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Endocrine disruption: From a whole-lake experiment to a calibrated ecosystem model

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ABSTRACT

The unique Canadian whole-ecosystem study on the impact of 17α-ethinylestradiol (EE2) on a freshwater food web (Karen et al., 2014) provides evidence of the value of whole-ecosystem experiments for understanding indirect effects of endocrine disrupting compounds (EDCs) and other aquatic stressors. To further explain the indirect effects of EE2 observed in the experimental lake, an ecosystem model based on AQUATOX equations was successfully developed and calibrated. Discussions with the scientists who gathered the experimental data were necessary to ensure the consistency of the parameters used in the model and the realism of the biomass dynamics observed in such ecosystems. The prediction results helped further explain how the other fishes were impacted by the fathead minnow collapse. This study also suggests that a mix of reduced gamete production, increased gamete mortality and fish mortality is a potential mechanism for the collapse of the fathead minnow population.

1. Introduction

Endocrine disrupting compounds (EDCs), one category of MPs of great concern, might affect the health of humans and animal species by either mimicking or blocking the behavior of natural hormones. Endocrine disruption was first observed in 1994 in caged trout exposed to sewage effluents (Purdom et al., 1994) and since then, has attracted much interest (Arlos et al., 2018; Auriol et al., 2006; Gross et al., 2017; Ternes et al., 1999). EDCs, include hormones, pharmaceuticals and personal care products (PPCPs), pesticides, industrial chemicals, combustion by-products and surfactants. Numerous chemicals present in the environment still remain unidentified and are considered suspicious as potential EDCs (Fuhrman et al., 2015).

Many laboratory experiments and field measurements have been performed to characterize the biological processes involved in endocrine disruption and their consequences on aquatic and terrestrial species (Chang et al., 2009; Chen and Hsieh, 2017; Tetreault et al., 2011; Bahamonde et al., 2013). Endocrine disruption has first been highlighted in fish and then, in the whole food web, i.e. invertebrates, amphibians, reptiles, birds and mammals (Clotfelter et al., 2004; De Castro et al., 2015; Fent et al., 2006). Despite the growing concern towards EDCs, impact on wild populations and consequences on whole ecosystems remain unclear. Indeed, experimental approaches to characterize ecological impacts are costly and time-consuming and thus, single-species tests are often preferred. The problem is that such data alone may not be suitable for specifically addressing the question of environmental effects, and subsequently the hazard and risk assessment.

Ruhí et al. (2016), studied a river food web composed of macroinvertebrates and put forward the notion that both waterborne exposure and trophic interactions need to be taken into account when assessing the potential ecological risks of emerging pollutants in aquatic ecosystems. With the exception of the few studies reviewed in Arnold et al. (2014), little research has been conducted on higher-trophic levels of wildlife species under natural conditions in the receiving environment or under simulated environmental exposure to EDCs.

Probably the best known example of EDCs affecting wildlife species is the Canadian multi-year whole-ecosystem study performed at an experimental lake with exposure of well-defined fish and lower-trophic-level populations to environmentally-relevant concentrations of the synthetic hormone 17α-ethinylestradiol (EE2) (Kidd et al., 2007). EE2 was chosen because it is one of the most widespread and potent EDCs and its environmental concentration is known to impact the endocrine system and the reproductive functions of aquatic organisms. For the first time, both direct and indirect effects of EE2 on the abundance of fish populations were demonstrated, with *fathead minnow* declining dramatically after 2 years of EE2 addition (Kidd et al., 2014). However, little evidence of direct effects of this synthetic oestrogen were observed on lower-trophic-level organisms, which is unlikely expected at low nanogram per litre concentrations of EE2. Still, increases in some taxa,

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such as the insect Chaoborus, crustacean zooplankton, rotifers and total invertebrates occurred during the experiment. Those changes could be explained as indirect effects based on a reduction in predation by fishes in the lake food web but these explanations remained speculative. The results of their study provide evidence of the value of whole-ecosystem experiments for understanding indirect effects of endocrine disrupting compounds (EDCs) and other aquatic stressors.

Mechanistic models can help further understand the impact of contaminants on aquatic environments and assess their ecological risk. Indeed, ecological models have been increasingly developed for environmental risk assessment (ERA) (De Laender et al., 2008; Galic et al., 2010; Pastorok et al., 2008). Since the goal of ERA is to maintain ecosystem functions and services, Preziosi and Pastorok (2008) called attention to the need for greater incorporation of food web analysis. Carlman et al. (2015) also highlighted that ecosystem models are fundamental for sustainable decision-making. However, implementation to decision making has been very slow, mainly due to the high uncertainty accompanying ecosystem models (Gal et al., 2014), even if some authors offer a framework and guidance to confronting uncertainties in models (Refsgaard et al., 2007).

This study takes up the challenge to develop and calibrate an ecosystem model that will help further explain the indirect effects of EE2 observed on a freshwater food web during the Canadian whole-lake experiment with EE2 (Kidd et al., 2014). Indeed, ecosystem models have been reported in the literature for assessing the impact and risk of different anthropogenic stressors (Gilboa et al., 2014; Grechi et al., 2016; Sourisseau et al., 2008; Taffi et al., 2015) but none has been found for EDCs. The developed ecosystem model includes the reproductive and development endpoints affected by endocrine disruption in fish and is able to predict the indirect effects on the whole ecosystem through ecological interactions, i.e feeding and competition, along with the lake stratification and physico-chemical dynamics. The aim of this paper is to describe the results of both the development and the calibration of the ecosystem model and to analyze the prediction results of the indirect effects of EE2 on a freshwater food web with the experimental data.

2. Materials and methods

2.1. Step 1: collecting experimental data

The unique Canadian whole-lake experiment with EE2 (Kidd et al., 2014) has provided numerous data, including biomass concentration and diet composition, that are well suited for the development and calibration of an ecosystem model. Therefore, the experimental data used for the model come from Lake 260 at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (Kidd et al., 2007). This experimental lake is oligotrophic (high oxygen and low nutrient concentrations) and typical of boreal shield lakes. Six other experimental lakes in the same area were also studied as reference systems. The knowledge and experience of the scientists who worked on those lakes were used to better understand the biological and physico-chemical dynamics of Lake 260.

The study started in 1999 with baseline data collected until 2000. Each year of 2001–2003, EE2 was added to the epilimnion for 20–21 weeks during lake stratification. Seasonal mean concentrations for the summer were 5.0, 6.1 and 4.8 ng/l for 2001 through 2003, respectively. Lower concentrations were measured under the ice during winter. Details on the additions, water sampling and analyses to quantify EE2 are given in Palace et al. (2006). The study continued 7 years after EE2 addition was stopped to measure ecosystem stability and recovery after stressor removal (Blanchfield et al., 2015). The physico-chemical data were collected monthly during the open-water season.

Phytoplankton and zooplankton samples were collected biweekly. Fish abundance data were based on catch-and-release methods using trap nets (spring and autumn, all species) and short (30 min) evening gill net sets on spawning shoals for lake trout (autumn). Biomass of the minnow species was estimated as the product of abundance and mean size from minnow trap captures, standardized by lake area. Mark-and-recapture techniques were used to estimate the abundance of lake trout (autumn data) and white sucker (spring data). Biomass was estimated as the product of abundance estimates and mean size, standardized by lake area.

2.2. Step 2: selecting the modelling approach

The US EPA model AQUATOX (Park and Clough, 2010) is an integrated fate and effects model combining water quality, food web interactions, chemical fate and ecotoxicological processes. AQUATOX is probably the best known tool in risk assessment that accounts for the complexity of communities and ecosystems. While the studies using AQUATOX are, at the very least, based on qualitative biomonitoring, none are supported by a comprehensive quantitative analysis of the biomass and diet composition of the modelled organisms (Lombardo et al., 2015). In most existing AQUATOX studies, only simple verification checks or partial calibrations of the model are typically possible. Indeed, AQUATOX is an open source model and has become very complex with time, making the parametrization step difficult.

A dynamic ecosystem model was previously constructed to predict the effects of metals and pesticides on lentic ecosystems, in an object-oriented framework using simplified AQUATOX equations and the software package WEST (MikebyDHI.com) (De Laender et al., 2008). The simulation results were compared to experimental results obtained from micro- and mesocosm studies. The model was successful in predicting ecological effects of chemicals by considering direct effects but also ecological interactions (feeding and competition relationships). In this study, the simplified AQUATOX model of De Laender et al. (2008) was used. This model was already implemented in the software package WEST, and the equations were modified when it was needed to describe the lake food web dynamics.

2.3. Step 3: selecting the model structure

A simplified food web was built with the most relevant populations of plankton and fish naturally present in the experimental Lake 260 (Fig. 1). The different species of plankton were grouped according to their annual dynamics (i.e. blooms) (Group 1: chlorophyte, dinoflagellates, cyanophyta; Group 2: crysophyta, cryptophyta; Group 3: diatoms). The main fish species selected for the model are characterized by different spawning periods and habitats (hypo- or epilimnion, offshore, bottom or littoral). Based on back and forward discussions with the ecologists who gathered the experimental data, *lake trout* was decided to be the only species spending most of the time in the hypolimnion, but still feeding on the fishes that mainly live in the epilimnion and move around the lake for food.

