978	1962445.36	284049.6	79.50	2456.088
9	Taro	203.973	569899.95	82488.85	55.73	2952.196
10	Oglio	166.176	1405426.85	203425.3	45.40	13494.31
11	Mincio	135.739	822029.42	118982.7	37.09	12973
12	Panaro	54.906	1352663.80	195788.2	15.00	79887.91
Total river length		652	15916707	2303827		115972.3

Remained TCS load is about 5 % of the initial total load. Actual concentration in Pontelagoscuro section results 0.926 ng/L, that is smaller than the concentration in UK rivers (50 ng/L, from Price et al., 2010).

2.9 Initial conditions, input loadings and chemical scenarios

Initial conditions and input loadings used in Po river modeled are summarized in Table 2.63.

Table 2.63 - Main input loadings and initial condition in Po river model.

Variable	Loading from upstream	Initial condition in water column	Initial condition in river bed
DO	Oxygen loads and washout turned off	10 mg/L	/
CO_2	Constant input: 0.12 mg/L	0.01 mg/L	/
NH_4-N	Time varying from observed data	0.29 mg/L	/
NH_3-N	Time varying from observed data	3.02 mg/L	/
TSP (Total soluble Phosphorous)	Time varying from observed data	0.12 mg/L	/
TOC	Time varying from observed data	2.36 mg/L	0 mg/L
TSS	Time varying from observed data	36.5 mg/L	/
Cyclotella (mg dry/L)	Equal to washout	0.12	/
Chromulina (mg dry/L)	Equal to washout	0.0296	/
Brachionus (g dry /m^2)	Equal to washout	0.1	/
Amphipoda (g dry /m^2)	0	0.003	/
Chironomus (g dry /m^2)	0	0	/
Oligochaeta (g dry /m^2)	0	0.3	/
Trichoptera (g dry /m^2)	0	0.054	/
Gastropoda (g dry /m^2)	0	0.0002	/
Odonata (g dry /m^2)	0	0.006	/
Bleak (g dry /m^2)	0	0.3	/
Chub (g dry /m^2)	0	2.07	/
Wels catfish (young) (g dry /m^2)	0	2.7	/
Wels catfish (adult) (g dry /m^2)	0	2.8	/

The model is run for 1 year (from 01/01/1989 to 01/12/1989) and then for 3 years (from 01/01/1988 to 01/12/1990). For both run periods and for each of the two chemicals tested (LAS and TCS) four scenarios are simulated:

- Control scenario: no chemical input;
- Scenario 1: constant daily chemical input equal to the actual assessed chemical concentration in river Po;
- Scenario 2: constant daily chemical input concentration equal to the lowest EC50 of Po river modelled organisms;
- Scenario 3: constant daily chemical input concentration equal to the lowest LC50.

Table 2.64 summarized the input values of the four scenarios.

Table 2.64 - simulation scenarios for LAS and TCS.

Chemical	**Control**		**Scenario 1**		**Scenario 2**		**Scenario 3**	
	Initial condition	Constant inflow concentration	Initial condition	Constant daily inflow concentration	Initial condition	Constant daily inflow concentration	Initial condition	Constant daily inflow concentration
LAS (µg/L)	-	-	3.22	3.22	460	460	770	770
TCS (µg/L)	-	-	0.000926	0.000926	1.61	1.61	16.1	16.1

2.10 Ecological risk assessment indicators

River health definition is one of the most complicate, debated and subjective environmental challenges. Karr (1999) defines river quality concept as composed by two factors:

- ecological value: a river has an high value if there is an high biodiversity, if it is resistant to stress, if it is in good functional and structural conditions;
- anthropic value: a river has an high value if it has a good capacity to provide services and goods to society.

The instruments for "measure" in a conventional way the river quality are the indices, or indicators. They can be based on the evaluation of the composition and structure of the biota community, or on the evaluation of the functionality of the river from an anthropic point of view. Several indices have been derived during these years (i.e. IBE (Indice Biotico Esteso), LIM (Livello di Inquinamento da Macrodescrittori), IBMR (Indice

Biologique Macroftitique en Rivière), IFF (Indice di Funzionalità Fluviale), II (Indice Ittico)), but currently, in practice, many uncertainties are present, especially concerning the choice of the indicator, and the monitoring procedure (Fenoglio & Bo, 2009).

To compare the control with perturbed simulation results and quantify the effects and the risk of xenobiotics on the ecosystem, the same indicators used in river Thames study by Lombardo are chosen (see Lombardo, 2013):

1) Objective variation of average biomass

For each species the average biomass in control simulation is compared with the average biomass in perturbed simulation:

$$\bar{\varepsilon} = \sum_{i=1}^{N} \frac{\bar{B}_{i\,PERT} - \bar{B}_{i\,CONT}}{B_{i\,CONT}} \cdot 100 \cdot \frac{1}{N} \quad (27)$$

Where:

$\bar{\varepsilon}$ = average objective perturbation in the system;

N = number of biological species/groups modelled in the system;

$\bar{B}_{i\,PERT}$ = average biomass of species "i" during the perturbed simulation (g dry/m^2);

$\bar{B}_{i\,CONT}$ = average biomass of species "i" during the control simulation (g dry/m^2).

2) Ecosystem maturity indicator

AQUATOX calculates the total primary production of the system and the community respiration. By comparing production/respiration ratio (28) of control and perturbed simulation, an idea on the change in maturity level of the system is given.

$$\frac{P}{R} = \frac{GPP}{R_{comm}} \quad (28)$$

Where:

P/R = production-respiration ratio;

GPP = gross primary production ($gO_2/(m^2\ d)$);

R_{comm} = community respiration ($gO_2/(m^2\ d)$).

3) Shannon index (community diversity indicator)

It is a diversity index that mathematically measure the relative abundance of a species or group over the others (29). Compared the index values of control and perturbed simulations will give an idea of the xenobiotics effects on community diversity.

$$SD = -\sum_{i=1}^{N} p \cdot \ln p \tag{29}$$

$$p = \frac{\overline{B_i}}{\overline{B_{tot}}} \tag{30}$$

$$\overline{B_i} = \frac{\sum_{i=0}^{t} B_{it}}{T} \tag{31}$$

$$\overline{B_{tot}} = \sum_{i=1}^{N} \overline{B_i} \tag{32}$$

Where:

SD = Shannon index;

N = number of biological species/groups modelled in the system;

$\overline{B_i}$ = average of the values of biomass of organism "i" at the time "t" for the entire period of simulation;

$\overline{B_{tot}}$ = total average biomass of the system.

4) Ecological Quality Ratio (WFD index)

The overall rationale of the WFD is to set the values of several biological, hydro-morphological, physico-chemical and pollutants parameters so that, through them, it is possible to determine water body status among the five established “Quality classes” for all water types. In Annex V, Section 1.1.1 of the Directive the quality elements for the classification of the rivers are listed as follows:

- Biological elements: composition and abundance of aquatic flora, benthic invertebrates, fish fauna;
- Hydromorphological elements: hydrological regime, river continuity, morphological conditions;
- Chemical and physico-chemical conditions;
- Specific pollutants: pollution by priority substances and other important substances discharged in the water body.

For each quality element, the Directive provides a definition of high, good and moderate status. This is based on the calculation of the Ecological Quality Ratio (EQR), that is the relationship between the observed biological parameters and the reference optimum values. Ratios are classify into five classes, the boundary values should be decided by Member States on the basis of intercalibration exercise that currently is not yet completed. EQR equal to 1 represent a high status, EQR equal to 0 represent a bad status (Annex V, Section 1.4.1). EQRs simplify the ecosystem into a single number that is an indicator of quality and it ensures the comparability between results from different assessment methods.

Regarding the specific case of the Po river, on the basis of the WFD indications and according to the Italian Environmental law D.Lgs. 152/06, a management plan was elaborated in 2010. The objective of this plan is to reach by 2015, and in some cases by 2027, a good quality status of the Po river and of other artificial or natural channel into the catchment (Autorità di bacino del fiume Po, 2010b).

The Directive does not provide reference values for this calculation, thus, as done in Thames river study, the control simulation is considered the reference status of Po river, to compared with perturbed system status. Organisms are grouped in Plants, Zooplankton & Macroinvertebrates and Fishes according to Annex V of WFD. The comparison is done evaluating organisms biomass variation from control and perturbed simulation and to each relative biomass variation value is associated a EQR (Table 2.65).

Table 2.65 -Ecological quality ratio value classification.

Relative biomass variation	**Ecological Quality Ratio**	**Classification**
0 ÷ 5 %	0.5 ÷ 1	No visible perturbation
5 ÷ 15 %	0.25 ÷ 0.5	Low perturbation
15 ÷ 25 %	0.15 ÷ 0.25	Moderate perturbation
25 ÷ 50 %	0.05 ÷ 0.15	Moderate-High perturbation
50 ÷ 100 %	0 ÷ 0.05	High perturbation

5) Ecological service index

To evaluate the differences between the level of quality of control and perturbed systems from a human services point of view, two indicators are chosen according to river Thames study: Secchi depth and Fish catch quality. Secchi depth is a measure of water turbidity and it could be used a measure of the pleasantness of the ecosystem from the human point of view. Fish catch quality is a measure of the goodness of the ecosystem as a service for fishery and it is evaluated calculating the overall fishes biomass variation between control and perturbed simulation. If fish biomass decreases because of a system perturbation, less fishes remain to be caught and the fishing service of the river gets worse.

3 Results

Results of *control* (with no chemicals) and *perturbed* (with chemicals) simulations are presented in this Chapter.

The control simulation results consist in the biota time-varying biomass curves, corresponding to a given "best" set of parameters that best approximate the observed data points. The goodness of the calibration results is measured mathematically with Pearson linear correlation coefficient and with Nash–Sutcliffe efficiency (NSE) coefficient. This control model represents the unperturbed river Po model that will be compared with the perturbed one in order to assess the chemical risk through the calculation of various biological and ecological service indices as objective biomass variation indicator, ecosystem maturity indicator, Shannon index, Ecological Quality Ratio, Ecological service index. In Paragraph 3.1, control simulation results are presented and discussed for each organism simulated, in Paragraph 3.2, perturbed simulation results are presented for LAS and TCS chemicals and risk analysis is done.

3.1 Control ecosystem

The control ecosystem is the reference point, it represents the Po river segment without chemicals and it will be compared with the perturbed system in order to assess the effects of different LAS and TCS concentrations in the aquatic system. Data discussed in Chapter 2 are used to implement the control ecosystem that is stabilized and calibrated for periods of time of 1 year and 3 years. The goodness of calibration is mathematically assessed with coefficients described in § 2.1.3.

Because of the huge amount of parameters to calibrate and scarce data availability, for some organisms the calibration is done trying to simulate at least the correct annual mean. Also a biomass variation pattern over the year should be present for each organisms, if the biomass results to be constant this is a sign of a gap in the model. Pearson and Nash–Sutcliffe efficiency (NSE) coefficients are not calculated for three years model because there are few observed data in 1988 and 1990 to compared with simulated ones.

In this paragraph calibration results of 1 year simulation are commented for physico-chemical data and for each organism simulated. The year chosen is the 1989 because to this year are referred most of observed data. Results of 3 years simulation are presented in the last paragraph.

3.1.1 Physico-chemical data

Calibrated physico-chemical data referred to 1989 are here presented in order to facilitate the discussion of biomass calibration results. Nutrients (P, NH_3 N-NH_4), CO_2, NO_3, and Oxygen are plotted in Figures 3.1 and 3.2. Total soluble phosphorus and nitrate are more or less constant during the year with an average concentration respectively of 0.05 mg/L and 2.3 mg/L. The Carbon dioxide has values between 0.2 and 0.8 mg/L, with an average value of 0.5 mg/L, it has a peak in July, probably due to a bloom of some organisms that increase community respiration (see Figure 3.3). The presence of nutrients NH_3 & N-NH_4 in the Po river can be due to the use of fertilizers generally applied in Autumn-Winter or to the sewage discharges. NH_3 & N-NH_4 has values between 0.02 mg/L and 0.7 mg/L, with an average value of 0.3 mg/L. The higher concentrations in the cold period can be related to the higher discharge in the basin in condition of low water flow and also to the reduced bacterial activity as assessed by Tartari et al. (1991). Nutrients decrease appreciably in April and September when temperatures are medium, organisms consume more and inputs are lower, as well as due to the dilution effect of the peak flow of the Po river observed in these months. Dissolved oxygen has values between 5.5 and 13 mg/L, with an average value of 9.14 mg/L, denoting a discrete oxygenation level, it increases in low temperature periods probably because of the temperature effects on the dissociation process of the oxygen (saturation is higher in cold water).

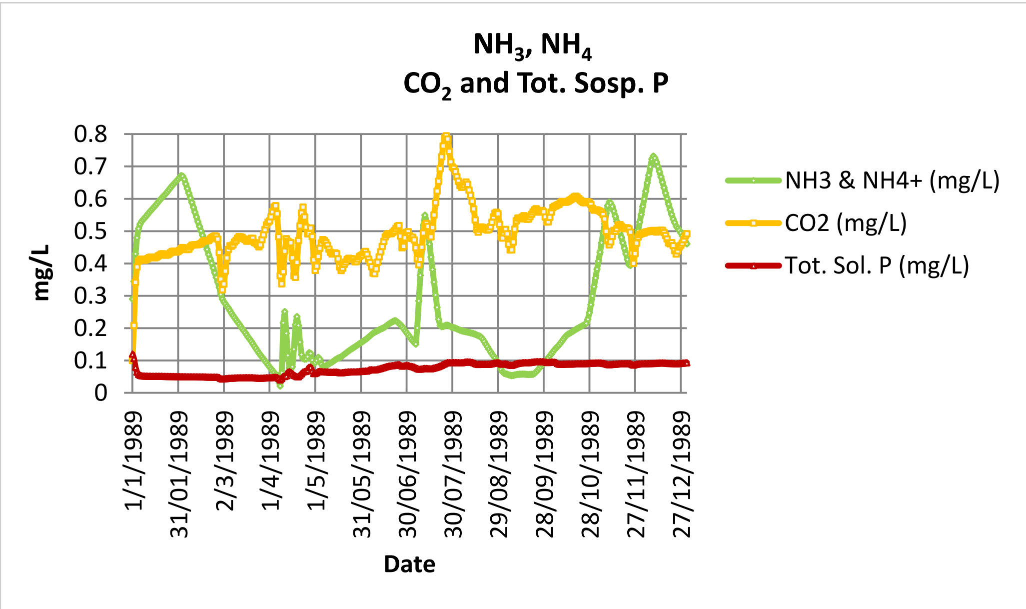


Figure 3.1 - NH_3 & N-NH_4, CO_2 and total soluble Phosphorous trends in one year control simulation (on the x-axis labels are every 30 days).

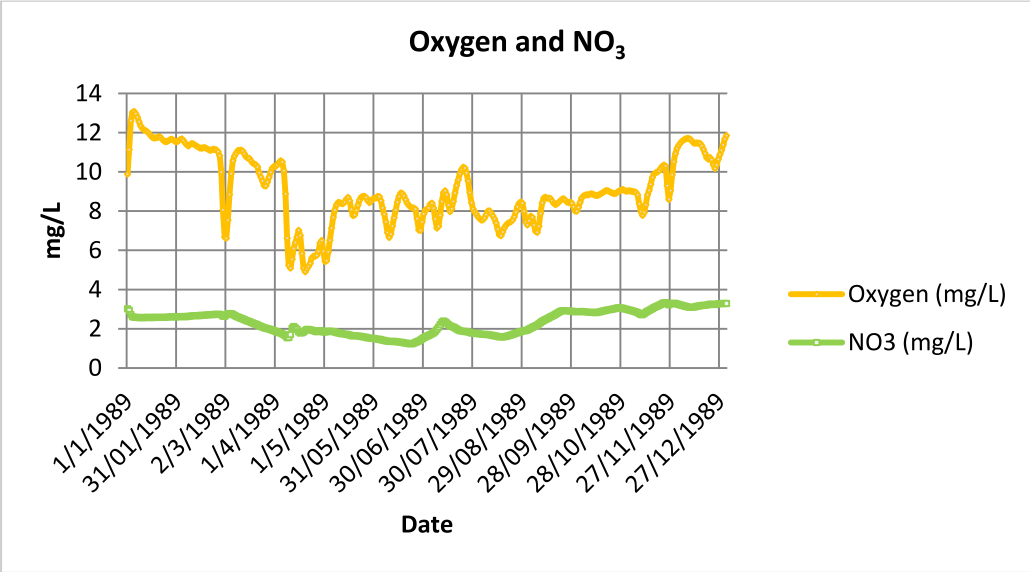


Figure 3.2 - NO_3 and oxygen trends in one year control simulation (on the x-axis labels are every 30 days).

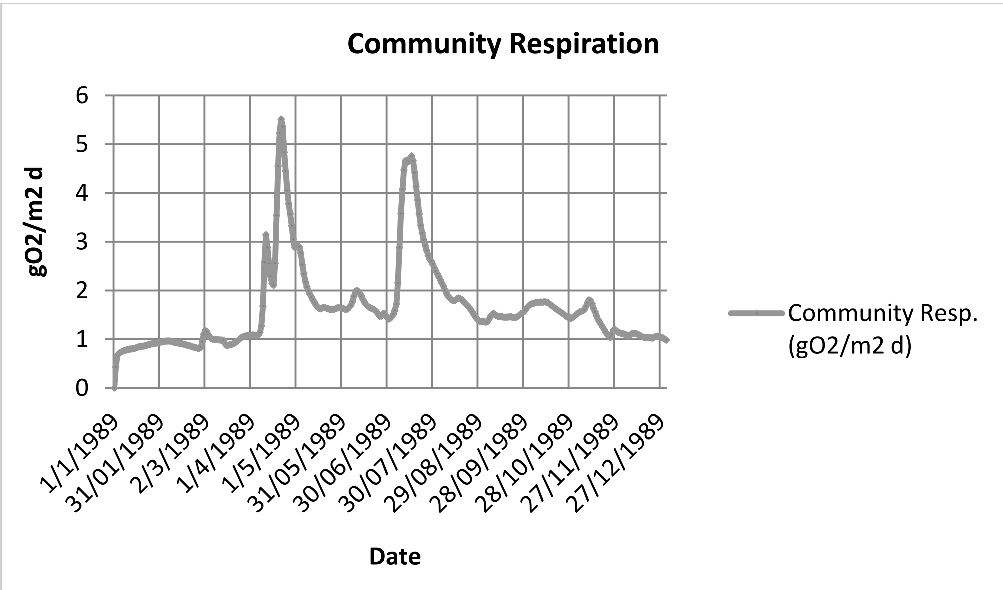


Figure 3.3 - Community respiration (g O2/m2 d) in 1 year control simulation (on the x-axis labels are every 30 days).

Inorganic solids and detritus trends are plotted in Figures 3.4, 3.5, the resulting Secchi depth is reported in Figure 3.6. The total suspended solids are mainly composed by inorganic solids, in fact particulate detritus concentrations are very low. Sedimented detritus are mainly labile, while particulate and dissolved detritus are more or less labile and refractory in the same proportion. Detritus have a pick in April probably because there is a high consumption and more organic material is egested. According to Secchi depth, water turbidity is reduced in July when TSS decreased, and it increases over the rest of the year when Secchi depth assumes an average value of 20 cm.

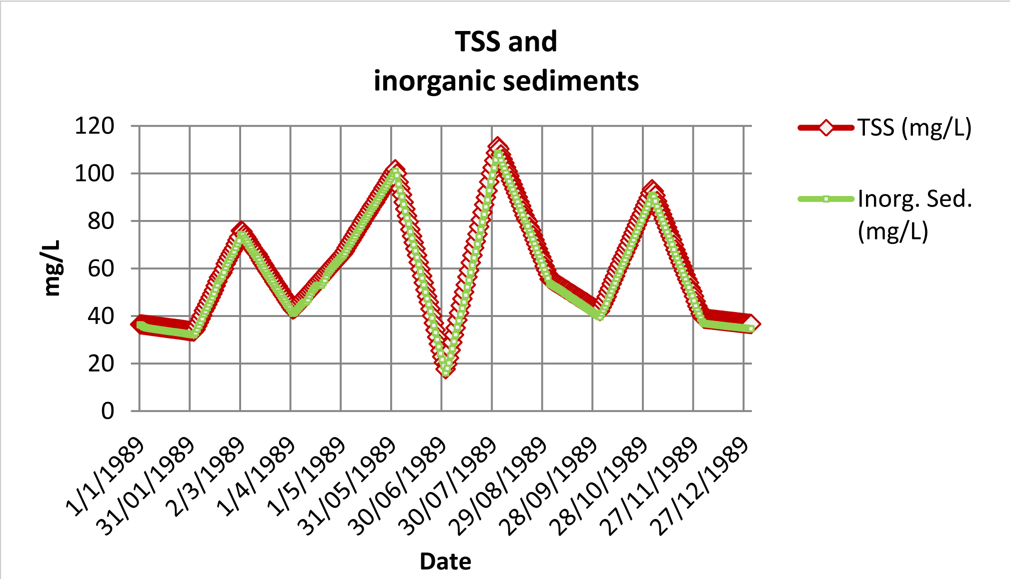
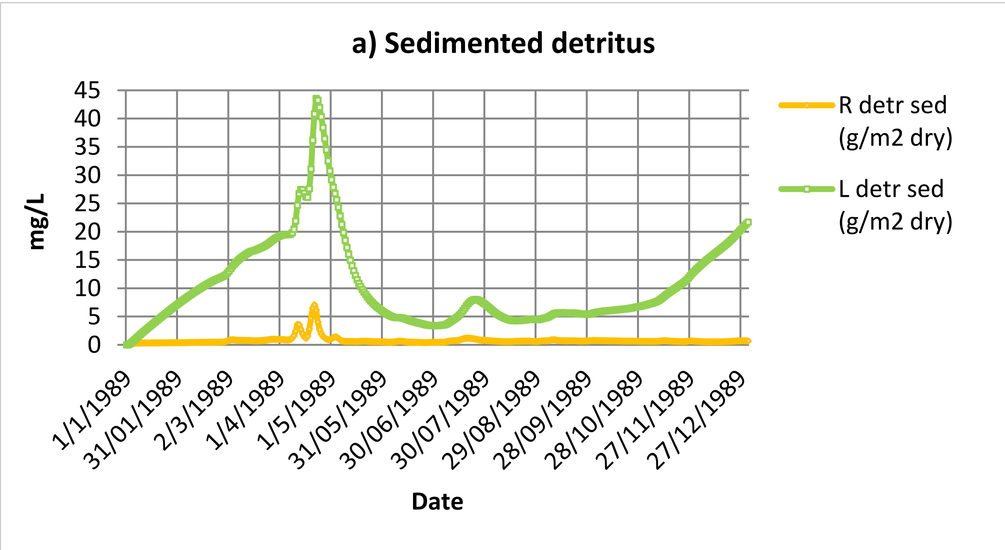
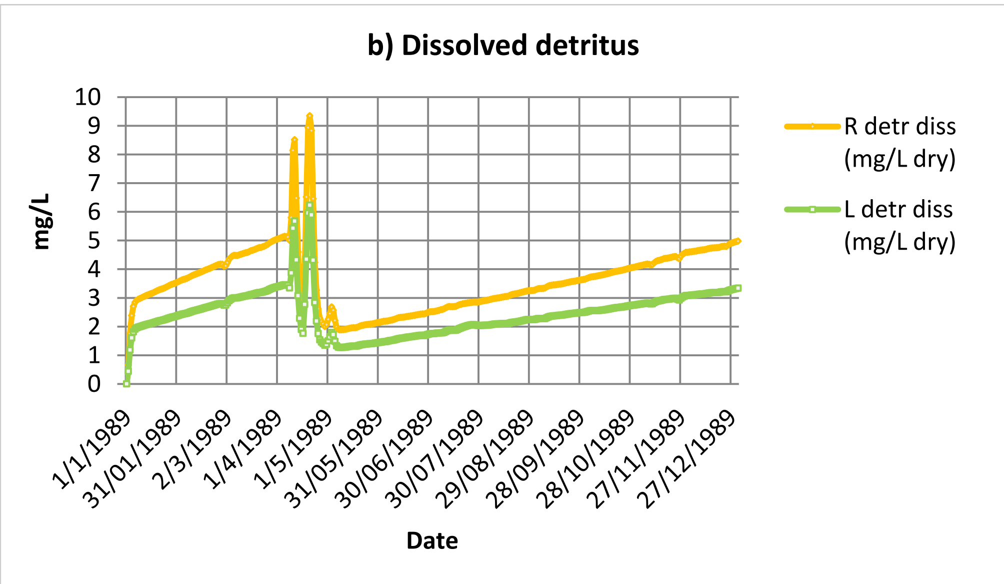


Figure 3.4 - Total Suspended Solids and Inorganic Sediments trends in one year control simulation (on the x-axis labels are every 30 days).





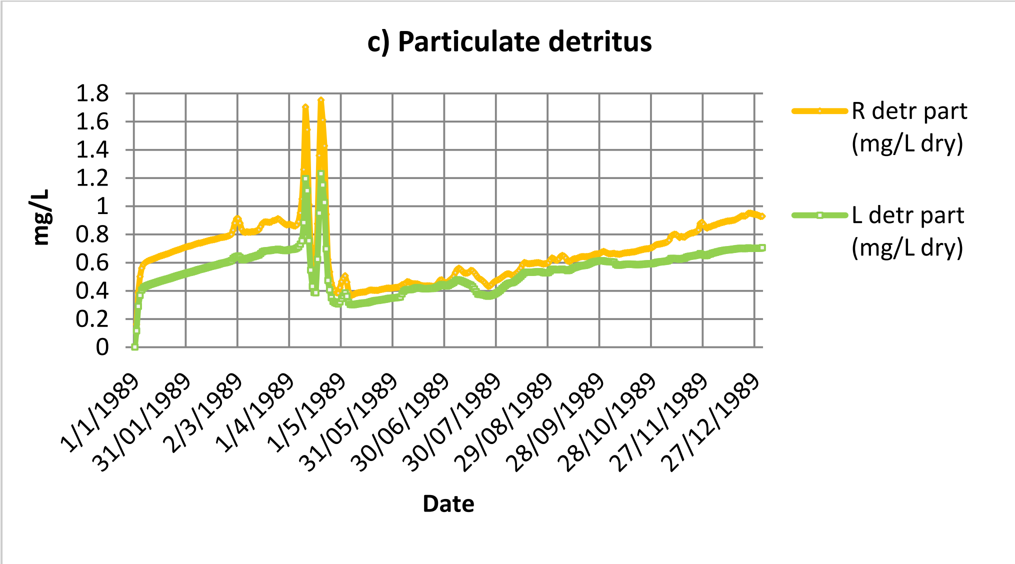


Figure 3.5 - Trends in one year control simulation of a) Sedimented detritus, b) Dissolved detritus and c) Particulate detritus (on the x-axis labels are every 30 days).

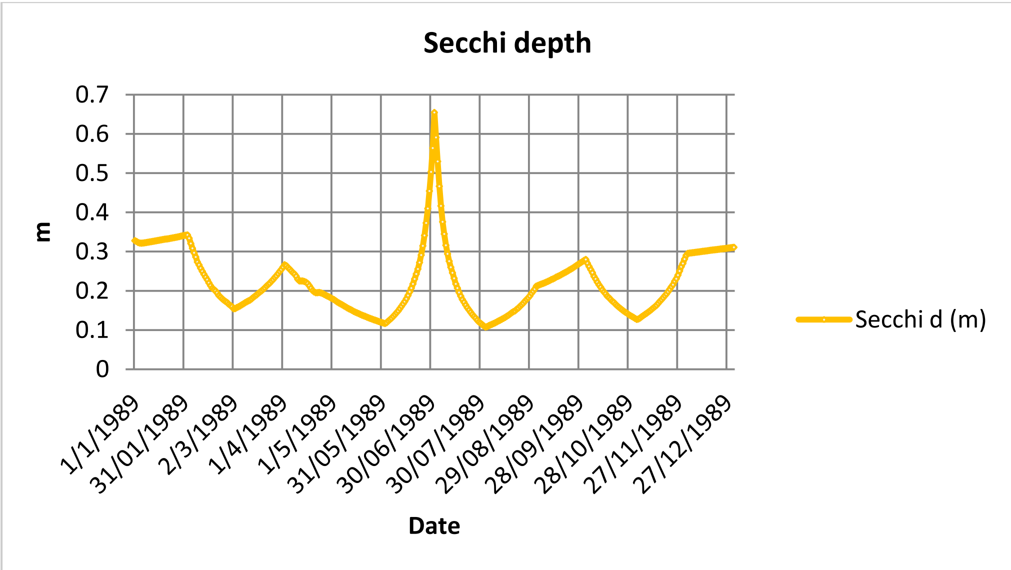


Figure 3.6 - Secchi depth trend in one year control simulation (on the x-axis labels are every 30 days).

3.1.2 Food web

Preference matrix percentages are calibrated together with organisms parameters to stabilize the model. Substantially changes of the pre-calibration values involve phytoplankton predation, in fact the preference percentage of organisms that feed on phytoplankton (mainly Bleak, Rotifer, Trichoptera) have been reduced, to avoid collapse of primary producers. Another change is the addition of particulate detritus and Odonata in adult Wels catfish diet and Rotifers in the young Wels catfish diet (Table 3.4), in order to stabilize macroinvertebrates and Cyclotella predation (by reducing the biomass of their predators Odonata and Rotifer) that would otherwise be too high.

Regarding egestion coefficients, for several organisms the value of 0.2 is decreased to 0.15 in order to enhance biomass production that otherwise would not have achieved the observed one (Table 3.5).

Table 3.4- Calibrated preference matrix for Po river model (numbers are %).

Prey	Predator											
	Amph.[+]	Esternal insects	Chiron. (larvae)	Oligoch.	Trich.	Rotifer	Gastrop.	Odon.	Bleak	Chub	Yonug wels catfish	Adult wels catfish
R detr sed	2.0		5.6	10.0			3.5					
L detr sed	47.0		38.9	90.0			69.2					
R detr part	2.0		5.6		2.6	9.7	3.5			13.4		0.9
L detr part	47.0		38.9		56.1	43.7	5.8			13.4		0.9
Cyclotella	1.0		9.3		25.2	3.0	9.3		8.5			
Chromulina	1.0		1.9		16.2	43.7	8.7		53.1			
Macrophytes												
Amphipoda								29.6	4.7		27.2	11.9
Esternal insects									1.9	20.8	12.0	4.4
Chironomid								19.3	4.0	20.1	10.2	3.2
Oligochaeta								19.1	1.8		13.2	
Trichopter								13.9	3.8	10.7		
Rotifer (Brachionus)									13.9		15.7	
Gastropod								18.0			9.0	4.4
Odonata									8.3	16.7	11.1	2.9
Bleak										4.9	1.6	44.5
Chub												26.9
Young wels catfish												
Adult wels catfish												

[+] Unchanged from the initial preference matrix

Table 3.5- Calibrated egestion coefficient for Po river model.

Prey	Predator										
	Amph.[+]	**Esternal insects**	**Chiron. (larvae)** [+]	**Oligoch.** [+]	**Trich.**	**Rotifer**	**Gastrop.** [+]	**Odon.** [+]	**Bleak**	**Chub**	**Yonug wels catfish**	**Adult wels catfish**
R detr sed	1		1	1			1					
L detr sed	0.1		0.2	0.3			0.1					
R detr part	1		1		1	1	1			1		1
L detr part	0.1		0.2		0.2	0.2	0.2			0.2		0.2
Cyclotella (Diatom)	0.8		0.2		0.35	0.3	0.2		0.15			
Chromulina (Chrysophyte)	0.8		0.2		0.30	0.3	0.2		0.1			
Macrophytes												
Amphipoda								0.2	0.15		0.2	0.15
Esternal insects									0	0.15		0.15
Chironomid (larvae)								0.2	0.15	0.15	0.2	0.15
Oligochaeta								0.2	0.15		0.2	
Trichopter (larvae)								0.2	0.15	0.15	0.2	
Rotifer (Brachionus)									0.16		0.2	
Gastropod								0.2	0.15		0.2	0.15
Odonata (larvae)									0.15	0.15	0.15	0
Bleak										0.2	0.15	0.15
Chub												0.15
Young wels catfish												
Adult wels catfish												

[+] Unchanged from the initial egestion coefficients

3.1.3 Phytoplankton

The new calibrated parameters of phytoplankton are listed in Table 3.6 for Cyclotella (Diatom) and Chromulina (Chrysophyte). For both organisms, respiration rate is decreased in order to reduce biomass losses due to the respiration process, otherwise biomass will rapidly go to zero. Light extinction coefficient is reduced assuming that light extinction process is already accounted by site-specific parameters chosen in chapter 2 (Table 3.6). Cyclotella appears strictly correlated to Bleak (predator), increasing Bleak biomass Cyclotella decreases. Chromulina varies very little when predator biomass varies because its concentration is on average 0.04 mg/L and most organisms start feed at higher prey concentrations.

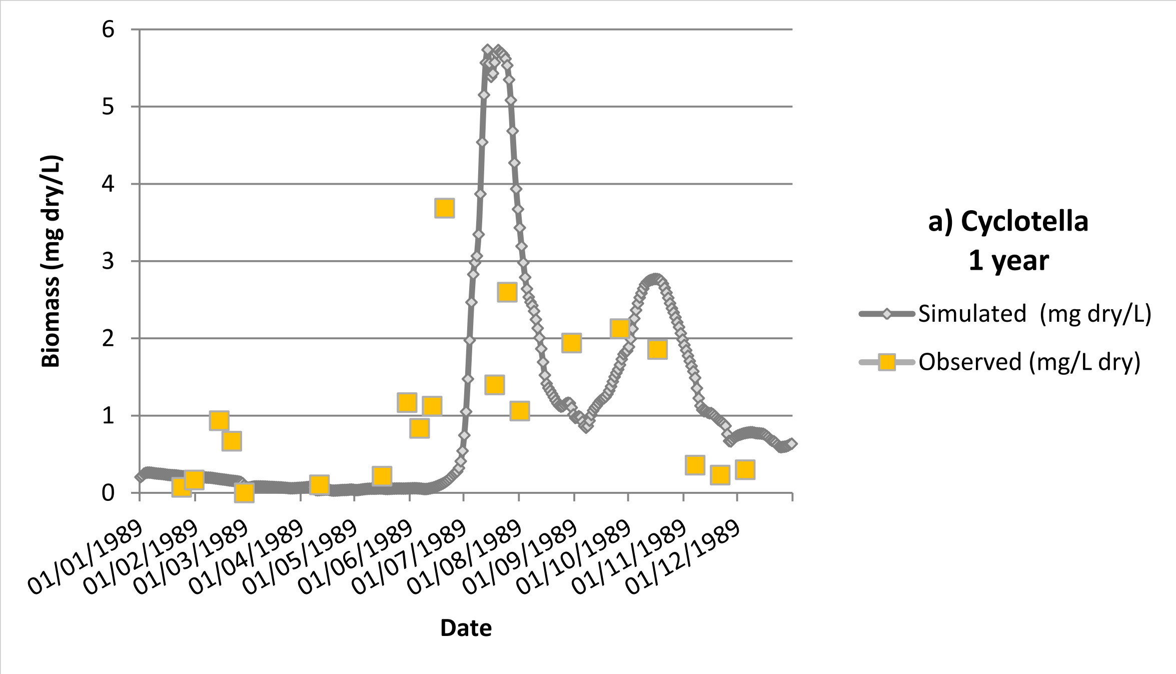
Table 3.6 - Cyclotella and Chromulina default and calibrated parameters (the star highlights changed parameters).

Parameter	Cyclotella		Chromulina	
	Initial	**Calibrated**	**Initial**	**Calibrated**
Saturating light (Ly/d)	22.5	22.5	67	67
Max. saturating light (Ly/d)	300	Not used		Not used
Min. saturating light (Ly/d)	22.5	Not used		Not used
P half-saturation (mg/L)	0.055	0.05 *	0.046	0.046
N half-saturation (mg/L)	0.117	0.117	0.006	0.006
Inorg. C half-saturation (mg/L)	0.054	0.054	0.054	0.054
Temp. response slope	1.8	1.8	2	1.8
Optimum temperature (°C)	20	23.5 *	25	20 *
Maximum temperature (°C)	35	30 *	35	35
Min. adaptation temperature (°C)	2	7 *	2	2
Max. Photosynthetic rate (1/d)	1.87	1.87	2	2
Photorespiration coefficient (1/d)	0.026	0.026	0.026	0.026
Respiration rate at 20°C (g/g-d)	0.08	0.0752 *	0.2	0.0483 *
Mortality coefficient (g/g-d)	0.001	0.001	0.01	0.009 *
Exponential mortality coefficient (g/g-d)	0.05	0.01 *	0.04	0.01 *
P : Organics	0.007	0.007	0.007	0.007
N : Organics	0.059	0.059	0.059	0.059
Light Extinction	0.14	0.02 *	0.144	0.01 *
Wet to Dry	5	5	5	5
Sedimentation rate (KSed) (m/d)	0.005	0.005	0.31	0.31
Exp sedimentation coefficient	0.05	0.05	0.693	0.693

Calibration results are graphically represented in Figures 3.7 and 3.8 for one year and three years simulation periods. Cyclotella has biomass observed values between 0 mg dry/L and 3.7 mg dry/L, while the simulated values are between 0 mg dry/L and 5.7 mg dry/L, the average biomass is 1.042 mg dry/L. The observed peaks in June and September are shifted in July and October in the simulation. Chromulina has biomass observed values between 0 mg dry/L and 0.16 mg dry/L, while the simulated values are between 0 mg dry/L and 0.13 mg dry/L, the average biomass is 0.042 mg dry/L. It has peaks in June and October and decreases in Spring, this trend is reproduced also in the simulation. As assessed by Garibaldi (1991), algae concentration variation seems to be independent from the water flow and from temperature, in fact the peak of Cyclotella is observed in Summer and Autumn, but in general Diatoms are peculiar of cold seasons. This can be due to the interaction of many factors including nutrients availability or predation. This trend is in line with pH variation (Figure), in fact the increase of pH in Summer can be caused by the increase in consume of CO_2 by algae, this shifts the balance between carbonate ion (CO_3^{2-}), deriving from the calcium carbonate of limestone rocks, and carbonic acid (HCO_3^-), deriving from the dissolution of CO_2 in water, towards right (37). Thus more hydroxide ions are formed and pH increases.

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \qquad (37)$$

For both species, seasonal trends are more or less maintained in three years simulation. For Cyclotella the peaks are in July in every year but their values increase respect one-year simulation until reaching the value of 9 mg dry/L in the second year (1989). For Chromulina in every year there is a peak of biomass in Autumn as in one year model, but the peak decrease progressively during years until reaching the value of 0.017 mg dry/L. For both species, analyzing the year 1989, it can be found that there are changes from one to three year simulation periods. This is probably due to different upstream values of nutrients or plankton that are generated in the three-years model with respect to one year simulation.



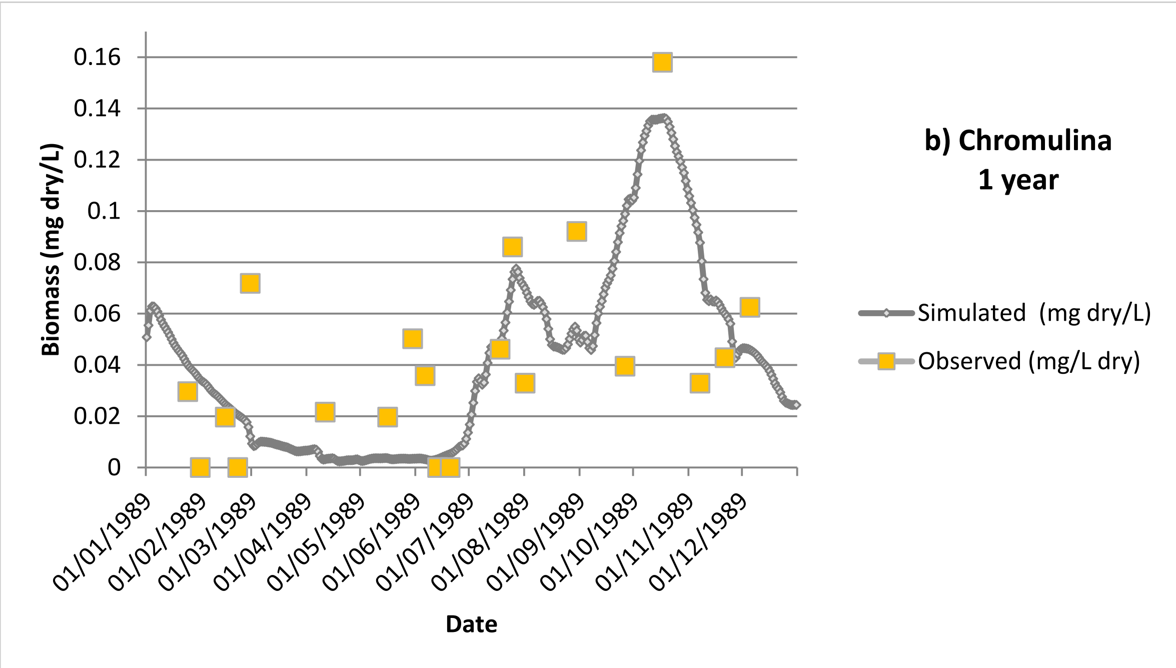
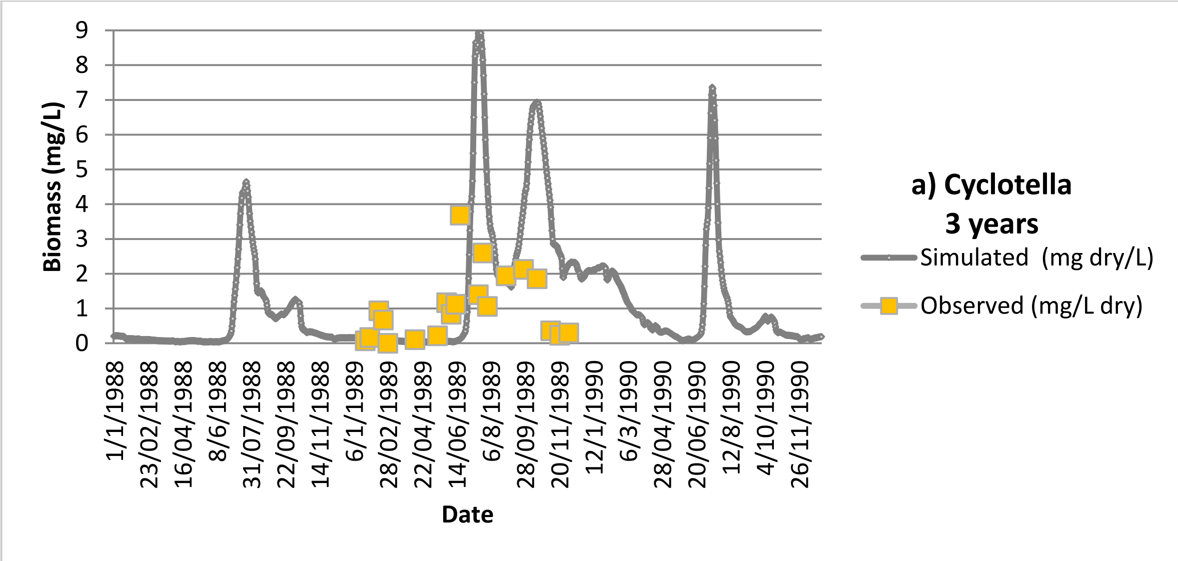


Figure 3.7 - One year (1989) control simulation results for a) Cyclotella and c) Chromulina.



