Modelling population dynamics in mesocosms using an individual-based model coupled to a bioenergetics model

Viviane David, Sandrine Joachim, Cléo Tebby, Jean-Marc Porcher, Rémy Beaudouin

Modelling population dynamics in mesocosms using an individual-based model coupled to a bioenergetics model

Viviane David[1,2], Sandrine Joachim[2], Cleo Tebby[1], Jean-Marc Porcher[2], Rémy Beaudouin[1,2]

[1] Unit of Models for Ecotoxicology and Toxicology (METO), INERIS, 60550 Verneuil en Halatte, France. Tel: +33344618238; e-mail: remy.beaudouin@ineris.fr

[2] UMR-I 02 SEBIO, INERIS, Parc ALATA, BP2, 60550 Verneuil-en-Halatte, France

Abstract: Developing population models is of great interest as these models enable to extrapolate toxicity observed at the molecular or individual levels to the population level, and thus improve environmental risk assessment of chemical substances. For this purpose, accounting for natural variations of environmental conditions and prey dynamics along with the chemical stress is needed. In this study, an individual-based model (IBM) coupled to a Dynamic Energy Budget (DEB) model was developed in order to predict three-spined stickleback population dynamics in semi-controlled stream experiments (in mesocosms). Datasets obtained in mesocosms offer the opportunity to develop and evaluate the model predictions. The most sensitive parameters of the DEB-IBM were identified by sensitivity analyses and were calibrated based on data from two independent mesocosm experiments. The predictive capacities of our model were subsequently evaluated using three independent mesocosm datasets under different environmental scenarios. Finally, our model was applied to a theoretical case of toxic effects to show an example of application of the model in a regulatory context. While the most uncertain population processes (in particular, competition for food in mesocosms) in our three-spined stickleback DEB-IBM could be modelled more accurately, our model can already serve to assess the impacts of toxicants at the population level by improving the analyses of mesocosm experiments, in decreasing the uncertainty of the experimental results. Therefore, in a second step, it could be used to predict the consequences on viability of a population exposed to a contaminant under various environmental and exposure scenarios.

Key words Individual-based model, bioenergetics, three-spined stickleback, mesocosm, risk assessment

1. Introduction

In a regulatory context, predicting chronic exposure and effects of pollutants on aquatic ecosystem endpoints is of a great interest (Adams, 2002; Ruden et al., 2016). However, as

ecosystems are dynamic entities which exhibit a complex network of interactions among organisms and between organisms and their environment, current ecological risk assessment approaches often fail to predict toxicant effects accurately on population and ecosystem endpoints (Adams, 2002). Indeed, toxicant effects are mainly studied at an individual level and extrapolation towards a higher level of organization is often too simplistic (Forbes et al., 2017). Ecological risk assessment should be a comprehensive and integrated approach able to assess toxicant impacts in complex ecosystems. In particular, a focus should be made on the bottom-up and top-down processes that occur in aquatic ecosystems and make risk assessment a challenge (Galic et al., 2010).

For this purpose, ecological modeling has been suggested as a relevant tool to improve the understanding of ecosystem functioning and interactions between biocenosis and biotope. Indeed, the potential of models towards enhancing ecological realism in current risk assessment is increasingly recognized (Beaudouin et al., 2015; Forbes et al., 2011; Forbes et al., 2017; Galic et al., 2010; Grimm et al., 2017). Furthermore, individual-based modelling (IBM) has demonstrated its ability to predict population dynamics by modelling the interactions between individuals and their environment (Grimm and Railsback, 2005). Furthermore, including individual energy budgets in IBMs was suggested to be essential for realistic modelling of populations affected by food availability (Sibly et al., 2013). In particular, in risk assessment, among the different existing approaches for energy budgets, embedding a Dynamic Energy Budget (DEB) model (Kooijman, 2010) into an IBM is a convenient way of extrapolating impacts of toxicants from organism to population level (Beaudouin et al., 2012a; Beaudouin et al., 2015; Martin et al., 2013) thus providing considerable insight into the possible range of effects of chemicals (Galic et al., 2017).

Although demand for DEB-IBMs in ecotoxicology is rising, special attention should be paid to their outcomes so that the models may be relevant tools for risk assessment and decision-making. Indeed, the model should be able to accurately predict real-world ecosystem and the population properties, under a variety of environmental conditions (food availability and seasonal variations of the environment) without chemical stress. Unfortunately, the predictive capacities of DEB-IBMs are rarely evaluated, because there are rarely enough data to both calibrate and evaluate these models. Furthermore, detailed and accurate comparisons between simulations and observations often require a sound knowledge of the populations characteristics (size of the population, sex, length of the individuals, data on the state of maturity, etc.); these

data are often scarce because multiple samplings in the same environment are costly, logistically difficult and time-consuming.

Mesocosm experiments are of a great interest because they are a convenient tool to perform high tier ecotoxicological studies (i.e. studies performed at the population level or higher) (EFSA, 2013). Among species used for mesocosm studies, fish species are of a specific interest for assessing impacts of contaminants in the environment (van der Oost et al., 2003). Specifically, the teleost fish three-spined stickleback (*Gasterosteus aculeatus*) is one of the fish species that can be used in mesocosms (OECD, 2006). This species is native of the Northern Hemisphere and its ecology and biology have been well studied (Bell and Foster, 1994; Wootton, 1984). Moreover, effects of toxicants have been studied both in laboratory and mesocosms on stickleback individuals and populations (de Kermoysan et al., 2013a; Maunder et al., 2007; Naslund et al., 2017; Roussel et al., 2007). Mesocosm experiments have the advantage of mimicking the complexity of a natural ecosystem by exhibiting a high level of biological organization (Caquet, 2002; Caquet et al., 2000) and they can provide interesting datasets for developing ecological models. However, these studies are mostly performed using few replicates and exhibit an important variability of the results due to the natural variability inherent of high levels of biological organization (EFSA, 2013; Sanderson, 2002). Consequently, these experiments are characterized by a low statistical power when comparing the control and the chemical-exposed populations which could be improved by using IBMs (Beaudouin et al., 2012b).

Consequently, developing an IBM to predict the stickleback population dynamics in mesocosm would help to analyze the ecotoxicological experiment, and offers the opportunity to evaluate an ecological model later applied to risk assessment. An IBM has recently been developed for sticklebacks in field conditions (Mintram et al., 2018) to predict endocrine disruption. However, this model does not integrate some ecological factors like photoperiod, water temperature, and food availability, nor a bioenergetic model which would describe physiological processes at the organism level. Hence, as suggested by the authors, a more mechanistical approach which would take into account interactions between the effect of a toxicant and ecological factors would help to improve the realism of the IBM predictions.

In this paper, we developed a DEB-IBM to predict three-spined stickleback population dynamics in mesocosms. We used this model to analyze data from mesocosm experiments that included relevant information on the environmental conditions (food density, temperature and photoperiod). The predictive capacities of our model were assessed by calibrating and

evaluating it on several independent datasets of mesocosm experiments. In this study, we showed how individual-based modeling can provide realistic simulated population dynamics in mesocosms. Furthermore, to illustrate usage in a risk assessment context, we used our model to assess the impacts of hypothetical toxicants on stickleback population dynamics in mesocosm experiments.

2. Material and methods

2.1. Mesocosm experiments.

2.1.1. Experimental design.

Five independent experiments from 2010 to 2014 with an identical protocol were performed using artificial streams of 20-m long and 1-m wide, located in the North of France (INERIS, Verneuil-en-Halatte, France). The two first mesocosm experiments (2010 and 2011) were conducted with 9 and 8 replicates of mesocosms respectively whereas experiments performed in 2012, 2013 and 2014 were conducted with 3 replicates. The same experimental design was used for each experiment. A detailed description is given in de Kermoysan et al. (2013a). Briefly, the same protocol of species introduction was used for each experiment. Each mesocosm was set up with sediments, macrophytes and macroinvertebrates between October and December. The experiments started in March when 15 females and 10 male sticklebacks with homogenous length (See Appendix Part I.1.2. Tables A.1 and A.2) were introduced (after called the founders). The sex identification of the founders was performed with the model developed by de Kermoysan et al. (2013b). Founders were individually marked with 1.2 mm x 2.7 mm alphanumeric tags (VI Alpha Tags, Northwesr Marine Technology, Shaw Island, WA, USA).

The different species as well as the physico-chemical parameters of the environment varied naturally throughout the experiments and the freshwater temperature and stickleback prey abundances were monitored over time. Water temperature was recorded every 10 minutes by two temperature sensors (HOBO0257, Prosensor, Amanvilliers, France) placed in the surface at 5 m from the inlet of water and at 15 m at a water depth of 70 cm. Macroinvertebrates were sampled every four weeks by specific sampling devices, *i.e* tubes, tiles or mesocosm walls. Zooplankton were also sampled every four weeks with a Perspex tube of 5 cm in diameter and 0.8 m in height (See David et al. (2018) for more details on temperature measurements and prey samplings).

Mesocosms were emptied in October and fish were killed by an overdose of MS-222 and stored in 4% formalin before being individually measured and weighed. Fish shorter than 26 mm in length were assumed to be immature and thus classified as juveniles (de Kermoysan et al., 2013a). Sexes of fish longer than 26 mm were determined by visual observations. The ethics committee of the National Institute of Industrial Environment and Risks (INERIS) approved all the experiments performed and described in this manuscript.

2.1.2. Descriptive variables of the stickleback populations.

The descriptive variables of each fish population at the end of the experiments were the population abundance (N.tot), female and juvenile frequencies (F.F and F.J) and the mean lengths and coefficients of variation of the lengths of five categories of individuals, male and female founders (L.M.C00, L.F.C00, CV.M.C00, CV.F.C00) males and females born in the mesocosms (L.M.CXX, L.F.CXX, CV.M.CXX, CV.F.CXX), and juveniles (L.J, CV.J). Finally, observations of gonad maturity of males (immature vs mature) were also made and the frequency of mature males among the total number of males (F.M.m) was calculated as well as the mean length of mature males and the coefficient of variation of their lengths (L.M.m and CV.M.m). Observed endpoint values for each year can be found in Appendix Part I.1.3. Tables A.3 and A.4.

2.2. DEB-IBM description.

The DEB theory is based on a mathematical description of the energy fluxes within the organism. It describes the rates at which the organism assimilates energy from food and uses it for maintenance, growth and reproduction. Rates of ingestion and allocation depend on body size and temperature (Kooijman, 2010). Individual-based modelling simulates population dynamics by describing the interactions between individuals as well as with their environment (Grimm and Railsback, 2005). Our DEB-IBM description follows the ODD (Overview, Design concepts, Details) protocol (Grimm et al., 2010). The detailed description of the Design concepts, initialization, input data and submodels are provided in Appendix Part II.1. The model was implemented under Netlogo 6.0.2 and the script of the model is provided in Appendix Part IV.