In AQUATOX, the model consists of a set of objects, and each object describes the growth of a model population in terms of its biomass concentration using differential equations including biological processes such as assimilation, photosynthesis, respiration, consumption or mortality, and additional processes such as migration, diffusion or loading. By connecting different objects and defining feeding relationships between them, a customized food web can be designed (Fig. 2). The number of populations that can be modelled is unlimited and available objects are: phytoplankton, zooplankton, planktivorous fish and piscivorous fish.

Intrinsically identical objects (e.g. various groups of phytoplankton) can be differentiated by parameter tuning (e.g. spring vs. Summer populations). Dynamic driver variables, also called ‘forcing functions’ (indicated on Fig. 1, at the top of the “big box”), are used as external factors. Daily values of the dynamic driver variables are contained in an input file which is read by the ecosystem model during simulation.

The important toxicological endpoint for modelling endocrine

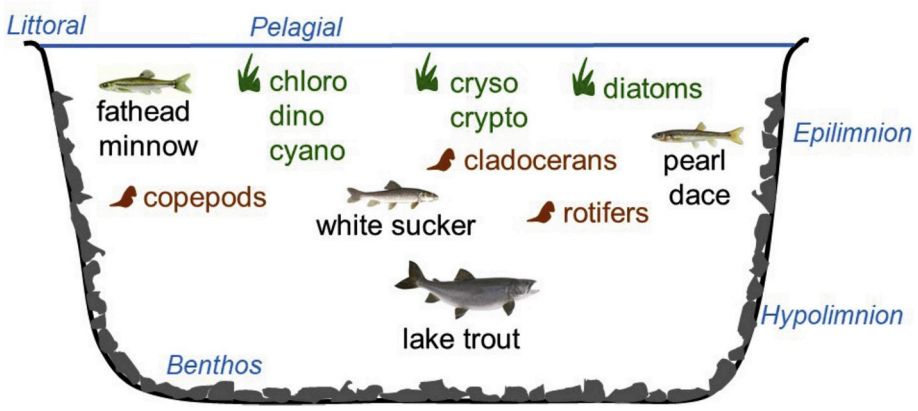

Fig. 1. Conceptual model of the simplified food web used for the model. (Abbreviations: chloro = chlorophyte; dino = dinoflagellates; cyano = cyanophyta; cryso = crysophyta; crypto = cryptophyta).

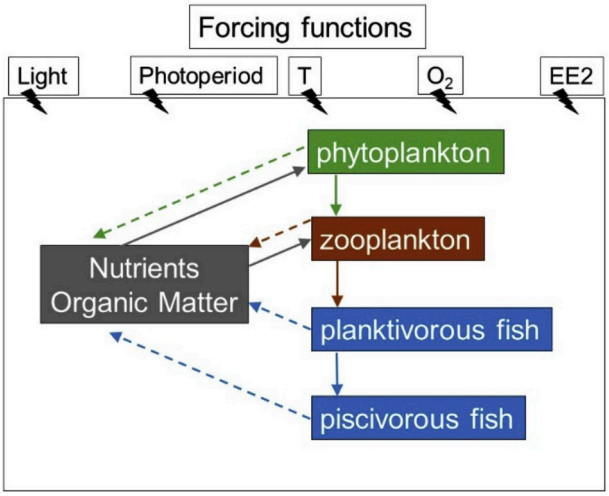

Fig. 2. Simplified framework of the ecosystem model built in WEST with AQUATOX equations. (Full arrow: consumption terms; Dashed arrow: loss terms).

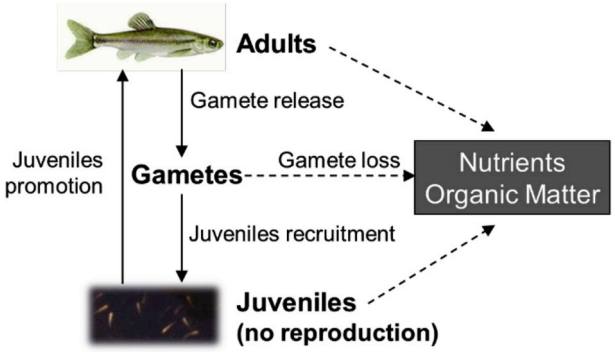

Fig. 3. Conceptual model of the fish life cycle.

disruption is the reproductive ability of fish. For this purpose, two fish classes are used in the model: juveniles and adults (Fig. 3). Intersex fish are not considered explicitly because some of them are assumed to still be able to reproduce. Females and males are not differentiated explicitly either but the sex ratio is and it is important because it determines the gamete quantity that is produced and, thus, the juveniles being recruited (the newly adopted equations are presented and discussed in the *Results* section). Among the gametes released by the adults, some are lost to the detritus and some turn into new fish, which

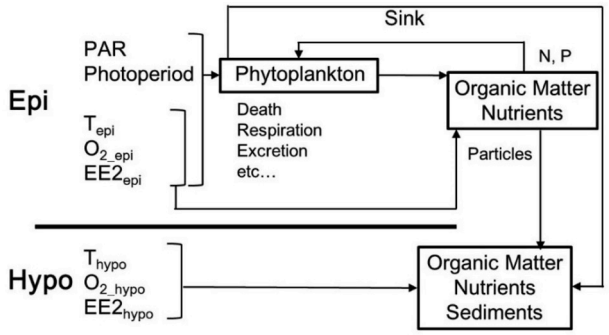

Fig. 4. Conceptual model of the exchanges between the two stratified layers. (Abbreviations: Epi = Epilimnion; Hypo = Hypolimnion; PAR = Photoactive Radiation; T = Temperature; O_2 = Oxygen Concentration; EE2 = EE2 concentration; N = Nitrogen Concentration; P = Phosphorus Concentration).

is called “Juveniles recruitment”. The juveniles become adults when they can reproduce, which is called “Juveniles promotion”.

The model consists of (i) state variables, such as biomass dynamics and also dissolved organic matter (DOM), particulate organic matter (POM), nitrogen (N) and phosphorus (P), (ii) forcing functions, which are measured time series used as model input, such as photoactive radiation (PAR), photoperiod, temperature (T), conce ikntrations of oxygen (O_2) and 17α-ethinylestradiol (EE2), and (iii) exchanges between the two stratified layers (Fig. 4). Supported by the experimental data collected after lake stratification in the spring until shortly before turnover in the autumn, two stratified layers are modelled (epilimnion and hypolimnion) with different biological and physico-chemical compositions. The epilimnion is defined as the surface layer of water with uniform temperature (ignoring any shallow temporary stratification phenomena), while the hypolimnion is defined as the bottom layer of water, also with uniform temperature.

2.4. Step 4: calibrating the model

The forcing functions used to calibrate the ecosystem model (Table 1) come from data collected from the lake in 2000, from May 2nd to October 29th, before the addition of EE2. On May 2nd, 2000, the lake was already stratified, and the overturn of the lake started on October 8th, so most of the simulation results correspond to the dynamics of a stratified lake. The biomass concentrations measured in the experimental lake in May 2nd, 2000, were used as initial biomass concentrations and were not changed during calibration.

The model calibration was conducted following a stepwise procedure (Fig. 5), similar to the procedure adopted by Corominas et al.

Table 1
Forcing functions used for the ecosystem model. (Frequency = Measurement frequency).

Input	Unit	Frequency	Definition
PAR	cal/m^2.d	Daily	Photosynthetically active radiation
Photoperiod	–	Daily	Fraction of day with sunlight
T[Epi]	°C	Weekly	Water temperature in the epilimnion
T[Hypo]	°C	Weekly	Water temperature in the hypolimnion
O[Epi_Wat]	g/m^3	Biweekly	Oxygen concentration in the water column of the epilimnion
O[Epi_Sed]	g/m^3	Biweekly	Oxygen concentration at the water-sediment interface of the epilimnion
O[Hypo_Wat]	g/m^3	Biweekly	Oxygen concentration in the water column of the hypolimnion
O[Hypo_Sed]	g/m^3	Biweekly	Oxygen concentration at the water-sediment interface of the hypolimnion

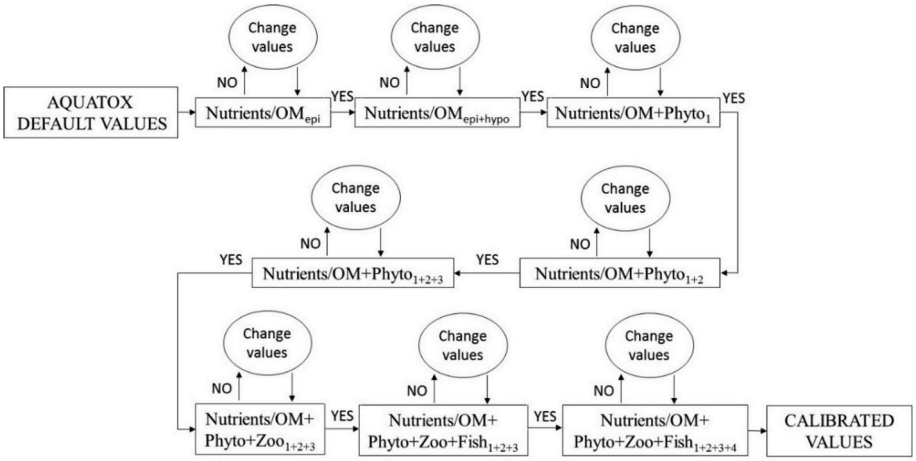

Fig. 5. Proposed stepwise procedure for the model calibration. (Abbreviations: Epi = Epilimnion; Hypo = Hypolimnion; OM = Organic Matter; Phyto = Phytoplankton; Zoo = Zooplankton; 1 + 2+ … = Group 1 + Group 2+ …).