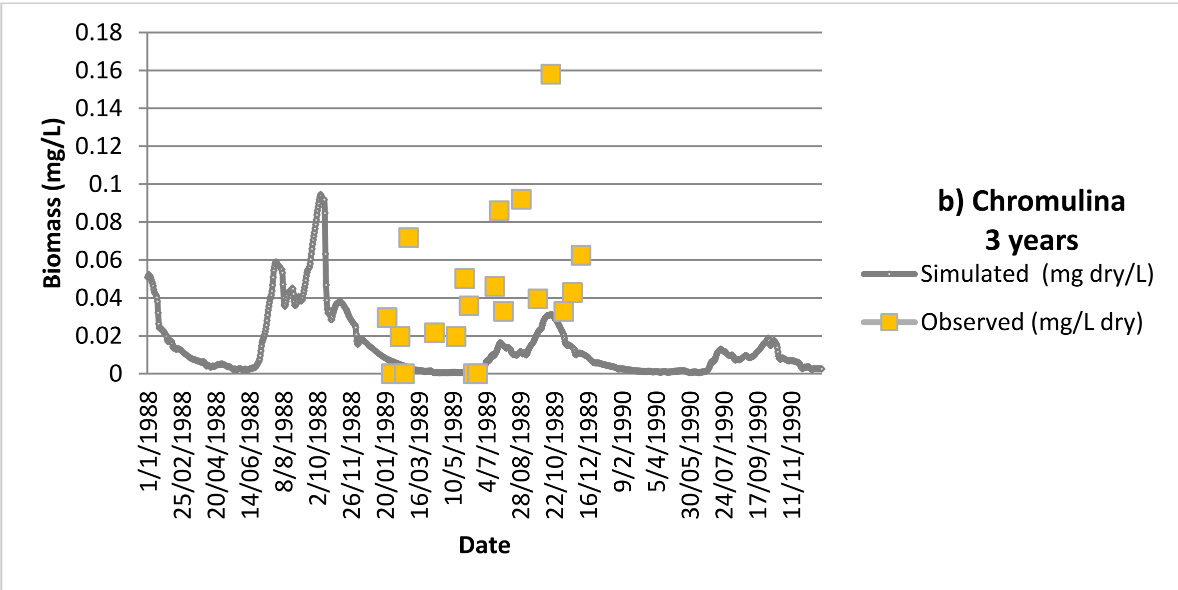


Figure 3.8 - Three years (1988-1990) control simulation results for a) Cyclotella and b) Chromulina.

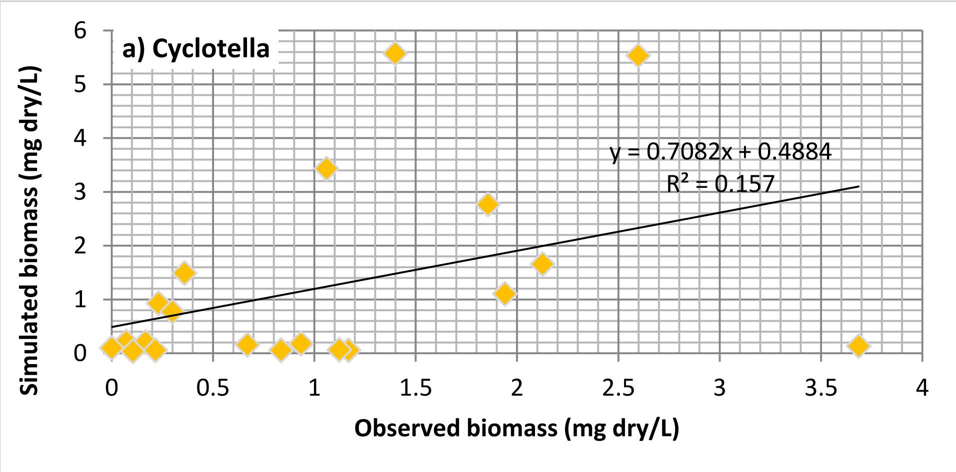
In Table 3.7 the resulting indices ε_i, $\varepsilon_{i(1-3)}$, r and E are shown and in Figure 3.9 the linear correlation between observed and simulated values is graphically represented. Regarding one year simulation, ε_i is less than 1 for both Cyclotella and Chromulina, this means that the model simulates correctly the average annual biomass. According to Table 3.3, $r_{(1)}$ Cyclotella value indicates that the correlation is regular, while for Chromulina is high. The model efficiency measured with NSE is not satisfactory for both Cyclotella and Chromulina, this can be caused by the fact that NSE coefficient is sensitive to extreme values because of squared differences.

Regarding three year simulation, the average biomass is of the same order of magnitude of one year average biomass ($\boldsymbol{\varepsilon_{i(1-3)}}$ <100) for both Cyclotella and Chromulina, this means that the three years model is at least stable.

In conclusion, the model simulates in a satisfactory way algal biomass trends in 1 year and 3 years.

Table 3.7 - Indices for the assessment of the goodness of the model for plants.

Taxa	Observed average biomass (mg dry/L)	1 year simulation average biomass (mg dry/L)	3 years simulation average biomass (mg dry/L)	ε_i (%)	$\varepsilon_{i(1-3)}$ (%)	r (1)	E (1)
Cyclotella	1.0421	1.0418	1.198	0.0249	15	0.396	-1.815
Chromulina	0.04205	0.04202	0.014	0.078	67.6	0.642	0.297



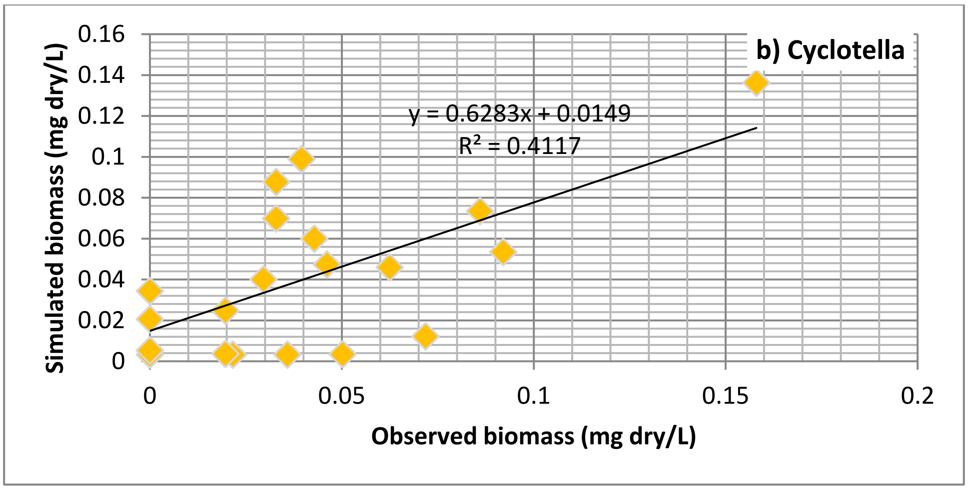


Figure 3.9 - Graphical correlation between observed and simulated biomass of a) Cyclotella and b) Chromulina for 1-year model.

3.1.4 Zooplankton

The new calibrated parameters of zooplankton are listed in Table 3.8 for the rotifer Brachionus. Parameters modified are mainly those connected to consumption as half saturation feeding, maximum consumption and minimum prey for feeding carrying capacity, and also the temperature. Zooplankton consumption is strictly connected to detritus formation, thus, if community consumption increases, more sediments are produced and also rotifer biomass increases. Moreover, Rotifer biomass varies accordingly to the variation of Cyclotella biomass because they feed on algae.

Table 3.8 - *Brachionus calyciflorus* default parameter from animal AQUATOX library (search scientific name Brachionus) and calibrated parameters values (the asterisk means parameters changed).

Parameter	Initial	Calibrated
Half saturation feeding (mg/L)	1	0.5 *
Maximum consumption (g/g*d)	3.4	3.355 *
Min. prey for feeding (g/m^2)	0.1	0.09 *
Temp. response slope	2	2
Optimum temperature (°C)	18	23 *
Maximum temperature (°C)	25	29 *
Min. adaptation temperature (°C)	5	4 *
Mean wet weight (g wet)	1.2 * 10^(-7)	1.2 * 10^(-7)
Endogenous respiration (1/d)	0.34	0.33 *
Specific dynamic action (unitless)	0	0
Excretion : respiration	0.17	0.17
N : Organics (frac dry)	0.09	0.09
P : Organics (frac dry)	0.014	0.014
Wet to dry	4.7	4.7
Gametes : biomass	0.18	0.18
Gamete mortality (1/d)	0.06	0.06
Mortality coefficient (1/d)	0.25	0.25
Sensitivity to sediments	zero	zero
Carrying capacity (g/m^2)	4	8 *
Vel max. (cm/s)	400	400
Mean lifespan (d)	4	4
Fraction that is lipid (wet wt.)	0.012	0.012

Calibration results are graphically represented in Figures 3.10 and 3.11 for one year and three years simulation periods. Brachionus has biomass observed values between 0 mg dry/L and 0.3 mg dry/L, while the simulated values are between 0 mg dry/L and 0.52 mg dry/L, the average biomass is 0.048 mg dry/L. Its biomass has a peak in July that corresponds to the Cyclotella peak, in fact, as said before, Rotifer feeds on algae and its trend is strictly related to Cyclotella biomass trend. Brachionus biomass slightly increases in April, this is due to the particulate detritus peak. In three years simulation the peak in July is maintained in every year, but in the second year it reach the value of 0.75 mg dry/L that is higher than relative simulated value in one year model (0.52 mg dry/L). As observed for phytoplankton this is probably due to different upstream values that are generated in the three-years model respect to one year model.

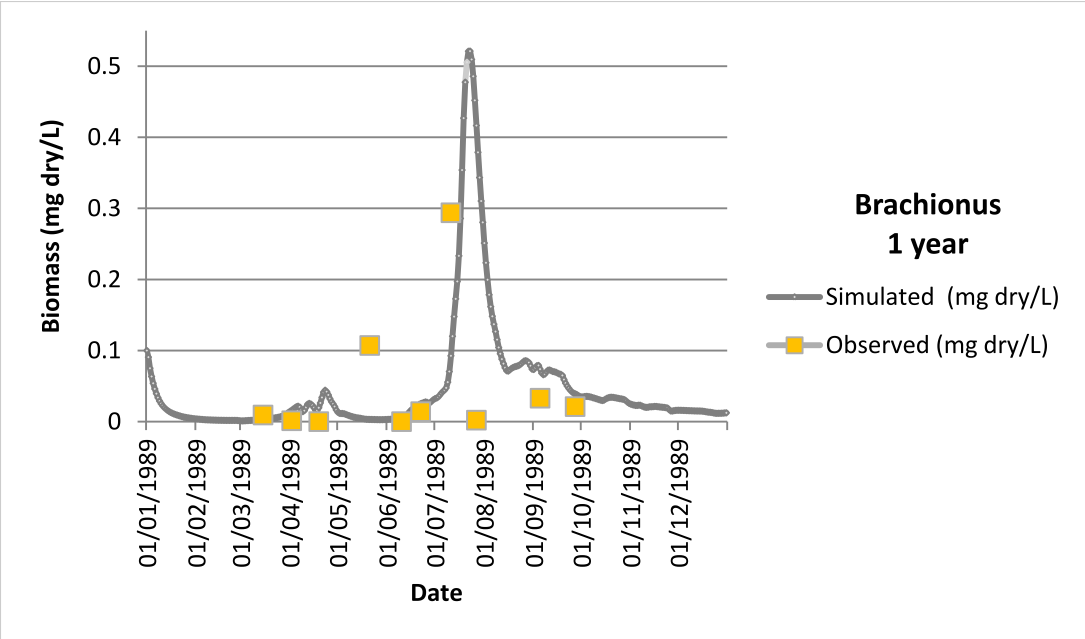


Figure 3.10- One year (1989) control simulation results for Brachionus rotifer.

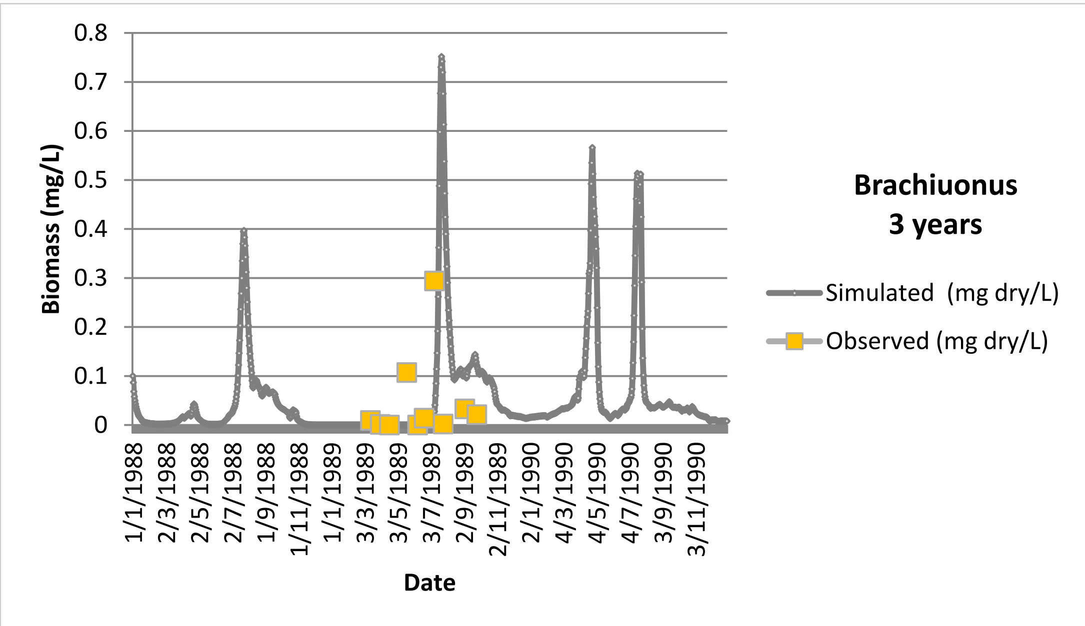


Figure 3.11 - Three years (1988-1990) control simulation results Brachionus rotifer.

In Table 3.9 the resulted indices ε_i, $\varepsilon_{i(1-3)}$, r and E are shown and in Figure 3.12 the linear correlation between observed and simulated values is graphically represented. Regarding one year simulation, ε_i is about 2 %, thus the model slightly underestimates the average annual biomass, this means that parameters values must be corrected, but in this case the result is satisfactory considering that observed biomasses are subjected to measure errors and also are referred to 1990 and not to 1989. Thus it is satisfactory that at least the trend is reproduced by the model. $r_{(1)}$ value indicates that there is no correlation between observed and simulated data, thus the model has to be improved from the point of view of seasonality. Efficiency measured with NSE is not satisfactory, this can be caused by the fact that NSE coefficient is sensitive to extreme values because of squared differences, and can also be related to the abovementioned problems with seasonality.

Regarding three year simulation, the average biomass is of the same order of magnitude of one year average biomass ($\boldsymbol{\varepsilon_{i(1-3)}}$ <100), this means that the three years model is at least stabile.

Table 3.9 - Indices for the assessment of the goodness of the model for rotifer Brachionus.

Taxa	Observed average biomass (mg dry/L)	1 year simulation average biomass (mg dry/L)	3 years simulation average biomass (mg dry/L)	ε_i (%)	$\varepsilon_{i(1-3)}$ (%)	$r_{(1)}$	$E_{(1)}$
Brachionus	0.048177	0.047075	0.054941	2.29	14.04	-0.0132	-1.956

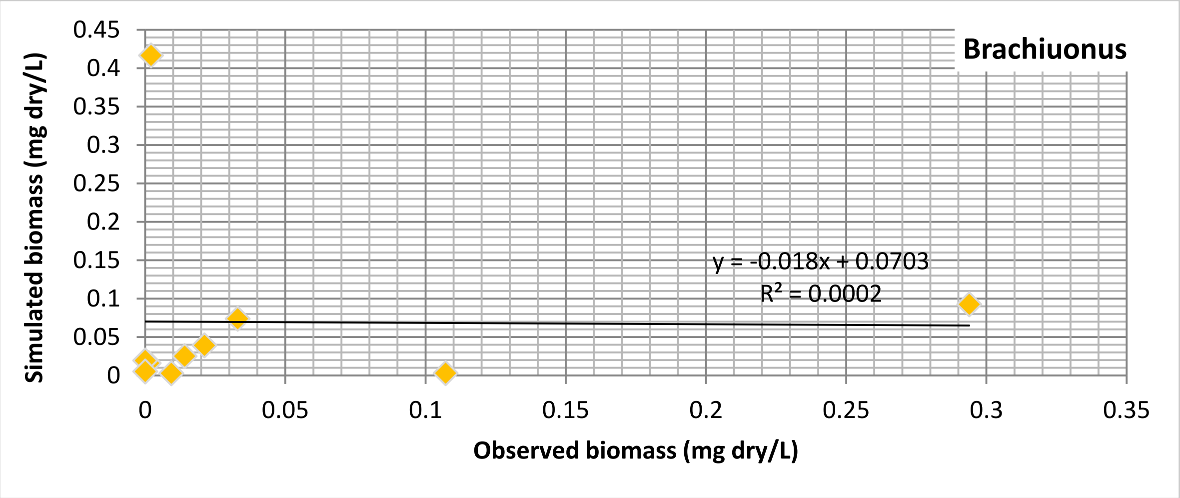


Figure 3.12 - Graphical correlation between observed and simulated biomass of Brachionus rotifer.

3.1.5 Macroinvertebrates

The new calibrated parameters for macroinvertebrates are listed in Table 3.10. Changes regard mainly parameters related to consumption and temperature.

Table 3.10 - Calibrated parameters values for macroinvertebrates (the asterisk means parameters changed).

Parameter	**Species**					
	Amphipoda	**Chironomus (larvae)**	**Trichoptera (larvae)**	**Oligochaeta**	**Gastropoda**	**Odonata (larvae)**
Half saturation feeding (mg/L)	0.9*	0.4*	0.05*	0.5*	0.5*	0.2*
Maximum consumption (g/g*d)	0.12*	0.6*	0.23*	0.36*	0.074*	1.4*
Min. prey for feeding (g/m^2)	0.9*	0.9*	0.1	0*	0.9*	0.05*
Temp. response slope	2*	1.62	2*	1.7*	1.4	2*
Optimum temperature (°C)	20	23*	22*	9*	20	22*
Maximum temperature (°C)	28*	35*	35	23.9*	38	25*
Min. adaptation temperature (°C)	5	4*	7.35*	5	3*	7.35*
Mean wet weight (g wet)	0.000037	0.0075	0.06	0.06	0.33	0.08
Endogenous respiration (1/d)	0.005	0.035	0.013	0.01	0.008	0.0286*
Specific dynamic action (unitless)	0.18	0.18	0	0.18	0.25	0.18
Excretion : respiration	0.17	0.17	0.17	0.17	0.17	0.17
N : Organics (frac dry)	0.09	0.014	0.09	0.014	0.09	0.09
P : Organics (frac dry)	0.014	0.014	0.01	0.014	0.01	0.014
Wet to dry	3.64	4.7	4.7	4.83	4.7	4.7
Gametes : biomass	0.01	0	0	0.09	0.1	0
Gamete mortality (1/d)	0.01	0	0	0.01	0.9	0
Mortality coefficient (1/d)	0.02	0.001	0.0004*	0.001	0.000189	0.002
Sensitivity to sediments	Zero	Zero	Zero	Zero	Zero	Zero
Carrying capacity (g/m^2)	0.1	25	10*	11*	30	5
Vel max. (cm/s)	400	125	250	200	400	400
Mean lifespan (d)	182	365	365	1000	1825	365
Fraction that is lipid (wet wt.)	0.012	0.013	0.0013	0.0075	0.013	0.013

Calibration results are graphically represented in Figures 3.13 and 3.14 for one year and three year simulation period.

Analysing 1 year simulation, Amphipoda have biomass observed values between 0.001 g dry/m^2 and 0.01 g dry/m^2, while the simulated values are between 0.002 g dry/m^2 and 0.0063 g dry/m^2, the average biomass is 0.00376 g dry/m^2. There is a peak in November and December, and a decrease in March. Amphipoda trend is strongly influenced by the maximum consumption parameter value, while it is slightly influenced by predation because Amphipoda concentrations are most of time lower than the "minimum prey for feeding" value of their predators. In three years simulation the annual average biomass is not maintained, an evident accumulation of biomass takes place followed by a bloom occurred in April 1990 when the concentration increases up to 0.05. This behaviour is probably connected to the fact that Amphipoda predation is underestimated by the model and thus it is connected to "minimum prey for feeding" value of predators.

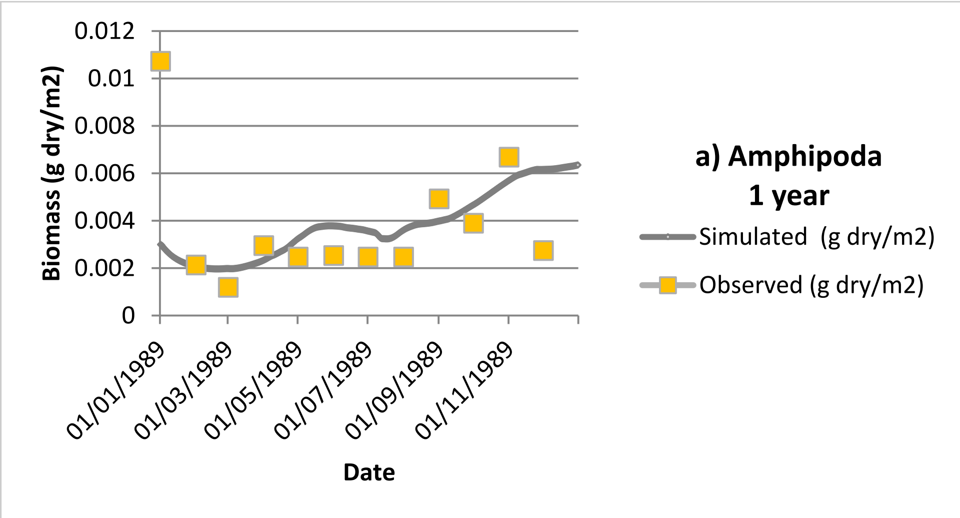
Chironomids have biomass observed values between 0 g dry/m^2 and 1.44 g dry/m^2, while the simulated values are between 0 g dry/m^2 and 1.05 g dry/m^2, the average biomass is 0.15 g dry/m^2. The simulated peak does not correspond to the observed one, this is because in the model Chironomids feed mainly on detritus and in fact the modelled peak is in May when system detritus increase. In three years simulation the annual biomass trend is maintained.

Oligochaeta have biomass observed values between 0 g dry/m^2 and 0.7 g dry/m^2, while the simulated values are between 0 g dry/m^2 and 0.65 g dry/m^2, the average biomass is 0.15 g dry/m^2. The simulated peak does not correspond to the observed one, this is because, as said before for Chironomids, in the model Oligochaeta feed mainly on detritus. In three years simulation the peak in May is maintained in every year but its value increases progressively in the three years until reaching 1.5 g dry/m^2 in the last year.

Trichoptera have biomass observed values between 0.0065 g dry/m^2 and 0.098 g dry/m^2, while the simulated values are between 0.03 g dry/m^2 and 0.06 g dry/m^2, the average biomass is 0.047 g dry/m^2. There is a decrease in June and a peak in August, this behaviour is probably due to Odonata predation. In three years simulation there is a biomass decrease in the first year, in the second year the trend is similar to one year simulation and in the third year there is a peak in July.

Gastropoda have biomass observed values between 0 g dry/m^2 and 0.0053 g dry/m^2, while the simulated values are between 0 g dry/m^2 and 0.0055 g dry/m^2, the average biomass is 0.0015 g dry/m^2. Observed biomass trend is not well reproduced by the model for the same reasons written for Amphipoda, in fact biomass increases exponentially because predation is not simulated as organism biomass concentration is too low. In three years simulation the annual biomass trend is not maintained for the same reasons written for Amphipoda, and Gastropoda biomass have an high increase from January to May of the second year, then it stabilizes.

Odonata have biomass observed values between 0 g dry/m^2 and 0.33 g dry/m^2, while the simulated values are between 0 g dry/m^2 and 0.4 g dry/m^2, the average biomass is 0.066 g dry/m^2. It has a peak in May 1989. Observed biomass trend is not well reproduced by the model. I the simulation biomass concentration is influenced by prey concentration, in fact Odonata curve has the same behaviour of Chironomus, Oligochaeta curves. In three years simulation the annual biomass trend is maintained in the first year when there is a peak in March, while in the third year the peak is in January, moreover peaks values are higher than those simulated in the one-year model.



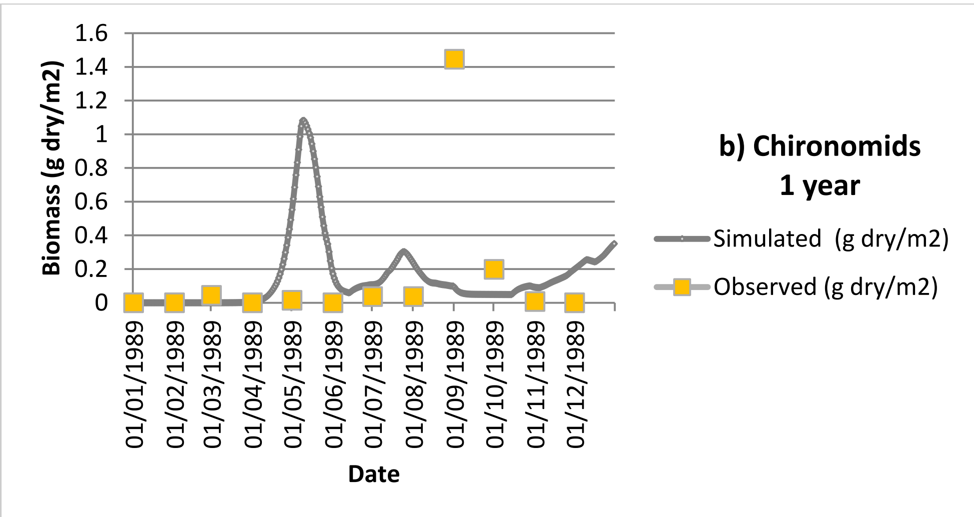
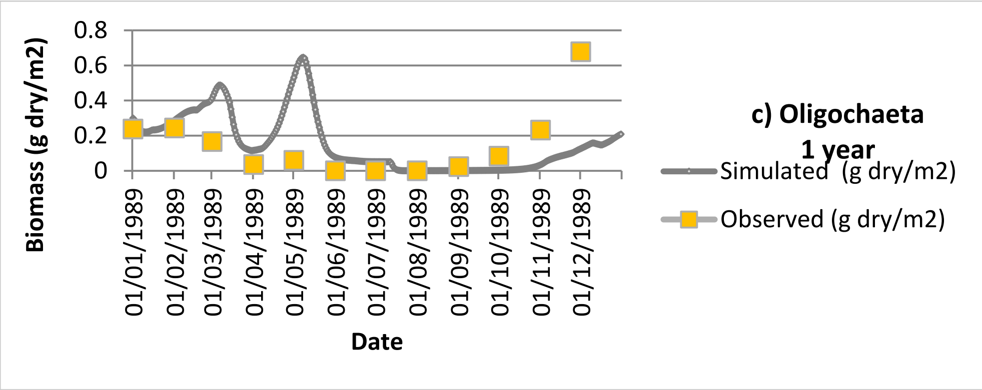
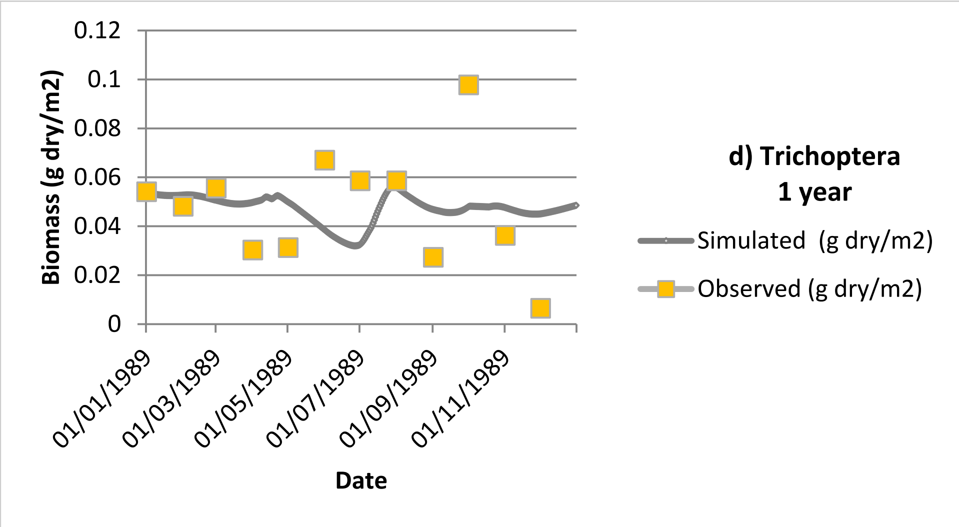


Figure 3.13 - One year (1989) control simulation results for a) Amphipoda, b) Chironomus.





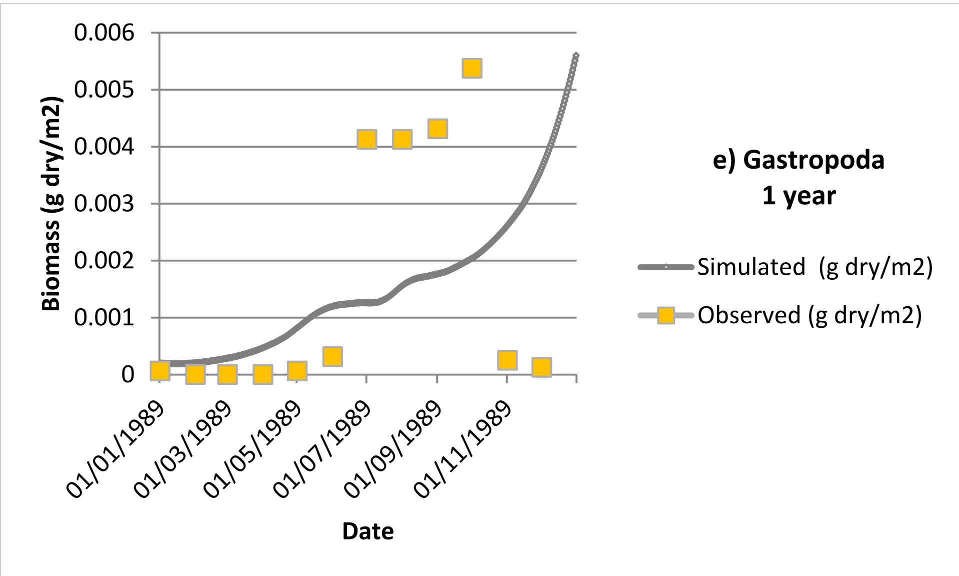


Figure 3.13 - One year (1989) control simulation results for c) Oligochaeta,d) Trichoptera, e) Gastropoda.

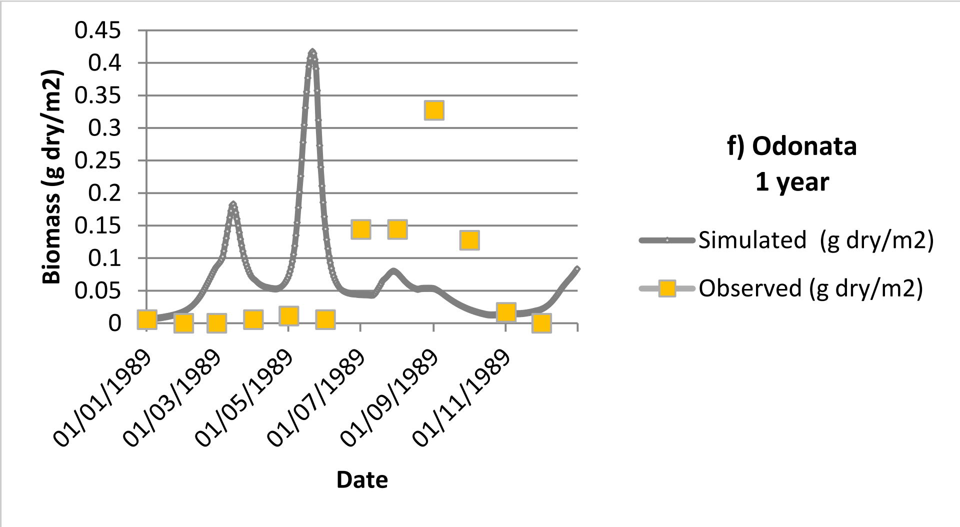
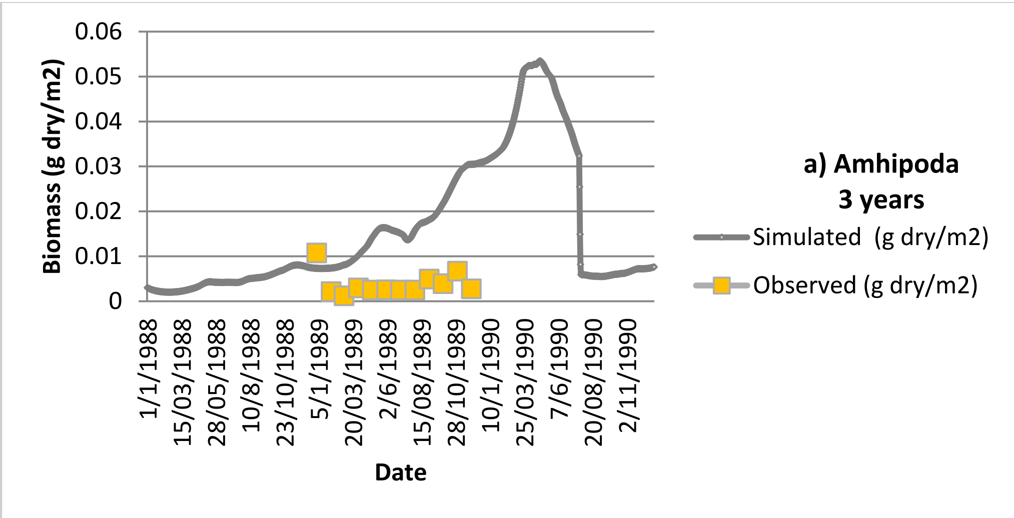


Figure 3.13 - One year (1989) control simulation results for f) Odonata



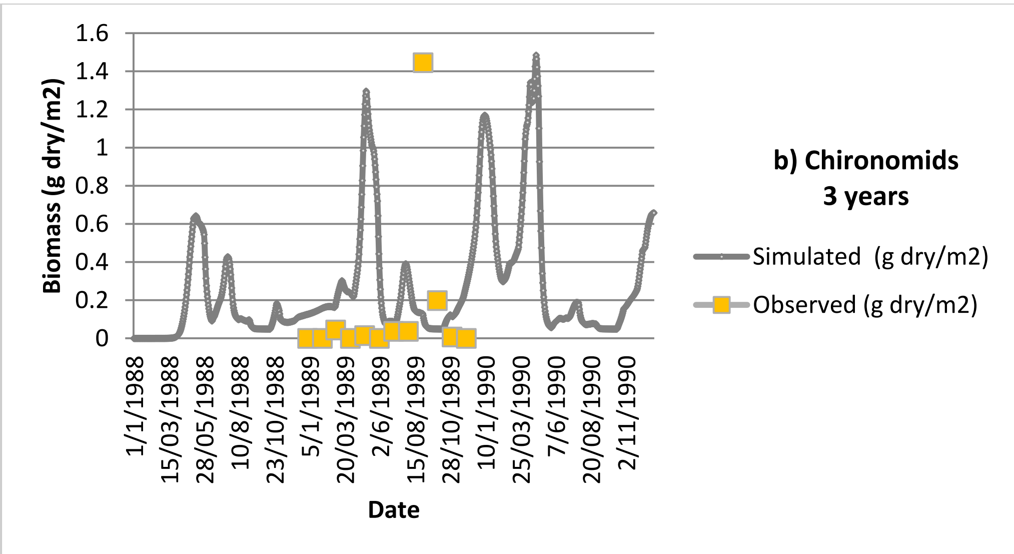
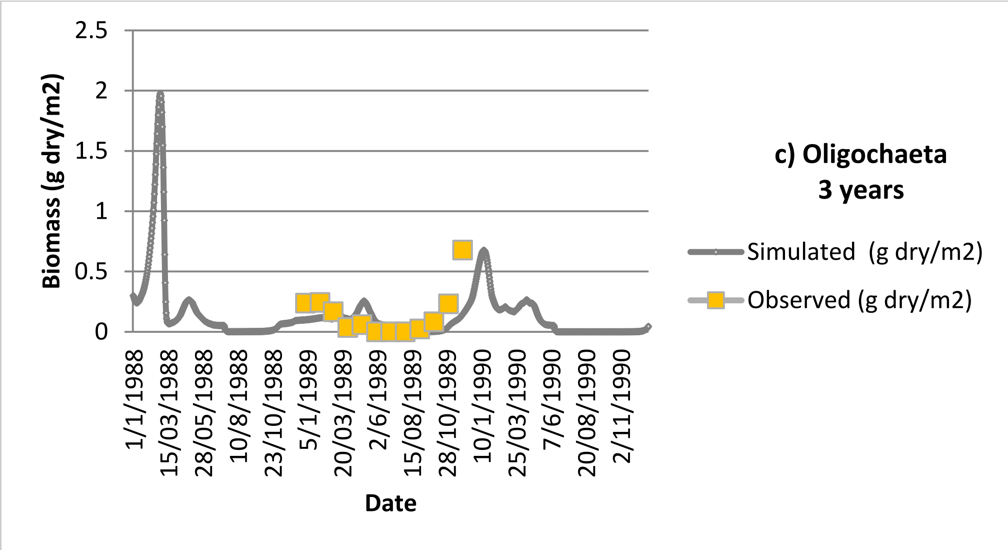
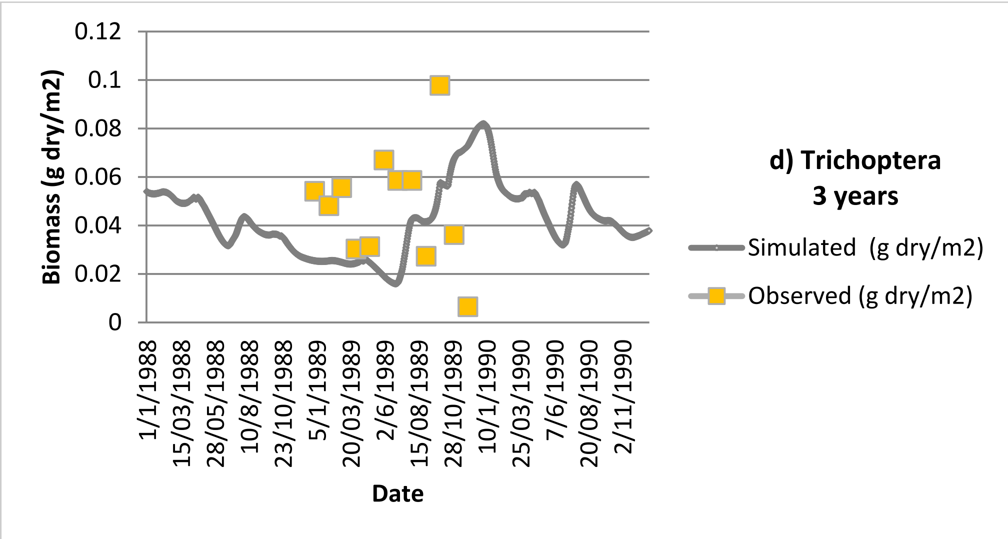


Figure 3.14 - Three years control simulation results for a) Amphipoda, b) Chironomus.





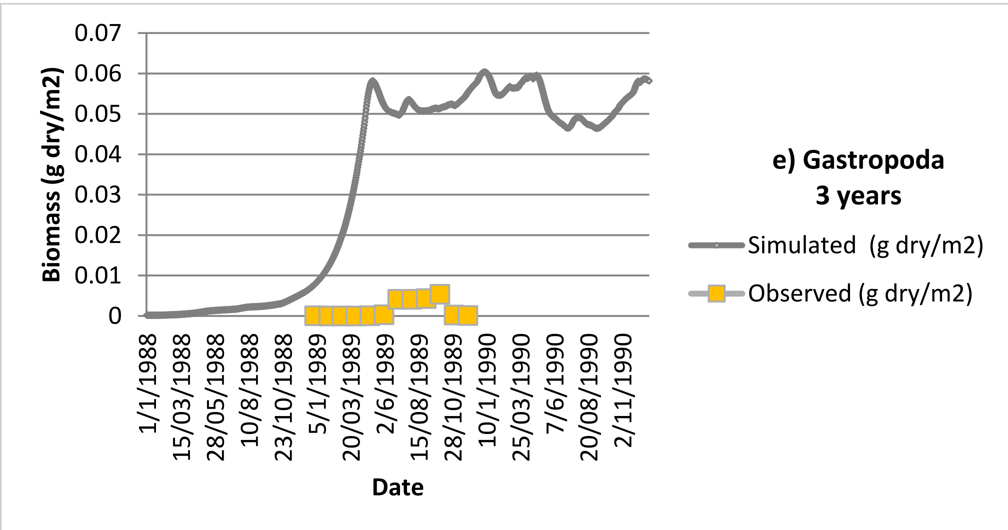


Figure 3.14 - Three years control simulation results for, c) Oligochaeta, d) Trichoptera, e) Gastropoda.

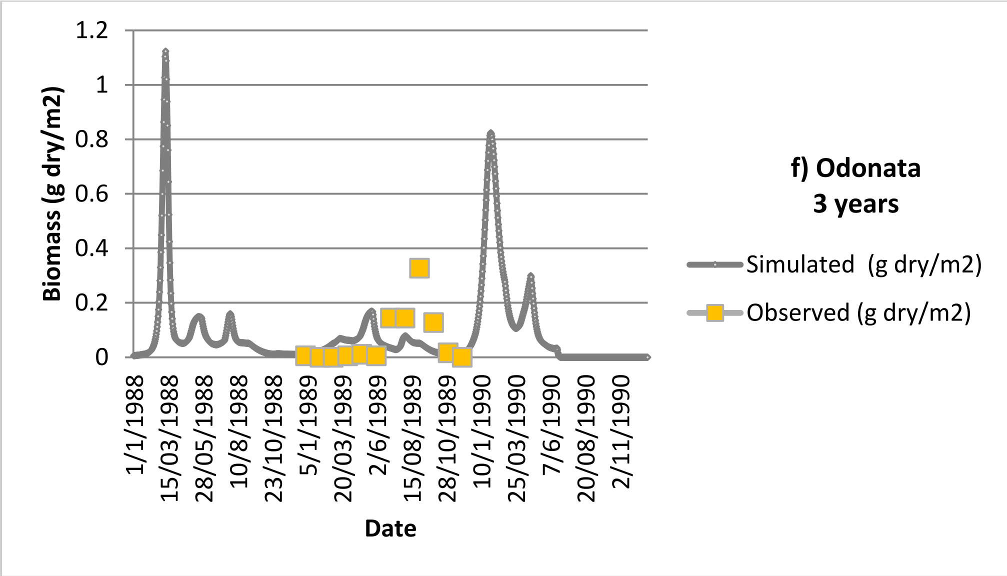


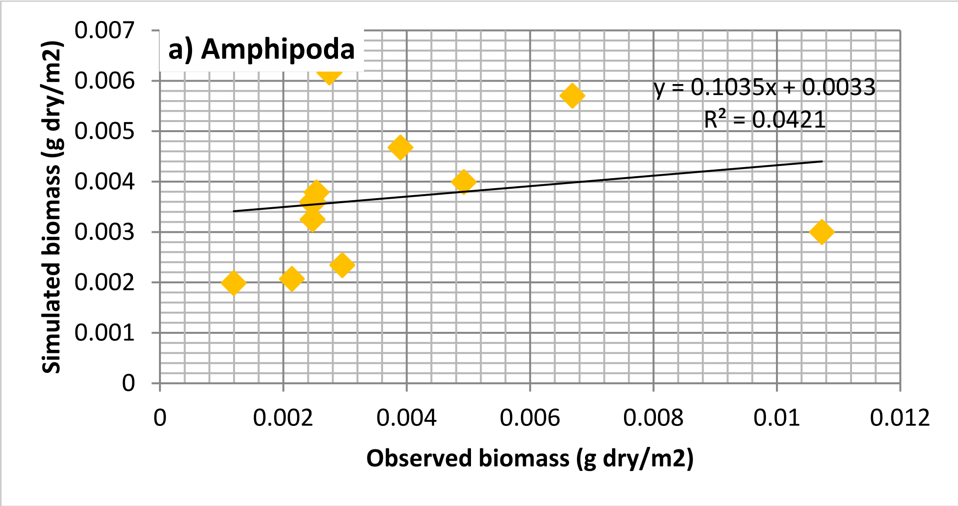
Figure 3.14 - Three years control simulation results for f) Odonata.

