Ecotoxicology and Environmental Safety 156 (2018) 463–475

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Estimation of population-level effect of the endocrine disruptor pyriproxyfen in *Daphnia magna* by using changes in sex ratio and reproductive output

Yoshinari Tanaka[a,b,*], Kensei Nakamura[a], Shigeto Oda[a], Haruna Watanabe[a], Norihisa Tatarazako[a,c]

[a] Center for Health and Environmental Risk Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki, Japan
[b] Sophia University, Graduate School of Global Environmental Studies, 7-1 Kioicho, Chiyoda-ku, Tokyo, Japan
[c] Ehime University, Graduate School of Agriculture, 10-13 Dogo-Himata, Matsuyama, Ehime, Japan

ARTICLE INFO

Keywords:
Endocrine disrupting chemicals
Daphnia magna
Sex change
Reproductive inhibition
Population-level effect
Ecological modeling

ABSTRACT

Here we developed an analytical means of estimating population-level effects of endocrine disruptors on *Daphnia magna*. Our approach was based on the fact that the endocrine-disrupting juvenile hormone analogs induce the production of male neonates if they are exposed to the analogs during a particular period in their prenatal development; the method also assumed that the abnormal production of male neonates in the sake of production of female neonates reduces population growth. We constructed a linear toxicodynamics model to elucidate the period in which *D. magna* neonates are sensitive to exposure to the analog and also the probability of an individual neonate changing sex under specific exposure concentrations. The proposed model was applied to *D. magna* reproduction test data obtained under time-varying exposure to pyriproxyfen to derive the maximum-likelihood estimates and the posterior distributions of the model parameters. To quantitatively assess the ecological risk at the population level, we conducted a population dynamics simulation under two time-varying exposure scenarios (i.e., constant or pulsed exposure) by using an age-structured population model. When the change in sex ratio was based on the time-weighted average concentration during the period of sensitivity, change in sex ratio caused approximately equivalent population-level effects as did reproductive inhibition (i.e., reduction in the total number of neonates per female parent) regardless of the exposure scenario. In contrast, when change in sex ratio was based on maximum concentration during the sensitive period, change in sex ratio caused only half the population-level effects as did reproductive inhibition under constant exposure, whereas it caused a much larger population-level effect than did reproductive inhibition under pulsed exposure.

1. Introduction

To assess the ecological risks posed by endocrine disruptors (EDs), amendment of the *Daphnia magna* reproduction test to include the additional endpoint of sex ratio has been proposed (OECD, 2012). This approach is based on the assumption that the abnormal production of male neonates, which is induced by endocrine disruption especially by juvenile hormone analogs (Baldwin et al., 2001; Olmstead and LeBlanc, 2003; Oda et al., 2005a, 2005b; Tatarazako and Oda, 2007; Matsumoto et al., 2008; Dang et al., 2012; Ginjupalli and Baldwin, 2013), brings about ecological risk in natural populations. However, there are no analytical procedures available to estimate ecological risk from observed changes in sex ratio. Furthermore, there is currently no means of comparing the ecological risks of endocrine disruption as measured by changes in sex ratio with those measured by using other endpoints in terms of ecologically relevant criteria. Therefore, a means of converting individual-level responses induced by EDs to population-level effects is urgently needed.

Several ecotoxicological and endocrinological studies have shown that the determination of neonate sex in cladoceran species is limited to within the early developmental stage, and *D. magna* neonates respond to EDs during this period of sensitivity by changing sex, which produces an increased number of male neonates (Oda et al., 2005b; Wang et al., 2005; Tatarazako and Oda, 2007; Matsumoto et al., 2008; Kato et al., 2011; Ginjupalli and Baldwin, 2013). Therefore, given that the period of sensitivity to EDs is limited, the population-level effects of EDs are likely to reflect whether the concentration of an ED changed over time or whether exposure occurred in a pulse-like manner (Ashauer et al., 2007).