2.2.1. Purpose.

The DEB-IBM was developed to predict three-spined stickleback population dynamics in mesocom experiments. To this end, the model includes relevant information about the environment (i.e. temperature, photoperiod and prey dynamics). The DEB model was previously developed with laboratory data on life history traits of three-spined sticklebacks (Leloutre et al., 2018) and validated for founder sticklebacks in mesocosms (David et al., 2018). The objective of the model is to help improve current risk assessment of chemicals, by improving the analyses of mesocosm experiments (Beaudouin et al., 2012b).

2.2.2. Entities, state variables, and scales.

The model is based on daily timesteps and the simulated mesocosm is a channel of 20 m^2 based on the mesocosm surface areas at INERIS); the depth is not taken into account.

The model includes two entities, individuals and environment. Individuals are divided into three categories, juveniles, female and male adults. Individuals are characterized by variables describing their physiology in terms of sex, length, mass, energetic processes, maturity and reproductive states. The eggs and larvae are not explicitly modelled as individuals because they still use their energy from their yolk sac and thus do not assimilate food. However, they are taken into account in the reproduction processes: the eggs, larvae and nests are modelled as a whole entity and are represented as an attribute of males.

The second entity in the model, environment, is defined by three main variables, temperature, photoperiod and mesocosm food density. These variables are updated daily from the data recorded during the experiments. Briefly, temperature and food scenarios were respectively defined daily from the freshwater temperature measurements (averaged per day and per location of the sensors) and the prey samplings (abundance data converted into energy) made in mesocosms using the same framework than in David et al. (2018). Daily photoperiod data were from the Astronomical Applications Department of the U.S. Naval Observatory (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php). Figures A.1 and A.2 present the inputs for each of the five years and details are given in Appendix Part I.1.1.

2.2.3. Process overview and scheduling.

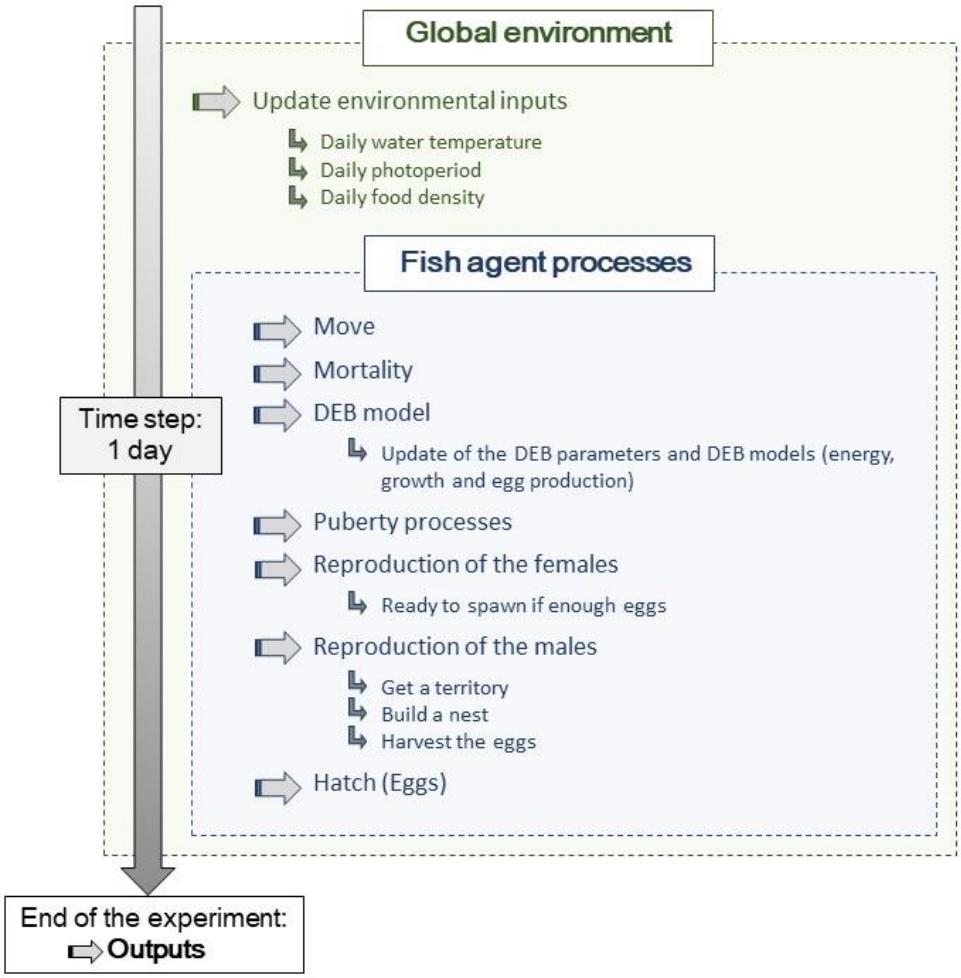

Figure 1: Conceptual diagram of the overall scheduling of events within one timestep in the DEB-IBM

Full details of all processes and the associated equations (Eq. (A.1) to (A.16)) can be found in the ODD protocol provided in Appendix Part II.1. Figure 1 presents the overall scheduling of events within one timestep. At each timestep, the environment global variables are updated (temperature, photoperiod and food density) followed by the fish variables (see Figure 1, "global environment" box). Individuals can move in their environment (Figure 1, "move") and their growth, energy for maturity and reproduction dynamics are described by the individual DEB models (Figure 1, "DEB model"). To introduce inter-individual variability, the $\{\dot{p}Am\}, \varphi, E_H^p$ and E_H^b DEB parameters are individual-specific and are lognormally distributed as suggested by Kooijman et al. (1989) and explained in Appendix Part I.1.1.

The amount of food foraged depends on the individual predation distance (R_ref and L_ref parameters), on the prey size an individual has access to (a_food, b_food, ratio_f), and on the boldness of males (Bold.M). Competition for food is modeled taking into account the total fish biomass and modulates the intake of energy from food (K_dens, a_Kdens) (see Table 1).

When they reach 26 mm-long, juveniles are classified as males or females as in the experimentally collected data. Sexual maturity of the individuals is predicted by the DEB model (Figure 1, "puberty processes"). Furthermore, when males have mature gonads, they need to accumulate enough energy to support the reproduction processes. Then, reproduction processes are modelled for females based on the clutch sizes they can produce and the fraction of eggs they can spawn in a nest (Figure 1, "reproduction of the females"). The reproductive behavior of males is more complex (Figure 1, "reproduction of the males") as they must defend a territory if enough space is available, build a nest, attract females, harvest eggs and take care of them until hatching (Wootton, 1984). Each nest has a daily probability to be destroyed. Males can make several nests until the end of the breeding period. Besides the season, the end of the reproduction processes for males also depends on the number of successful nests (Mori, 1993). At the end of the incubation period in the nest, the number of surviving larvae determines the number of newly created juveniles (Figure 1, "hatch"). Individuals can die due to the temperature- and density-dependent stochastic mortality sub-model (Figure 1, "mortality"). Finally, inter-mesocosm variability is included on the mortality and the food density and inter-annual variability is included on one parameter responsible for competition for food. The simulations run for the same length of time as the real mesocosm experiments and the DEB-IBM simulation outputs are the endpoints monitored in the mesocosm experiments (see 2.1.2).

Table 1: Parameter names, values, units and descriptions of the IBM.

Parameter	Description	Value	Unit	Equation	References
Reproduction parameters					
Photop.Thr	Minimum day duration for beginning of breeding period	11.305	h		de Kermoysan. 2013
Breeding.Period	Duration of the breeding period	117.23	d		**Fitted on population data**
Adult.Thr	Minimum length for fish sex determination	26	mm		de Kermoysan. 2013
L0	Standard length of juveniles at hatching	5.72	mm		de Kermoysan. 2013
L_mat_founder	Standard length of mature fish for the equations	33.01	mm		de Kermoysan. 2013
a_R.max	Parameter for calculation of the clutch size depending on female length	5.37	1/mm	A.10	Laboratory observations
b_R.max	Parameter for calculation of the clutch size depending on female length	16.8	-	A.10	Laboratory observations
P.OL	Survival probability of eggs	0.936	-		Poulin & FitzGerald. 1989; Mori. 1987; Santos. 2013; Kynard. 1978
A.clutch.max	Maximal duration females keep eggs	2	d		Wootton. 1984
Proba.Stop	Probability of stopping the reproduction processes for a male	0.09	-		**Fitted on population data**
Part_Rmax	Fraction of eggs laid by a female	0.703	-		de Kermoysan. 2013
a_Nb.Egg	Parameter in relationship between maximum number of eggs in a nest and male length	58.8	-	A.11	Laboratory observations
b_Nb.Egg	Parameter in relationship between maximum number of eggs in a nest and the male length	-437	-	A.11	Laboratory observations
Time_harvest_mean	Harvest mean duration for males	12.7	%		Kraak et al 1999
Time.Acc	Length of time for male founder sticklebacks to acclimate to their new environment	-7	degree/d		Laboratory observations
Female_Select	Number of females that can spawn in a given nest	4	-		Van Iersel 1953 ; Moodie. 1972 ; Wootton 1984
Time_Dvpt_Eggs	Duration of egg development	6.9	degree/d		de Kermoysan. 2013
R_min	Minimal energy for males to support the reproduction processes	1586.56	J		Smith 1992
R_diff	Length of time between two breeding periods	11.14	degree/d		**Fitted on population data**
A.territori.min	Minimal territory size for a male	0.226	m²		van den Assem 1967; Candolin & Voigt. 2001; Mori. 1987
Gamma.Compete	Competition parameter for getting a territory	0.15	-		Rowland. 1994
Food parameters					
a_food	Parameter in Length - Mouth size relationship	0.297	-	A.4	**Fitted on population data**
b_food	Parameter in Length - Mouth size relationship	-0.743	mm	A.4	**Fitted on population data**
ratio_f	Ratio prey size/mouth size	0.6	-	A.5	Gill & Hart. 1994
K_dens	Parameter of density dependence for the food	8702.2	mg/m²	A.8	**Fitted on population data**
a_Kdens	Parameter of density dependence for the food	0.437	-	A.8	**Fitted on population data**
R_ref	Reference radius in calculation of the radius of the water column	57.60	mm	A.3	**Fitted on population data**
L_ref	Reference length in calculation of the radius of the water column	23.54	mm	A.3	**Fitted on population data**
Bold.M	Percentage of boldness for males during foraging	0.17	-		**Fitted on population data**
Survival parameters					
m_dens	Parameter for density dependent mortality	0.0000086	-	A.16	**Fitted on population data**
mr	Malus for the survival of males in reproduction	0.000856	-		Laboratory observations
Mu	Natural mortality rate at unit weight	0.00323	1/d	A.15	Laboratory observations
bN	Allometric scaling factor	- 0.0516	1/d	A.15	Laboratory observations
DP50_m	50% of the density dependence	4000	mg/m²	A.13	Laboratory observations
M.nn	Daily mortality of the nests	0.0191	-		Laboratory observations
a_t	Parameter in mortality rate - temperature relationship	3.54	-	A.14	Laboratory observations
b_t	Parameter in mortality rate - temperature relationship	-90.8	-	A.14	Laboratory observations
P.Dest.nid	Probability of a nest being destroyed after an attack	0.01	-	A.12	Laboratory observations
Variability parameters					
cv	Inter-individual variability	0.061	-		**Fitted on population data**
CV.c_M	Coefficient of variation of the mortality (inter-mesocosm variability)	23.92	%		**Fitted on population data**
CV.c_F	Coefficient of variation of the food (inter-mesocosm variability)	14.94	%		**Fitted on population data**
Year-dependent parameters					

Inter-annual variability for K_dens	Year 2010	Year 2011	Year 2012	Year 2013	Year 2014
	0.974	1.026	0.759	0.628	0.724

Laboratory observations mean that data are from observations or previous experiments made one sticklebacks in INERIS (See Appendix Part I.3. Figures A.3 to A.6).