(2011) and Mannina et al. (2011), by which the fit to multiple relevant variables is incrementally pursued. In essence, nutrients are fitted first and subsequently, the trophic levels are added to the multivariate fitting objective. Each time a trophic level is added, the parameters related to that level are updated to achieve a best fit to the data corresponding to that level. However, since this addition of trophic level may affect the trophic levels already present, a recalibration is done of the other lower trophic levels according to the same sequence as they were added. Multiple iterations over these trophic levels may be necessary before the next trophic level can be added and the model calibration goes forth. The fine tuning of the parameters was performed following a trial and error approach based on the comparison of the simulation results with the experimental results. The quality of the model fits to the data was evaluated by performing the Mean Square Relative Error (MSRE) statistical test (Hauduc et al., 2015). The AQUATOX default parameter values were selected as starting point for the model calibration (Park and Clough, 2010). Moreover, the parameter values that were used can be found back in the source code that is coming with the paper.

The first simulations were performed with the nutrients (NH_4, NO_3, PO_4) and organic matter (OM) present in the epilimnion. A sensitivity analysis was performed by visual inspection of the simulation results after perturbation of the parameter under study. By comparing the effect of different parameters, a ranking of the most influential parameters was obtained. For this purpose, each parameter was separately multiplied or divided by two, then changed by ± 20% to study the consequences of smaller changes, and the consequences on the concentrations of nutrients and organic matter observed. From there, the most influencing parameters were selected and tuned until the simulation results were sufficiently close to the experimental data.

The sensitivity analysis was performed by visual inspection of the simulation results after perturbation of the parameter under study. By comparing the effect of different parameters, a ranking of the most influential parameters was obtained.

In the second round of simulations, the nutrients in the hypolimnion were added and connected to the epilimnion through the stratification and mixing processes naturally occurring in lakes. After performing the sensitivity analysis and tuning the most influencing parameters for nutrients, phytoplankton were added, one group at a time (1. chryso- and cryptophyta 2. diatoms 3. chloro- and dinophyta, cyanobacteria). The model calibration continued following the same procedure with zooplankton, fish in the epilimnion (1. *fathead minnow*, 2. *pearl dace*, 3. *white sucker*) and finally fish in the hypolimnion (4. *lake trout*). After each group is added, a sensitivity analysis is performed, and the most influencing parameters tuned before adding the next group.

The simulation results were compared to the experimental data and, back and forward discussions with the scientists who gathered the data were necessary to ensure the consistency of the physico-chemical and biological parameters used in the model and the realism of the biomass dynamics in such ecosystems.

2.5. Step 5: predicting indirect effect of EE2

During the unique Canadian whole-lake experiment with EE2 (Kidd et al., 2014), the strongest direct effect of EE2 was observed on fathead minnow, with a collapse of the fish species in the second year of adding EE2 due to endocrine disruption. The objective of the developed ecosystem model is to further explain the indirect effects of EE2 observed on the food web of the experimental lake. Therefore, specific parameters were calibrated in fathead minnow in order to describe the direct effect of EE2 on fathead minnow, and then the indirect effect on other fish species was analyzed. After back and forth discussions with the scientists who gathered the experimental data, the parameters involved in reducing gamete production, increasing gamete mortality and/or increasing fish mortality were identified as potential hypotheses. The experimental data collected during the second year of

adding EE2 to the lake (Kidd et al., 2007) were used to fine tune the selected parameters.

3. Results and discussion

The aim of this paper is to describe the results of both the development and the calibration of the ecosystem model and to compare the prediction results of the indirect effects of EE2 on a freshwater food web with the experimental data. The ecosystem model was built according to the different compartments found in the experimental lake; in addition to the biota, the physical and chemical characteristics were also considered. The developed ecosystem model is inspired by the simplified AQUATOX equations of De Laender et al. (2008) (see their paper for a full list of equations, parameters, state variables and forcing functions that form the basis of the model presented here). In the Results and Discussion section, only the simplified AQUATOX equations of De Laender et al. (2008) that were changed and the equations that were added are presented.

Regarding the calibration, results present the best fit obtained between the experimental data and the simulation outputs, including all the boxes of Fig. 5 (the sections "Model Parameters" and "Calibration Results" correspond to the last box of Fig. 5 called "Calibrated Values"). The final model has a considerable number of parameters, distributed over 16 objects (fishes, organic matter, phyto- and zooplankton species). For each object, a number of parameters did not have to be calibrated because they represent constant lake characteristics (volume, pH, depth, etc.) and detritus ratios (ratio of phosphorus to organic matter, ratio of nitrogen to ammonia, etc.). For the other parameters, a sensitivity analysis was performed to find the limited subset of parameters that still allows getting a good model fit to the data.

3.1. Physico-chemical characteristics

3.1.1. Stratification

The percentage of sedimented organic particles ($\%_{sed}$) from the epilimnion to the hypolimnion is calculated using the following equation, where $\%_{sed}$ is assumed to be constant over time:

$$\%_{Sed} = \frac{V_{Offshore}}{V_{Epi}} \tag{1}$$

where $V_{Offshore}$ (m^3) is the volume from where the particles sediment to the hypolimnion and V_{Epi} (m^3) is the total volume of the epilimnion (Fig. 6).

In AQUATOX, the stratification is considered to occur when the mean water temperature exceeds 4 °C and the difference in temperature between the epilimnion and hypolimnion exceeds 3 °C. When those two conditions are not met, the two layers mix, which normally occurs during the spring and the fall. The equations used in the ecosystem model for the mixing of organic matter and nutrients were presented by Vallet et al. (2014):

If $T_{mean}(t) > 4\,°C$ and $(T_{epi}(t)\text{-}T_{hypo}(t)) > 3\,°C$,

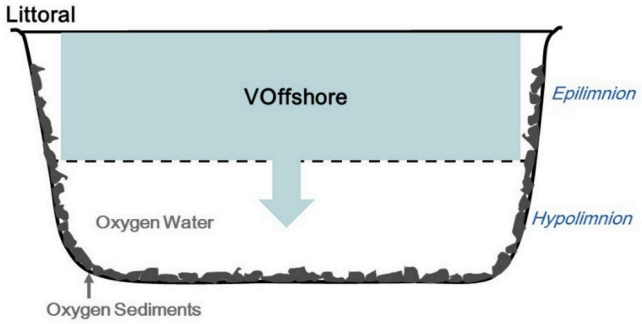

Fig. 6. Conceptual model of the particles sedimentation in the stratified lake.

$$then\ Mix(t) = 0 \tag{2}$$

where Mix (g/m^3.d) is the lake overturn rate, T_{mean} (°C) the mean water temperature, T_{epi} (°C) the water temperature in the epilimnion and T_{hypo} (°C) the water temperature in the hypolimnion.

$$\text{else } Mix(t) = \frac{Qmix}{Vtot}(X_{in}(t) - X(t)) \tag{3}$$

where Mix $(g/m^3.d)$ is the lake overturn rate, Q_{mix} (m^3/d) is the mixing flow, V_{tot} (m^3) the total volume of the lake, X (g/m^3) the concentration of the variable being modelled (Nutrient (NH4, NO3 or PO4) or Organic Matter (DOM, POM or SOM)), X_{in} (g/m^3) the concentration of the variable entering the modelled layer (epilimnion or hypolimnion).

3.1.2. Oxygen and temperature

Oxygen concentrations in the sediments are different from the oxygen concentrations in the water (Fig. 6). Thus, a correction factor was applied, both in the epilimnion and the hypolimnion, to differentiate the reactions happening in the sediments from the ones in water:

$$DO_{corr}(t) = \frac{O_2(t)}{O_2(t) + HalfSatO} \tag{4}$$

where DO_{corr} $(unitless)$ is the oxygen correction factor, O_2 (g/m^3) the dissolved oxygen concentration and $HalfSatO$ (g/m^3) the half-saturation constant for oxygen.

Temperature is another important controlling factor in the model, involved in the stratification of the lake, but also in the biotic and chemical fate processes. The same correction factor as the one used by De Laender et al. (2008) was applied to the corresponding equations in order to calculate the temperature correction for microbial processes.

3.1.3. Transport

A genetic screening of the *fathead* population studied during the recovery phase of the EE2 lake experiment demonstrated that there was no fish migration from surrounding lakes (Blanchfield et al., 2015). Besides, the lake inflow and outflow were shown to be negligible compared to the total volume of the lake and because it is an experimental area, there was no fishing either. Therefore, the lake could be modelled as a closed system with no exchanges with the outside. Consequently, the transport equations used in AQUATOX for the biota, nutrients and organic matter, such as loading, washout, migration, fishing, etc., could be removed from the model.

