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TK-TD Model of *Lemna* Populations (MoLePo) Version 1

TRACE documentation

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This TRACE document ("TRAnsparent and Comprehensive model Evaludation") provides supporting evidence that the model TK-TD Model of *Lemna* Populations (MoLePo) V1 was thoughtfully designed, correctly implemented, and thoroughly tested for its intended purpose.

The rationale of this document follows:

Schmolke A, Thorbek P, DeAngelis DL, Grimm V. 2010. Ecological modelling supporting environmental decision making: a strategy for the future. *Trends in Ecology and Evolution* 25: 479-486.

and uses the updated standard terminology and document structure in:

Grimm V, Augusiak J, Focks A, Frank B, Gabsi F, Johnston ASA, Kułakowska K, Liu C, Martin BT, Meli M, Radchuk V, Schmolke A, Thorbek P, Railsback SF. 2014. Towards better modelling and decision support: documenting model development, testing, and analysis using TRACE. Ecological Modelling 280:129-139

and

Augusiak J, Van den Brink PJ, Grimm V. 2014. Merging validation and evaluation of ecological models to 'evaludation': a review of terminology and a practical approach. Ecological Modelling 280:117-128.

We also followed the EFSA PPR Scientific Opinion on good modelling practice (EFSA PPR 2014). In the reports describing the use of the model for a specific plant protection product, the summary sheet proposed in the Scientific Opinion is used.

EFSA PPR Panel (2014). Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA Journal 12.3, p. 3589.

The *MoLePo* model itself was based on the model by Schmitt et al. (2013):

Schmitt W, Bruns E, Dollinger M, Sowig P. 2013. Mechanistic TK-TD-model simulating the effect of growth inhibitors on *Lemna* populations. *Ecol Model* 255:1– 10.

but partly refined, e.g. in order to be able to consider the exposure to dynamic mixtures of two active substances. Refinements of the original model are indicated in this documentation.

We thank Walter Schmitt for support during the implementation of the model.

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Definitions/Abbreviations

AGD	Aquatic Guidance Document (EFSA PPR Panel 2013)
dw	dry weight
ECx	Effective concentration for x % effects
EFSA	European Food Safety Authority
ERO	Ecological Recovery Option (see AGD)
ETO	Ecological Threshold Option (see AGD)
FN	Frond Number
FOCUS	FOrum for international Coordination of pesticide fate models and their Use
fw	fresh weight
ODD	Overview, Design concepts, Details
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted Environmental Concentration (e.g. by FOCUS scenarios)
TD	Toxicodynamics
TK	Toxicokinetics
TRACE	TRAnsparent and Comprehensive model Evaludation (Grimm et al. 2014)

1   Problem formulation

This TRACE element provides supporting information on: The decision-making context in which the model will be used; the types of model clients or stakeholders addressed; a precise specification of the question(s) that should be answered with the model, including a specification of necessary model outputs; and a statement of the domain of applicability of the model, including the extent of acceptable extrapolations.

Summary

The ultimate aim of building and 'evaludating'[1] (Augusiak et al. 2014) this model is to provide a complimentary tool which can serve as a virtual laboratory for the population-level risk assessment (ERA) of plant protection products on macrophytes in edge-of-field water bodies, especially for time variable exposure concentrations and environmental conditions expected or edge of field water bodies in the EU. The model species, *Lemna* sp., was selected as a sensitive standard test species.

The decision making context of the herein presented TK-TD population model of *Lemna* sp.*,* is the authorization of plant protection products in the EU under European Regulation No 1107/2009 and following the Aquatic Guidance Document (AGD, EFSA PPR Panel 2013). In this AGD the specific protection goals for aquatic macrophytes are described as follows: 'Aquatic vascular plants will be protected at the population level by considering their growth and/or abundance/biomass in edge-of-field surface waters. Option 1 (ETO) allows negligible effects only. Option 2 (ERO) allows medium effects as long as the duration of the effect on the abundance and/or biomass of vulnerable populations of macrophytes is not longer than weeks or small effects when they last for a few months. In option 2, the acceptable magnitude of effects is small to medium since large effects are not desirable even if recovery can be demonstrated.'

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[1] 'We introduce the term 'evaludation', a fusion of 'evaluation' and 'validation', to describe the entire process of assessing a model's quality and reliability. Considering the iterative nature of model development, the modelling cycle, we identified six essential elements of evaludation: (i) 'data evaluation' for scrutinising the quality of numerical and qualitative data used for model development and testing; (ii)'conceptual model evaluation' for examining the simplifying assumptions underlying a model's design;(iii) 'implementation verification' for testing the model's implementation in equations and as a computer programme; (iv) 'model output verification' for comparing model output to data and patterns that guided model design and were possibly used for calibration; (v) 'model analysis' for exploring the model's sensitivity to changes in parameters and process formulations to make sure that the mechanistic basis of main behaviours of the model has been well understood; and (vi) 'model output corroboration' for comparing model output to new data and patterns that were not used for model development and parameterization.' Augusiak at al. (2014).

Note that, because for each substance the TK-TD for *Lemna* model has to be parameterized separately, some of the evaludation elements in this TRACE doc are only demonstrated with example data sets but have to be done also for the application of the model for a specific compound (e.g. model output verification (including calibration) and corroboration).

The model aims to extrapolate from effects on and recovery of growth in laboratory tests to effects of other, not tested, time variable exposure conditions (i.e. PEC time series as predicted by FOCUS step 3 or 4 modelling) under laboratory or field conditions in the EU (e.g. constant or time variable water temperature, light intensity, nutrient concentrations).

The model species was selected; because *Lemna sp.* is the standard macrophyte test organism (EFSA PPR Panel 2013). So, data on its sensitivity are available from standardized laboratory tests (e.g. OECD Test guideline 221) and additional data, e.g. for other exposure patterns, can easily by produced (Tier 2 C. Refined exposure tests, according to EFSA PPR Panel 2013). With respect to its vulnerability *Lemna* can be considered as 'likely exposed' because it is a typical species in in edge of field lentic or slowly flowing water bodies in agricultural landscape, especially ditches and ponds. As a species floating at the surface, it might be exposed to drift entries onto the fronds and uptake of dissolved toxicants from the water column. *Lemna* is often among the most sensitive macrophytes species tested (Giddings et al. 2013) and for a given chemical tests with other macrophyte species can be used to assess the relative sensitivity of *Lemna* (Tier 2A and 2 B. EFSA PPR Panel 2013). Due to its high growth rate and the easy transportation, e.g. by water fowl, *Lemna*'s internal and external recovery potential can be considered to be relatively high. Thus, modelling the effects of dynamic exposure on *Lemna* populations will in most cases be used for the ETO-option, unless there is evidence that other macrophytes, e.g. rooted ones, are clearly less sensitive than *Lemna*.

2   Model description

This TRACE element provides supporting information on: The model. Provide a detailed written model description. For individual/agent-based and other simulation models, the ODD protocol is recommended as standard format. For complex submodels it should include concise explanations of the underlying rationale. Model users should learn what the model is, how it works, and what guided its design.

Summary:

This section presents the complete model description following the ODD format (Overview, Design concepts, Details). Originally, the ODD is a protocol for describing individual-based population models (Grimm et al., 2006; Grimm et al., 2010). The *Lemna* model is not an individual based model because the population dynamics is described by its total biomass per vessel or area only (differential equation model). Nevertheless, ODD was used to structure this section. The *Lemna* model links a one compartment TK-TD model which links internal concentrations of one or two toxicants to inhibition of growth) to a growth model which includes the influence of temperature, irradiance and nutrient concentrations in the water on photosynthesis as well as the impact of temperature on respiration if field populations should be modelled. The state variables are internal concentrations of toxicants in the plants and biomass per vessel (simulating laboratory tests) or per area (simulating field populations).

2.1   Purpose

The proximate purpose of this *Lemna* model is to reproduce the population dynamics (biomass) of the duckweed *Lemna* sp. under time variable exposure conditions to one or two chemical stressors. The ultimate purpose is to use the model to predict the effects of exposure profiles predicted for FOCUS step 3 and 4 scenarios or similar profiles on *Lemna* populations under laboratory or field conditions.

2.2   Entities, state variables and scales

The model's entities are the biomass of the *Lemna* population, described by the state variable biomass and the internal concentration of one or two active substances, and the environment, defined by the water concentrations of one or two active substances, water temperature, light intensity, and nitrogen and phosphorus concentrations (Table 1). The variables describing the environment can be considered as forcing function, because in the model they are only a function of time and not of any of the other state variables. However, for simplicity we will use them as state variables here.

The model is not spatially explicit. The basic unit for biomass is g dw for simulating of laboratory tests and g dw/m² for field tests. By the use of factor, biomass can be transformed to frond numbers.

Table 1:   State variables characterizing the population and the environment

	Meaning	Unit
State variables for the population		
s_BM	Biomass density (dry weight)	mg dw / vessel (lab) or g dw/m² (field) (Fixed ratios to fresh weight and frond number assumed).
s_Cint1	Internal conc. a.s. 1	µg/L
s_Cint2	Internal conc. a.s. 2	µg/L
State variables of the environment		
s_Cext1	Water concentration a.s. 1	µg/L
s_Cext2	Water concentration a.s. 2	µg/L
s_Temp	Water temperature	°C
s_Rad*	Radiation intensity	kJ/(m²d)
s_Nitro	Nitrogen concentrations	mg N/L
s_Phos	Phosphorus concentration	mg/ P/L

The model can simulate growth inhibition tests over one or a few weeks and field dynamics over a year or more.

The differential equations are solved in intervals depending on the algorithm used (see section 5). Usually, the change of biomass is calculated at least once per hour. Input files of exposure concentration can include data per hour or day. Environmental factors like temperature, irradiance or nutrient concentrations are expected per day.

2.3   Process overview and scheduling

The TK-TD Lemna model can be used for two cases: simulating a population in a laboratory growth inhibition tests or population growing in the field. For the laboratory population, the control growth is as assumed to be exponential with a constant rate. For the field population, the control growth is assumed to be dependent on temperature, irradiance, nutrient concentrations and density dependence. In both cases, the effect of the exposure to one or two toxicants is modelled via the same TK-TD model.

The scheduling of the different processes in the model is shown in Figure 1. In the initialization process, the initial biomass of the *Lemna* plant is determined and the internal concentration(s) of toxicant(s) is set to zero. Thus, each simulation starts with 'clean' plants. Then, for simulation of field populations, every day the environmental conditions (temperature, irradiance, nutrient levels) for that day are taken from an input file (missing values are interpolated). Based on these conditions, factors describing their impact on photosynthesis or respiration rates are calculated. Conditions in growth inhibition tests in the laboratory are usually kept constant at values allowing high exponential growth of the (control) plants. Therefore, the growth is not modelled explicitly for simulation of laboratory tests but a fixed exponential growth rate for the control is used.

FOCUS surface water models provide the predicted environmental concentration (PEC) on an hourly basis. So, exposure is updated at each simulated time step. If the time step selected is smaller than the interval for which the exposure data are provided, the concentrations are linearly interpolated.

From the actual concentration in the water, the TK part of the model calculates the internal concentration in the plants. The internal unbound concentration drives the inhibition of the biomass production rate (TD module). This is due to the fact that toxicant bound to structural plant material (described via partition plant:water) is considered to reach the target sites for growth inhibition. In the next step, the *Lemna* biomass is updated considering the inhibition due to the toxicant and – for field populations only – the impact of the environmental factors.

Per default, the state variables as well as the external concentration and other environmental factors are saved once per simulated day. However, it can also be decided to save results on an hourly basis. At the end of the simulation the program creates some summary statistics in an additional output file.
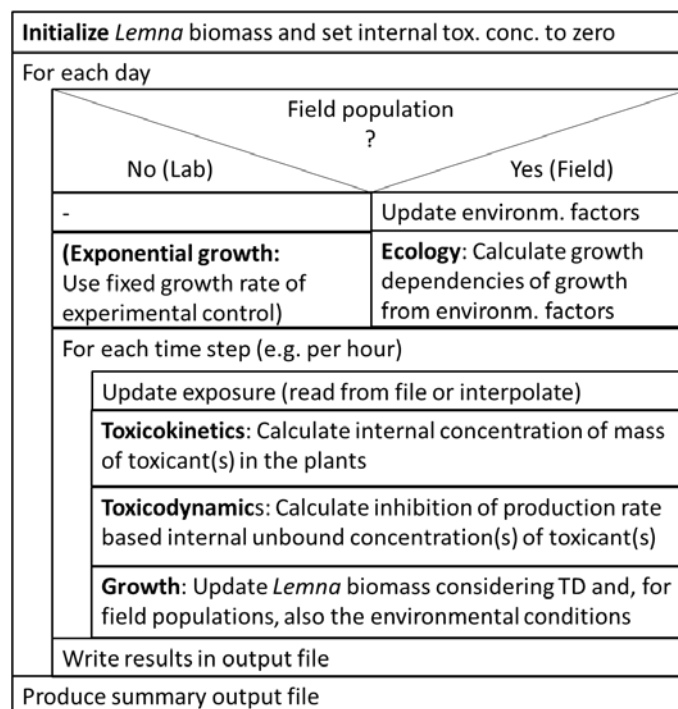
Figure 1:   Structogram of the *Lemna* Model.

2.4   Design concepts

2.4.1   Basic Principles

The *Lemna* model is a differential equation model that describes the dynamic development of biomass based on effective photosynthesis and respiration rates linked to a one-compartment TK-TD model (Figure 2).
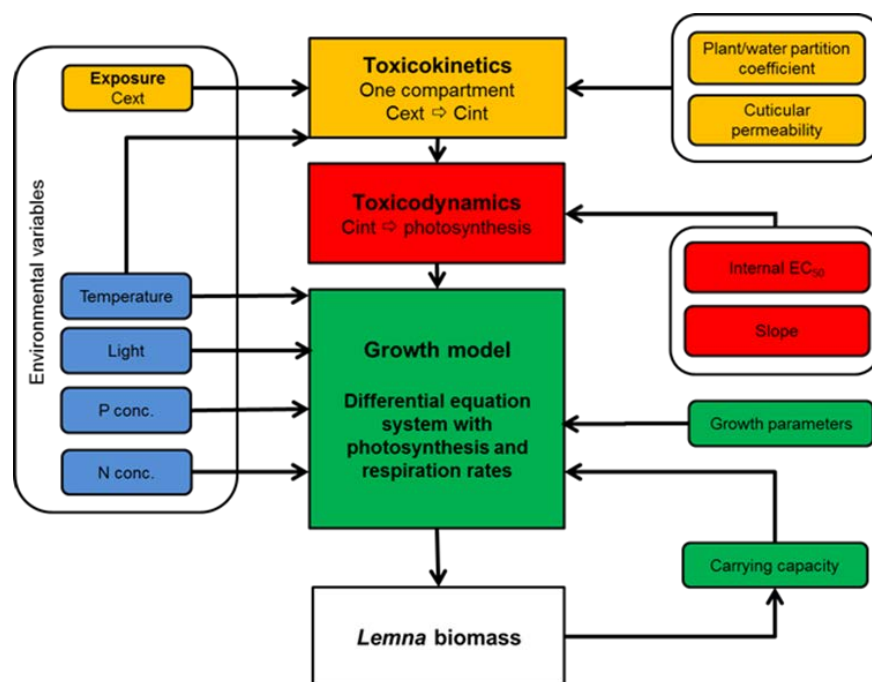
Figure 2:   Basic principles used to model *Lemna* growth

Cext = external concentration (in the water), Cint = internal concentration (in the plant)

The TK model translates the external (herbicide) exposure concentration of one or two active substance into internal concentrations in the plant tissue considering (in a simplified approach) uptake, metabolism and elimination. From the internal concentrations, the inhibition of the photosynthesis is calculated via a dose-response function described by substance specific internal $EC50_{int}$ and slope parameters (TD).

For simulating field populations, the effective photosynthesis rate is calculated by multiplying the optimal photosynthesis rate with factors, which depend on environmental conditions such as temperature, light, nutrient concentrations (nitrogen and phosphorus), and biomass (density dependence). The actual respiration rate is calculated from a default respiration rate affected by temperature. This allows modelling changes in biomass to realistic and temporally variable environmental conditions including exposure.

For simulation of growth inhibition tests in the laboratory, the growth model is simplified. Instead of explicitly modelling the influence of temperature, irradiance and nutrient concentrations, a fixed exponential growth rate is used which is derived directly from a fit to the control data. This constant control growth rate is then only affected by the toxicant.

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2.4.2 Emergence

Emergence occurs at the population level, where long-term dynamics emerge from the variable environmental conditions including the concentrations of the toxicant(s), the TK and TD parameters and the density dependence of growth.

2.4.3 Interaction

No interaction of individual plants is modelled, except for the density dependence of the growth, which in reality can be caused by self-shading of overlapping fronds.

2.4.4 Stochasticity

The model is deterministic, it includes no stochastic elements.

2.4.5 Observation

The actual inhibition of the photosynthesis by the internal concentrations is used as an additional observer to the state variables.

The model produces a time series of the state and observer variables in the form of tables and plots over time.

Details on selecting and exporting the different outputs are given in the user manual.

2.5 Initialization

To initialize the model a start biomass has to be entered or defined in an input file (see user manual). The internal concentration in the plant is set to zero.

2.6 Input data

The environmental variables (exposure in the form of concentrations of the toxicant in the water and temperature, irradiance and nutrient concentrations) are either set to fixed values (e.g. to simulate a laboratory test) or read from an input file. Model parameters are read from a database but they can also be entered or edited directly in the user interface. For parameters, which should be calibrated or which should be varied within Monte-Carlo simulations, also the ranges and the type of distribution can be entered.

2.7 Sub-models

The text to follow describes the sub-models of the *MoLePo* model. The basic description is cited from Schmitt et al. (2013) and indicated by the use of *italic* font. Only the numbering of the equations is different from the original paper. This description is followed by additional explanations and descriptions of model refinements if needed.

2.7.1   Toxikokinetic model

As mentioned above, the major purpose of the model is the extrapolation to exposure scenarios differing in their temporal variation. Experimental results indicate that uptake of organic chemicals into and release from aquatic macrophytes are not instantaneous and may take hours to days (Gobas et al., 1991). Therefore, this process needs to be considered explicitly in a TK submodel. Because of the simple structure of Lemna fronds and due to the fact that all parts of the plant are in direct contact with the contaminated water, the kinetics of distribution within the plant need not to be considered. It was assumed that uptake through the plant cuticle is the time limiting process of toxicokinetics, recognising the protection function of this outer cover of the plant. Consequently, a simple one-compartment model, as given in mass balance Eq. (1), was chosen to describe the toxicokinetics.

Equation 1:   Simple one compartment model for toxicokinetics (Schmitt et al. 2013)

$$\frac{d}{dt} M_{int}(t) = \Phi_{in} - \Phi_{out} - k_{met} M_{int}(t)$$

Here, M_{int} = mass of substance in the plant; Φ_{in}, Φ_{out}t = substance fluxes (mass/time) into and out of the plant; k_{met} = metabolic degradation rate, and t = time.

In- and out-fluxes are permeation processes across the membranes covering the plant. In case of Lemna, this will primarily be the leaf cuticle. Such permeation fluxes can be expressed in terms of permeability (P), available surface area (A), and the concentration of the permeate:

Equation 2:   Flux of substance through into and out of the plan (Schmitt et al. 2013)

$$\Phi = A \cdot P \cdot C$$

Inserting Equation 2 into Equation 1 and transforming Equation 1 into an equation for concentration instead of masses by dividing by the volume of the plant compartment leads to the final TK-equation implemented in the model:

Equation 3:   Basic TK equation of the *Lemna* model (Schmitt et al. 2013)

$$\frac{d}{dt} C_{int}(t) = \frac{P \cdot A}{V} \left(C_{ext}(t) - \frac{C_{int}(t)}{K_{p:w}} \right) - k_{met} C_{int}(t)$$

It should be noted that the term for the flux out of the plant compartment is based on the concentration in the internal water $C_{int,wat} = C_{int}/K_{p:w}$ given by the total internal concentration divided by the steady state partition coefficient (=bioconcentration factor) between plant and water $K_{p:w}$. This is because only that substance solved in the water phase is available for permeation.

Notes on and refinements of the original model

Check of consistency of units

Concentrations C_{ext} and C_{int} are given in mass per volume, e.g. µg/L. The permeability P is given in cm/d.