2.3. Sensitivity analysis.

In order to highlight the parameters affecting the most the model outputs, and to prepare the calibration process, local and global sensitivity analyses of the IBM were conducted. A sensitivity analysis for the DEB model had already been performed in Leloutre et al. (2018). For the IBM, a local sensitivity analysis was first performed using 5 % variation on the parameters in order to assess the direct effects of the parameters on the model outputs according to the method described in Ginot et al. (2006). To account for the interactions between parameters, a sensitivity analysis using the Morris method was also performed (Morris, 1991). In case of complex models, this method allows to sort the factors according to their influence using a specific exploration process. This method is described in Beaudouin et al. (2015). Briefly, 80 trajectories were carried out using 30 replicates of each set of parameters (see Appendix Part II.2.2.). Finally, the variance-based Sobol method (Saltelli et al., 2010; Sobol' et al., 2007) was used to obtain detailed information on the interactions between the most important parameters that had been identified previously using the local and Morris sensitivity analysis. The Sobol method was performed with 10 % variation on the parameter values.

2.4. Model calibration.

Calibration of the DEB model had already been performed with laboratory experiments in Leloutre et al. (2018) and the relevance of the model for predicting the physiological processes of founder sticklebacks was presented in David et al. (2018). Values and descriptions of the DEB parameters is given in Appendix Part II.3. Table A.6. Only one DEB parameter representing energy at puberty (J) was refitted to better predict the maturity of males in mesocosms. Calibration of the population model was an iterative process using a genetic algorithm available within the software BehaviorSearch (Grimm and Railsback, 2005). The distance to minimize was the weighted sum of least squares (WLS) between the predictions and the observations of the endpoints of the population (Eq. 1).

$$WLS = \frac{1}{n}\sum_{i=1}^{n}\frac{(Sim_i - \mu_{obs_i})^2}{{\sigma_{obs_i}}^2} \quad \text{(Eq. 1)}$$

With n the number of observed outputs, Sim_i the value of the simulated outputs, μ_{obs} the mean of the observed outputs and σ_{obs} the standard deviation of the observed outputs.

Uncertainty range of the parameters to calibrate were specified based on the parameter values either from the literature (see Table 1) or from historical laboratory experiments on three-spined sticklebacks performed at INERIS (De Kermoysan, 2013); they are provided in

Appendix Part II.3. Table A.7. When necessary, ratios between parameters were introduced to cope with collinearity. The parameters for the generic algorithm were 0.01, 100, 0.7, 3 respectively for the mutation rate, the population size, the crossover rate and the tournament size. The model was calibrated on two datasets obtained in control mesocosms (experiments 2010 and 2011, 9 and 8 populations respectively) joined together, thus the final function to minimize was the sum of the WLS for both years. The parameters with the higher impacts on the outputs according to the sensitivity analyses were calibrated. Five independent calibrations were performed, final parameters values were kept when less than 5% of variations in parameter values between the independent calibrations was found.

Finally, after the mean dynamics of the populations were calibrated, the inter-mesocosm variability was calibrated. To this purpose, the CV of the food scenarios and the mortality (see Table 1) were calibrated minimizing the weighted sum of least squares (WLS) between the predictions (CV of the outputs calculated on 100 simulations) and the observations of the CV of the outputs for both experiments. To assess the relevance of the calibration, the comparison of the predicted distributions of the descriptive variables of the stickleback population in mesocosms to the observed data for both years was made using a Kolmogorov–Smirnov test.

A focus was made on the data used for the calibration. Indeed, when the mesocosms were emptied, the recovery rate of the fry was lower than for other fish size classes due to multiple factors such as small length and cannibalism after collection. However, they represent a small fraction of the population (2.2 and 8.0 % of the population monitored in 2010 and 2011 respectively). We therefore excluded data relative to juveniles shorter than 15 mm to reduce the uncertainty linked to this specific class size during the calibration process.

2.5. Model evaluation.

The predictions of the model were assessed for three independent datasets (experiments 2012, 2013 and 2014) by comparing the observed and predicted values of the descriptive variables of the stickleback populations at the end of the experiments. 1000 model simulations were run for each year of experiments and were compared to the observed endpoints in mesocosms (in three replicates). The parameter values were the same in each simulation; the distributions of the descriptive variables of the populations is a result of the stochasticity of the model.

2.6. Model application to analyze mesocosm experiment

In order to demonstrate added values of the DEB-IBM in a context of mesocosms and risk assessment, we simulated mesocosm experiments testing hypothetical stressors that impact the physiological processes of sticklebacks and used the model to simulate the control distributions of the population endpoints for these experiments.

We tested the five DEBtox classical modes of action of toxicants (Billoir et al., 2008) but we chose the one with the strongest impacts on the model outputs: the so-called "synthesis cost impact" where reproduction and growth are both impacted by an increase of the cost of synthesis of a unit of structure [E_G] (Eq. 2). We presented another hypothetical toxicant which impacts directly the basal mortality M.n (Eq. 3) to model effects on survival.

$$[\mathrm{E}_G]_t = \frac{[\mathrm{E}_G]}{(1-s(Ct))} \text{ with } 0 < s(C_t) < 1 \quad \text{(Eq. 2)}$$

$$\mathrm{M.n}_t = \frac{\mathrm{M.n}}{(1-s(C_t))} \text{ with } 0 < s(C_t) < 1 \quad \text{(Eq. 3)}$$

With C_t the toxicant concentration and $s(C_t)$ the stress level.

These two modes of action were tested separately. The toxicant concentration C_t at time t was modeled to lead to a stress level $s(C_t)$ which we integrated in the model. The tested values for the stress level $s(C_t)$ ranged from 0 to 0.9 with an increment of 0.1. Inputs of year 2013 were used for the simulations. The stress levels of the toxicants during the simulation were modeled as constant.

As the experimental mesocosm platform comprises 12 artificial streams, one control and three chemical concentrations can be tested with three replicates per condition. To be representative of this experimental design, we designed hypothetical 1000 mesocosm experiments by simulating the population endpoints with three replicates of population per dose and three doses were kept leading to a stress level of 0.2, 0.5 and 0.8.

Then we compared two statistical methods to assess the toxicant effects on the population endpoints. First, the data in contaminated mesocosms was compared to the data in control conditions as usually made in mesocosm experiments (de Kermoysan et al., 2013a). This comparison was performed for each population endpoint at each concentration level with three replicates per condition using a Dunnett's post hoc test (hereafter referred as the classical methodology).

Secondly, the DEB-IBM was used to simulate the distribution of the control endpoints with 1000 simulations. Then, each population endpoint at each concentration level (with three replicates) was compared to the estimated distribution of the endpoints in control conditions using a Kolmogorov–Smirnov test (hereafter referred to as the methodology based on the estimation of the control endpoint distributions). Thus, the data collected in the control conditions of the mesocosm experiment would therefore not be used in this comparison.

We used both methodologies to determine the lowest observed effect concentrations (LOECs) for each endpoint. The level of significance for the tests was 5 %.

3. Results

3.1. Sensitivity analysis.

The local sensitivity analysis showed that the parameters that influenced the most the endpoints at the end of experiments were a parameter related to the male reproduction processes (Breeding.Period), the optimal temperature for the individual bioenergetic processes (CTO) and parameters related to the food: parameters determining the foraged water column volume (L_ref, R_ref), the prey size according to the mouth size (a_food, b_food, ratio_f) and the impact of the population density on the food availability (Appendix Part II.2.1. Figure A.7).

Based on the Morris sensitivity analysis (Appendix Part II.2.2. Figure A.8), we analyzed the importance of the different parameters on the outputs. Regarding population abundance, m_dens was clearly the most influential parameter. Regarding mean lengths and coefficients of variation, the parameters related to the food (R_ref, L_ref, a_food, b_food) and to a lesser degree K_dens, as well as parameters involved in male reproduction processes (R_diff, Proba.Stop) were the most influential parameters. Regarding the frequencies of females and juveniles, all parameters mentioned above were influent. These parameters presented both important means and standard deviations meaning that they have an overall strong influence on the outputs and present non-linear effects or strong interactions with other parameters.

Based on the local and Morris sensitivity analyses, the ten most influent parameters were prioritized for calibration: the density-dependent mortality (m_dens), the time between two reproduction events, the breeding period and the probability of stopping the reproduction processes for males (R_diff, Breeding.Period and Proba.Stop) as well as the food parameters (R_ref, L_ref, a_food, b_food, K_dens and a_Kdens). We also added two other parameters in the calibration: the inter-individual variability because it was only calibrated for founder

sticklebacks in mesocosms (David et al., 2018) and the parameter Bold.M responsible for the boldness of males in a foraging context as we had no information in literature.