3.2. Organic matter and nutrients

3.2.1. Model equations

The organic matter mass balance is composed of the losses coming from the different biota and is divided into 3 groups: (1) dissolved organic matter (DOM) (2) particulate organic matter (POM) (3) and sedimented organic matter (SOM). Compared to the simplified model developed by De Laender et al. (2008), a fraction of dead biota was added to the DOM, according to the equations from AQUATOX. For readability, the term (t) is not included on the right-hand side of differential equations.

$$\frac{dDOM}{dt} = Excret_{Biota} + Mortality_{Biota} \times F_{MortDOM} - Decomp_{DOM} + Mix \tag{5}$$

where $Excret_{Biota}$ $(g/m^3.d)$ is the total excretion rate of the biota, $Mortality_{Biota}$ $(g/m^3.d)$ the total dead biota rate, $F_{MortDOM}$ $(unitless)$ the fraction of dead biota transformed into DOM, $Decomp_{DOM}$ $(g/m^3.d)$ the DOM loss rate due to decomposition and Mix $(g/m^3.d)$ the lake overturn rate (see Equations (3) and (4)).

For the POM, the gametes lost by fish (not turning into juveniles, see Fig. 3) were added to the simplified AQUATOX equations of De Laender et al. (2008):

$$\frac{dPOM}{dt} = Mortality_{Biota} \times F_{MortPOM} + GameteLoss - Decomp_{POM} - Sed_{POM} - Ingest_{POM} \quad (6)$$

where $Mortality_{Biota}$ ($g/m^3.d$) is the total dead biota rate, $F_{MortalityPOM}$ (*unitless*) the fraction of dead biota transformed into POM, *GameteLoss* ($g/m^3.d$) the loss rate for gametes, $Decomp_{POM}$ ($g/m^3.d$) the POM loss rate due to decomposition, Sed_{POM} ($g/m^3.d$) the sedimentation rate of POM and $Ingest_{POM}$ ($g/m^3.d$) the consumption rate of POM by zooplankton.

For the SOM, the main difference with the simplified AQUATOX equations of De Laender et al. (2008) was to consider the lake stratification, according to the equation presented earlier (Equation (1)). Thus, when modelling the hypolimnion, part of the SOM coming from the epilimnion is considered.

The decomposition of the three organic matter groups is based on a maximum decay rate ($Decay_{Max}$, g/g.d), corrected for suboptimal temperature, dissolved oxygen and pH. The difference with the simplified AQUATOX equation used by De Laender et al. (2008), is that in the developed ecosystem model, the variation of oxygen concentrations between the water column and the water-sediment interface is considered (see Equation (4)). The DOM and POM are considered being decomposed in the water column only, while SOM also decomposes in the sediments.

A fraction of the decomposed organic matter is converted into nutrients (NH_4, NO_3, PO_4). The main additions made to the simplified AQUATOX equations used by De Laender et al. (2008) for the nutrient pool are the addition of NO_3 assimilation by phytoplankton and the nitrification/denitrification processes happening both in the water column and at the water-sediment interface:

$$\frac{dNH_4}{dt} = (Decomp_{OM} + Excret_{Biota} + Resp_{Biota}) \times N2OM - Assim_{NH4} - Nitrif_{water} - Nitrif_{sed} + Mix \quad (7)$$

where NH_4 (g/m^3) is the ammonia concentration, $Decomp_{OM}$ ($g/m^3.d$) the organic matter decomposition rate, $Excret_{Biota}$ ($g/m^3.d$) the total excretion rate of the biota, $Resp_{Biota}$ ($g/m^3.d$) the total respiration rate of the biota, N2OM (*unitless*) the N:OM ratio, $Assim_{NH4}$ ($g/m^3.d$) the NH_4 assimilation rate by phytoplankton, $Nitrif_{water}$ ($g/m^3.d$) the NH_4 nitrification rate in the water column, $Nitrif_{sed}$ ($g/m^3.d$) the NH_4 nitrification rate at the water-sediment interface and *Mix* ($g/m^3.d$) the lake overturn rate.

$$\frac{dNO_3}{dt} = Nitrif_{water} + Nitrif_{sed} - Denit_{water} - Denit_{sed} - Assim_{NO3} + Mix \quad (8)$$

where NO_3 (g/m^3) is the nitrate concentration, $Denit_{water}$ ($g/m^3.d$) the NO_3 denitrification rate in the water column, $Denit_{sed}$ ($g/m^3.d$) the NO_3 denitrification rate at the water-sediment interface and $Assim_{NO3}$ ($g/m^3.d$) the NO_3 assimilation rate by phytoplankton.

$$\frac{dPO_4}{dt} = (Decomp_{OM} + Excret_{Biota} + Resp_{Biota}) \times P2OM - Assim_{PO4} + Mix \quad (9)$$

where PO_4 (g/m^3) is the phosphate concentration, P2OM (*unitless*) the P:OM ratio and $Assim_{PO4}$ ($g/m^3.d$) the PO_4 assimilation by phytoplankton.

3.2.2. Calibration results

During the calibration of the nutrient and organic matter parameters, the most influencing parameters selected with the sensitivity analysis are related to the nitrification/denitrification processes and decomposition of organic matter (Table 2). The values presented were obtained after model fit (Fig. 7) and correspond to the final iteration of the calibration procedure (see Fig. 5, last box called "Calibrated values").

Maximum rates for the denitrification process are similar between the epi- and the hypolimnion, while nitrification happens at higher maximum rates in the epilimnion, which makes sense since the nitrification process requires aerobic conditions. When looking at the graph presenting final results after fine tuning of the calibrated parameters (Fig. 7A), it appears that NH4 mainly occurs in the epilimnion, where the nitrification is at its highest rate. The graph clearly shows that both NH4 and NO3 accumulated during the winter, when nitrification and denitrification rates are very low due to low temperatures, and then, as soon as spring starts, both NH4 and NO3 are eliminated through nitrification and denitrification processes. Early in the fall, when temperatures drop again, NH4 starts accumulating again. The simulation results obtained with the calibrated parameters succeeded at catching those dynamics.

Regarding SOM and DOM decomposition, the maximum rates appeared to be higher in the epilimnion, while higher in the hypolimnion for the POM. When looking at the POM graph (Fig. 7B), concentrations are higher in the hypolimnion, where the POM accumulates. Once again, the simulation results obtained with the calibrated parameters succeeded at matching the trend observed in the experimental data.

Back and forward discussions on the nutrient and organic matter dynamics with the freshwater system ecologists who gathered the experimental data confirmed that the calibrated parameters succeeded at simulating their dynamics during the open water season.

3.3. Phytoplankton

3.3.1. Model equations

The phytoplankton mass balance is described by the following equation:

$$\frac{dPhyto}{dt} = Photo - Resp - Excr - Mort - Sink - Pred \quad (10)$$

where *Phyto* (g/m^3) is the phytoplankton biomass, *Photo* ($g/m^3.d$) the rate of photosynthesis, *Resp* ($g/m^3.d$) the respiratory loss, *Excr* ($g/m^3.d$) the rate of excretion, *Mort* ($g/m^3.d$) the rate of non-predatory mortality, *Sink* ($g/m^3.d$) the loss due to sinking to the bottom and *Pred* ($g/m^3.d$) the consumption of phytoplankton by zooplankton.

Photosynthesis is modelled as a maximum rate, which is reduced by nutrient, temperature and light limitation factors (De Laender et al., 2008). For each species, optimal photosynthesis is reached at optimal temperature and depth, and is directly connected to the photosynthetically active solar radiation (PAR). Following AQUATOX equations, when there is no ice cover on the lake ($T_{mean} > 3\,^{\circ}C$), the light is entered in the ecosystem model as an input, using the measured values of Photoactive radiation (PAR) (Table 1). If there is an ice cover, a factor of 0.3 is applied to the values of PAR.

There are two main limiting factors to the light used for photosynthesis. The first one is the *Photoperiod*, representing the fraction of the day with daylight and entered in the ecosystem model as an input (Table 1). The second one is the extinction of light (*Extinct*) when entering the water, due to "self-shading" of the phytoplankton, organic particles and dissolved organic matter. Compared to the simplified model developed by De Laender et al. (2008), the light limitation factor was changed for the equation used in AQUATOX:

$$LtLimit(t) = 0.85 \times \frac{e \times Photoperiod(t) \times (LtAtDepth(t) - LtAtTop(t))}{Extinct(t) \times (Depth_{Bottom} - Depth_{Top})} \quad (11)$$

where *LtLimit* (*unitless*) is the light limitation, *0.85 (unitless)* the correction factor for daily formulation, *e* (*2.718, unitless*) the base of natural logarithms, *Photoperiod* (*unitless*) the fraction of the day with daylight, *LtAtDepth* (*unitless*) the limitation of algal growth due to light, *LtAtTop* (*unitless*) the limitation due to insufficient light, *Extinct (1/m)* the total light extinction from "self-shading" of the phytoplankton, organic particles and dissolved organic matter, $Depth_{Bottom}$ (*m*) the

Table 2
Calibrated values of the most influencing parameters for organic matter and nutrients in the epilimnion (Epi) and the hypolimnion (Hypo) (see Equations in De Laender et al. (2008)).