Thus, we get:

$$\mu g \, / \, L \, / \, d \qquad = cm \, / \, d \quad cm^2 \, / \, cm^3 \quad (\mu g/L - \mu g/L) - /d \quad \mu g/L = \mu g/L$$

The area A has to be calculated from the actual biomass using a transformation factor given by Schmitt as 1000 cm² / g dw. The volume of the plants is calculated from the fresh weight, using a factor of 16.7 g fw / g dw (Schmitt et al. 201) and assuming a density of 1 g/cm³. This value of density of 1 g/cm³ is not explicitly stated in the paper of Schmitt. In the R-code of the model provided the supplemental information you can find the statement C_int <- M_int/BM_fresh for calculating the internal concentration from the internal mass of the toxicant. However, to make the units consistent, the right part must include a density [e.g. g/mL]. Otherwise it becomes mass/mass and the concentration would have no dimension. Because no density parameter is used, its value is assumed to be 1 g/mL in the model by Schmitt et al.

$$A \, [cm^2] = BM_{dw} \, [g] * 1000 \, [cm^2/g] \text{ and } V \, [cm^3] = BMdw \, [g] * 16.7 \, [g_{fw}/g_{dw}]*1 \, [g/cm^3]$$

$C_{int}(t)$ indicates the total internal concentration in the plant, while $C_{int,water}$ is the internal unbound concentration, which is indicated later in the paper by Schmitt et al. (2013) and also in this report as $C_{int,unbound}$.

The kinetics of distribution within the plant is not to be considered as it is assumed the pesticide is homogenously distributed within the plant. The reason for this is the simple structure and small size of *Lemna* fronds. Hence, all parts of the plant are in direct contact with the contaminated water.

The partition coefficient between plant and water, $K_{p:w}$, is defined and measured as the concentration in the plant, related to fresh weight, divided by the concentration in the medium:

$$K_{p:w} = \frac{C_{int}}{C_{ext}}$$

However, Schmitt et al. (2013) assume

$C_{int, unbound} = C_{int} \, / \, K_{p:w}$ which is equivalent to $K_{p:w} = C_{int} \, / \, C_{int, unbound}$, but this is not a partition coefficient, because this should be something like $C_{int, bound} \, / \, C_{int, unbound}$. Therefore, it is questionable if $C_{int} \, / \, K_{p:w}$ is really the unbound concentration within the plant.

Schmitt calculates the unbound concentration in plant by dividing the total active substance concentration in plant by $K_{p:w}$.

$$C_{int_{unb}} = \frac{C_{int}}{K_{p:w}} \quad \Leftrightarrow \quad K_{p:w} = \frac{C_{int}}{C_{int_{unb}}}$$

This is an unusual definition of a plant water coefficient. Usually one would use the relation between bound and unbound as partition coefficient or as used in the original paper de Carvalho et al. (2007) the relation between internal and external concentration

$$PCF = \frac{C_{int}}{C_{ext}}.$$

Due to the reason that usually the partition coefficient is non-negative, we need the condition that $K_{p:w} \geq 0.94$.

Furthermore we can verify that if we regard the internal concentration that is defined as internal mass per volume. The plant volume can be calculated multiplying the current fresh biomass value with the plant density (equal to one, density of water is one).

$$C_{int} = \frac{M_{int}}{V} = \frac{M_{int}}{BM_{FW}} \Leftrightarrow M_{int} = C_{int} \cdot BM_{FW}.$$

In our case, we derive the fresh biomass by multiplying the dry biomass value BM to a conversion factor
$$k_{FW} = 16.7.$$

$$BM_{FW} = k_{FW} \cdot BM$$

The conversion factor is the ratio of fresh weight to dry weight, such that we obtain approximately 94 % of water in plant ($\frac{k_{FW}}{k_{FW}+1} = \frac{16.7}{16.7+1} \approx 0.94$) and 6 % of solid in plant. Problems can occur if $K_{p:w} < 0.94$ with the mass balance if we define the plant water coefficient as in Schmitt et al. 2013.

$$M_{int} = M_{int_{unb}} + M_{int_b}$$
$$\Leftrightarrow M_{int} = BM_{FW} \cdot 0.94 \cdot C_{int_{unb}} + M_{int_b}$$
$$\Leftrightarrow M_{int} = BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{P:w}} + M_{int_b}$$
$$\Leftrightarrow M_{int_b} = M_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{P:w}}$$
$$\Leftrightarrow M_{int_b} = BM_{FW} \cdot C_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{P:w}}$$
$$\Leftrightarrow M_{int_b} = BM_{FW} \cdot C_{int} \cdot \left(1 - \frac{0.94}{K_{P:w}}\right)$$

The internal mass has to be, due to physical reasons, greater than or equal to zero. Using the definition of a plant water coefficient given in Schmitt et al. 2013 leads to a contradiction in case that $K_{p:w}$ is smaller than 0.94 - as it is the case for metsulfuron-methyl.

$$M_{int_b} \geq 0$$
$$\Leftrightarrow BM_{FW} \cdot C_{int} \cdot \left(1 - \frac{0.94}{K_{P:w}}\right) \geq 0$$

The internal concentration C_{int} and BM_{FW} are non-negative, such that the inequality is fulfilled if the expression in the brackets is non-negative.

$$1 - \frac{0.94}{K_{P:w}} \geq 0$$
$$\Leftrightarrow -\frac{0.94}{K_{P:w}} \geq -1$$
$$\Leftrightarrow \frac{0.94}{K_{P:w}} \leq 1 \quad \Leftrightarrow 0.94 \leq K_{P:w}$$

In other words; a plant water coefficient smaller than 0.94 leads to a negative internal bounded mass. According to the calculation of a plant water coefficient concerning the herbicide MSM, we trust in the experimental values found in literature (EFSA 2015) concerning a pH of seven. The found log (Kow) values result in lower plant water coefficients (≈ 0.71), such that we have to take a $K_{p:w}$ of 0.94 for MSM.

The internal concentration can also be reduced by metabolic degradation characterised by the rate k_{met}. In the paper by Schmitt et al. (2013) k_{met} was set to zero and thus, the model was simplified by assuming no metabolism. In order to reduce the number of parameters to be calibrated, this is used as a default setting. Only if it is known that an active substance metabolised within the plant with a relevant rate or if without the assumption of metabolism, no acceptable fit to experimental data can be reached, k_{met} is parameterized.

In Equation 3 uptake and elimination are driven by the concentration gradient between external and internal unbound concentration. In addition, it is assumed that the unbound and bound internal concentrations are in equilibrium. In order to allow the simulation of situations where elimination is delayed, a rate k* was introduced into the model (Equation 4). A value of k* of 1 makes the model equivalent to Equation 3. A value of 0 means that there is no elimination at all. By default, k* is set to 1.

The introduction of k* does not affect the calculation of the masses. However, introducing k* is in contradiction to the assumption of an equilibrium at $C_{int} = K_{p:w} \cdot C_{ext}$.

Equation 4:   Modified TK equation including a delay factor for elimination

$$\frac{d}{dt}C_{int}(t) = \frac{P \cdot A}{V}\left(C_{ext}(t) - k^* \cdot \frac{C_{int}(t)}{K_{p:w}}\right) - k_{met}C_{int}(t)$$

For plant protection products with two active substances with the same mode of action no interaction between the two compounds is assumed. Thus, Equation 3 is just applied for each compound with compound specific parameters P, $K_{p:w}$ k* and k_{met}.

2.7.2   Toxikodynamics

For the simulation of toxic effects, the inhibition of photosynthesis rate is directly related to the unbound internal concentration, which is the concentration of the toxicant in the water phase of the plant. If the plant is in equilibrium with its environment, this unbound concentration is equal to that in the external water. The choice of the unbound concentration as the basis for the TD model has the advantage that respective EC$_x$ values are directly comparable to those determined experimentally. In cases of very fast uptake into the plant and establishment of equilibrium early in the testing period, both dose response

relationships based on internal and external concentration are the same. This was not the case if total concentrations were used as internal dose metric, because this is determined by the bioconcentration factor.

For the dose–response relationship, a Hill-function was employed:

Equation 5:   TD equation (Hill function, based on Schmitt et al. (2013)

$$f_{photo}\left(C_{int_{unb}}(t)\right) = 1 - E_{max} \cdot \frac{C_{int_{unb}}(t)^b}{EC50_{int}^b + C_{int_{unb}}(t)^b}$$

where E_{max} is the maximum effect (on the individual), EC_{50int} is the internal concentration causing 50% effect, and b is a shape parameter. The Hill-type relationship was preferred over other functions suitable for dose response relationships because its shape is independent of the EC50 value. Thus, it allows adjustment of the sensitivity in a simulation by changing the EC50 value without altering the shape of the concentration dependence.

Notes on refinements of the original model

E_{max} is usually (also in Schmitt et al. 2013) set to one (100 % inhibition of growth) and not calibrated in order to be able to simulate effects of concentrations above the range of test concentrations used for calibration. In some cases, the fit to the results of a growth inhibition test can be improved if the E_{max} is included in the fit. However, it should be carefully considered if a maximum inhibition < 100 % at high concentration is plausible.

The model describes an inhibition of photosynthesis. However, it can also be used for substances which affect growth rather than photosynthesis directly. The change in biomass is simplified as the difference between production of biomass due to photosynthesis and loss of biomass due to respiration (and dead) (see below).

The model does not differentiate between different types of organic material, e.g. sugars and structural material, within the plant. The parameters of the dose response function in Equation 5 are calibrated using data of growth inhibition tests (e.g. increase of frond numbers, respectively biomass over time) as these are the endpoints in the standard OECD test (OECD test guideline 221). The effect on photosynthesis rate is not measured directly in the usual growth inhibition tests.

Note, that it is assumed that the inhibition is directly dependent on the internal unbound concentrations. Thus, there is no damage and repair considered. In this aspect the model is similar to the reduced GUTS model (Jager & Ashauer 2018), recommended when no information on the internal concentration is given (e.g. from bioaccumulation tests). The internal unbound concentration corresponds to the internal scaled concentration in the GUTS terminology.

2.7.2.1 Modelling of the effects of binary mixtures

The original model by Schmitt et al. (2013) was set up for single active substances. However, some plant protection products contain two or more active substances. In the following, the model refinement to allow simulation of mixtures of two active substances is described.

Plummer & Short (1990) describe a method for identifying and quantifying departures from additivity (i.e., synergism and antagonism) when drugs are given in combination. We used their approach to describe the inhibition of photosynthesis caused by two active substances.

We rewrite the parameters of Equation 5 for simplicity to

Equation 6:   Simplified Hill equation

$$Y = 1 - E_{max} \frac{X^b}{X_{50}^b + X^b}.$$

We set *Emax* = 1 and regard the inverse to get a similar form of dose response-curve as described in Plummer and Short (1990)

Equation 7:   Dose-response function by Plummer and Short (1990)

$$Y = \frac{X^b}{X_{50}^b + X^b}$$

Conversion to logits of this equation leads to the following expression:

Equation 8:   Dose-response function by Plummer and Short (1990) expressed in logits

$$\log\left(\frac{Y}{Y-1}\right) = b \cdot \log(X) + b \cdot \log(X_{50}).$$

Assuming we have two active substances 1 and 2, we get equations describing the *logit* effect of each substance, whereas b_k, $k = 1,2$ are the Hill coefficients concerning substance 1 respectively 2. The parameters C_k, $k = 1,2$ describe the concentration of each substance. The constants $EC50_k$, $k = 1,2$ represent the respective single-agent doses of the two substances eliciting 50% effect.

Equation 9:   Dose-response written for two substances

$$Y_1 = b_1 \cdot \log(C_1) + b_1 \cdot \log(EC50_1)$$
$$Y_2 = b_2 \cdot \log(C_2) + b_2 \cdot \log(EC50_2)$$

To calculate the *logit* effect of the mixture we use equation 6 in (Kong and Lee, 2006).

Equation 10:   Logit effect of binary mixture

$$Y_{Mix} = b_1 \cdot \log(EC50_1) + b_1 \cdot \log\left(C_1 + \rho \cdot C_2 + \kappa\sqrt{C_1 \cdot \rho \cdot C_2}\right)$$
$$\text{with } \rho = \frac{EC50_1}{EC50_2}$$

The quantity *rho* is the relative potency of substance 2 versus substance 1, that implies nothing else but that one unit of substance 2 has the same effect as rho units of substance 1. The term $\sqrt{C_1 \cdot \rho \cdot C_2}$ is the geometric mean of C_1 and $\rho \cdot C_2$. The only parameter we do not know is *Kappa, which* represents the synergy-antagonism parameter:

Kappa < 0 Antagonism,

Kappa = 0 Additivity,

Kappa > 0 Synergism.

It is possible to make the equation more complex to incorporate a possible varying of the relative potency (Plummer and Short, 1990) or to capture local synergy, local additivity, or local antagonism (Kong and Lee, 2006). However, adding more unknown parameters leads to a higher degree of freedom and a higher need of experimental data describing the effect of the mixture. Therefore, we decided to keep the equation describing the *logit effect* of the mixture as simple as possible. To transform the *logit* effect to the effect *E* the following equation is used:

Equation 11:   Transformation of logit effect back to an inhibition factor

$$E = \frac{\exp(Y)}{1 + \exp(Y)} .$$

Before we can use the equation to describe the joint effect concerning on the photosynthesis rate, we have to invert the equation, such that we have

Equation 12: Transformation of effect to an inhibition factor

$$f_{photo}(E) = 1 - E$$

If we want to know the internal concentration in the plants we can read it out of above equation. The total internal concentration is nothing else but

Equation 13:   Total internal concentration for two active substances

$$C_{Mix} = C_1 + \rho \cdot C_2 + \kappa \sqrt{\rho \cdot C_1 \cdot C_2}.$$ The following is an idea how one can extend the logit effect concerning the mixture for more than two substances ($k = 2,...,n$). However, this has not been implemented.

Equation 14:   Logit effect for more than two active substances

$$Y_{Mix} = b_1 \cdot \log(EC50_1) + b_1 \cdot \log\left(C_1 + \sum_{k=2}^{n} \rho_k \cdot C_k + \kappa \cdot \sqrt[n]{C_1 \cdot \prod_{k=21}^{n} \rho_k \cdot C_k}\right)$$

$$\rho_k = \frac{EC50_1}{EC50_k}, \qquad k = 2,\cdots,n$$

2.7.3   Growth model for simulation laboratory growth inhibition tests

The *Lemna* growth inhibition test, according to OECD test guideline 221, is designed to achieve exponential growth of the plants in the controls over the test duration and to measure the inhibition of the growth rate depending on the concentration of the test item (which should be maintained over the test duration of 7 or 14 days). There temperature, light conditions and nutrient concentrations are kept constant at values allowing good growth of the plants as far as practical. This basic test design can be modified to allow determining of recovery after the standard exposure or of effects (and recovery) under shorter, pulse exposures (modified exposure tests, Tier 2A according to EFSA PPR Panel 2013). The first modification usually entails transferring exposed plants (often 12 or 15 fronds) into fresh clean medium at the end of the typical exposure period and following frond count's growth over a 7-14 d recovery period. In the 'pulsed exposure tests', the aim is to test shorter exposures than in the standard test but keep the test duration constant (e.g. 2 days of exposure followed by 5 days in untreated medium,). If several pulses are tested, the test can be prolonged accordingly.

Influences on plant growth, such as irradiation, temperature, nutrient concentrations, as well as density-dependence, are considered for simulating seasonal dynamics of *Lemna* populations in the field by means of the full growth model (see 2.7.4). However, in order to parameterize the TK-TD parameters or to test the TK-TD model by means of data from laboratory tests where environmental conditions are kept constant as far as practical, a simplified growth model was used.

Despite the fact that the intension of a standard *Lemna* test is to achieve constant exponential growth of the controls, nutrient depletion or other density dependent factors may result in growth rates that decrease at the end of the tests, especially in older tests with exposure duration without medium renewal over 14 days. To be able to account for this we use a logistic (sigmoid) growth model of the controls and fit the intrinsic growth rate r and the carrying capacity D_L to the control data in such cases. We used the function for density dependence which is also used in the full *Lemna* growth model by Schmitt et al. (2013, see 2.7.4.4). Exponential growth of the control over the test duration as achieved in most of the more recent tests can be achieved by a very large arbitrary value of D_L.

Thus, the following differential equation with the parameters r and D_L are used to describe growth of *Lemna* biomass BM in laboratory tests. They are fitted to the development in the experimental controls (see section 4.2). f_{photo} describes the inhibition of growth by one active substance according to Equation 5. If two active substances are present, f_{photo} is calculated as described in section 2.7.2.1.

Equation 15:   Differential equation used to model the growth o *Lemna* in laboratory tests depending on the internal unbound concentration of a toxicant

$$\frac{d}{dt} BM(t) = r \cdot \left(1 - \frac{1}{D_L}\right) \cdot f_{photo}\left(C_{int_{unb}}(t)\right) \cdot BM(t), \qquad BM(0) = BM_0$$

2.7.4   Growth model for field populations

The family of Lemnaceae is probably the best investigated group of aquatic macrophytes (Hillman, 1961; Landolt and Kandeler, 1987). Particularly, the growth of different Lemna spp. has been intensively investigated because these species are of interest for different potential applications due to their high growth rate, e.g., water body remediation (Ansari and Khan, 2008; Benjawan, 2008; Cheng, 2002) and

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feed supply for animal breeding. It is generally agreed that the development of Lemna biomass can be described well with a growth rate that depends on environmental factors such as temperature, irradiation, and nutrient supply (Landolt and Kandeler, 1987). Thus, growth of a Lemna population in terms of dry biomass can be simulated using a very simple model given by the following differential equation (Eq. (1)) (Driever et al., 2005).

Equation 16:   Differential equation to describe the growth of *Lemna* sp. depending on variable environmental factors

$$\frac{d}{dt}BM(t) = \left[k_{max} \cdot f_{photo}(\theta) - k_{resp} \cdot f_{resp}(\theta) \right] \cdot BM(t)$$

In this equation, BM = dry biomass, k_{max}= maximum photosynthesis rate, k_{resp} = respiration rate at reference temperature, f_{photo} = factor by which the maximum photosynthesis rate is reduced due to suboptimal conditions, f_{resp} = factor by which the maximum respiration rate is reduced, and θ = {T, I, P, N, D} is the set of environmental parameters potentially influencing the rates k_{max} and k_{resp}. f_{photo} and f_{resp} are scaling factors that depend on environmental parameters, which for the present model are temperature (T), irradiation (I), phosphate concentration (P), and nitrate concentration (N). In addition, f_{photo} is considered to depend on population density (D) (=biomass/area) resulting in a carrying capacity. Overall, fx(θ) is the product of functions depending on single parameters which are described below.

The toxic effect was included as an additional reduction factor. For growth inhibiting substances, the synthesis rate is reduced in relation to the internal concentration of the toxicant. For other modes of action, it might be desirable to increase the respiration rate in order to simulate increased mortality.'

Notes

There is a typo in Equation 1 of Schmitt et al. (2013) with the differential equation for biomass describing the change of biomass where BM is written as superscript after k_{max} while it should be a factor in the first term. In Equation 16 we set brackets [ ] to make clear that the actual biomass is multiplied with the difference of the actual production and respiration rate.

f_{resp} is not a 'factor by which the maximum respiration rate is reduced' because k_{resp} is not a maximum but a reference respiration rate. Thus, fresp can also be larger than 1 (see also 2.7.4.1).

The respiration term includes also losses due to mortality.

In the following, we describe the functions used to model the influence of temperature, light, nutrient concentrations and density dependence on photosynthesis or respiration. Here we just present the equations, the explanation by Schmitt et al. (2013) and additional remarks if needed. Figures of the shapes of these functions together with experimental data used to fit the functions are shown in section 'Data evaluation', section 3.2.

Biomass is decreasing ?	
No	Yes
-	Adapt current internal mass
Calculate internal mass for next time step	
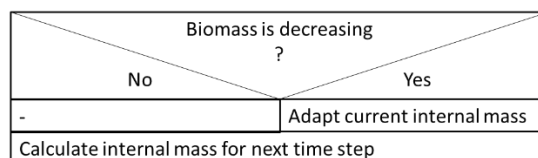
Additional refinement: How to handle shrinking plant cohorts? The model works well if the biomass is increasing. Nevertheless, if the biomass of *Lemna* population is decreasing e.g. due to temperature changes, the internal mass has also to be updated; otherwise the internal concentration is increasing while the biomass is shrinking. However, the internal concentration should stay the same, even then if parts of the plant cohort are dying respectively shrinking.

Hence, we have to add the condition that if the biomass is decreasing the internal mass has to decrease as well with the same factor as the biomass, such that the internal concentration stays the same.