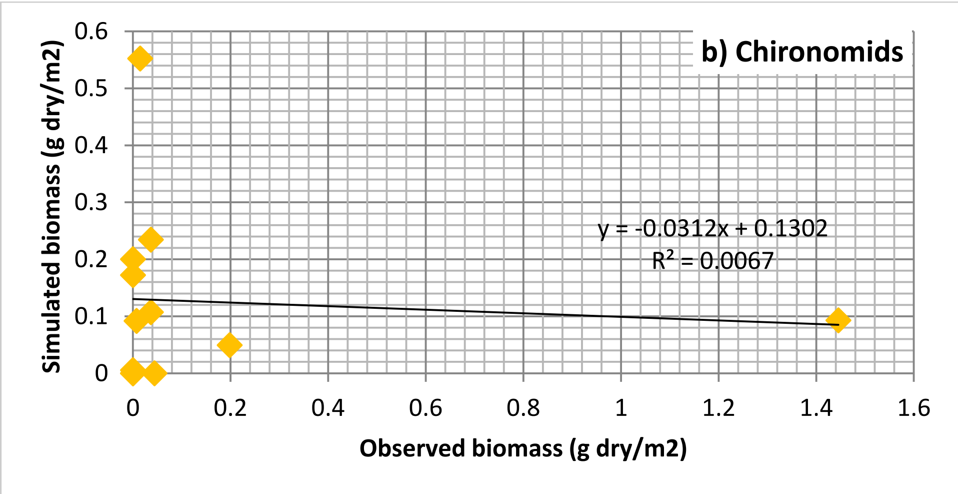
In Table 3.11 the resulting indices ε_i, $\varepsilon_{i(1-3)}$, r and E are shown and in Figure 3.15 the correlation between observed and simulated values is graphically represented. Regarding one year simulation, ε_i is less than 1 % for Amphipoda, Trichoptera, Oligochaeta, Gastropoda, Odonata, this means that the model simulates at least the average annual biomass. Only for Chironomids ε_i is about 3 %, the model slightly overestimates the average annual biomass, this means that parameters values must be corrected, but in this case the result is satisfactory considering that observed biomasses are subjected to measure errors. For Amphipoda, Trichoptera, Oligochaeta and Gastropoda, r $_{(1)}$ value indicates that there is very low correlation between observed and simulated data, while for Chironomids and Odonata that there is no correlation, thus the model has to be improved with regard to seasonality. Efficiencies measured with NSE are not satisfactory, this can be caused by the fact that NSE coefficient is sensitive to extreme values because of squared differences.

Regarding three year simulation, for Chironomids, Trichoptera, Oligochaeta and Odonata the average biomass is of the same order of magnitude of one year average biomass ($\boldsymbol{\varepsilon_{i(1-3)}}$ <100), this means that the three years model is at least stable. For Amphipoda and Gastropoda in three years simulation there is a relatively large biomass overestimation that is due to the bloom of the two species described before.

Table 3.11 - Indices for the assessment of the goodness of the model for macroinvertebrates.

Taxa	Observed average biomass (g dry/m^2)	1 year simulation average biomass (g dry/m^2)	3 years simulation average biomass (g dry/m^2)	ε_i (%)	$\varepsilon_{i(1-3)}$ (%)	r (1)	E (1)
Amphipoda	0.00376	0.003801	0.0161	1.00	323	0.205	-0.049
Chironomus (larvae)	0.1486	0.153	0.269	2.95	76	-0.082	-0.211
Trichoptera (larvae)	0.0475	0.0473	0.0423	0.63	10.6	-0.0695	-0.117
Oligochaeta	0.147	0.153	0.139	4.40	9.1	0.145	-0.592
Gastropoda	0.001563	0.001547	0.0329	0.985	2003	0.263	0.008
Odonata (larvae)	0.065604	0.066104	0.086	0.76	31.6	-0.036	-0.204





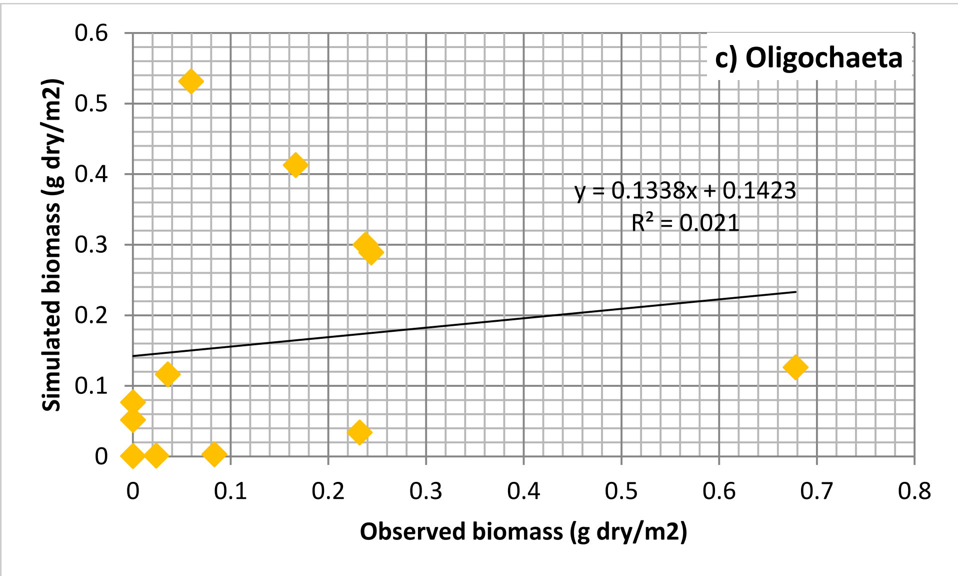
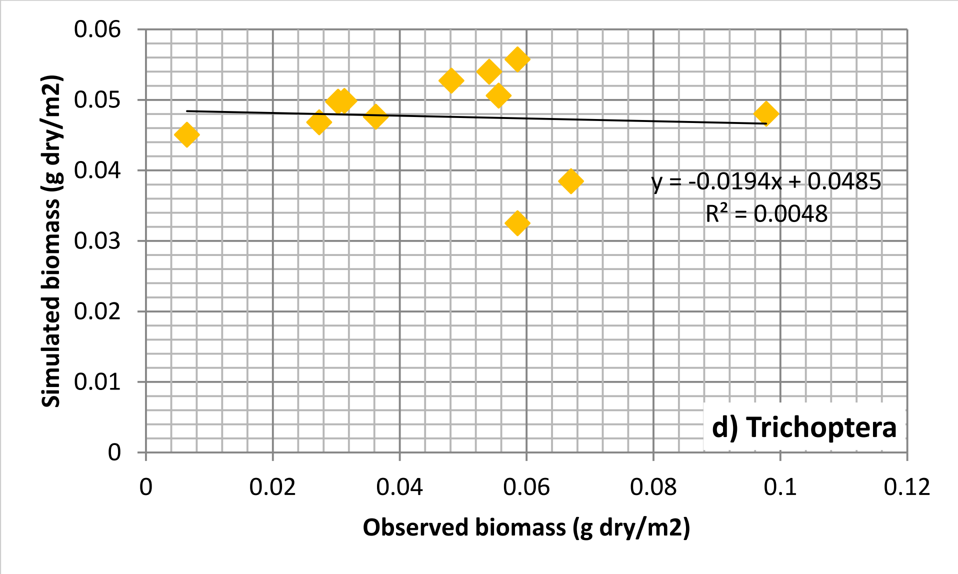
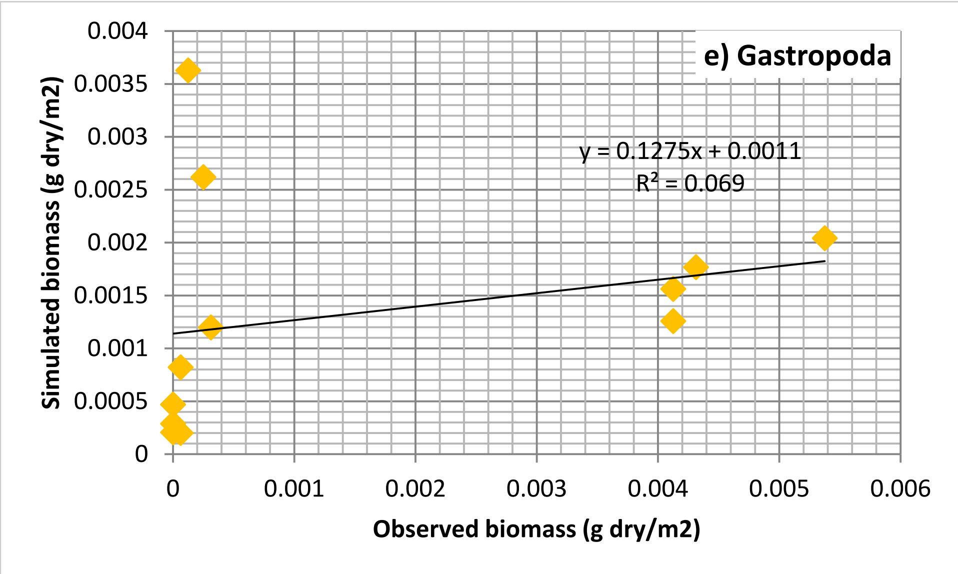


Figure 3.15 - Graphical correlation between observed and simulated biomass of a) Amphipoda, b) Chironomus, c) Oligochaeta.





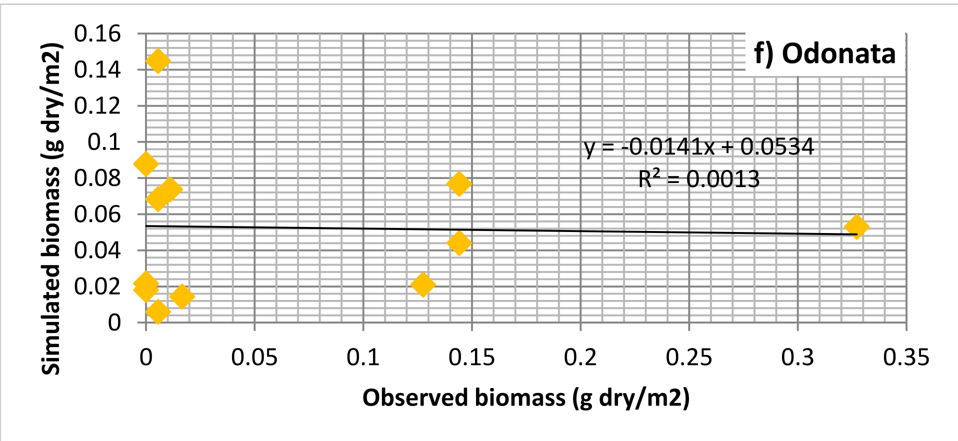


Figure 3.15 - Graphical correlation between observed and simulated biomass of c) Trichoptera, , e) Gastropoda, f) Odonata.

3.1.6 Fishes

The new calibrated parameters for fishes are listed in Table 3.12. Variations involve mainly consumption-related parameters and temperature values.

Table 3.12 - Calibrated parameters values for fishes (the asterisk means parameters changed).

Parameter	Species			
	Bleak	**Chub**	**Young Wels catfish**	**Adult Wels catfish**
Half saturation feeding (mg/L)	0.05*	0.01*	0.9*	1
Maximum consumption (g/g*d)	0.14447*	0.54*	0.733*	20*
Min. prey for feeding (g/m^2)	0.3*	1*	0.05*	2.25*
Temp. response slope	2*	2*	2*	2*
Optimum temperature (°C)	24*	17*	20*	17*
Maximum temperature (°C)	30*	30*	30*	28*
Min. adaptation temperature (°C)	3*	3*	2*	3*
Mean wet weight (g wet)	3.6	329	43.76	3150
Endogenous respiration (1/d)	0.025	0.008*	0.0004	0.004
Specific dynamic action (unitless)	0.15	0.1*	0.172	0.172
Excretion : respiration	0.05	0.05	0.05	0.05
N : Organics (frac dry)	0.01	0.03	0.03	0.03
P : Organics (frac dry)	0.025	0.0149	0.031	0.031
Wet to dry	3.7	3.7	4.5	3.7
Gametes : biomass	0.09	0.09	0.1	0.3
Gamete mortality (1/d)	0.9	0.9	0.9	0.01
Mortality coefficient (1/d)	0.006	0.0008*	0.01	0.0003
Sensitivity to sediments	Zero	Zero	Zero	Zero
Carrying capacity (g/m^2)	4*	0.8	0.1*	0.1886*
Vel max. (cm/s)	400	400	400	400
Mean lifespan (d)	730	365	730	730
Fraction that is lipid (wet wt.)	0.02	0.02	0.03	0.03

Calibration results are graphically represented in Figures 3.16 and 3.17 for one year and three years simulation periods.

Analysing 1 year simulation, Bleak has biomass observed values between 0.044 g dry/m^2 and 0.86 g dry/m^2, while the simulated values are between 0.24 g dry/m^2 and 1.07 g dry/m^2, the average biomass is 0.56 g dry/m^2. There is a peak in August and September, possibly connected to the bloom of diatoms, as Bleak feed on them. In three years simulation there is a pick in July of the first year, another in September of the second year and the last in July of the third year. Chub has biomass observed values between 2.07 g dry/m^2 and 3.27 g dry/m^2, while the simulated values are between 2.07 g dry/m^2 and 2.7 g dry/m^2, the average biomass is 2.52 g dry/m^2. Simulated trend has slight peaks in Spring and Autumn, aligned with observed peaks that are however more accentuated. In three years simulation the annual biomass trend is maintained as biomass remains almost constant. Young wels catfish has biomass observed values between 1 g dry/m^2 and 13 g dry/m^2, while the simulated values are between 2.75 g dry/m^2 and 8.8 g dry/m^2, the average biomass is 5.73 g dry/m^2. There is a peak in April due to the peak of preys (macroinvertebrates), another peak results in November but this is in contrast with observed data, this is possibly a model problem due to error in calibrating Young wels catfish consumption parameters, or to some ecological process relevant for describing its biomass dynamics which is not included in the model. In three years simulation the annual biomass fluctuate with slight variation around the value of 8 g dry/m^2 with a peak in November of the first year. Adult wels catfish has biomass observed values between 0.96 g dry/m^2 and 12.8 g dry/m^2, while the simulated values are between 2.3 g dry/m^2 and 25 g dry/m^2, the average biomass is 5.58 g dry/m^2. Biomass has a peak in April that corresponds to the peak of preys (macroinvertebrates). In three years simulation the annual biomass trend is maintained with a peaks in April in every year, with the exception of a pick in October of the first year.

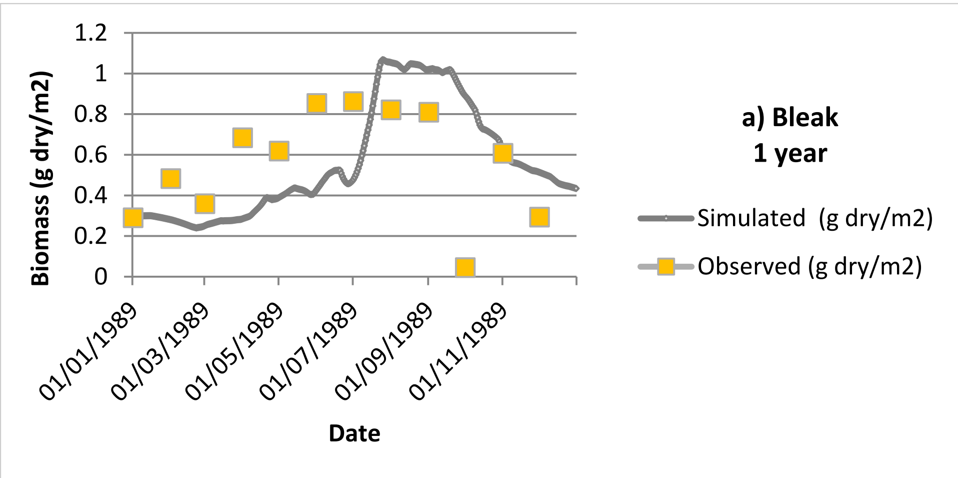


Figure 3.16 - One year (1989) control simulation results for a) Bleak.

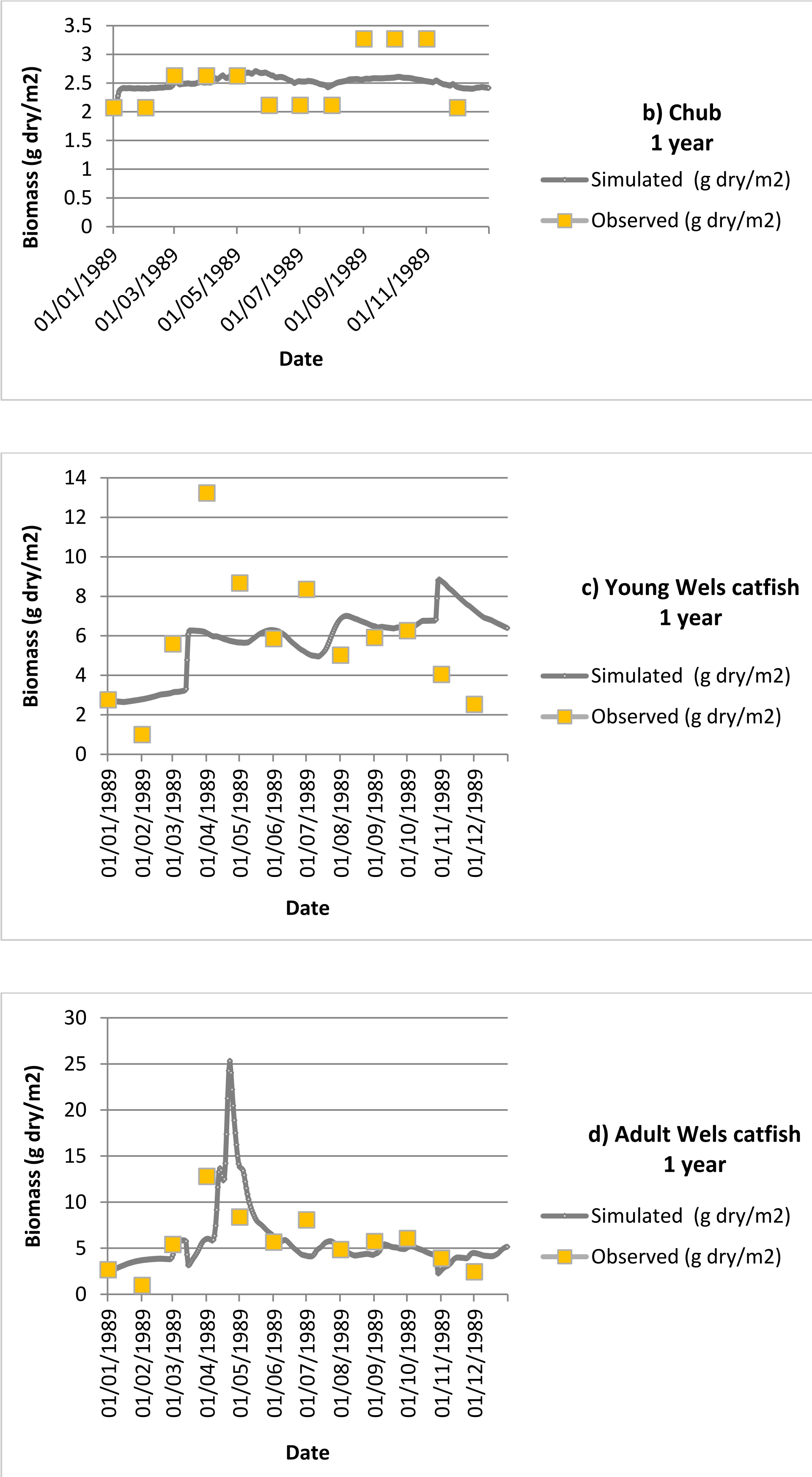


Figure 3.16 - One year (1989) control simulation results for b) Chub, c) young and d) adult Wels catfish.

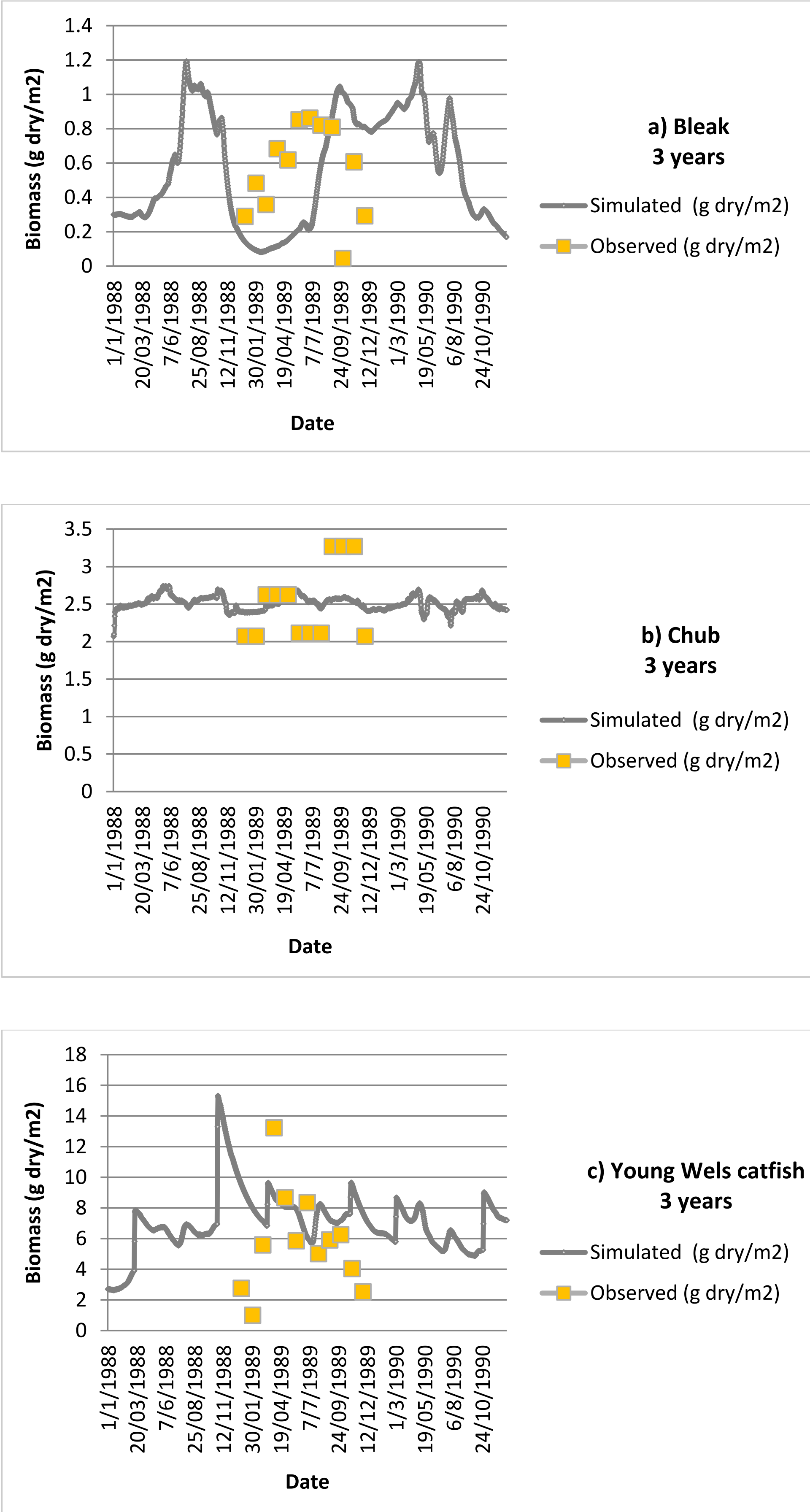


Figure 3.17 - Three years control simulation results for a) Bleak, b) Chub, c) young Wels catfish.

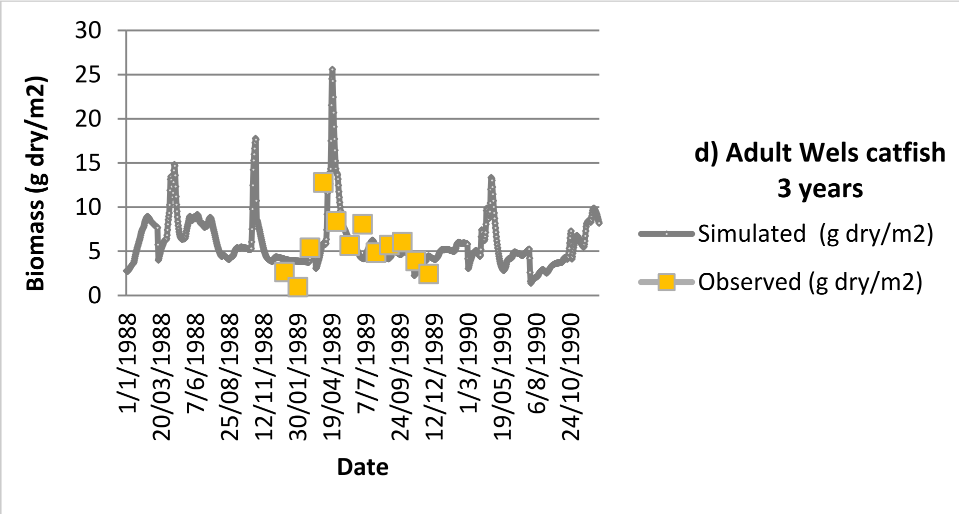


Figure 3.17 - Three years control simulation results for d) adult Wels catfish.

In Table 3.13 the resulted indices ε_i, $\varepsilon_{i(1-3)}$, r and E are shown and in Figure 3.18 the linear correlation between observed and simulated values is graphically represented. Regarding one year simulation, ε_i is less than 1 % for all the fishes, this means that the model simulates reliably the average annual biomass. For all the species of fish, r $_{(1)}$ value indicates that there is a relatively low correlation between observed and simulated data, thus the model seasonality could be improved. Efficiencies measured with NSE are not satisfactory, this can be caused by the fact that NSE coefficient is sensitive to extreme values because of squared differences, or to the abovementioned seasonality issue.

Regarding three year simulation, for all the fishes the average biomass is of the same order of magnitude of one year average biomass ($\boldsymbol{\varepsilon_{i(1-3)}}$ <100), this means that the three years model is, at least, stable.

Table 3.13 - Indices for the assessment of the goodness of the model for fish.

Taxa	Observed average biomass (g dry/m^2)	1 year simulation average biomass (g dry/m^2)	3 years simulation average biomass (g dry/m^2)	ε_i (%)	$\varepsilon_{i(1-3)}$ (%)	r (1)	E (1)
Bleak	0.56	0.559	0.56	0.206	0.576	0.159	-0.852
Chub	2.52	2.52	2.516	0.043	0.147	0.425	0.167
Young wels catfish	5.767	5.733	7.043	0.599	22.11	0.21	-0.095
Adult wels catfish	5.579	5.58	5.87	0.023	5.16	0.475	0.009

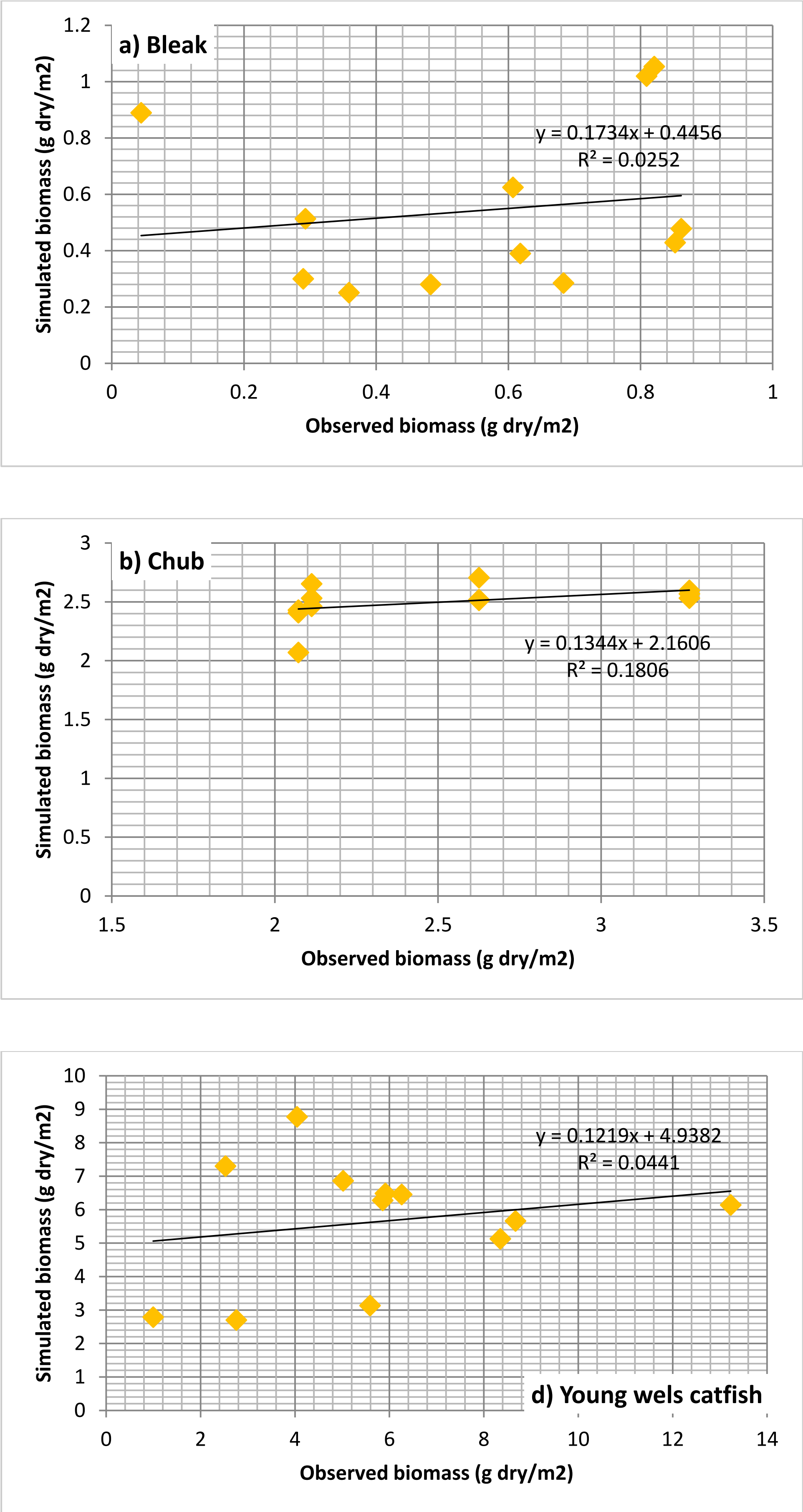


Figure 3.18 - Graphical correlation between observed and simulated biomass of a) Bleak, b) Chub, c) young and d) adult Wels catfish.

3.2 Perturbed ecosystem

In this paragraph the results of perturbed ecosystem are presented and compared with the control results of Paragraph 3.1. The one year and three years models are perturbed with three different input concentrations values of LAS and TCS as described in Paragraph 2.9, in order to assess the effects of the two chemicals on the ecosystem for a short term (1 year) and a longer term (3 years) simulations. The results are mathematically analyzed with methods described in Paragraph 2.10.

3.2.1 1 year simulation

The 1 year model described in Paragraph 3.1 is perturbed with different concentrations of LAS and TCS. Two distinct simulations are done for each chemical in order to avoid interaction or synergies and to assess the effects of the single substance in the environment.

3.2.1.1 LAS perturbation

As described in § 2.9, the system is perturbed with three different constant concentrations inputs of LAS: LAS 1 (C = 3.22 μg/L) that corresponds to the assessed actual concentration in the Po river segment, LAS 2 (C = 460 μg/L) that is equal to the EC50 of the most sensitive organism (adult wels catfish) and LAS 3 (C = 770 μg/L) that is equal to the LC50 of the most sensitive organism (adult wels catfish). In Figure 3.19 results of perturbed scenarios are shown and compared with control trends. For all organisms simulated, LAS 1 concentration input seems to have no effects on biomass trend, as the resulted curves overlap the control ones. For this reason the analysis is focused only on LAS 2 and LAS 3 scenarios.

Algal peaks progressively decrease in scenarios 2 and 3. Both Cyclotella and Chromulina have high LC50 and EC50 values, thus their biomass reduction is probably mainly due to indirect effects as a slight increase in predators biomass (see Bleak and Chironomids) (Figure 3.19 -a, b).

Brachionus rotifer presents a slight increase in biomass in April and September and a slight decrease in July for both scenarios (Figure 3.19 - c).

Amphipoda biomass does not change in scenario 2, while in scenario 3 it shows a slight increase in June (Figure 3.19 - d).

Chironomids biomass does not change in scenario 2, while in scenario 3 it shows a bloom in the month of May, where the density is almost the double with respect to the unperturbed scenario. This is probably related to the decrease, in the same period, of the biomass of Odonata and Chub, the principal Chironomids predators (Figure 3.19 -e).

Oligochaeta and Gastropoda biomass progressively decreases in scenarios 2 and 3, this can be due to direct effects of LAS (Figure 3.19 - f, h). Trichoptera biomass slightly increases from January to July and visibly decreases from July to December for both scenarios (Figure 3.19 - g). Odonata biomass has an opposite trend respect to Trichoptera, it visibly decreases from January to July and slightly increases from July to December for both scenarios (Figure 3.19 - i). As Odonata is the principal Trichoptera predator, this suggest that Trichoptera biomass trend is influenced by Odonata trend (a so called “top down” trophic control”), but the contrary is not true, so if Odonata biomass increases , predation upon Trichoptera increases too and Trichoptera biomass decreases.

Bleak biomass increases from January to July and slightly decreases from July to December (Figure 3.19 - j). As Bleak is one of the principal Odonata predation, it can explain the Odonata biomass trend: if Bleak biomass increases, predation upon Odonata increases too and Odonata biomass decreases. Bleak biomass trend is possibly a result of changes in predation by Chub, and young-adult Wels catfish. Chub biomass does not change in scenario 2 while in scenario 3 it visibly decreases from January to May. This can be due to the absence of Odonata that is one of the principal prey of the fish (Figure 3.19 - k). Young and adult Wels catfish show a progressive decrease in biomass for both scenarios, this is because they are the most sensitive organisms to the studied chemical (Figure 3.19 - l, m).

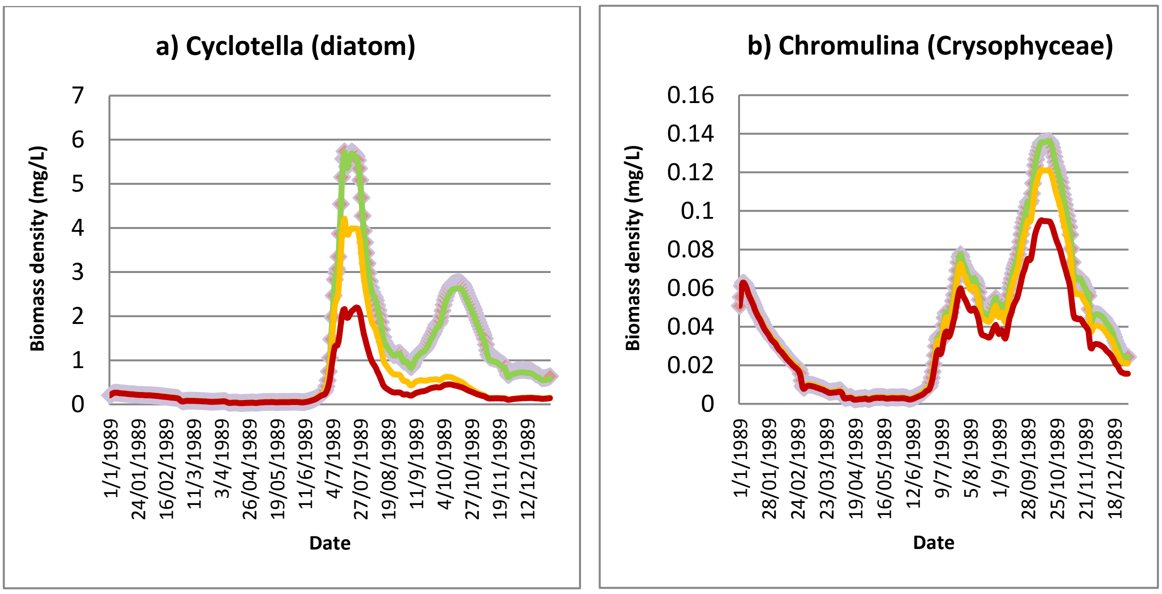


Figure 3.19 - Biomass density trends in 1 year simulation with four different scenarios:

control LAS 1 (C = 3.22 µg/L) LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)

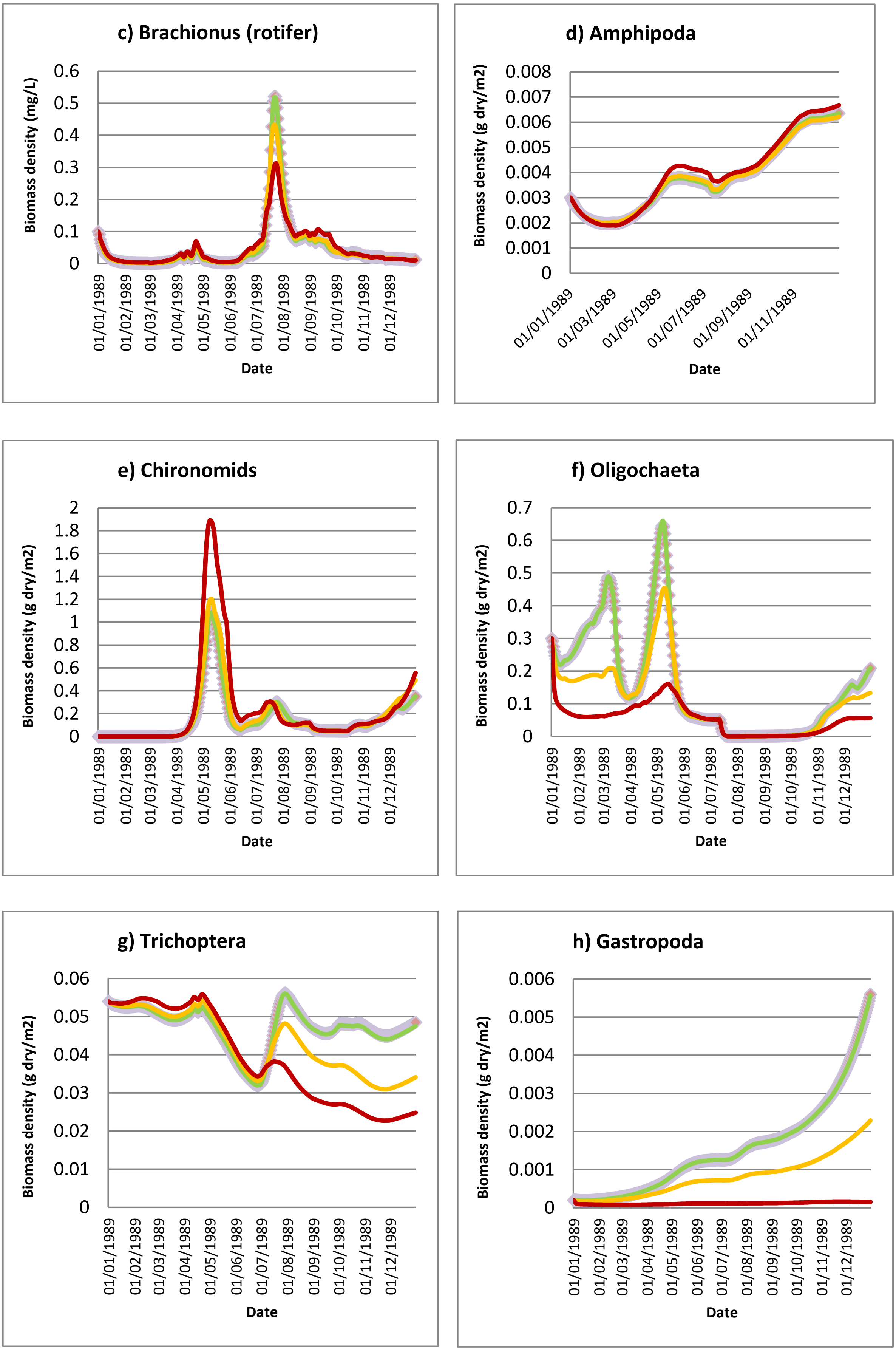


Figure 3.19 - Biomass trends in 1 year simulation with four different scenarios:

control LAS 1 (C = 3.22 µg/L) LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)

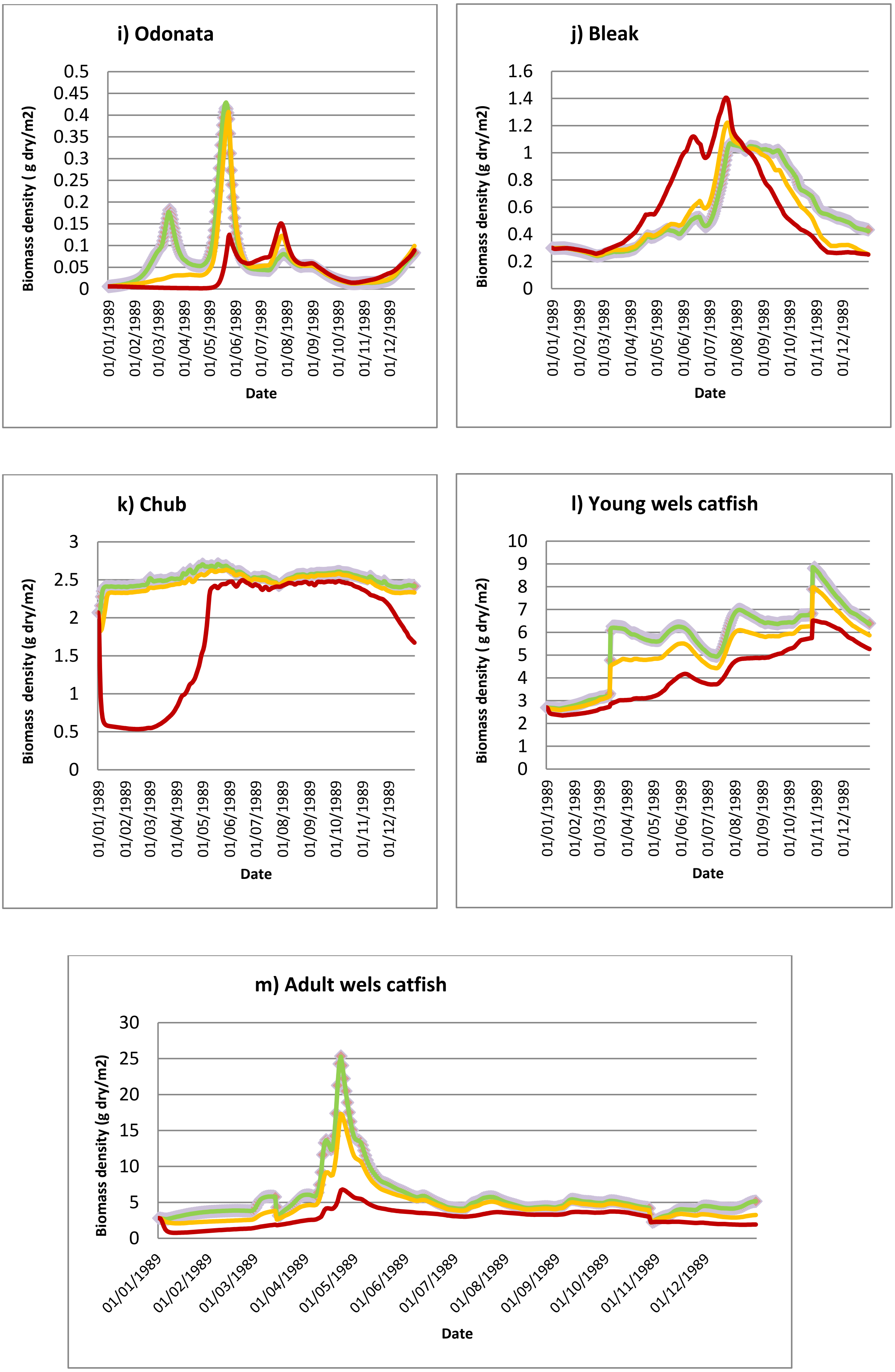


Figure 3.19 - Biomass trends in 1 year simulation with four different scenarios:

control   LAS 1 (C = 3.22 µg/L)   LAS 2 (C = 460 µg/L)   LAS 3 (C = 770 µg/L

1) Objective biomass variation indicator

The overall average biomass variation in the ecosystem is assessed with the objective biomass variation indicator (Formula 27, § 2.10). Percentages in Figure 3.20 indicate that the average biomass of living groups does not change compared to control model biomass in scenario 1, while it decreases of about 15 % in scenarios 2, and 22 % in scenario 3. There is clearly an objective average perturbation of the ecosystem due to LAS.