Parameter	Unit	Epi	Hypo	Definition
kDNit[Wat]	g/g.d	0.008	0.009	Maximum denitrification rate in the water column
kDNit[Sed]	g/g.d	0.008	0.008	Maximum denitrification rate at the water-sediment interface
kNit[Wat]	g/g.d	0.6	0.005	Maximum nitrification rate in the water column
kNit[Sed]	g/g.d	0.2	0.001	Maximum nitrification rate at the water-sediment interface
$Decay_{Max}$[SOM_Sed]	g/g.d	0.1	0.001	Maximum decomposition rate of SOM at the water-sediment interface
$Decay_{Max}$[DOM_Wat]	g/g.d	0.03	0.0001	Maximum decomposition rate of DOM in the water column
$Decay_{Max}$[POM_Wat]	g/g.d	0.001	0.002	Maximum decomposition rate of POM in the water column

maximum depth and $Depth_{Top}$ *(m)* the depth of the top layer.

Nitrogen and phosphorus compounds are assimilated during the photosynthesis process, which is modelled in the nutrient equations (see Equations (7)–(9)). Regarding the assimilation of nitrogen, because only 23 percent of the weight of nitrate is nitrogen and 78 percent of ammonia is nitrogen, this results in an apparent preference for ammonia. Thus, compared to the simplified model developed by De Laender et al. (2008), a preference factor (*NH4Pref*) inspired by AQUATOX was added:

$$NH4Pref\,(t) = \frac{\frac{14}{18} \times NH4(t) \times \frac{14}{62} \times NO3(t)}{\left(KN + \frac{14}{18} \times NO3(t)\right) \times \left(KN + \frac{14}{62} \times NO3(t)\right)} + \frac{\frac{14}{18} \times NH4(t) \times KN}{\left(\frac{14}{18} \times NH4(t) + \frac{14}{62} \times NO3(t)\right) \times \left(KN + \frac{14}{62} \times NO3(t)\right)} \quad (12)$$

where *NH4Pref* *(unitless)* is the ammonia preference factor, *14/18 (unitless)* the ratio of nitrogen to ammonia, *14/62 (unitless)* the ratio of nitrogen to nitrate, *KN* (gN/m^3) the half-saturation constant for nitrogen uptake, *NH4* (g/m^3) the concentration of ammonia and *NO3* (g/m^3) the concentration of nitrate.

Regarding the other biotic processes (i.e. respiration, excretion, mortality, sinking and predation), the equations used in the developed ecosystem model are described in De Laender et al. (2008), except for the respiratory loss, which was replaced by the AQUATOX equation. Respiratory loss is exponential with temperature and since the developed ecosystem model is to be applied to stratified lakes, implying changes of temperature, the AQUATOX equation was preferred over the simplified equation of De Laender et al. (2008) that was used for non-stratified micro- and mesocosms:

$$Resp(t) = Resp20 \times 1.045^{(Temp(t)-20)} \times Phyto(t) \quad (13)$$

where *Resp* $(g/m^3.d)$ is the respiratory loss, *Resp20* *(g/g.d)* the respiration rate at 20 °C, *1.045* (/°C) the exponential temperature coefficient, *Temp* (°C) the ambient water temperature and *Phyto* (g/m^3) the phytoplankton Biomass.

3.3.2. Calibration Results

During the calibration of the phytoplankton parameters, the most influencing parameters selected with the sensitivity analysis are mainly related to photosynthesis (Table 3). Group 1 and Group 3 seem to assimilate better P than N, while Group 2 assimilates P and N similarly. Group 3 has the lowest maximum photosynthesis rate. Parameters for mortality, sedimentation and temperature were also selected as influencing parameters.

Experimental data for phytoplankton biomass suffered from high variability, because of the many species in each group, but also because of the experimental uncertainty resulting in high variability of the data collected. The values presented in Table 3 were obtained after model fit within the error bars of the experimental data (Fig. 8) and correspond to the final iteration of the calibration procedure for the three groups of phytoplankton (see Fig. 5, last box called “Calibrated values”).

Back and forward discussions on the phytoplankton biomass dynamics with the scientists who gathered the experimental data confirmed that the calibrated parameters managed to simulate relatively well the main trends during the open water season, considering the high variability of the experimental data and except for the initial decline. The simulation results captured that Group 1 organisms were more abundant than Group 2, and that Group 3 had the lowest concentrations. Besides, Group 1 is characterized by a bloom between April and June, which explains the high initial biomass concentration, followed by a drop (Fig. 8A). Blooms for Group 2 and Group 3 start later, which can be seen with lower drops on the graphs (Fig. 8B and 8C).

3.4. Zooplankton

3.4.1. Model equations

The zooplankton mass balance is described by the following equation (De Laender et al., 2008):

$$\frac{dZoo}{dt} = Cons - Def - Resp - Exc - Mort - Pred \quad (14)$$

where *Zoo* (g/m^3) is the zooplankton biomass, *Cons* $(g/m^3.d)$ the consumption of phytoplankton and POM, *Def* $(g/m^3.d)$ the defecation of unassimilated food, *Resp* $(g/m^3.d)$ the respiratory loss, *Excr* $(g/m^3.d)$ the excretion of dissolved organic matter, *Mort* $(g/m^3.d)$ the non-predatory mortality and *Pred* $(g/m^3.d)$ the consumption of zooplankton by planktivorous fish.

3.4.2. Calibration results

During the calibration of the zooplankton parameters, the most influencing parameters selected with the sensitivity analysis are mainly related to the consumption of phytoplankton and POM (Table 4).

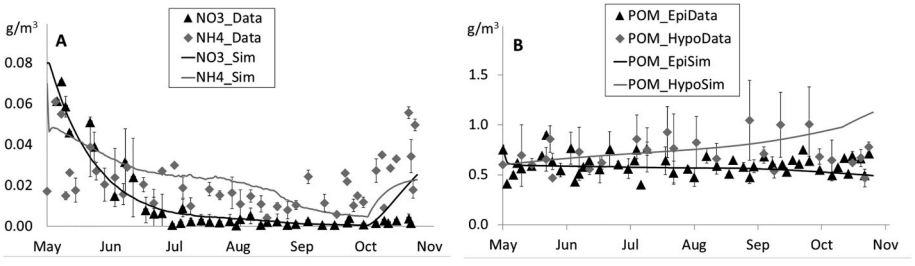

Fig. 7. Calibration results for (A) nitrate and ammonia in the epilimnion and (B) the particulate organic matter in the whole lake. MSRE values (g/m^3): NH4 = 0.0002; NO3 = 0.001; POM_epi = 0.015; POM_hypo = 0.055. (Abbreviations: Epi = Epilimnion; Hypo = Hypolimnion; Data = Experimental Data; Sim = Simulation Results).

Table 3
Calibrated values of the most influencing parameters for phytoplankton. (Abbreviation: Group 1: crysophyta, cryptophyta; Group 2: diatoms; Group 3: chlorophyte, dinoflagellates, cyanophyta) (see Equations in De Laender et al. (2008)).

Parameter	Unit	Group 1	Group 2	Group 3	Definition
PS[Max]	/d	5	6	3.9	Maximum photosynthesis rate
Light[Sat]	cal/m^2.d	60	65	150	Light saturation level for photosynthesis
K[N]	g/m^3	0.003	0.06	0.004	Half saturation constant for N uptake
K[P]	g/m^3	0.03	0.05	0.03	Half saturation constant for P uptake
k[Mort]	g/g.d	0.0001	0.001	0.0001	Intrinsic mortality rate
E[Mort]	g/g.d	0.001	0.01	0.05	Approximate fraction killed per day with total limitation
k[Sed]	m/d	0.01	0.1	0.01	Sinking rate
E[Sed]	–	0.01	0.1	0.001	Exponential factor for accelerated sinking when stressed (light/nutrients)
Resp[20]	/d	0.05	0.08	0.2	Intrinsic respiration at 20 °C
T[Resp]	°C	2	1.8	2	Temperature coefficient for respiration
T[Opt]	°C	13	13	20	Optimum temperature
T[Ref]	°C	2	2	4	Minimum adaptation temperature

