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Abstract: Bisphenol A (BPA), a well-known endocrine-disrupting chemical, is ubiquitously present in the aquatic environment. Its impacts at the population level on three-spined sticklebacks have been studied in artificial streams with low-dose BPA concentrations. The causes explaining the observed effects remained unclear. Here, we used an individual-based model coupled to a Dynamic Energy Budget model to (i) assess the potential of modelling to predict impacts at the population level using individual level laboratory ecotoxicological endpoints and (ii) provide insight on the mechanisms of BPA toxicity in these mesocosms. To do that, both direct and indirect effects of BPA on three-spined sticklebacks were incorporated in the model. Indeed, direct BPA effects on fish have been identified based on literature data whereas indirect effects on sticklebacks have been taken into account using sampling data of their prey from the exposed artificial streams. Results of the modelling showed that direct BPA effects on fish (impacts on gonad formation, growth, male reproductive behavior, eggs and larvae survival) mainly explained the three-spined stickleback population structure in the mesocosms, but indirect effects were not negligible. Hence, this study showed the potential of modelling in risk assessment to predict the impacts on fish population viability from behavioural and physiological effects measured on organisms.

Keywords: bisphenol A, individual-based model, risk assessment, mesocosms, three-spined sticklebacks

1. Introduction

Bisphenol A (BPA) is of a scientific and public interest due to its potential adverse effects on exposed organisms and its ubiquity in the environment (Flint et al. 2012, Im and Löffler 2016). This chemical is mainly used for the synthesis of polycarbonate and epoxy resins (Beausoleil et al. 2018). Furthermore, BPA is one of the most investigated substances for its endocrine disruptor properties and the regulatory consideration of this substance has been a challenge during the last decades (Beausoleil et al. 2018).

Many studies have shown that BPA can impact different aquatic organisms as effects on growth, reproduction and survival were observed for macrophytes, mollusks, crustaceans, insects, zooplankton and amphibians (Flint et al. 2012, Canesi and Fabbri 2015). Furthermore, BPA can also impact different fish species as shown by several studies at the individual level (Flint et al. 2012, Canesi and Fabbri 2015). More specifically, BPA induces synthesis of vitellogenin at concentrations ranging from 10 to 1360 µg/L depending on the fish species (Ishibashi et al. 2001, Hatef et al. 2012, Mihaich et al. 2012, Villeneuve et al. 2012, Allner et al. 2016, Negintaji et al. 2018) and fish gonads could also present abnormal structure starting from 1 µg/L (Mandich et al. 2007, Molina et al. 2013, Chen et al. 2017). Effects on eggs and embryos were observed such as hatching delay, embryo mortality, embryo abnormality and disruption of the hatching success starting from 10 µg/L (Shioda and Wakabayashi 2000, Aluru et al. 2010, Staples et al. 2011, Bhandari et al. 2015, Fan et al. 2018). Other impacts on physiological processes, such as growth, development and general metabolism, were also shown in fish (Flint et al. 2012, Canesi and Fabbri 2015).

In a long-term experiment, effects of BPA on three-spined stickleback (*Gasterosteus aculeatus*) population dynamics have been tested in artificial streams (*i.e.* mesocosms) using environmentally realistic exposure conditions (1, 10 and 100 µg/L) (de Kermoysan et al. 2013).

Mesocosm experiments have the advantage to test a chemical in relevant environmental conditions and investigate the effects at different levels of biological organization (Caquet et al. 2000, EFSA 2013). In de Kermoysan et al. (2013), both individual and population level effects on three-spined sticklebacks were observed. Indeed, gonad morphology of females and males was impacted respectively at 1 µg/L and 10 µg/L respectively. Concerning population level endpoints, the fish length frequencies distribution shifted to higher lengths compared to the control at the 100 µg/L BPA treatment. However, the causes leading to the observed population effects remained unclear. Hence, to improve environmental risk assessment, it is essential to provide a robust understanding of ecosystem functioning and population dynamics under toxicant stress, which is one of the challenges of ecological modelling (Goussen et al. 2016, Forbes et al. 2017).

Individual-based models (IBMs) allow to extrapolate ecotoxicological effects from individuals to populations (Forbes et al. 2008). Furthermore, coupling an IBM to a Dynamic Energy Budget (DEB) model offers great flexibility for integrating individual impacts on bioenergetic processes such as growth and reproduction (Beaudouin et al. 2012, Martin et al. 2013). A DEB-IBM has been developed to describe three-spined stickleback population dynamics in mesocosms under control conditions (David et al. 2019). The mesocosm experimental design considered in the model was the same as the one used in de Kermoysan et al. (2013). Thus, this model can be adapted to predict a toxicant exposure and the potential impacts on the biotic and abiotic compartments in order to improve the understanding of the population responses found in the artificial streams. Hence, this would help to validate the modelling approach for predicting toxicant effects which is increasingly needed in risk assessment (Galic et al. 2010, Hommen et al. 2010, Forbes et al. 2017)

Accordingly, we aimed at integrating BPA effects in the DEB-IBM developed by David et al. (2019) to (i) assess the potential of modelling to predict impacts on population viability using individual level ecotoxicological endpoints and (ii) provide insights on the mechanisms of BPA

toxicity during the artificial stream experiment. Thus, both direct and indirect effects of BPA on three-spined sticklebacks were incorporated in the model. Direct effects were integrated via dose-response curves on physiological processes of fish whereas indirect effects were integrated by using prey abundances measured in the exposed artificial streams. Finally, the model predictions were compared to the experimental fish population data observed in the mesocosm experiment in order to assess the accuracy of the predictions and conclude on BPA impacts at the population level.

2. Material and methods

2.1. Mesocosm experiment

A complete detailed description of the experimental design and the data is given in de Kermoysan et al. (2013). Briefly, the experiment was performed using twelve artificial streams of 20-m long and 1-m wide, locating in the North of France (INERIS, Verneuil-en-Halatte, France). Three nominal concentrations of BPA were tested (1, 10 and 100 µg/L) with 3 replicates as well as three controls. Before exposure, each mesocosm was set up with sediments, macrophytes and macroinvertebrates between October and December 2011. On March 5th, 2012, 15 female and 10 male sticklebacks (after called the founders) with similar length were introduced in each artificial stream (mean lengths of 42.95 ± 1.40 mm and 44.36 ± 2.50 mm, respectively for females and males). Exposure began on April 15th, 2012 and ended on September 28th, 2012. Treatment for each mesocosm was randomly chosen. BPA water concentrations were monitored every month at different locations (0, 5 and 19 m from the inlet of water). Water temperature was measured every 10 minutes using temperature sensors (HOBO0257, Prosensor, Amanvilliers, France). Abundance and diversity of macroinvertebrates and zooplankton communities were sampled every four weeks (details on the methods used for temperature measurements and prey samplings are provided in David et al. (2018)).

Mesocosms were emptied on October 1st, 2012 and, all the fish of the 12 populations were recovered, measured and weighed. Fish with a length inferior to 26 mm were assumed to be immature and thus classified as juveniles. Sexes of fish with a length superior to 26 mm were determined by visual observations of the gonads. Descriptive variables of the populations were the population abundance (N.tot), the number of female and male founders at the end of the experiment (N.F.C00, N.M.C00), female and juvenile frequencies (F.F and F.J) and mean lengths and the coefficients of variation of the lengths of five categories of individuals: male (L.M.C00 and CV.M.C00) and female founders (L.F.C00 and CV.F.C00), males (L.M.CXX and CV.M.CXX) and females born in the mesocosms (L.F.CXX and CV.F.CXX) and juveniles (L.J, CV.J). Finally, observations of the maturity of male gonads(immature vs mature) were also made and the frequency of mature 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).

2.2. Model description

The DEB-IBM predicting the population dynamics of three-spined sticklebacks in mesocosms in control conditions was already published in David et al. (2019). Thus, a full detailed description of the model according to the ODD protocol (Grimm et al. 2010) is provided there. However, all parameter values of the DEB-IBM in control conditions can be found in the supplementary Information (SI) (Table S1). The model in control conditions was calibrated on two mesocosm experiment datasets and was successfully evaluated on three others: all the prediction evaluations and datasets are detailed in David et al. (2019). In particular, the control populations of the BPA mesocosm experiment were amongst the validation datasets.

Here, we adapted the model in control conditions to predict the stickleback population dynamics in the BPA mesocosm experiments by integrating the exposure scenarios and BPA direct and indirect impacts on sticklebacks. Briefly, the model includes relevant information of the environment such as temperature, photoperiod and prey density over time. The stickleback

preys considered in the model are zooplankton, higher crustacea (*Gammarus pulex* and *Asellus aquaticus*) and a diptera family (Chirominidae). The inputs of the model for the simulations are the water temperature, the photoperiod and the food scenarios (given per day) as well as the lengths of the 15 female and 10 male founders that were introduced in each artificial stream. The time step of the model was set to 1 day and the simulation runs lasted the same amount of time as the real BPA experiment (211 days). The model outputs were the endpoints which were determined for the experimental populations. The model was implemented under Netlogo 6.0.2 (language Logo). The script of the DEB-IBM is provided in SI.