Here we propose an analytical method of evaluating the ecological risk posed by EDs by examining the effects of an ED on sex ratio (i.e., the abnormal production of male neonates). Although we applied our

* Corresponding author at: Sophia University, Graduate School of Global Environmental Studies, 7-1 Kioicho, Chiyoda-ku, Tokyo, Japan.
E-mail address: y-tanaka-fo5@sophia.ac.jp (Y. Tanaka).

https://doi.org/10.1016/j.ecoenv.2018.03.044
Received 16 November 2017; Received in revised form 27 February 2018; Accepted 19 March 2018

approach to the standard *D. magna* reproduction test (OECD Test Guideline No. 211, Annex 7) with the insecticide pyriproxyfen as the test substance, our approach is applicable to the substances that induce endocrine disruption especially for parthenogenetically reproducing organisms. The aim of this study was to develop a means of converting changes in sex ratio (as a consequence of an individual-level effect on sex change) into a population-level effect to allow comparisons between the ecological risks estimated by using different endpoints or chemicals (Forbes and Calow, 1999; Tanaka and Nakanishi, 2000).

Previous studies have reported that juvenile hormones and their agonists (analogs), which have been developed as insect growth regulators, induce the production of males in some cladoceran species including *D. magna* (Olmstead and LeBlanc, 2002, 2003; Tatarazako et al., 2003; Oda et al., 2005a, 2005b). More current studies have also described the time- and age-dependent effects of pyriproxyfen (Ginjupalli and Baldwin, 2013; Abe et al., 2014), one of the major juvenile hormone analogs. This compound has two distinct effects to change sex (the production of males) and to reduce the reproductive capacity (the total number of neonates) in *D. magna* (Watanabe et al., 2018). We have chosen pyriproxyfen as the test substance for the present study, because this compound is repeatedly demonstrated to have the multiple effect on the change of sex and reproduction. Elucidation of ecological effects and development of risk assessment method for pyriproxyfen have practical importance as well, since this compound is widely used in household, and for agricultural and horticultural applications to control insect pests (Ishaaya and Horowitz, 1995). Nonetheless, our main purpose is to present a general framework of an ecologically sound procedure of risk estimation for EDs that have the adverse effects on daphnids similar to those pyriproxyfen induces.

The framework in the present study was composed of three parts including the final part in which the risk estimates were attempted to be compared between the sex ratio change and the reproductive inhibition induced by the same compound. The first part was a toxicodynamic model that predicted both the probability of a change of sex (from female to male) to occur for a particular neonate and the second part was to estimate the reduction of age-specific fecundity (the number of offspring with either sex) of that neonate when it matures due to time-dependent exposure to a chemical. All model parameters critical for predicting these responses were estimated by using Markov chain Monte Carlo (MCMC) simulations within a Bayesian framework (McCarthy, 2007; Billoir et al., 2008).

The third part of the framework was designed to convert the predicted change in sex ratio and age-specific fecundity to a population-level effect by using a matrix population model (Liess et al., 2006; Hanson and Stark, 2011). Using the estimated posterior distributions of the model parameters, we estimated the population-level effects and the probability distribution of the chemical with regard to specific exposure scenarios causing a reduction in the intrinsic population growth rate.

Finally, the population-level effects that were reflected by either the change in sex or the inhibition of reproduction or both were compared in terms of population growth rate. *D. magna* responds exposure to pyriproxyfen by reducing the total number of neonates produced by a female as well as by changing neonates' sex into male (Oda et al., 2005b; Watanabe et al., 2018), and the exposure concentrations which induce these responses noticeably overlap (Watanabe et al., 2018). Therefore, the insecticide pyriproxyfen provided a good system in which we could demonstrate the availability of a population-level risk analysis in measuring ecological risks induced by different modes of action by chemicals.

2. Materials and methods

2.1. Daphnia magna extended reproduction data

We used chronic reproduction data using *D. magna* that were obtained in our research project and has been published elsewhere (Watanabe et al., 2018). Here we outline the experimental procedure and the major results.

All experiments were conducted in accordance with OECD Test guideline 211. We used the NIES strain of *D. magna* (Oda et al., 2005b) as the test organism. In brief, less than 24-h-old offspring obtained from 2-week-old daphnids were exposed to each concentration of the test chemical with the semi-static procedure for 21 days. The rearing media including the food and the test chemical was renewed three times a week. Ten replicate glass vessels each containing a single neonate in 50 mL of the test solution were kept at 21 ± 1 °C under a 16-h light and 8-h dark photoperiod. The daphnias were fed daily with approximately 0.1 mg carbon of freshwater alga *Chlorella vulgaris*. All offspring produced were removed to be counted every day, and were morphologically sexed after the removal under a stereomicroscope on the basis of the length of the first antennae, as described in Annex 7 of OECD Test Guideline 211 (OECD, 2012).