Pseudo- Code: Adapting internal mass in case that the biomass is shrinking

Let $0 = t_0 < t_1 < \cdots < t_n$ be discrete time steps. We assume we have a constant positive step size $h > 0$ and non-negative initials for the dry biomass and internal mass $M_{int}(t_0) = M_{int_0} \geq 0$, and $BM(t_0) = BM_0 > 0$.

For $j = 0, \dots, n-1$

$$t_{j+1} \leftarrow t_j + h$$

$$BM_{j+1} \leftarrow BM_j + h \cdot f_{BM}\left(t_j, M_{int_j}, BM_j\right)$$

If $BM_{j+1} - BM_j < 0$ **then**

$$M_{int_j} \leftarrow M_{int_j} - M_{int_j} \cdot \frac{BM_{j+1} - BM_j}{BM_j}$$

end if

$$M_{int_{j+1}} \leftarrow M_{int_j} + h \cdot f_{M_{int}}\left(t_j, M_{int_j}, BM_j\right)$$

End for

2.7.4.1 Temperature dependence

Experimentally, usually only the net growth rate of Lemna is determined which shows an asymmetric bell shaped temperature dependence (van der Heide et al., 2006; Lasfar et al., 2007). The net growth rate is, however, the difference between photosynthesis rate and the respiration rate which are known to have different temperature dependencies (Criddle et al., 1997).

The photosynthesis rate is assumed to show a maximum at the temperature T$_{opt}$ and its temperature dependence is therefore described by the following asymmetric bell shaped relation composed of two sigmoidal functions:

Equation 17: Temperature dependence of photosynthesis of *Lemna* sp. according to Schmitt et al. (2013)

$$f_{photo}\big(T(t)\big) = \begin{cases} \exp\left(-\ln(10)\cdot\dfrac{(T(t)-T_{opt})^2}{(T_{min}-T_{opt})^2}\right) & \text{if } T(t) \leq T_{opt} \\[3mm] \exp\left(-\ln(10)\cdot\dfrac{(T(t)-T_{opt})^2}{(T_{max}-T_{opt})^2}\right) & \text{if } T(t) > T_{opt} \end{cases}$$

In this function, T_{min} is used when $T < T_{opt}$ and T_{max} when $T > T_{opt}$.

Respiration is a metabolic process and is therefore assumed to depend exponentially on temperature following the Arrhenius law for thermally activated processes. Therefore, the temperature dependence can be expressed by van't Hoff's rule with a Q10 (=relative change caused by 10 ◦C temperature change) of about two (Atkin et al., 2002). A Q10 of the same size is also supported by data on the life span of L. minor fronds that halves with a 10 ◦C temperature increase (Wangermann and Ashby, 1951):

Equation 18: Temperature dependence of respiration of *Lemna* sp. according to Schmitt et al. (2013)

$$f_{resp}\big(T(t)\big) = Q_{10}^{(T-T_{ref})/10}$$

where T_{ref} is the temperature for which an experimentally determined value for k_{ref_resp} is available.

Notes

The function f_{resp} is an exponential function and so, it has its co-domain in the range [0, ∞] and not [0, ∞] as the functions influencing the photosynthesis rate (see below). The reason is that f_{resp} is multiplied with a reference respiration rate at a given temperature and not a maximum respiration rate.

2.7.4.2 Light dependence

In outdoor studies, it has been observed that the photosynthesis rate increases linearly with irradiation up to saturation value I_{sat} and stays constant beyond that (Hodgson, 1970). Respectively, the dependence is described as:

Equation 19: Light dependence of photosynthesis of *Lemna* sp. according to Schmitt et al. (2013)

$$f_{photo}\big(I(t)\big) = \begin{cases} \alpha\cdot I(t) + \beta & \text{if } I(t) \leq I_{sat} \\ 1 & \text{if } I(t) > T_{sat} \end{cases}$$

2.7.4.3 Nutrient dependence

Since Lemna is considered to be useful for remediation of eutrophic water bodies, its potential for uptake of nutrients has been extensively investigated (Cheng et al., 2002; Benjawan et al., 2008) and a large number of publications exist on this topic. However, due to its specific focus, this published information cannot easily be used for the present model since usually only untypically high nitrate and phosphate concentrations have been investigated.

Moreover, the focus has been on the uptake of nutrients rather than on the relationship between growth rate and nutrient concentration, which is of interest here.

The influence of an environmentally relevant range of nutrient concentrations on growth-rate was extensively investigated by Lüönd (1983). For both nitrogen and phosphorus, a steep increase at low concentrations and a subsequent levelling out with a further decrease at very high concentrations was observed. Since the decrease only occurs under conditions that are atypical for natural surface waters, it was disregarded here and the dependence was described by a simple Monod-type relationship:

Equation 20:   Effect on a nutrient on photosynthesis of *Lemna* sp. according to Schmitt et al. (2013)

$$f_{photo}\big(N(t)\big) = \frac{N(t)}{N(t) + N_{50}}$$

where [N] = nitrogen concentration and [N]50 is the concentration at which half the maximum is reached.

The same equation was also used for phosphorus using the respective concentration.

Notes

According to Liebig's law of the minimum, the two f_{photo} for N and P should not be multiplied but only the minimum of both functions should be used to describe the effects of nutrient concentrations on plant growth. For example, if phosphorus inhibits growth by 50 % it doesn't matter if nitrogen would inhibit growth by 20 or 40 %, the resulting inhibition would be 50 %.

Therefore, we used the following equation:

Equation 21:   Effect of nitrogen and phosphorus on photosynthesis of *Lemna* sp. following Liebig's law of minimum

$$f_{photo}(N(t), P(t)) = Min[\frac{N(t)}{N(t) + N_{50,}} \quad , \quad \frac{P(t)}{P(t) + P_{50}}]$$

Note that in the R-code provided as supplemental information to the paper of Schmitt et al. (2013), a slightly different formula is used instead of Equation 20.

Equation 22:   Effect on a nutrient on photosynthesis of *Lemna* sp. according to R-code

$$f_{photo}\big(N(t)\big) = \frac{N(t)^{a_N}}{N(t)^{a_N} + N_{50}{}^{a_N}} \cdot \frac{KiN}{KiN + \text{N(t)}}$$

The calculation of nutrient dependencies is similar for both nitrate and phosphate. Again N is the nitrogen concentration, and N_{50} is the concentration at which half the maximum is reached. The valued of N_{50} respectively P_{50} are the same in the R-code and in Schmitt et al. (2013). The Hill coefficients a_N and a_P are set equal to one. The values of KiN is 604, the value of KiP 101.

The presentation of nutrient response in the R-code is similar to the equation used in Lasfar et al. (2007) to describe the intrinsic growth rate in dependence of nitrogen and phosphate concentration.

In Lasfar et al. (2007) the reason of the additional term (nutrient inhibitions constants) is to take into account any inhibition effect at higher nutrient concentrations. The values of KIN and KIP in Lasfar et al.

2007 are equal to the values given in the R-code. However, the saturation constants in Lasfar et al. 2007 are totally different (N50 = 0.95 mgL-1, P50 = 0.31 mgL-1).

Since the differences of Equation 22 and Equation 20 are small for realistic nutrient concentrations in water, we used the simpler Equation 20.

2.7.4.4 Density dependence

Investigations of Lemna growth rates at different mat densities (=biomass/area) showed a clear dependence (Monette et al., 2006; Driever et al., 2005) and it was suspected that inhibition of growth at high densities occurs due to self-shading of fronds overlapping at the surface. It was shown that the density dependence can mathematically best be described by a linear relationship with a limit density D_L:

Equation 22:   Density dependence of photosynthesis of *Lemna* sp. according to Schmitt et al. (2013)

$$f_{photo}\big(BM(t)\big) = \begin{cases} 1 - \frac{1}{D_L} \cdot BM(t) & \text{if } BM(t) \leq D_L \\ 0 & \text{if } BM(t) > D_L \end{cases}$$

3   Data evaluation

This TRACE element provides supporting information on: The quality and sources of numerical and qualitative data used to parameterize the model, both directly and inversely via calibration, and of the observed patterns that were used to design the overall model structure. This critical evaluation will allow model users to assess the scope and the uncertainty of the data and knowledge on which the model is based.

Summary:

TK-TD parameters are compound specific. In most cases, direct measurements are not available and they have to be estimated from physico-chemical properties of the substance or calibrated using data from laboratory toxicity tests. Here we present the general approach of such an inverse parameterization of the TK-TD model. For the general growth model we use per default the parameters provided by Schmitt et al. (2013), which are based on a literature review and some general assumptions (direct parameterization).

3.1   TK-TD parameters

The TK-TD parameters are compound and species specific and usually not available from direct measurements. In such a case, they have to be estimated from physico-chemical properties of the substance or they are calibrated using results of growth inhibition tests ('inverse parameterization').

These growth inhibition tests are often conducted in the context of regulatory risk assessment following requirements of Good Laboratory Practise (GLP). Thus, the quality of the documentation of the test conditions and the results is usually high. Note that data from tests which also include a recovery period are more suitable for calibration of the TK-TD parameters than data sets without such an elimination phase. A standard test following OECD test guideline 221 can be supplemented with a recovery period by transferring plants after the end of the standard exposure period into fresh uncontaminated medium and measuring their growth.

The calibration of TK-TD parameters comprises the following steps:

1.   Estimating parameters from physico-chemical properties of the substance

2.   Preparation of the experimental data from growth inhibition tests

3.   Calibrating the growth parameters

4.   Calibrating the TK-TD parameters to data of one or more tests

5.   Calculating confidence intervals for the fitted parameters

An example of this approach to parameterize the TK-TD model is given in section 6.1 using data for Metsulfuron-methyl (MSM).

3.1.1   Estimating TK-parameters from physico-chemical properties

Carvalho et al. (2007) measured plant-water partition coefficient $K_{p:w}$ for *Lemna* for a set of substances with different log K_{ow} values and calculated the following regression model. Note that a log K_{ow} below 1 has only very small effects on the $K_{p:w}$ estimated by Equation 23 (see Figure 3).

Equation 23:   Regression of $K_{p:w}$ from Log K_{ow} by Carvalho et at. (2007)

$$\log(K_{p:w} - 0.71) = 0.73 \cdot \log Kow - 1.37$$

$$\Leftrightarrow K_{p:w} = 10^{0.73 \cdot logKow - 1.37} + 0.71$$
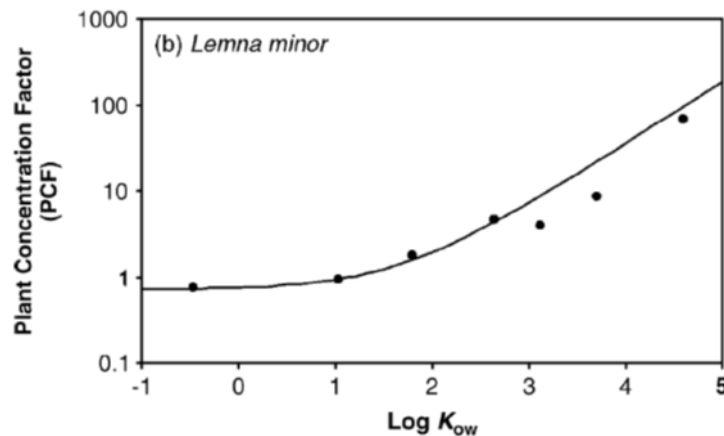
Figure 3:   Water:Plant partition coefficient $K_{p:w}$ as a function of log Kow (copied from Carvallho et al 2013) according to the regression in Equation 23

In order to reduce the number of parameters to be calibrated using data from growth inhibition tests (see below), $K_{p:w}$ can first be calculated by this regression and calibrated only if no satisfying fit can be achieved. Schmitt et al. (2013) have also done this for metsulfuron-methyl.

Data on permeability *P* of a substance via the plant cuticle are available for *Myriophyllum spicatum* and a set of 16 substances (Figure 4, Heine et al. 2015). Because we are interested here in the permeability for *Lemna*, we suggest to include this parameter in the calibration procedure (see below) but to use the fitted exponential relation between log K_{ow} and *P* (Figure 4) to get a starting value for the calibration of *P*. A reliable range of permeability for the calibration is considered to be 0.0001 – 10 cm / d.

Equation 24:   Regression of P [cm/s] from Log K$_{ow}$ [-] based on data of Heine et al. (2015) for Myriophyllum spicatum

$$P = 0.0061 \cdot e^{0.7492 \log Kow}$$
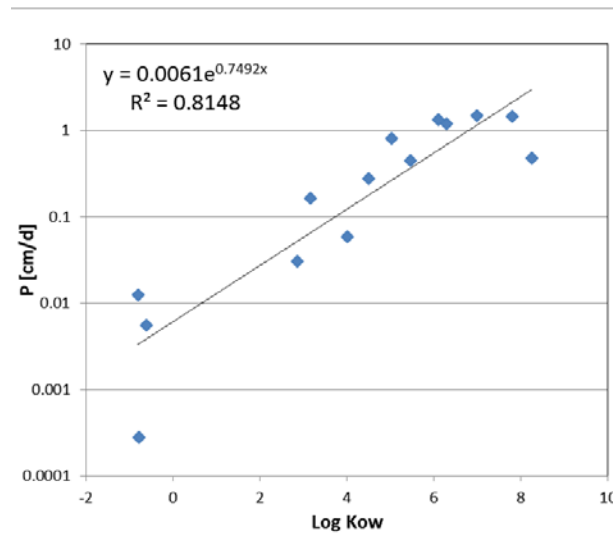
Figure 4:   Permeability *P* as a function of Log K_{ow} using data of Heine et al. (2015) for *Myriophyllum spicatum*

3.1.2 Preparation of experimental data sets for calibration

Given that the repeated count data from *Lemna* growth inhibition tests are given as frond numbers while biomass is usually only measured at the beginning and end of a test, the calibration and validation of the model is done using the frond number and not the biomass data. Therefore, the predicted biomass BM(t) (as dry weight) is converted into frond numbers by dividing the biomass by a fixed mean frond weight of 0.1 mg dw/frond for *L. gibba (*reported by Schmitt et al. (2013) for all comparisons of observations and model predictions. Thus, it is assumed that the toxicant or other factors have no effect on the size respectively biomass per frond. Because we use a constant factor, its exact value is not relevant for the determination of goodness of fit. However, another value than the default by Schmitt et al. (2013) can also be used.

By default, the means of the experimental frond numbers per treatment level and observation day are used to calculate the goodness of fit of the model (see examples later).

In prolonged refined exposure tests, a small number of fronds (usually 12 or 15) per vessel are put once a week (in some cases biweekly) into fresh medium to keep the plants in the exponential growth phase and to avoid a density dependent decline of growth rate. By doing this, experiments can be conducted over several weeks if needed. In the model, the water surface area can be virtually unlimited and so, exponential growth can be simulated over several weeks. For the calibration and validation examples of the model in this report, we decided not to simulate the 'reset' of the populations done in some tests but to extrapolate the data to continuous growth using the observed growth rates after the 'reset'. The following figure gives a simplified example. This was done to be consistent with the use of the model by Schmitt et al. (2013). However, the implementation of the model is also able to simulate experimental 'resets' of frond numbers if the data sets are provided in this way.

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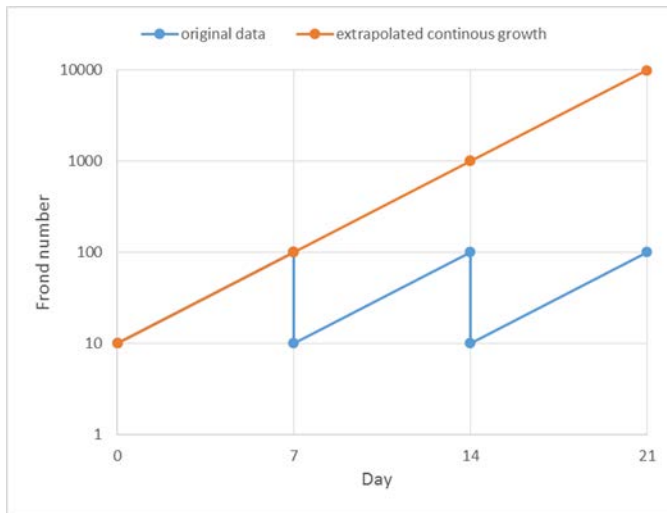
Day	Original frond numbers	Extrapolated frond numbers
0	10	10
7	100	100
7	10	100
14	100	1000
14	10	1000
21	100	10000

Figure 5:   Example data for extrapolated continuous growth for an experiment where each week 10 fronds were transferred into vessels with fresh medium

3.1.3   Calibrating the growth parameters

As outlined in section 2.7.3, a simple exponential or logistic growth model is used for simulation of laboratory tests with constant temperature and light conditions and a surplus of nutrients. The two growth parameters of Equation 15, *r* and D_L, are fitted to the observed mean dynamics of the controls for each specific test. Thus, differences between tests with respect to laboratory conditions and plant quality are considered by the calibration of the control growth rate <u>before</u> the TK-TD parameters are calibrated based on the growth of the exposed plants. By doing so, the control growth is fitted as good as possible and the calibration and testing of the TK-TD model is not affected by a bad prediction of the control growth.

If the growth of the control is exponential (which should be usually the case), *r* can be obtained by log linear regression of the mean frond number of the controls over time (in Microsoft Excel) and DL can be set to an arbitrary high value so that density dependence is not relevant. When control growth in the test is apparently not exponential, non-linear fitting with a Downhill Simplex algorithm (Nelder & Mead 1965) is used to minimize the residual sum of squares (within the program for the *Lemna* model).

3.1.4   Calibrating the TK-TD parameters

After the modelled growth of the control is adjusted to the growth observed in a specific test, the TK-TD parameters are fitted by minimizing the sum of squared residuals between experimental and predicted (frond number) data in one or more tests. Therefore the residual sum of squares or the χ^2 (to down-weight deviations at high abundances) between observed and modelled frond numbers of all dates, treatment levels and, if more than one test was considered for the calibrations, tests) are minimized. For this the Downhill Simplex method (Nelder-Mead-Algorithm) provided by the NelderMeadSolver Class routine of the Microsoft Solver Foundation package in Visual Basic .NET Framework (Version 4.7.02558) is used.

For the calibration procedure it must be specified which parameter should be calibrated, which starting values should be used, and in which ranges the algorithm should search for the optimum parameter values.

We suggest to set the metabolism and elimination delay rates, k_{met} and $k*$, to 0 and 1, respectively, to keep the TK model simple. Only if no satisfying fit is achieved, these parameters should be included in the calibration. However, it should be kept in mind to avoid 'overfitting', i.e. fitting a model with many parameters to a small experimental data set.

The potential to reach 100 % inhibition of growth at high concentrations of a toxicant is considered a reliable and protective assumption. Thus, the maximum effect E_{max} is by default set to 1 and not calibrated. However, it can be selected to calibrate E_{max}, too.

The start values for calibration of EC_{50} and slope b of the concentration response based on the internal unbound concentration can be estimated from a growth inhibition test under the assumption that after exposure over 7 or even 14 days, external and internal concentrations are in equilibrium. The experimental EC50 and slope can be calculated using the observed inhibition of growth rate over e.g. 7 days and then using e.g. the Solver function in Excel to minimize the residual sum of squares for the Hill equation (see Equation 6 with E_{max} set to 1). To keep the fitted parameter in a realistic range, we suggest setting the lower and upper limit for the calibration to 0.1 and 10 times the values derived for the experimental data.

If plant protection products with two active substances have to be modelled, first the TK-TD models for each substance are parameterized and tested separately. Then, it should be tried to model the effect of the product with the default assumption of concentration addition (kappa = 0). If concentration addition is not able to predict the observations reasonable well, kappa should be calibrated to observations in tests with the product without changing the TK-TD parameters for the single substances.

3.1.5   Calculation of confidence intervals for the calibrated parameters

We use the likelihood function to generate confidence intervals for the calibrated parameter values. For the background, see Jager (2016). The basic principle is to start with the calibrated values of one parameter and increase successively the interval around. The interval is defined by all parameter values that are not rejected in the ratio test (Likelihood-ratio criterion: X^2, df = 1, α = 0.05). We use a degree of freedom of one, because one parameter is fixed, and α = 0.05 to calculate the 95% confidence interval. This yields to a critical X^2 value of approximately 3.841433.

Two options are implemented:

a. Calculation of asymptotic standard errors. If the values for the specific parameter are varied, the other values are kept fixed. This approach provides always symmetric confidence intervals.

b. Profiling the likelihood. This approach is more robust and more suited for smaller data sets (Jager 2016). Here, for each modified parameter value, the other parameter values are calibrated before the likelihood ratio test is conducted. In each simulation step an optimization (calibration of the non-fix parameters) is done, this costs time. Therefore, this approach needs much more calculations then the asymptotic standard error approach.