2.3. Integration of the BPA exposure and ecological scenarios

Mean BPA concentrations for each treatment and sampling date were calculated using the measured concentrations made at the three sampling locations. Then, the daily BPA concentrations were linearly interpolated between the sampling dates. Thus, three BPA concentration scenarios were estimated, one for each BPA treatment (1, 10 and 100 µg/L) which gave the daily BPA concentration in water. These BPA scenarios were used as an additional input in the DEB-IBM.

The food scenarios were calculated using the measured abundance of macroinvertebrates and zooplankton monitored in each BPA treatment (averaged on the replicates), which was then converted into energy data and linearly interpolated between the sampling dates as described in David et al. (2018). Thus, changing the food scenario input of the model allowed to assess the indirect BPA effects on the prey density.

As no difference of the daily mean water temperature was found between the BPA treatments (de Kermoysan et al. 2013), the temperature scenarios were not changed in the DEB-IBM between the BPA treatments and the control.

2.4. Integration of the direct BPA impacts on sticklebacks

Direct effects of BPA on the physiological processes of three-spined sticklebacks were integrated in the model using dose-response equations. To do that, BPA effects on fish were identified at the individual level by reviewing the literature (See table S2 in SI). Only the studies using an exposure via water and reporting measured concentrations in water were kept. Only one article was kept even if the measured concentrations were not provided as this study provided toxicological information directly measured on three-spined sticklebacks (Jolly et al. 2009).

Based on literature and the available data, four physiological processes of fish were impaired by BPA exposure: growth, gonad maturity, eggs and embryo survival and male reproductive behavior.

As shown in Lindholst et al. (2003), the kinetics in the organism of BPA proceeds very fast and the steady state is reached within 24 h for zebrafish and rainbow trout. Consequently, we directly linked the BPA concentration in water to the effects on sticklebacks in the model as the internal concentration was assumed to be proportional to the external concentration.

Finally, as the end of the BPA exposure was very close to the emptying date of the mesocosms (three days difference), we did not introduce delayed effects of BPA between the end of the BPA exposure and the emptying date.

2.4.1. Growth inhibition

Several studies showed that BPA could affect growth of the F0 and F1 generations of several fish species such as medaka, fathead minnow, sheepshead minnow and rainbow trout (Sohoni et al. 2001, Warner Kara and Jenkins Jeffrey 2009, Yokota et al. 2009, Sadoul et al. 2017). Two dose-response curves were calculated: the first one for individuals exposed only at the adult stage such as the founders fish and the second for individuals that were exposed during their

entire life cycle. BPA impacts on growth were introduced by decreasing the energy conductance $\dot{v}$ in the DEB model leading to a reduction of the amount of reserves mobilized as suggested by Sadoul et al. (2019).

2.4.2. Embryo and larvae mortality

First, we supposed that BPA had no impact on the number of produced eggs by the founder females. Indeed, BPA concentrations from 1.75 to 5.00 µg/L delayed ovulation in brown trout but had no effect on the quality of eggs (egg mass and fertility) (Lahnsteiner et al. 2005). Furthermore, in a long-term experiment (444 days), Staples et al. (2011) showed that the number of eggs per female and per day was impacted at BPA concentrations higher than 640 µg/L for both F0 and F1 fathead minnow reproducing adults. In another long-term experiment (164 days), no statistically significant changes in number of eggs and spawns were found for adult fathead minnow at BPA concentrations ranging from 1 to 640 µg/L (Mihaich et al. 2012) although induction of vitellogenin was found for both males and females starting from 64 µg/L. Finally, Sohoni et al. (2001) found a statistical decrease of the total number of eggs spawned per female fathead minnows after 164 days of exposure for a BPA concentration of at least 1280 µg/L.

Nevertheless, BPA can impact the percentage of viable eggs and larvae when eggs and embryos were directly exposed to BPA as shown for zebrafish (Saili et al. 2012). Furthermore, the percentage of viable eggs and larvae can also be impacted by BPA when only adults were exposed (not the eggs directly) from 20 µg/L as shown for *Gobiocypris rarus* and zebrafish (Fan et al. 2018, Guo et al. 2019). Finally, in two long-term experiments where fathead minnow reproducing adults and offsprings were both exposed to BPA, the hatching success was reduced at 160 µg/L (Staples et al. 2011) and 640 µg/L (Sohoni et al. 2001). Here, only studies where adults and offsprings were both exposed to BPA, as in our mesocosms, were kept. We modeled this impact by decreasing the number of eggs and larvae leaving the nest.

2.4.3. Male reproductive behavior

One study on three-spined sticklebacks showed an inhibition of the spiggin production (Jolly et al. 2009) for BPA concentrations ranging from 2.5 pg/L to 250 µg/L. Indeed, in natural conditions, this protein is only secreted during the breeding season by male sticklebacks to use it as a glue during nest building (Wootton 1984). As the DEB-IBM does not directly model the spiggin production, we extrapolated this BPA effect by reducing the length of the breeding period. Thus, the number of built nests will decrease as males will stop reproductive processes sooner.

2.4.4. Gonad malformations

In the mesocosm experiment, malformations of the gonads (decrease of the ratio between the length of the gonads and the standard fish length) were observed for both male and female sticklebacks (de Kermoysan et al. 2013). A decrease of the gonadosomatic index (GSI) due to BPA exposure for both males and females have also been found in two studies (Sohoni et al. 2001, Mihaich et al. 2012). We introduced this effect on the energy allocated to sexual maturity and reproduction (predicted by the DEB model). However, we did not integrate this effect for the founders as they already had mature gonads.

2.5. Calibration of the dose-response parameters

The stress level for each type of effect integrated in the DEB-IBM was determined with a Hill equation (Eq. 1).

$$s_{(t)} = \frac{\mathrm{C}_{(t)}{}^{n_{tox}}}{EC_{50}{}^{n_{tox}} + \mathrm{C}_{(t)}{}^{n_{tox}}} \quad \text{(Eq. 1)}$$

With $C(t)$ the exposure concentration at time t, EC_{50} the concentration leading to 50 % of effect, n_{tox} the Hill coefficient and $s(t)$ the stress level at time t. n_{tox} and EC_{50} values are specific to the type of BPA effect integrated in the model.

First, these dose-response parameters were fitted on the literature data using the R package "drc" (Ritz et al. 2016).

Second, to assess if the main BPA impacts were integrated in the DEB-IBM, the dose-response parameters were recalibrated on the observed experimental population data with an iterative process using a genetic algorithm available within the software BehaviorSearch. 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).

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

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

2.6. Sensitivity analyses of the dose-response parameters

Sensitivity analysis by using the variance-based Sobol' method (Sobol' et al. 2007, Saltelli et al. 2010) were performed on the dose-response parameters varying 10 % of their values for the highest exposure scenario. The Sobol's method identifies the contribution of each parameter (First order index) as well as the interactions between parameters (Total index). To assess the impacts of parameter variations over time of the experiment, three sensitivity analyses were made at different time of simulations: during the beginning of the breeding period (70 days), during the end of the breeding period (140 days) and at the end of the experiment (211 days). The sensitivity analyses were made with the "sobol2007" function implemented in the R package "sensitivity" (Iooss et al. 2012) which uses the Mauntz estimators recommended for small first-order and total indices.

2.7. Model simulations

2.7.1. Predictive capacity of the model using the dose-responses fitted on literature data

To assess the predictive capacity of the model, population endpoints were simulated with the DEB-IBM using the individual dose-responses fitted on literature data for each BPA treatment. 1000 simulations were performed to account for the stochasticity of the model: inter-mesocosm environmental variability, inter-individual performance variability, and stochastic processes (for example survival). Simulation results were then compared to the observed experimental data. The relative errors (RE) and normalized root-mean-square deviations (NRMSD) between the simulations and the observations in the artificial streams were calculated to conclude on the predictive capacity of the model.

2.7.2. Explanation of the observed effects at the population level

Before integrating BPA effects into the model, population endpoint data from each treatment were compared to the simulated or observed control endpoints. The LOECs were calculated in two different ways using the methodology described in David et al. (2019). First, they were calculated using the observed control endpoints in the artificial streams as a reference and a Dunnett's post hoc test as in de Kermoysan et al. (2013). Second, they were estimated comparing the distribution of the control endpoints estimated by the DEB-IBM to the observations made in the exposed mesocosms using a Kolmogorov–Smirnov test. The level of significance for all tests was 5 %. To estimate the distribution of the control endpoints, 1000 simulations of the DEB-IBM were performed.

Then, in order to gain more knowledge on the observed BPA impacts, 1000 simulations of the DEB-IBM were made for each BPA treatment with the recalibrated dose-responses on the observed population data in the artificial streams. The simulation results were compared to the simulations using the dose-responses fitted on literature data to evaluate the added value of this recalibration by calculating the RE and NRMSD between the simulations and the observations in the mesocosms.

Finally, the contribution of either direct or indirect BPA effects was assessed with the DEB-IBM with the recalibrated dose-response curves. Four types of simulations were made for the 100 µg/L BPA treatment: (i) without any BPA effects, (ii) integrating only indirect effects of BPA, (iii) integrating only the direct effects of BPA on fish and finally, (iv) integrating both direct and indirect effects. The RE and NRMSD for each type of simulation were compared to conclude on the contribution of direct and indirect effects. Furthermore, the ratios between the root-mean-square deviations of the simulations with the different BPA effects and the simulations without BPA effects for each output were calculated to conclude on the added benefit of the models.