As the test substance, we used pyriproxyfen (CAS 95737-68-1, 99.0% purity) and prepared a 10,000-fold stock solution for each test concentration in dimethylformamide and added it to the M4 medium at a concentration of 0.01% (v/v).

Experiments in the present study consists of two major parts that have alternative schemes of exposure, the constant exposure and the pulsed exposure. The pulsed-exposure experiment consists further of several subschemes of pulsed exposure: four kinds of single-pulse regime (one of the exposure regimes, which was the single-pulse exposure during the first two days at the start of experiment, "P1-1" see below, was not used for the analysis of sex change since it induced no male production) and one multiple-pulse regime (see Graphical abstract of Watanabe et al. (2018) for graphical representation of the exposure schemes used in this study).

For the constant exposure experiment, we conducted a standard 21-day toxicity assay to obtain a concentration–response relationship. This experiment was performed at nominal pyriproxyfen concentrations of 25, 74, 222, 677 and 2000 ng/L. Measured concentrations in these treatments, which were estimated using GC/MS (GCMS-QP2010, Shimadzu Co., Kyoto, Japan), are available in Watanabe et al. (2018; Supplementary Material). Three measurements were conducted at the start and at the end of the renewal period (2 days) for each nominal concentration, resulting in average values at the start of renewal as 28.7, 83.7, 230, 713 and 2290 ng/L. The measured concentrations faded rapidly during the renewal period, as indicated by the time-weighted average concentration was 59% of the nominal concentrations on average across concentrations. We used the nominal concentrations for modeling the response data including those obtained with the pulsed-exposure regime, because the measured concentrations did not show impermissible discrepancies with the nominal concentrations.

For the single-pulse exposure treatment, the daphnias were exposed to 525 ng/L pyriproxyfen for 2 days at 4 different age-classes; Day 0–1 (neonate), Day 5–6 (juvenile), Day 10–11 (adult), and Day 15–16 (adult), the scheme of which was respectively denoted as P1-1, P1–2, P1–3 and P1–4. For the multiple-pulse exposure treatment, which was denoted as P4, the daphnids were exposed to 131 ng/L pyriproxyfen for 2 days in each four age-class (Day 0–1, Day 5–6, Day 10–11, and Day 15–16), and then the total duration of exposure was 8 days.

All of the pulsed-exposure regimes were standardized to 50 ng/L in terms of time-weighted average concentration during the 21-day duration of the experiment, in order to exclude the effect of total amount of exposure that animals received from effects resulting from differences in exposure regimes.

From the constant exposure experiment EC_{50} was estimated as 137 ng/L [95% confidence interval (95% CI): 126–148 ng/L] for fecundity and 238 ng/L (95% CI: 173–302 ng/L) for proportion of male offspring. The constant exposure of 50 ng/L pyriproxyfen did not affect either the fecundity or the proportion of male offspring, while a single-

pulse exposures (P1-1, P1–2, P1–3 and P1–4) did not reduce fecundity, but the proportion of male offspring increased depending on the four subcategories (the timing of exposure in terms of the age of the parent). The multiple-pulse exposure (P4) resulted in a decrease in fecundity and the highest proportion of male offspring (Watanabe et al., 2018).

2.2. The sex change model

To predict the effect of time-specific exposure to pyriproxyfen on the sex of *D. magna* neonates, we constructed a simple toxicodynamics model (*the sex change model*) which assumed there was a limited period of sensitivity during prenatal development. Receptors to pyriproxyfen were supposed to be activated with exposure to pyriproxyfen only during this time and induce a change in sex at a later developmental stage.