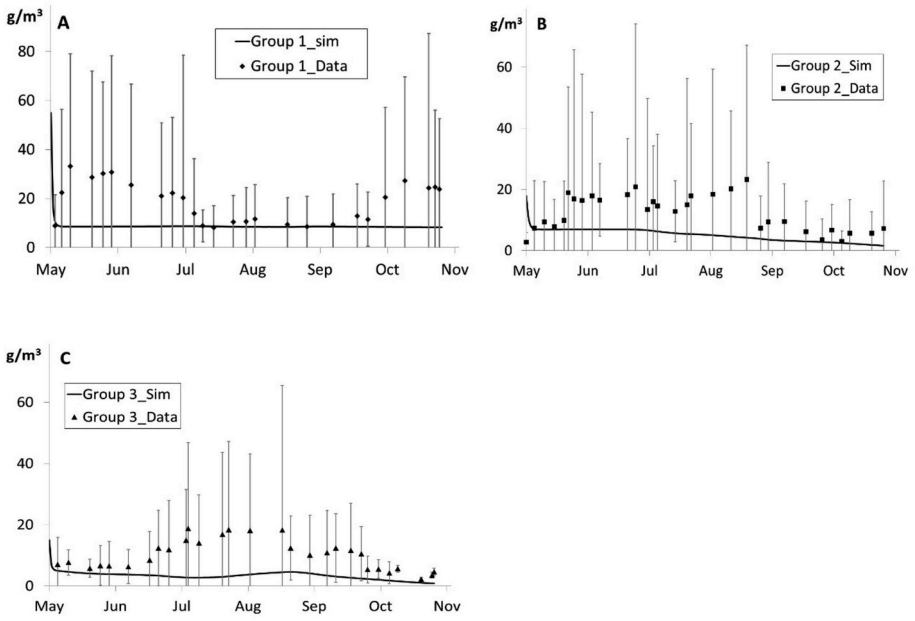

Fig. 8. Calibration results for (A) Group 1 (crysophyta, cryptophyta) (B) Group 2 (diatoms) and (C) Group 3 (chlorophyte, dinoflagellates, cyanophyta). MSRE values (g/m^3): Group 1 = 165; Group 2 = 82; Group 3 = 71. (Abbreviations: Data = Experimental Data; Sim = Simulation Results).

Table 4
Calibrated values of the most influencing parameters for zooplankton (see Equations in De Laender et al. (2008)).

Parameter	Unit	rotifer	cladocera	copepod	Definition
C[Max]	/d	4	2	4	Maximum consumption rate
Pref[Phyto1]	–	0	0.2	0.2	Preference for phyto – Group 1
Pref[Phyto2]	–	0	0.2	0.2	Preference for phyto – Group 2
Pref[Phyto3]	–	0	0.2	0.2	Preference for phyto – Group 3
Pref[POM]	–	1	0.4	0.4	Preference for POM
B[Min_Phyto1]	g/m^3		10	8	Minimum concentration to begin feeding: phyto – Group 1
B[Min_Phyto2]	g/m^3		7	15	Minimum concentration to begin feeding: phyto – Group 2
B[Min_Phyto3]	g/m^3		6	5	Minimum concentration to begin feeding: phyto – Group 3
B[Min_POM]	g/m^3	0.4	0.5	0.55	Minimum concentration to begin feeding: POM
F[HalfSat_Phyto1]	g/m^3		1	1	Half saturation constant for consumption of phyto – Group 1
F[HalfSat_Phyto2]	g/m^3		1	1	Half saturation constant for consumption of phyto – Group 2
F[HalfSat_Phyto3]	g/m^3		1	1	Half saturation constant for consumption of phyto – Group 3
F[HalfSat_POM]	g/m^3	0.5	1	1	Half saturation constant for consumption of POM
Egest[Coeff_Phyto1]	–	0.1	0.1	0.2	Egested fraction of consumed phyto – Group 1
Egest[Coeff_Phyto2]	–	0.1	0.1	0.2	Egested fraction of consumed phyto – Group 2
Egest[Coeff_Phyto3]	–	0.1	0.1	0.2	Egested fraction of consumed phyto – Group 3
Egest[Coeff_POM]	–	0.05	0.1	0.1	Egested fraction of consumed POM
K[Excr]	g/g.d	0.0001	0.001	0.01	Proportionality constant for excretion:respiration
Resp[0]	/d	0.05	0.014	0.014	Intrinsic respiration
dK[Resp]	–	0.001	0.01	0.01	Fraction of energy lost to dynamic action
k[Mort]	g/g.d	0.0001	0.0001	0.002	Intrinsic mortality rate
T[Opt]	°C	16	14	18	Optimum temperature

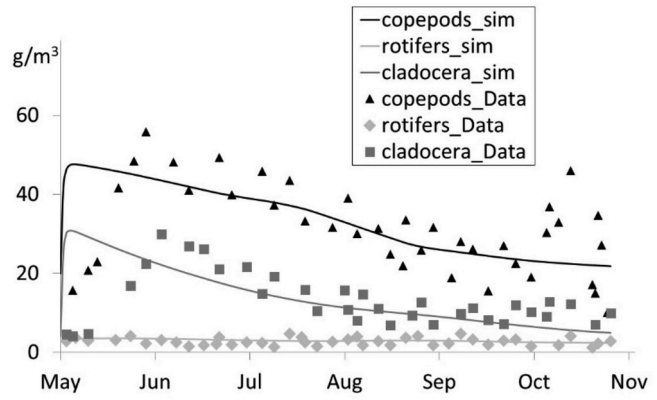

Fig. 9. Calibration results for zooplankton. MSRE values (g/m^3): copepods = 77; rotifers = 0.99; Cladocera = 110. (Abbreviations: Data = Experimental Data; Sim = Simulation Results).

Parameters for excretion, respiration, mortality and temperature were also selected as influencing parameters. The values presented were obtained after model fit for the three groups of zooplankton (Fig. 9) and correspond to the final iteration of the calibration procedure (see Fig. 5, last box called "Calibrated values").

The final values of the parameters resulted in rotifers only eating POM. While rotifers normally eat some phytoplankton, their main diet consists of dead or decomposing organic material, due to their microscopic size. The scientists who gathered the experimental data confirmed that it was an acceptable simplification of the model.

When looking at the graph presenting final results after fine tuning of the calibrated parameters (Fig. 9), it appears that the simulation results succeeded at predicting the zooplankton dynamics during the open water season, except for a faster growth of cladocera and copepods in May. Back and forward discussions on the zooplankton biomass dynamics with the scientists who gathered the experimental data confirmed that when looking at experimental data for other years or in the reference lakes, cladocera and copepods have earlier growth peaks, starting early May for copepods and mid-May for cladocera.

3.5. Fish

3.5.1. Model equations

For the fish mass balance, De Laender et al. (2008) used the same equation as for the zooplankton mass balance presented in Equation (14). In order to model endocrine disruption, two fish classes were added (Juveniles & Adults, see Fig. 3) and three reproductive terms added to the mass balance, accordingly to AQUATOX:

Juveniles:

$$\frac{dFish_{juv}}{dt} = Cons - Def - Resp - Exc - Mort - Pred - Promo + Recruit \quad (15)$$

Adults:

$$\frac{dFish_{adult}}{dt} = Cons - Def - Resp - Exc - Mort - Pred - GameteLoss + Promo \quad (16)$$

where $Fish_{juv}$ (g/m^2) is the juvenile biomass, $Fish_{adult}$ (g/m^2) is the adult biomass, *Cons* $(g/m^2.d)$ the consumption of POM, *Def* $(g/m^2.d)$ the defecation of unassimilated food, *Resp* $(g/m^2.d)$ the respiratory loss, *Excr* $(g/m^2.d)$ the excretion of dissolved organic matter, *Mort* $(g/m^2.d)$ the non-predatory mortality, *Pred* $(g/m^2.d)$ the consumption of fish by piscivorous fish, *GameteLoss* $(g/m^2.d)$ the loss of gametes during spawning, *Promo* $(g/m^2.d)$ the promotion from juveniles to adults and *Recruit* $(g/m^2.d)$ the recruitment from viable gametes to juveniles.

Eggs and sperm, modelled as gametes, can be a significant fraction of adult biomass. Because only a small fraction of these gametes results in viable young when shed at the time of spawning, entered as parameters in the model (*SpawningStart and SpawningEnd*), the remaining fraction is lost to detritus (*GameteLoss*).

If SpawningStart ≤ t ≤ SpawningEnd, **then**:

$$GameteLoss(t) = (GMort + IncrMort) \times FracAdults(t) \times PctGamete \times Bio(t) \quad (17)$$

Else: $GameteLoss(t) = 0$where *GameteLoss* $(g/m^2.d)$ is the loss of gametes during spawning, *SpawningStart (d)* the date when spawning starts, *SpawningEnd (d)* the date when spawning ends, *GMort (1/d)* the gamete mortality, *IncrMort (1/d)* the increased gamete and embryo mortality due to toxicant, *FracAdults (unitless)* the fraction of biomass that is adult, *PctGamete (unitless)* the fraction of adult biomass that is in gametes and *Bio* (g/m^2) the biomass.

As the biomass of a fish population reaches its carrying capacity, which is the maximum sustainable biomass, reproduction is usually reduced due to stress. In the model, this results in assuming the proportion of adults and the fraction of biomass in gametes at a maximum.

$$FracAdults(t) = 1 - \left(\frac{Capacity(t)}{KCap}\right) \quad (18)$$

If $Bio_{fish}(t) < KCap$, **then**:

$$Capacity(t) = KCap - Bio_{fish}(t) \quad (19)$$

Else: $Capacity(t) = 0$where *FracAdults (unitless)* is the fraction of biomass that is adult, *Capacity* (g/m^2) the biomass capacity, *KCap* (g/m^2) the carrying capacity and *Bio* (g/m^2) the biomass.