2.7.3. Populational dose-response curves

Population dose-response curves were extrapolated between the 0 and 100 µg/L BPA treatments. To do that, the food scenarios were linearly interpolated between the actual tested BPA concentrations. The chosen extrapolated concentrations were 0.5, 1.5 and 2 µg/L and from 5 to 70 µg/L with a step of 5 µg/L. 1000 model simulations were made for each extrapolated concentration. The population dose-response curves were calculated by linearly interpolated between the median of the simulations per concentrations. They were then compared to the ones calculated on the observed data using a Hill model available in the R package “drc” (Ritz et al. 2016).

3. Results

3.1. BPA exposure and ecological scenarios

The mean concentration throughout the experiment for each treatment were 0.97, 4.53 and 76.6 µg/L respectively for the 1, 10 and 100 µg/L BPA treatments. Figure 1 presents the three BPA exposure scenarios for the different treatments. Globally, BPA concentrations in water were lower than expected for the 10 and 100 µg/L treatments (between 46.5 and 91.8 % of the expected concentration for the 100 µg/L treatment and between 19.5 and 80.0 % for the 10 µg/L

treatment). For the 1 µg/L treatment, concentrations were either lower or higher than the expected values (between 39.7 and 148.9 %).

Concerning the food scenarios, Figure 2 presents the total energy calculated using all stickleback preys considered in the model and Figure S1 in SI presents the energy for each prey type. The peak of total energy (around 110 days of the experiment) of prey observed in the control and the 1 µg/L BPA treatment was lower in the 10 µg/L treatment and did not occurred in the 100 µg/L BPA treatment (Figure 2). As shown in Figure S1, this result was mainly due to the dynamics of the macroinvertebrates.

3.2. Integration of the direct BPA effects and sensitivity analyses

Figure 3 presents the dose-responses fitted on literature data or on population observed data in the artificial streams for the four integrated BPA impacts in the DEB-IBM and Table 1 presents the dose-response parameter values. Furthermore, the dose-response curve fitted on literature data for the number of produced eggs by females showed no effect of BPA at 100 µg/L (Figure S9). Thus our assumption of neglecting this effect sounds justified. Figure 4 shows the results of the Sobol sensitivity analysis of the different types of outputs: female frequency, frequency of mature males, mean length of founder females and juveniles. The results for the other outputs can be found in SI Figure S4. Overall, the Sobol's total indices were important meaning that the interactions between the toxic parameters were strong.

The EC50 parameters of the effect on the duration of the breeding period had strong effects on the female frequency (Figure 4a). However, for the frequency of mature males, the parameter that had the most influence was the EC50 of the effect on the growth of non-founder fish (Figure 4b). Similarly, the juvenile mean length was strongly impacted by a variation of the EC50 and the Hill coefficient parameter values relative to the duration of the breeding period (Figure 4d). However, the parameters relative to growth of founders strongly impacted the mean length of founder females (Figure 4c). Thus, globally all toxic parameters had strong influence on all

outputs, except the parameters relative to the effect on egg survival which had low impacts (Figures 4 and S4).

Furthermore, the influence of the parameters was dependent on the simulation time (Figure S5). For example, the parameters related to effects on growth strongly influenced the mean length of juveniles at the beginning and middle of the experiment (at 70 and 140 days) whereas those related to a reduction of the duration of the breeding period had more influence at the end of the experiment.

3.3. Model predictive capacity

The comparison of the model simulations using the dose-response curve fitted on literature data with the mesocosms observations for each BPA treatment can be found in Figure S2. The model gave relatively good predictions of the observed population endpoints (RE = 26 % and NRMSD = 0.31 for all endpoints in the 100 µg/L BPA treatment). Nevertheless, the decrease of the frequency of mature males in the 100 µg/L BPA treatment was not predicted. Furthermore, the mean length of females and males born in the mesocosms were slightly overestimated. For the other endpoints, the model predicted well the population dynamics under BPA stress. Concerning the length frequencies, the model gave good predictions for the control and the 1 µg/L treatment (Figure S3a,b). However, the DEB-IBM overestimated the length frequencies of the fish for the first cohorts for the 10 µg/L and 100 µg/L treatments at the end of the experiment (Figure S3c,d).

3.4. Explanation of the observed effects at the population level

Table S3 presents the comparison of the LOECs using the observed control endpoints in the artificial streams or using the distribution of the control endpoints estimated by our model as references for the statistical tests. Both approaches identified a LOEC at 100 µg/L for the juvenile mean length and the CV of their lengths. The LOEC for the frequency of mature males (F.M.m) was decreased to 10 µg/L and LOECs equal to 10 or 100 µg/L were found for four other endpoints (L.F.C00, L.F.CXX, L.M.CXX and CV.F.CXX) using the distribution of the

control endpoints estimated by our model. All other endpoints had a LOEC above 100 µg/L. Consequently, our model showed that seven endpoints were found to have a LOEC at 10 or 100 µg/L compared to three using only the observed mesocosm data.

After recalibration of the toxic parameters (see Table 1), the dose-response curves for the growth and the effects on gonads were shifted to the right which implies an increase of the BPA stress levels at the studied concentrations (Figure 3). No major differences were found for the effects on eggs and larvae survival and spiggin inhibition. This recalibration improved all the predictions of the endpoints (RE = 14 % and NRMSD = 0.22 for all endpoints in the 100 µg/L BPA treatment) (Figure 5), and especially the one concerning the frequency of mature males. Finally, the length frequencies were still well predicted for the control and 1 µg/L treatment (Figure 6 a,b) and were strongly improved for the 100 µg/L BPA treatment (Figure 6d). However, predictions for the 10 µg/L BPA treatment could still be improved as fish lengths of the first cohort were still slightly overestimated (Figure 6c).

Figure 7 shows the comparison between the model predictions including either direct and/or indirect effects of BPA for the 100 µg/L treatment. Integrating only indirect effects improved the model predictions for some endpoints like the juvenile mean length (RE = 27 % and NRMSD = 0.32 for all endpoints in the 100 µg/L BPA treatment) compared to the predictions of the model without BPA effects (RE = 29 % and NRMSD = 0.34). Furthermore, considering only direct effects of BPA on the physiological processes of sticklebacks improved other population endpoints like the mean length of founders or the frequency of mature males (RE = 16 % and NRMSD = 0.24). Finally, including both direct and indirect effects in the model strongly improved the predictions of all population endpoints (RE = 14 % and NRMSD = 0.22).

Figure 8 presents the benefit of the model considering the indirect and/or direct BPA effects compared to the one without BPA effects. First, the average benefit on all the population endpoints is 20.7 % with the model using both type of BPA effects against 16.5 % and 6.1 % for the model using direct or indirect effects alone respectively. Indeed, the benefit of the model

using indirect effects alone was lower than the one using direct effects alone for most endpoints. For example, almost no indirect effects could explain the frequency of mature males (0.86 %) whereas for other endpoints like the female frequency, the benefit of the model considering the indirect effect alone was not negligible (25.8 %). Finally, the benefit of the model, regardless of the BPA effects, were negative for two endpoints (total abundance and the CV of the length of mature males).

3.5. Population dose-response curves

Figure S6 in SI presents the extrapolated food scenarios and Figure 9 the extrapolated dose-response curves at the population level with the DEB-IBM compared to the dose-responses calculated on the observed data for the number of female founders, the CV of the length of the males born in artificial streams and the frequency of mature males. Figure S7 presents the results for all population endpoints. First, the model showed no impacts on total abundance and the number of male and female founders at the end of the experiment whereas the dose-responses fitted on the observed data suggested an effect (Figure 9a and S7). On the contrary, no effect on the juvenile frequency was shown with the dose-responses fitted on the observed data whereas the model showed one (Figure S7). For the juvenile mean length and the frequency of mature males, the shape of the extrapolated dose-response curves with the DEB-IBM showed a less noticeable effect between the 10 and 100 µg/L treatments compared to the dose-responses calculated on the observed data (Figure 9b and S7). Furthermore, dose-response curves having a slope which changed direction within the range of the tested treatments (*i.e.* non-monotonic dose-responses) were found for the CV of the lengths of both males and females born in mesocosms as well as the CV of the lengths of the mature males (Figure 9c and S7). Finally, for the other endpoints, the extrapolated dose-responses with the DEB-IBM presented the same shape than the ones calculated on the observed data (Figure S7).

4. Discussion

Population modelling in risk assessment was suggested to be a powerful tool to gain more knowledge on the mechanisms of chemical toxicity on populations (Forbes et al. 2011) and to extrapolate from ecotoxicological observations to relevant ecological effects (Forbes et al. 2008). Hence, it would help to improve decision-making and ecological management (Goussen et al. 2016). Here, we proposed a DEB-IBM to predict BPA effects on the population dynamics of three-spined sticklebacks in artificial streams. This model was based on a DEB-IBM previously developed for predicting population dynamics of three-spined sticklebacks without toxicant stress (David et al. 2019).

4.1. Integration of the direct BPA effects on sticklebacks

The development of the individual dose-responses describing BPA effects was not straightforward to handle due to the heterogeneity of the literature studies (different studied species and exposure design). The exposure route via food was neglected in our model, while some studies have showed an accumulation of BPA in invertebrates (Nurulnadia et al. 2014) suggesting that this exposure route could be possible for sticklebacks. However, the only study on fish that tested the effects of contaminated food with BPA (Drastichová et al. 2005) showed effects at higher concentrations than those studied in the mesocosm experiment, thus the results were not considered.