In the sex change model, time (τ) for a neonate individual, which is discrete and measured in days, was set 0 at the day of birth (release from its parent) and counted backward through the prenatal development stage (for example, $\tau = 3$ means three days before birth). We assumed that a hypothetical receptor for pyriproxyfen was distributed during the developmental period with a relative density of $n_R(\tau)$. For simplicity, we assumed a uniform distribution for $n_R(\tau)$ with median m and the range of $2d + 1$.

$$n_R(\tau) = \begin{cases} 1 & if\ m - d \le \tau \le m + d \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

The relative amount of ED molecules bound to the receptor at time τ, which is denoted as f_τ, was assumed to follow a stepwise linear model (a linear function with a threshold and a ceiling),

$$f_\tau = n_R(\tau) \min[h \max(x_{t-\tau} - \theta, 0), 1], \quad (2)$$

in which x_t is the exposure concentration (in the logarithmic scale) of the chemical at time t (the time in chronology when the neonate was released), θ is the minimum threshold concentration at which the change in sex occurs, h is the hazard coefficient indicating how steeply the response increases with toxicant concentration, and min() and max () denote the minimum and the maximum values in the parenthesis. Thus, a set of two parameters, θ and h, determined the chemical-specific rate at which the sex changed in test organisms and indicated the toxicity of the chemical as regards to the sex change (i.e., smaller θ indicates that the response can be triggered by lower concentrations of the chemical, and larger h indicates that the response is elevated more quickly as the concentration increases). The stepwise linear function was chosen for simplicity and relevance for parameter estimation. We had attempted to use a more informative kinetics function, like the Michaelis-Menten equation, for finding a predictive function. However, it ended up with non-convergent results of posterior distribution for the parameters. We speculated that the sample size and the number of test concentrations of the data we analyzed were not large enough for projecting non-linear concentration-response relations.

The strength of the physiological response to the ED was assumed to be proportional to the mean value of f in the sensitive period, $\overline{f}$ (referred to as $\overline{f}$-model). However, the maximum f values during the sensitive period, $f_{\max}$, may be more important than the mean value if the endocrine-disrupting effect of chemicals produces an irreversible physiological response in sex change. To include such an effect, we made an alternative assumption for the strength of the physiological response to the exposure (referred to as $f_{\max}$-model). The probability of sex change p under a particular exposure regime was assumed to be

$$p \cong \overline{f} \quad (3a)$$

in the $\overline{f}$-model, and

$$p \cong f_{\max} \quad (3b)$$

in the $f_{\max}$-model. Here, $f_{\max}$ is the maximum f value during the period of sensitivity, and $\overline{f}$ is the mean value of f, that is, $\overline{f} = \frac{1}{1+2d}\sum_{\tau=m-d}^{m+d} f_\tau$.

2.3. Reproductive inhibition model

In accord with the dynamic energy budget (DEB) model (Kooijman and Metz, 1984; Kooijman and Bedaux, 1996), we established the following equation for the body length at age a relative to the asymptotic maximum body length, which is equivalent to the well-known von Bertalanffy equation,

$$l_a = 1 - (1 - l_0)e^{-\gamma a}, \quad (4)$$

where l_0 is the body length at birth relative to the maximum length, and γ is the growth rate coefficient (Appendix A). In simulations we used numerical solutions for the difference equation of body length (Appendix A), which approximated the differential of Eq. (4) as

$$l_{a+1} = l_a + \gamma(1 - l_a). \quad (5)$$

We further assumed that the reproductive output (the total energy spent for reproduction at a particular time), denoted w_a as being relative values to the maximal reproductive output, was equal to the squared relative body length: $w_a = l_a^2$, following the assumption made in the classical DEB framework (Kooijman and Metz, 1984).

Cladoceran species reproduce asexually by releasing broods of neonates several times throughout their lifespans. We assumed that each brood size was determined by the reproductive output accumulated after the previous release or by the age of reproductive maturity. The zth brood size B_z is predicted by

$$B_1 = F_{\max} \sum_{a=\alpha}^{t_1-1} w_a \text{ and } B_z = F_{\max} \sum_{a=t_{z-1}}^{t_z-1} w_a, \quad (6)$$

in which t_z is the day when the zth brood was released, $F_{\max}$ is the maximal fecundity per day when the body length reaches the maximum, and α is the age of reproductive maturity, which was assumed to be 3 days before the day of the first observed reproduction throughout this study.

The body length of *D. magna* at birth relative to the maximal length, l_0, was determined to be 0.13 from published information (Appendix B) and was treated as a fixed parameter throughout this study. The model prediction of reproductive outputs based on the *Daphnia* reproduction data was fairly insensitive to l_0.