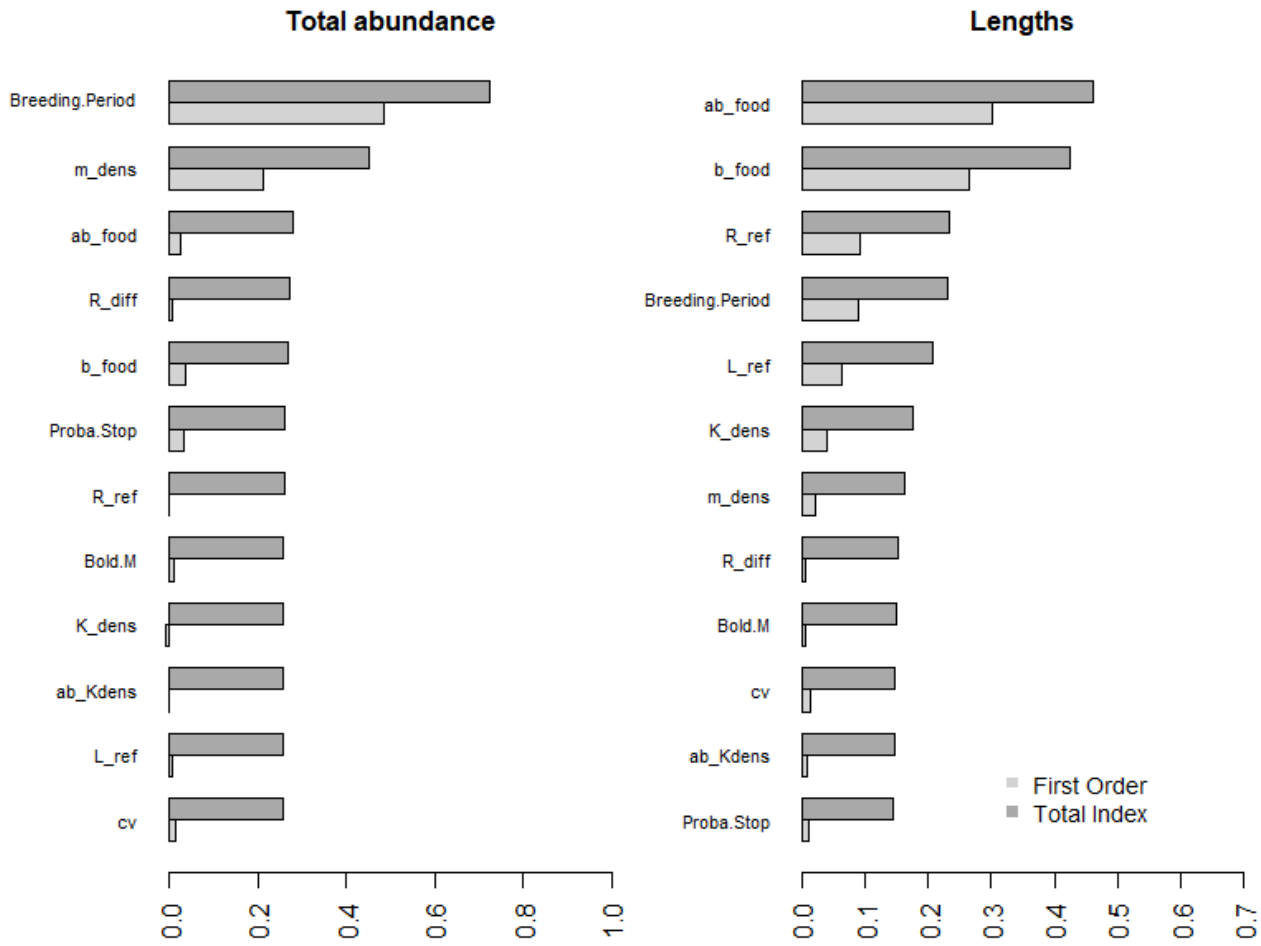

Figure 2: Results of the sensitivity analysis using the Sobol method. The graph on the left represents the first order and total index of the total abundance output and the graph on the right represents the first order and total index of the length outputs (averaged over each category of individuals). To avoid correlation between some parameters, a ratio between some parameters were used for the sensitivity analysis (ab_food for a_food/b_food and ab_Kdens for K_dens/a_Kdens corresponding respectively to the sensitivity analysis of a_food and a_Kdens).

Results of the sensitivity analyses using the Sobol method on the twelve chosen parameters (Figures 2 and A.9) highlighted the fact that interactions between these parameters were very important, suggesting that the calibration should be performed keeping all the chosen parameters together.

3.2. Model calibration.

The five independent calibrations of the parameters including the calibrations of the CV of the outputs converged to the same values which can be found in Table 1 and A.7. Figure 3 shows that the predictions of the population endpoints were consistent with the observations as all points were lined up on the identity line. The statistical analyses showed no significant differences (p-value > 0.05) between the observed and the simulated distributions of the descriptive variables of the population except for L.F.C00, L.F.CXX and CV.F.CXX of 2010. However, for these endpoints, the medians of the simulations were still included in the interval of confidence of the observed medians (see Appendix Part II.3.2. Figure A.10). Furthermore, if the 36 outputs for both years are considered to be independent binomial variates, 0 to 5 false positive results are expected ($\alpha = 0.05$). Distributions of the length frequencies of all sticklebacks and of mature males were well predicted for both years as seen in Appendix Part II.3.2. Figures A.11 and A.12.

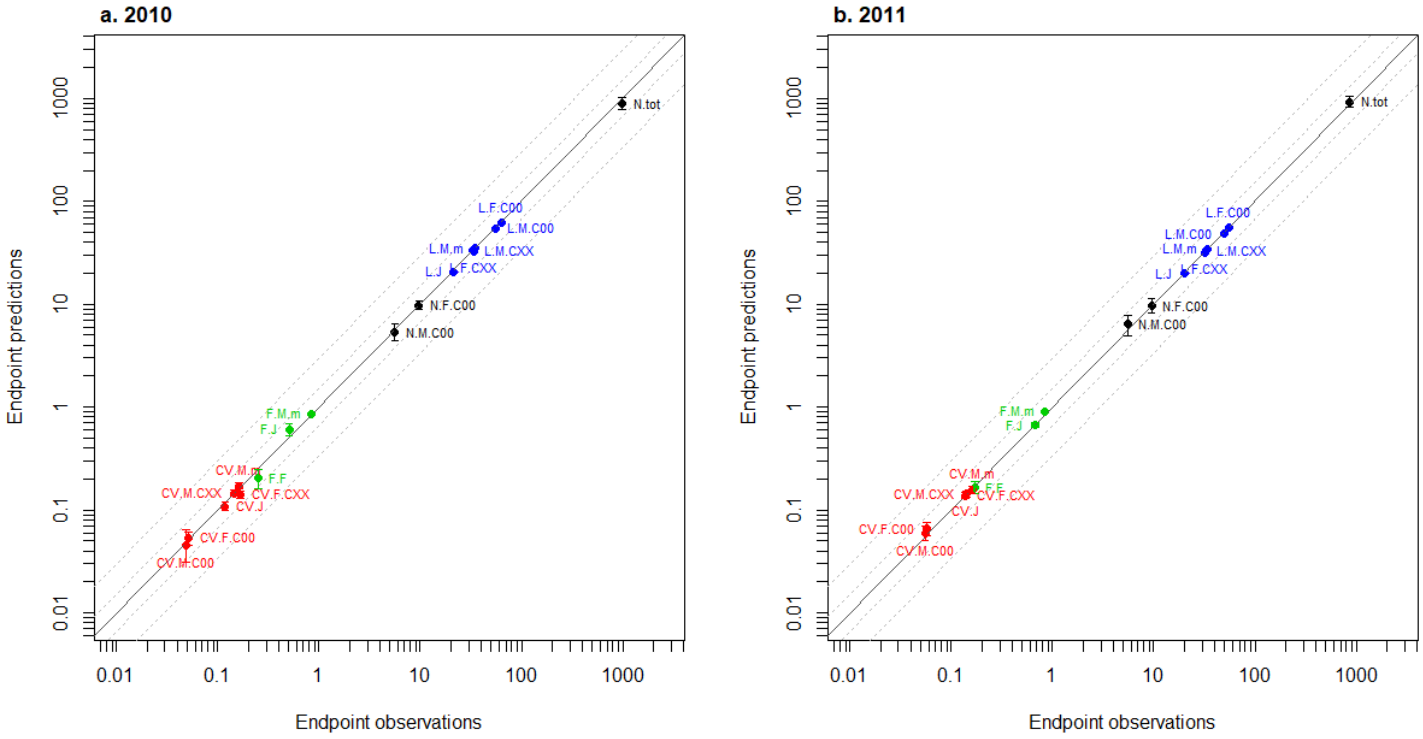

Figure 3: Results of the calibration for 2010 (a) and 2011 (b). The colors represent the type of endpoints: the abundance is in black, the mean lengths in blue, the frequencies in green and the CV of lengths in red. Dotted lines represent the 1.5-fold and 3-fold changes. The full line represents the identity line.

3.3. Model evaluation.

Daily mean temperatures follow the same dynamics in the 2012, 2013, and 2014 mesocosm experiments (see Appendix Part I.1.1.2. Figure A.2a). The largest variation was observed in 2013 when the most extreme temperatures were observed (4.3 and 21.3°C). Daylight data were the same every year (see Appendix Part I.1.1.2. Figure A.2b). Concerning data on food availability, zooplankton dynamics were similar in all three experiments (see Appendix Part I.1.1.2. Figure A.2c). A peak of zooplankton energy occurred during late spring and early summer every year, about 100 days after the beginning of the experiment. Energy from macroinvertebrates was lower in 2014 for both small (< 5 mm) and large (> 5 mm) macroinvertebrates. Finally, a peak was observed for both small and large macroinvertebrates during spring, 80-100 days after the beginning of the experiments, only in 2013.*

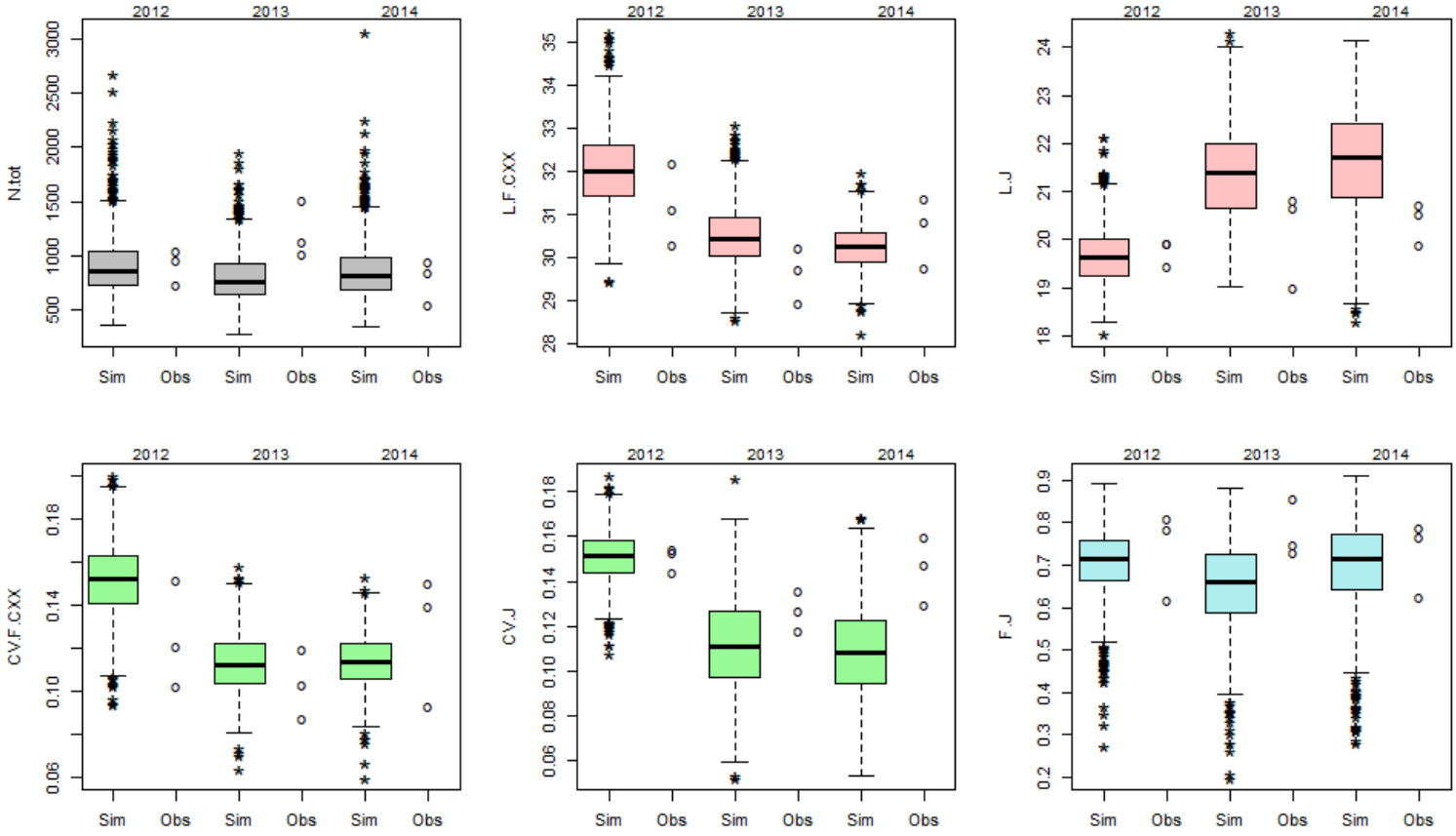

Figure 4: Predicted and observed stickleback population endpoints for each year of experiments. Boxplots represent the 1000 simulations and the points represent the observations in the control mesocosms.