From literature, four main BPA effects on fish appeared to be interesting to include in the model (effects on growth, gonad maturity, eggs and embryo survival and male reproductive behavior). However, the BPA effects on the reproductive behavior of males were more complex to incorporate as very scarce literature data was available on the mating behavior of fish. One study showed a decrease of male locomotion and behavior during courtship in zebrafish (Li et al. 2017) at a low-dose BPA concentration but, to our knowledge the measured concentrations in water were not available. We thus extrapolated this effect by using the study on three-spined sticklebacks which showed an inhibition of the spiggin, protein used for nest building (Jolly et al. 2009). In the model, spiggin was correlated to the breeding period (inhibition of spiggin

implies a reduction in the duration of the breeding season) which would thus decrease the success rate of the reproductive processes of males (in particular a decrease of the number of nests) (Mori 1993). Other DEB-IBM parameters could have been impacted instead of the duration of the breeding period, like the nest mortality or the probability of stopping the reproduction processes. However, impacting the breeding period allows to take in account different possible physiological and behavioral effects such as a reduction of the overall courtship and nest building. Furthermore, this parameter influenced the most the outputs of the model as shown in David et al. (2019).

Globally, the DEB-IBM with the toxic parameters fitted on literature data at the individual level gave overall good predictions of the population endpoints, especially for the mean length and CV of the lengths of juveniles as well as the female frequency. Hence, this supports the idea that individual-based modelling may be a sound approach for predicting toxicant effects at the population level from the toxicological data recorded at the individual level (Galic et al. 2010, Hommen et al. 2010, Forbes et al. 2017).

4.2. Explanation of the observed effects at the population level

The sensitivity analyses made for the 100 µg/L BPA treatment revealed that the influence of the toxic parameters was strongly dependent on the studied outputs and the time of the simulation (Figures 4, S4 and S5). First, the mean length of juveniles was mostly impacted by the variations of the parameter values relative to a reduction of the duration of the breeding period whereas the male and female mean lengths were most sensitive to changes in the toxic parameters related to growth. Furthermore, the female and juvenile frequencies and the total fish abundance (Figures 4a and S4 a,b) were mostly impacted by a variation of the toxic parameters related to a reduction of the duration of the breeding season. Indeed, as the last reproductive events were inhibited, less juveniles were present in the artificial streams at the end of the experiment which directly impacted the juvenile frequency as well as the mean length of the juveniles. Shortened reproductive season was already shown to impact cohort abundances

with another IBM for zebrafish (Hazlerigg et al. 2014) and the duration of the breeding period was already one of the critical parameter for the DEB-IBM developed without toxicant stress (David et al. 2019). Finally, the sensitivity of the population endpoints was clearly dependent on the type of BPA effects. Thus, it was necessary to include multiple BPA effects to accurately predict all population endpoints which highlights the complexity of mechanisms of toxicity that could have occurred in the mesocosm experiment.

The DEB-IBM simulations of the descriptive variables of the stickleback populations for each BPA treatment with the recalibrated dose-response curves on population data were close to the observed endpoints. Thus, this suggests that all main BPA effects have been integrated in the model and explained the population structure found in the 100 µg/L treatment. However, some predictions, such as the length frequency of the fish of the first cohort, could still be improved, especially for the 10 µg/L BPA treatment, suggesting that other effects could have happened in the artificial streams, and consequently decreased the growth rate of the first cohort. For example, BPA was shown to have complex neurobehavioral effects. Indeed, it altered the locomotion and can induce hypoactivity as shown on fish larvae at concentrations between 2 and 200 µg/L (Inagaki et al. 2016, Guo et al. 2019). BPA was also shown to reduce the aggressive behavior of adult zebrafish as well as induce a weaker adaptability to new environments (Wang et al. 2015). On the contrary, Saili et al. (2012) showed that low-dose BPA exposure levels led to early zebrafish life-stage hyperactivity and adults exhibited learning deficits. Thus, BPA effects on fish locomotion and activity are not fully understood but could impact the predation processes and growth density-dependence driven by fish interactions.

4.3. Contribution of the indirect and direct BPA effects

The comparison of the DEB-IBM simulations integrating direct and/or indirect effects showed that we obtained simulations closer to the observed endpoints when both direct and indirect impacts were taking into account for the 100 µg/L BPA treatment (Figure 7). Indeed, as seen in Figure 2, total energy of stickleback preys was lower at this treatment. This result

was not surprising as BPA impacts on invertebrates have been widely described (Flint et al. 2012, Canesi and Fabbri 2015). In particular for the stickleback main preys, BPA was shown to affect growth and feeding of *Asellus aquaticus* during acute and chronic exposures to high BPA concentrations (Plahuta et al. 2015). Reproduction and mortality of *Gammarus fossarum* was also impacted starting from 5 µg/L of BPA (Ladewig et al. 2006, Schirling et al. 2006). Developmental inhibition induced by BPA exposure was observed at very low exposure concentrations (10 ng/L) on *Chironomus riparius* larvae (Watts et al. 2003). Finally, zooplankton species such copepods were also impacted at low BPA concentrations at 1 and 10 µg/L (Marcial et al. 2003).

Assessing the impacts of species loss in an ecosystem requires to understand the processes that occur across trophic levels in order to consider possible cascading effects (Duffy et al. 2007, O'Connor et al. 2013). Here, as explained in de Kermoysan et al. (2013), indirect effects could explain the shift of the population structure towards higher lengths at the 100 µg/L BPA treatment. The model predictions of the mean length of juveniles were for example improved when including only the food scenario for the 100 µg/L treatment (Figure 7). The growth of the individuals could be reduced as less food was available leading to a larger number of fish in the length class of [20; 26 mm[that did not reach the adult stage. Another explanation is that density-dependent mechanisms for growth were lower as the decrease of the food resource indirectly also reduced fish reproduction. As seen in Figure 8, the benefit of the model including indirect effects alone was 17.7 % which was not negligible compared to the benefit including direct effects alone (31.5 %).

On the contrary, the decrease of the frequency of mature males is mainly explained by direct BPA effects (Figure 8). Indeed, the decrease of the food resource in the environment could have explained a decrease of the maturity as described in the DEB model (Kooijman 2010) but the results showed that it was clearly not sufficient to explain the observed effect. Concerning total abundance (Figure 8), the benefits of the models including the different types of BPA effects

were negative. However, the large coefficient of variation of the observations (26.7 % for the 100 µg/L treatment) induces great uncertainty and then, the benefit may probably be smaller than the noise. Indeed, as seen in Figure 5, it is not possible to conclude on the relevance of the model predictions for total abundance as the three replicates were in the predictive interval of the simulations for the 100 µg/L treatment.

Globally, the combination of both direct and indirect effects gave better predictions (Figures 7 and 8), highlighting the complex interactions between the trophic levels. Thus, this BPA case study highlights that modelling food availability as well as the effects on the physiology of the organisms would help to provide relevant chemical exposure–response relationships at the population level which is necessary for ecological risk assessment (Forbes et al. 2017).

4.4. Extrapolation of the ecotoxicological effects at the population level

In risk assessment, the use of the DEB theory (Kooijman 2010) coupled to an IBM was suggested to be a convenient approach to extrapolate the effect of toxicants measured on the individual life history traits to effects at higher levels of biological organization (Beaudouin et al. 2012, Martin et al. 2013).

However, as the literature data concerned different fish species exposed to BPA with diverse experimental designs, this study gives an example of different extrapolation issues that could happened. More specifically, extrapolating effects between species adds uncertainties in predictions. Here, the comparison of the calibrated dose-response curves on population responses in artificial streams and the dose-response curves fitted on literature data showed that the BPA effects on growth and gonad maturity were stronger than assumed by literature data. Furthermore, the assumption of the extrapolations between literature data and the way of integrating the effect in the DEB-IBM could also induce a discrepancy between the stress level calculated on the dose-response curve and the population observed responses. For example, the effect of BPA on the male reproductive behavior was extrapolated from the response of spiggin, an androgen and antiandrogen biomarker (Katsiadaki et al. 2002). Nevertheless, we found that

the recalibration of the dose-response parameters did not strongly change the dose-response curve of the spiggin inhibition on sticklebacks suggesting that including this effect on the duration of the breeding season was a relatively acceptable extrapolation. Finally, to go further on the modelling of the exposure, the population model could be coupled to toxicokinetic models that provide a quantitative mechanistic framework to predict the time-course of the concentration of chemicals in an organism (Grech et al. 2019).

Furthermore, adverse outcome pathways (AOPs) have emerged as a useful framework to assess impacts of chemicals at the molecular level. AOPs describe mechanisms linking molecular initiating events to outcomes at an individual level. Here the concept could be applied to the case of sticklebacks as spiggin synthesis in sticklebacks is assumed to be regulated by a renal androgen receptor. Indeed, the inhibition of the spiggin production could occur if antiandrogens occupy the androgen receptor without activating it. Thus, spiggin inhibition here is a good example of how AOPs could be used to identify mechanisms of toxicity and extrapolate them from the molecular to the population level with a modelling approach.