Pseudo-code: Profiling the likelihood

Assume we have calibrated $m \in \mathbb{N}$ different parameters. Each parameter has a given possible range $p_k \in [a_k, b_k], k = 1, \cdots, m,$ whereas $a, b \in \mathbb{R}$ with $a < b$. Concerning the calculation of likelihood a normal distribution is assumed (normal log likelihood function).

1. We set one parameter fix: Let's say $p_k \in [a_k, b_k]$. Assuming we want to perform $N \in \mathbb{N}$ simulations, we have a step size of $h = \frac{1}{N-1}$. In each simulation run, the fix parameter gets an updated value (equidistant decomposition of the interval).

2. In every simulation maximizing the log likelihood respectively minimizing the minus log likelihood concerning all other parameters $p_j, j = 1, \cdots, k-1, k+1, \cdots, m,$ expect of the fix one is performed.

3. Calculate the approximate χ^2- distribution value of each run

   a. Finding the best objective function value (Likelihood) of all simulations ℓ^*

   b. The approximate distribution can be calculated as $\widetilde{\chi^2} \approx 2 \cdot (\ell(i) - \ell^*)$ for each simulation $i = 1, \cdots, n$.

4. Calculate the bounds of the confidence interval of the fixed parameter p_k : The interval is defined as all those values of the parameter that are not rejected in a likelihood-ratio test at confidence level α.

The calculation of the asymptotic standard error is much faster, because in every simulation step the log likelihood function to do the likelihood-ratio test is calculated but not calibrated as by profiling the likelihood.

However, the calculation is approximate and the confidence interval does not reflect the probability that the observed interval contains the true value of the parameter.

It is possible to calculate also a joint confidence region of all fitted parameters. Therefore, the likelihood-ratio has to be modified. Instead of a degree of freedom of one, we have a $df = m$: The number of calibrated parameters defines the critical X² distribution value: Assuming we have two parameters we get $\chi^2 \approx 5.991488$, in case of three parameters X²≈ 7.814714 at confidence level α=0.05 and so on.

The borders of the joint confidence region are defined of those values of the parameters not rejected in the likelihood-ratio test.

More information can be found in the refresher document by Tjalling Jager available online at http://www.debtox.info/downloads/coursemat/refresher.pdf or Moerbeek et al (2004), Meeker and Escobar (1995) and Pawitan (2001).

3.2   Growth model for simulating field populations

We use the parameters reported by Schmitt et al. (2013) as the default parameter set for the growth model to simulate field populations (Table 3): *'The parameters of the growth model were defined by evaluating data taken from different literature sources. If data were available in tables, these were used directly. In cases where only graphs were found in the publications, the data were derived from those by digitising the graphs, using software developed in-house.*

All of the parameters are species dependent. Since most literature information is found for L. minor, it was decided to compile a consistent parameter set for this species. Thus, wherever possible, data for L. minor were chosen. In the remaining cases, data from an alternative Lemna species were used. However, when selecting datasets from the literature, priority was first given to suitability and reliability of the data and second to the species.

Values of the parameters of the model … were taken from the publications if explicitly reported. In other cases they were derived by fitting the equations to respective data using nonlinear optimisation methods. Details can be found in the supporting information provided with this publication.'

The default parameter set of Schmitt et al. (2013) is given in Table 2. The selection of the parameter values and the shapes of the functions to describe effects of temperature, irradiance, nutrient concentrations and density dependence are discussed in the following section.

3.2.1   Maximum photosynthesis rate and reference respiration rate

In laboratory growth inhibition tests the conditions are optimized (as far as practical) for the growth of *Lemna*. The validity criterion for the OECD 221 growth inhibition test with *Lemna* sp. is that the control should show an average growth rate of at least 0.275 /d. However, in tests the growth of the control is often higher and can reach values close to or even above 0.4 /d. For example, see Figure 16 where a growth rate of 0.39 / d was found. Therefore, a maximum photosynthesis rate of 0.42 /d and a reference respiration rate of 0.05 /d, which result in a maximum growth rate of approximately 0.37 seems to be reasonable settings. Peeters et al. (2013) used very similar maximum rate of 0.4 /d for their model of duckweed population dynamics. They also used a loss rate of 0.05/d. However, this loss rate is assumed to be constant, and not temperature dependent as in the model by Schmitt et al. (2013).

3.2.2   Temperature dependencies

Regarding the temperature dependence of photosynthesis and respiration Schmitt et al. (2013) written in the supplemental information (Numbers of figures and tables were updated to refer to this TRACE document):

Table 2:   Parameters of the growth model including the values used by Schmitt et a. (2013).

Parameter	Value	Unit	Description	Reference
Equation 16				
r_0	0.42	1/d	Maximum photosynthesis rate	Lasfar (2007)
k_{ref_resp}	0.05	1/d	Respiration rate at reference temperature	Claus (1972)
Equation 17				
T_{min}	8.0	°C	Minimumgrowth temperature	Lasfar (2007)
T_{max}	40.5	°C	Maximum growth temperature	Lasfar (2007)
T_{opt}	26.7	°C	Optimum growth temperature	Lasfar (2007)
Equation 18				
T_{ref}	25	°C	Reference temperature for respiration rate	Claus (1972)
Q_{10}	2		Q10 for respiration rate	Wangermann & Ashby (1951)
Equation 19				
I_{sat}	15000	kJ/(m² d)	Saturating global radiation	Hodgson (1970)
α	5E-5	1/(kJ/m² d)	Slope of radiation dependence	Hodgson (1970)
β	0.25	-	Intercept of radiation dependence[1]	Hodgson (1970)
Equation 21				
P_{50}	0.0043	mg/L	P-conc. where growth rate is halfened [1]	Lüönd (1983)
N_{50}	0.034	mg/L	N-conc. where growth rate is halfened	Lüönd (1983)
Equation 22				
D_L	176	g_dw/m²	Limit density	Monette (2006)
Additional scaling factors, not explicitly occurring in the equations				
A_/_DW	1000	cm²/g dw.	Frond area / dry weight	Landolt (& Kandeler 1987), (40 mm²/frond estimated from photograph)
DW_/_frond	0.1	mg_dw./frond	Dry weight / frond	Determined for *L. gibba* (Schmitt et al. 2013)
FW_/_DW	16.7	g fw/g_dw.	Fresh weight/ dry weight	Determined for *L. gibba* (Schmitt et al. 2013)

[1] There is a typo in Table 1 of Schmitt et al. (2013) where β is called the slope and α the intercept. In addition, the intercept has to be dimensionless.

The temperature dependence of the net growth rate of Lemna populations has been investigated for various species in several early publications (Hillman, 1961; Landolt, 1987). It was consistently found that maximum growth occurs roughly at temperatures between 25 °C and 30 °C. The minimum temperature at which growth is observed varies somewhat more between different species and lies between 4 °C and 18 °C. Detailed data about growth rates of L. minor at different temperatures are given by van de Heide (2006) and Lasfar (2007). In both cases a very similar characteristic of the temperature dependence was found. Because the data set of Lasfar (2007) contains more data points it was chosen here as basis for deriving an equation for calculating $k_{photo}(T)$.

The observed rates are net growth rates and need therefore to be separated into the photosynthesis rate and the respiration rate. For this purpose the respiration rate has been estimated as the inverse of the typical life span of Lemna fronds that is about 20 days at temperatures around 25 °C with little variation between species (Claus, 1972), but also longer life spans have been observed (Landoldt, 1987; Wangermann, 1950). A respiration rate of 0.05 d-1 at 25 °C is assumed, which was scaled to temperature using Equation 18 and subtracted from the measured growth rates. The remaining growth rates are considered the true photosynthesis rates and Equation 17 was fitted to the experimental values. The result is presented in Figure 6, and the resulting parameter values are listed in

Table 2.
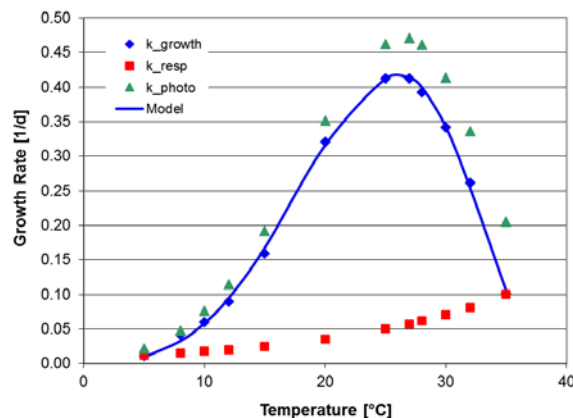
Figure 6:   Dependence of observed (◆) and calculated (line) net growth rate k_{growth} of *L. minor* in dependence of temperature. Symbols (■) show the respiration rates k_{resp} calculated with Equation 18 and (▲) the photosynthesis rates $k_{photo} = k_{growth} + k_{resp}$. Equation 17 was fitted to k_{photo} (copied from Schmitt et al. 2013, supplemental data)

Notes

The fit for k_{growth} looks reasonable and is based on a large number of data points. The respiration rate was estimated from the average life-time. This makes sense because the respiration rate includes also mortality in the model and the estimated values results in zero biomass when the photosynthesis is set to zero over 20 days. The modelling of the respiration rate based on van't Hoff's rule with a Q10 of 2 is a plausible approach to consider the increased metabolism costs with increasing temperature.

In Figure 6 k_{photo} reaches values of approximately 0.46 /d while the maximum photosynthesis rate reported in the list of parameters is 0.42 /d (see Table 3). We also use this value of 0.42 /d as default because it is the more protective of the values for predictions of recovery (of abundance).

Peeters et al. (2013) used similar minimum and optimum temperature (i.e. 8 and 26 °C, respectively) but a lower maximum temperature of 32.5 °C compared to the 40 °C used by Schmitt et al. (2013). They also assumed a linear relation of the growth rate from temperature below and above the optimum temperature which results in a different pattern as in the model by Schmitt et al. (2013), especially at temperature below 10 and above 26 °C.
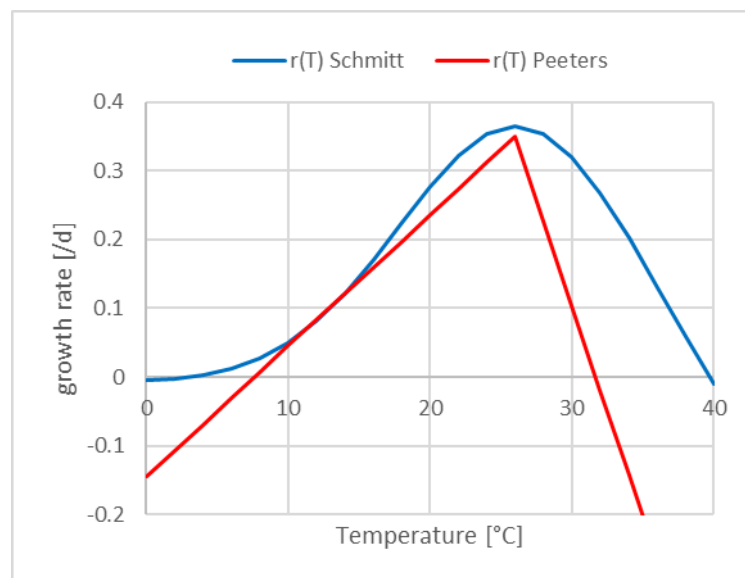
Figure 7:   Comparision of the effect of temperature on the photosynthesis rate in the models of Schmitt et al. (2013) and Peeters et al. (2013). Note that for this comparison not inhibition due to light, nutrients nor density dependence was considered.

3.2.3   Light dependence

In the supplemental data Schmitt et al. (2013) write (numbers of figures and equations were updated for this document):

The influence of irradiation on Lemna growth has been investigated in laboratory (Landoldt 1987; Lasfar 2007; Anderson, 2005) and outdoor (Hodgson, 1969) studies. The laboratory studies show that the relative growth rate increases with radiation intensity (Landoldt, 1987) and length of photo period (Lasfar, 2007) up to a maximum and declines at even stronger or longer irradiation, probably due to photo respiration. Also an influence of the wavelength of the irradiating light is given (Anderson, 2005). Although this information is comprehensive and very detailed, it can hardly be used for deriving parameters for a model suitable to simulate behavior under daylight conditions due to complications in translating intensities between light sources with different spectra. Therefore only the data of Hodgson (1969) were considered, which relate the growth rate of L. minor directly to global irradiation. These data show a linear relationship

between radiation and growth rate up to a saturation value from which on the rate remains constant at the maximum level up to the highest radiation values observed in the study (Figure 8). Fitting Equation 19 to the data yields the parameters listed in Table 1.
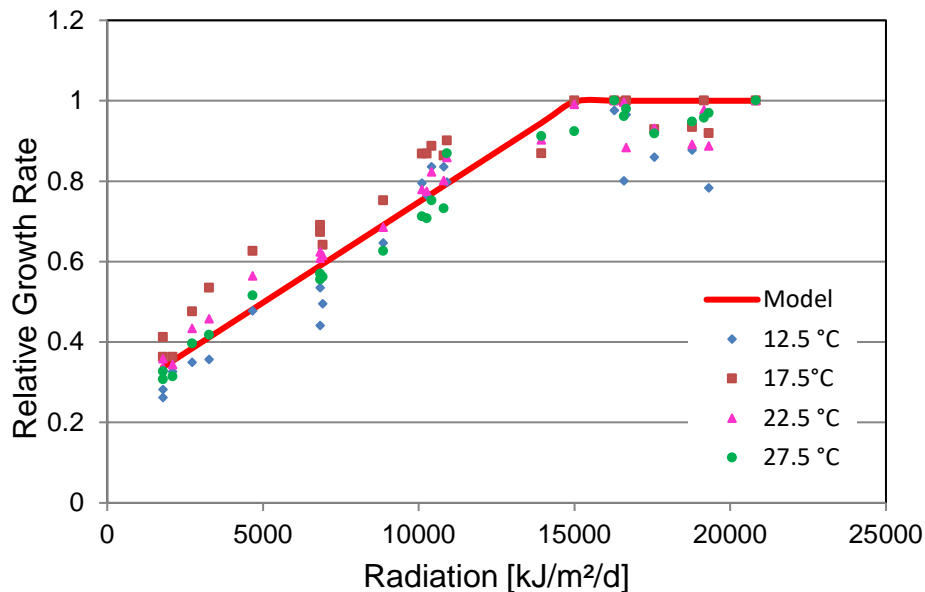
Figure 8:   Growth rates of *L. minor* observed at different temperatures under natural daylight. The line shows the dependence on global radiation evaluated by fitting Eq. 4 to the data (copied from Schmitt et al. 2013, suppl. data).

Notes

Weather data in the FOCUS models also include irradiance as global radiation per day (FOCUS 2001). Thus, it makes much sense to use a function for light dependence including this measure as argument. The effect of temperature on this relationship seems to be relatively small and thus, it is reasonable to ignore it for simplicity. Despite that, light dependence can be affected also by other factors (e.g. the wavelength) this simple modelling approach can be considered sufficient to consider scenario specific and time variable light conditions into time variable photosynthesis rates for modelling field populations.

The model does not consider an inhibition of photosynthesis at very high light intensities, i.e. > 20 000 kJ/m²/d. Thus, the model might overestimate growth under such conditions. However, it has to be considered case by case how often such light conditions occur in simulated exposure scenarios.

Peeters et al. (2013) describe the dependence of growth from light also as a linear function up to a saturation value given a 450 µmol /m²/s. According to Sattin et al. (1997) this corresponds to 237 J/m²/s or 13642 kJ/m²/d under the assumption of 16 h of light on a summer day. Thus, this values is similar to the one used by Schmitt et al. (2013).

3.2.4   Nutrients dependence

In the supplemental data, Schmitt et al. (2013) write on the parameterization of the dependence of photosynthesis from nutrient concentrations (numbers of equations, figures and tables were updates for this document):

The influence of nutritional conditions on growth of Lemna has extensively been investigated under the aspect of remediation of eutrophicated water bodies and biomass production (Cheng, 2002; Pharlin, 1987; Ansari 2008; Benjawan, 2008). Due to the nature of the questions underlying these investigations the focus was on high levels of nitrogen and phosphorus while water bodies adjacent to agricultural fields, which are of interest for pesticide risk assessments, today will typically have low to medium concentrations of nutrients (Jarvie, 2003; Neal, 2006; Garnier, 2005). Moreover it is difficult to derive exact quantitative information on the relation between growth rate and nutritional conditions from the results because usually nutrient concentrations change during the study. Furthermore only nutrient uptake rates are reported and not growth rates.

In some studies, however, relative growth rates have been determined under controlled nutritional conditions (Ericsson, 1981; Lüönd, 1983; Cedergreen, 2002; Lasfar, 2007). Growth of Lemna may either be limited by the rate of nutrient supply if the biomass is large enough to deplete the water from nutrients by further growing (Ericsson, 1981) or at very low nutrient concentrations the relative growth rate gets concentration dependent (Lüönd, 1983; Cedergreen, 2002; Lasfar, 2007).

The most comprehensive dataset is found in Lüönd (1983), where four different species have been investigated. To derive a quantitative relationship between growth rate and nitrogen and phosphorus concentrations for L. minor Equation 20 was fitted to the respective data of Lüönd (1983). The results are shown in Figure 9 and Figure 10 where curves calculated with the fitted equation are shown in comparison to the observed data. Optimized values of the parameters are given in Table 2.
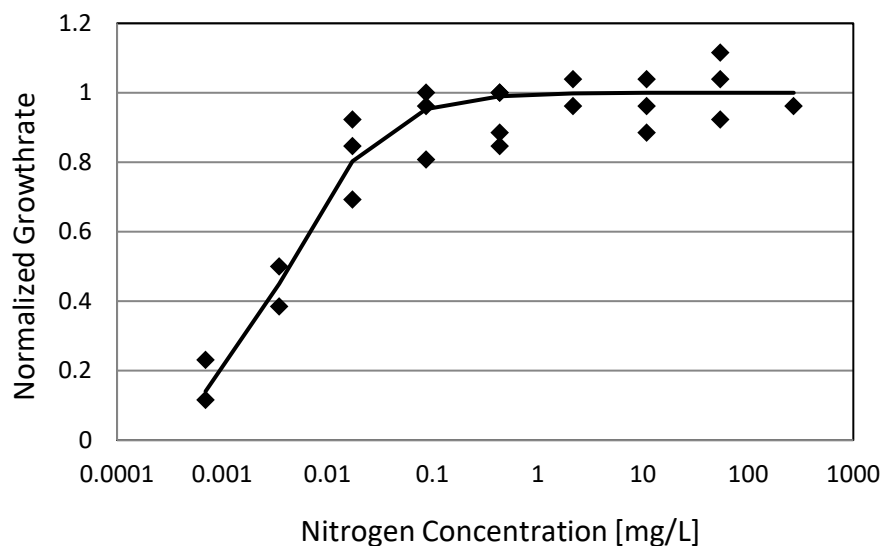
Figure 9:   Growth rates of *L. minor* in dependence of nitrogen concentration. The line represents Equation 20 fitted to the data (copied from Schmitt et al. 2013, suppl. data).
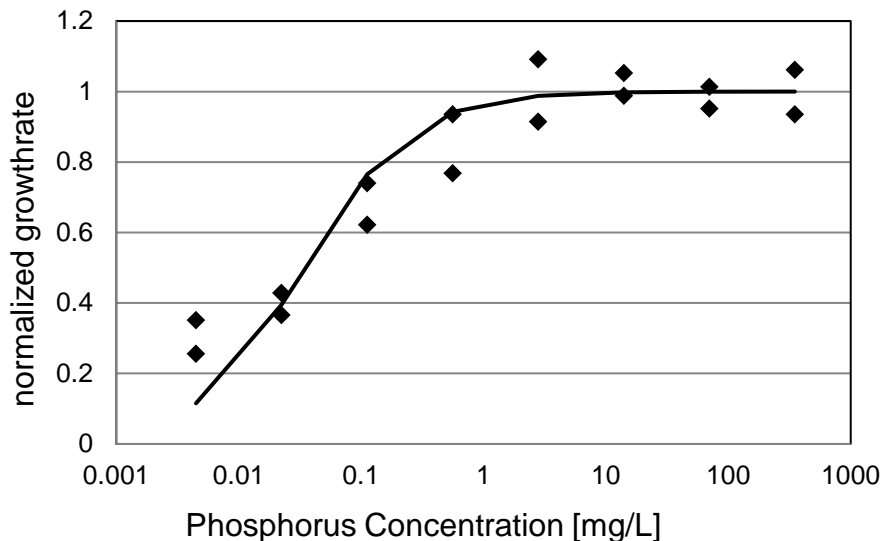
Figure 10:   Growth rates of *L. minor* in dependence of phosphorus concentration. The line represents Equation 20 fitted to the data (copied from Schmitt et al. 2013, suppl. data).