The model gave overall good predictions of the population endpoints (Figures 4 and A.14). The model was able to reproduce differences in the population endpoints including inter-year variations between 2012, 2013 and 2014. Indeed, the observed endpoints were within the

interval range of the predictions. However, mean lengths of the founders of 2012 were slightly overestimated and the frequency of mature males in 2013 were overestimated (Appendix Part II.6. Figure A.14). Furthermore, our DEB-IBM gave overall good predictions of the length distributions (Figure 5) as all observations were within the predicted interval and the medians followed the shape of the observed frequencies. In details, a shift of the juvenile frequency peak was however observed in 2014 (observed peak at 18 mm and simulated peak lower, at 22 mm) which could be explained by the last breeding events at the end of the breeding period occurring latter than predicted (Figure 5c).

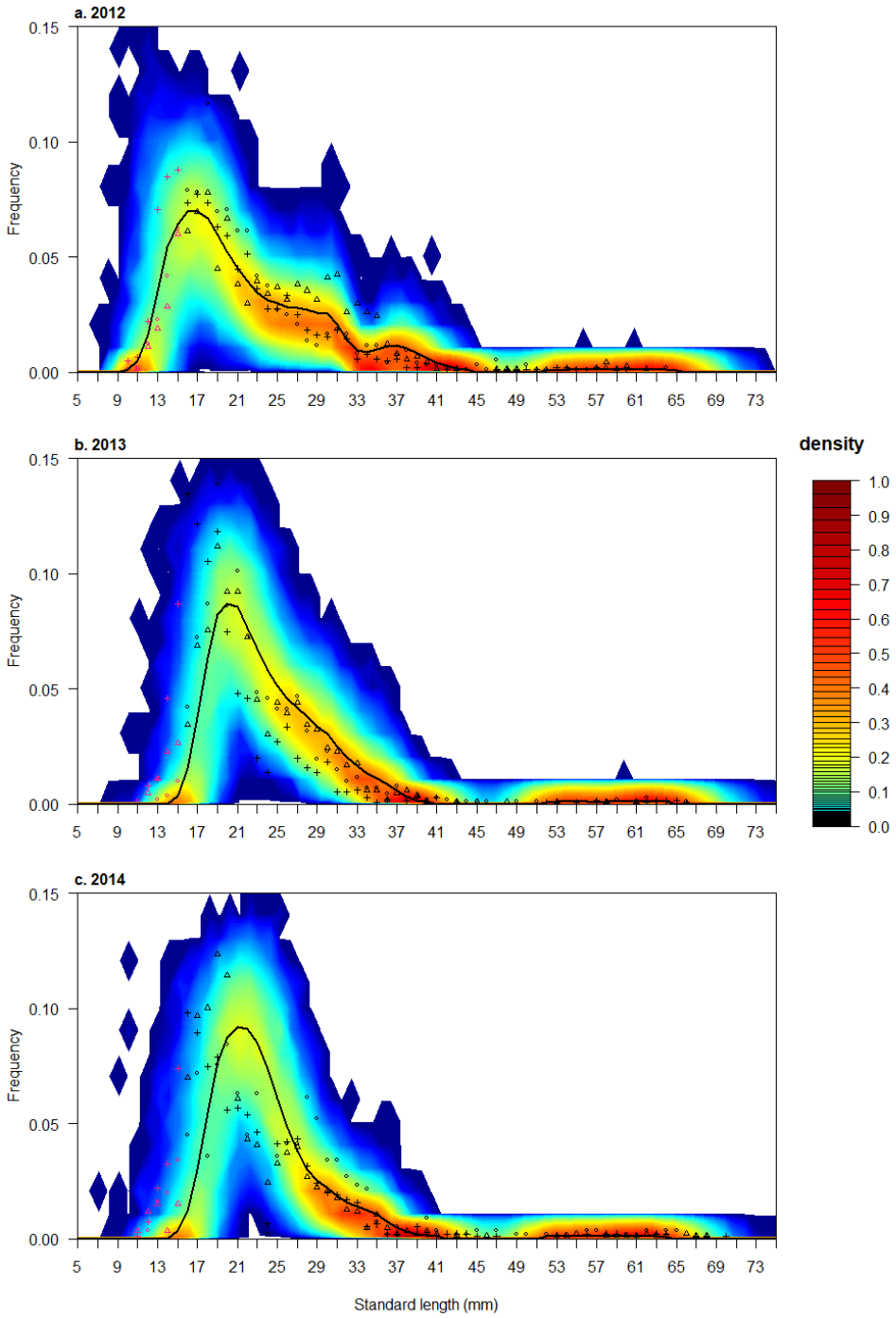

Figure 5: Probabilistic distributions of the length frequency predicted by the model length compared to frequency distributions observed in the control mesocosms for 2012 (a), 2013 (b) and 2014 (c). Circles, crosses and triangles represent the length frequency distributions of the three observed populations. Red points correspond to the class size that were excluded from the calibration process (individuals with a length smaller than 15 mm). Full black lines represent the median length frequency distributions of the simulated populations. Color level represents the frequency of simulated populations (n = 1000) having a given percentage of individuals for a given class length. Frequency inferior to < 1e-04 are represented in white.

3.4. Model application to analyze mesocosm experiment

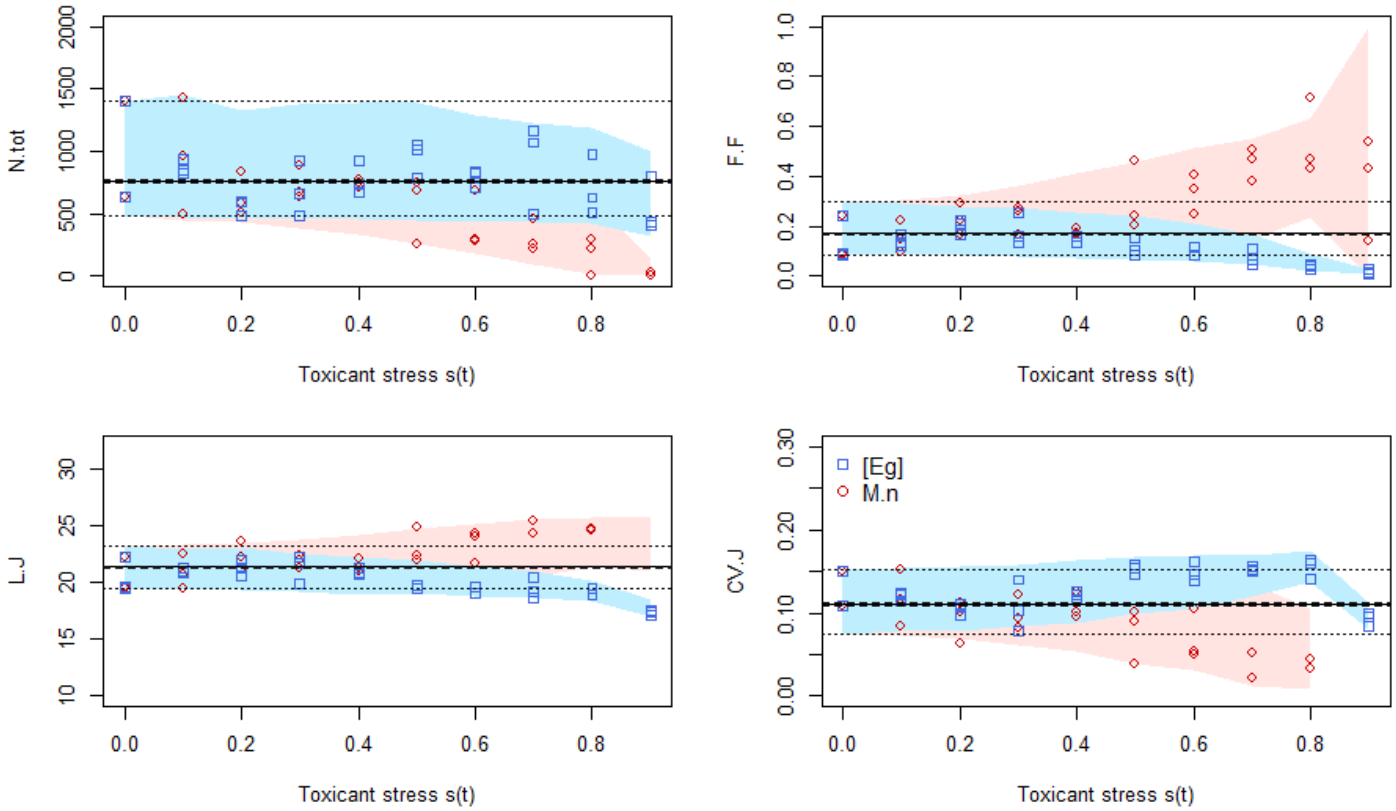

Figure 6: Effects of hypothetical toxicants with the two different tested modes of action and with 3 simulations. Red circles represent the simulations with the toxicant affecting the basal mortality and blue squares represent the simulations with the toxicant affecting the cost of synthesis of a unit of structure. Dashed grey lines represent the 95 % prediction interval of the median of the simulations in control conditions (1000 simulations) and dotted grey lines represent the 95 % prediction interval of the endpoints of the simulations in control conditions. Light red zone represents the 95 % prediction interval (1000 simulations) of the endpoints with the toxicant affecting the basal mortality and light blue zone represents the one from the toxicant affecting the cost of synthesis of a unit of structure.