During spawning, gametes are lost from the adults and the juveniles gain the viable gametes through recruitment, which is, in other words, the biomass gained from successful spawning:

If SpawningStart ≤ t ≤ SpawningEnd, **then**:

$$Recruit(t) = (1 - (GMort + IncrMort)) \times FracAdults(t) \times PctGamete \times Bio(t) \quad (20)$$

Else: $Recruit(t) = 0$where *Recruit* $(g/m^2.d)$ is the recruitment from viable gametes to juveniles, *SpawningStart (d)* the date when spawning starts, *SpawningEnd (d)* the date when spawning ends, *GMort (1/d)* the gamete mortality, *IncrMort (1/d)* the increased gamete and embryo mortality due to toxicant, *FracAdults (unitless)* the fraction of biomass that is adult, *PctGamete (unitless)* the fraction of adult biomass that is in gametes and *Bio* (g/m^2) the biomass.

The juveniles promoted to adults is determined in the model by the rate of growth, considered as the sum of consumption and the loss terms other than mortality.

$$Promo(t) = KPro \times (Cons(t) - Def(t) - Resp(t) - Exc(t)) \quad (21)$$

where *Promo* $(g/m^2.d)$ is the promotion from juveniles to adults, *KPro* *(unitless)* the fraction of growth that goes to promotion, *Cons* $(g/m^2.d)$ the consumption of phytoplankton and POM, *Def* $(g/m^2.d)$ the defecation of unassimilated food, *Resp* $(g/m^2.d)$ the respiratory loss and *Excr* $(g/m^2.d)$ the excretion of dissolved organic matter.

3.5.2. Calibration results for fathead minnow and pearl dace

During the calibration of the *fathead minnow* and *pearl dace* parameters, the most influencing parameters selected with the sensitivity analysis are mainly related to reproduction and food consumption (Tables 5 and 6). Parameters for excretion, respiration, mortality and temperature were also selected as influencing parameters.

Experimental data were collected in the spring (May 16) and the adult fish concentrations were 0.23 g/m^2 and 0.28 g/m^2 for *fathead minnow* and *pearl dace* respectively. The simulation results were quantitatively discussed with ecological specialists, in order to validate the biomass dynamics obtained during the open water season for both juveniles and adults. The values presented In Tables 5 and 6 correspond

Table 5
Calibrated values of the most influencing parameters for fathead minnow and pearl dace (*same parameters for juveniles and adults*) (see Equations in De Laender et al. (2008) and AQUATOX).

Parameter	Unit	minnow	dace	Definition
Spawning[Start]	d	50	1	Date when spawning starts
Spawning[End]	d	80	15	Date when spawning ends
G[Mort]	1/d	0.9	0.9	Gamete mortality rate
Incr[Mort]	1/d	0	0	Increase gamete mortality rate
K[Cap]	g/m^2	0.5	1	Carrying capacity
CA	1/d	0.36	0.36	Max ingestion rate for a 1-g fish at optimal temp
Pref[clado]	–	0.7	0.7	Preference for cladocera
Pref[cop]	–	0.2	0.2	Preference for copepods
Pref[POM]	–	0.1	0.1	Preference for POM
F[HalfSat]	g/m^2	0.1	0.025	Half saturation constant for consumption of food
Egest[Coeff]	–	0.3	0.2	Egested fraction of consumed food
RA	1/d	0.0148	0.0148	Basal respiration rate
RB	–	−0.2	−0.2	Slope of the allometric function
T[Opt]	°C	22	21	Optimum temperature
T[Max]	°C	24	27	Maximum temperature tolerated
T[Ref]	°C	12	10	Minimum adaptation temperature
XM	°C	1	5	Maximum acclimation allowed
Z	m	2	3	Mean depth where fish lives

to the final iteration of the calibration procedure (see Fig. 5, last box called “Calibrated values”) and were obtained after the model was fitted with the experimental data and the experts validated the biomass dynamics Fig. 10).

For *fathead minnow* (Fig. 10A), the experimental data (0.23 g/m^2 on May 16) was collected before the spawning period, which lasted from the end of June to the end of July. The beginning of spawning can be seen on the graph with a drop of the adult biomass occurring after June 21st, correlated to an increase of the juvenile biomass. Before spawning, the adult biomass increases because they are producing gametes and then, when spawning starts, the gametes are lost, which explains the decrease in the adult biomass. Conversely, the juvenile biomass increases when spawning starts, because viable gametes turn into young fish. After spawning, from July 21st to the fall, biomass of both adults and juveniles increases first, due to high temperature and food availability during summer, and then decreases when fall arrives, due to a decrease in temperature and food availability.

For *pearl dace* (Fig. 10B), the spawning period starts earlier (mid-April) and ends around mid-May, which means the experimental data (0.28 g/m^2 on May 16) was collected just at the end of the spawning period (May 16). The same pattern than for *fathead minnow* occurs but earlier. The decrease of adult biomass and increase of juvenile biomass started before the beginning of the graph and ended around June 17th, when spawning ended.

3.5.3. Calibration results for white sucker and lake trout

During the calibration of the *white sucker* and *lake trout* parameters, the most influencing parameters selected with the sensitivity analysis are mainly related to reproduction and food consumption (Tables 7 and 8). Parameters for excretion, respiration, mortality and temperature were also selected as influencing parameters.

Experimental data were collected in the spring (May 16) for adult *white sucker* and the biomass concentration was measured at 7.42 g/m^2. For the adult *lake trout*, the data were collected in the fall (October 3rd) and the biomass concentration was measured at 4.04 g/m^2. The simulation results were quantitatively discussed with ecological specialists, in order to validate the biomass dynamics obtained during the open water season for both juveniles and adults. The values presented In Tables 7 and 8 correspond to the final iteration of the calibration procedure (see Fig. 5, last box called “Calibrated values”) and were obtained after the model had been fitted with the experimental data and the experts validated the biomass dynamics (Fig. 11).

Adult *white sucker* have the same spawning period as adult *pearl dace*, which is between mid-April and mid-May. Thus, similar dynamics can be observed on both graphs (Figs. 10B and 11C). However, due to higher mean weights (Tables 7 and 8), the biomass changes observed in *white sucker* are of lower magnitude compared to *pearl dace*, both for adults and juveniles.

The piscivorous fish, *lake trout* (Fig. 11D), have a later and longer spawning period (mid-August to mid-October), compared to the other planktivorous fishes (*fathead minnow*, *pearl dace* and *white sucker*). Nevertheless, a similar pattern occurs, which is an increase in both adult and juveniles biomass in the summer, and then, a decrease in adult biomass when spawning starts, due to gamete loss.

3.5.4. Endocrine disruption

After having successfully calibrated the ecosystem model with a unique set of experimental data collected in 2000, from May 2nd to October 29th, before the addition of EE2, the experimental data collected during the second year of adding EE2 to the lake (Kidd et al., 2007) were used to help further explain the indirect effects on the lake food web. The experimental results showed that the strongest direct effect of EE2 was observed on fathead minnow, with a collapse of the fish species in the second year of adding EE2 due to endocrine disruption. Therefore, discussions with experts in ecotoxicology and EDCs helped identify specific parameters to be modified in *fathead minnow* in order to test a potential hypothesis for the biological processes involved in endocrine disruption in *fathead minnow* (Table 9).

When looking at the graph presenting final results after fine tuning of the selected parameters (Fig. 12E and 12F), it appears that a mix of reduced gamete production (*PctGamete*), increased gamete mortality (Incr[Mort]) and increase of both adults and juveniles mortality (k[Mort_Adult] and k[Mort_Juv]) is a potential hypothesis for explaining the collapse of both adult and juvenile fathead minnow due to EE2 addition (Kidd et al., 2007).

The simulation results also help further explain how the other fishes were impacted by the collapse of *fathead minnow*. Indeed, endocrine disruption in *fathead minnow* did not only affect its own population but also other fish populations (Kidd et al., 2014). After three summers of EE2 addition, the experimental results showed a 58% reduction of the *pearl dace* population. However, a reduction of the pearl dace population was also observed in the reference lakes and, thus, no conclusion on the link with EE2 could be made. The model prediction results provided a new insight regarding a potential link with EE2, with 16%

Table 6
Calibrated values of the most influencing parameters for fathead minnow and pearl dace (Juveniles [J] and Adults [A]) (see Equations in De Laender et al. (2008) and AQUATOX).