The predictive capacity of our model was assessed on one reproductive season and thus, should be also evaluated for more than one season. To do that, the ecological scenarios would need to be extrapolated. This could be done by including a specific model of the aquatic food webs describing the dynamics over time. Furthermore, BPA effects on reproduction can be supposed to be long lasting or even irreversible as seen for example with the alterations of the gonadotroph cells in zebrafish at BPA concentrations of 100 and 1000 µg/L (Molina et al. 2018). In a study on rainbow trout egg exposed to BPA, Sadoul et al. (2017) also showed long-term effects on growth and gene expressions related to metabolism. Consequently, to make long-term predictions of BPA effects, it would be highly interesting to consider long-lasting effects of BPA on stickleback growth as well as on gonad maturity. However, according to the parsimonious principle, as the end of the mesocosm experiment was really close to the end of the exposure, the BPA long-lasting effects were neglected in our model.

To go further, integrating landscape characteristics (*e.g.* habitat, vegetation patches) in addition to species interactions and environmental conditions (e.g., temperature, food availability) in the ecological scenarios could be interesting as they may also be impacted by toxicants (Rico et al. 2016). Furthermore, other ecological responses, like, for example, a shift of diet to other available species in response to the absence of the preys (Allen and Wootton 1984) was not considered although this could minimize the indirect BPA effects.

4.5. Added value of ecological modelling to estimate toxicant thresholds

We here analyzed the power of modelling to estimate toxicant thresholds. First, the model without including toxicant effects was used to assess the LOECs (methods presented in David et al. (2019)) and showed that 39 % of the monitored population endpoints had a LOEC at 10 or 100 µg/L (only 17 % based on simple statistical comparisons (de Kermoysan et al. 2013)) (see Table S3). Moreover, the minimum LOEC value was estimated at 100 µg/L in de Kermoysan et al. (2013), whereas it was lowered to 10 µg/L using data analysis based on population dynamics modelling. Consequently, our approach improved environmental risk assessment by decreasing the risk of false negative results when analyzing data from artificial streams.

Furthermore, our study offers the opportunity to compare dose-responses at the population level extrapolated by the DEB-IBM to the dose-responses calculated on the observed data with a Hill model (Figure 9 and S7). We found that in most cases, the extrapolated dose-response curves found with the model were consistent with the ones fitted on the observed data. However, the model helped to refine the shape of the dose-responses between the tested concentrations. For example, the dose-response estimated by the model showed a slower decrease of the frequency of mature males. Furthermore, the model showed non-monotonic dose-response relationships, as frequently found for endocrine disruptors (Vandenberg et al. 2013). This was specially the case for the CV of the lengths of females and males born in the mesocosms and the mature males. Such unconventional dose-responses represents a challenge

to current risk assessment (Lagarde et al. 2015). Hence, population models could add value to ecological risk assessment, extrapolating dose-responses at population level, which is essential for improving decision-making and protection of ecosystems (Forbes et al. 2008, Galic et al. 2010, Forbes et al. 2011).

5. Conclusions

The predictive capacity of our DEB-IBM in context of risk assessment of chemicals was assessed on stickleback population data from a mesocosm experiment where the artificial streams were exposed to BPA. We showed that including BPA effects on stickleback gonad formation, growth, male reproductive behavior and eggs and larvae survival gave us a relevant predictive model of the effects at the population level. Recalibration of the dose-response curves of the BPA effects on fish on experimental population data showed that the main BPA effects were integrated in the model. Indirect effects were not negligible and participated to the stickleback population structure observed in the 100 µg/L BPA treatment. Therefore, ecological models, such as ours, have a strong potential for improving environmental risk assessment.

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Figures and tables

Table 1. Description and parameter values of the dose-response relationships fitted on literature data or recalibrated on stickleback population observations in the mesocosms (artificial streams).

Parameter	Description	Unit	Recalibrated	Fitted on literature	IC 95%
EC50_B.Period	Concentration leading to 50 % of effect on the duration of the breeding period	µg/L	14.8	14.1	[12.7 ; 15.6]
n_B.Period	Hill coefficient for the effect on the duration of the breeding period	-	0.11	0.12	[0.10 ; 0.13]
EC50_P.OL	Concentration leading to 50 % of effect on the mortality of eggs and larvae	µg/L	6.71	6.38	[5.74 ; 7.01]
n_P.OL	Hill coefficient for the effect on the mortality of eggs and larvae	-	2.00	2.17	[1.95 ; 2.38]
EC50_R	Concentration leading to 50 % of effect on the energy allocated to maturity and reproduction	µg/L	0.50	6.67	[0 ; 8.00]
n_R	Hill coefficient for the effect on the energy allocated to maturity and reproduction	-	0.68	0.73	[0.58 ; 0.87]
EC50_gr.NF	Concentration leading to 50 % of effect on the growth for non founders	µg/L	7.02	12.5	[8.72 ; 16.19]
n_gr.NF	Hill coefficient for the effect on the growth for non founders	-	0.43	0.47	[0.37 ; 0.56]
EC50_gr.F	Concentration leading to 50 % of effect on the growth for founders	µg/L	6.80	10.1	[6.00 ; 13.00]
n_gr.F	Hill coefficient for the effect on the growth for founders	-	0.89	0.84	[0.70 ; 1.04]

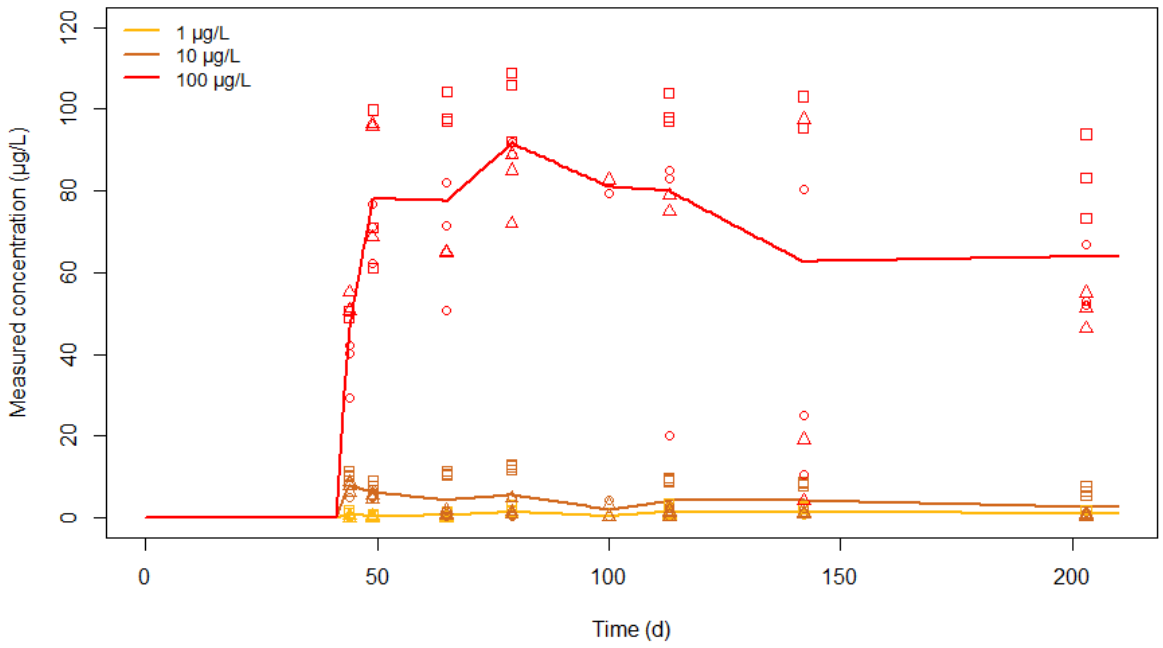

Figure 1. BPA exposure scenarios based on the measured concentrations made in the mesocosms for the three treatments (yellow: 1 µg/L, orange: 10 µg/L and red: 100 µg/L). Squares, circles and triangles represent respectively the measured BPA concentrations in the three replicate mesocosms at 0, 5 and 19 m from the inlet of the water. Full lines represent the extrapolated scenarios for each BPA treatment.

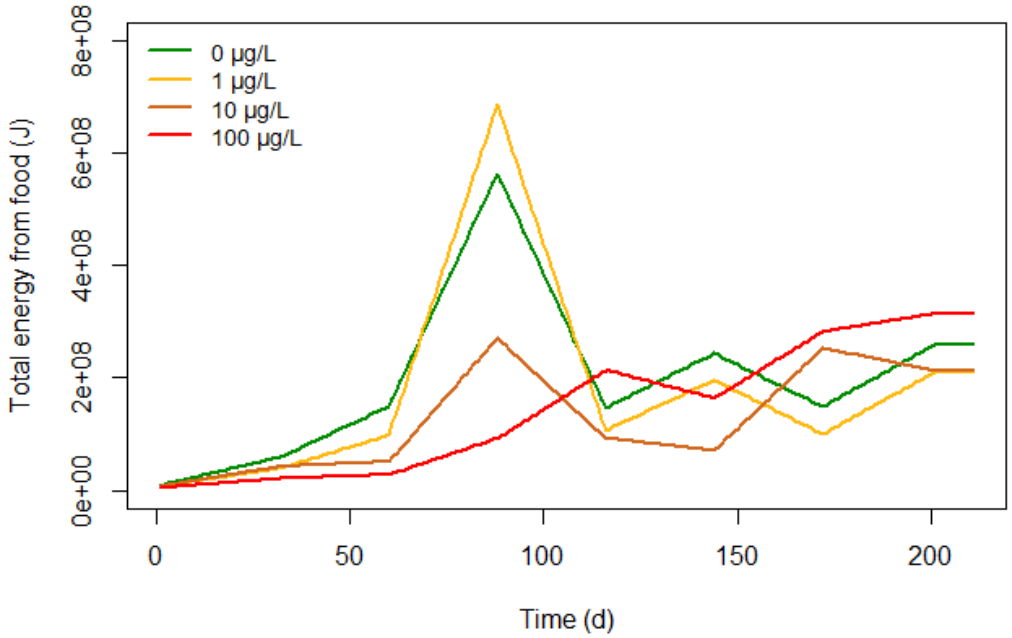

Figure 2. Food scenarios throughout time for each BPA treatment (green: 0 µg/L, yellow: 1 µg/L, orange: 10 µg/L, red: 100 µg/L) calculated using the results of zooplankton and macroinvertebrates samples.