Figures 6 and A.15 show predictions in an example of mesocosm experimental design (three simulated population dynamics), where stickleback populations were exposed to a hypothetical toxicant with increasing stress levels, as well as the 95 % prediction interval computed on 1000 model simulations in control conditions. Overall, the hypothetical toxicants impacted the population endpoints at stress levels greater than 60 %, the toxicant targeting the survival having greater impacts than the one targeting the cost of synthesis of a unit of structure (Figure 6). Figure 7 presents the comparison of the LOEC for the total abundance endpoint using the data in the three control replicates or using the estimation of the distribution of the abundance in control conditions, in the case of a toxicant targeting basal mortality. The mean LOEC

assessed using the methodology based on the estimation of the control endpoint distribution by the model was lower (0.72) than using the classical methodology (0.80). Furthermore, in 26 % of the cases, the classical methodology would have led us to conclude that there was no effect of the toxicant whereas there is one, whereas this occurred in 4.8 % of cases with the methodology presented here, based on the estimation of the control endpoint distributions. The results were the same for the other population endpoints (see Appendix Part III.2. Figures A.16 and A.17).

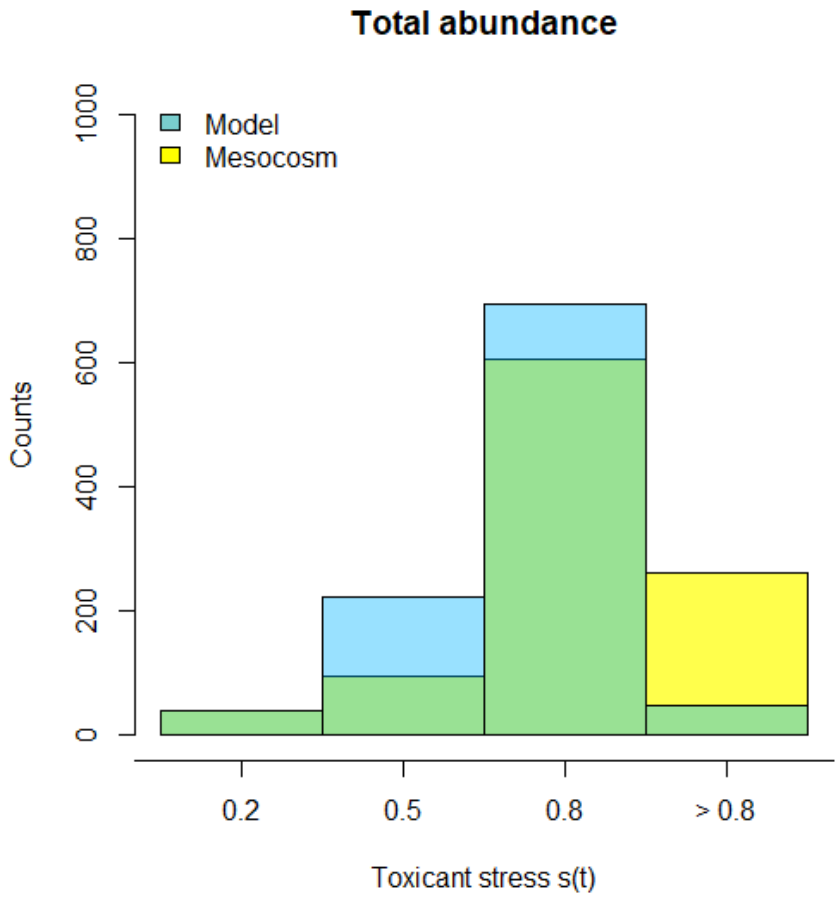

Figure 7: Comparison of the LOEC distributions calculated for the total abundance endpoint having a toxicant stress impacting the basal mortality. In yellow, the control was constituted by the three replicates available with the mesocosm experiment design. In blue, the distribution of the control endpoint was estimated by the model simulations (1000 simulations).

4. Discussion

The objective of this study was to develop a relevant tool to analyze data from mesocosm experiments and show its potential to better understand effects of hypothetical toxicants on population endpoints. For this purpose, we developed a DEB-IBM which describes stickleback

population dynamics in mesocosms based on the DEB model developed by Leloutre et al. (2018) and evaluated for founders in mesocosm (David et al. 2018). This original modelling approach integrates some ecological factors (photoperiod, water temperature, and food availability) and their interactions with the physiological processes at the organism level. Consequently, our model can help us explore the interactions between biotic, abiotic conditions as well as chemical exposure in mesocosm and improve the data analyze of these high tier ecotoxicological studies.

4.1. Model calibration

The DEB-IBM was calibrated on two independent datasets of population dynamics and was evaluated on three others. These datasets included data on stickleback population endpoints and the environmental conditions (climatic and physico-chemical conditions as well as data on the food webs communities) and were independent. Calibration of IBMs is not straightforward as they generally have many parameters with complex and interacting effects on outputs (Beaudouin et al., 2008; Grimm and Railsback, 2005) and using two datasets exhibiting different environmental conditions (high and low food level in 2010 and 2011 respectively, see Appendix Part I.1.1.1. Figure A.1) increases the difficulty of the calibration but avoids making the model too specific to some environmental conditions. To facilitate the calibration processes, we reduced the number of parameters to calibrate to twelve. The choice of parameters was based on the results of the sensitivity analyses and the level of knowledge of these parameters (Beaudouin et al., 2008; Grimm and Railsback, 2005). Thus, some parameters which appeared to be important in the sensitivity analyses were not recalibrated. For example, the optimal temperature was not refitted as it has been extensively studied and thus was well known (Hovel et al., 2015). Finally, the expected values of the endpoints of the calibration datasets were accurately predicted by our model. Furthermore, the inter-mesocosm variability of food density and mortality were calibrated and the overall variability of the endpoints resulting from inter-individual and inter-mesocosm variability was found to be realistic.

4.2. Density-dependent processes and inter-annual variability

Density-dependent mortality and growth were widely recognized as key mechanisms for fish population dynamics and were extensively studied (Hazlerigg et al., 2012; Lorenzen and Enberg, 2002; Webster, 2004). In our model, density-dependent mortality was calculated based

on the total number of individuals in the environment whereas effects of density-dependence on growth were dependent on the fish biomass as suggested by Lorenzen and Enberg (2002) and Hazlerigg et al. (2012). It is also well documented that in fish populations, competitive interactions between size classes produce complex dynamics (Claessen et al., 2000; Persson, 1988; Persson et al., 2000; Webster, 2004). Especially, when attacking the same prey, power relationships are unequal between adult and juvenile sticklebacks (Gill and Hart, 1996) as they do not have the same foraging capacities, which explains why larger sticklebacks could be more dominant during competition for food. Furthermore, intense intra-cohort competition may occur for juveniles because of their high density in mesocosms compared to the adult cohorts (Claessen et al., 2000), as observed for length frequencies (Figures 5 and A.11). To take into account these characteristics, a stronger density-dependent effect on juvenile growth was introduced compared to adults for a given population biomass.

In order to use the model in a regulatory context, robust validation is essential. However, by comparing data on stickleback populations over time, we observed that the mean lengths of the adult sticklebacks born in mesocosms decreased from 2010 to 2014 (see Appendix Part II.5. Figure A.13). This result could not be linked either to the food level, which was not particularly low in 2012, 2013 and 2014, or to the temperature scenarios, or to the founder lengths at the beginning of the experiment which were in the same range as 2011. To capture this process, as in several IBMs (Beaudouin et al., 2008; Cao et al., 2016; Kellner and Swihart, 2017), a year-dependent variation was integrated on one parameter (K_dens, level of density-dependence for growth) and this strongly improved the predictions of the model for 2012 to 2014. Indeed, as seen in figure 5, the model successfully captured the length frequencies of these years, meaning that the different cohorts of sticklebacks were well predicted. This parameter re-adjusted for each year is representative of the level of density-dependence for growth and thus, is indirectly linked to the environmental and biological conditions in which the sticklebacks evolved. Another solution could have been to readjust the DEB parameters influencing growth. For example, increasing the energetic cost per unit of structure would decrease the individual growth (Billoir et al., 2008). However, we did not keep this solution as individual growth data in mesocosms would be required to recalibrate the DEB model and the observed decrease in length might be reversible if the environment changes.

Another IBM for sticklebacks shows the same limits as our model regarding the difficulty of quantifying density-dependent growth for sticklebacks, as a readjustment of the IBM was required (Mintram et al., 2018). Several hypotheses could explain these inter-annual

differences. First, the annual variability of the natural vegetation establishment or colonization of the preys (*e.g*. Chironomidae) and predators (*e.g.* Dytiscidae) in mesocosms probably resulted in inter-annual variability of the habitat complexity and heterogeneity as well as differences in the food availability. Secondly, as this decrease seems to be linear over time, another explanation could be an acclimatization of the reared stickleback strains used for the mesocosm experiments as sticklebacks are known to rapidly evolve depending on their environment (Barrett et al., 2011; Crivelli and Britton, 1987; Mori and Takamura, 2004; Spence et al., 2013).

4.3. Model improvements

Globally, the mechanisms that contribute to population regulation are still not fully integrated in our DEB-IBM and represent a source of uncertainty. To improve the modeling, it would be interesting to acquire more information on population dynamics with different density levels in mesocosms as well as on genetic selective changes that could occur in captivity even when there is a new introduction of wild organisms (Ford, 2002) and on the phenomena of phenotypic plasticity (Via and Lande, 1985). In three-spined sticklebacks, it has been shown that both phenotypic plasticity and standing genetic variation could interact with traits related to the biomechanics of feeding showing a stronger genetic predisposition, whereas traits related to locomotion are mainly plastic (Lucek et al., 2014). Mori and Takamura (2004) also showed changes in stickleback body size but did not conclude about whether these morphological changes were related to adaptive phenotypic plasticity or to genetic divergence.

Other processes relative to food foraging and some reproduction processes could represent a source of uncertainty in the model. Indeed, the development of our model showed that modeling predation processes and stickleback foraging behavior was essential. As revealed by the sensitivity analyses, parameters related to food foraging had a strong influence on the outputs and exhibited strong interactions with other parameters. With increasing size, fish can become more explorative, exploiting different ecological niches and generally increase the mean size of prey that is eaten (Persson, 1988). Therefore, in our DEB-IBM, the size of the prey a stickleback can have access to varied linearly as a function of the mouth length of the stickleback as suggested by Gill and Hart (1994). Furthermore, as explained in David et al. (2018), we calculated the available food density each day and for each individual. Juveniles had a reduced predation distance as they tend to be hidden in the vegetation as a refuge from

cannibalism and thus have a less bold and explorative behavior during foraging (Foster et al., 1988). Inversely, males were considered to be slightly more bold than females as they show greater exploration of their environment in a foraging context (King et al., 2013). Accordingly, the adult male lengths results from two opposing processes: greater boldness of males leading to a faster growth of the young males (*i.e.* males born in mesocosms had slighter higher mean lengths than females in all experiments) and the parental cares leading to a lower growth for the active reproductive males (male founders in mesocosms).