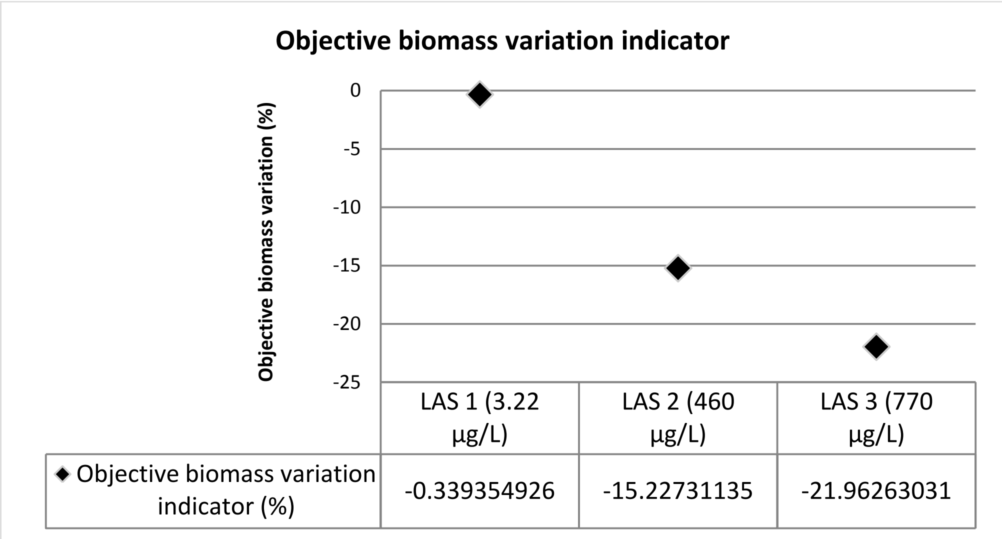


Figure 3.20 - Percentages of objective overall biomass variation for the three TCS perturbation scenarios (Cyclotella and Trichoptera blooms excluded).

To highlight what are the organisms more sensitive to the LAS perturbation, the relative biomass variation in perturbed scenarios compared to the biomass in the control one is calculated with Formula 38.

$$\varepsilon_{i\,LASj} = \frac{|B_{Ai\,LASj} - B_{Ai\,cont}|}{B_{Ai\,cont}} * 100 \tag{38}$$

Where:

$\varepsilon_{i\,LASj}$ = relative biomass variation of the organism "i" from control to perturbed model with scenario LAS_j;

$B_{Ai\,cont}$ = average biomass of the organism "i" in the control model;

$B_{Ai\,LASj}$ = average biomass of the organism "i" in the perturbed model with scenario LAS_j.

The plants with the highest relative biomass variation is Cyclotella, that decreases of about 48 % in LAS 2 scenario and of about 68 % in LAS 3 scenario (Figure 3.21). The fact that it decreases progressively in the two scenarios means that even if Cyclotella has the highest LC50 value (290000 µg/L), it is indirectly influenced by LAS concentration and probably its reduction is due to an increase of biomass predators as Bleak and Chironomids. Chromulina biomass variation reach 24 % in scenario 3 (Figure 3.21), this is connected to the increase in LAS concentration but also to an increase of predators (Bleak, Chironomids), the variation percentage is not so high as Cyclotella because Chromulina has a concentration of an order of magnitude lower, and predation stops when biomass is too low (see "Maximum consumption" parameter).

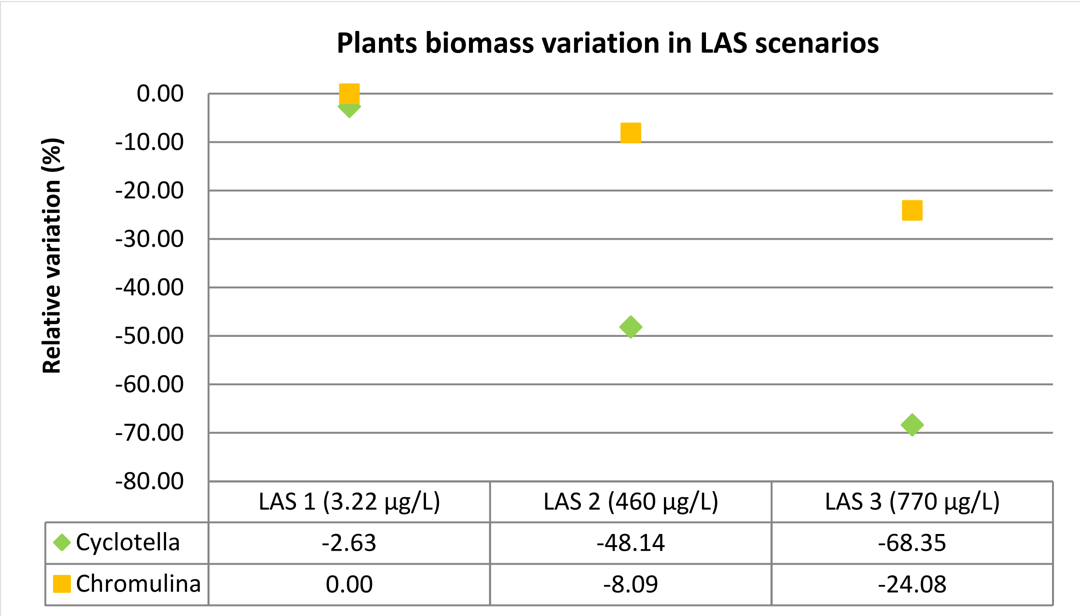
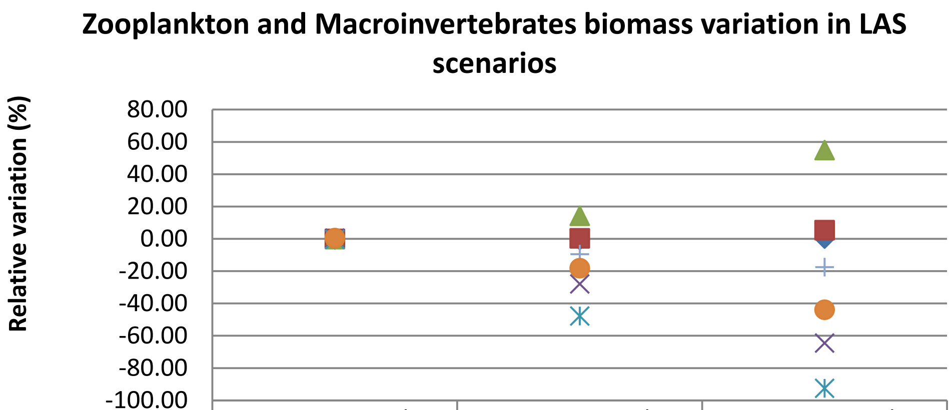


Figure 3.21 - Average biomass relative variation between control and LAS-perturbed models for plants Cyclotella and Chromulina.

Zooplankton biomass relative variation are negligible (Figure 3.22), this is connected to the negligible variation of labile particulate detritus that are the principal food item of Rotifers (Figure 3.24). Brachionus LC50 is high (3340 µg/L) with respect to the concentrations simulated I the scenario, in fact LAS does not seem to have effects on this organism.

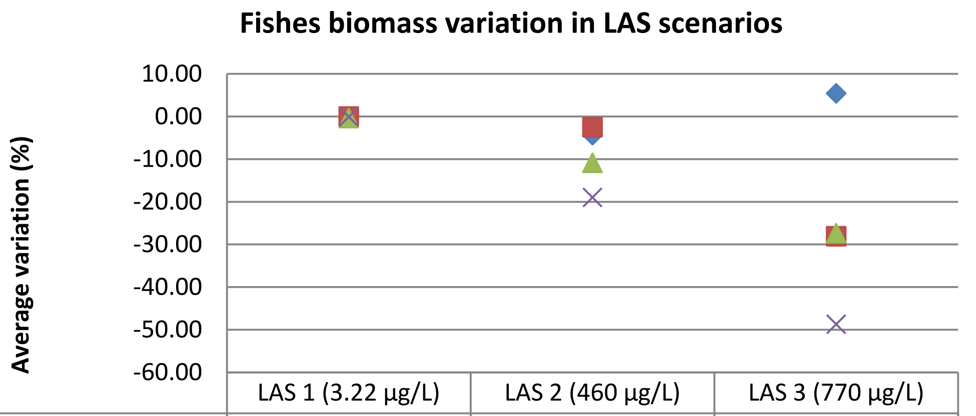
Regarding aquatic invertebrates (Figure 3.22), Gastropoda group has the highest decrease in biomass (93% in scenario 3), followed by Oligochaeta (65%) this is definitely due to indirect ecological effects of LAS (the direct effect of LAS would be a biomass decrease due to its toxicity), also due to the fact that the LC50 is low (1020 µg/L), but also to indirect effects due to an increase of predator biomass (Bleak). Trichoptera group reaches -18% of relative biomass variation in scenario 3, the reduction is less stronger than in Gastropoda and Oligochaeta probably because the LC50 is higher (8600 µg/L). Chironomids are the organisms less sensitive to LAS perturbation, they have the highest LC50 (8600 µg/L) among macroinvertebrates and during perturbation their biomass increases because the principal predator, Chub, biomass decreases. Amphipoda relative biomass variation is negligible in scenario 1 and 2, equal to 5% in scenario 3, the increase in biomass is probably due to a decrease in predators (Odonata and young Wels catfish) biomasses. The invertebrates predator Odonata biomass decreases in scenario 2 (18%) and its relative variation reaches -44 % in scenario 3. Odonata LC50 is high (equal to 2400 µg/L), this mean that its biomass variation is not so directly influenced by LAS concentration but mainly by indirect effects as the little presence of food (Oligochaeta, Trichoptera, Gastropoda).



	LAS 1 (3.22 µg/L)	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Rotifer Brachionus (%)	-0.30	-0.72	0.12
Amphipod (%)	-0.08	0.26	5.31
Chironomids (%)	-0.21	14.22	54.91
Oligochaeta (%)	0.38	-27.97	-64.57
Trichoptera (%)	-0.47	-9.56	-17.55
Gastropoda (%)	-0.39	-47.81	-92.56
Odonata (%)	0.42	-18.22	-43.94

Figure 3.22 - Average biomass relative variation between control and LAS-perturbed models for zooplankton and macroinvertebrates.

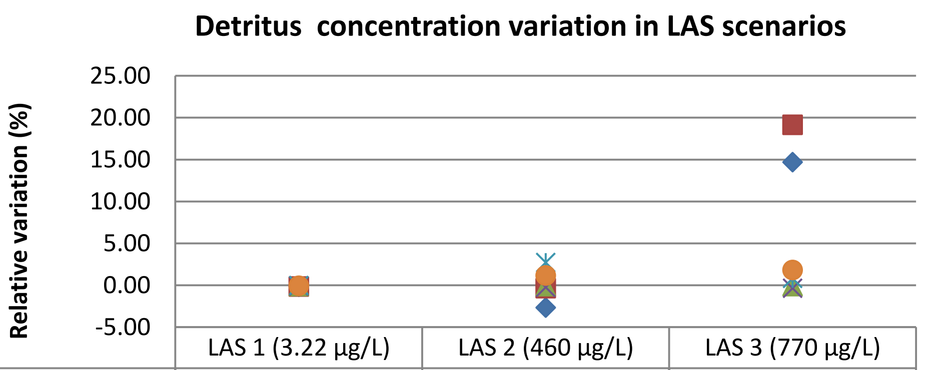
Adult Wels catfish is the most LAS-sensible organism in the ecosystem (LC50 = 770 µg/L), its relative biomass variation reaches -19% in scenario 2 and -49% in scenario 3. It is followed by Chub and young Wels catfish which reach respectively the 28 % and 27 % of relative biomass variation in scenario 3. This visible decrease of biomass of the principal ecosystem predators affect mainly the small sized Bleak which presents an increase of biomass.



	LAS 1 (3.22 µg/L)	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Bleak (%)	-0.34	-4.37	5.44
Chub (%)	0.00	-2.50	-28.08
Young wels catfish (%)	-0.36	-10.85	-27.37
Adult wels catfish (%)	-0.08	-18.97	-48.71

Figure 3.23 - Average biomass relative variation between control and LAS-perturbed models for fishes.

The average biomass relative variation is evaluated also for detritus because it is the main food items of some macroinvertebrates and its modification can influence the entire ecosystem. Dissolved and particulate detritus does not change while sedimented detritus slightly increase in scenario 3 (Figure 3.24).



	LAS 1 (3.22 µg/L)	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
R detr sed (g/m2 dry)	-0.16	-2.65	14.70
L detr sed (g/m2 dry)	-0.14	-0.33	19.16
R detr diss (mg/L dry)	-0.09	-0.09	-0.09
L detr diss (mg/L dry)	-0.09	-0.23	-0.35
R detr part (mg/L dry)	-0.01	2.73	0.83
L detr part (mg/L dry)	-0.05	1.18	1.83

Figure 3.24 - Average biomass relative variation between control and LAS-perturbed models for detritus.

It is interesting also to compare LC50 values with relative average biomass variation in the three different scenarios (Table 3.14, Figure 3.25) in order to verify if there is a relation between ecotoxicology parameters and objective perturbation. The most sensitive organisms, with the lower LC50 (adult Wels catfish), present an high objective perturbation but not the highest one. The higheSt objective perturbation is reached by Gastropoda that has a LC50 of 1020 (µg/L).

The inverse relationship between LC50 and objective perturbation is not present for all organisms. For example Cyclotella presents the highest LC50 value but has a high objective perturbation. (68%), this is in contrast with what could be expected from direct toxic effects of LAS. Oligochaeta and Gastropoda groups have the same LC50 value but the objective perturbation of Gastropoda is almost the double of Oligochaeta one in scenario 3. The same is true for Trichoptera and Chironomids: both are characterised by the same sensitivity to LAS, but the latter displays an increase in biomass while Trichoptera shows a decrease. This is the demonstration that toxicological effects do not necessarily dominate model outputs, but also other ecological processes play an important role.

Table 3.14 - Organisms LC50 values for LAS compared to relative biomass variation for the three different LAS perturbation scenarios.

Organism	**LC50 (µg/L)**	**EC50 (µg/L)**	**LAS 1 (3.22 µg/L) (%)**	**LAS 2 (460 µg/L) (%)**	**LAS 3 (770 µg/L) (%)**
Cyclotella	290000	29000	-2.63	-48.14	-68.35
Chromulina	9100	910	0.00	-8.09	-24.08
Rotifer Brachionus	3340	2000	-0.30	-0.72	0.12
Amphipod	7600	1700	-0.08	0.26	5.31
Chironomids	8600	8000	-0.21	14.22	54.91
Oligochaeta	1020	610	0.38	-27.97	-64.57
Trichoptera	8600	5150	-0.47	-9.56	-17.55
Gastropoda	1020	610	-0.39	-47.81	-92.56
Odonata	2400	1440	0.42	-18.22	-43.94
Bleak	3200	2400	-0.34	-4.37	5.44
Chub	835	500	0.00	-2.50	-28.08
Young wels catfish	1670	2000	-0.36	-10.85	-27.37
Adult wels catfish	770	460	-0.08	-18.97	-48.71

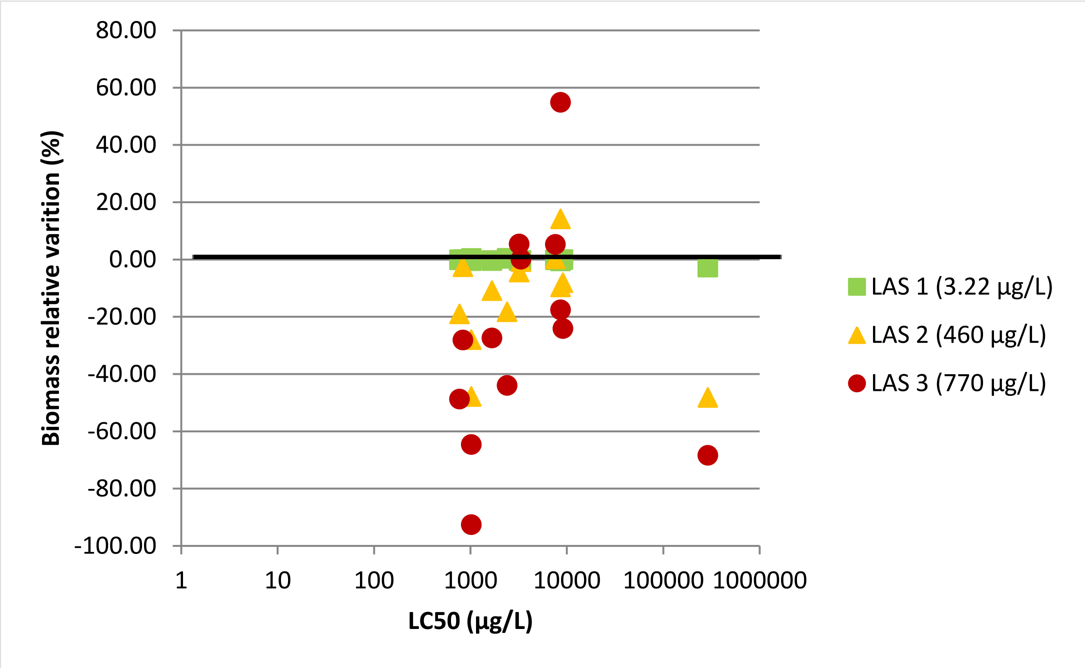


Figure 3.25 - Relative biomass variation compared to LC50 values.

2) Ecosystem maturity indicator

By comparing production/respiration ratio (Formula 28, § 2.10) of control and perturbed simulation, an idea on the change in maturity level of the system is given. In Table 3.15 and Figure 3.26 P/R ratio of control and perturbed simulation are presented. In scenario 1 production and respiration of the community do not change form control simulation, in scenario 2 and 3 the ratio decreases respectively till 0.169 and 0.119.

Table 3.15 - GPP (gross primary production ($gO_2/(m^2\ d)$), community respiration ($gO_2/(m^2\ d)$) and production-respiration ratio average over one year.

Scenario	**GPP (gO2/m2 d)**	**Community Resp. (gO2/m2 d)**	**P/R (frac)**
Control	0.556	1.644	0.267
LAS 1 (3.22 µg/L)	0.547	1.638	0.263
LAS 2 (460 µg/L)	0.346	1.483	0.169
LAS 3 (770 µg/L)	0.207	1.389	0.119

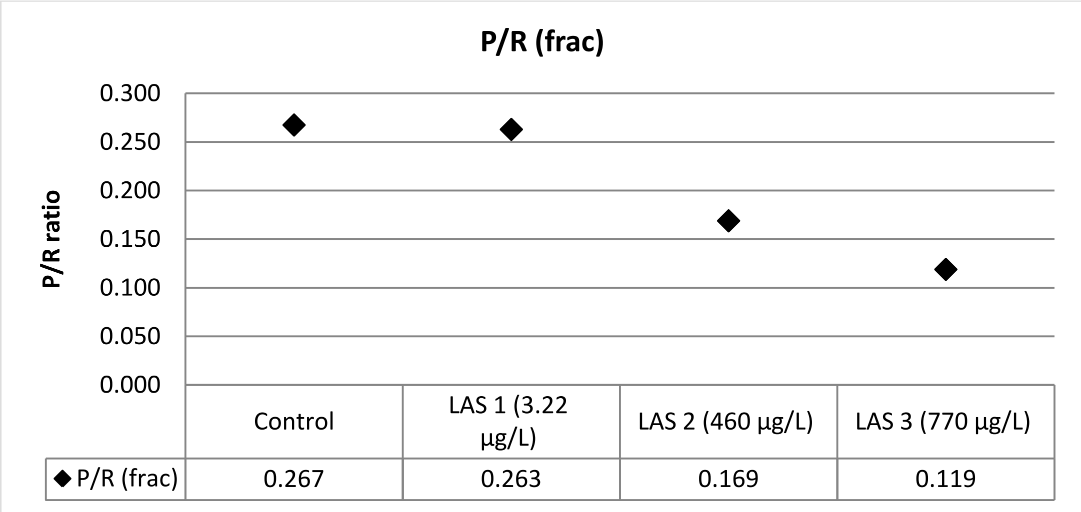


Figure 3.26 - Production-respiration ratios for control and perturbed scenarios.

3) Shannon index (community diversity indicator)

It is a diversity index that mathematically measure the relative abundance of a species or group over the others (Formula 29, § 2.10). Compared the index values of control and perturbed simulations will give an idea of the xenobiotics effects on community diversity (Figure 3.27). Shannon index value does not change sensibly between the different scenarios, in scenario 1 and 2 Shannon index slightly decreases according to the ecological theory that perturbation decreases the ecosystem diversity but in scenario 3 it slightly increases.

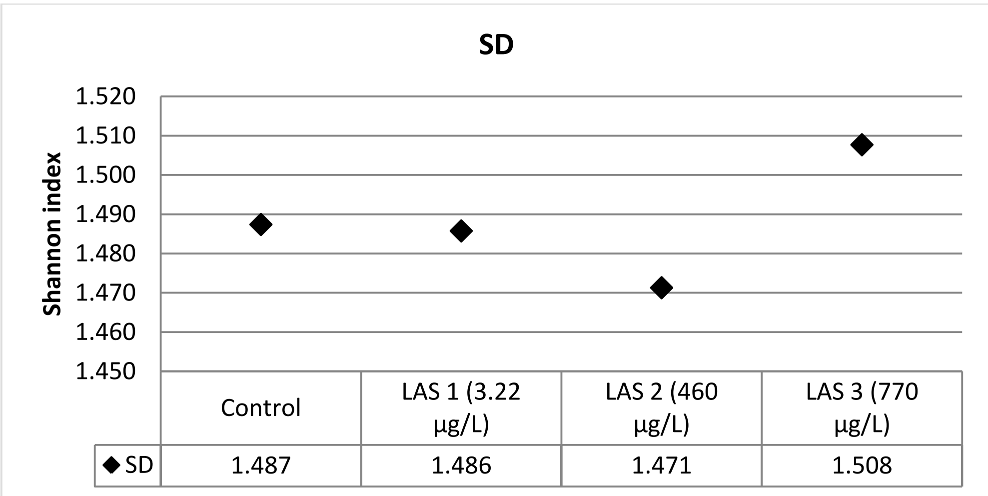


Figure 3.27 - Shannon index of control and perturbed scenarios.

4) Ecological Quality Ratio (WFD index)

The ecological quality ratio is calculated dividing the organism biomass in perturbed scenarios by the organism biomass in control model, in other words by using relative average biomass variations calculated in point 1). In this way, the control simulation average biomass is considered the reference status of Po river, to compared with perturbed system biomass. Organism biomasses are grouped in the following functional categories: plants, zooplankton & macroinvertebrates, fishes. Then also the total ecosystem biomass is considered. Then ratios are classified into five classes (as recommended by WFD), according to Table 2.65. In general an increase of LAS concentration in water brings to an decrease of ecological quality ratio. In scenario 1 there is no visible perturbation for any organism in the system. A high perturbation level is shown only by plants in scenario 3. Zooplankton and macroinvertebrates present low perturbation. Fishes show moderate-high perturbation only in scenario 3. Overall the system resulted not perturbed in scenario 1, lowly perturbed in scenario 2 and moderately-highly perturbed in scenario 3.

Table 3.16 - Relative biomass variation and respective classification for each organisms functional groups and for the total ecosystem.

Ecosystem group	LAS 1 (3.22 µg/L)		LAS 2 (460 µg/L)		LAS 3 (770 µg/L)	
	Relative biomass variation	Classification	Relative biomass variation	Classification	Relative biomass variation	Classification
Plants	-0.0253	No visible perturbation	-0.4659	Moderate-High perturbation	-0.6663	High perturbation
Macroinv. &zooplan.	0.0003	No visible perturbation	-0.0821	Low perturbation	-0.1133	Low perturbation
Fishes	-0.0019	No visible perturbation	-0.1229	Low perturbation	-0.3450	Moderate-High perturbation
Total	-0.0034	No visible perturbation	-0.1450	Low perturbation	-0.3599	Moderate-High perturbation

5) Ecological service index

To evaluate the differences between the level of quality of control and perturbed systems from a human services point of view, Secchi depth and Fish catch quality indicators are used. Secchi depth does not present important variation from control to perturbed situations (Figure 3.28). Instead, average fishes biomass in control and LAS 1 scenarios is almost the double of the biomass in perturbed scenarios 3.

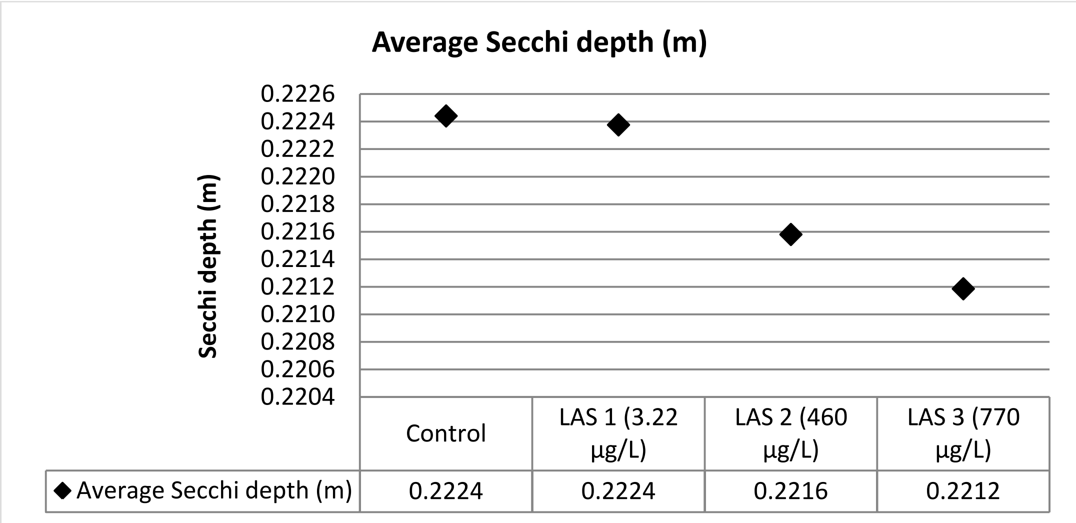


Figure 3.28 - Average Secchi depth in control and LAS-perturbed simulations.

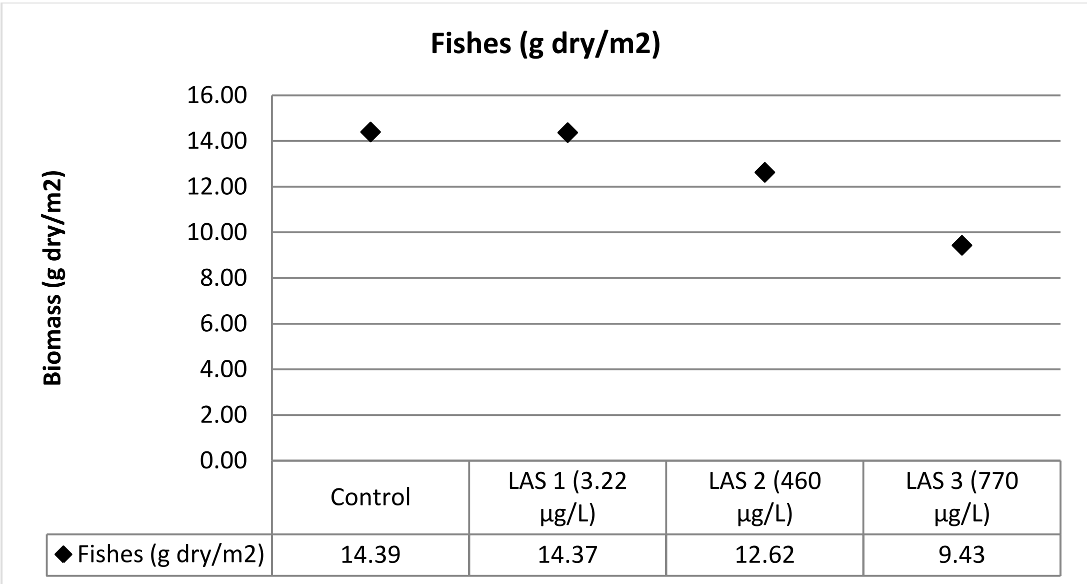


Figure 3.29 - Average fish biomasses in control and LAS-perturbed simulations.

3.2.1.2 TCS perturbation

As described in § 2.9, the system is perturbed with three different constant concentrations input of TCS: TCS 1 (C = 0.000926 μg/L) that corresponds to the assessed actual concentration in the section of the Po river, TCS 2 (C = 1.61 μg/L) that is equal to the EC50 of the most sensitive organism (phytoplankton) and TCS 3 (C = 16.1 μg/L) that is equal to the LC50 of the most sensitive organism (phytoplankton). In Figure 3.30 results of perturbed scenarios are shown and compared with control trend.

For all organisms simulated, TCS 1 concentration input seems to have no effects on biomass trend, as the resulted curves overlap the control ones. For this reason the analysis is focused only on TCS 2 and TCS 3 scenarios.

Plants are the most sensible organisms in TCS perturbation, their picks decrease increasing TCS concentration, but with some differences: Cyclotella biomass rapidly goes to zero in both scenarios TCS 2 - TCS 3, while Chromulina biomass has a strong decrease in scenario 2 and goes to zero in scenario 3. Even if the two algae have the same LC50, their behaviour is different because there are differences in the predation they undergo. In fact Cyclotella, having an higher concentration, is more subjected to predation, moreover predators biomass slightly increases in the third scenario (see Bleak and Chironomids) (Figure 3.30 - a, b). Brachionus rotifer biomass presents the same trend for scenario 2 and 3, it shows a slight increase in biomass in April and a slight decrease in July. In scenario 3 there is a slight increase in biomass (Figure 3.30 - c). This is probably due to the decrease in biomass of one of the principal Rotifer predator (young Wels catfish). Amphipoda, Gastropoda, Oligochaeta and Odonata biomasses decrease in scenario 2 but increase in scenario 3 (Figure 3.30 -d, f, h, i). Chironomids biomass decreases in July in scenarios 2, while increases in July and December in scenario 3, probably because the predator young Wels catfish decrease in biomass (Figure 3.30 - e). Trichoptera biomass decrease from July to December in both scenarios (Figure 3.30 -g), probably because the predators Odonata and Bleak increase in biomass. Regarding fishes, Chub does not show any perturbation, young and adult Wels catfish show a decrease in biomass mainly in scenario 3, while Bleak shows an increase, this is probably due to the decrement of predators (Chub and Wels catfish) (Figure 3.30 - j, k, l, m).

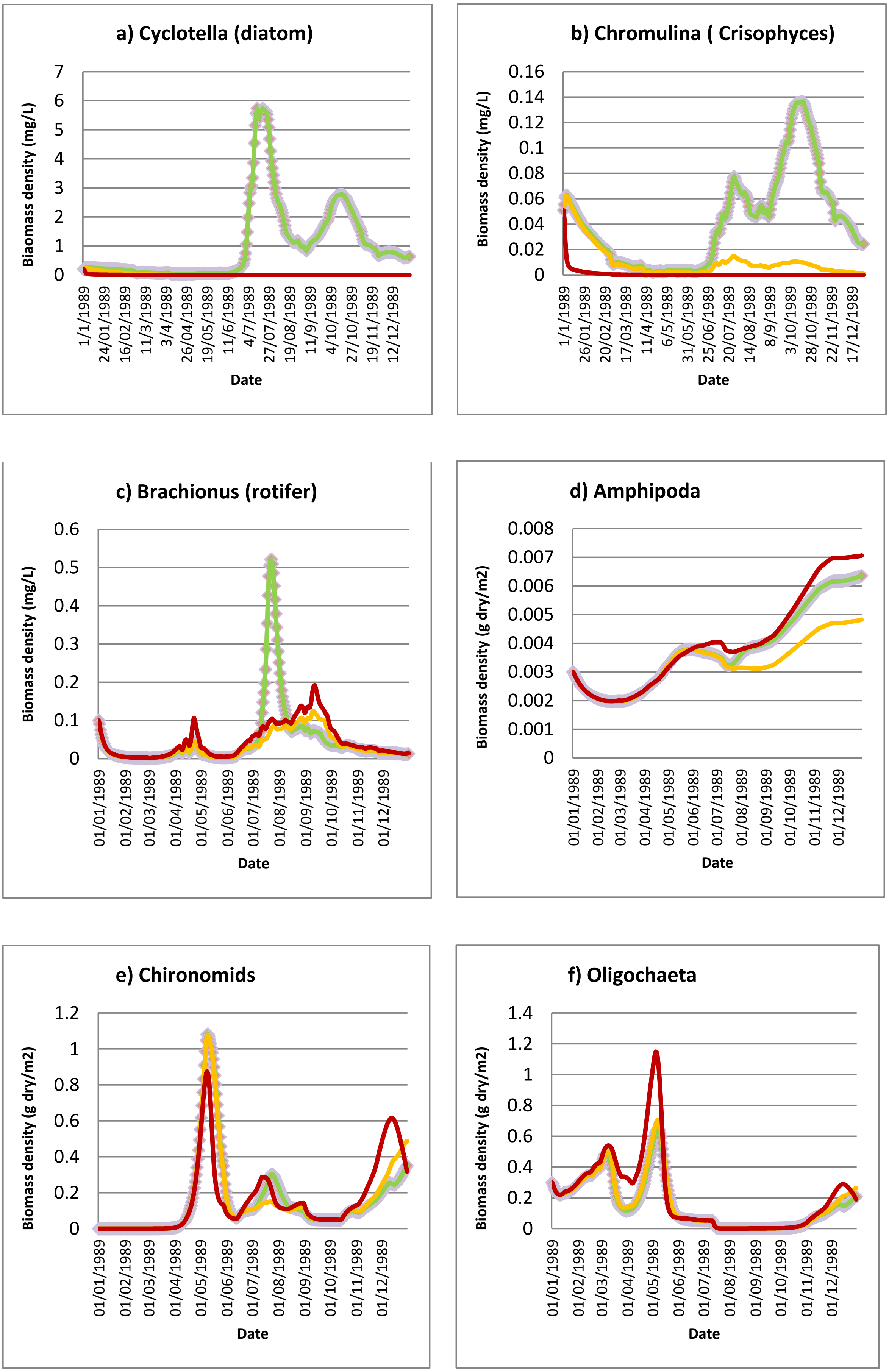


Figure 3.30 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 1 (C = 0.926 µg/L) TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)

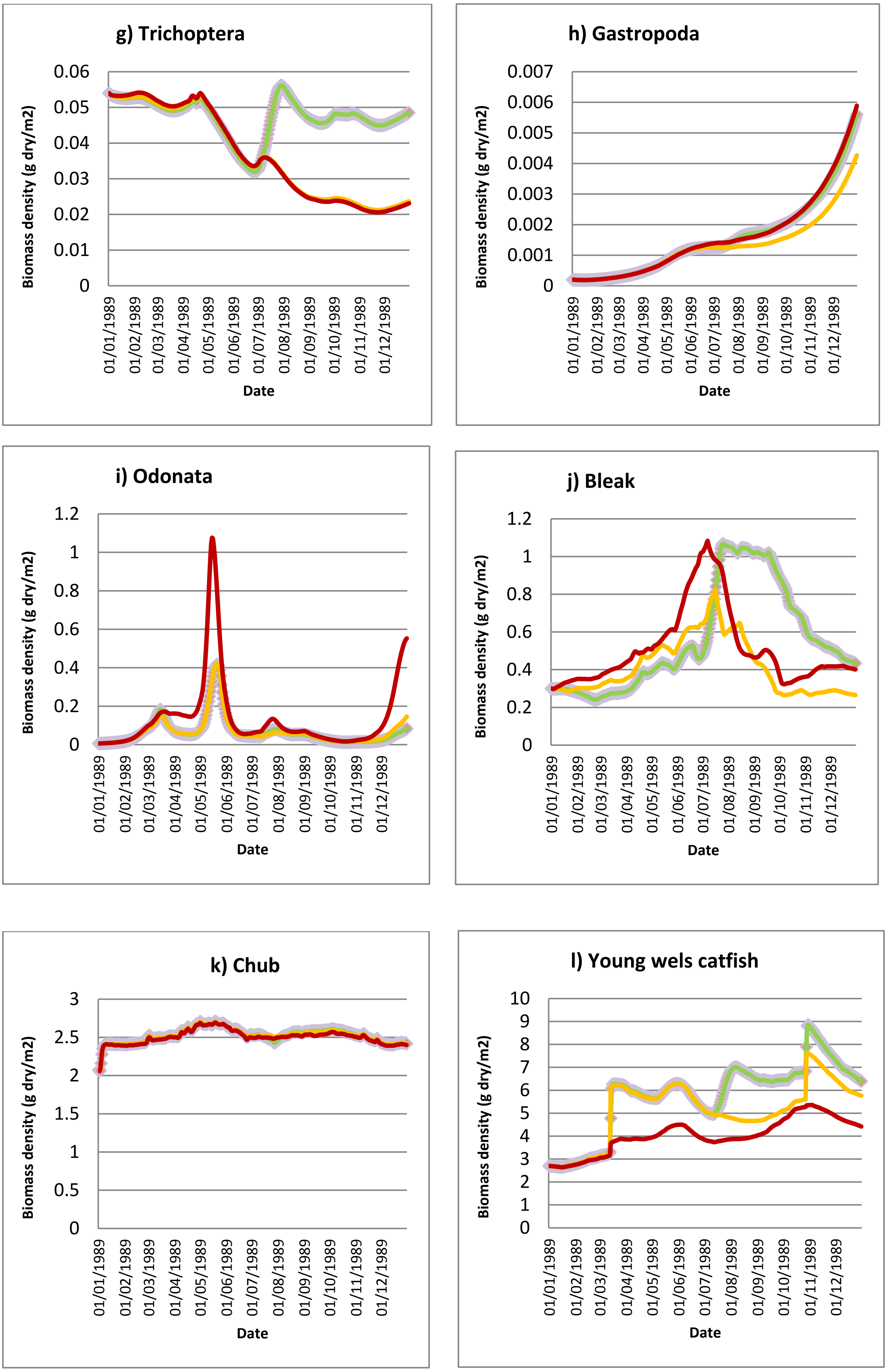


Figure 3.30 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 1 (C = 0.926 µg/L) TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)

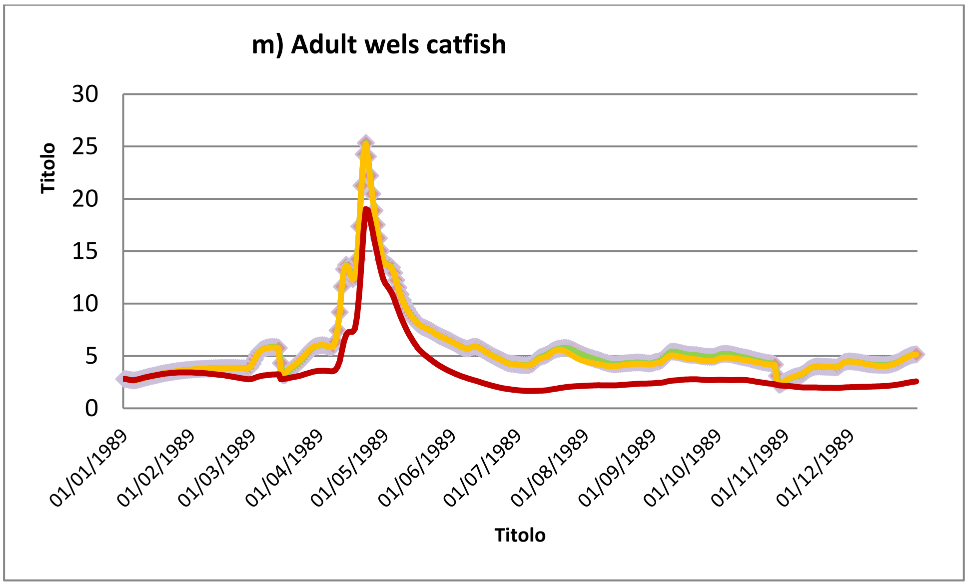


Figure 3.30 - Biomass trends in 1 year simulation with four different scenarios:
control TCS 1 (C = 0.926 µg/L) TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)

1) Objective biomass variation indicator

The overall average biomass variation in the ecosystem is assessed with the objective biomass variation indicator (Formula 27, § 2.10). Percentages in Figure 3.31 indicate that the average group biomass decreases more strongly in scenario 2 than in scenario 3. So at the highest TCS input concentration does not correspond the highest average biomass variation. This demonstrates that indirect effects are influencing perturbation results and, thus, risk assessment, in an unpredictable and apparently counterintuitive manner.