Notes

The function used is a typical function to describe the effect of a resource on a process (e.g. in enzyme kinetics (Michaelis-Menten-Kinetics). The data sets used to calibrate the function for N and P included a sufficient number of data.

In contrast to Schmitt et al. (2013) we do not use the product of both functions but the minimum (see Equation 21) following Liebig's law of minimum which is considered the more realistic approach.

Coors et al. (2006) write that 'according to Landolt and Kandeler (1987), *L. minor* requires as minimum 0.07 mg N/L and 0.0034 mg P/L to achieve half the maximum growth rate'. The values for N is twice as high as the one used by Schmitt et al. (2013) (0.034 mg N/L) while the value for P is similar (0043 mg P/L). Peeters et al. (2013) used half saturation constants of 0.05 mg P/ L and 0.04 mg N / L. based on Lüönd (1980). Thus, they assumed a much higher P demand of *Lemna.* We kept the values used by Schmitt et al. (2013) because they were based on a newer paper of the same author Lüönd (2013).

3.2.5   Density dependence

Schmitt et al. (2013) assume a linear density dependence for the photosynthesis rate up to the density limit (Equation 22). Above the density limit, $f_{photo}(BM),$ and so also the photosynthesis rate, is set to zero (Figure 11, left).

The value of 176 g/m² for the density limit D_L is taken from Monette (2006) for *L. minor.* In Peeters et al (2013), a similar value is given (180 g/m² dry weight). If the photosynthesis rate and the respiration rate are kept constant, this results in a classical logistic population growth (dN/dt = r N(1-N/K).
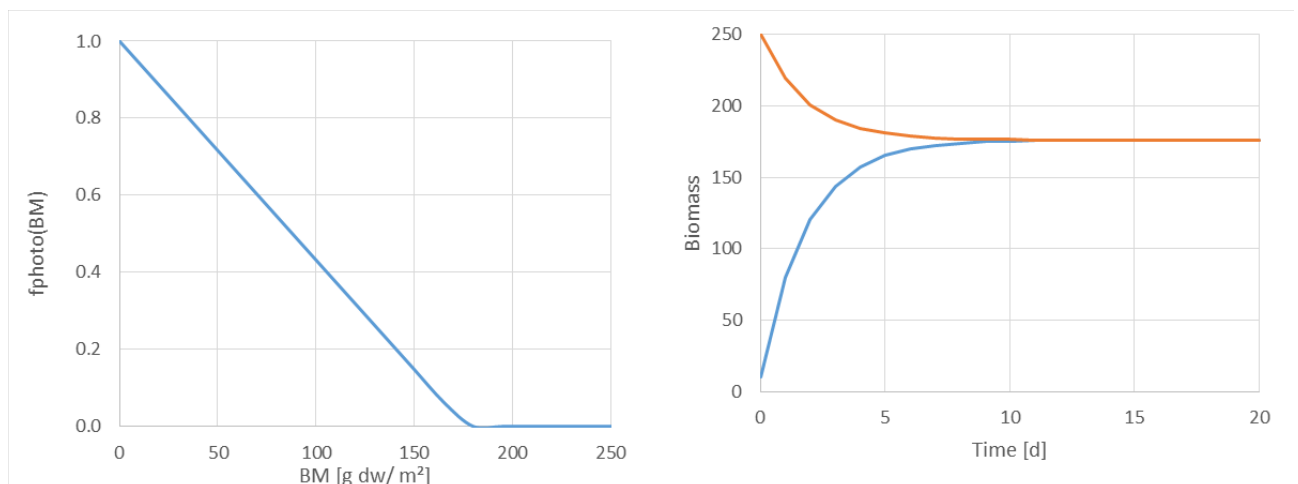
Figure 11:   Densitiy dependence of *Lemna* sp. with the density limit DL set to 176 g dw/m² (left) and resulting growth for a growth rate of 0.37 /d for two start densities and k_{photo} = 0.42 /d and k_{resp} = 0.05 /d (right).

4   Conceptual model evaluation

This TRACE element provides supporting information on: The simplifying assumptions underlying a model's design, both with regard to empirical knowledge and general, basic principles. This critical evaluation allows model users to understand that model design was not ad hoc but based on carefully scrutinized considerations.

Summary:

The TK-TD model is kept simple to allow an inverse parameterization by means of typically available growth inhibition tests with *Lemna*. Nevertheless, this should allow modelling the effects of many growth inhibitors sufficiently well. In other cases, the model can be made more complex by using additional parameters '(e.g. the metabolisation rate) refinements of the TK-TD model based on information on the specific compound. The design of the growth model reflects data availability and is in line with general principles and theory in the field of macrophytes ecology. As a population model, the *Lemna* model does not explicitly consider interactions with other species (e.g. grazing, completion) or environmental factors (e.g. nutrients). However, by changing the growth parameter values or the input data for the environmental variables, effects of such interactions can be approximated.

4.1   The TK-TD model

The TK-TD model is a single compartment model assuming homogenous distribution of the toxicants within the plant (Figure 12). Considering the relatively simple structure of *Lemna* (compared to other macrophytes with roots, rhizome, stems, and leaves) and its small size this seems justified. In order to reduce the number of parameters to be calibrated the toxicokinetic in its most simple form is characterized by only two parameters, the permeability P through the cuticle as the probably most relevant transport step, and the partition coefficient between plant and water $K_{p:w}$ to estimate the internal unbound concentration which can result in the inhibition of growth. The toxicodynamic part is simplified to just relating the effect to the internal unbound concentration via a dose-response relation. So, damage and repair processes are not explicitly modelled; growth reacts directly to the internal concentration.
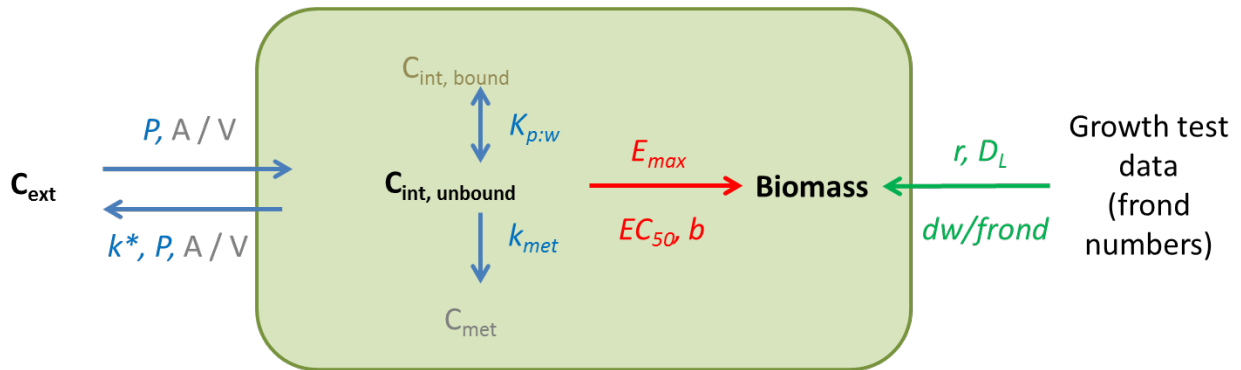
Figure 12:   Conceptual diagram of the TK-TD model for *Lemna* and its relation to growth inhibition tests.

Note that k_{met} and k* are set to neutral values (0 and 1) in the most simple form of the TK-TD model.

The introduction of a metabolic degradation rate $k_{met} > 0$ allows considering situations where effects are smaller or recovery is faster than to be expected from only the elimination of the substance.

The modelled TK-TD is not dependent of temperature because no data are available to parameterize such a relationship. It can be assumed that permeability increases with higher temperature. Because laboratory tests are conducted usually at relatively high temperature (24 ± 2 °C according to OECD test guideline 212), the uptake (but also the elimination) in the field can be expected to be lower in most cases (at least for scenarios representing Central or North Europe). The effects of exposure to two active substances has been implemented by the introduction of a single additional parameter *kappa*, which determines the degree of syngergism, antogonism or just concentration addition. The mathematics are based on a peer-reviewed paper on the combination effect of drugs. It might be possible that for specific compounds, the implemented TK-TD model is not appropriate and the results of laboratory tests cannot be modelled sufficiently well. In such a case, refinements of the TK-TD model should be considered.

The model simulates inhibition of photosynthesis and photosynthesis is used directly to model the production of biomass. The TK-TD parameters are calibrated by means of ecotoxicological tests where growth and not photosynthesis is measured. Thus, the model is applicable to modes of actions related to growth inhibition in general, not only inhibition of photosynthesis. Schmitt et al. (2013) also applied the model for a sulfuron herbicide, which inhibits cell division.

4.2   The growth model for simulating field populations

The growth model is based on general ecological principles with the intension to keep the model as simple as possible but to be able to simulate typical population dynamics resulting from the seasonal variation of temperature, light conditions, nutrients and density dependence (Figure 13). The model concept is very

similar to the one developed independently by Peeters et al. (2013) to model the effects of global warming on floating plants.

The change of biomass is described by a production (via photosynthesis) and a loss term (respiration including biomass losses to mortality). Changes of biomass due to immigration (e.g. transport by water fowl or via flowing water from upstream), emigration (here also passively by transport), grazing, parasitism, illness, or harvesting are not included. The ignorance of immigration is conservative with respect to recovery. As a population model, the *Lemna* model does not explicitly consider grazing, competition, parasitism etc. Therefore, the model would have to be extended to a community or ecosystem model, which would need much more effort for parameterization. However, effect of competition can for example be simulated by assuming low nutrient concentrations, losses by e.g. grazing can be simulated by increasing the reference respiration rate or directly reducing the biomass. Thus, by changing model parameters, the effects of interaction with other species can be approximated.

Temperature, light, nutrients and density dependence are modelled using general principles such as van Hoff's rule, Liebig's law of minimum, and the usual assumption of linear density dependence. These submodels were parametrized by experimental data (see 3.2).

Photosynthesis was described as a function of the global daily radiation, expressed in KJ/m²/d. So, the temporal scale for this sub-model is one day and photoperiod and daily variation of light is not considered. This is acceptable with respect to much longer time scale (e.g. one year) for which the model should predict the biomass dynamics. It is not the purpose of the model to describe diurnal variation of photosynthesis but daily changes of biomass. In addition, irradiance data for the FOCUS scenarios are also available as global radiation per day (in Langley /m²/d or kJ/m²/d) and their seasonal variability is sufficient to result in plausible seasonal dynamics of the *Lemna* biomass.

Nutrients were simulated as forcing functions, i.e. like temperature and light not affected by *Lemna*. In reality the nutrients are used by the plants and therefore nutrient concentrations and the growth of *Lemna* affect each other. To keep the model simple, these interactions (and also internal nutrient concentrations in the plants) were not explicitly modelled. For simulating laboratory populations, the medium is renewed in intervals short enough to avoid growth inhibition due to depletion of nutrients. In the field, the nutrient levels in the water are not only affected only by *Lemna* but also by other macrophytes, algae, decomposition processes and exchange processes with the sediment and, last but not least, inputs from the adjacent agricultural areas. Thus, instead of modelling these complex interactions, time series of nutrient concentrations are used as model inputs and can be designed to represent different nutrient scenarios.

The density limit D_L (or the carrying capacity) is used as a parameter in the model, independent of environmental factors. It can be assumed that the density limit is not affected by temperature because temperature is not a resource. It should only affect how fast the density limit is reached. In contrast to this, light intensity might have an effect on the thickness of the *Lemna* mat at its density limits. The more light, the lower the shading effect of overlying fronds in a mat. On the other hand, very high light intensities might result in photoinhibition, which could balance this effect. So, for the purpose of the model, producing a realistic pattern of seasonal dynamics, the simplified submodel should be sufficient. The density limit is expected to be dependent on the available nutrients but this is included in the nutrient dependence model of the photosynthesis.
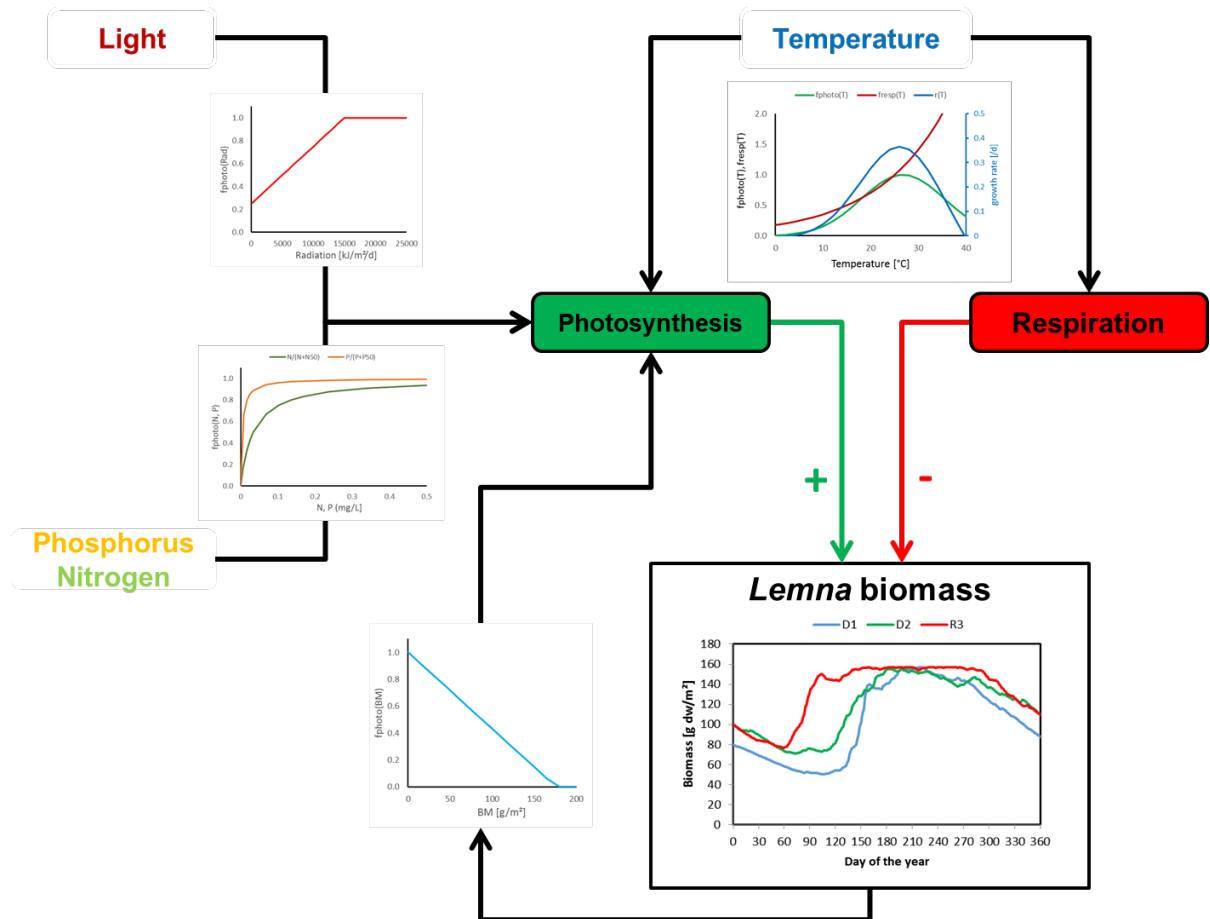
Figure 13:   Conceptual diagram of the growth model including the effects of environmental factors and density dependence. Diagrams represent the dependence of photosynthesis or respiration from environmental factors and the examples of resulting biomass dynamics over a year

5   Implementation verification

This TRACE element provides supporting information on: (1) whether the computer code implementing the model has been thoroughly tested for programming errors, (2) whether the implemented model performs as indicated by the model description, and (3) how the software has been designed and documented to provide necessary usability tools (interfaces, automation of experiments, etc.) and to facilitate future installation, modification, and maintenance.

Summary:

To make sure that the computer code actually works as specified in section 2, a series of tests has been performed during the model implementation process. In addition, results of the implemented model were compared to results obtained by the implementations of Schmitt et al. (2013). The implementation of the TK-TD model as well as the seasonal growth model described here revealed very similar results to the ones reported by Schmitt et al. (2013) for their implementation. Thus, the implementation is considered to be correct.

5.1   Software

The model has been implemented in Microsoft Visual Studio Professional (2012). The model implementation, a user manual and the input and output files of the MSM example used throughout this documentation are available on request (judith.klein@ime.fraunhofer.de or udo.hommen@ime.fraunhofer.de).

The interface of the program with its main features calibration, sensitivity analysis, validation (in the sense of comparing model predictions with experimental data) and prediction are described in the user manual.

5.2   General code testing

- Syntax Checks: Microsoft Visual Studio Professional 2012 automatically checks for syntax errors and prompts the programmer to fix them.

- Code revision: the program has been reviewed by the authors to check for logical errors and possible mistakes and to ensure that it agrees with the model formulation.

- Print statements and output comparison with excel calculations: print statements (commands inserted in the main code or given out using the command center in the NetLogo user interface) were used to output values for variables of interest as a way to observe what was going on, to check whether variables had values within expected ranges and to print out a notification of error otherwise. In order to make sure that a coded equation calculated values correctly, output values were also compared to values recalculated in excel. Examples are supplied in Appendix C. Therein, all details are specified to allow replication, including statements written in the command center and debug code inserted in the main code

- Visual checks: The correctness of some implemented processes has been checked using the relevant produced plots which were described in section 2.4.5.

  o With constant environmental conditions, the model predicts a (sigmoid) logistic growth curve, with the carrying capacity determined by the parameter DL.

  o If environmental variables are modified in direction of sub-optimal conditions, the growth rate decreases and the carrying capacity is reached later.

  o Below certain limits, low nutrient levels decrease also the carrying capacity.

5.3   Comparison of results with the results of the implementation by Schmitt et al. (2013)

The original model by Schmitt et al. (2013) was implemented in R but has been re-implemented here as a stand-alone program including a user-interface which also offers model calibration and testing. Therefore, it was possible to test the new implementation by comparing results with results obtained with the implementation by Schmitt.

5.3.1   Testing the implementation of the TK-TD and the calibration

Schmitt et al. (2013) do not describe in detail the calibration method they used to parameterize the TK-TD parameters for MSM. 'The permeability together with the TD parameters EC_{50int}, E_{max} and b were determined by non-linear optimisation. The whole model was fitted to results of a toxicological study with 7-d semi-static exposure and subsequent recovery in uncontaminated medium using *Lemna gibba*. For the optimisation process, the model was parameterised as described above with the exception that the respiration rate was set to zero. This is justified because the continuous observation period for the population was 7 d after which a quasi-renewal occurred due to the selection and transfer of only 15 fronds into the uncontaminated nutrient solution. This period is significantly shorter than the half-life of 20 d and therefore no decay of frond numbers was expected.'

In Schmitt et al. (2013) a $K_{p:w}$ value of 0.75 is used. Due to the remarks in section 2.7.1 the plant water coefficient $K_{p:w}$ has to be greater than or equal to 0.94. Therefore, we choose a value of 0.94.

To be consistent with Schmitt et al. (2013) we also included E_{max} in the calibration here for comparison. For the parameters to be fitted we used the values found by Schmitt et al. (2013) as starting values. The growth rate of the control was set to 0.39 /d as obtained from a log-linear fit to the data.

The parameter values revealed by the calibration of Schmitt et al. (2013) and the calibration here are given in the Table 3. With our calibration, we received slightly different values. All parameter values are higher than the parameter values of Schmitt et al. (2013). Schmitt et al. (2013) do not provide a measure for the goodness of fit but the calibrated dynamics look very similar (Figure 11). The model efficiency is 0.999.
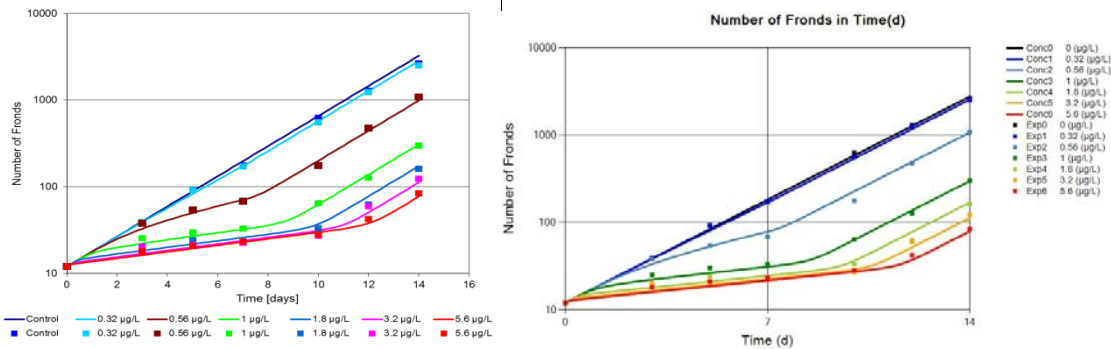
Figure 14:   Calibration of the TK-TD model to a test with 7 day constant exposure to MSM followed by 7 days of recovery in uncontaminated medium. 'Note that in the experiment the number of fronds was reduced to 15 at day seven, but the data were recalculated from day seven on by multiplication with the respective reduction factor (Schmitt et al. 2013).Left: Copied from supplemental data in Schmitt et al. (2013): Right: Calibration using the implementation described in this report.