Concerning the reproduction processes, egg production of females was described by the DEB model and the size of the clutch was estimated as a linear function of the female length fitted on laboratory data (Appendix Part I.3.1. Figure A.3 and Eq. (A.10)). However, female sticklebacks can retain their eggs for a short time if they do not find a nest to spawn. Afterward, they can spawn the clutch even if a male is not present or, occasionally, develop overripe eggs (Lam et al., 2011; Sokołowska, 2006; Wootton, 1984). For more realism, this phenomenon was introduced in the model. However, uncertainties on the process are important as it is still poorly understood. In particular, the length of time a female can keep the eggs in the ovarian cavity is not well known. Nevertheless, the sensitivity analyses of our model revealed that the length of time during which females can keep eggs had a low impact on the model outputs.

In comparison to females, stickleback males exhibit an important energy investment in reproductive behavior (Smith and Wootton, 1999; Van Iersel, 1953; Wootton, 1984). In our DEB-IBM, an approach to link the energy expenditure in reproductive behavior to the energy for reproduction predicted by the DEB model was developed. To do that, we extrapolated the amount of energy needed for the duration of one reproduction success based on the study of Smith and Wootton (1999) and Mori (1993) to use it as a threshold for males. Thus, males which do not have enough energy to accomplish one reproduction process would not acquire their reproductive behavior (*i.e* get a territory, build a nest, harvest and take care of the eggs). Furthermore, males in the field need to be at least 1 or 2 years old to start the reproduction processes (Baker, 1994; Dufresne et al., 1990). This was consistent with the absence of sexual dimorphism observed in our mesocosm between young adult female and male sticklebacks (*i.e.* adults born in mesocosm) due to the absence of reproductive behavior during the first year in young males. In our modeling of their reproductive behavior, males born in mesocosms do not start the reproduction processes during their first spring. In addition, another interest of using the energy for reproduction predicted by the DEB model for both males and females is that the

reproduction performances would depend on environmental conditions as widely described in literature (Borg, 1982; Hopkins et al., 2010; Wootton, 1973, 1984).

Although it has been shown that temperature and a photoperiod threshold seem to induce the beginning of the breeding period (Wootton, 1984), factors influencing the end of this period are still not well understood. As the breeding period typically takes place between late March and early August (Sokołowska, 2006; Wootton, 1984), the duration of the breeding period was introduced as a parameter in the model to control reproduction. However, the understanding of the environmental causes influencing the end of the reproduction should be increased, because, as seen in the sensitivity analyses, the parameter Breeding.Period had a strong influence on the model outputs. Another factor controlling the end of the reproduction is the number of successful nests (Mori, 1993). The number of successful nests predicted by the model, between one and four for the mesocosm experiments of 2010 and 2011, is consistent with the observations of Mori (1993) in field (*i.e.* between one and three successful nests during the breeding period).

Finally, improving our DEB-IBM by modeling spatial variations of the environment in addition to the temporal variations of some variables would be highly interesting to account for specific interactions between individuals and their environment (nursery areas, refuge from predation, deferential exploitation of the food patches, etc.). Spatially-explicit bioenergetics IBMs have already been developed for fish populations (Boyd et al., 2018; Politikos et al., 2015) and performed well in modelling the fish habitat as well as the movements towards favorable feeding areas. Furthermore, modelling fish distribution in response to an environmental change would be interesting in environmental management and risk assessment.

4.4. Model application to analyze mesocosm experiment

The predictions of our model in control conditions could represent a relevant baseline for determining magnitude and statistical significance of the effects in exposed populations and could help to better understand toxicant impacts on stickleback populations. Indeed, the small number of mesocosm replicates combined with the important variability due to the high levels of biological organization (EFSA, 2013; Sanderson, 2002) have been presented as a major drawback for detecting significant effects of chemicals in mesocosms as it is a source of false negative results (i.e. concluding a chemical has no effect when it really has) (Shaw et al., 1994; Beaudouin et al. 2008, EFSA, 2013).

Here, we illustrated an application of our DEB-IBM to mesocosm experiments by integrating effects of hypothetical toxicants having different modes of action (Galic et al., 2017; Martin et al., 2013). Overall, a toxicant which would affect the basal mortality as well as the cost of synthesis of a unit of structure would have strong effects on the population structure. By comparing the classical methodology to the methodology based on the estimation of the control endpoint distributions, we showed that the LOECs calculated with the model simulations were lower than the ones calculated using the three replicates of the control. This highlights the interest of our approach which could improve environmental risk assessment by decreasing the risk of false negative results when analyzing data from mesocosms. Indeed, using IBMs to simulate the endpoint distributions of populations in mesocosms allows to estimate the descriptive variables of the populations (i.e. the shape, expectation and variance) compared to a simple estimation from the observed data from mesocosms (Beaudouin et al., 2012b). Furthermore, we assumed here only one type of effect by toxicants explaining why the populational effects have been mainly seen with a high stress level (Figure 5). However, toxicants could have multiple impacts (i.e. combined effects on survival and reproduction for example) which could increase the populational responses.

Finally, although the ecological realism of mesocosm experiments is strongly improved compared to laboratory tests, theses aquatic model ecosystems are characterized by a reduction in size and complexity when compared to natural aquatic ecosystems (Caquet et al., 2000; EFSA, 2013) as they are designed to answer to a specific question. Consequently, other natural processes could occur in natural ecosystems, as for example competition between several fish species, which does not occur in our mesocosm experiments and thus, are not modelled in our IBM.

5. Conclusions

A model which accurately predicts population dynamics over time and takes into account relevant information on the environment, is of great interest for ecological management, long-term decision making and risk assessment. Here, we developed a DEB-IBM for predicting stickleback population dynamics in mesocosms taking into account the main environmental factors. The model was successfully calibrated and the predictions of the population dynamics in control conditions were accurate. Hence, as seen with the case study of hypothetical toxicants, the model can help extrapolate from individual mechanistic responses to the population level. The DEB-IBM should then help us to have a closer understanding of toxicant

effects on stickleback population dynamics and improve environmental risk assessment by lowering the uncertainty of the toxic thresholds.

Acknowledgments

The authors wish to thank G. De Kermoysan and all the interns (Y. Guénand, P. Miguet, D. Georges, N. Torchaire, V. Martz, E. Phyron, H. Guillot, M. Palos-Ladeiro, M. Lonjaret, M.Blachon, L.Roy and A. Bernard) who contributed to the success of the mesocosm experiments. This study was supported by the 190 program of the Ministry of Ecology and DOREMIPHARM, funded by ANSM (French National Agency for Medicines and Health Products Safety) as well as by the European Regional Development Fund (ERDF). The authors also thank the three anonymous reviewers for their helpful suggestions for improvement and their constructive remarks.

References

Adams, S.M., 2002. Biological indicators of aquatic ecosystem stress. American Fisheries Society.

Baker, J.A., 1994. Life history variation in female threespine stickleback., in: Publications, O.S. (ed.), The Evolutionary Biology of the Threespine Stickleback, pp. 144–187.

Barrett, R.D., Paccard, A., Healy, T.M., Bergek, S., Schulte, P.M., Schluter, D., Rogers, S.M., 2011. Rapid evolution of cold tolerance in stickleback. Proc. Biol. Sci. 278, 233-238.

Beaudouin, R., Dias, V., Bonzom, J.M., Pery, A., 2012a. Individual-based model of *Chironomus riparius* population dynamics over several generations to explore adaptation following exposure to uranium-spiked sediments. Ecotoxicology 21, 1225-1239.

Beaudouin, R., Ginot, V., Monod, G., 2012b. Improving mesocosm data analysis through individual-based modelling of control population dynamics: a case study with mosquitofish (*Gambusia holbrooki*). Ecotoxicology 21, 155-164.

Beaudouin, R., Goussen, B., Piccini, B., Augustine, S., Devillers, J., Brion, F., Pery, A.R., 2015. An individual-based model of zebrafish population dynamics accounting for energy dynamics. PLoS One 10, e0125841.

Beaudouin, R., Monod, G., Ginot, V., 2008. Selecting parameters for calibration via sensitivity analysis: An individual-based model of mosquitofish population dynamics. Ecol. Modell. 218, 29-48.

Bell, A.M., Foster, S.A., 1994. Introduction to the evolutionary biology of the threespine stickleback. The evolutionary biology of the threespine stickleback, vol. Chapter 1. Oxford University Press, pp. 1-28.

Billoir, E., Delignette-Muller, M.L., Pery, A.R., Geffard, O., Charles, S., 2008. Statistical cautions when estimating DEBtox parameters. J. Theor. Biol. 254, 55-64.

Borg, B., 1982. Seasonal effects of photoperiod and temperature on spermatogenesis and male secondary sexual characters in the three-spined stickleback, *Gasterosteus aculeatus L.* Can. J. Zool. 60, 3377-3386.

Boyd, R., Roy, S., Sibly, R., Thorpe, R., Hyder, K., 2018. A general approach to incorporating spatial and temporal variation in individual-based models of fish populations with application to Atlantic mackerel. Ecol. Modell. 382, 9-17.

Cao, J., Guan, W., Truesdell, S., Chen, Y., Tian, S., 2016. An individual-based probabilistic model for simulating fisheries population dynamics. Aquac. Fish. 1, 34-40.

Caquet, T., 2002. Use of aquatic mesocosms in ecotoxicology, state of the art and perspectives. Radioprotection - Colloques 37.

Caquet, T., Lagadic, L., Sheffield, S.R., 2000. Mesocosms in ecotoxicology (1): Outdoor aquatic systems. Rev. Environ. Contam. Toxicol. 165, 1-38.

Claessen, D., Roos, A.M.d., Persson, L., 2000. Dwarfs and Giants: Cannibalism and Competition in Size-Structured Populations. Am. Nat. 155, 219-237.

Crivelli, A.J., Britton, R.H., 1987. Life history adaptations of *Gasterosteus aculeatus* in a Mediterranean wetland. Environ. Biol. Fishes 18, 109-125.

David, V., Goussen, B., Tebby, C., Joachim, S., Porcher, J.M., Beaudouin, R., 2018. Modelling historical mesocosm data: Application of a fish bioenergetics model in semi-natural conditions. Ecol. Freshw. Fish 27, 1101-1113.

De Kermoysan, G., 2013. Caractérisation de la dynamique de population de l'épinoche à trois épines, *Gasterosteus aculeatus*, dans un mésocosme lotique : Application à l'évaluation des effets du bisphénol A dans un contexte écosystémique. AgroParisTeach, p. 253.

de Kermoysan, G., Joachim, S., Baudoin, P., Lonjaret, M., Tebby, C., Lesaulnier, F., Lestremau, F., Chatellier, C., Akrour, Z., Pheron, E., Porcher, J.M., Pery, A.R., Beaudouin, R., 2013a. Effects of bisphenol A on different trophic levels in a lotic experimental ecosystem. Aquat. Toxicol. 144-145, 186-198.

de Kermoysan, G., Pery, A.R., Porcher, J.M., Beaudouin, R., 2013b. A non-invasive method based on head morphology to sex mature three-spined stickleback (*Gasterosteus aculeatus L.*) in rearing conditions. Math. Biosci. 244, 148-153.