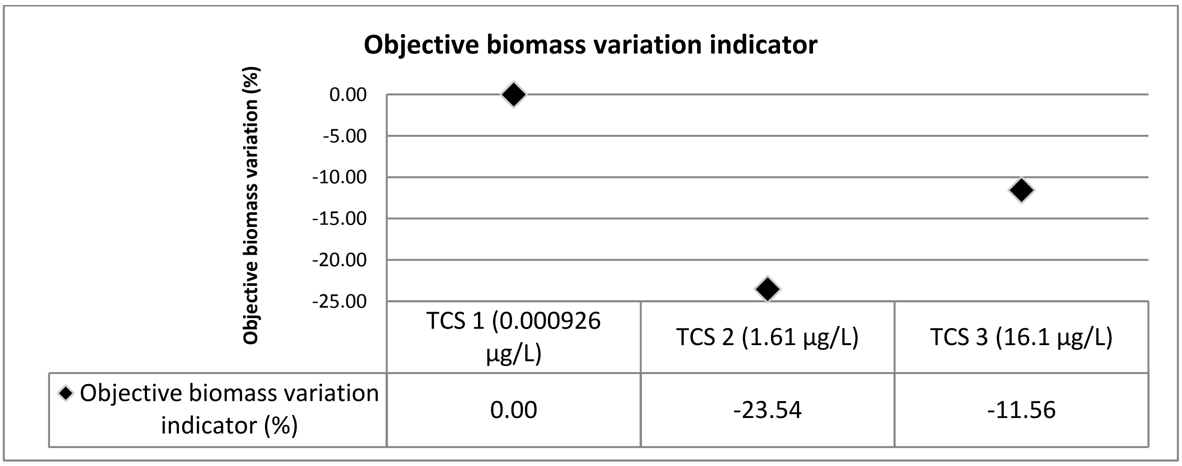
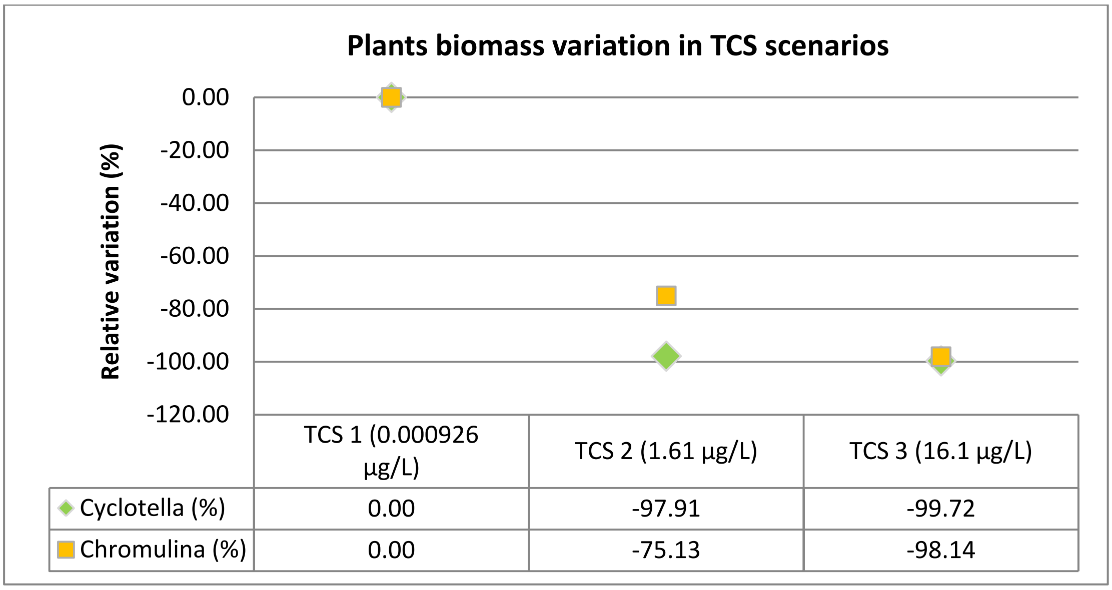


Figure 3.31 - Percentages of objective overall biomass variation for the three TCS perturbation scenarios.

To highlight what are the organisms more sensitive to TCS perturbation, the relative biomass variation of perturbed scenarios compared to the biomass in the control one are calculated with Formula 37.

Plants are the most TCS-sensible organisms in this study. The plants with the highest relative biomass variation is Cyclotella, that decreases of about 98% in TCS 2 scenario and of about 99.72 % in TCS 3 scenario (Figure 3.32). The fact that it decreases progressively in the two scenarios suggests that it is directly influenced by TCS concentration. Chromulina biomass variation reach 98% in scenario 3 (Figure 3.21), but its variation is more gradual with respect to Cyclotella. This is probably due to the fact that Cyclotella biomass variation is influenced also by predation, while Chromulina predation stops because biomass is too low (see "Maximum consumption" parameter).

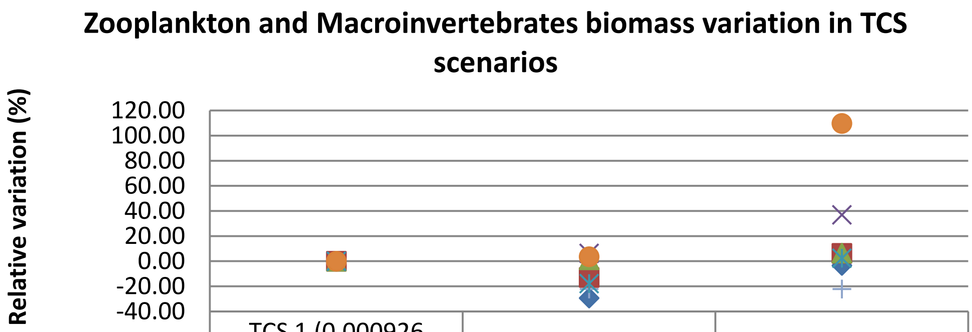


	TCS 1 (0.000926 μg/L)	TCS 2 (1.61 μg/L)	TCS 3 (16.1 μg/L)
Cyclotella (%)	0.00	-97.91	-99.72
Chromulina (%)	0.00	-75.13	-98.14

Figure 3.32 - Average biomass relative variation between control and TCS-perturbed models for plants Cyclotella and Chromulina.

Zooplankton biomass relative variation reaches about 30% in scenarios 2 but it is negligible (3%) in scenario 3 (Figure 3.33). This demonstrates that Rotifer biomass variation is not directly influenced by TCS, in particular in scenario 3 the biomass trend is probably due to the decrease in biomass of one of the principal Rotifer predator (young Wels catfish).

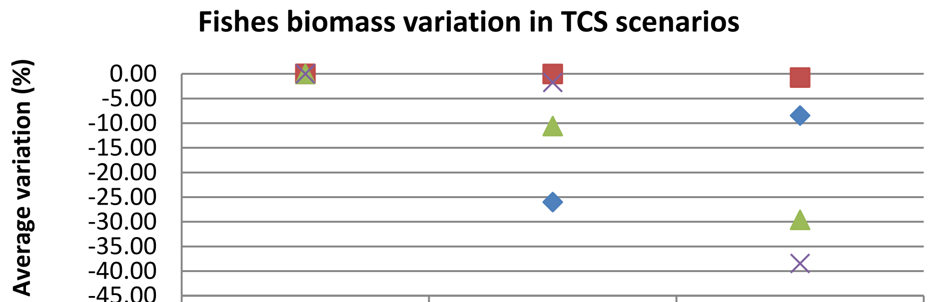
Regarding aquatic invertebrates (Figure 3.33), Trichoptera group has the highest decrease in biomass that is more or less equal to 22% in both scenarios. The fact that biomass variation is almost the same in the two scenarios, demonstrates that Trichoptera are influenced by indirect ecological effects. Amphipoda and Gastropoda biomass decreases in scenario 2 until respectively 13% and 18% but increases in scenario 3 until 6% and 2%. The increase in biomass is probably due to a decrease in predators (Wels catfish). Oligochaeta, Chironomids and Odonata biomass increases in both scenarios until respectively 37%, 6% and 109%, this is probably due to a decrease in predation.



	TCS 1 (0.000926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Rotifer Brachionus (%)	0.00	-29.32	-3.35
Amphipod (%)	0.00	-12.90	6.26
Chironomids (%)	0.00	1.15	6.50
Oligochaeta (%)	0.00	6.30	36.96
Trichoptera (%)	0.00	-22.09	-22.13
Gastropoda (%)	0.00	-17.79	2.41
Odonata (%)	0.00	3.62	109.75

Figure 3.33 - Average biomass relative variation between control and TCS-perturbed models for zooplankton and macroinvertebrates.

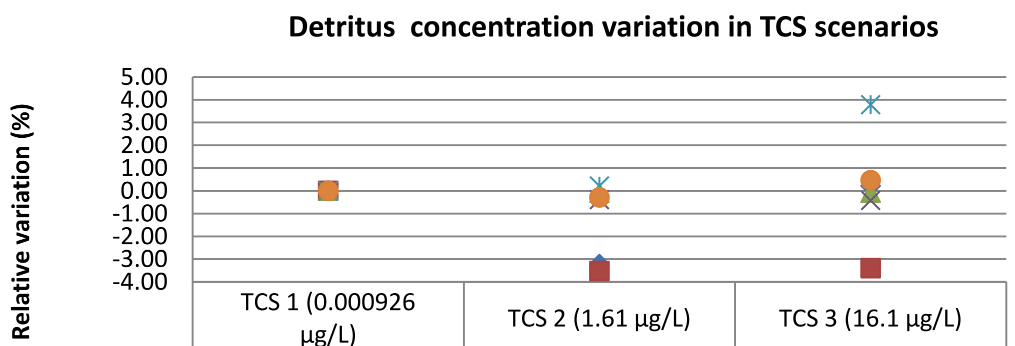
Bleak relative biomass variation reaches the maximum value of 26% in scenarios 2, while in scenario 3 the biomass variation is 9%. This demonstrates that Bleak is not directly influenced by TCS that much, in particular in scenario 3 the biomass trend is probably due to the decrease in biomass of one of the principal predators (adult Wels catfish). Chub biomass does not change in perturbed scenarios. Wels catfish relative biomass variation reaches -30% for young and -38% for adults and it becomes progressively stronger with increasing TCS concentration, this demonstrates that probably there is a direct effect of TCS on Wels catfish.



	TCS 1 (0.000926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Bleak (%)	0.00	-26.00	-8.46
Chub (%)	0.00	-0.03	-0.75
Young wels catfish (%)	0.00	-10.59	-29.60
Adult wels catfish (%)	0.00	-1.74	-38.45

Figure 3.34 - Average biomass relative variation between control and TCS-perturbed models for zooplankton and macroinvertebrates.

Detritus concentration variation reaches the maximum value of 3%. Dissolved and particulate detritus do not change, an exception occurs in scenario 3 where refractory particulate detritus increase. Sedimented detritus slightly decrease in the second scenario and increase in scenario 3 (Figure 3.35).



	TCS 1 (0.000926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
R detr sed (%)	0.00	-3.22	0.22
L detr sed (%)	0.00	-3.52	-3.39
R detr diss (%)	0.00	-0.09	-0.08
L detr diss (%)	0.00	-0.39	-0.42
R detr part (%)	0.00	0.21	3.78
L detr part (%)	0.00	-0.29	0.47

Figure 3.35 - Average biomass relative variation between control and TCS-perturbed models for zooplankton and macroinvertebrates.

It is interesting also to compare the LC50 values of organisms with relative average biomass variation in the three different scenarios (Table 3.17, Figure 3.36).

Organisms with the lowest LC50 (phytoplankton) present the highest objective perturbation. Rotifer present the highest LC50 value and has n low objective perturbation. Bleak and Chub groups have the same LC50 value but different objective perturbations. The same is for Young and adult Wels catfish. This demonstrates the important effect of indirect ecological interactions in driving ecosystem dynamics.

Table 3.17 - Organisms LC50 values for TCS compared to relative biomass variation for the three different TCS perturbation scenarios.

Organism	**LC50 (µg/L)**	**TCS 1 (0.000926 µg/L) (%)**	**TCS 2 (1.61 µg/L) (%)**	**TCS 3 (16.1 µg/L) (%)**
Cyclotella	16.1	0.00	-97.91	-99.72
Chromulina	16.1	0.00	-75.13	-98.14
Rotifer Brachionus	1544	0.00	-29.32	-3.35
Amphipod	200	0.00	-12.90	6.26
Chironomids	400	0.00	1.15	6.50
Oligochaeta	1260	0.00	6.30	36.96
Trichoptera	400	0.00	-22.09	-22.13
Gastropoda	1260	0.00	-17.79	2.41
Odonata	400	0.00	3.62	109.75
Bleak	260	0.00	-26.00	-8.46
Chub	260	0.00	-0.03	-0.75
Young wels catfish	370	0.00	-10.59	-29.60
Adult wels catfish	370	0.00	-1.74	-38.45

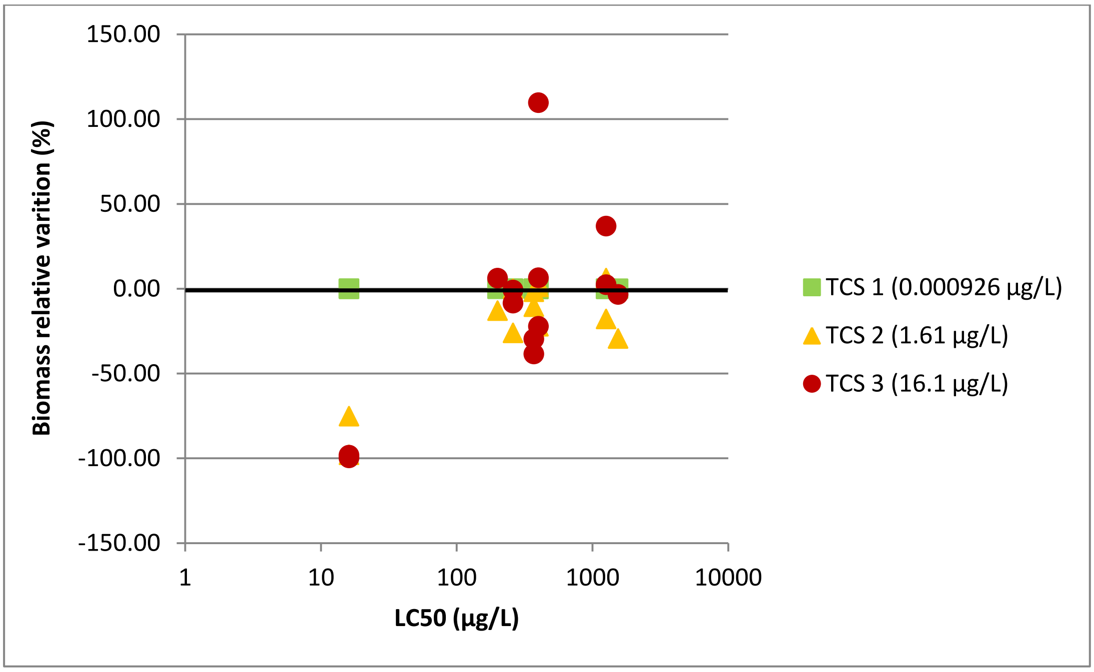


Figure 3.36 - Relative variation of annual biomass compared to LC50 values for simulated organisms.

2) Ecosystem maturity indicator

By comparing production/respiration ratio (Formula 28, § 2.10) of control and perturbed simulation, an idea on the change in maturity level of the system is given. In Table 3.18 and Figure 3.37 P/R ratio of control and perturbed simulation are presented. Production and respiration of the community decrease from scenario 2 to scenario 3 and P/R ratio reaches values respectively of 0.016 and 0.014.

Table 3.18 - GPP (gross primary production ($gO_2/(m^2$ d)), community respiration ($gO_2/(m^2$ d)) and production-respiration ratio average over one year.

	GPP (gO2/m2 d)	Community Resp. (gO2/m2 d)	P/R (frac)
Control	0.556	1.644	0.2675
TCS 1 (0.000926 µg/L)	0.556	1.644	0.2675
TCS 2 (1.61 µg/L)	0.0194	1.47	0.0155
TCS 3 (16.1 µg/L)	0.0172	1.337	0.0143

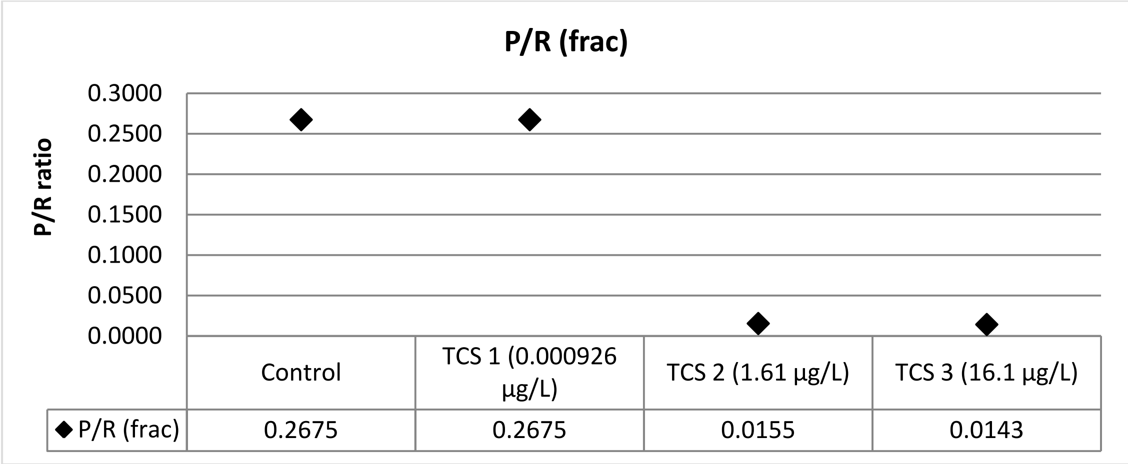


Figure 3.37 - Production-respiration ratios for control and TCS-perturbed scenarios.

3) Shannon index (community diversity indicator)

It is a diversity index that mathematically measure the relative abundance of a species or group over the others (Formula 29, § 2.10). Compared the index values of control and perturbed simulations will give an idea of the xenobiotics effects on community diversity (Figure 3.38). Shannon index value does not change sensibly between the different scenarios, in scenario 2 Shannon index slightly decreases according to the ecological theory that perturbation decreases the ecosystem diversity, but in scenario 3 it slightly increases.

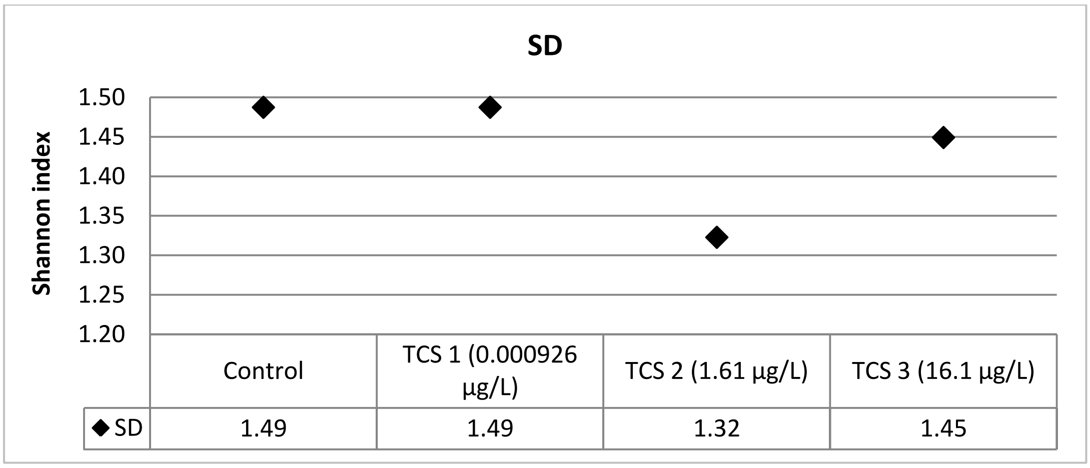


Figure 3.38 - Shannon index of control and TCS-perturbed scenarios.

4) Ecological Quality Ratio (WFD index)

The ecological quality ratio is calculated dividing the organism biomass in perturbed scenarios by the organism biomass in control model, in other words by using relative average biomass variations calculated in point 1). In this way, the control simulation average biomass is considered the reference status of Po river, to be compared with perturbed system biomass. Organism biomasses are grouped in the following functional categories: plants, zooplankton & macroinvertebrates, fishes. Then also the total ecosystem biomass is considered. Then ratios are classified into five classes (as recommended by WFD), according to Table 2.65. Plants show a high perturbation level in scenarios 2 and 3. Zooplankton and macroinvertebrates do not present visible perturbation. Fishes show moderate-high perturbation only in scenario 3. Overall the system resulted lowly perturbed in scenario 2 and moderately-highly perturbed in scenario 3.

Table 3.18 - Relative biomass variation and respective classification for each organisms functional groups and for the total ecosystem.

Ecosystem group	**TCS 1 (0.000926 μg/L)**		**TCS 2 (1.61 μg/L)**		**TCS 3 (16.1 μg/L)**	
	Relative biomass variation	Classification	Relative biomass variation	Classification	Relative biomass variation	Classification
Plants	0	No visible perturbation	-0.9703	High perturbation	-0.9966	High perturbation
Macroinv. &zooplan.	0	No visible perturbation	-0.0238	No visible perturbation	0.2698	No visible perturbation (positive)
Fishes	0	No visible perturbation	-0.0591	Low perturbation	-0.2716	Moderate-High perturbation
Total	0	No visible perturbation	-0.1199	Low perturbation	-0.3048	Moderate-High perturbation

5) Ecological service index

To evaluate the differences between the level of quality of control and perturbed systems from a human services point of view, Secchi depth and Fish catch quality indicators are used. Secchi depth variation is negligible. Fishes biomass variation is marked only in scenario 3.

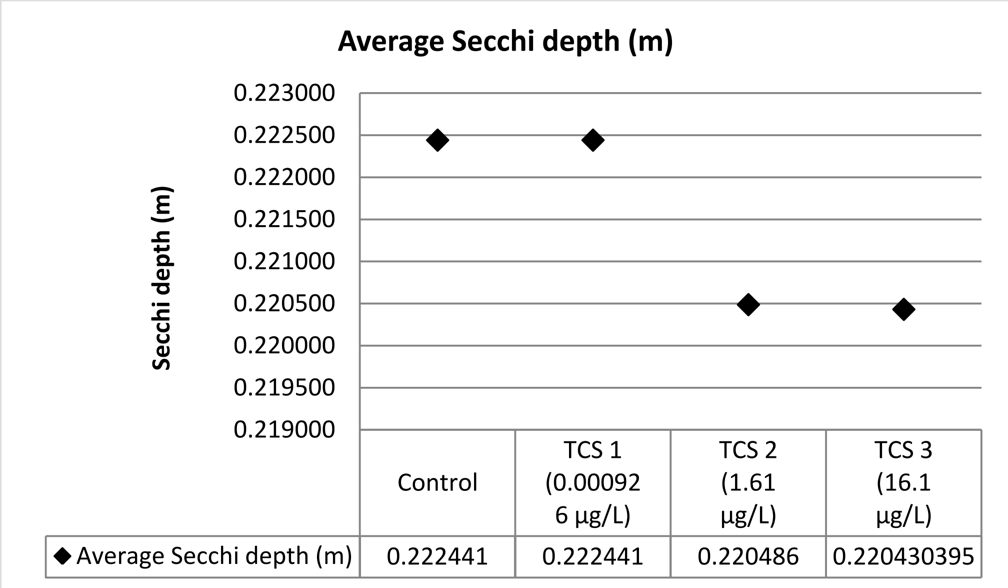


Figure 3.39 - Average Secchi depth in control and TCS-perturbed simulations.

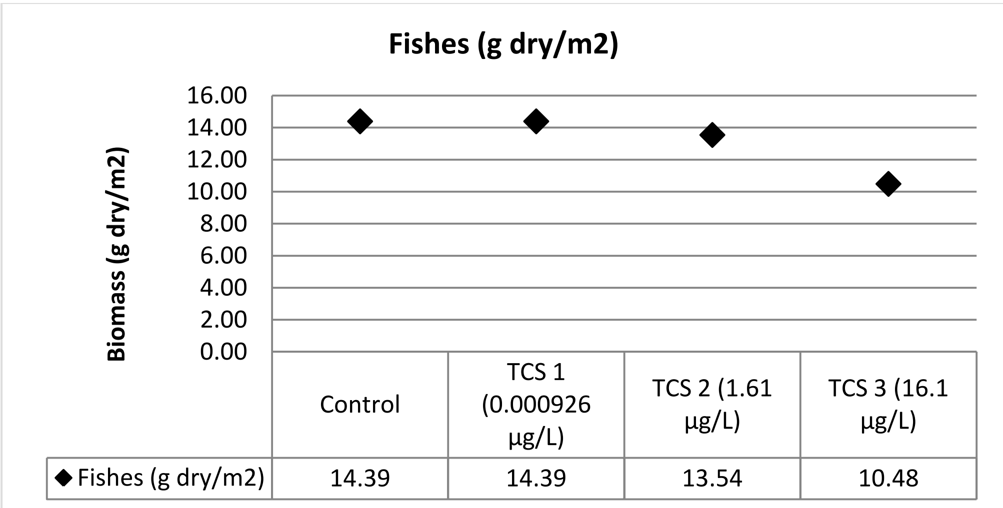


Figure 3.40 - Average fish biomasses in control and TCS-perturbed simulations.

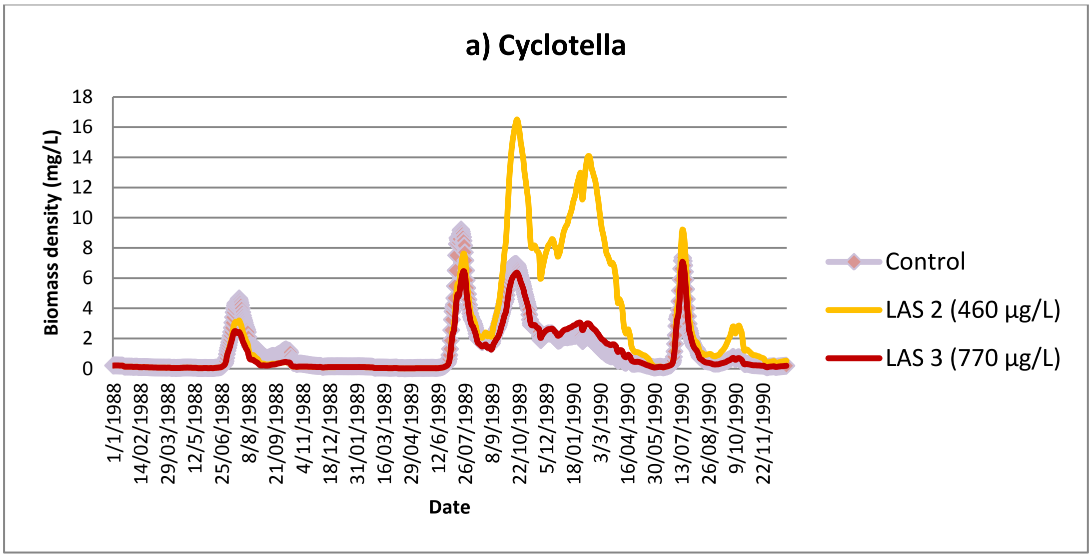
3.2.2 3 years simulation

The 3 years model described in Paragraph 3.1 is perturbed with different concentrations of LAS and TCS. This simulations results are needed to understand the effects of long-term perturbations. Two distinct simulations are done for each chemical in order to avoid interaction or synergies and to assess the effects of the single substance in the environment. Comparisons between control and perturbed systems are done only on a graphical basis and current concentration scenarios of LAS (LAS 1) and TCS (TCS 1) are not analysed because it has been demonstrated in the previous paragraph that total average biomass variation is less than 1%, not appreciable with a graphical analysis.

3.2.2.1 LAS perturbation

In Figure 3.41 the trends of simulated organisms biomass in control, LAS 2 (C = 460 µg/L) and LAS 3 (C = 770 µg/L) scenarios are shown for a 3 year simulation period.
Cyclotella shows no visible perturbation in scenario 3 but in scenario 2 its biomass has peaks that reach 16 mg/L in October 1989 and February 1990. This corresponds to the decrease in Bleak biomass in the same period. There is a different trend in the year 1989 respect to the 1 year simulation period result. Chromulina shows no visible perturbation in scenario 2, but in scenario 3 its biomass decreases reaching the minimum in the third year.
Brachionus shows a peak in April 1990 in scenario 2 and 3 and maintains the trend of control simulation in the remaining months.
Amphipoda shows no visible perturbation in both perturbed scenarios. Chironomids show no visible perturbation in scenario 2 but in scenario 3 the biomass show peaks of 1.5-2 g dry/m^2 in May 1988, May 1989, March 1990. Oligochaeta biomass decreases progressively in scenario 2 and 3, it almost extinct in the third year for both scenario. Trichoptera shows a peak in January 1990 in scenario 2 in which biomass is tripled respect control scenario, in scenario 3 biomass decreases from August 1989 to June 1990 and then it stabilizes to the values of unperturbed scenario. Gastropoda biomass decreases in scenario 2 from January to November 1989, then it stabilizes to the values of unperturbed scenario, while in scenario 3 it goes to zero for all the three years. Odonata show peaks where biomass is double (0.4 g dry/m^2) respect the control in May 1988 and May 1989 in scenario 2, while in scenario 3 there are higher (1 g dry/m^2) peaks in May 1989 and March 1990.
Bleak shows in scenario 2 a decrease from 0.8 to 0.4 g dry/m^2 in November 1989, while in scenario 3 it shows an increase in July 1988 then it stabilizes to the values of unperturbed scenario. Chub shows a decrease from January to March every year in

scenario 3, while in scenario 2 shows no visible perturbation. Young and adult Wels catfish biomass decreases progressively in scenario 2 and 3 maintaining the trend of control simulation curve.



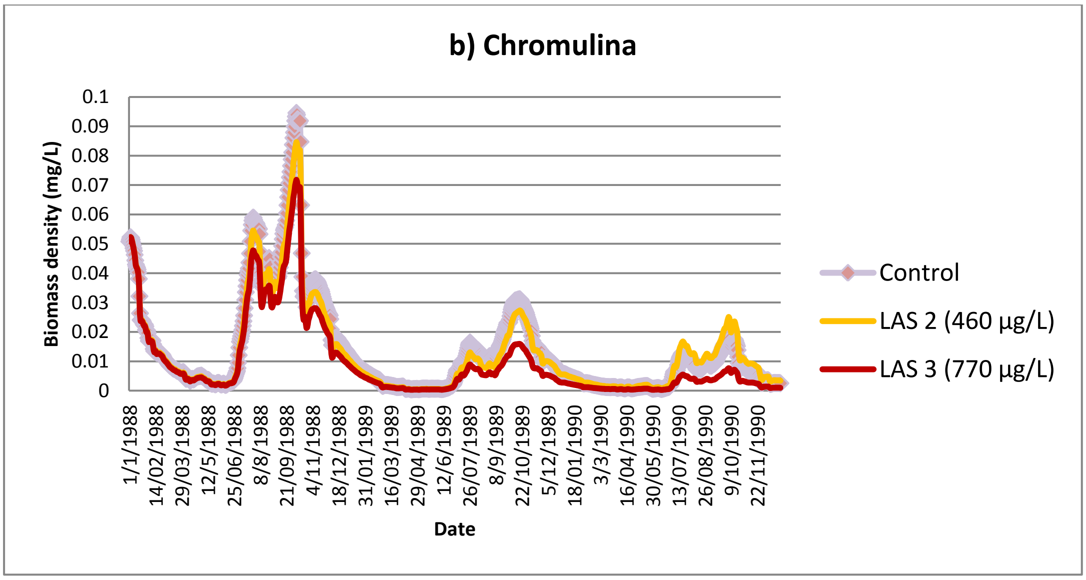
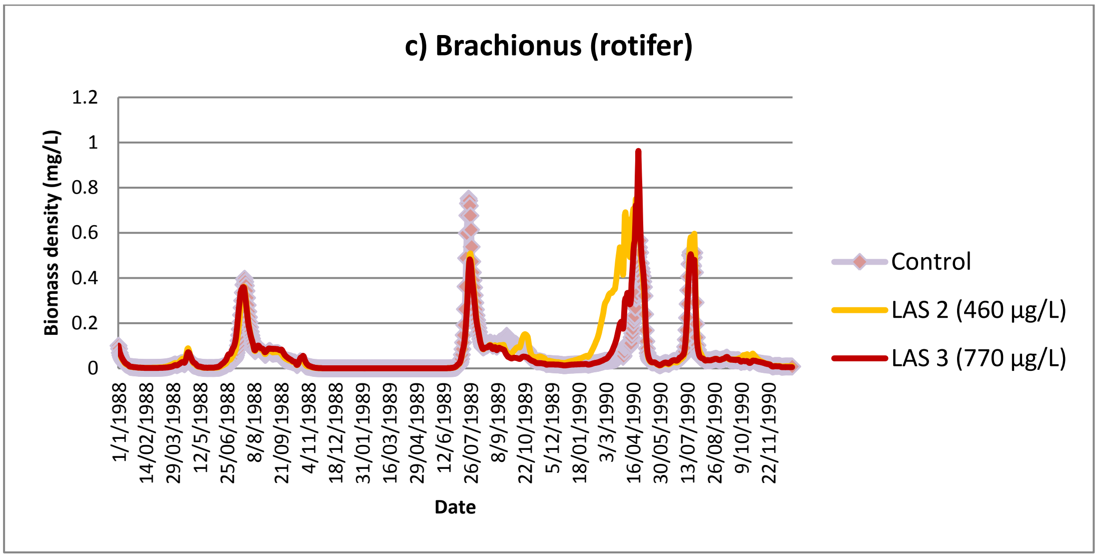
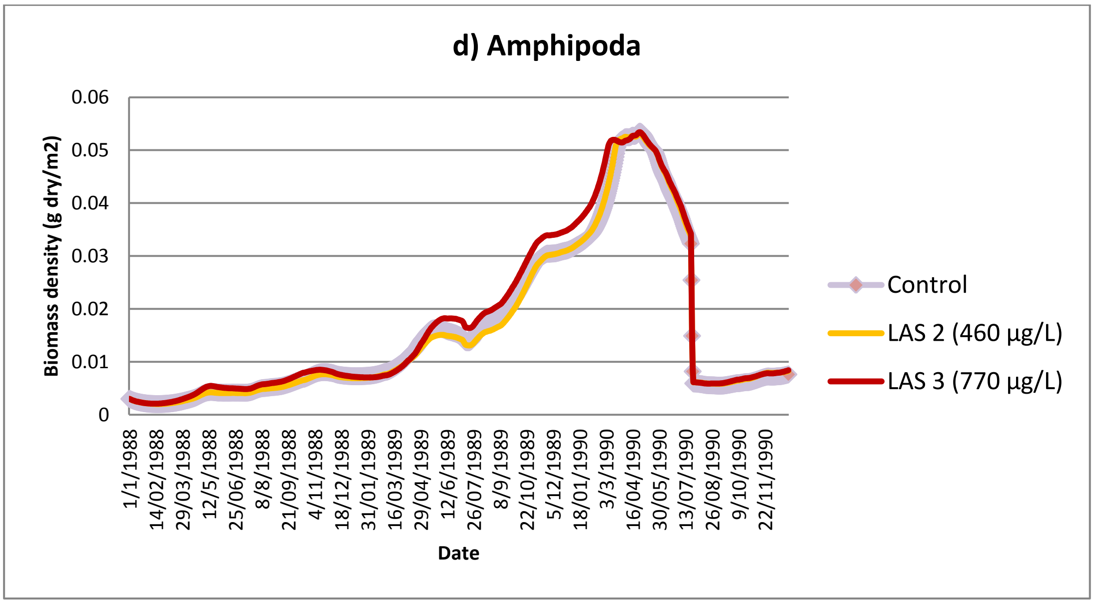


Figure 3.41 - Biomass trends in 3 year simulation for three different scenarios:
control LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)





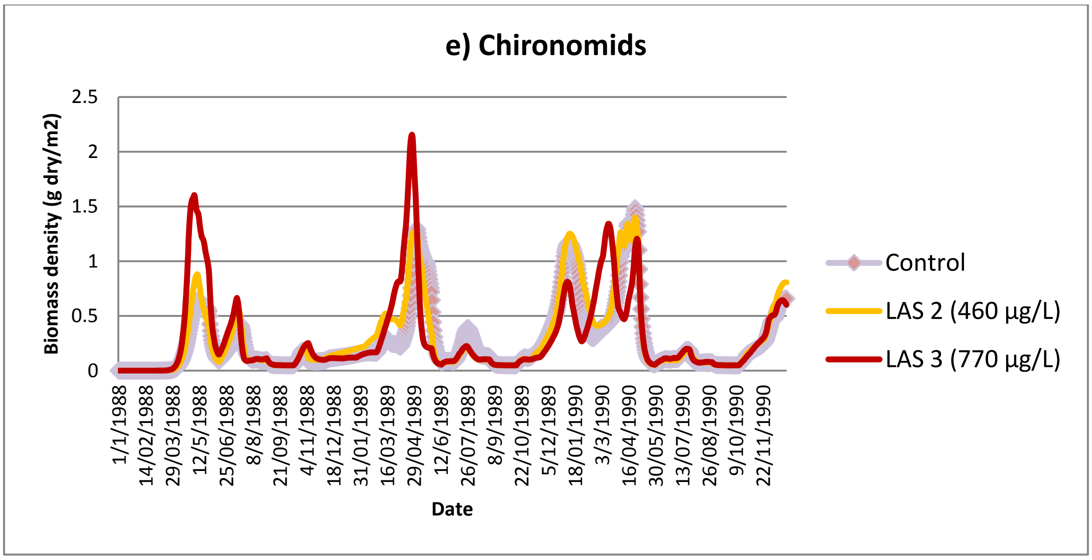
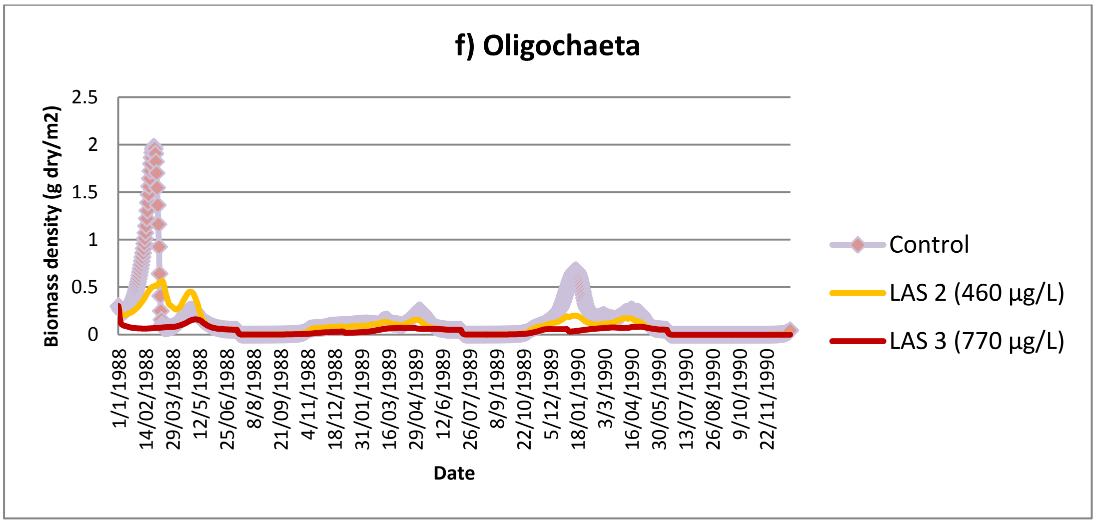
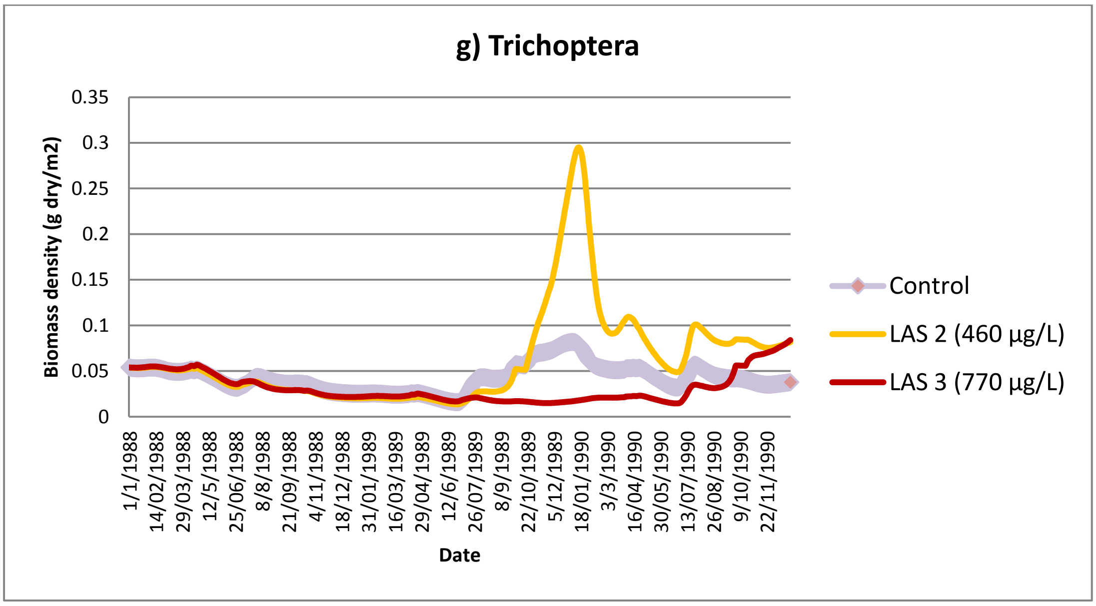


Figure 3.41 - Biomass trends in 3 year simulation for three different scenarios: control LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)