Parameter	Unit	minnow [J]	minnow[A]	dace[J]	dace [A]	Definition
K[Pro]	–	0.3	0	0.1	0	Fraction of growth that goes to promotion
PctGamete	–	0	0.1	0	0.08	Fraction of adult biomass in gametes
k[Excr]	g/g.d	0.1	0.05	0.05	0.05	Proportionality constant for excretion:respiration
k[Mort]	g/g.d	0.11	0.06	0.1	0.02	Intrinsic mortality rate
Activity	–	0.07	0.03	0.05	0.06	Factor for respiratory loss due to swimming
Weight[Mean]	g	0.3	3	1	5	Mean weight

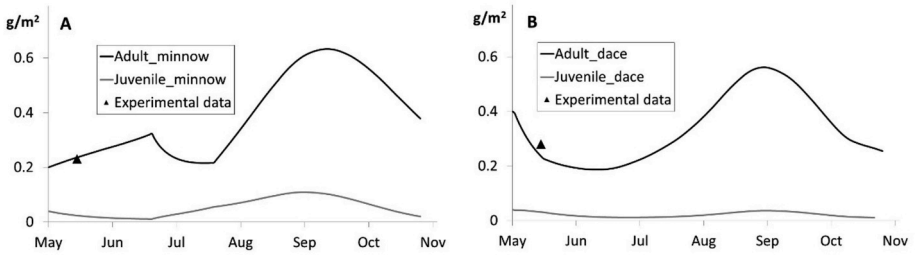

Fig. 10. Simulation results from the calibration procedure for (A) fathead minnow and (B) pearl dace.

Table 7
Calibrated values of the most influencing parameters for white sucker and lake trout (*same parameters for juveniles and adults*) (see Equations in De Laender et al. (2008) and AQUATOX).

Parameter	Unit	sucker	trout	Definition
Spawning[Start]	d	1	165	Date when spawning starts
Spawning[End]	d	8	180	Date when spawning ends
PctGamete	–	0.03	0.02	Fraction of adult biomass that is in gametes
G[Mort]	1/d	0.9	0.9	Gamete mortality rate
Incr[Mort]	1/d	0	0	Increase gamete mortality rate
K[Cap]	g/m^2	10	6	Carrying capacity
CA	1/d	0.15	0.589	Max ingestion rate for a 1-g fish at optimal temp
Pref[minnow]	–	0	0.4	Preference for adults fathead minnow
Pref[dace]	–	0	0.4	Preference for adults pearl dace
Pref[sucker]	–	0	0.2	Preference for juveniles white sucker
Pref[clado]	–	0.1	0	Preference for cladocera
Pref[cop]	–	0.1	0	Preference for copepods
Pref[POM]	–	0.8	0	Preference for POM
RA	1/d	0.0274	0.00463	Basal respiration rate
RB	–	−0.348	−0.295	Slope of the allometric function
T[Opt]	°C	20	17	Optimum temperature
T[Max]	°C	26	21	Maximum temperature tolerated
T[Ref]	°C	2.5	5	Minimum adaptation temperature
XM	°C	2	2	Maximum acclimation allowed
Z	m	3	8	Mean depth where fish lives

reduction of the adult pearl dace population and no significant changes for the juveniles population due to EE2 (Fig. 12G). Regarding the white sucker population, the experimental results did not indicate significant changes, which corresponded with the simulation results that predicted no changes for the adult population. However, a 62% reduction was predicted for the juveniles population, but this may have gone unnoticed experimentally since they represent the population class that is most difficult to sample due to the smaller sizes (Fig. 12G). Regarding the *lake trout* population, the experimental results and the model predictions both showed a reduction of around 25% (juveniles and adults) (Fig. 12H).

Back and forward discussions with the scientists who gathered the data and experts in ecology validated the simulation results. Since *lake trout* could no longer feed on *fathead minnow*, they turned to *pearl dace* and *white sucker*. The consequence was a decrease of both prey biomass (Fig. 12G) and a change in lake trout biomass (Fig. 12H). Therefore, the calibrated model was successful in predicting the indirect effects of EE2 on the lake food web, and thus further explain the experimental data.

4. Conclusion

An ecosystem model that can help further explain indirect effects of endocrine disruption in fish in a lake food web was successfully developed and calibrated with a unique set of experimental data coming from the whole-lake study of Kidd et al. (2007, 2014). Back and forward discussions with the scientists who gathered the data and experts in ecology were necessary to ensure the consistency of the physico-

Table 8
Calibrated values of the most influencing parameters for white sucker and lake trout (Juveniles [J] and Adults [A]) (see Equations in De Laender et al. (2008) and AQUATOX).

Parameter	Unit	sucker[J]	sucker[A]	trout[J]	trout [A]	Definition
K[Pro]	–	0.1	0	0.05	0	Fraction of growth that goes to promotion
F[HalfSat]	g/m^2	0.5	1	0.1	0.1	Half saturation constant for consumption of food
Egest[Coeff]	–	0.1	0.1	0.2	0.3	Egested fraction of consumed food
k[Excr]	g/g.d	0.001	0.01	0.01	0.001	Proportionality constant for excretion:respiration
k[Mort]	g/g.d	0.001	0.001	0.001	0.015	Intrinsic mortality rate
Activity	–	0.0001	0.002	0.009	0.0064	Factor for respiratory loss due to swimming
Weight[Mean]	g	120	600	800	400	Mean weight

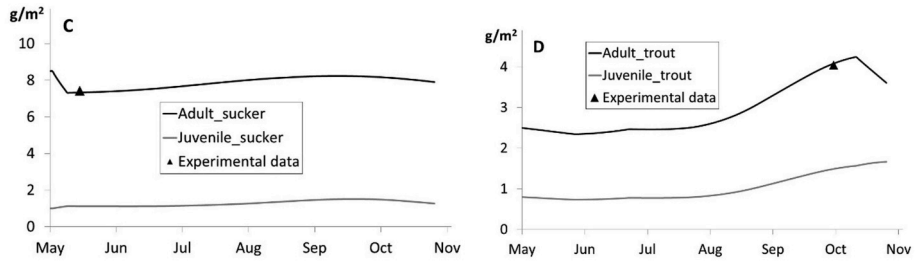

Fig. 11. Simulation results after the calibration for (C) white sucker and (D) lake trout.

Table 9
Calibrated parameters of fathead minnow to describe direct effects of EE2 (see Equations in De Laender et al. (2008) and AQUATOX).

Parameter	Unit	Without EE2	With EE2	Definition
PctGamete	–	0.1	0.01	Fraction of adult biomass that is in gametes
Incr[Mort]	1/d	0	0.1	Increase gamete mortality rate
k[Mort_Adult]	g/g.d	0.06	0.18	Intrinsic mortality rate of adults
k[Mort_Juv]	g/g.d	0.11	0.15	Intrinsic mortality rate of juveniles

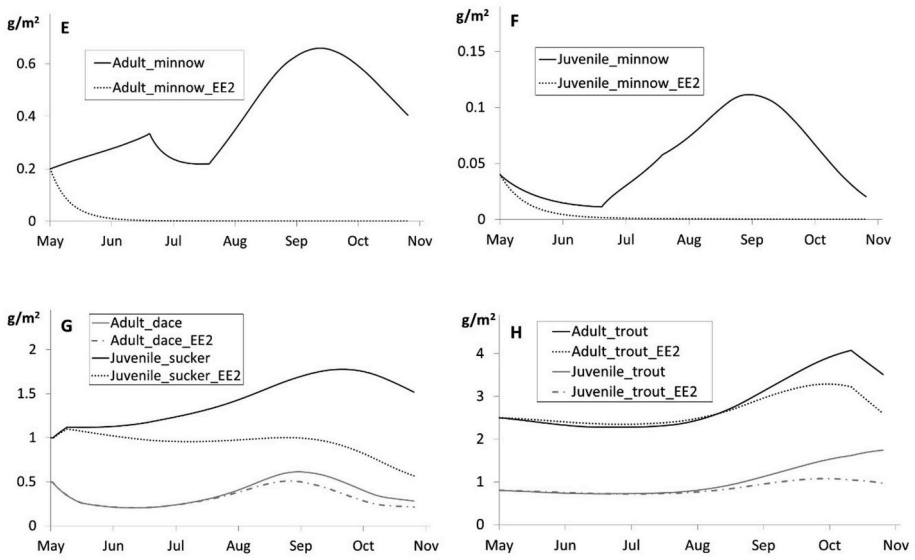

Fig. 12. Simulation results with and without EE2 in the lake for (E) adult fathead minnow, (F) juvenile fathead minnow, (G) adult pearl dace and juvenile white sucker, (H) adult and juvenile lake trout.

chemical and biological parameters used in the model and the realism of the biomass dynamics in such ecosystems. This study suggests that a mix of reduced gamete production, increased gamete mortality and fish mortality produced a similar pattern in fathead minnow as observed in the second year of exposure to EE2. The simulation results also helped explain how the other fishes were impacted by the collapse of fathead minnow.

To further investigate the indirect effect of EE2, an EDC of great concern, the ecosystem model will be used to better understand the ecological interactions and how endocrine disruption in fishes, such as *fathead minnow,* can impact a whole lake food web. Indeed, more research is needed to develop ecosystem models, like the one presented in this paper, that can support ERA of EDCs and other aquatic stressors.

Software availability

Name of the software: ELA_Fish model library.

Software requirements: WEST 3.7.6 (or higher).

Program Language: Model Specification Language (MSL) (see Vanhooren et al. (2003), for an explanation on MSL code).

Program Size: approximately 25 MB.

Availability: The source code for the ELA_Fish model library can be obtained via GitHub at the following URL: https://github.com/modelEAU/ELA_msl.git.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envsoft.2019.01.013.

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