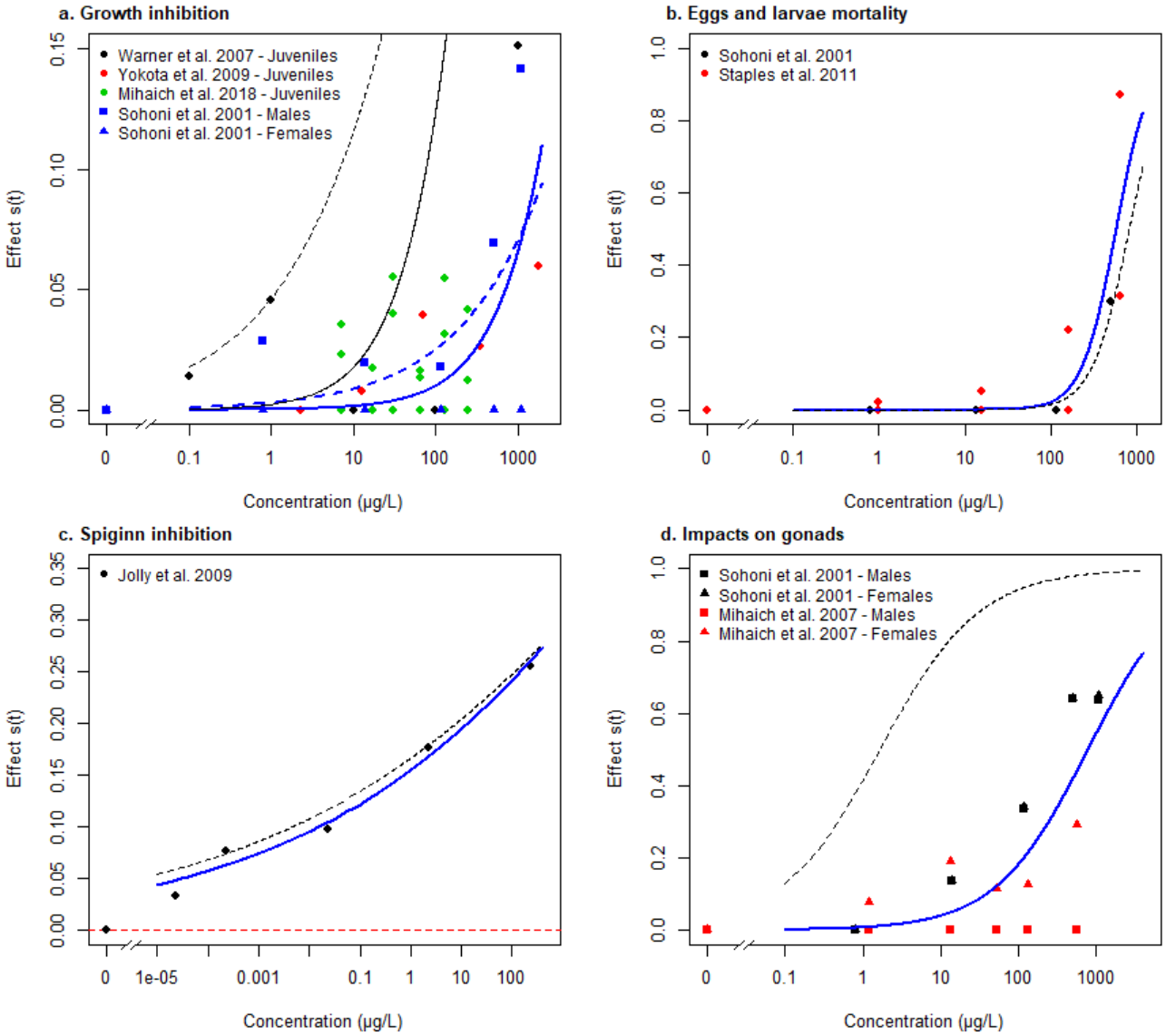

Figure 3. Dose-responses of the effects of BPA on growth (a), egg and larvae mortality (b), spiggin production (c) and gonads (d). Points represent data from literature, the blue lines represent the dose-responses fitted on the data points. The black lines represent the recalibrated dose-response curves on the artificial stream population data. For growth inhibition, the full lines represent the dose-responses for adults (males and females) whereas the dashed lines represent the dose-response for juveniles.

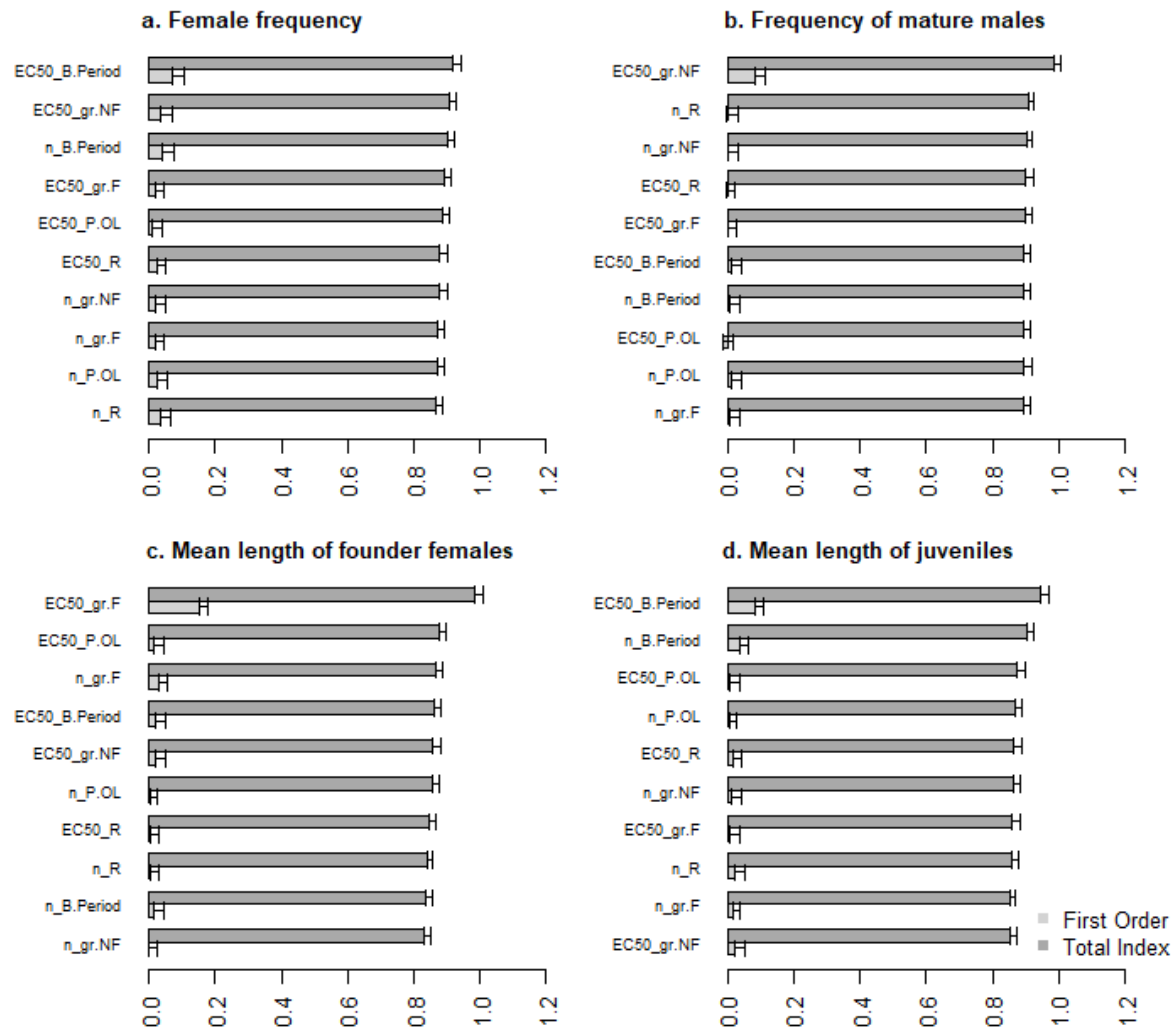

Figure 4. Results of the Sobol sensitivity analysis for the female frequency (a), the frequency of mature males (b), the mean length of founder females (c) and the mean length of juveniles (d). The first order (light grey) and total index (dark grey) were represented on each graph.