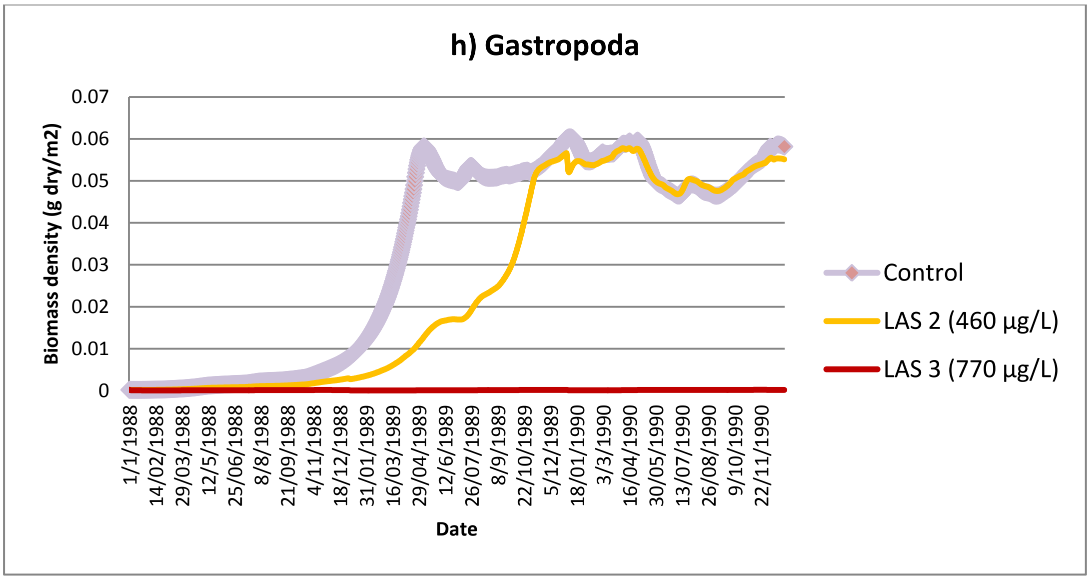
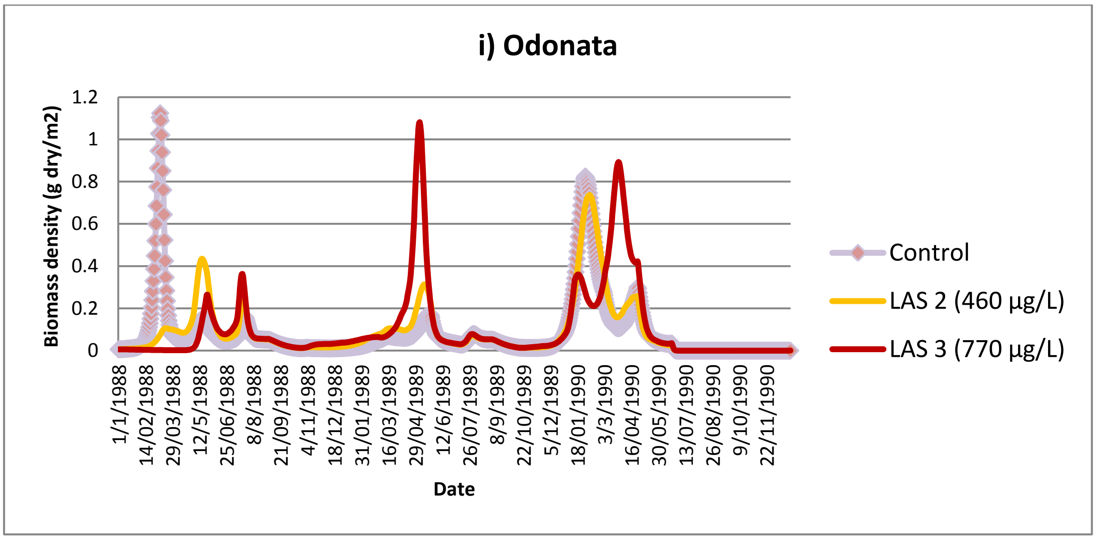
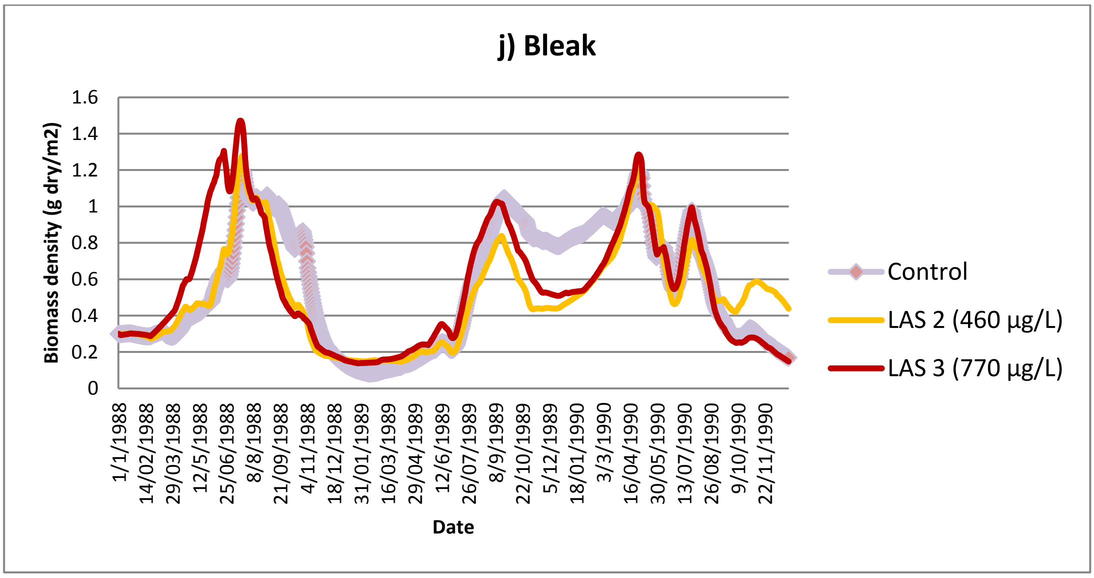


Figure 3.41 - Biomass trends in 3 year simulation for three different scenarios:
control LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)





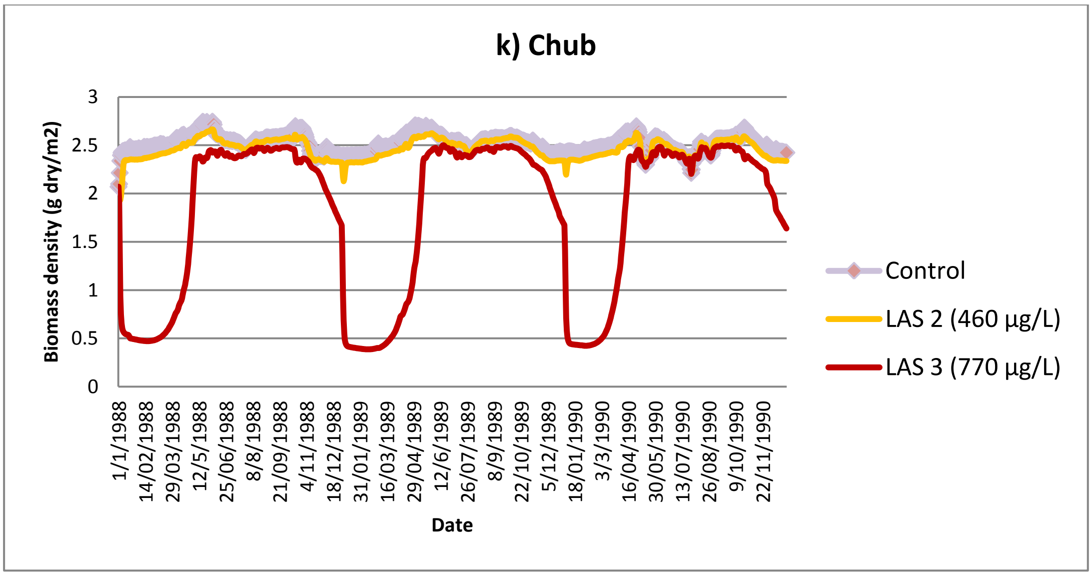
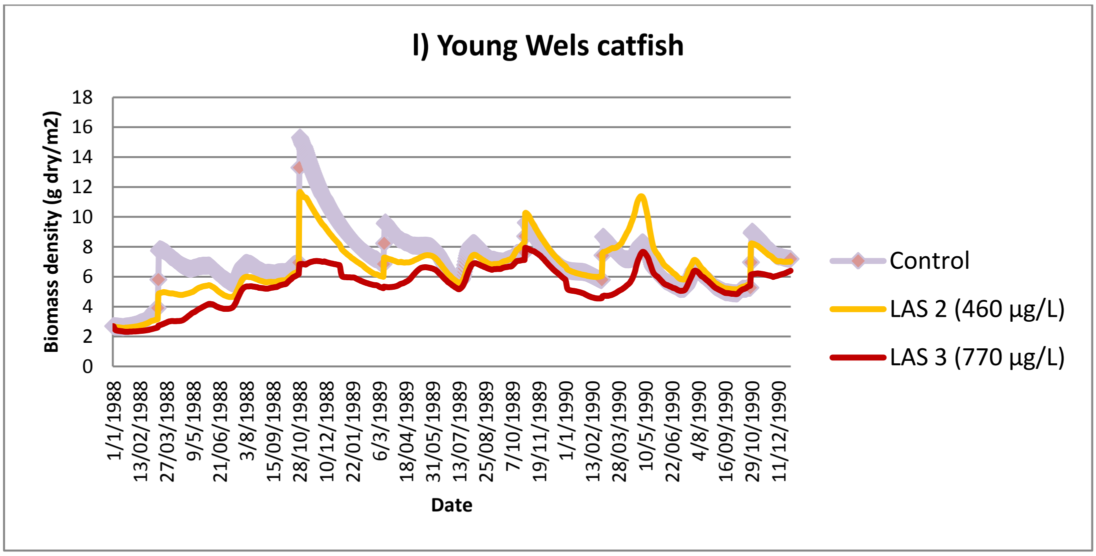


Figure 3.41 - Biomass trends in 3 year simulation for three different scenarios:

control LAS 2 (C = 460 μg/L) LAS 3 (C = 770 μg/L)



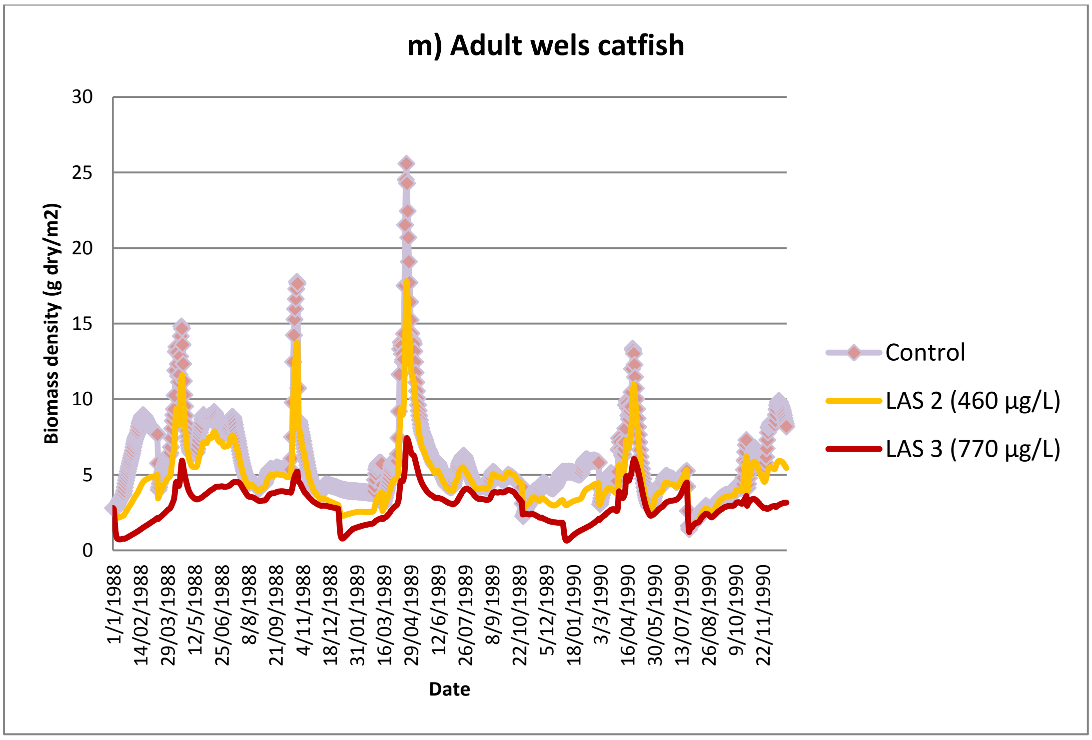


Figure 3.41 - Biomass trends in 3 year simulation for three different scenarios:
control LAS 2 (C = 460 µg/L) LAS 3 (C = 770 µg/L)

3.2.2.2 TCS perturbation

In Figure 3.42 the trends of simulated organisms biomass in TCS 2 (C = 1.61 µg/L) and TCS 3 (C = 16.1 µg/L) scenarios are shown for a 3 year simulation period.

Cyclotella and Chromulina are extinct in scenarios 2 and 3. Brachionus shows a visible decrease in the first and second years in scenarios 2 and 3. In the third year it shows a peak in scenario 3 and maintains the trend of control simulation in the following months.

Amphipoda shows no visible perturbation in perturbed scenarios for all the three years. Chironomids show no visible perturbation in perturbed scenarios for all the three years, but it shows a peak at the end of the third year in scenario 3. Oligochaeta shows no visible perturbation in perturbed scenarios for all the three years. Trichoptera is extinct in the third year in all the perturbed scenarios. Gastropoda shows no visible perturbation in perturbed scenarios for all the three years. Odonata show no visible perturbation in scenario 2, while it shows peaks in scenario 3 every year.

Bleak shows in scenario 2 and 3 a decrease of biomass from July 1989 to April 1990, in scenario 3 it shows a peak in July, then it stabilizes to the values of unperturbed scenario. Chub shows no visible perturbation in perturbed scenarios for all the three years. Young and adult Wels catfish biomass decreases progressively in scenario 2 and 3 maintaining the trend of control simulation curve.

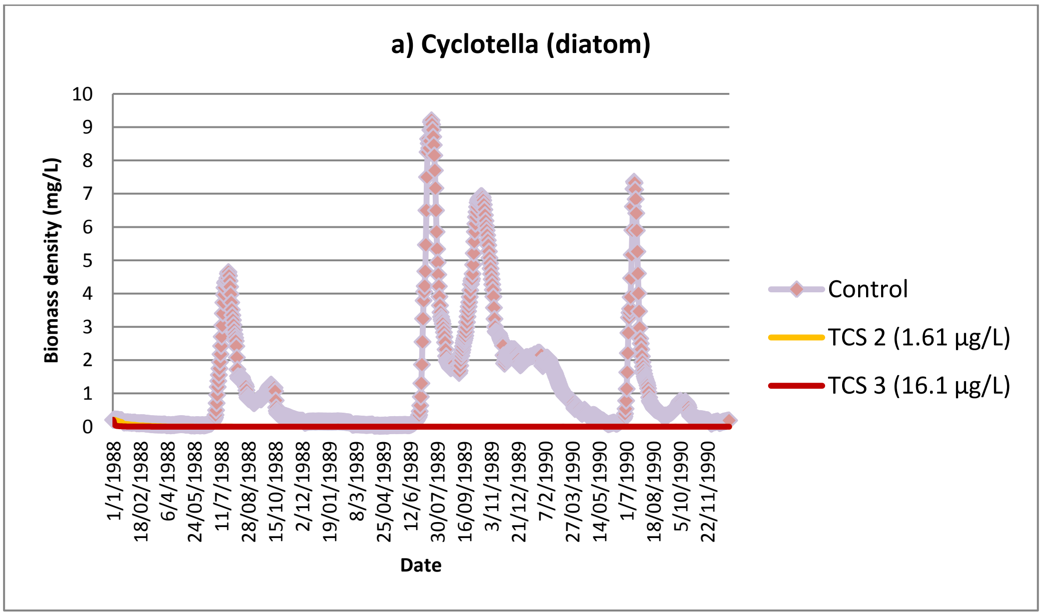
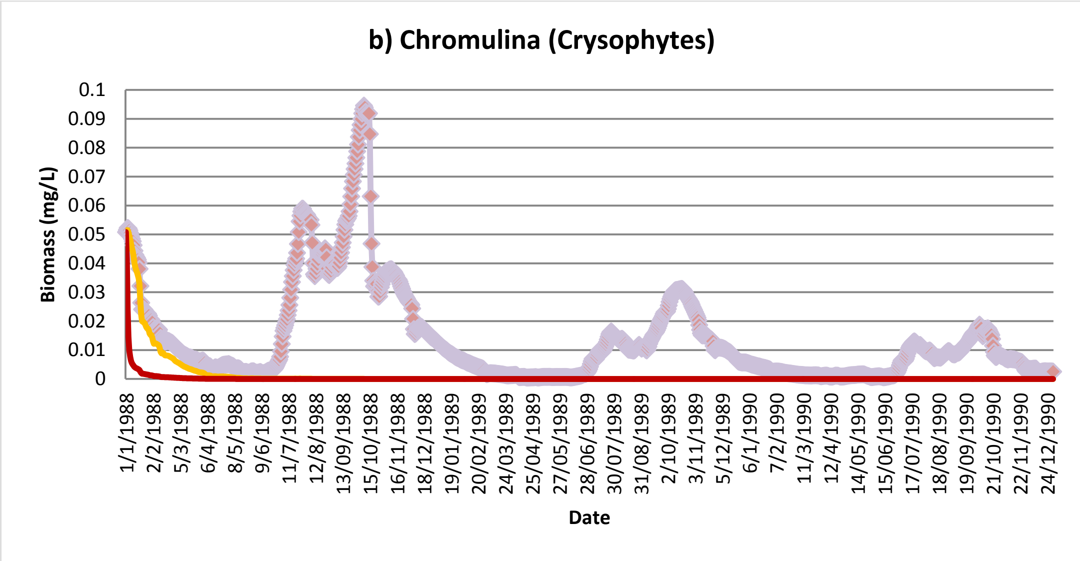
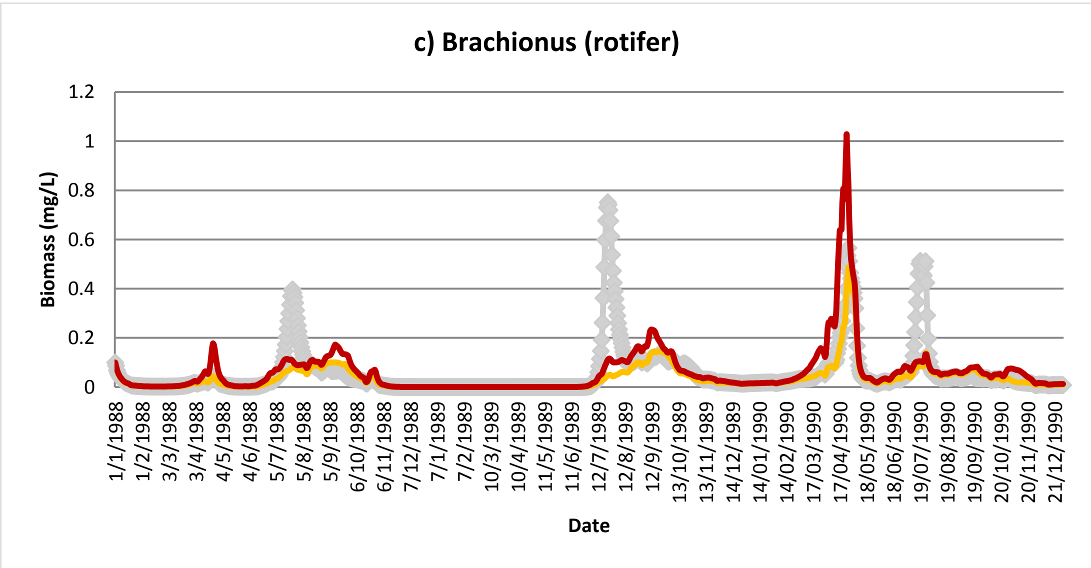


Figure 3.42 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)





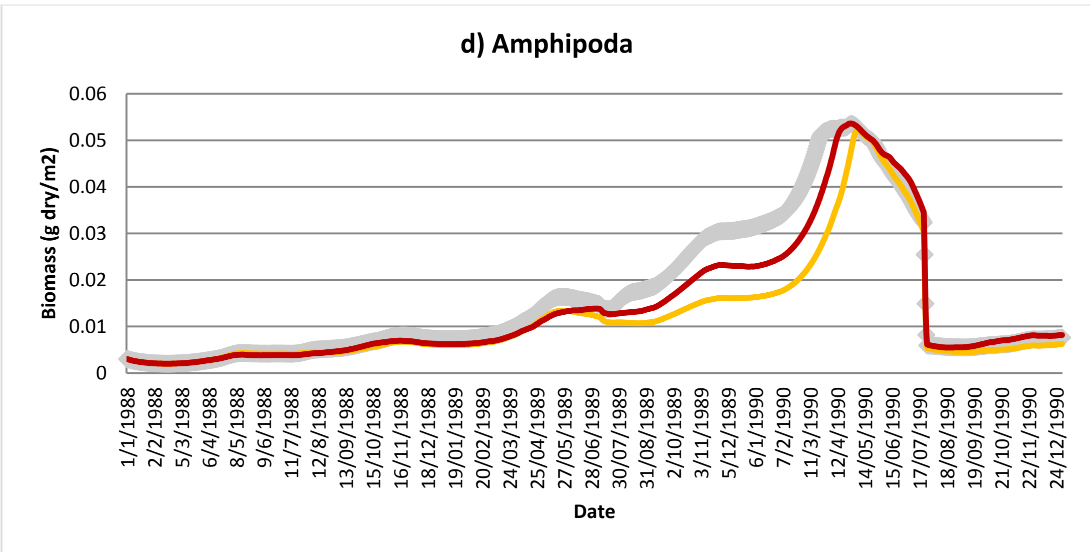
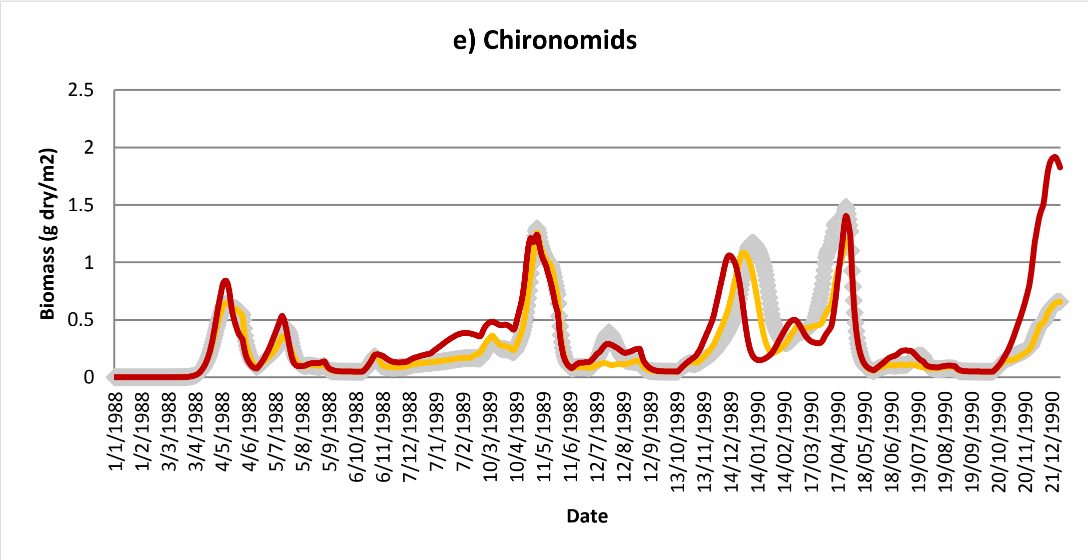
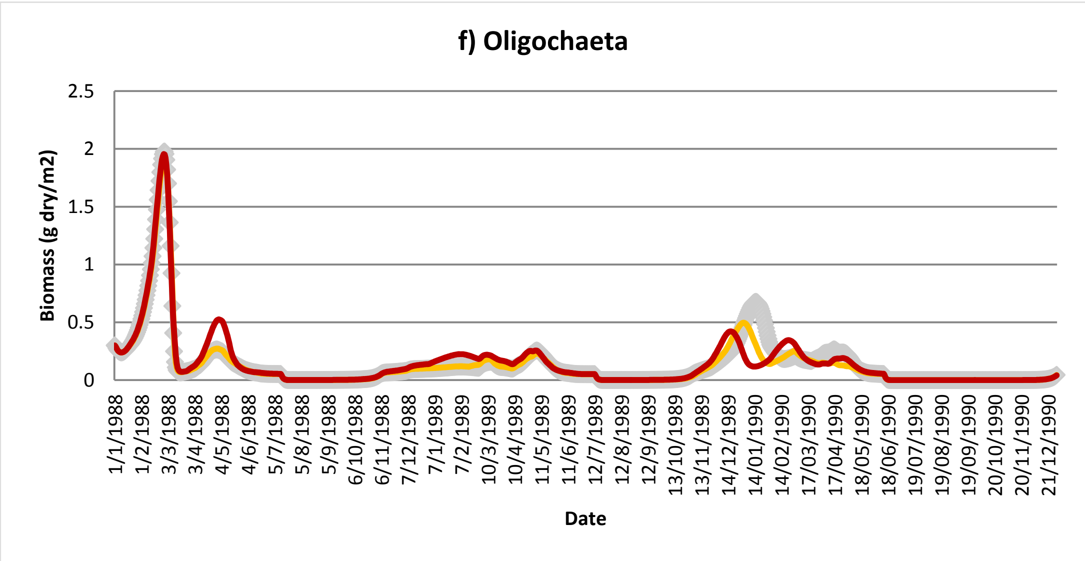


Figure 3.42 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)





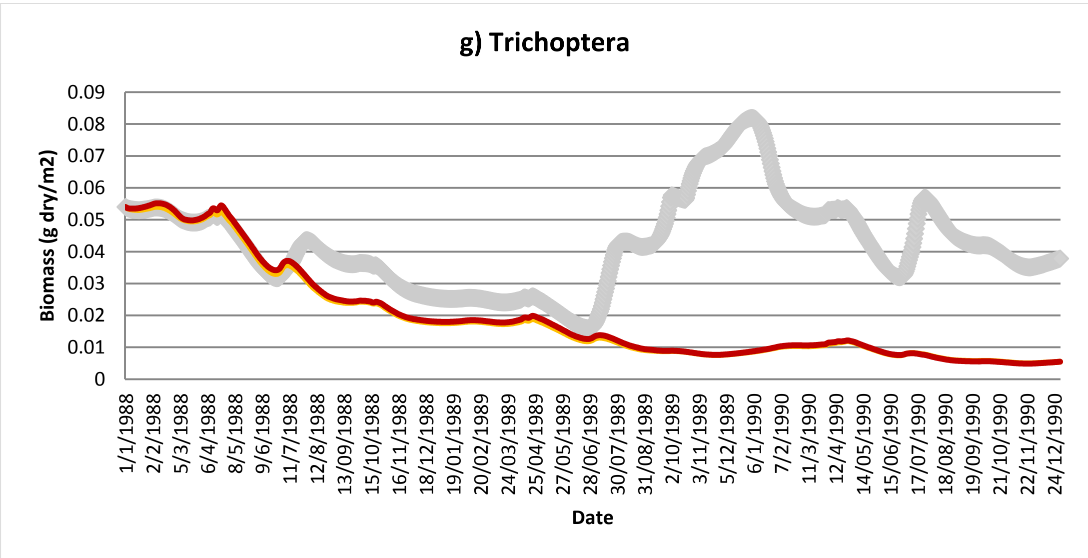
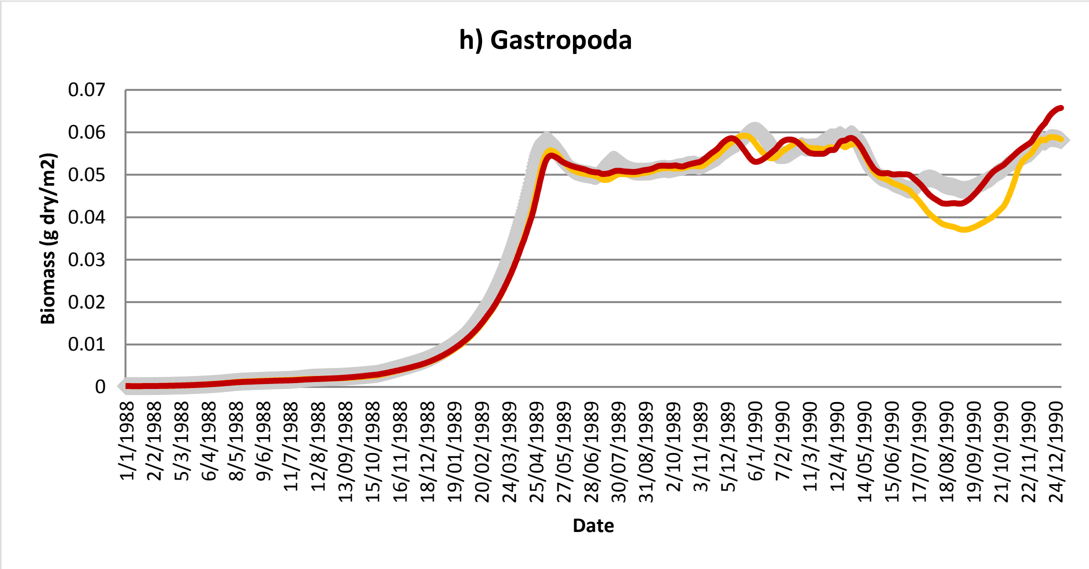
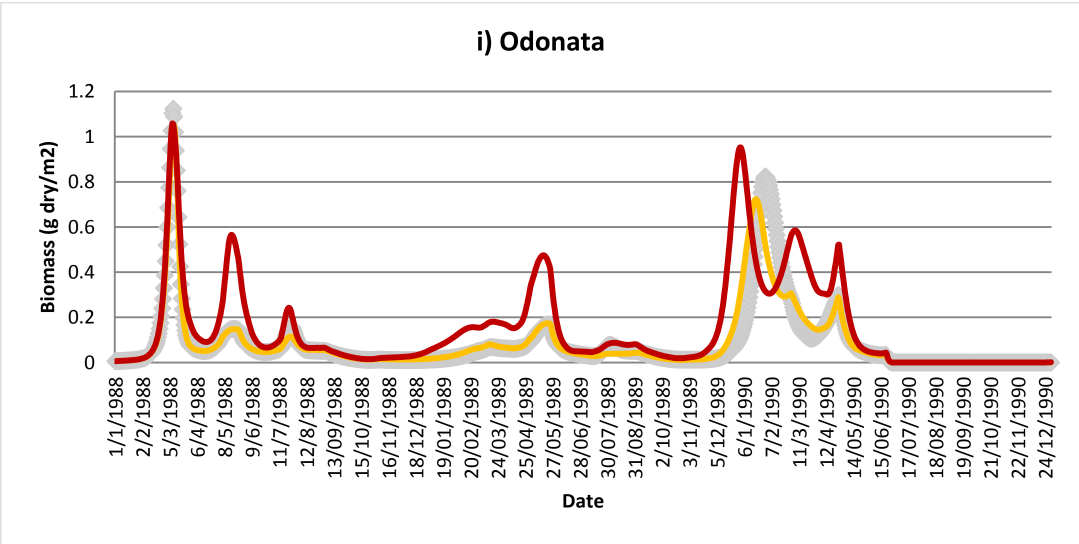


Figure 3.42 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)





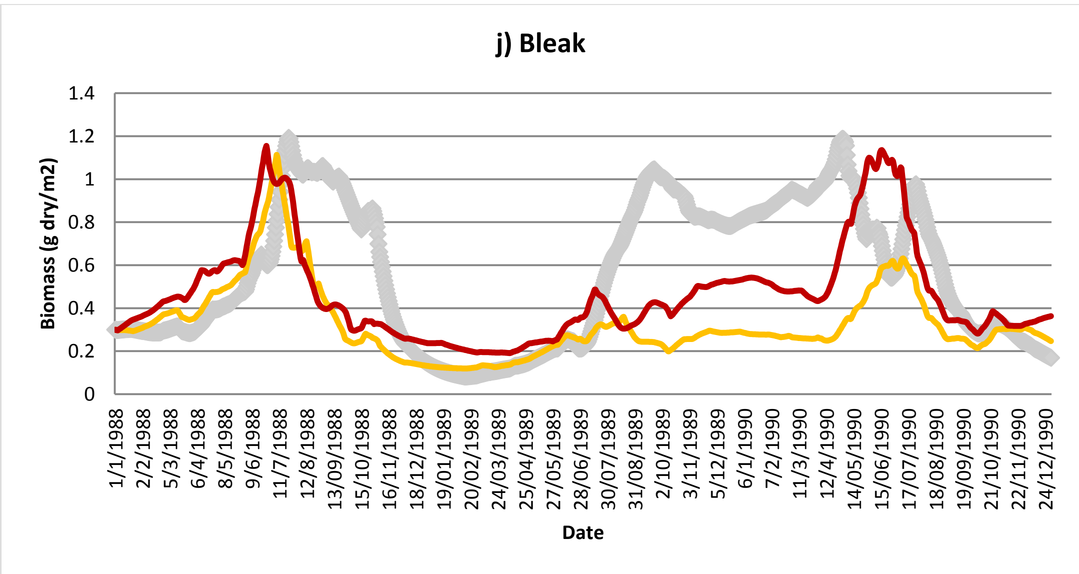
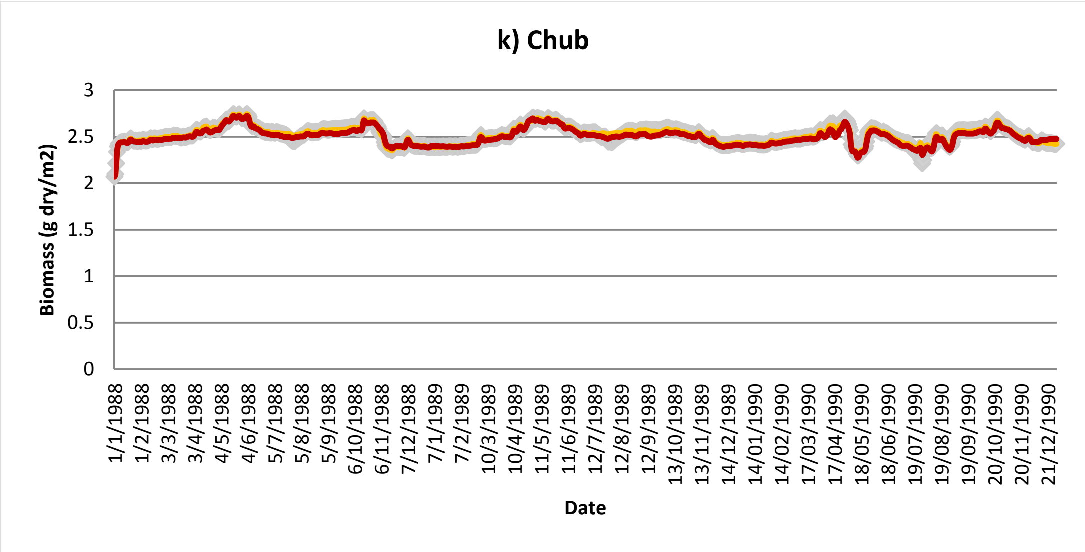
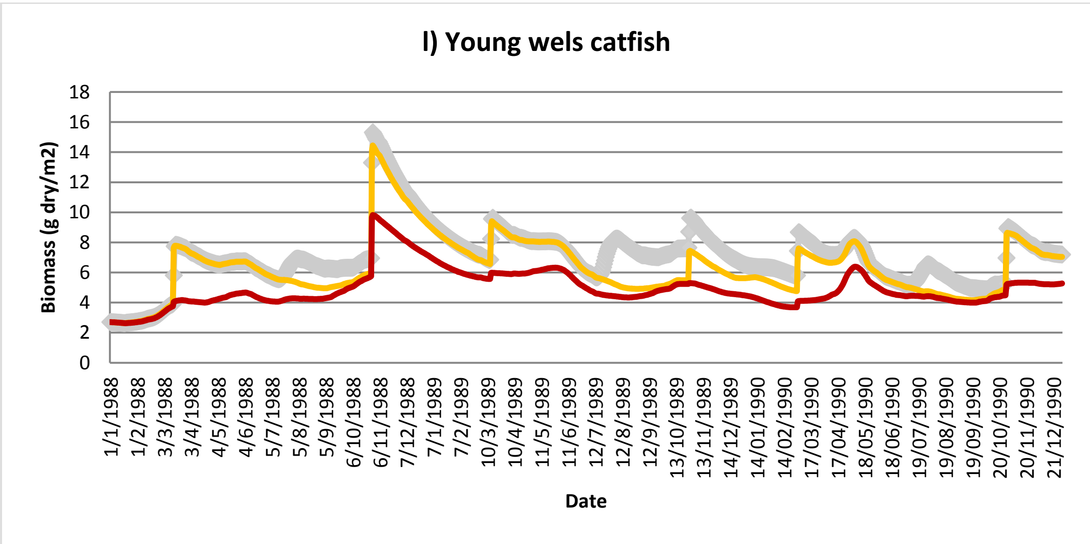


Figure 3.42 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)





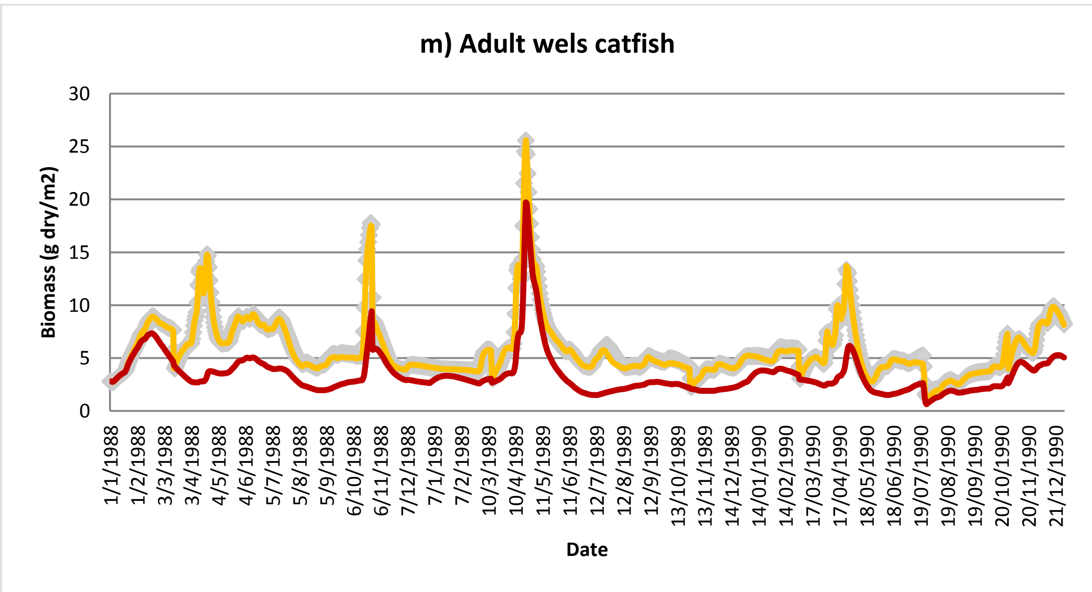


Figure 3.42 - Biomass trends in 1 year simulation with four different scenarios:

control TCS 2 (C = 1.61 µg/L) TCS 3 (C = 16.1 µg/L)

4 Discussion

In this Chapter the results of control and perturbed simulations are discussed and commented, trying to give an opinion about the goodness of the control model and about the results of perturbed model. Paragraph 4.1 concerns control simulation, Paragraph 4.2 is about LAS perturbed simulation and 4.3 is about TCS perturbed simulation.

4.1 Control ecosystem

Analyzing average biomass relative variation, Pearson coefficient and NSE coefficient (Table 4.1), an opinion on the control model quality can be derived. The one-year model simulates in a satisfactory way the annual average biomass of all organisms as the relative variation is less than 1%, with the exception of Brachionus, Chironomus and Oligochaeta groups that have a relative variation respectively of 2.29%, 2.95% and 4.4%. However, even these prediction errors can be considered acceptable because the observed data set of the three groups are affected by uncertainties, for example Brachionus data set is referred to 1990, while the year of the simulation is 1989, Chironomus and Oligochaeta data set are derived on the basis of average organisms weight and also the individual density is calculated assuming a sample surface that can bring to biomass density values affected by errors.

Pearson coefficient of the one-year control model gives a measure of the correlation between observed and simulated data. Chromulina shows the highest correlation, Chub and adult Wels catfish a regular correlation, Cyclotella, Amphipoda, Gastropoda and young Wels catfish a low correlation, Oligochaeta and Bleak shows a very low correlation. No correlation is found for Brachionus, Chironomus, Trichoptera and Odonata. Therefore, the model does not simulate in a satisfactory way the seasonal changes of most of organisms biomass, this is possibly due to the huge amount of parameter to calibrate, in particular consumption, optimum temperature parameters and preference matrix values, as well as to the uncertainty characterizing observations, to limitations in the model and to possibly inappropriate modelling assumptions (e.g. a zero dimensional ecosystem is simulated but this may be inappropriate for a river). More precise and quantitative information are needed particularly on organisms diets in order to improve the model efficiency.

Nash–Sutcliffe efficiency (NSE) coefficients are lower than 0, this means that, for most the organisms, observed mean is a better predictor of the model results. The model has surely to be improved, but also the very low values of NSE can be caused by the fact that it is sensitive to extreme values because of squared differences.

Analysing three year simulation model and comparing it with one year model, it can be found in some cases that the general organism seasonal trend is maintained with some variation in peak values, in other cases (Amphipoda and Gastropoda) the biomass trend and average biomass is completely different in the two models. Variation in peaks values can be attributed to different upstream inputs of nutrients, detritus or plankton generated in the three-years model respect to one year model. The special behaviour of Amphipoda and Gastropoda is due to the fact that predation is not simulated as organisms biomass concentration is too low.

Table 4.1 - Summary of the indices for the assessing of the goodness of control model. ε_i is the average biomass relative variation from observed and 1-year simulation models, $\varepsilon_{i(1-3)}$ is the average biomass relative variation from 1-year simulation and 3-years simulation models, r $_{(1)}$ is Pearson coefficient, E $_{(1)}$ is NSE coefficient.

Taxa	Observed biomass (mg dry/L)	1 year simulation biomass (mg dry/L)	3 years simulation biomass (mg dry/L)	ε_i (%)	$\varepsilon_{i(1-3)}$ (%)	r $_{(1)}$	E $_{(1)}$
Cyclotella	1.0421	1.0418	1.198	0.0249	15	0.396	-1.815
Chromulina	0.04205	0.04202	0.014	0.078	67.6	0.642	0.297
Brachionus	0.048177	0.047075	0.054941	2.29	14.04	-0.0132	-1.956
Amphipoda	0.00376	0.003801	0.0161	1.00	323	0.205	-0.049
Chironomus	0.1486	0.153	0.269	2.95	76	-0.082	-0.211
Trichoptera	0.0475	0.0473	0.0423	0.63	10.6	-0.0695	-0.117
Oligochaeta	0.147	0.153	0.139	4.40	9.1	0.145	-0.592
Gastropoda	0.001563	0.001547	0.0329	0.985	2003	0.263	0.008
Odonata	0.065604	0.066104	0.086	0.76	31.6	-0.036	-0.204
Bleak	0.56	0.559	0.56	0.206	0.576	0.159	-0.852
Chub	2.52	2.52	2.516	0.043	0.147	0.425	0.167
Young wels catfish	5.767	5.733	7.043	0.599	22.11	0.21	-0.095
Adult wels catfish	5.579	5.58	5.87	0.023	5.16	0.475	0.009

The control model should be appreciated because it is the first attempt, to my knowledge, to integrate all the numerous, existing ecological information on the Po river and analyse ecosystem functioning. However, it should be considered as a preliminary model of the River Po segment ecosystem mainly for two aspects that have to be improved:

1) the quality of observed data (many uncertainties and assumptions forced by lack of data);

2) the quantity and quality of parameters (too many parameters to calibrate).

To develop a good model with AQUATOX it is fundamental to have a good starting data set, in the case of the Po river from the hydrologic, morphologic and physico-chemical point of view the data were sufficient for a good simulation, while data on biota were absolutely incomplete and too few for a correct and satisfactory simulation. Moreover recent data on Po river biota are absent in the literature, this is a gap that should be filled because biota monitoring is very important for an accurate risk assessment.

Another aspect to be improved is the quantity and quality of parameters to calibrate. As in AQUATOX there are a lot of parameters that can be changed during calibration, the higher is the number of known parameter values form experiments or from literature, the lower is the number of parameter to be changed during calibration, the easier and more rapid is the calibration and results interpretation. In this study the lack of quantitative data about organisms diets is the main issue regarding parameters.