Table 3:   Calibrated parameters of the TK/TD for metsulfuron-methyl data in Schmitt et al. (2013). Results from Schmitt et al. (2013) are copied from Table 2 in Schmitt et al. (2013).

| Parameter | Unit | Calibration by Schmitt et al. 2013 | | Calibration here | | |
		Value	CV	Value	95 % CI	Description
P	cm/d	0.0054	0.00017	0.0075	0.0074-0.0077	Permeability
E_{max}	–	0.784	0.007	0.8	0-1	Maximum effect
EC_{50int}	µg/L	0.3	0.0052	0.34	0.1 - 1000	Internal EC50 based on unbound conc.
b	–	4.16	0.48	4.46	0.1 – 502.232	Slope of internal concentration response function

Note: It is not specified how the coefficients of variation (CV) were calculated. Usually CV is defined as standard deviation divided by the mean. Therefore, they are relative values. However, here the CV seem to present absolute values because a CV of e.g. 0.017 % for P seems to be very small. In contrast to this, our method provides confidence intervals (for details see section 3.1.5).

5.3.2   Testing of the implementation of the seasonal growth model

The model implementation of Schmitt et al. (2013) was used in one of the case studies of the MODELINK workshop to demonstrate how such a model could be used within a regulatory risk assessment (Hommen

et al. 2016). For this purpose three FOCUS exposure scenarios have been modelled, i.e. D1 - Lanna, D2 – Brimstone and R3 Bologna. The FOCUS weather data were used as inputs into the *Lemna* model. Note that the FOCUS scenarios include data for global radiation (which can be expressed in KJ/m²/d, and air temperature. The air temperature were directly used in the *Lemna* model, despite that water temperature should be less variable than air temperature. However, as a floating plant, *Lemna* is directly in contact with the air. Therefore, the use of the air temperature is considered acceptable to compare the effect of different time series of temperature and radiation. It was further assumed for the MODELINK simulations, that nutrients were not limiting the growth.

In order to test our implementation of the seasonal growth model, we tried to reproduce the control runs for these three scenarios using the related temperature and irradiance data (see Section 6.2).
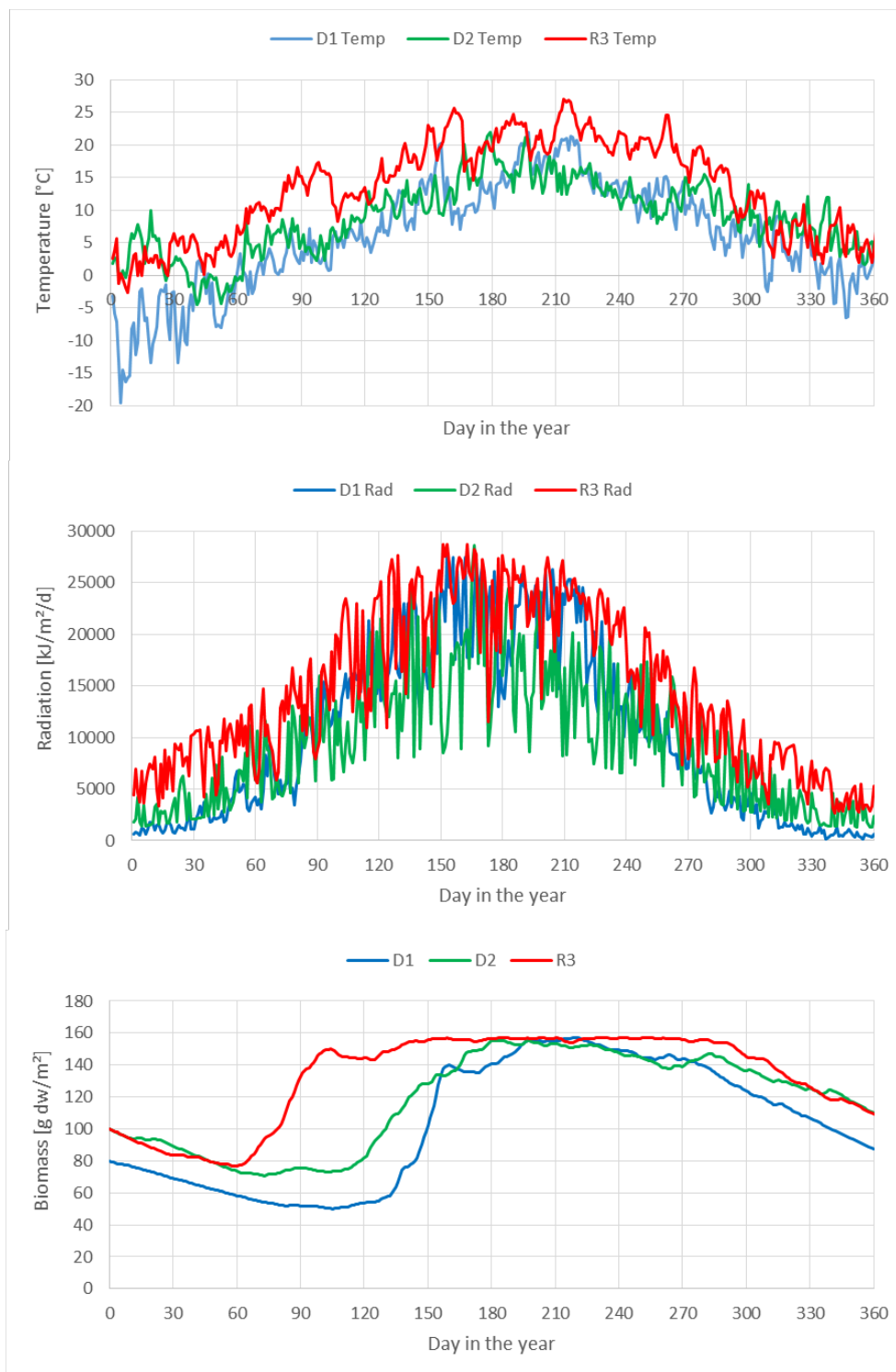
Figure 15:   Weather data and resulting seasonal dynamics of *Lemna* biomass without exposure to a toxicant for three FOCUS scenarios (D1 = Lanna, D2 = Brimstone, R3 = Bologna) simulated for the MODELINK workshop (Hommen et al. 2016).

6   Model output verification

This TRACE element provides supporting information on: (1) how well model output matches observations and (2) how much calibration and effects of environmental drivers were involved in obtaining good fits of model output and data.

Summary:

The substance specific TK-TD model should always be verified by comparing predictions with experimental data not used for the calibration, if such data are available. Here we present an example for MSM. No calibration of the seasonal growth model parameters was performed. Only the direct parameterization was performed as described in section 3.2, data evaluation. For verification of the predicted dynamics of field populations we refer to the example in Schmitt et al. (2013) and other data sets.

6.1   Output verification of the TK-TD model

We use Metsulfuron-methyl (MSM) as an example substance to demonstrate how the TK-TD model can be parameterized as outlined in section 3.1. The underlying data are taken from Schmitt et al. (2013). The settings for the calibration are summarized in Table 4.

For a log K_{ow} of -1.87 or -1.7 (EFSA 2015, pH=7) the regression model of deCarvalho et al. (2007) predicts a plant-water partition coefficient of 0.71. Due to the fact, that this value is lower than 0.94 (section 2.7.1), we set K_{ow} equal to 0.94. This parameter was not further calibrated while the TK-TD parameters *P, EC$_{50}$* and b were calibrated using results of a test with *L. gibba*, including a constant exposure period over 7 days followed by a recovery period in new uncontaminated medium of also 7 days (Figure 16). Note that in the experiment, the number of fronds was reduced to 15 at Day 7, but the data were recalculated from Day 7 on by multiplication with the respective reduction factor (Schmitt et al. 2013).
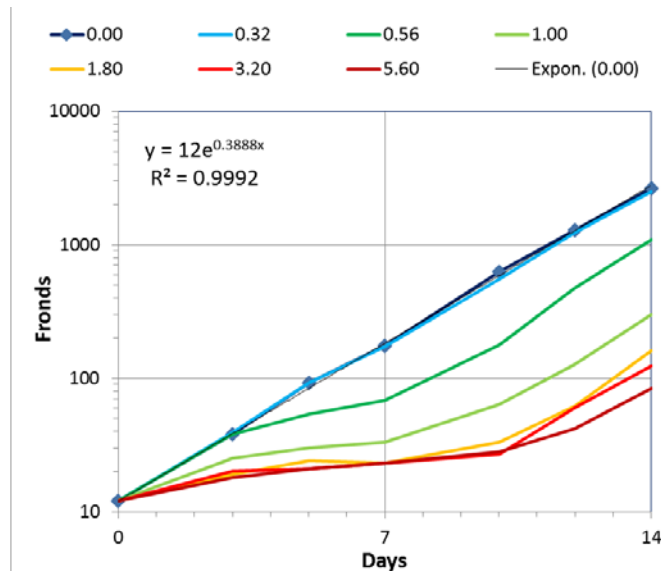
Figure 16:   Experimental data on growth of *L. gibba* exposed to different concentrations of MSM in µg/L (redrawn from Schmitt et al. 2013). The equation provides the results of a log-linear fit for the control growth.

The control showed exponential growth over the full 14 days with an intrinsic rate of increase $r = 0.389$/d. This rate was used to simulate the growth according to Equation 15. The density limit D_L was set to 1 000 000 to exclude relevant density dependent growth inhibition over the 14 days.

A starting value for the permeability P of 0.0019 cm/d was estimated from the log Kow of -1.37 using Equation 24. It was assumed that metabolic degradation and delayed elimination were not relevant (k_{met} = 0, k* = 1) and thus, they were not calibrated.

Start values for the parameters EC_{50} and b were derived from a regression of the observed inhibition of growth rate over the 7 days of exposure. The Hill function (Equation 5) was fitted by means of the Solver Function in Excel. If E_{max} was fixed to 1, an EC_{50} of 0.97 µg/L and a slope b of 1.35 were found to give the best fit (Figure 17). If E_{max} was also optimized, the fit was better and revealed values of 0.75 for E_{max}, 0.60 µg/L for $EC_{50,}$ and 3.89 for b. However, because we also consider a maximum inhibition of 75 % for higher concentrations unrealistic, we use the two-parameter fit here.
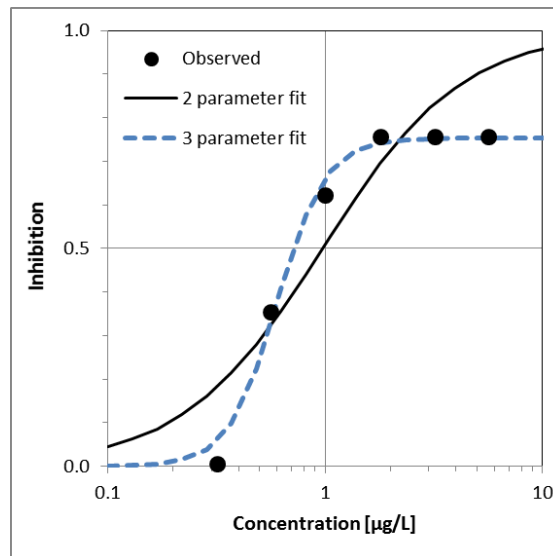
Figure 17:   Fitted dose response functions for the inhibition of growth rate over the 7 days of exposure to MSM in the study shown in Figure 16

Table 4:   MSM example: Settings for the calibration of the TK-TD parameters. The calibrated parameters are indicated in bold.

Parameter	Symbol	Unit	Starting or default value	Proposed range
Plant water partition coefficient	$K_{p:w}$	-	0.94	Estimated from log Kow = -1.7 leads to kpw equal to 0.71, which is lower as the lower bound (0.94) 0.1 - 1000, based on range found by Carvalho et al. (2007)
Permeability	P	cm/d	0.0017	Estimated from Log Kow 0.0001 - 10, based on data for *Myriophyllum* (Heine et al. 2015)
Metabolism rate	k_{met}	/d	0	Fixed, no relevant metabolic degradation assumed
Elimination delay rate	k^*	-	1	Fixed, no delay of elimination assumed

Maximum effect	E_{max}	-	1	Fixed , up to 100 % inhibition assumed	
Medium effect concentration	$EC50_{int}$	µg/L	0.97	0.097 -9.7	
Slope of conc-response function	b	-	1.35	0.135 - 13.5	
Growth rate of control	r	/d	0.39	from log-linear fit (Figure 16)	
Density limit	D_L	mg	1 000 000	Set to achieve exponential growth	

Table 5: Result of least square fit. No transformation of data.

Parameter	Initials	Lower	Upper	Fit	Result Value
K_wp	0.94	0.1	1000	no	0.94
P	0.0017	0.0001	10	yes	0.024
Emax	1	0	1	no	1.00
EC50int	0.97	0.097	9.7	yes	0.53
b	1.35	0.135	13.5	yes	4.39

Without fitting Emax, the growth inhibition during the exposure period, especially at the higher concentration 1.8 µg/L, 3.2 µg/L and 5.6 µg/L is overestimated by the model. This is an effect of the calibration objective, which minimizes the residual sum of squares, which is affected more by the deviation later in the test, when the frond numbers in the controls are larger. Thus, the extrapolation to continuous exponential growth over the two weeks, instead of considering the reset of the populations to a small number of fronds after the first week, has an effect on the relevance of the different data points for the calibration.
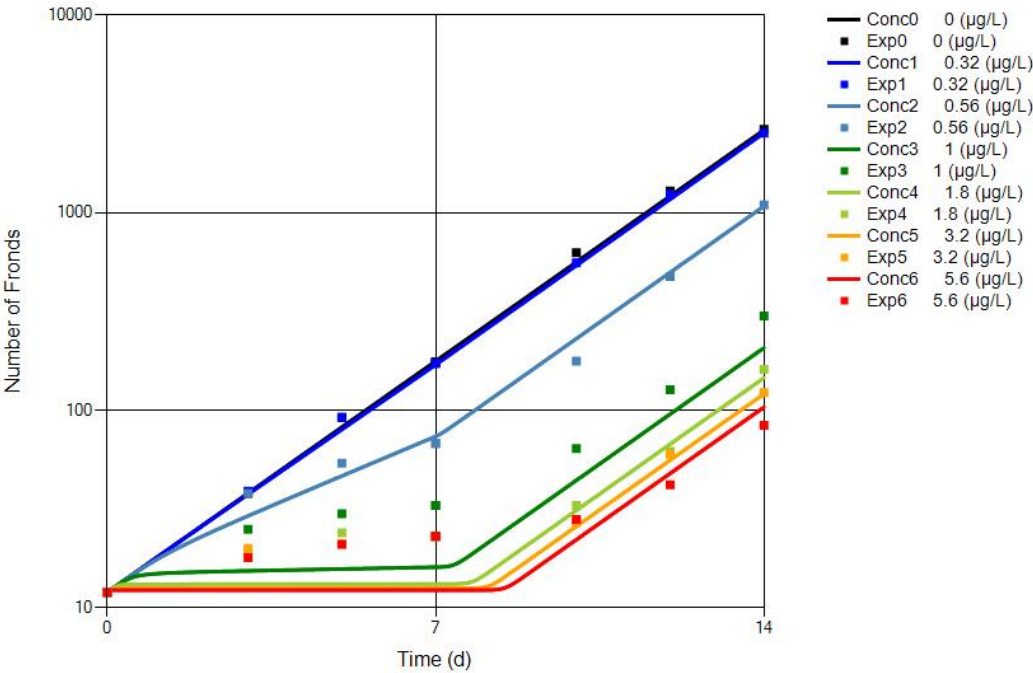
Figure 18: Measured and predicted Date in log scale.
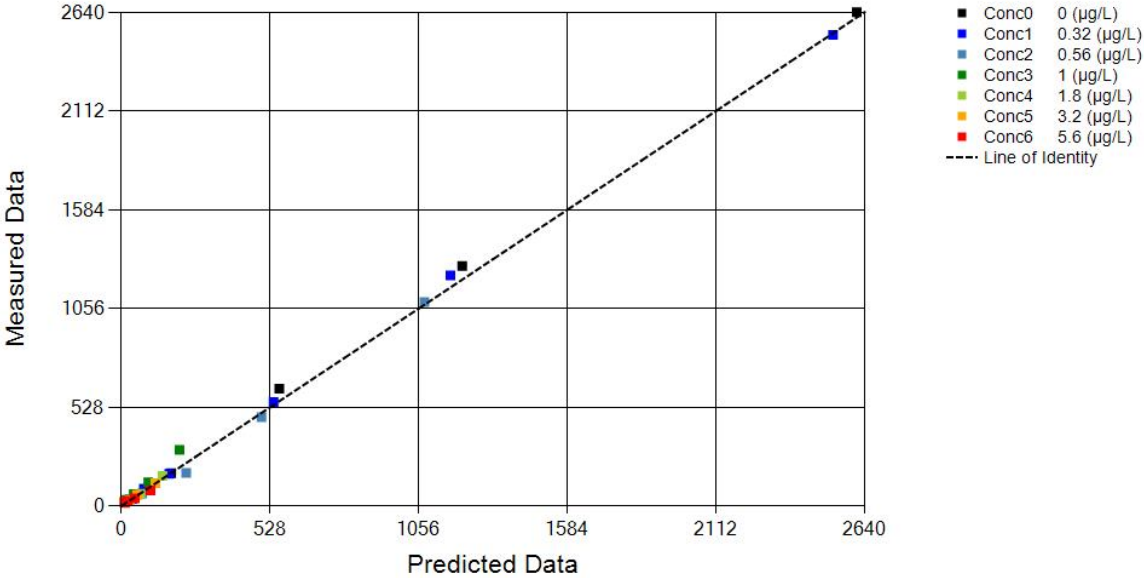
Figure 19: Measured and predicted Number of Fronds

Table 6: Statistical measurements concerning the goodness of the calibration fit.

	Conc 0	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6
Chi-Quadrat	12.445	5.519	20.804	61.883	12.775	11.205	17.024
Model Error (χ^2)	4.538	3.169	6.862	38.225	15.039	12.541	25.879
Coefficient of Determination	0.999	0.999	0.996	0.996	0.983	0.995	0.993
Model efficiency	0.998	0.999	0.996	0.813	0.966	0.969	0.783
Absolute Residuals	178.138	105.52	111.526	185.027	48.794	33.46	56.51
Squared Residuals	10541.01	4635.35	3697.948	10633.345	509.084	256.321	678.284
Scaled Root Mean Squared Error	0.052	0.036	0.078	0.436	0.172	0.143	0.295
Scaled Total Error	0.037	0.023	0.059	0.32	0.152	0.122	0.262

Number of Fitted Parameters: 3. Note that the control growth is not affected by fitting the TK-TD parameters.

The following table presents the result of the calculation of confidence intervals. The intervals of all three parameters, the permeability P and the medium effect concentration EC50int and the Hill coefficient are rather small. The predicted dynamics of the total internal concentration is shown in Figure 20.

Table 7:   Result of the Calculation of Confidence Intervals based on Profiling the Likelihood function without fitting.