Dufresne, F., FitzGerald, G.J., Lachance, S., 1990. Age and size-related differences in reproductive success and reproductive costs in threespine sticklebacks (*Gasterosteus aculeatus*). Behav. Ecol. 1, 140-147.

EFSA, 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 11, 3290.

Forbes, V.E., Calow, P., Grimm, V., Hayashi, T.I., Jager, T., Katholm, A., Palmqvist, A., Pastorok, R., Salvito, D., Sibly, R., Spromberg, J., Stark, J., Stillman, R.A., 2011. Adding Value to Ecological Risk Assessment with Population Modeling. Hum. Ecol. Risk. Assess. 17, 287-299.

Forbes, V.E., Salice, C.J., Birnir, B., Bruins, R.J.F., Calow, P., Ducrot, V., Galic, N., Garber, K., Harvey, B.C., Jager, H., Kanarek, A., Pastorok, R., Railsback, S.F., Rebarber, R., Thorbek, P., 2017. A Framework for Predicting Impacts on Ecosystem Services From (Sub)Organismal Responses to Chemicals. Environ. Toxicol. Chem. 36, 845-859.

Ford, M.J., 2002. Selection in Captivity during Supportive Breeding May Reduce Fitness in the Wild. Conserv. Biol. 16, 815-825.

Foster, S.A., Garcia, V.B., Town, M.Y., 1988. Cannibalism as the cause of an ontogenetic shift in habitat use by fry of the threespine stickleback. Oecologia 74, 577-585.

Galic, N., Grimm, V., Forbes, V.E., 2017. Impaired ecosystem process despite little effects on populations: modeling combined effects of warming and toxicants. Glob. Chang. Biol. 23, 2973-2989.

Galic, N., Hommen, U., Baveco, J.M., van den Brink, P.J., 2010. Potential application of population models in the European ecological risk assessment of chemicals II: Review of models and their potential to address environmental protection aims. Integr. Environ. Assess. Manag. 6, 338-360.

Gill, A.B., Hart, P.J.B., 1994. Feeding behaviour and prey choice of the threespine stickleback, the interacting effects of prey size, fish size and stomach full. Anim. Behav. 47.

Gill, A.B., Hart, P.J.B., 1996. Unequal competition between three-spined stickleback, *Gasterosteus aculeatus*, *L.*, encountering sequential prey. Anim. Behav. 51, 689-698.

Ginot, V., Gaba, S., Beaudouin, R., Aries, F., Monod, H., 2006. Combined use of local and ANOVA-based global sensitivity analyses for the investigation of a stochastic dynamic model: Application to the case study of an individual-based model of a fish population. Ecol. Modell. 193, 479-491.

Grimm, V., Ayllón, D., F. Railsback, S., 2017. Next-Generation Individual-Based Models Integrate Biodiversity and Ecosystems: Yes We Can, and Yes We Must. Ecosystems 20, 229–236.

Grimm, V., Berger, U., DeAngelis, D.L., Polhill, J.G., Giske, J., Railsback, S.F., 2010. The ODD protocol A review and first update. Ecol. Modell. 221, 2760-2768.

Grimm, V., Railsback, S.F., 2005. Individual-based Modeling and Ecology, Princeton ed, New Jersey.
Hazlerigg, C.R., Lorenzen, K., Thorbek, P., Wheeler, J.R., Tyler, C.R., 2012. Density-dependent processes in the life history of fishes: evidence from laboratory populations of zebrafish *Danio rerio*. PLoS One 7, e37550.

Hopkins, K., Moss, B.R., Gill, A.B., 2010. Increased ambient temperature alters the parental care behaviour and reproductive success of the three-spined stickleback (*Gasterosteus aculeatus*). Environ. Biol. Fishes 90, 121-129.

Hovel, R.A., Beauchamp, D.A., Hansen, A.G., Sorel, M.H., 2015. Development of a Bioenergetics Model for the Threespine Stickleback. Trans. Am. Fish. Soc. 144, 1311-1321.

Kellner, K.F., Swihart, R.K., 2017. Simulation of oak early life history and interactions with disturbance via an individual-based model, SOEL. PLoS One 12, e0179643.

King, A.J., Fürtbauer, I., Mamuneas, D., James, C., Manica, A., 2013. Sex-Differences and Temporal Consistency in Stickleback Fish Boldness. PLoS One 8, e81116.

Kooijman, S.A., 2010. Dynamic Energy Budget for metabolic organization, Third Edition ed. Cambridge University Press, Cambridge.

Kooijman, S.A.L.M., Van Der Hoeven, N., Van Der Werf, D.C., 1989. Population Consequences of a Physiological Model for Individuals. Funct. Ecol. 3, 325-336.

Lam, T.J., Nagahama, Y., Chan, K., Hoar, W.S., 2011. Overripe eggs and postovulatory corpora lutea in the threespine stickleback, *Gasterosteus aculeatus L.*, form trachurus. Can. J. Zool. 56, 2029-2036.

Leloutre, C., Péry, A.R.R., Porcher, J.-M., Beaudouin, R., 2018. A bioenergetics model of the entire life cycle of the three-spined stickleback, *gasterosteus aculeatus.* Ecol. Freshw. Fish 27, 116-127.

Lorenzen, K., Enberg, K., 2002. Density-dependent growth as a key mechanism in the regulation of fish populations: evidence from among-population comparisons. Proc. Biol. Sci. 269, 49-54.

Lucek, K., Sivasundar, A., Seehausen, O., 2014. Disentangling the role of phenotypic plasticity and genetic divergence in contemporary ecotype formation during a biological invasion. Evolution 68, 2619-2632.

Martin, B.T., Jager, T., Nisbet, R.M., Preuss, T.G., Hammers-Wirtz, M., Grimm, V., 2013. Extrapolating ecotoxicological effects from individuals to populations: a generic approach based on Dynamic Energy Budget theory and individual-based modeling. Ecotoxicology 22, 574-583.

Maunder, R.J., Matthiessen, P., Sumpter, J.P., Pottinger, T.G., 2007. Impaired reproduction in three-spined sticklebacks exposed to ethinyl estradiol as juveniles. Biol. Reprod. 77, 999-1006.

Mintram, K.S., Brown, A.R., Maynard, S.K., Liu, C., Parker, S.-J., Tyler, C.R., Thorbek, P., 2018. Assessing population impacts of toxicant-induced disruption of breeding behaviours using an individual-based model for the three-spined stickleback. Ecol. Modell. 387, 107-117.

Mori, S., 1993. The Breeding System of the 3-Spined Stickleback, *Gasterosteus-Aculeatus* (Forma-Leiura) with Reference to Spatial and Temporal Patterns of Nesting Activity. Behaviour 126, 97-124.

Mori, S., Takamura, N., 2004. Changes in morphological characteristics of an introduced population of the threespine stickleback *Gasterosteus aculeatus* in Lake Towada, northern Japan. Ichthyol. Res. 51, 295-300.

Morris, M.D., 1991. Factorial Sampling Plans for Preliminary Computational Experiments. Technometrics 33, 161-174.

Naslund, J., Fick, J., Asker, N., Ekman, E., Larsson, D.G.J., Norrgren, L., 2017. Diclofenac affects kidney histology in the three-spined stickleback (*Gasterosteus aculeatus*) at low mug/L concentrations. Aquat. Toxicol. 189, 87-96.

OECD, 2006. Guidance document on simulated freshwater lentic field tests, in: OECD, Environment, Directorate (Eds.), Paris, p. 37.

Persson, L., 1988. Asymmetries in Competitive and Predatory Interactions in Fish Populations, in: Ebenman, B., Persson, L. (Eds.), Size-Structured Populations. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 203-218.

Persson, L., Bystrom, P., Wahlstrom, E., 2000. Cannibalism and Competition in Eurasian Perch: Population Dynamics of an Ontogenetic Omnivore. Ecology 81, 1058-1071.

Politikos, D., Somarakis, S., Tsiaras, K.P., Giannoulaki, M., Petihakis, G., Machias, A., Triantafyllou, G., 2015. Simulating anchovy's full life cycle in the northern Aegean Sea (eastern Mediterranean): A coupled hydro-biogeochemical–IBM model. Prog. Oceanogr. 138, 399-416.

Roussel, H., Joachim, S., Lamothe, S., Palluel, O., Gauthier, L., Bonzom, J.M., 2007. A long-term copper exposure on freshwater ecosystem using lotic mesocosms: individual and population responses of three-spined sticklebacks (*Gasterosteus aculeatus*). Aquat. Toxicol. 82, 272-280.

Ruden, C., Adams, J., Agerstrand, M., Brock, T.C., Poulsen, V., Schlekat, C.E., Wheeler, J.R., Henry, T.R., 2016. Assessing the relevance of ecotoxicological studies for regulatory decision making. Integr. Environ. Assess. Manag.

Saltelli, A., Annoni, P., Azzini, I., Campolongo, F., Ratto, M., Tarantola, S., 2010. Variance based sensitivity analysis of model output. Design and estimator for the total sensitivity index. Comput. Phys. Commun. 181, 259-270.

Sanderson, H., 2002. Pesticide studies. Environ. Sci. Pollut. Res. 9, 429-435.

Sibly, R.M., Grimm, V., Martin, B.T., Johnston, A.S.A., Kułakowska, K., Topping, C.J., Calow, P., Nabe-Nielsen, J., Thorbek, P., DeAngelis, D.L., 2013. Representing the acquisition and use of energy by individuals in agent-based models of animal populations. Methods Ecol. Evol. 4, 151-161.

Smith, C., Wootton, R.J., 1999. Parental energy expenditure of the male three-spined stickleback. J. Fish Biol. 54, 1132-1136.

Sobol', I.M., Tarantola, S., Gatelli, D., Kucherenko, S.S., Mauntz, W., 2007. Estimating the approximation error when fixing unessential factors in global sensitivity analysis. Reliab. Eng. Syst. Safe. 92, 957-960.

Sokołowska, E.a.K., E., 2006. Annual reproductive cycle in two free living populations of three-spined stickleback, patterns of ovarian and testicular development. Oceanologia 48, 103-121.

Spence, R., Wootton, R.J., Barber, I., Przybylski, M., Smith, C., 2013. Ecological causes of morphological evolution in the three-spined stickleback. Ecol. Evol. 3, 1717-1726.

van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57-149.

Van Iersel, J.J.A., 1953. An Analysis of the Parental Behaviour of the Male Three-Spined Stickleback (*Gasterosteus Aculeatus L.*). Behaviour. Supplement, III-159.

Via, S., Lande, R., 1985. Genotype-Environment Interaction and the Evolution of Phenotypic Plasticity. Evolution 39, 505-522.

Webster, M.S., 2004. Density Dependence via Intercohort Competition in a Coral-Reef Fish. Ecology 85, 986-994.

Wootton, R.J., 1973. The effect of size of food ration on egg production in the female three-spined stickleback, *Gasterosteus aculeatus L.* J. Fish Biol. 5, 89-96.

Wootton, R.J., 1984. A Functional Biology of Sticklebacks, Peter Calow, Department of Zoology ed, University of Sheffield, England.