4.2 LAS perturbation

The Po river ecosystem presents no visible changes if perturbed with actual LAS concentration of 3.22 µg/L (LAS 1 scenario) both in short and long term perturbation. Relative biomass variation with respect to the control model is less than 1%. Instead, the ecosystem perturbed with higher concentration (C=460 µg/L, C= 770 µg/L) shows visible changes. Gastropoda results the organism subject to the higher impact, followed by Cyclotella and adult Wels catfish. The interesting point is that these results cannot be explained if the attention is focused only on LC50 and EC50 values, i.e. on the direct effect of toxicity. In fact adult Wels catfish should be the most LAS-sensitive organism according to its LC50 value, while the most sensitive organism resulted to be Gastropoda, moreover Cyclotella has the highest LC50 value but it is still very sensitive to LAS perturbation. This can be explained only if indirect ecological effects triggered by chemical toxicity are taken into account. Moreover, this is an important proof that demonstrated the idea that an accurate risk assessment should be based not only on direct effects and ecotoxicological tests results but also on indirect effects assessment, e.g. through models.

In the following paragraphs LAS 1 and LAS 2 perturbed scenarios are discussed for one year simulation period. Discussion of three year simulation period is not carried out because no quality indices are calculated, the comments in Paragraph 3.2.2 are considerate sufficient for the thesis objective. An important consideration has to be made regarding ecotoxicological parameter: in this discussion uncertainties on the main ecotoxicological values of LC50, EC50, BCF, K_1 and K_2 are not considered but a change in the value of these parameters could influence in profound manner the perturbation effects. In this contest the objective of this thesis is to evaluate the power of modelling approach in risk assessment and not to evaluate the overall toxicity effects of LAS. For this reason the attention is not focused on the question "is LAS toxicant and at which concentrations?", but it is focused on the question "are indirect effects fundamental to understand the risk due to LAS presence in the system?".

4.2.1 Perturbed scenario LAS 2 (C = 460 μg/L)

Cyclotella (diatom) behavior depends mainly on the effect that LAS produce on predators (Bleak and Chironomids). In fact it has high LC50 and EC50 values, but its biomass decreases until 48% (highest decrease). As reported in Appendix C, Figure C 1 (a-b), photosynthesis and respiration remains constant but predation increases, thus the biomass reduction is certainly due to indirect effects as a slight increase in predators biomass (see Bleak and Chironomids) (Figure 3.19 -a, b). Since Cyclotella has the highest LC50 value (290000 mg/L), in indices-based risk assessment this value will brings to zero risk for the organisms, but with a modelling approach it can be seen that indirect effects are compelling. Thus for Cyclotella LC50 value cannot be considered as a useful parameter to have an idea of the risk of LAS on the organisms in the ecosystem.

Chromulina behavior is different because its predation mortality is absent in control and perturbed scenarios, moreover photosynthesis and respiration remain constant, thus the slight reduction of 8% is due to direct effect of LAS perturbation.

Brachionus rotifer presents a negligible decrease in biomass, this is the result of a decrease of consumption (connected to Cyclotella biomass decrease), a decrease of predation (connected to young Wels catfish biomass decrease) and an increase of defecation (probably due to toxicant), in conclusion it can be considered that LAS concentration equal to 460 μg/L produces no effects on zooplankton (see Appendix C, Figure C1 (c)). This is in line with LC50 value (3340 mg/L, higher than the lower LC50) that can be considered as a useful parameter to have an idea of the risk.

Amphipoda biomass presents a negligible increase, this is due to an increase in consumption connected to the increase of detritus biomass, its predation is absent in all scenarios (see Appendix C, Figure C1 (d)). It can be concluded that LAS has no effects on Amphipoda at concentration equal to 460 μg/L.

Chironomids biomass increases, this is due to a decrease in predators biomass (Odonata, Bleak, Chub, young and adult Wels catfish). It can be concluded that LAS has no effects on Chironomids at concentration equal to 460 μg/L. LC50 value (8600 mg/L, higher than the lower LC50) can be considered as a useful parameter to have an idea of the risk.

Oligochaeta biomass decreases due to a decrease in consumption related to sedimented detritus decrease and an increase in defecation related to toxicant effects. Predation also decrease but the effect of defecation increase is more effective. In this case it can be concluded that LAS has direct effects on Oligochaeta and LC50 value (1020 mg/L, very near to the lower LC50) can be considered as a useful parameter to have an idea of the risk.

Trichoptera presents a consumption decrease related to Cyclotella biomass decrease and not to direct LAS effects. Moreover Trichoptera biomass trend is influenced by Odonata trend.

Gastropoda biomass decreases as there is an increase in defecation, due to toxicant, and a decrease in consumption due to Cyclotella biomass trend.

Odonata biomass has an opposite trend with respect to Trichoptera (Figure 3.19 - i). As Odonata is the principal Trichoptera predator, this demonstrates that Trichoptera biomass trend is influenced by Odonata trend, not the contrary, so if Odonata biomass increases, Trichoptera predation increases too and Trichoptera biomass decreases. Odonata has an average decrease in consumption, defecation and predation, the decrease in consumption determinates the overall biomass decrease and this is due to a decrease in prey biomass (Trichoptera, Oligochaeta). As Bleak is one of the principal Odonata predation, it can be explain the Odonata biomass trend: if Bleak biomass increases, Odonata predation increases too and Odonata biomass decreases.

Bleak biomass slightly decreases. Its trend is a result of a increase in predation by Chub, and young-adult Wels catfish (see Appendix C, Figure C1 (j)). Chub biomass slightly decreases, this can be due to the absence of Odonata that is one of the principal prey of the fish (Figure 3.19 - k), but also to direct effect of LAS. Young and adult Wels catfish show a progressive decrease in biomass, this is due to an increase in defecation related to toxicant presence, this is because they are the most sensitive organisms and direct effects of LAS are visible (see Appendix C, Figure C1 (l-m)).

Table 4.2 - LC50, EC50 and relative biomass variation in perturbed LAS scenario 2.

Organism	LC50 (µg/L)	EC50 (µg/L)	LAS 2 (460 µg/L) (%)
Cyclotella	290000	29000	-48.14
Chromulina	9100	910	-8.09
Rotifer Brachionus	3340	2000	-0.72
Amphipod	7600	1700	0.26
Chironomids	8600	8000	14.22
Oligochaeta	1020	610	-27.97
Trichoptera	8600	5150	-9.56
Gastropoda	1020	610	-47.81
Odonata	2400	1440	-18.22
Bleak	3200	2400	-4.37
Chub	835	500	-2.50
Young wels catfish	1670	2000	-10.85
Adult wels catfish	770	460	-18.97

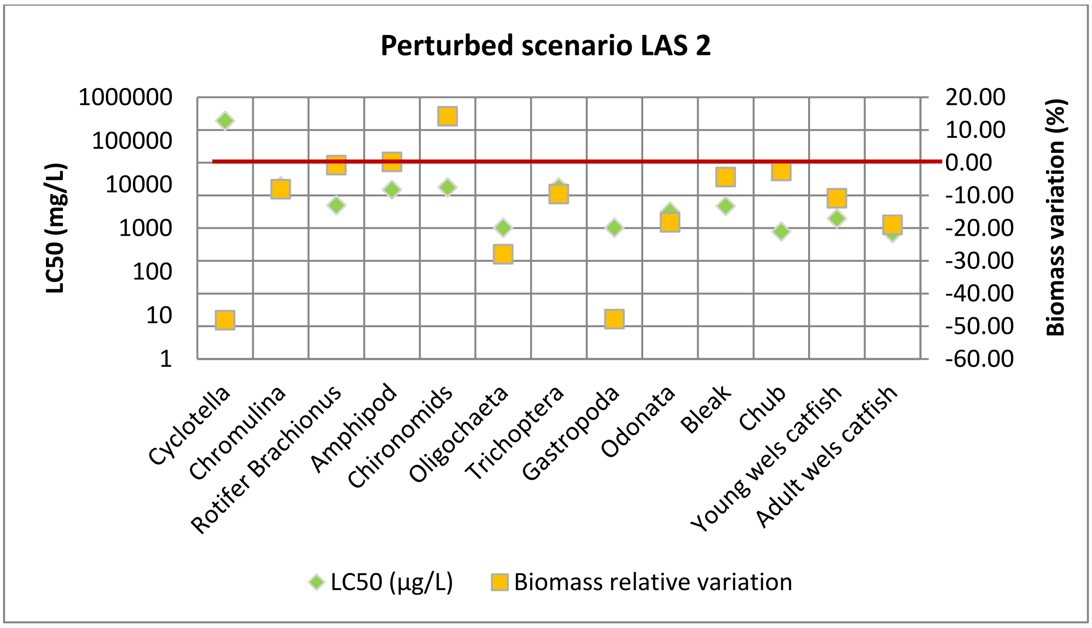


Figure 4.1 - LC50 vs Biomass relative variation for simulated organisms in perturbed scenario LAS 2.

4.2.2 Perturbed scenario LAS 3 (C = 770 µg/L)

With an higher LAS concentration the ecosystem dynamics do not change with respect to scenario 2 but effects are amplified (Table 4.2). Gastropoda are almost extinct, other organisms negatively affected by LAS perturbation are Cyclotella (-68%), Oligochaeta (-64%), adult Wels catfish (-49%), Odonata (-44%), Chub (-28%), young Wels catfish (-27%), Chromulina (-24%), Trichoptera (17%). Chironomids, Bleak, Amphipod and Rotifer are positively affected by LAS perturbation because of indirect effects.

Table 4.3 - LC50, EC50 and relative biomass variation in perturbed LAS scenario 3.

Organism	**LC50 (µg/L)**	**EC50 (µg/L)**	**LAS 3 (770 µg/L) (%)**
Cyclotella	290000	29000	-68.35
Chromulina	9100	910	-24.08
Rotifer Brachionus	3340	2000	0.12
Amphipod	7600	1700	5.31
Chironomids	8600	8000	54.91
Oligochaeta	1020	610	-64.57
Trichoptera	8600	5150	-17.55
Gastropoda	1020	610	-92.56
Odonata	2400	1440	-43.94
Bleak	3200	2400	5.44
Chub	835	500	-28.08
Young wels catfish	1670	2000	-27.37
Adult wels catfish	770	460	-48.71

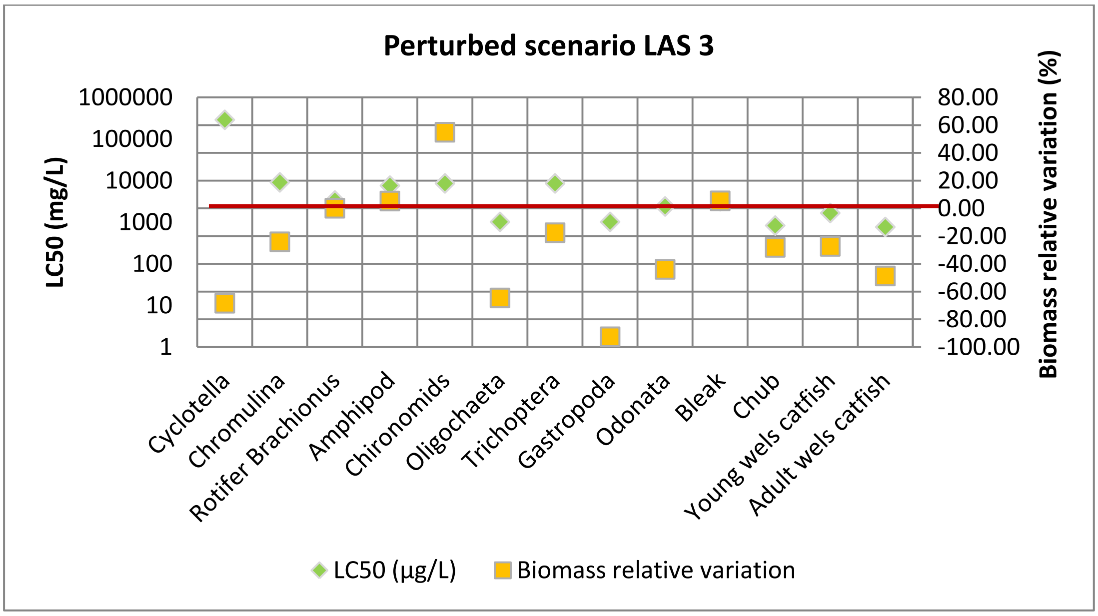


Figure 4.2 - LC50 vs Biomass relative variation for simulated organisms in perturbed scenario LAS 3.

4.2.3 Ecological indicators

The overall biomass variation is stronger as LAS concentration increases in water (Figure 3.20). This larger change is due to direct and indirect effects as described in detail in the previous paragraphs.

The decrease in production/respiration of the community increasing LAS concentration in water is mainly the result of a decrease in primary production (Figure 3.26), that is connected to indirect effects of LAS on Cyclotella. Gross primary production is the total production of organic matter through photosynthesis, while community respiration is the metabolism of organic matter by animals. P/R is a common measure of the trophic status of a system, P/R equal to 1 correspond to a mature ecosystem in which there is a balance between what is produces and what is consumed in terms of organic matter, if P/R is less than 1 the system is heterotrophic, if P/R is higher than 1 the system is autotrophic. In the case of the Po river, the system is heterotrophic and with LAS perturbation this status is accentuated.

Ecosystem biodiversity, measured with Shannon index (Figure 3.27), slightly decrease in LAS 2 scenario and slightly increase in LAS 3 scenario, variation are due to the decrease in presence in term of biomass of some species (Cyclotella and adult wels catfish) and increase of others (young wels catfish, Chub). But in conclusion the biodiversity is not so affected by LAS perturbation because Shannon index variation is negligible.

The ecological quality variation is described in Table 3.16. In scenario 1 there is no visible perturbation for any organism in the system. A high perturbation level is shown only by plants in scenario 3. Zooplankton and macroinvertebrates present low perturbation. Fishes show moderate-high perturbation only in scenario 3. Overall the system resulted no perturbed in scenario 1, low perturbed in scenario 2 and moderate-high perturbed in scenario 3.

Regarding ecological services indices, turbidity is not affected by LAS perturbation, while fishes biomass is reduce by 12% in LAS 2 scenario and by 34% in LAS 3 scenario, therefore in principle chemical pollution could cause inefficiency and problems for the fisheries present in the Po river.

4.3 TCS perturbation

Po river ecosystem presents no visible changes if perturbed with current TCS concentration of 0.000926 µg/L (TCS 1 scenario) that is very low and closed to zero. The ecosystem perturbed with higher concentration (C = 16.1 µg/L) shows higher variations for all organisms. Effects are mainly on phytoplankton that is the most TCS-sensitive group. But also zooplankton and macroinvertebrates present some visible effects.

In this perturbation case the organisms with the lower LC50 are also the organisms with the higher biomass reduction. But the inverse relation between LC50 and biomass reduction is not respected for example for Brachionus, that has the higher LC50 but presents a biomass reduction of about 30% in scenario 2. As written for LAS perturbation, this is an important proof that demonstrated the thesis idea that an accurate risk assessment should be based not only on direct effects and ecotoxicological tests results but also on indirect ecological effects assessment through models.

In the following paragraphs TCS 2 and TCS 3 perturbed scenarios are discussed for one year simulation period. Discussion of three year simulation period is difficult because no quality indices are calculated, the comments in Paragraph 3.2.2 are considerate sufficient for the thesis objective.

4.3.1 Perturbed scenario TCS 2 (C = 1.61 µg/L)

Cyclotella and Chromulina are the most TCS-sensitive organisms, their biomasses decrease respectively by 97% and 75%. As reported in Appendix C, Figure C 2 (a-b), photosynthesis decreases drastically while respiration remains constant and predation decreases. The mortality is certainly due to TCS presence. In this case LC50 value can be considered as a useful parameter to have an idea of the risk of TCS on the organisms in the ecosystem.

Brachionus rotifer presents a decrease in biomass (-30%) even if its LC50 value is the higher. This is the result of a decrease in biomass of one of the principal Rotifer predator (young Wels catfish).

Amphipoda biomass presents a decrease of about 12%, this is due to a slight decrease in consumption connected to the slight decrease of detritus biomass, its predation is absent in all scenarios (see Appendix C, Figure C2 (d)). Also direct effects are involved.

Chironomids biomass slightly increases, this is due to a decrease in predators biomass (Bleak, young and adult Wels catfish). It can be concluded that TCS has no effects on Chironomids and LC50 value (8600 mg/L, higher than the lower LC50) can be considered as a useful parameter to have an idea of the risk.

Oligochaeta biomass increases due to an increase in consumption of detritus. In this case it can be concluded that TCS has no effects on Oligochaeta and LC50 value (1260 mg/L, higher than the lower LC50) can be considered as a useful parameter to have an idea of the risk.

Trichoptera and Gastropoda biomasses decreases because consumption decreases. This is related to Cyclotella biomass decrease and to predator (Odonata) increase and not to direct TCS effects.

Odonata biomass increases because its consumption increases. As Bleak is one of the principal Odonata predation, it can be explained the Odonata biomass trend: if Bleak biomass increases, Odonata predation increases too and Odonata biomass decreases.

Bleak biomass decreases. Its trend is a results of a decrease in prey (Cyclotella and Chromulina) and not a direct effect of TCS (see Appendix C, Figure C2 (j)). Chub biomass decrease is negligible, it can be concluded that TCS have no effects on it. Young and adult Wels catfish show a progressive decrease in biomass, this is due to a decrease in consumption because of the prey (Bleak) decrease and an increase in defecation related to toxicant presence (see Appendix C, Figure C2 (l-m)).

Table 4.4 - LC50, EC50 and relative biomass variation in perturbed TCS scenario 2.

Organism	**LC50 (µg/L)**	**TCS 2 (1.61 µg/L) (%)**
Cyclotella	16.1	-97.91
Chromulina	16.1	-75.13
Rotifer Brachionus	1544	-29.32
Amphipod	200	-12.90
Chironomids	400	1.15
Oligochaeta	1260	6.30
Trichoptera	400	-22.09
Gastropoda	1260	-17.79
Odonata	400	3.62
Bleak	260	-26.00
Chub	260	-0.03
Young wels catfish	370	-10.59
Adult wels catfish	370	-1.74

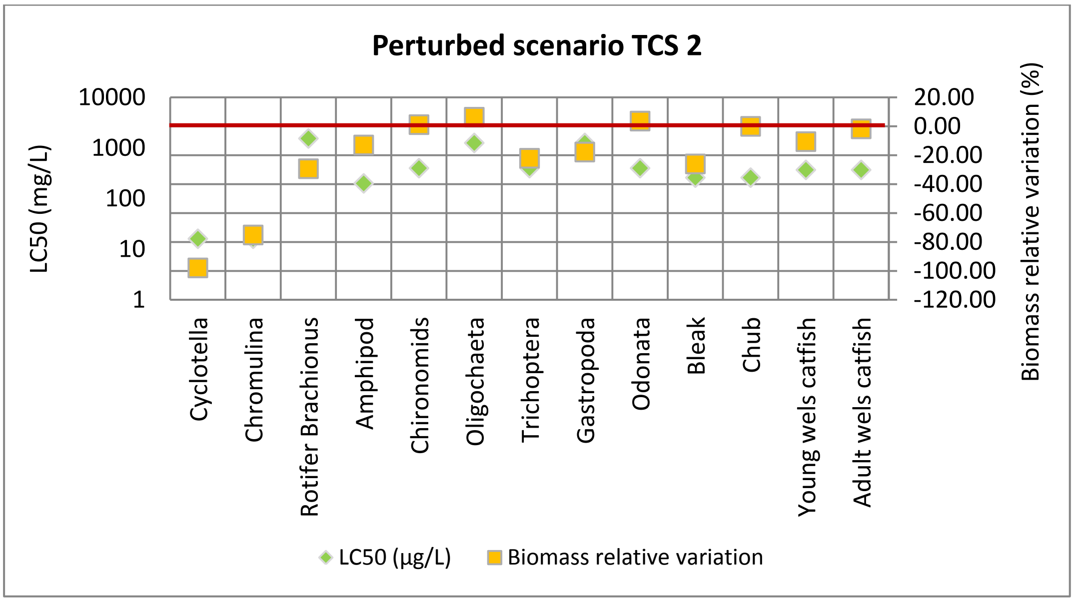


Figure 4.3 - LC50 vs Biomass relative variation for simulated organisms in perturbed scenario TCS 2.

4.3.2 Perturbed scenario TCS 3 (C = 16.1 µg/L)

With a higher TCS concentration the ecosystem dynamics do not visibly change respect to scenarios 1 and 2 but effects are amplified (Table 4.2). Cyclotella and Chromulina are almost extinct, other organisms negatively affected by LAS perturbation are adult Wels catfish (-38%), young Wels catfish (-29.6%), Trichoptera (-22%), Bleak (-8%), Rotifer (-3%). Amphipoda, Chironomids, Oligochaeta, Gastropoda and Odonata are positively affected by LAS perturbation because of indirect effects (Table 4.5 and Figure 4.4).

Table 4.5 - LC50, EC50 and relative biomass variation in perturbed TCS scenario 3.

Organism	**LC50 (µg/L)**	**TCS 3 (16.1 µg/L) (%)**
Cyclotella	16.1	-99.72
Chromulina	16.1	-98.14
Rotifer Brachionus	1544	-3.35
Amphipoda	200	6.26
Chironomids	400	6.50
Oligochaeta	1260	36.96
Trichoptera	400	-22.13
Gastropoda	1260	2.41
Odonata	400	109.75
Bleak	260	-8.46
Chub	260	-0.75
Young wels catfish	370	-29.60
Adult wels catfish	370	-38.45

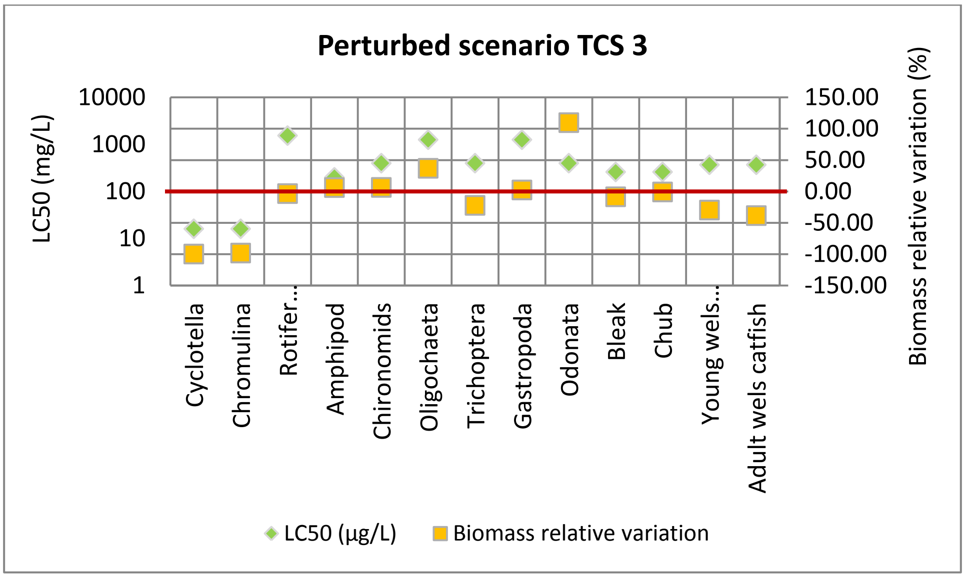


Figure 4.4 - LC50 vs Biomass relative variation for simulated organisms in perturbed scenario TCS 3.

4.3.3 Ecological indicators

Overall biomass variation indicator decreases in scenario 2, but increases in scenario 3. Thus, there is not a direct relation between overall average biomass decrease and TCS concentration increase. This can be explained by the presence of direct and indirect effects as described in detail in the previous paragraphs.

The decrease in production/respiration (Figure 3.37) of the community increasing TCS concentration in water is mainly the result of a decrease in primary production, that is connected to indirect effects of LAS on Cyclotella. In the case of the Po river, the system is heterotrophic and with TCS perturbation this status is accentuated.

Ecosystem biodiversity, measured with Shannon index (Figure 3.38), slightly decrease in TCS 2 scenario and slightly increase in TCS 3 scenario, variation are due to the decrease in presence in term of biomass of some species and increase of others. But in conclusion the biodiversity is not so affected by TCS perturbation because Shannon index variation is negligible.

The ecological quality variation is described in Table 3.18. Plants show a high perturbation level in all scenarios. Zooplankton and macroinvertebrates do not present visible perturbation. Fishes show moderate-high perturbation only in scenario 3. Overall the system resulted low perturbed in scenario 1 and 2 and moderate-high perturbed in scenario 3.

Regarding ecological services indices, turbidity is not affected by TCS perturbation, while fishes biomass is reduce by 6% in TCS 2 scenarios and by 27% in TCS 3 scenario. Therefore, in principle, chemical pollution could affect the fisheries of the Po river according to these results.

5 Conclusions

The Po river ecotoxicological model developed in this thesis work is a useful tool to demonstrate the importance of ecosystem models in risk assessment. Comparison between unperturbed and perturbed simulation results shows that chemical effects on organisms cannot be attributed only to individual toxicity effects (expressed with LC50 and EC50 toxicity parameters) but also to biota direct and indirect interactions within the entire ecosystem. Some organism with a high LC50 (high resistance to chemicals) displaya high sensitivity in the perturbed model (for example Cyclotella in LAS perturbation), and in some cases organisms displaying the same LC50 have completely different behaviours. This highlights the fact that an accurate risk assessment should be based not only on direct effects measured with ecotoxicological tests (PEC/PNEC approach) but also on the assessment of indirect ecological effects through models.

The model perturbed with current LAS and TCS concentrations does not present visible perturbation both in short and long term simulations, thus the two chemicals at actual concentration do not seem represent a risk for Po river ecosystem, at least if they act in isolation from other chemicals as in the case of these simulations. The same result is found by Lombardo (2013) for the river Thames.

The ecosystem perturbed with high LAS concentrations (C=460 µg/L, C= 770 µg/L) shows visible changes. Gastropoda results the organism subject to the highest impact, followed by Cyclotella and adult Wels catfish.

Results of TCS perturbed scenario demonstrate the same result reached by Lombardo for the river Thames, i.e. that 1 µg/L concentration is enough to significantly reduce phytoplankton communities, creating an overall imbalance in the ecosystem.

The Po river ecosystem is more resistant to LAS because even at high concentrations (40 or 70 times the current concentration) no animal is extinct in the short and long term, while for TCS phytoplankton becomes extinct at high concentrations.

Problems encountered in the thesis work regard mainly the development of the control model, because of the lack of observed data for calibration and the huge amount of parameters to calibrate. The one-year model simulates in a satisfactory way the annual average biomass of all organisms but it does not simulate in a satisfactory way most of the time series of organisms biomass. To improve the control model quality a better quantitative knowledge of organisms biomass variation in time and of organisms diet is needed. The more accurate are organisms data the more efficient the model can be. In particular, for the Po river, a biota monitoring network should be developed in order to register biomasses variations over time, and models should be used to analyse these variations.

Appendices

5.1 Appendix A - Physical and chemical variables

Table A.1 - Flow data

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27/11/1990	252288000
28/11/1990	241056000
29/11/1990	190944000
30/11/1990	158112000
01/12/1990	91584000
02/12/1990	127008000

03/12/1990	119232000
04/12/1990	110592000
05/12/1990	104544000
06/12/1990	100224000
07/12/1990	95904000
08/12/1990	91584000
09/12/1990	89856000
10/12/1990	91584000

11/12/1990	155520000
12/12/1990	188352000
13/12/1990	167616000
14/12/1990	145152000
15/12/1990	133920000
16/12/1990	121824000
17/12/1990	113184000
18/12/1990	105408000

19/12/1990	100224000
20/12/1990	97632000
21/12/1990	95040000
22/12/1990	94176000
23/12/1990	91584000
24/12/1990	87264000
25/12/1990	81993600
26/12/1990	79401600

27/12/1990	76809600
28/12/1990	75513600
29/12/1990	75945600
30/12/1990	75081600
31/12/1990	74304000

Table A.2 - Monthly averaged daily light.

Data	**(MJ/m2 giorno)**	**kWh/m2/d**	**Observed Ly/d**
01/1988	5.3	1.47	126.61
1/02/1988	8.2	2.28	195.89
1/03/1988	13.7	3.81	327.28
1/04/1988	17.4	4.83	415.67
1/05/1988	21.1	5.86	504.06
1/06/1988	23.1	6.42	551.83
1/07/1988	23.3	6.47	556.61
1/08/1988	19.8	5.5	473
1/09/1988	15.1	4.19	360.72
1/10/1988	10.1	2.81	241.28
1/11/1988	6	1.67	143.33
1/12/1988	4.3	1.19	102.72
		Max	556.61
		Min	102.72
		Annual light range	453.89

Table A.3 - Daily light intensity function, computed with AQUATOX.

Julian day	Solar AQUATOX (Ly/d)
1.00	127.55
2.00	126.96
2.99	126.43
3.99	125.96
4.99	125.56
5.99	125.22
6.99	124.94
7.98	124.73
8.98	124.58
9.98	124.49
10.98	124.47
11.98	124.51
12.97	124.61
13.97	124.78
14.97	125.01
15.97	125.31
16.97	125.66
17.96	126.08
18.96	126.57
19.96	127.11
20.96	127.72
21.95	128.39
22.95	129.13
23.95	129.92
24.95	130.78
25.95	131.70
26.94	132.68
27.94	133.72
28.94	134.82
29.94	135.98
30.94	137.20
31.93	138.48
32.93	139.83
33.93	141.22
34.93	142.68
35.93	144.20
36.92	145.77
37.92	147.40
38.92	149.09
39.92	150.83
40.92	152.63
41.91	154.48
42.91	156.39
43.91	158.35
44.91	160.36
45.91	162.43
46.90	164.55
47.90	166.72
48.90	168.94
49.90	171.21
50.90	173.53
51.89	175.90
52.89	178.32
53.89	180.78
54.89	183.29
55.89	185.84
56.88	188.44
57.88	191.09
58.88	193.78
59.88	196.51
60.88	199.28
61.87	202.09
62.87	204.94
63.87	207.83
64.87	210.76
65.86	213.73
66.86	216.73
67.86	219.77
68.86	222.84
69.86	225.95
70.85	229.09
71.85	232.26
72.85	235.46
73.85	238.69
74.85	241.95
75.84	245.24
76.84	248.56
77.84	251.90
78.84	255.26
79.84	258.65
80.83	262.07
81.83	265.50
82.83	268.95
83.83	272.43
84.83	275.92
85.82	279.43
86.82	282.96
87.82	286.50
88.82	290.06
89.82	293.62
90.81	297.21
91.81	300.80
92.81	304.40
93.81	308.01
94.81	311.63
95.80	315.26
96.80	318.89
97.80	322.53
98.80	326.16
99.80	329.81
100.79	333.45
101.79	337.09
102.79	340.73
103.79	344.37
104.78	348.01
105.78	351.64
106.78	355.27
107.78	358.89
108.78	362.50
109.77	366.10
110.77	369.70
111.77	373.28
112.77	376.85
113.77	380.41
114.76	383.95
115.76	387.48
116.76	390.99
117.76	394.48
118.76	397.96
119.75	401.42
120.75	404.85
121.75	408.27
122.75	411.66
123.75	415.03
124.74	418.37
125.74	421.69
126.74	424.98
127.74	428.24
128.74	431.47
129.73	434.68
130.73	437.85
131.73	441.00
132.73	444.11
133.73	447.18
134.72	450.22
135.72	453.23
136.72	456.20
137.72	459.13
138.72	462.03
139.71	464.88
140.71	467.70
141.71	470.48
142.71	473.21
143.70	475.90
144.70	478.55
145.70	481.15
146.70	483.71
147.70	486.23
148.69	488.70
149.69	491.12
150.69	493.49
151.69	495.81
152.69	498.09
153.68	500.31
154.68	502.49
155.68	504.61
156.68	506.69
157.68	508.70
158.67	510.67
159.67	512.58
160.67	514.44
161.67	516.24
162.67	517.99
163.66	519.68
164.66	521.32
165.66	522.90
166.66	524.42
167.66	525.88
168.65	527.29
169.65	528.63
170.65	529.92
171.65	531.15
172.65	532.32
173.64	533.42
174.64	534.47
175.64	535.46
176.64	536.38
177.64	537.24
178.63	538.04
179.63	538.78
180.63	539.46
181.63	540.08
182.63	540.63
183.62	541.12
184.62	541.54
185.62	541.91
186.62	542.21
187.61	542.44
188.61	542.62
189.61	542.73
190.61	542.77
191.61	542.76
192.60	542.67
193.60	542.53
194.60	542.32
195.60	542.05
196.60	541.72

197.59	541.32
198.59	540.86
199.59	540.34
200.59	539.75
201.59	539.11
202.58	538.40
203.58	537.62
204.58	536.79
205.58	535.89
206.58	534.94
207.57	533.92
208.57	532.84
209.57	531.70
210.57	530.50
211.57	529.24
212.56	527.92
213.56	526.54
214.56	525.11
215.56	523.61
216.56	522.06
217.55	520.45
218.55	518.79
219.55	517.06
220.55	515.29
221.55	513.45
222.54	511.57
223.54	509.62
224.54	507.63
225.54	505.58
226.53	503.48
227.53	501.33
228.53	499.13
229.53	496.88
230.53	494.58
231.52	492.23
232.52	489.83
233.52	487.38
234.52	484.89
235.52	482.35
236.51	479.77
237.51	477.14
238.51	474.47
239.51	471.75

240.51	468.99
241.50	466.20
242.50	463.36
243.50	460.48
244.50	457.57
245.50	454.61
246.49	451.63
247.49	448.60
248.49	445.54
249.49	442.45
250.49	439.32
251.48	436.16
252.48	432.97
253.48	429.75
254.48	426.50
255.48	423.22
256.47	419.91
257.47	416.58
258.47	413.23
259.47	409.85
260.47	406.44
261.46	403.02
262.46	399.57
263.46	396.10
264.46	392.62
265.45	389.11
266.45	385.59
267.45	382.06
268.45	378.50
269.45	374.94
270.44	371.36
271.44	367.77
272.44	364.18
273.44	360.57
274.44	356.95
275.43	353.33
276.43	349.70
277.43	346.06
278.43	342.42
279.43	338.78
280.42	335.14
281.42	331.50
282.42	327.86

283.42	324.22
284.42	320.58
285.41	316.94
286.41	313.32
287.41	309.69
288.41	306.08
289.41	302.47
290.40	298.87
291.40	295.29
292.40	291.71
293.40	288.15
294.40	284.60
295.39	281.07
296.39	277.55
297.39	274.05
298.39	270.57
299.39	267.10
300.38	263.66
301.38	260.24
302.38	256.84
303.38	253.46
304.38	250.11
305.37	246.78
306.37	243.48
307.37	240.21
308.37	236.96
309.36	233.75
310.36	230.56
311.36	227.41
312.36	224.28
313.36	221.20
314.35	218.14
315.35	215.12
316.35	212.14
317.35	209.19
318.35	206.28
319.34	203.41
320.34	200.58
321.34	197.79
322.34	195.04
323.34	192.33
324.33	189.67
325.33	187.05

326.33	184.47
327.33	181.94
328.33	179.46
329.32	177.02
330.32	174.63
331.32	172.28
332.32	169.99
333.32	167.75
334.31	165.55
335.31	163.41
336.31	161.32
337.31	159.28
338.31	157.29
339.30	155.36
340.30	153.48
341.30	151.66
342.30	149.89
343.30	148.18
344.29	146.52
345.29	144.92
346.29	143.38
347.29	141.90
348.28	140.47
349.28	139.10
350.28	137.79
351.28	136.54
352.28	135.35
353.27	134.22
354.27	133.15
355.27	132.15
356.27	131.20
357.27	130.31
358.26	129.49
359.26	128.73
360.26	128.03
361.26	127.39
362.26	126.81
363.25	126.30
364.25	125.85
365.25	125.46

Table A.4 - Water temperatures.

Date	Temperature (°C)
13/12/1988	6.1
31/01/1989	5.9
09/03/1989	9.9
11/04/1989	12.8
16/05/1989	16
20/06/1989	23.6
18/07/1989	24
30/08/1989	22
26/09/1989	21.8
03/11/1989	14.5
05/12/1989	6
23/01/1990	6.1
27/03/1990	13.1
03/05/1990	18.7
27/06/1990	25.8
24/07/1990	28.2

Table A.5 - Water pH

Date	pH
13/12/1988	8.03
31/01/1989	7.84
09/03/1989	7.85
11/04/1989	7.76
16/05/1989	7.91
20/06/1989	8.61
18/07/1989	8.72
30/08/1989	8.13
26/09/1989	8.02
03/11/1989	7.83
05/12/1989	7.89
23/01/1990	7.81
27/03/1990	8.18
03/05/1990	8.07
27/06/1990	8.73
24/07/1990	8.21

Table A.6 - Loadings of NH_4^+ and NO_3

Date	NH_4^+ (mg/L)	NO_3 (mg/L)
08/01/1988	0.29	3.02
03/02/1988	0.18	3.00
02/03/1988	0.22	2.93
06/04/1988	0.16	3.10
04/05/1988	0.12	2.71
06/07/1988	0.08	2.05
03/08/1988	0.07	1.38
07/09/1988	0.08	2.03
14/10/1988	0.076923	2.16667
17/10/1988	0.128205	1.60256
18/10/1988	0.205128	1.51282
19/10/1988	0.141026	1.42308
20/10/1988	0.089744	1.4359
24/10/1988	0.089744	1.25641
27/10/1988	0.038462	1.38462
04/11/1988	0.076923	1.66667
07/11/1988	0.089744	1.73077
08/11/1988	0.102564	1.73077
07/12/1988	0.37	1.21
04/01/1989	0.56	2.52
01/02/1989	0.72	2.56
01/03/1989	0.29	2.74
07/04/1989	0.010803	1.60071
08/04/1989	0.089912	1.62888
09/04/1989	0.361328	1.82337
10/04/1989	0.196847	2.33855
11/04/1989	0.134931	1.95578
12/04/1989	0.060025	2.03489
13/04/1989	0.049559	1.85759
14/04/1989	0.064565	1.74439
15/04/1989	0.079739	1.79802
17/04/1989	0.252963	1.94578
18/04/1989	0.242329	1.96095
20/04/1989	0.118663	1.90122
21/04/1989	0.082556	1.89092
24/04/1989	0.089281	1.83338
26/04/1989	0.119294	1.90185
28/04/1989	0.072383	1.85477
02/05/1989	0.107104	1.86385
04/05/1989	0.060362	1.81728
21/06/1989	0.26	1.16
05/07/1989	0.16	1.84
10/07/1989	0.70	2.33
20/07/1989	0.19	1.83
16/08/1989	0.19	1.55
31/08/1989	0.05	1.98
20/09/1989	0.04	2.93
11/10/1989	0.19	2.80
25/10/1989	0.23	3.06
08/11/1989	0.66	2.70
22/11/1989	0.40	3.33
07/12/1989	0.80	3.02
20/12/1989	0.57	3.25
10/01/1990	0.37	3.20
16/01/1990	0.81	2.94
29/01/1990	0.87	3.06
12/02/1990	0.60	2.95
15/02/1990	0.74	3.12
28/02/1990	0.23	3.14
12/03/1990	0.20	2.30
24/03/1990	0.589744	3.97436
25/03/1990	0.448718	3.70513
26/03/1990	0.02	2.21
27/03/1990	0.205128	3.39744
28/03/1990	0.102564	3.32051
30/03/1990	0.089744	2.76923
31/03/1990	0.089744	2.4359
02/04/1990	0.064103	2.39744
03/04/1990	0.089744	2.25641
05/04/1990	0.141026	2.26923
06/04/1990	0.512821	2.80769
08/04/1990	0.435897	3.37179
09/04/1990	0.564103	3.15385
11/04/1990	0.384615	3.46154
12/04/1990	0.205128	2.97436
14/04/1990	0.24359	2.96154
15/04/1990	0.179487	2.98718
16/04/1990	0.115385	2.80769
18/04/1990	0.115385	2.64103
20/04/1990	0.089744	2.52564
22/04/1990	0	4.29297
23/04/1990	0	4.25956
24/04/1990	0	4.02103
25/04/1990	0	3.73506
26/04/1990	0	3.33856
27/04/1990	0	3.30522
01/05/1990	0.029817	2.81222
03/05/1990	0.105169	2.63508
07/05/1990	0.03	1.24
21/05/1990	0.15	1.60
13/06/1990	0.03	1.53
25/06/1990	0.14	1.59
27/06/1990	0.16	1.51
11/07/1990	0.01	1.08
25/07/1990	0.00	0.51
08/08/1990	0.01	1.15
20/08/1990	0.01	1.46
12/09/1990	0.01	1.73
26/09/1990	0.04	2.55
08/10/1990	1.37	3.54
24/10/1990	0.09	2.61
07/11/1990	0.07	2.25
21/11/1990	0.23	2.75
12/12/1990	0.49	3.83
20/12/1990	0.34	2.86

Table A.7 - Loadings of Total Soluble Phosphorous

Date	Tot. Sol. P (mg/L)
14/10/1988	0.123227
17/10/1988	0.069029
18/10/1988	0.075703
19/10/1988	0.07591
20/10/1988	0.08581
24/10/1988	0.070512
27/10/1988	0.061488
04/11/1988	0.105141
07/11/1988	0.06065
07/04/1989	0.040585
08/04/1989	0.050832
09/04/1989	0.061028
10/04/1989	0.055355
11/04/1989	0.059202
12/04/1989	0.075746
13/04/1989	0.057429
14/04/1989	0.061277
15/04/1989	0.055603
17/04/1989	0.057626
18/04/1989	0.055178
20/04/1989	0.062874
21/04/1989	0.060426
24/04/1989	0.062501
26/04/1989	0.082944
28/04/1989	0.05578
02/05/1989	0.06805
04/05/1989	0.059877
23/03/1990	0.108197
25/03/1990	0.081967
26/03/1990	0.065574
28/03/1990	0.059016
31/03/1990	0.052459
01/04/1990	0.062295
03/04/1990	0.04918
04/04/1990	0.062295
06/04/1990	0.068853
07/04/1990	0.07541
09/04/1990	0.088525
10/04/1990	0.095082
12/04/1990	0.07541
14/04/1990	0.12459
15/04/1990	0.068853
16/04/1990	0.062295
18/04/1990	0.068853
22/04/1990	0.130432
23/04/1990	0.123151
24/04/1990	0.115869
25/04/1990	0.085611
26/04/1990	0.084885
27/04/1990	0.077604
29/04/1990	0.066319
01/05/1990	0.058283
03/05/1990	0.046997

Table A.8 - Loadings of soluble and suspended TOC

Data	TOC sosp	TOC sol	TOC tot	% particulate
	mg/l	mg/l	mg/l	
14/10/1988	0	2.35884	2.35884	0
17/10/1988	1.57712	5.48744	7.06456	22.32439
18/10/1988	4.52188	4.39368	8.91556	50.71897
19/10/1988	1.76068	3.74774	5.50842	31.96343
20/10/1988	1.11561	3.48756	4.60317	24.23569
24/10/1988	1.41921	2.89377	4.31298	32.90555
27/10/1988	0	2.11177	2.11177	0
04/11/1988	0.346425	1.88451	2.230935	15.52824
07/11/1988	0.525902	1.42299	1.948892	26.98467
08/11/1988	0	1.61121	1.61121	0
07/04/1989	3.18633	2.60756	5.79389	54.99466
08/04/1989	4.1697	3.01547	7.18517	58.03203
09/04/1989	7.59308	3.6141	11.20718	67.75192

10/04/1989	4.15191	3.89376	8.04567	51.60428
11/04/1989	1.47857	3.46641	4.94498	29.90042
12/04/1989	1.37184	2.65597	4.02781	34.0592
13/04/1989	0.49727	2.35851	2.85578	17.41276
14/04/1989	0.390535	2.50662	2.897155	13.47995
15/04/1989	0.477826	2.40154	2.879366	16.59483
18/04/1989	7.98569	3.36546	11.35115	70.35137
19/04/1989	6.59648	3.06636	9.66284	68.26647
20/04/1989	6.57703	2.92032	9.49735	69.25121
21/04/1989	2.23813	1.98163	4.21976	53.03927
24/04/1989	0.508853	1.9837	2.492553	20.41493
26/04/1989	0.295383	2.02838	2.323763	12.71141
28/04/1989	0.021099	2.13718	2.158279	0.977575
02/05/1989	0.750041	2.35314	3.103181	24.17007
04/05/1989	0.408324	1.62833	2.036654	20.04877
24/03/1990	0.443038	6.20253	6.645568	6.666669
25/03/1990	0.316456	5	5.316456	5.952386
27/03/1990	1.07595	4.68354	5.75949	18.68134
28/03/1990	0.443038	4.05063	4.493668	9.859162
30/03/1990	0.759494	5.06329	5.822784	13.04349
31/03/1990	13.9873	3.16456	17.15186	81.54976
02/04/1990	0.126582	3.60759	3.734172	3.389828
03/04/1990	0.506329	3.48101	3.987339	12.69842
05/04/1990	4.43038	3.29114	7.72152	57.37704
06/04/1990	1.07595	4.17722	5.25317	20.48192
08/04/1990	1.4557	4.68354	6.13924	23.7114
09/04/1990	0.443038	5.25316	5.696198	7.777784
11/04/1990	1.58228	4.36709	5.94937	26.59576
12/04/1990	7.34177	6.4557	13.79747	53.21099
13/04/1990	0.063291	4.36709	4.430381	1.42857
15/04/1990	3.10127	4.11392	7.21519	42.98251
17/04/1990	1.39241	4.43038	5.82279	23.91311
18/04/1990	5.37975	5.37975	10.7595	50
20/04/1990	4.87342	3.35443	8.22785	59.23078
22/04/1990	2.74916	6.72352	9.47268	29.02199
23/04/1990	8.89604	4.79348	13.68952	64.98431
24/04/1990	0.555741	4.5301	5.085841	10.92722
25/04/1990	5.22798	3.30491	8.53289	61.26857
26/04/1990	1.50279	3.04153	4.54432	33.06963
27/04/1990	1.43172	2.77787	4.20959	34.01091
29/04/1990	0.007246	4.75084	4.758086	0.152296
01/05/1990	1.40329	3.19816	4.60145	30.49669
03/05/1990	0.0432	4.78707	4.83027	0.894352

Table A.9 - Loadings of TSS

Date	**TSS (mg/L)**	**Date**	**TSS (mg/L)**	**Mean**
01/01/2010	45	01/01/2011	28	36.5
01/02/2010	45	01/02/2011	22	33.5
01/03/2010	111	01/03/2011	42	76.5
01/04/2010	57	01/04/2011	28	42.5
01/05/2010	93	01/05/2011	40	66.5
01/06/2010	40	01/06/2011	164	102
01/07/2010	28	01/07/2011	6	17
01/08/2010	172	01/08/2011	51	111.5
01/09/2010	71	01/09/2011	40	55.5
01/10/2010	64	01/10/2011	20	42
01/11/2010	158	01/11/2011	29	93.5
01/12/2010	49	01/12/2011	29	39

5.2 Appendix B - Biota observed time-series and parameters

Table B.1 - Numeric density of Cyclotella (Diatom) and Chromulina (Chrysophyte) in the Po river section of Pontelagoscuro from 10/9/1988 to 27/03/1990 (from Garibaldi L., 1991).