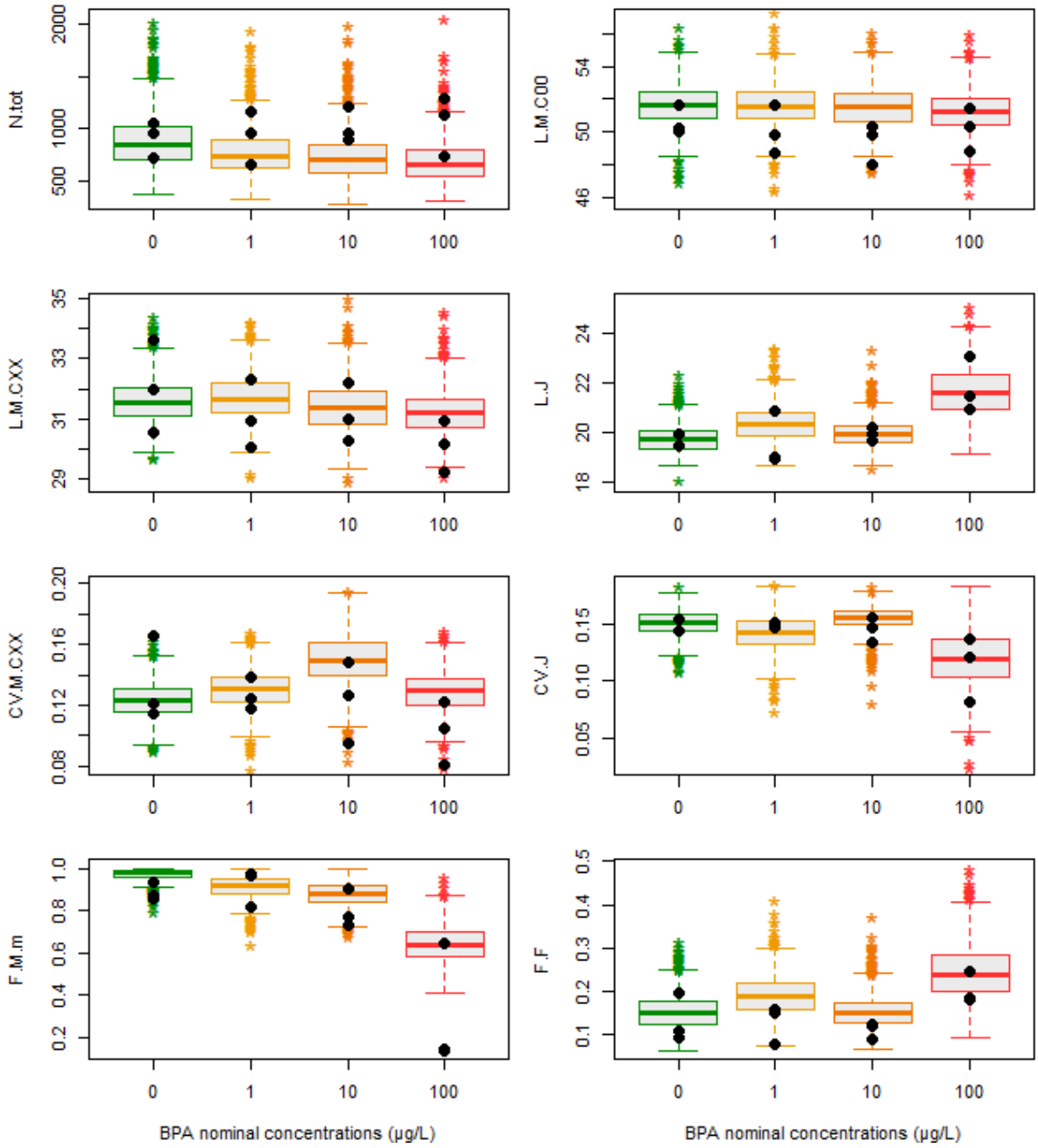

Figure 5. Simulation of the toxicity of BPA using calibrated dose-responses on physiological processes of sticklebacks. Boxplot represent the model predictions for each BPA treatment (green: 0 µg/L, yellow: 1 µg/L, orange: 10 µg/L and red: 100 µg/L) and the different population endpoints (N.tot: Total abundance, L.M.C00: Mean length of the founder males, L.M.CXX: Mean length of the males born in mesocosm, L.J: Mean length of juveniles, CV.M.CXX: CV of lengths of males born in mesocosms, CV.J: CV of lengths of juveniles, F.M.m: Frequency of mature males, F.F: Frequency of females). Black points represent the observations made in the mesocosms.

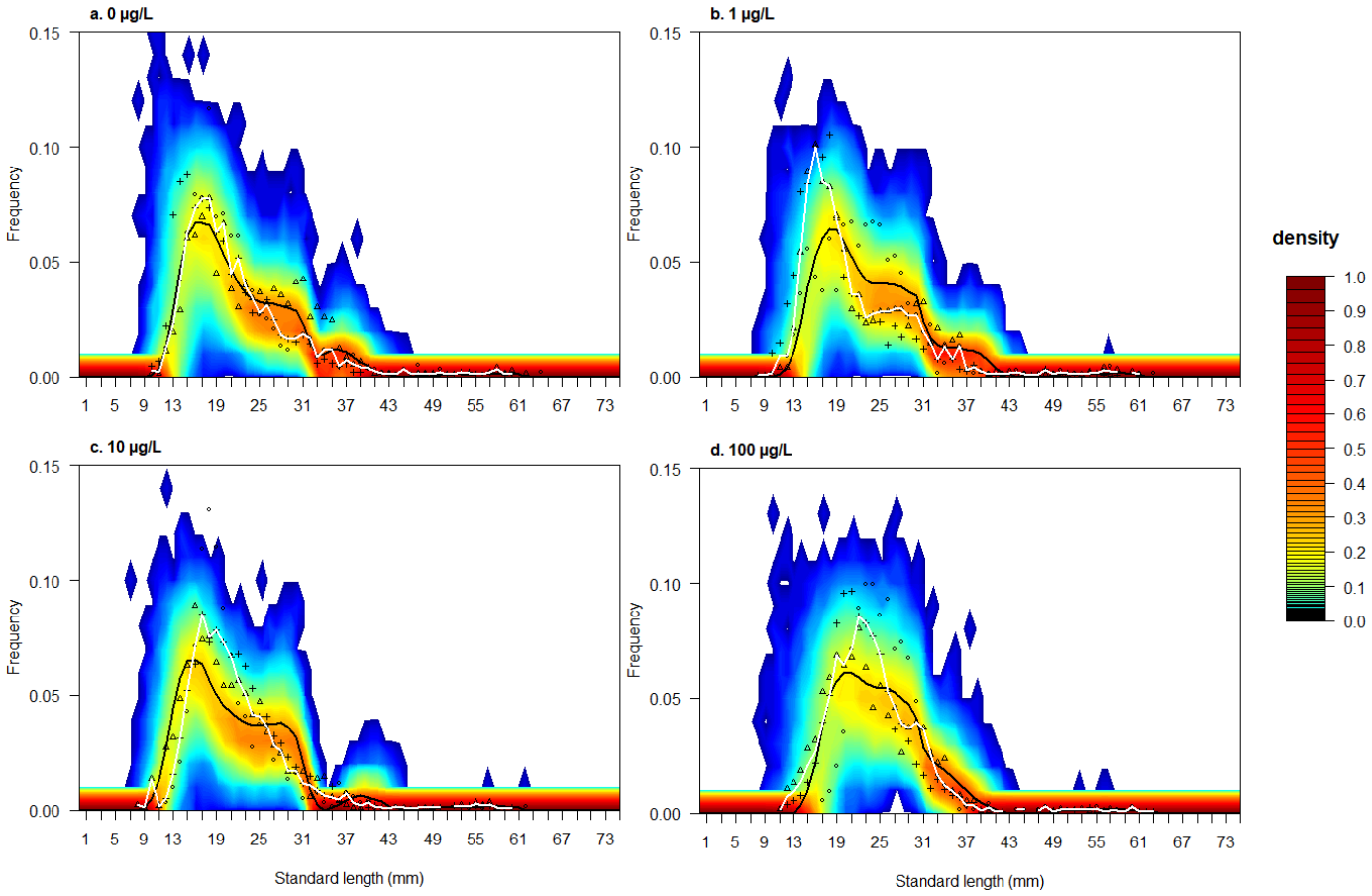

Figure 6. Probabilistic distributions of the length frequency predicted by the model compared to the length frequency distributionobserved for each BPA treatment: 0 µg/L (a), 1 µg/L (b), 10 µg/L (c) or 100 µg/L (d) after recalibration of the dose-response parameters on the artificial streams population data. Different point types represent the length frequency distributions of the different observed populations. Full black and white lines represent the median length frequency distributions of the simulated and observed populations respectively. 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.