	Value	Lower Bound	Upper Bound
P	0.0254	0.0245	0.0265
EC50int	0.526	0.526	0.534
b	5.586	5.207	5.588
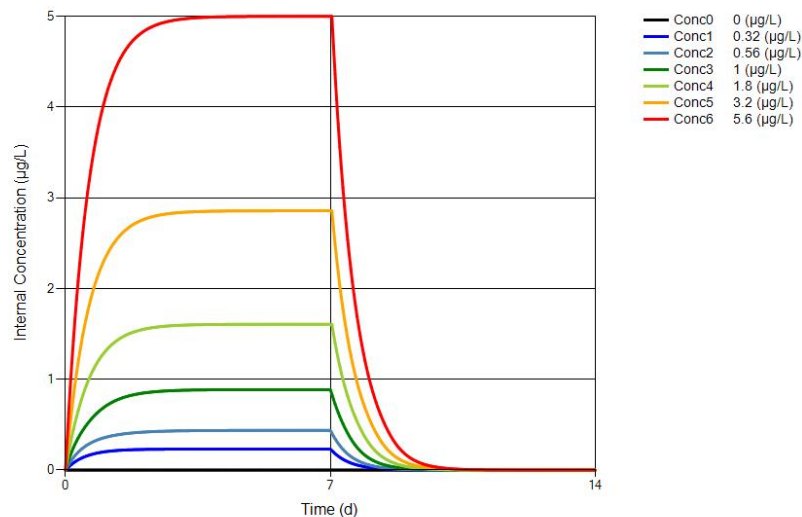
Figure 20:   Predicted internal concentration for the MSM example (Date Schmitt et al 2013).

A calibrated TK-TD model should always be tested by comparing its predictions for other exposure patterns (e.g. pulsed exposure) to experimental data not used for the calibration. An example will be shown in 8.1.

6.2   Output verification of the seasonal growth model

The seasonal growth model should allow simulating population dynamics of *Lemna* field populations. These dynamics are expected to include a growth period in spring when temperature and light conditions become more favourable. The growth is expected to become limited due to nutrient limitation (caused by *Lemna* itself or by other macrophytes or algae) and / or competition for surface area and thus, light. So, without relevant competition or grazing a period of a closed mat of *Lemna* can be expected. Later in the year, when temperature and light condition inhibit more and more the photosynthesis, the losses by e.g. respiration become larger than the production and the biomass declines down to low values during the winter season. This basic pattern is affected by the given light and temperature conditions. For populations in the North of Europe we would expect a later increase of biomass in spring and probably an earlier decrease in autumn than for populations more in the South.

The model as shown produces this general pattern here for three weather sets of FOCUS scenarios: Lanna in Sweden (D1), Brimstone in the UK and Bologna in Italy (R3). For the simulated year, the weather was quite different with e.g. a mean temperature of 5.9 °C in Lanna but 13.2 °C in Bologna and Brimstone in between (Figure 21; for the simulated temperature and radiation time series see Figure 15). However, the annual mean radiation was slightly higher in Lanna than in Brimstone. For all three scenarios, the nutrient concentrations set to 0.3 mg phosphorus / L and 0.6 mg nitrogen /L representing almost non limiting concentrations ($f_{photo}(N) = 0.95$).

The predicted dynamics by MoLePo (Figure 21) correspond to the expected pattern from the model of Schmitt et al. (2013; see Figure 15). The earliest population growth and the longest period of abundance close to the density limit is predicted for the Bologna scenario (R3) while the Lanna weather (D1) shows the latest onset of population growth in the season and shortest period of stable population abundance. Nevertheless, in all scenarios, the same maximum biomass is reached. Due to a slight inhibition of growth by the nitrogen concentration of 0.3 mg/L the density limit of 176 g /m² is not reached.

	Mean T [°C]	Mean Rad [kJ/m²/d]
Lanna (D1)	5.9	10252
Brimstone (D	8.9	9489
Bologna (R3)	13.2	14397
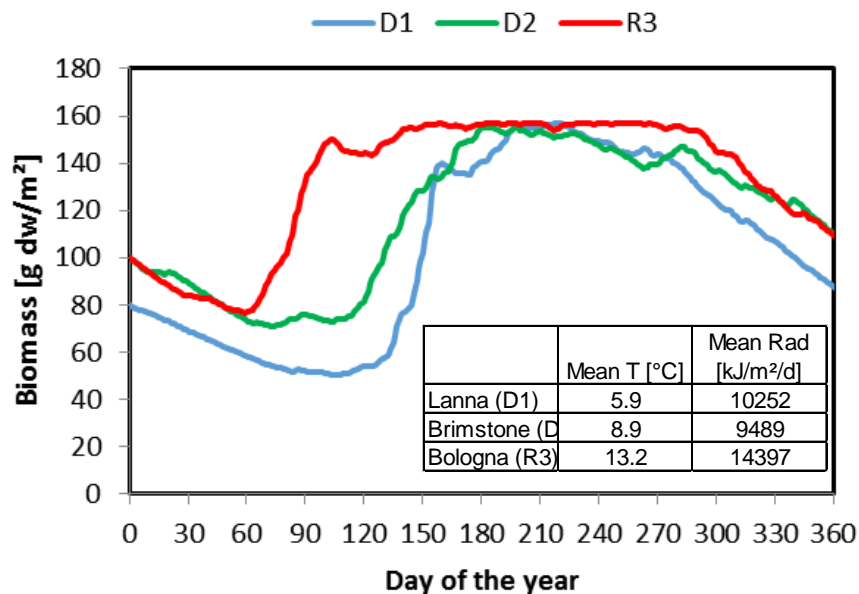
Figure 21:   Dynamics of *Lemna* for three weather scenarios. The inlay shows annual mean temperature and radiation per scenario. Nutrient concentrations were kept constant at the same values in all three scenarios

The Brimstone scenario run was used to test the effect of nutrient concentrations. Because only the most limiting nutrient affects the growth, only phosphate concentrations were varied as an example. As to be expected, the nutrient level affects the slope of the biomass increase in spring and also the maximum biomass reached in summer (Figure 22).
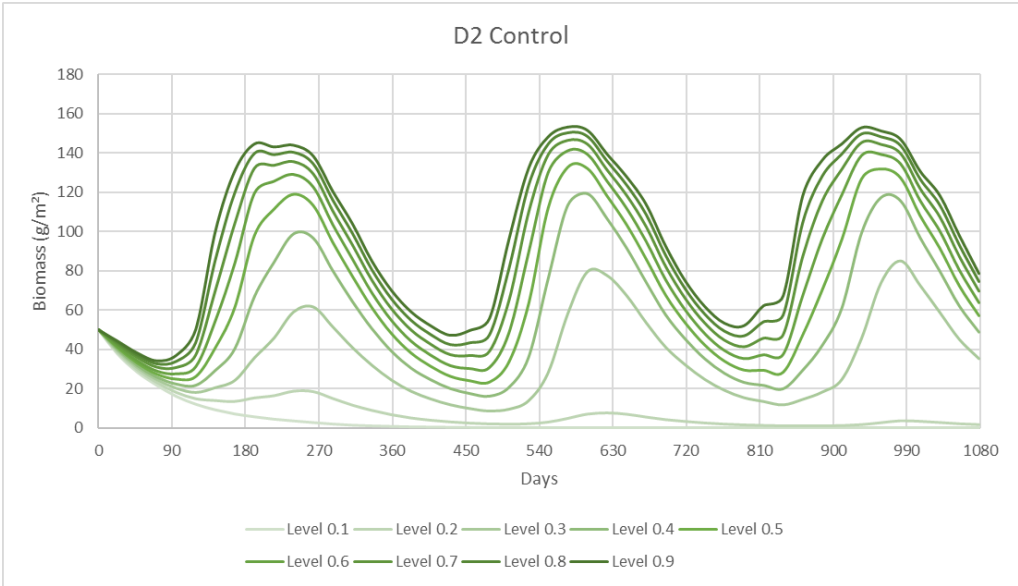
Figure 22:   D2 control scenario different nutrient concentrations, initial biomass 50 g/m².

7   Model analysis

This TRACE element provides supporting information on: (1) how sensitive model output is to changes in model parameters (sensitivity analysis), and (2) how well the emergence of model output has been understood.

Summary:

As the TK-TD model is substance specific, a local sensitivity analysis for the TK-TD parameters was performed for the MSM example to demonstrate the approach. In this case, the internal EC50 had the strongest effect on the growth limitation for constant exposure. The slope of the concentration response was less important. For short-term (1 day) exposure, the permeability was the most relevant TK-TD parameter. A local sensitivity analysis of the parameters of the seasonal growth model revealed a low sensitivity (coefficients < 1) for all model parameters. The highest sensitivity of the mean annual biomass was found for the parameters related to respiration (as the summarizing loss term of the model). Finally, a global sensitivity analysis by Schmitt et al. (2013) of predicted effects and recovery, by means of Monte-Carlo simulations of the seasonal growth model, revealed nearly no variability of the maximum effect and only a very small effect (in the range of 20%) on the delay of development in a drainage scenario. For two simulations considering run-off exposure and constant environmental conditions, variation of the parameters led to about 1.5–2-fold variability of maximum reduction of biomass and delay of development if the effect occurred during the growth phase. Initial biomass and photosynthesis rate were significantly correlated with the both endpoints while the duration of the effect was significantly correlated to respiration rate. Both size and duration of the effect were most sensitive to respiration rate. If an effect occurred after carrying capacity was reached, it was mainly correlated to respiration rate to which it also showed the highest sensitivity.

We propose to conduct a sensitivity analysis on the TK-TD parameters after calibration and output verification to provide additional information on how the calibrated parameters affect the model outputs. As this analysis is substance specific, we demonstrate a possible approach here for the MSM example. The influence of the parameters of the growth model were analysed for typical seasonal dynamics of an unexposed population. We also provide the results of Schmitt et al. (2013) analysing the sensitivity of the magnitude of effect and the time to recovery to some model parameters in three scenarios.

7.1   Sensitivity of the TK-TD model

We analysed the influence of the TK-TD parameters for the MSM parameter set (see Section 6.1) on the growth rate over 7 days as the primary endpoint of the standard OECD test (OECD 221). We conducted

two scenarios: exposure over the 7 days to 7 d- EC50 and exposure only on the first day to the 7 times the standard EC50 followed by 6 days of recovery.

For a local sensitivity analysis each TK-parameter used in the model (i.e. $P, K_{p:w}, EC50_{int}, b$) plus growth rate of the control was varied in the range considered reliable for the calibration (see Table 3) in 100 step (Alternatives +'/- 50 % around the calibrated values) while the others were kept fixed.

The growth rate over the full test (i.e. (ln(n7) – ln(n0))/7 served as the endpoint for the sensitivity analysis and the relative change of the growth rate was plotted over the relative change of the parameter. Due to this standardisation to relative changes, the point (1, 1) in the resulting graph corresponds to the default setting, i.e. the fitted parameter set resulting in an inhibition of the growth rate by 50 %. The line of K$_{p:w}$ is shown for a scaled value greater than or equal to 0.94, because the plant water coefficient has to be greater than 0.94 per definition for the given fresh weight to dry weight ratio.

In addition, we calculated sensitivity coefficients as suggested by EFSA PPR Panel (2014). Therefore, the relative change of the growth rate if a parameter was changed by 10 % was divided by the 10 %. As the parameter can be changed in both directions, the mean of the two resulting coefficients was used. A sensitivity above 1 indicates that the model output (here: growth rates) reacts to a greater degree than the parameter has been changed, and vice versa. A negative sensitivity coefficient indicates the output reaction inverse to the parameter change. For example, a sensitivity coefficient of -0.5 indicates that an increase (decrease) of the analysed parameter by 10 % resulted in a decrease (increase) of the growth rate by 5%.

For the 7-d exposure scenario (Figure 26), the EC50 was found to be the most relevant parameter with a sensitivity coefficient of 3.104 (Table 8). The higher the EC50, the smaller the inhibition of growth rate and, thus, the higher the growth rate. The slope of the dose response function is much less sensitive because it is only relevant during the uptake phase but not when the internal concentration is in equilibrium with the external concentration. Therefore, even large changes of b have only small effects on the growth rate in this scenario. Also Kp:w and P were insensitive (sensitivity coefficients close to 0.5 respectively -0.5). Increasing Kp:w values result in a higher proportion of the toxicant bound to plant matter and not available for binding to the target. Increasing permeability means increased speed of the uptake. Thus, it is more relevant if the uptake is slow. The shorter the time until equilibrium with the external concentration is reached, the lower the relevance of the permeability.

Figure 23:   Results of a local sensitivity of the growth rate over 7 d for the TK-TD parameters with exposure over the full 7 d of the simulated test. The parameters scaled to 1 result in a 50 % inhibition of the growth rate.

For the short-term (1 d) exposure scenario, the picture is different (Figure 24) because the speed of the elimination and the effect during the elimination become more important. The sensitivity to the EC50 is much less pronounced (sensitivity coefficient of 0.18) than in the constant exposure scenario because the inhibition is restricted only to a part of the 7 days. The slope b is still not sensitive (-0.01). The permeability shows an inverse pattern to the constant exposure because higher permeability also means faster elimination in the TK-TD model (and thus, a smaller effect in the growth rate over the full week). However, the sensitivity coefficient is still relatively low. Higher Kp:w means that at the end of the exposure period more toxicant is bound to the plant and will be released into the plant during the recovery period. As for the constant exposure scenario, P and Kp:w show similar (low) sensitivity, but in different directions.
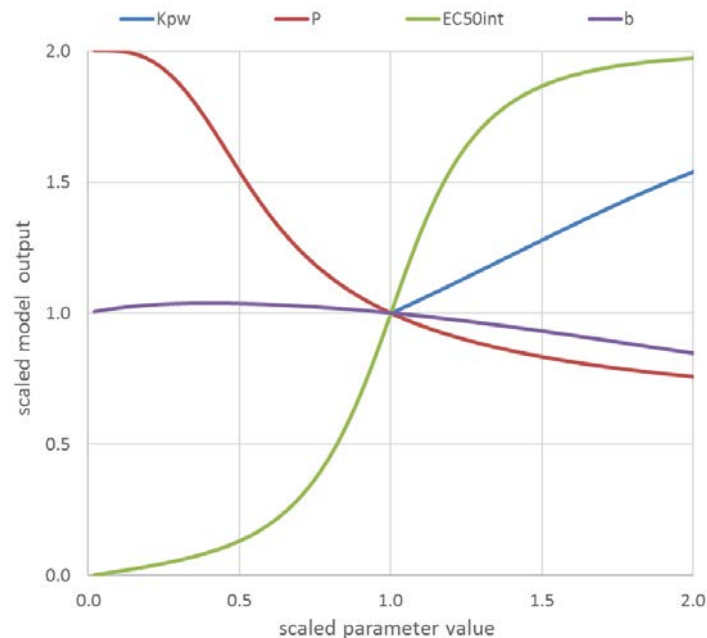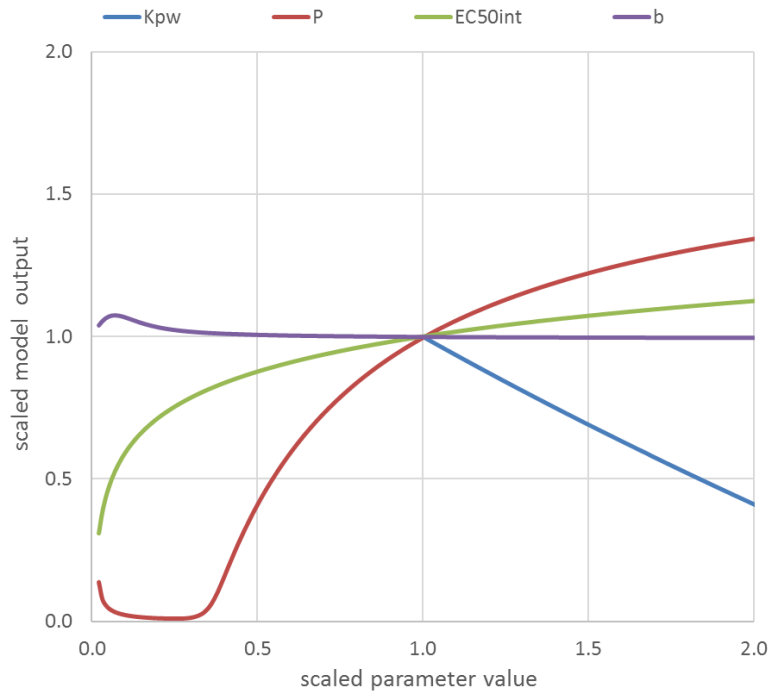
Figure 24:   Results of a local sensitivity of the growth rate over 7 d for the TK-TD parameters. Exposure over the first day with a recovery period of six days. The parameters scaled to 1 result in a 50 % inhibition of the growth rate.

Table 8.   Sensitivity coefficients calculated as means of the relative change of the 7-d growth rate related to an increase and a decrease of the calibrated parameter by 10 %

Scenario	Kp:w	P	EC50	b
7-d exposure	0.551	-0.536	3.104	-0.109
1-d exposure	-0.65	0.66	0.18	-0.01

7.2   Sensitivity of the seasonal growth model

For the FOCUS D2 weather scenario, a local sensitivity analysis of the growth related parameters was conducted. Nutrient concentrations were set to high level (resulting in 10 % growth inhibition). The default parameters were changed by + or -10 %. Maximum and mean biomass over the year were used as model outputs. Sensitivity coefficients were calculated as the relative change of the model output divided by the relative change of the model parameter. The coefficients for increase and decrease were averaged to describe the sensitivity of a specific parameter. Thus, a sensitivity coefficient of 1 means that the relative change of maximum or mean biomass was the same as for the model parameter. Values larger 1 indicate that the model output reacted stronger than the parameter while values

between 0 and 1 indicate that the model was relatively insensitive to changes of the parameter. Values below zero mean that if the parameter was increased, the model output decreased, and vice versa.

The results are summarized in Table 9. All coefficients were between -1 and 1. Thus, the maximum and mean annual biomass is relatively robust against small changes in the model parameters. The highest sensitivity (1.0) was calculated for the density limit, since this parameter directly defines the maximum biomass which can be reached in the model (sensitivity coefficient = 1). A relatively high sensitivity of the mean biomass was found for the parameters describing the temperature dependence of the respiration (respectively, the rate of biomass loss). This is caused by the fact that the respiration rate drives the decline of the biomass, which is especially relevant in autumn and which determines the minimum biomass reached in winter.

Table 9: Sensitivity coefficients of all growth parameters. We consider the D2 control scenario.

Parameter	Max Biomass	MeanBiomass
kmax_photo	0.19	0.40
kref_resp	-0.17	-0.48
T_min	-0.09	-0.58
T_max	0	0
T_opt	-0.29	-0.26
T_ref	0.30	0.84
Q_10	0.12	0.77
I_sat	0	0
beta	0.02	0.12
alpha	0.04	0.18
P_50	-0.01	-0.02
N_50	-0.01	-0.02
D_L	1	0.80
BM_0	0	0.20

7.3   Sensitivity of effects and recovery

Schmitt et al. (2013) analysed the variability of predicted effects of time variable exposure by means of Monte-Carlo simulations (global sensitivity analysis according to EFSA PPR Panel 2014). We cite from the supplemental data:

In the Monte Carlo (MC) simulation for variability and sensitivity assessment maximum photosynthesis rate (k_{photo}^{max}), respiration rate (k_{resp}^{max}), limit density (DL) and initial biomass (BM$_0$) were varied. Though, in the case of late application the latter two were kept constant since they will have no influence if the effect occurs when the population is already in the capacity limit. From varying temperature and radiation it was abstained. Both factors determine the effective growth rate and thus their influence is already implicitly

captured by varying the maximum rate. Considering them in addition would only lead to cross-correlations difficult to evaluate. The limit density was included because it is possibly an effect of self-shading of fronds in different layers and may thus in a natural environment depend on site specific lighting conditions.

The real variability of the parameters is not known. Though for the maximum photosynthesis rate it seems to be rather low since reported observed values are very consistent. The same can be assumed for the respiration rate. Therefore in the MC simulation for all parameters but the initial biomass a normal distribution with a standard deviation of 10% was assumed. The initial biomass was assumed to be evenly distributed in an interval between 0.01 and 5 g dry weight/m² (see Tab. 5). In total 100 parameter sets were sampled from the distributions and respective simulations performed.

Table 10: Parameters of the Monte-Carlo simulation (Tab. 5 in Schmitt et al. 2013)

Parameter		Mean	SD / Interval	Type of distribution
k_{photo}^{max}	*[g dw/d]*	*0.47*	*0.1*	*normal*
k_{resp}^{max}	*[g dw/d]*	*0.05*	*0.1*	*normal*
BM_0	*[g dw/m²]*	*2.5*	*[0.01 , 5]*	*even*
D_L	*[g dw/m²]*	*176*	*0.1*	*normal*

Since the incidence of a significant effect is prerequisite for the variability assessment the 100x exposure level was chosen for the MC simulation. From the resulting biomass curves the maximum effect and the time to recovery were determined as endpoints. The latter was defined as the interval between the first deviation between control and effect curve larger than 1% of the control and the time point where this deviation decreased below 1% again.