Date	Numeric density ((ind/10^3)/L)	
	Diatoms	Chrysophytes (Other Algae)
	Cyclotella (comensis)	Chromulina globosa
10/9/1988	18567	0
20/9/1988	50695	169
18/10/1988	845	169
25/10/1988	1548	442
8/11/1988	710	237
23/11/1988	778	0
13/12/1988	338	0
24/1/1989	406	152
31/1/1989	942	0
14/2/1989	5276	101
21/2/1989	3778	0
28/2/1989	0	369
11/4/1989	590	111
16/5/1989	1217	101
30/5/1989	6598	258
6/6/1989	4718	184
13/6/1989	6341	0
20/6/1989	20816	0
18/7/1989	7897	237
25/7/1989	14671	442
1/8/1989	5986	169
30/8/1989	10957	473
26/9/1989	12006	203
17/10/1989	10484	812
7/11/1989	2029	169
21/11/1989	1302	220
5/12/1989	1691	321
23/1/1990	930	609
27/2/1990	7423	220
27/3/1990	67067	5715

Table B.2 - Cyclotella and Chromulina calculated biomass time-series

Taxa	Cyclotella (mg/L)	Chromulina globosa (mg/L)
10/9/1988	3.2876	0
20/9/1988	8.976404	0.032885
18/10/1988	0.149621	0.032885
25/10/1988	0.274099	0.086008
8/11/1988	0.125717	0.046117
23/11/1988	0.137758	0
13/12/1988	0.059849	0
24/1/1989	0.071889	0.029577
31/1/1989	0.166797	0
14/2/1989	0.934205	0.019653
21/2/1989	0.668959	0
28/2/1989	0	0.071803
11/4/1989	0.104469	0.021599
16/5/1989	0.21549	0.019653
30/5/1989	1.168287	0.050204
6/6/1989	0.835401	0.035804
13/6/1989	1.122781	0
20/6/1989	3.685823	0
18/7/1989	1.398297	0.046117
25/7/1989	2.597748	0.086008
1/8/1989	1.059922	0.032885
30/8/1989	1.940121	0.09204
26/9/1989	2.125865	0.039501
17/10/1989	1.856369	0.158005
7/11/1989	0.359269	0.032885
21/11/1989	0.230541	0.042809
5/12/1989	0.29942	0.062463
23/1/1990	0.164672	0.118504
27/2/1990	1.314367	0.042809
27/3/1990	11.87534	1.11207

Table B.3 - Zooplankton time-series from Antonietti et al. (1995)

Sample station: Torricella di Sissa (PR) (after Serafini Island)					
Date	**Brachionus calyciflorus (Ind/L)**	**Polyarthra sp. (Ind/L)**	**Synchaeta sp. (Ind/L)**	**Filinia gr. Longiseta-terminalis (Ind/L)**	**Asplancna girodi-brightwelli (Ind/L)**
15/03/1990	18.1864	0	42.4346	0	0
02/04/1990	0	29.2795	49.7909	0	0
19/04/1990	0		8.73088	0	0
21/05/1990	265.328	40.5218	43.3523	8.90146	1.23367
10/06/1990	0	35.3019	10.0979	0	0
22/06/1990	49.9344	22.2184	22.0349	10.2588	0
11/07/1990	947.597	436.388	54.9569	25.0471	1.10403
27/07/1990	13.8175	132.422	563.557	441.503	29.996
05/09/1990	119.221	58.9078	36.4718	34.136	1.45993
27/09/1990	65.4293	28.9758	34.2702	19.2278	0.925475

Sample station: Casalmaggiore (CR) (after Serafini Island)					
Date	**Brachionus calyciflorus (Ind/L)**	**Polyarthra sp. (Ind/L)**	**Synchaeta sp. (Ind/L)**	**Filinia gr. Longiseta-terminalis (Ind/L)**	**Asplancna girodi-brightwelli (Ind/L)**
15/03/1990	43.891	0	99.0341	0	0
02/04/1990	8.30215	36.9698	42.4432	0	0
19/04/1990	0	11.3084	42.1023	0	0
21/05/1990	448.878	33.0478	64.0909	6.48	1.14443
10/06/1990	0	73.4296	23.0682	0	0
22/06/1990	43.7958	24.8552	50.3977	10.26	0
11/07/1990	1011.48	403.537	45.1136	7.41	2.8128
27/07/1990	0	342.27	422.955	449.243	32.888
05/09/1990	100.573	33.6852	23.9773	6.39	0.700785
27/09/1990	75.3811	16.4489	26.875	14.28	1.36535

Calculated average values between the two sampled stations				
Taxa	**Brachionus calyciflorus (Ind/L)**	**Polyarthra sp. (Ind/L)**	**Synchaeta sp. (Ind/L)**	**Filinia gr. Longiseta-terminalis (Ind/L)**
15/03/1990	31.0387	0	70.73435	0
02/04/1990	4.151075	33.12465	46.11705	0
19/04/1990	0	5.6542	25.41659	0
21/05/1990	357.103	36.7848	53.7216	7.69073
10/06/1990	0	54.36575	16.58305	0
22/06/1990	46.8651	23.5368	36.2163	10.2594
11/07/1990	979.5385	419.9625	50.03525	16.22855
27/07/1990	6.90875	237.346	493.256	445.373
05/09/1990	109.897	46.2965	30.22455	20.263
27/09/1990	70.4052	22.71235	30.5726	16.7539

Calculated biomass time-series				
Taxa	**Brachionus calyciflorus (mg/L)**	**Polyarthra sp. (mg/L)**	**Synchaeta sp. (mg/L)**	**Filinia gr. Longiseta-terminalis (mg/L)**
15/03/1990	0.009312	0	0.014147	0
02/04/1990	0.001245	0.001656	0.009223	0
19/04/1990	0	0.000283	0.005083	0
21/05/1990	0.107131	0.001839	0.010744	0.00323
10/06/1990	0	0.002718	0.003317	0
22/06/1990	0.01406	0.001177	0.007243	0.004309
11/07/1990	0.293862	0.020998	0.010007	0.006816
27/07/1990	0.002073	0.011867	0.098651	0.187057
05/09/1990	0.032969	0.002315	0.006045	0.00851
27/09/1990	0.021122	0.001136	0.006115	0.007037

Table B.4 - Zooplankton time-series from Ferrari et al. (1989).

Sampled data						
Date	**Brachionus calyciflorus (Ind/L)**	**Brachionus Bennini (Ind/L)**	**Brachionus Quadridentatus (Ind/L)**	**Brachionus Angularis (Ind/L)**	**Brachionus Budapestinensis (Ind/L)**	**Brachionus Family (Ind/L)**
27/07/1988	1123.01	247.303	157.38	248.523	74.9159	1851.132
29/07/1988	696.838	107.601	129.583	74.7019	48.7981	1057.522
30/07/1988	1066.3	97.7288	202.055	64.5562	54.363	1485.003
31/07/1988	1070.01	87.8564	229.526	61.8562	52.512	1501.761
01/08/1988	852.345	70.4866	211.758	73.5331	65.6611	1273.784
02/08/1988	1247.76	60.6142	231.609	78.0215	63.8101	1681.815
03/08/1988	1165.98	50.8497	183.84	67.7472	91.9591	1560.376
04/08/1988	769.902	70.9665	128.451	65.0471	90.1082	1124.475
05/08/1988	756.598	60.986	141.043	98.8039	103.257	1160.688
06/08/1988	504.253	58.5029	85.7745	81.2125	63.8221	793.565
08/08/1988	95.0927	53.8606	50.4775	75.6838	45.2043	320.3189
09/08/1988	405.234	51.3775	55.2087	87.4893	50.7692	650.0787
11/08/1988	506.434	76.6164	57.4117	213.411	92.0673	945.9404
12/08/1988	314.068	149.214	47.504	107.757	127.716	746.259
13/08/1988	326.281	394.142	67.2352	105.186	215.781	1108.625
14/08/1988	117.121	384.27	64.5867	95.0401	296.43	957.4478
15/08/1988	282.225	284.322	54.3178	157.808	362.163	1140.836
16/08/1988	720.611	169.379	51.6693	213.645	442.644	1597.948

17/08/1988	324.318	107.025	63.9005	144.705	313.209	953.1575
18/08/1988	361.396	89.6557	68.752	149.065	348.858	1017.727
19/08/1988	322.574	109.557	81.1035	124.156	226.923	864.3135
20/08/1988	547.219	92.0789	70.8347	231.084	187.572	1128.789
21/08/1988	159.433	104.698	68.1862	294.109	245.637	872.0632
22/08/1988	120.611	169.691	72.9173	151.999	183.786	699.0043
23/08/1988	183.424	99.6241	62.7689	75.9994	84.2668	506.0832
24/08/1988	161.832	74.865	60	73.0422	59.9159	429.6551

Calculated biomass series		
Rotiferi	**Brachionus Family (mg/L)**	**Brachionus calyciflorus (mg/L)**
27/07/1988	0.55534	0.336903
29/07/1988	0.317257	0.209051
30/07/1988	0.445501	0.31989
31/07/1988	0.450528	0.321003
01/08/1988	0.382135	0.255704
02/08/1988	0.504544	0.374328
03/08/1988	0.468113	0.349794
04/08/1988	0.337342	0.230971
05/08/1988	0.348206	0.226979
06/08/1988	0.23807	0.151276
08/08/1988	0.096096	0.028528
09/08/1988	0.195024	0.12157
11/08/1988	0.283782	0.15193
12/08/1988	0.223878	0.09422
13/08/1988	0.332588	0.097884
14/08/1988	0.287234	0.035136
15/08/1988	0.342251	0.084668
16/08/1988	0.479384	0.216183
17/08/1988	0.285947	0.097295
18/08/1988	0.305318	0.108419
19/08/1988	0.259294	0.096772
20/08/1988	0.338637	0.164166
21/08/1988	0.261619	0.04783
22/08/1988	0.209701	0.036183
23/08/1988	0.151825	0.055027
24/08/1988	0.128897	0.04855

Table B.4 - Macroinvertebrates data set from Cironi and Ruffo (1981)

River bottom		
Station: Monte isola de Pinedo (before Serafini Island)		
Taxa	**Oligochaeta (Ind/m^2)**	**Diptera (Ind/m^2)**
01/06/1974	184500	31.9842
01/07/1974	38995.2	161.7705
01/08/1974	23123.25	469.5225
01/09/1974	38282.83	1077.68
01/10/1974	77359	829.3455
01/11/1974	108689.5	232.765
01/12/1974	126829.5	463.963
01/01/1975	121268.6	618.421
01/02/1975	118222.7	868.0305
01/03/1975	117941.2	1207.045
01/04/1975	86030.9	640.94
01/05/1975	75289.65	17.5
01/06/1975	42773.45	69.219
01/07/1975	24660.28	95.625
01/08/1975	11105.58	778.25
01/09/1975	16054.35	1636.864
01/10/1975	7853.5	1068.169
01/11/1975	5040.4	230.3421

01/12/1975	7507	277.969
01/01/1976	7375	304.375
01/02/1976	7210.05	255.781
01/03/1976	9693.1	157.1875
01/04/1976	56913	59.844
01/05/1976	77883	35.9375
01/06/1976	107961	491.903
01/07/1976	112893.3	975.5935

01/08/1976	117842.1	2115.023
01/09/1976	80619.5	861.305
01/10/1976	48808.4	437.6895
01/11/1976	3674.24	0
01/12/1976	3343.219	21.875
01/01/1977	8308.333	73.281
01/02/1977	10608.9	74.844

From sampled macrophytes analysis							
Date	**Efemerotteri (ind/m2)**	**Date**	**Amphipoda (ind/m2)**	**Date**	**Odonati (ind/m2)**	**Date**	**Ditteri (ind/m2)**
01/06/1974	55.3529	01/06/1974	80.9087	01/06/1974	70.8824	01/06/1974	122.85
01/08/1974	34	01/08/1974	77.2765	01/08/1974	51.4706	01/09/1974	134.096
01/10/1974	2.94118	01/10/1974	100.221	01/11/1974	109.706	01/11/1974	132.063
01/11/1974	32.0588	01/12/1974	132.734	01/02/1975	113.588	01/02/1975	149.024
01/12/1974	82.5294	01/02/1975	148.06	01/05/1975	100	01/06/1975	137.428
01/04/1975	78.6471	01/04/1975	152.003	01/09/1975	88.3529	01/07/1975	124.014
01/07/1975	90.2941	01/07/1975	138.803	01/11/1975	101.94	01/09/1975	101.038
01/09/1975	53.4118	01/08/1975	102.702	01/02/1976	107.765	01/01/1976	112.245
01/11/1975	67	01/09/1975	98.9369	01/07/1976	100	01/05/1976	163.433
01/01/1976	72.8235	01/11/1975	102.879	01/10/1976	115.529	01/08/1976	128.99
01/04/1976	90.2941	01/01/1976	110.587	01/01/1977	131.059	01/12/1976	102.12
01/07/1976	84.4706	01/02/1976	120.199	01/04/1977	115.53	01/02/1977	105.841
01/08/1976	51.4706	01/03/1976	127.862				
01/10/1976	1	01/04/1976	133.62				
01/11/1976	39.8235	01/06/1976	141.328				
01/02/1977	45.6471	01/08/1976	109.081				
		01/09/1976	105.315				
		01/10/1976	86.357				
		01/11/1976	84.4966				
		01/12/1976	86.4899				
		01/03/1977	90.4322				
		01/04/1977	94.2859				

Table B.5 - Calculated numerical density time-series for macroinvertebrates

Date	Amphipoda (Echinogammarus)	Diptera (Chironomus)	Oligochaeta	Trichoptera (Hydropsychidae)	Odonata (Nymphs)	Gastropoda
12/1988	73.51918	254.1071	0	68.75	0	1.25
1/1989	289.5525	0	47099767	136.25	0	1.25
3/1989	57.68428	0	1.29E+08	121.25	0	0
4/1989	32.23533	190.5803	37679814	140	0	0
5/1989	79.74003	0	18839907	76.25	0	0
6/1989	66.73279	63.52677	31399845	78.75	0	1.25
7/1989	68.42939	0	0	168.75	0	6.25
8/1989	66.73279	158.8169	0	147.5	0	82.5
9/1989	132.9001	6257.387	12559938	68.75	1165.629	86.25
11/1989	105.189	857.6114	31399845	246.25	442.1351	107.5
12/1989	180.4048	31.76339	1.22E+08	91.25	0	5
1/1990	74.08471	0	3.58E+08	16.25	0	2.5
3/1990	18.09703	0	7.47E+08	42.5	0	1.25
5/1990	212.6401	127.0535	1.35E+08	45	0	0
6/1990	278.8073	0	0	167.5	0	7.5
7/1990	26.58001	127.0535	97339519	52.5	0	2.5

Table B.6 - Calculated biomass densities time-series for macroinvertebrates

Date	Amphipoda (Echinogammarus)	Diptera (Chironomus)	Oligochaeta	Trichoptera (Hydropsychidae)	Odonata (Nymphs)	Gastropoda
12/1988	0.00272	0.04788	0	0.02729375	0	6.25E-05
1/1989	0.010713	0	0.300584	0.05409125	0.00545	6.25E-05
3/1989	0.002134	0	0.308098	0.04813625	0	0
4/1989	0.001193	0.03591	0.210409	0.05558	0	0
5/1989	0.00295	0	0.045088	0.03027125	0.00545	0
6/1989	0.002469	0.01197	0.075146	0.03126375	0.0109	6.25E-05
7/1989	0.002532	0	0	0.06699375	0.00545	0.000313
8/1989	0.002469	0.029925	0	0.0585575	0.141701	0.004125
9/1989	0.004917	1.179055	0.030058	0.02729375	0.321553	0.004313
11/1989	0.003892	0.161596	0.105204	0.09776125	0.125351	0.005375
12/1989	0.006675	0.005985	0.293069	0.03622625	0.01635	0.00025
1/1990	0.002741	0	0.856664	0.00645125	0	0.000125
3/1990	0.00067	0	1.788473	0.0168725	0	6.25E-05
5/1990	0.007868	0.02394	0.323128	0.017865	0.00545	0
6/1990	0.010316	0	0	0.0664975	0.00545	0.000375
7/1990	0.000983	0.02394	0.232952	0.0208425	0	0.000125

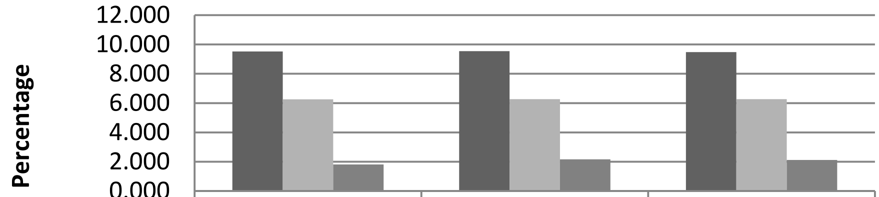
Table B.7 - Mean length and weight per year class of male and female chub (Vitali & Braghieri, 1984).

Age	% Females	% Males	Mixed sexes	Males	Female
			Weight (g)	Weight (g)	Weight (g)
0 +	68	32	5	6	8
1 +	60	40	39	33	39
2 +	60	40	164	166	163
3 +	68	32	329	327	330
4 +	80	20	434	416	446
5 +	92	8	547	468	567
6 +	95	5	800		800
7 +	100	0	800		800
Average				236	394.125

5.3 Appendix C - Useful results charts

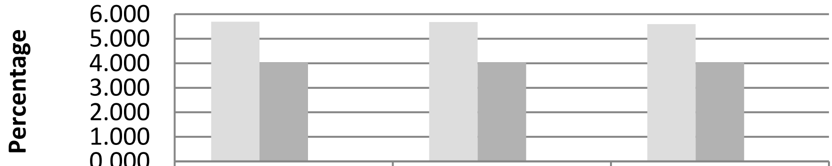
Figure C 1 - LAS effects on phytoplankton photosynthesis and respiration and on animals consumption and defecation. Predation is also reported.

a) Cyclotella



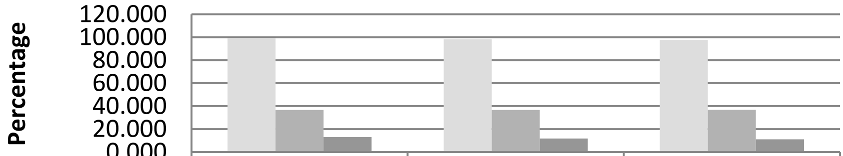
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Diatom Photosyn (Percent)	9.528	9.548	9.485
Diatom Respir (Percent)	6.258	6.273	6.273
Diatom Predation (Percent)	1.815	2.159	2.115

b) Chromulina



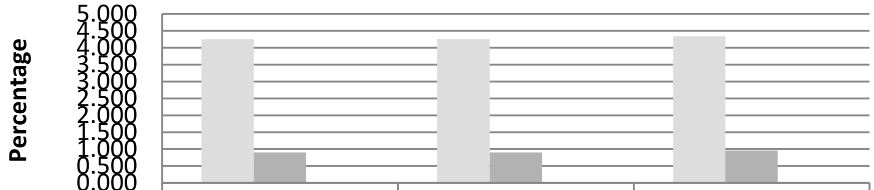
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Chrysophyte Photosyn (Percent)	5.692	5.680	5.596
Chrysophyte Respir (Percent)	4.022	4.029	4.029
Chrysophyte Predation (Percent)	0.000	0.000	0.000

c) Brachionus



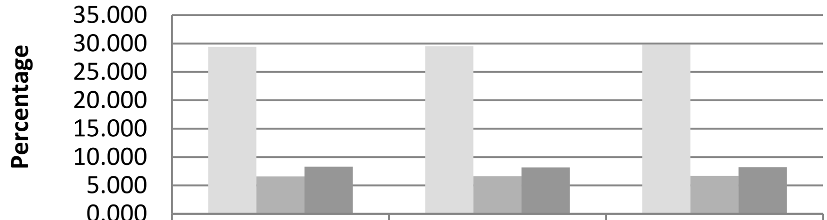
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Rotifer, Brachionus Consumption (Percent)	99.371	98.267	97.614
Rotifer, Brachionus Defecation (Percent)	36.626	36.709	36.824
Rotifer, Brachionus Predation (Percent)	13.010	11.757	11.043

d) Amphipoda



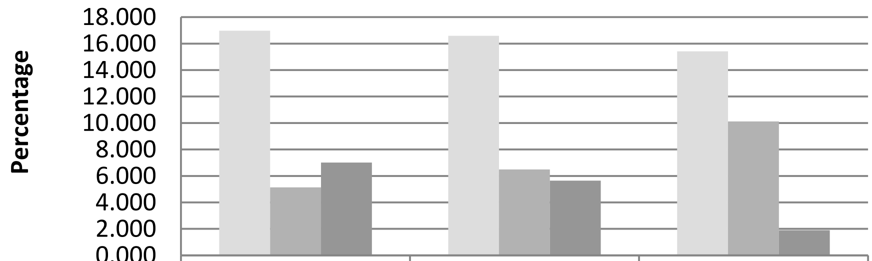
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Amphipod Consumption (Percent)	4.256	4.259	4.341
Amphipod Defecation (Percent)	0.901	0.903	0.958
Amphipod Predation (Percent)	0.000	0.000	0.000

e) Chironomids

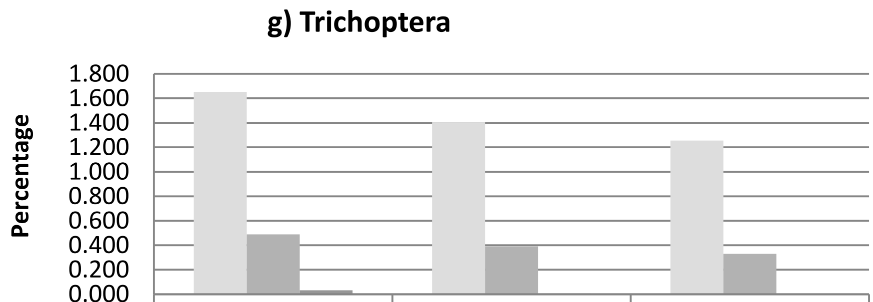


	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Young Chironomid Consumption (Percent)	29.421	29.527	29.802
Young Chironomid Defecation (Percent)	6.575	6.639	6.696
Young Chironomid Predation (Percent)	8.312	8.148	8.213

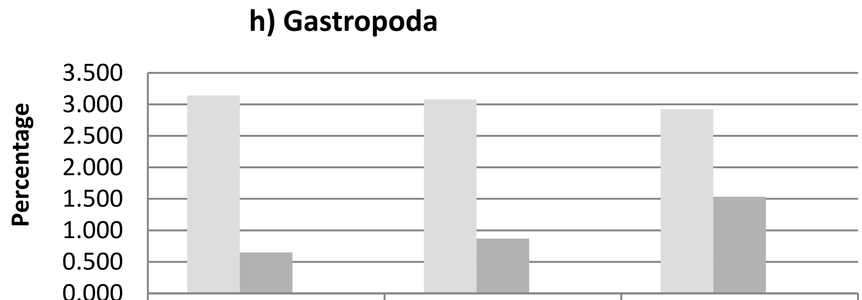
f) Oligochaeta



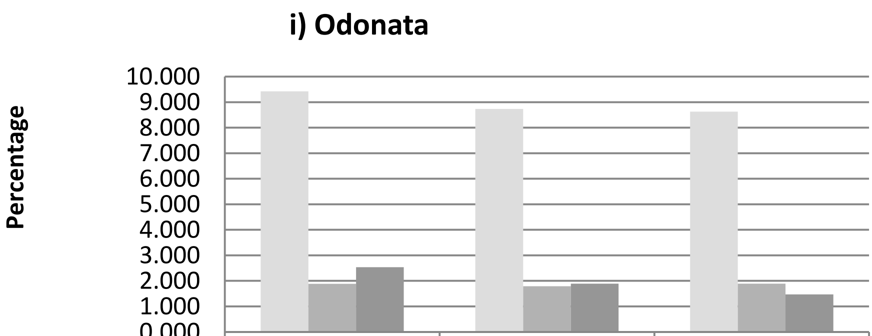
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Oligochaete Consumption (Percent)	16.980	16.582	15.415
Oligochaete Defecation (Percent)	5.138	6.485	10.119
Oligochaete Predation (Percent)	7.005	5.643	1.897



	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Caddisfly,Trichopter Consumption (Percent)	1.653	1.405	1.254
Caddisfly,Trichopter Defecation (Percent)	0.489	0.390	0.330
Caddisfly,Trichopter Predation (Percent)	0.032	0.006	0.003

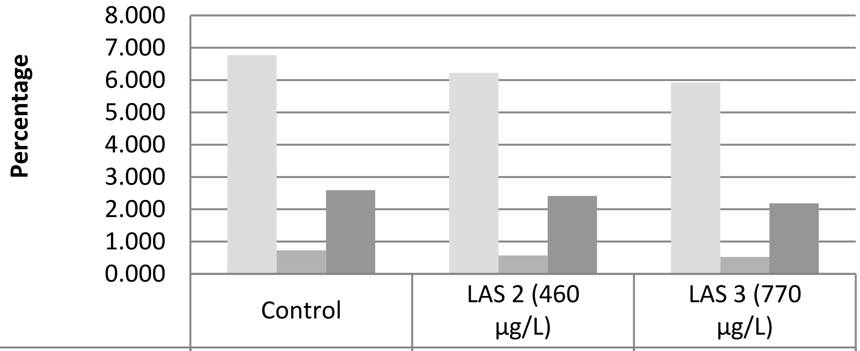


	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Gastropod Consumption (Percent)	3.144	3.080	2.924
Gastropod Defecation (Percent)	0.648	0.869	1.533
Gastropod Predation (Percent)	0.000	0.000	0.000



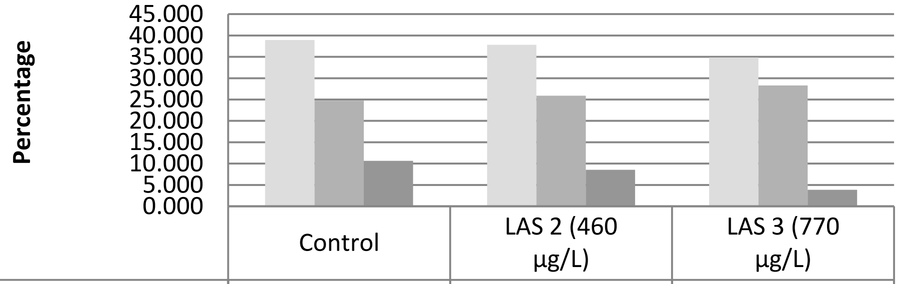
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Odonata Consumption (Percent)	9.428	8.738	8.631
Odonata Defecation (Percent)	1.886	1.787	1.891
Odonata Predation (Percent)	2.534	1.889	1.470

j) Bleak



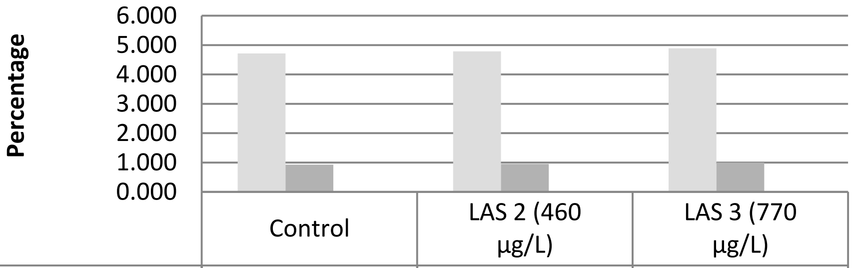
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Bleak Consumption (Percent)	6.772	6.224	5.924
Bleak Defecation (Percent)	0.730	0.569	0.525
Bleak Predation (Percent)	2.591	2.416	2.186

k) Chub



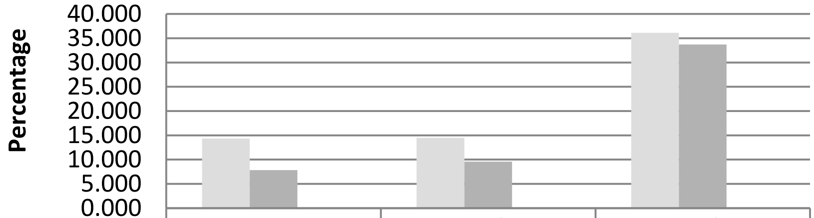
	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Chub - Cavedano Consumption (Percent)	38.956	37.806	34.863
Chub - Cavedano Defecation (Percent)	24.744	25.913	28.287
Chub - Cavedano Predation (Percent)	10.654	8.562	3.883

l) young Wels catfish



	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Siluro min di 32 cm Consumption (Percent)	4.717	4.787	4.888
Siluro min di 32 cm Defecation (Percent)	0.927	0.948	0.997
Siluro min di 32 cm Predation (Percent)	0.000	0.000	0.000

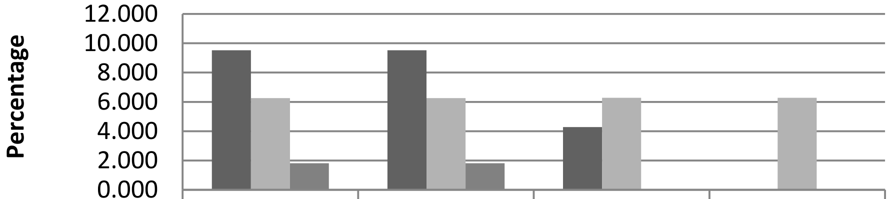
m) Adult Wels catfish



	Control	LAS 2 (460 µg/L)	LAS 3 (770 µg/L)
Siluro adult 32 cm Consumption (Percent)	14.331	14.484	36.129
Siluro adult 32 cm Defecation (Percent)	7.849	9.570	33.714
Siluro adult 32 cm Predation (Percent)	0.000	0.000	0.000

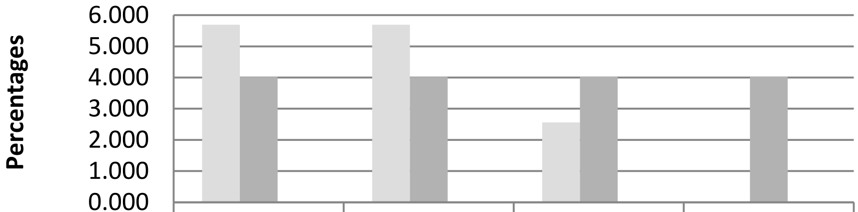
Figure C 2 - TCS effects on phytoplankton photosynthesis and respiration and on animals consumption and defecation. Predation is also reported.

a) Cyclotella



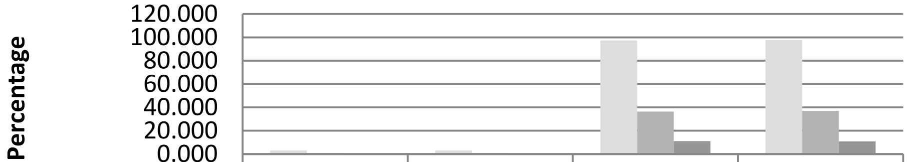
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Diatom Photosyn (Percent)	9.528	9.528	4.283	0.001
Diatom Respir (Percent)	6.258	6.258	6.279	6.279
Diatom Predation (Percent)	1.815	1.815	0.037	0.006

b) Chromulina



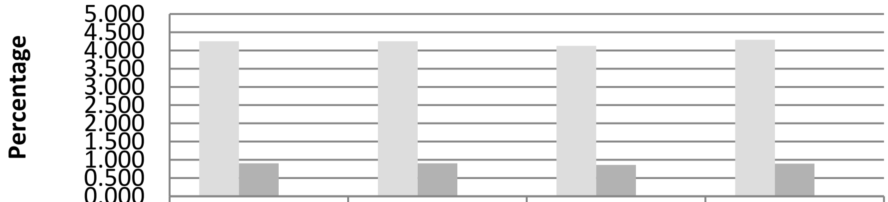
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Chrysophyte Photosyn (Percent)	5.692	5.692	2.559	0.000
Chrysophyte Respir (Percent)	4.022	4.022	4.033	4.033
Chrysophyte Predation (Percent)	0.000	0.000	0.000	0.000

c) Brachionus



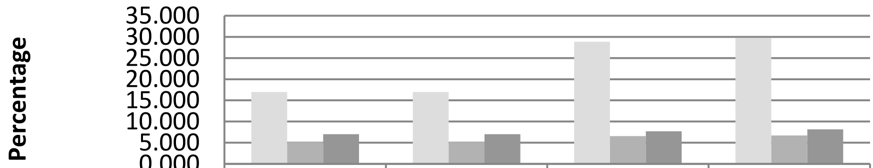
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Rotifer, Brachionus Consumption (Percent)	3.144	3.144	97.356	97.601
Rotifer, Brachionus Defecation (Percent)	0.648	0.648	36.432	36.956
Rotifer, Brachionus Predation (Percent)	0.000	0.000	11.149	10.836

d) Amphipoda



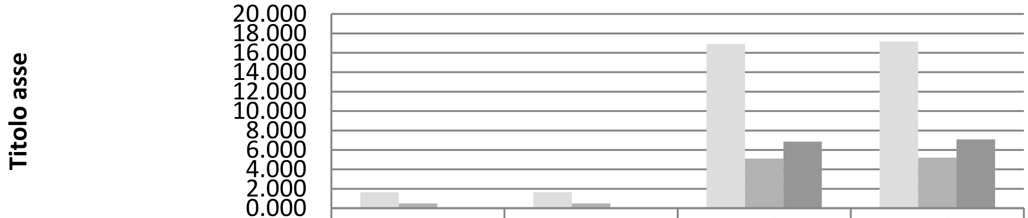
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Amphipod Consumption (Percent)	4.256	4.256	4.126	4.293
Amphipod Defecation (Percent)	0.901	0.901	0.858	0.892
Amphipod Predation (Percent)	0.000	0.000	0.000	0.000

e) Chironomids



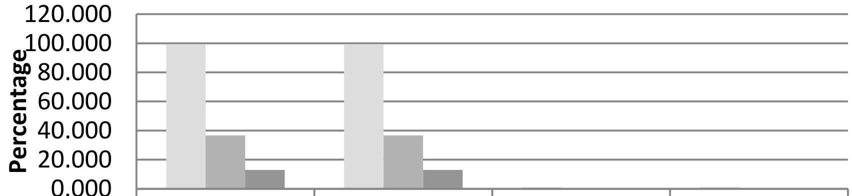
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Young Chironomid Consumption (Percent)	16.980	16.980	28.863	29.688
Young Chironomid Defecation (Percent)	5.138	5.138	6.550	6.709
Young Chironomid Predation (Percent)	7.005	7.005	7.678	8.151

f) Oligochaeta



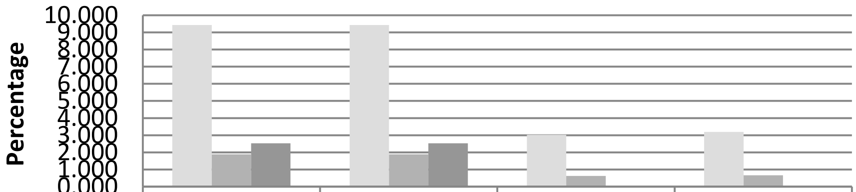
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Oligochaete Consumption (Percent)	1.653	1.653	16.906	17.147
Oligochaete Defecation (Percent)	0.489	0.489	5.116	5.204
Oligochaete Predation (Percent)	0.032	0.032	6.857	7.078

f) Trichoptera



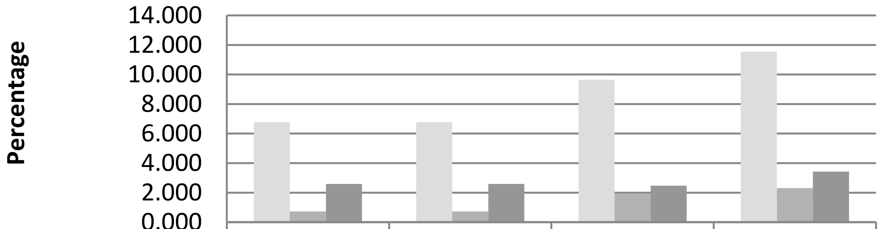
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Caddisfly,Trichopter Consumption (Percent)	99.371	99.371	1.230	1.245
Caddisfly,Trichopter Defecation (Percent)	36.626	36.626	0.309	0.316
Caddisfly,Trichopter Predation (Percent)	13.010	13.010	0.010	0.024

h) Gastropoda



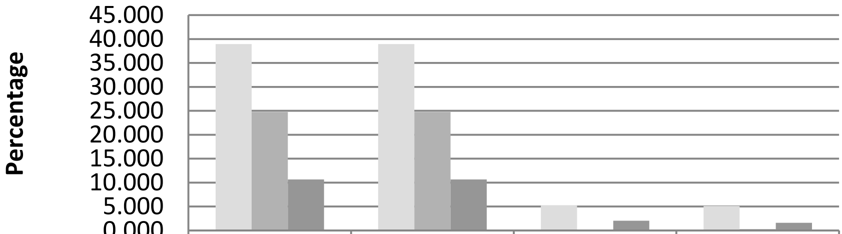
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Gastropod Consumption (Percent)	9.428	9.428	3.028	3.189
Gastropod Defecation (Percent)	1.886	1.886	0.626	0.663
Gastropod Predation (Percent)	2.534	2.534	0.000	0.000

i) Odonata



	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Odonata Consumption (Percent)	6.772	6.772	9.646	11.540
Odonata Defecation (Percent)	0.730	0.730	1.929	2.309
Odonata Predation (Percent)	2.591	2.591	2.473	3.422

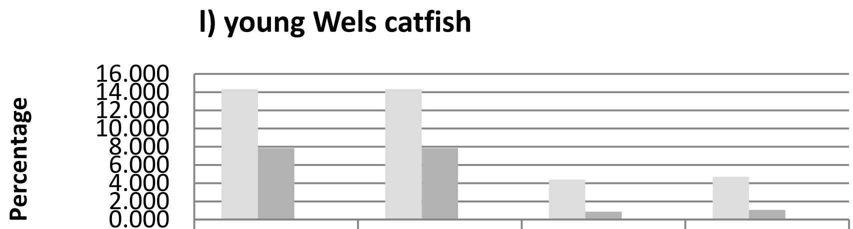
j) Bleak



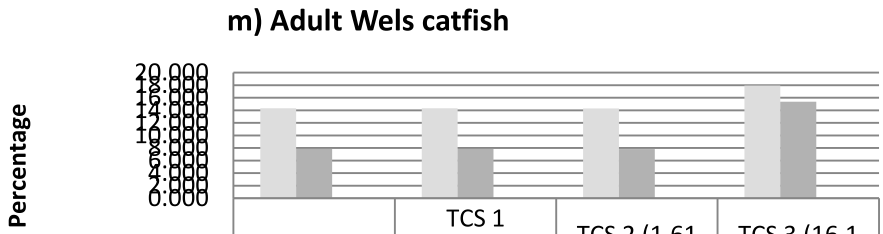
	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Bleak Consumption (Percent)	38.956	38.956	5.268	5.100
Bleak Defecation (Percent)	24.744	24.744	0.120	0.294
Bleak Predation (Percent)	10.654	10.654	2.024	1.595

k) Chub

	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Chub - Cavedano Consumption (Percent)	4.717	4.717	39.093	38.780
Chub - Cavedano Defecation (Percent)	0.927	0.927	24.875	25.303
Chub - Cavedano Predation (Percent)	0.000	0.000	10.658	10.007



	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Siluro min di 32 cm Consumption (Percent)	14.331	14.331	4.405	4.708
Siluro min di 32 cm Defecation (Percent)	7.849	7.849	0.869	1.066
Siluro min di 32 cm Predation (Percent)	0.000	0.000	0.000	0.000



	Control	TCS 1 (0.926 µg/L)	TCS 2 (1.61 µg/L)	TCS 3 (16.1 µg/L)
Siluro adult 32 cm Consumption (Percent)	14.331	14.331	14.297	17.945
Siluro adult 32 cm Defecation (Percent)	7.849	7.849	7.830	15.358
Siluro adult 32 cm Predation (Percent)	0.000	0.000	0.000	0.000

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Programs

Aquatox (US-EPA)
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