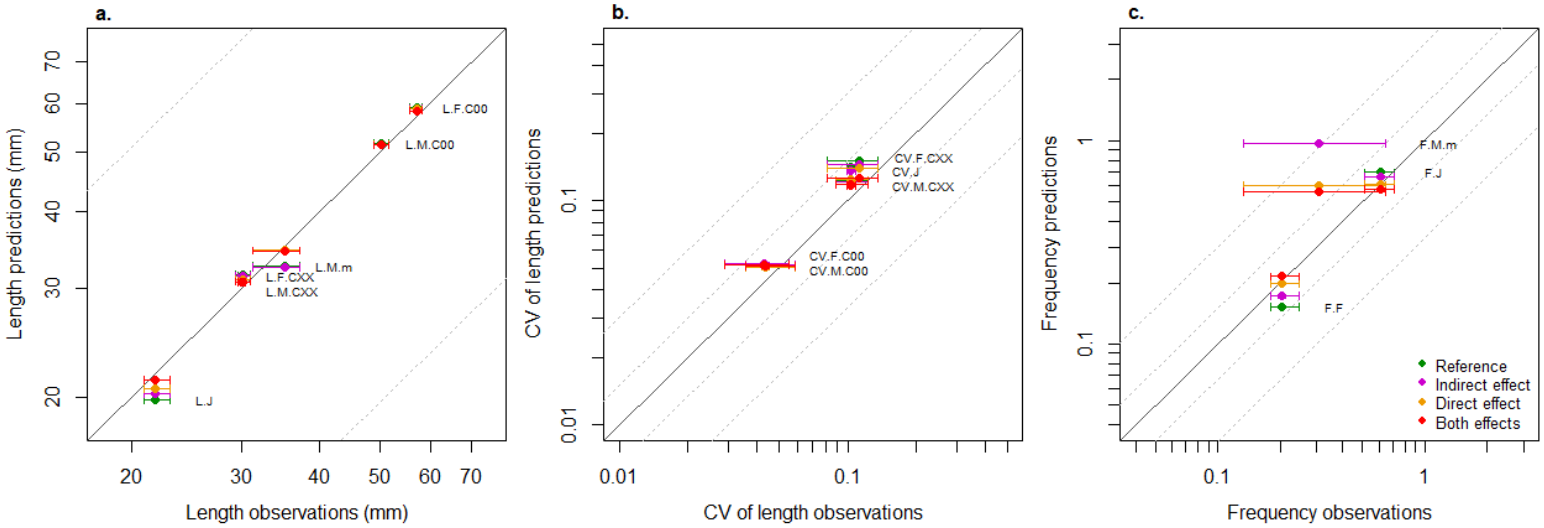

Figure 7. Predictions of the mean length of each category of individuals (a), the CV of their lengths (b) and the frequencies (c) compared to the observations made in the 100 µg/L BPA treatment. The green points represent the mean of the simulations of the model with direct and/or indirect BPA effects. The purple points represent the mean of the simulations of the model integrating only indirect BPA effects on the physiological processes of sticklebacks. The orange points represent the mean of the simulations of the model integrating only the direct BPA effects The red points represent the mean of the simulations integrating both direct and indirect effects. The error bars represent the 95 % confidence interval of the simulations and observations. Dotted lines represent the 1.5-fold and 3-fold changes and the full line represents the 1:1 line.

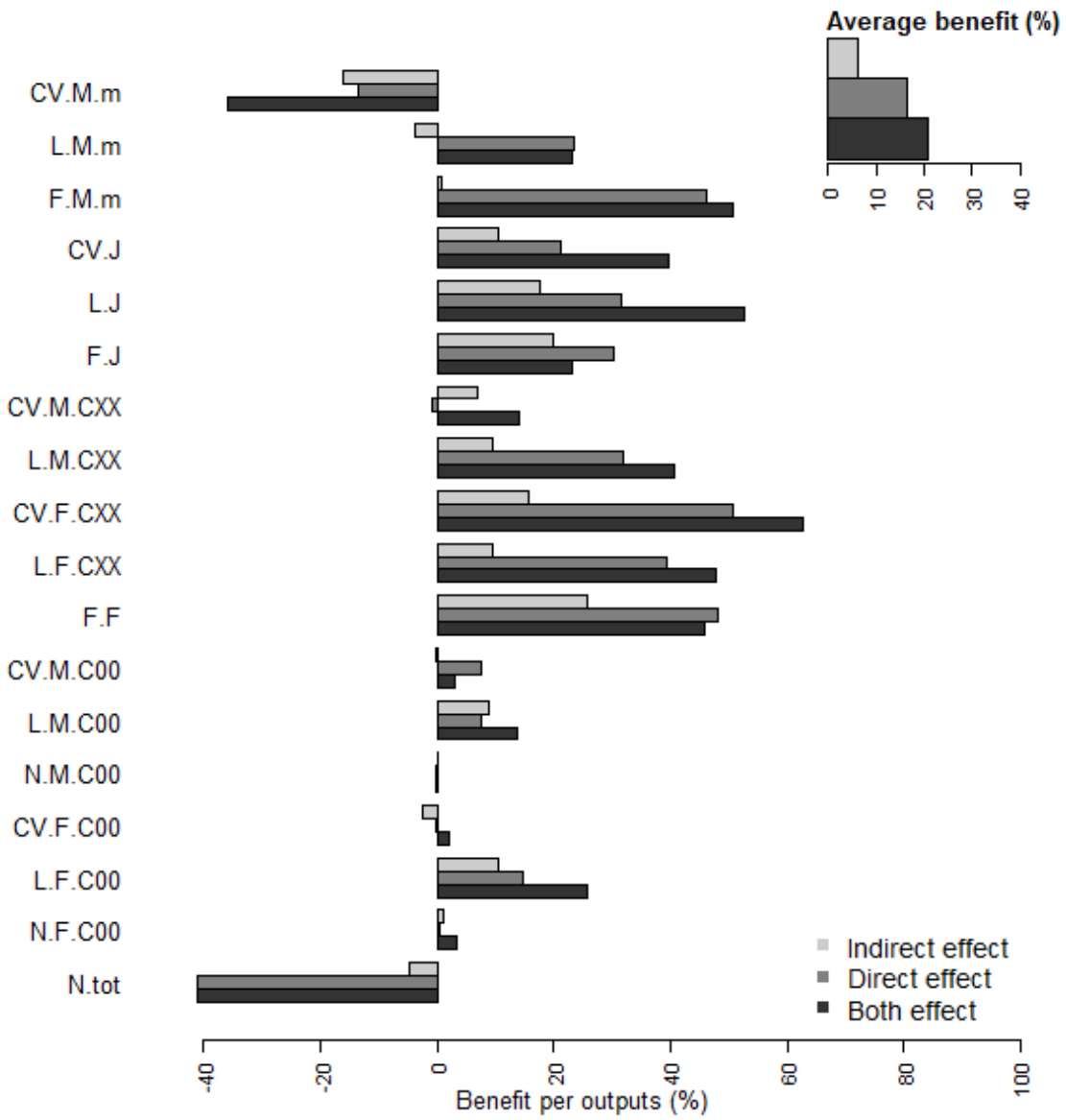

Figure 8. Added benefit of the models considering the indirect BPA effects alone (light grey), the direct BPA effects alone (medium grey) or both direct and indirect effects (dark grey) to predict the different descriptive variables of the stickleback population. The results are represented in term of ratios between the RMSE of these different models and the RMSE of the model without considering the BPA effects for each output. The average benefit on all the outputs is given on the top right position.

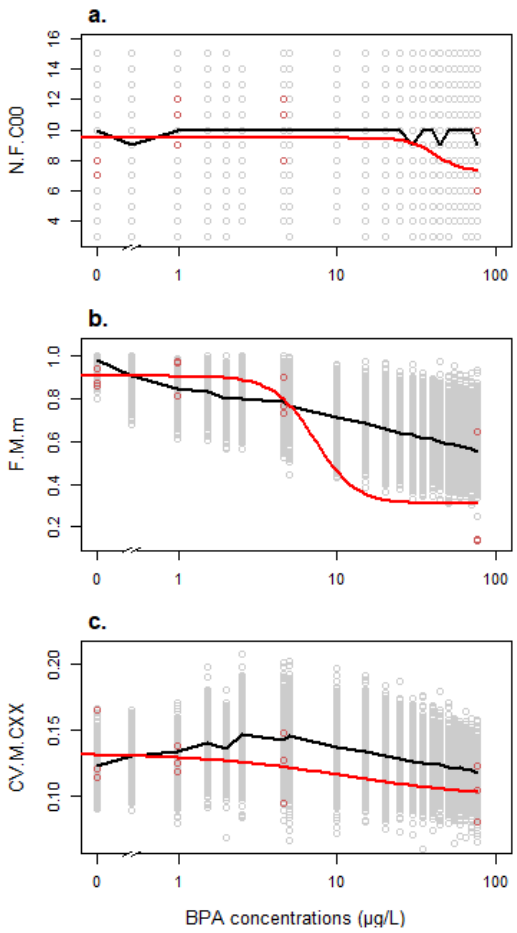

Figure 9. Dose-response curves at the population level for the number of female founders (a), the frequency of mature males (b) and the CV of lengths of the males born in the artificial streams (c). The red circles represent the real observations in the mesocosms for each BPA treatment and the red line the dose-response curve fitted on the observed data using a Hill model. The grey points represent the simulated endpoints with the DEB-IBM and the black line the extrapolated dose-response curves using the simulated data.