Variability of the endpoints was assessed by calculating their median, 5th and 95th percentile difference = 90% confidence interval). For evaluation of the sensitivity of the endpoints to the parameters varied in the simulation the respective correlation coefficients were determined and sensitivity coefficients were derived by calculating linear regression coefficients normalized to a 10% change of the respective parameter.

The results of the assessment are listed in Tab. 6. Variation of the parameters leads to an about 1.5 − 2 fold variability (= width of confidence interval) of the endpoints in three of four cases. For maximum effects arising in a population that reached the capacity limit, however, there is almost no variability.

In case of an effect occurring in the growth phase the initial biomass and photosynthesis rate show significant correlation (p < 0.05) with maximum effect and time to recovery while the latter is also significantly correlated to respiration rate. Both, size and duration of the effect are most sensitive to respiration rate. If an effect occurs after the capacity limit is reached it is mainly correlated to respiration rate to which it also shows the highest sensitivity. This is easily understandable since in this case during total inhibition of photosynthesis the deviation from control increases only according to the respiration rate. In addition it was found that maximum effect and time to recovery are almost 100% correlated for

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populations in the capacity limit. Thus very similar values for correlation and sensitivity to other factors were yielded.

Table 11:   Variability and sensitivity of size and duration of effects caused by short term exposure occurring during the growth phase or when the population has reached the density limit (Tab. 6 in Schmitt et al. 2013).

Effect during			Maximum effect			Time to recovery		
Growth phase	Variability							
	Median		0.21			63		
	5th %tile		0.15			47		
	95th %tile		0.31			91		
	Sensitivity							
	Parameter	R	p	Sens. Coeff.	R	p	Sens. Coeff.	
	k_{photo}^{max}	-0.16	0.01	-0.01	-0.64	<0.01	-0.07	
	k_{resp}^{max}	0.13	0.07	0.08	0.23	0.02	0.12	
	BM_0	-0.89	<0.01	-0.04	-0.50	<0.010	-0.02	
	D_L	0.10	0.33	0.06	0.00	0.97	0.08	
Capacity Limit	Variability							
	Median		0.07			28		
	5th %tile		0.06			22		
	95th %tile		0.08			46		
	Sensitivity							
	Parameter	R	p	Sens. Coeff.	R	p	Sens. Coeff.	
	k_{photo}^{max}	-0.13	0.19	-0.02	-0.58	<0.01	-0.15	
	k_{resp}^{max}	0.82	<0.01	0.11	0.58	<0.01	0.21	
	BM_0	-0.19	0.06	<-0.01	-0.13	0.21	-0.00	
	D_L	-0.03	0.78	0.01	-0.06	0.56	0.05	

Assessment for drainage scenario

Variability and sensitivity were only investigated for the simulation considering the climate conditions. It was performed in the same manner as for the run-off exposure. The parameters of the MC simulation were the same but the 5x exposure level was chosen as basis.

Tab. 7 shows the results of the analysis. In this case there is nearly no variability of the maximum effect and only a very small one of the time to recovery, which is in the range of 20%. Respectively the both endpoints are apparently insensitive to parameter variation. Thus the calculated correlation coefficients are more or less meaningless, though some are statistically reliable.

Table 12:   Variability and sensitivity of size and duration of effects caused by drainage entry. Time to recovery is calculated from the lag between control and affected biomass curves (see text). (Tab. 7 in Schmitt et al. 2013).

	Maximum effect			Time to recovery		
Variability						
Median	0.71			7		
5th %tile	0.69			4		
95th %tile	0.73			11		
Sensitivity						
Parameter	Corr. coeff.	p-value	Sens. Coeff.	Corr. coeff.	p-value	Sens. Coeff.
k_{photo}^{max}	-0.07	0.51	<0.001	-0.62	0.00	-0.01
k_{resp}^{max}	0.22	0.02	<0.001	0.23	0.02	0.01
BM_0	-0.92	<0.01	<-0.001	-0.25	0.01	<-0.001
D_L	0.16	0.12	<0.001	0.08	0.45	0.01

In the main paper (Schmitt et al. 2013), the results are summarized as follows:

'In the case of drainage exposure where realistic weather conditions were considered, there was nearly no variability of the maximum effect and only a very small effect (in the range of 20%) on the delay of development. Thus, both endpoints are apparently insensitive to parameter variation and the correlation coefficients calculated are of minor relevance.

For the other two simulations considering run-off exposure and constant environmental conditions, variation of the parameters led to about 1.5–2-fold variability (=width of confidence interval) of the endpoints. However, if effects occurred when the population had reached carrying capacity, there was almost no variability.

In the case of an effect occurring in the growth phase, the initial biomass and photosynthesis rates showed significant correlation (p < 0.05) with both endpoints while the duration of the effect was significantly correlated to respiration rate. Both size and duration of the effect were most sensitive to respiration rate. If an effect occurred after carrying capacity was reached, it was mainly correlated to respiration rate to which it also showed the highest sensitivity.'

8   Model output corroboration

This TRACE element provides supporting information on: How model predictions compare to independent data and patterns that were not used, and preferably not even known, while the model was developed, parameterized, and verified. By documenting model output corroboration, model users learn about evidence which, in addition to model output verification, indicates that the model is structurally realistic so that its predictions can be trusted to some degree.

Summary:

A TK-TD model calibrated to results of one or more laboratory growth inhibition tests should be tested by predicting the growth for other exposure conditions and comparing it to observations. We demonstrate this for the MSM example using a data set with exposure over one day followed by a recovery period of 6 days. Schmitt et al. (2013) compared predictions of the seasonal growth model to data from three ditches in the Netherlands. We show their findings but because we do not have access to the underlying data for these simulations, we did not reproduce them. However, as indicated by the comparison of simulations in section 5.3.2 it is very likely that our implementation would reveal similar results for these data.

8.1   Testing the calibrated TK-TD model by means of additional laboratory data sets

When a TK-TD model has been calibrated, it should be tested by predicting effects of exposure patterns, which have not been used for the calibration. The goodness of fit can be assessed visually from plots of predicted and observed frond numbers or biomass over time or from scatter plots of predicted versus observed data.

We suggest also to use the Model Efficiency EF as a statistical measure of goodness of fit of a model. According to FOCUS (2006), EF is similar to the Coefficient of Determination R^2 but applicable to non-linear models because it is based on deviations from the 1:1 line and not the regression line for plots of predictions versus observations. EF can be in range of minus infinity to 1. Larger values indicate a better agreement. If EF is negative the mean of the observed data is a better predictor than the model result. Positive EF values indicate the fraction of total variance of the data that is explained by the model.

Equation 25:   Model Efficiency EF (FOCUS 2006) with n = number of observations, Ci = ith calculated (predicted) value, Oi = ith observed value

$$EF = 1 - \frac{\sum_{i=1}^{n}(C_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2}$$

To demonstrate how a parameterized TK-TD model can be tested by predicting the outcome of laboratory growth inhibition tests not used for the calibration, we used a GLP laboratory growth inhibition tests with MSM with 4 d exposure followed by 3 d of recovery in clean growth medium (Ochoa Acuna 2016 (presentation at SETAC Nantes, data kindly provided by H. Ochoa-Acuna). Six concentrations have been tested (0.1 – 5.6 µg/L). The mean frond numbers of the three replicates per treatment level are given in

Table 13. The control showed a clear exponential growth (growth rate r = 0.364 /d) which was less than observed at the three lower test concentrations.

Table 13: Mean frond number measured at day 0, 3, 5, and 7 of the 96 h test.

Time d	Conc 0	Conc 1	Conc 2	Conc 3	Conc 4	Conc 5	Conc 6
	0	0.0135	0.045	0.15	0.5	1.7	5.6
0	12	12	12	12	12	12	12
3	36	33	35	36	31	20	16
5	73	76.33	75.67	76.33	54.67	20	16.33
7	154	176	174	181	127	25	17

The no or slight effects up to 0.5 µg/L are predicted well (Figure 25). Effects during exposure to 1.7 and 5.6 µg/L are over-predicted while the frond numbers at the end of the tests are slightly over-predicted. However, in total the model is able to predict the results reasonable well (model efficiency of 0.962, Figure 26).
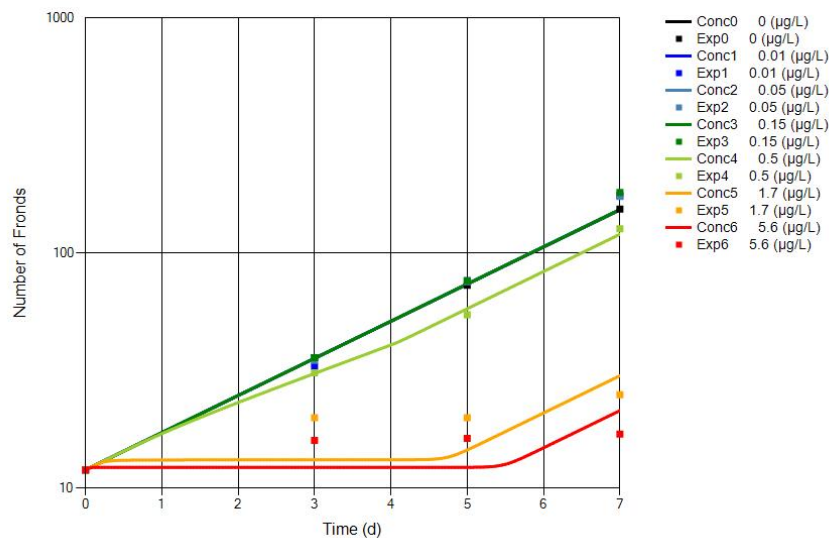

Figure 25:   Model prediction (lines) and observed data (dots) of a test with exposure to MSM over 96 h
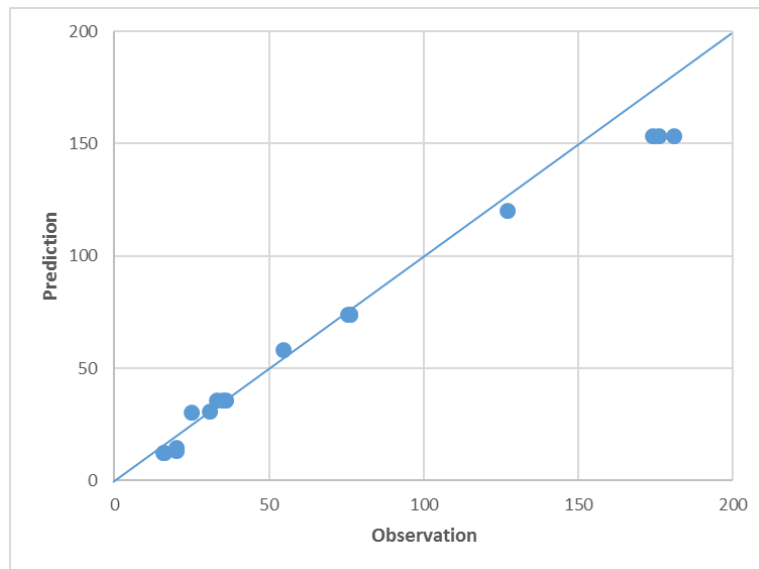
Figure 26:   Predicted over observed frond numbers of a 7 d test with 96 h exposure to MSM, model efficiency: 0.967.

For the Boxall data set used by Schmitt we do not have the real data, just information on the exposure patterns and the figures of the growth. So we can only compare patterns (Figure 27, Figure 28) but we could not plot predictions and observations within one plot nor calculate Model Efficiency. Overall the pattern is well predicted but at 1 µg/L the model overestimates the effect of the continuous exposure.
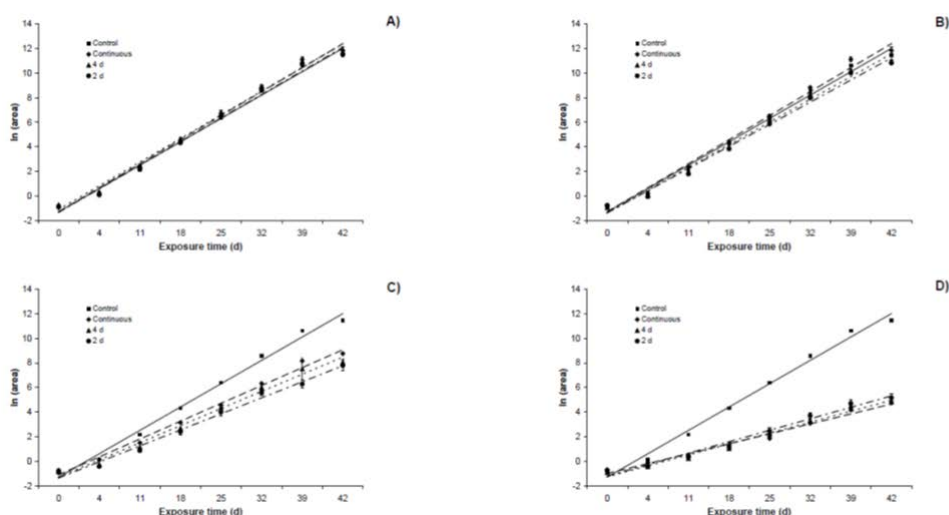
Figure 27:   Results of the experiments by Boxall et al. (2013) copied from DEFRA (2005).
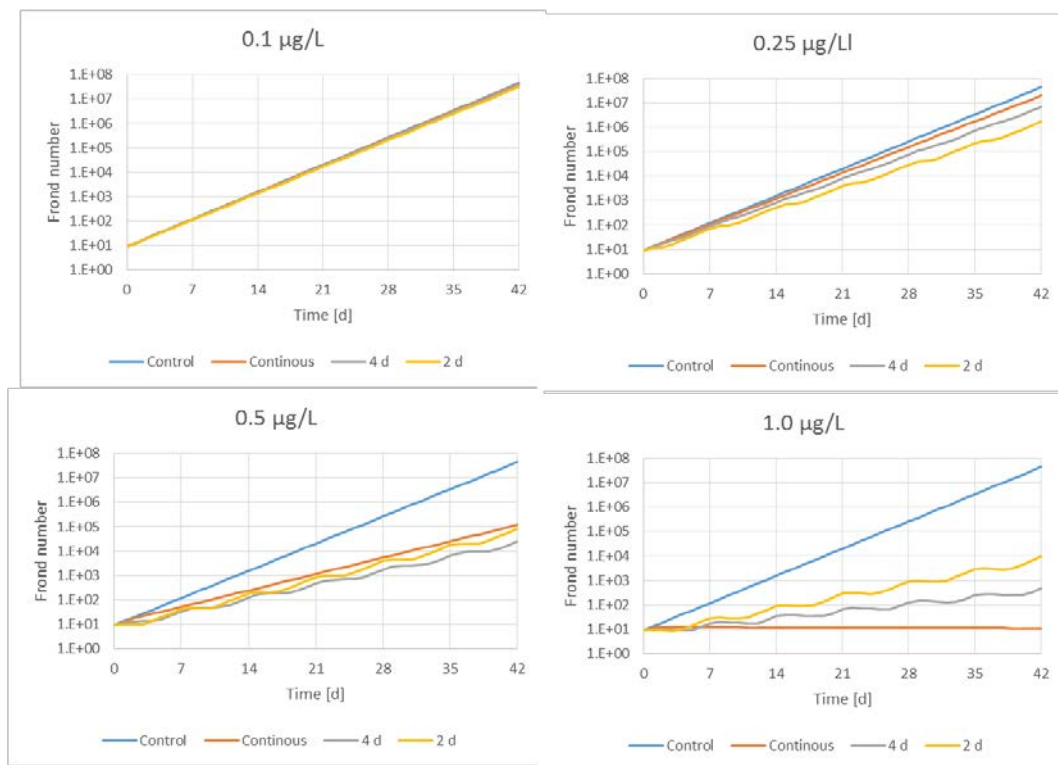

Figure 28:   Simulation of the experiments by Boxall et al. (2013), using the TK-TD parameter calibrated in run 3 and the default growth parameters (without density dependence)

8.1.1   Observed biomass dynamics of *Lemna* in ditches

Schmitt et al. (2013) provide the following model output corroboration:

For testing the growth model, it was necessary to demonstrate that the combination of the different dependences of the photosynthesis and respiration rates on environmental parameters leads to a realistic description of the development of Lemna biomass under natural environmental conditions. Unfortunately, the respective observed data are scarce. The data published by Driever et al. (2005) provide information about the development of biomass across time in three Dutch ditches. Although the observations are limited to a rather short period of about two months, this data set was taken for the validation due to the lack of more comprehensive data.

Lemna dry biomass was calculated for the course of a whole year starting on 1st January. Air temperature and global radiation were taken from the European meteorological data base MARS (JRC, 2004) choosing the datasets for the grid in which the observed ditches were located. For the observation period, the MARS temperature data were substituted by actual values recorded during the study. Nutrient concentrations in the ditches were reported but differed significantly between the three locations. Thus, the mean nitrogen and phosphorus concentrations were considered. In order to account for natural variation of the model parameters, a stochastic simulation was performed varying those parameters in a Monte-Carlo (MC) approach with 100 runs. For the biological parameters k_max_photo, k_ref_resp and D_L, a small variability with a standard deviation of 10% was assumed because from the available literature, it can be concluded that these parameters do not vary much. The site specific parameters BM0, [P]50 and [N]50 were considered to be more uncertain. Particularly, the initial value for the biomass depends on climate in the preceding period as well as on management of the ditches and may vary between almost zero and values close to the carrying capacity. A range of low initial values was used in order not to make the simulation meaningless by covering the whole potential dynamic range of biomass values right from the beginning. All parameters of the MC simulation are listed in Table 3.

Table 14: Parameter of the distributions considered for Monte-Carlo simulation (copied from Schmitt et al. 2013, Table 3)

Parameter	Mean	SD/interval	Unit	Type of distribution
k_{repro}^{max}	0.47	0.1	g d.w./d	Normal
k_{resp}^{max}	0.05	0.1	g d.w./d	Normal
BM_0	6	[2, 10]	g d.w./m^2	Uniform
D_L	176	0.1	g d.w./m^2	Normal
$[P]_{50}$	0.85	0.3	µg/L	Normal
$[N]_{50}$	0.46	0.3	µg/L	Normal

Qualitatively, the simulated Lemna populations show the expected behaviour (Fig. 3), such that in spring, the biomass increases rapidly due to favourable environmental conditions followed by a steady state phase with zero net growth in summer. Later in the year, biomass decreases when the respiration rate exceeds the photosynthesis rate which is reduced due to low temperature and light conditions.

Over all, predicted and observed distributions of Lemna biomass are in good agreement. More accurate predictions of single data series cannot be expected because important parameters are not known, particularly the actual initial biomass after the preceding winter. Considering these limitation, we concluded that the model is able to adequately simulate the development of Lemna populations under environmental conditions.
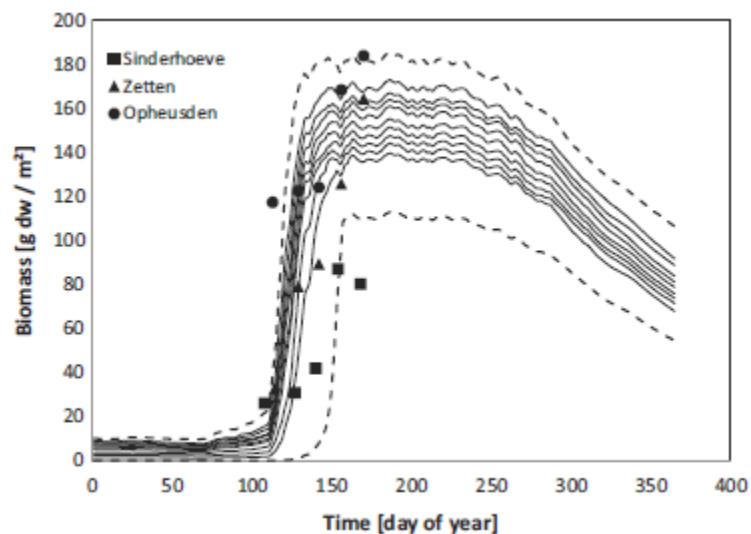

Figure 29:   Predicted *Lemna* growth (lines) over one year in comparison to observed field data from three Dutch ditches (symbols). The different lines show minimum and maximum (dashed) as well as the deciles of the biomass resulting from the Monte-Carlo simulation with variation of model parameters (copied from Schmitt et al. 2013, Fig. 3).

8.1.2   *Lemna* growth in outdoor microcosms

In cooperation with Wageningen Research and University, a long-term study is currently (2017 – 2019) being conducted to provide data sets on the growth of *Lemna* populations under field conditions in different seasons. The data will be used to test the *Lemna* population model including temperature, irradiance, nutrients and density dependence.

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