The toxic effect of pyriproxyfen on *D. magna* reproduction is assumed to be decomposed into two categories according to how the two model parameters characterize the toxic effect on reproduction, that is, the direct effect s_D and the indirect effect s_I. We assumed the same form of stress functions for the both effects:

$$s_D(t) = \min[h_D \max(x_t - \theta, 0), 1] \quad (7a)$$

and

$$s_I(t) = \min[h_I \max(x_t - \theta, 0), 1], \quad (7b)$$

in which x_t is the exposure concentration of the chemical at time t, θ is the threshold concentration, below which any toxic effects are not observed, and h_D and h_I indicate the hazard coefficients of the direct and the indirect effects. We treated the threshold concentration θ as a fixed parameter and defined it for simplicity as the no-observed effect concentration (NOEC) estimated for reproduction under the constant-exposure regime.

The direct effect on reproduction is supposed to result in immediate and reversible inhibition of reproduction, and exponentially reduce the relative reproductive output,

$$w_{a,t}^* = w_a \exp(-s_D(t)). \quad (8)$$

The asterisk denotes that the model parameter includes responses to the chemical.

It was further assumed that the indirect effect of the toxicant is to affect body growth of individuals. To be more specific, the toxicant was

supposed to affect the shape of the growth trajectory, determined by γ in Eq. (5) (Appendix A):

$$\gamma_t^* = \frac{\gamma}{1+s_I(t)}, \tag{9}$$

where γ_t^* indicates the growth rate coefficient under toxicant exposure at time t.

The relative body length under toxicant exposure is approximated by the following difference equation,

$$l_{a+1,t+1}^* = l_{a,t}^* + \gamma_t^*(1-l_{a,t}^*), \tag{10}$$

where $l_{a,t}^*$ indicates the body length of individuals aged a under the toxicant exposure at time t. Then, the relative reproductive output by individuals aged a at time t under both the direct and the indirect effects of the chemical is

$$w_{a,t}^* = (l_{a,t}^*)^2\exp(-s_D(t)). \tag{11}$$

Estimates of each brood size released by a female with a particular exposure history were obtained by Eqs. (6), (9), (10) and (11), with the date of reproductive maturity (α) determined directly from reproduction data.

2.4. Bayesian estimation of model parameters

Model parameters related to sex change and reproductive inhibition were separately determined with the Bayesian-based simulation (Markov chain Monte Carlo simulation, MCMC).

For sex change, the likelihood L of the model fitting the observed data was determined based on p, the probability of sex change. For maximum likelihood estimation of model parameters, especially those regarding the sensitivity period, m and d, the experimental design must include intermittent or pulsed-exposure regimes (experiments that include only constant-exposure regime are not relevant for estimating m and d). Each experimental replicate (i.e., the glass vessels that contained the test organisms) contained a parental female under a specific exposure regime, and the sex of all offspring at birth was recorded. The number of neonates that had changed sex (i.e., the number of males) among the offspring in the same replicate was assumed to be subject to the binomial probability distribution of sex change in the replicate, which was predicted using model parameters and exposure data according to the sex change model. Thus, the likelihood of the sex change model was defined as

$$L = \prod_{i=1}^{S} \frac{n_i!}{n_{f(i)}!\, n_{m(i)}!} p_i^{n_{m(i)}}\{1-p_i\}^{n_{f(i)}}, \tag{12}$$

where i is the index of the experimental replicate, S is the number of experimental replicates, p_i is the probability of sex change predicted for replicate i, $n_{f(i)}$ is the number of female offspring in replicate i, $n_{m(i)}$ is the number of male offspring in replicate i, and n_i is the total number of offspring in replicate i. Log-likelihoods cannot be evaluated when the prediction of p is exactly 0 or 1. Therefore, we assumed a floor and a ceiling for p, using

$$p = c\frac{exp(\varphi-0.5)}{1+exp(\varphi-0.5)} + (1-c)\varphi, \tag{13}$$

where $c = 0.01$ and $\varphi = \overline{f}$ or f_{max}. The right-hand side of the above equation converges to 0.996 or 0.00378 when φ converges to 1 or 0, and approximates φ very well as long as φ is not extremely close to 1 or 0.

In the above notation, the exposure regime is defined for each experimental replicate, and therefore all offspring of the same cohort in the same experimental replicate shared the exposure regime and day of birth. However, the exposure regime could be different between individuals that had different days of birth, even if the two individuals were produced by the same parent (in the same experimental replicate).

Once the sex and exposure regime were identified for all newborn neonates, we were able to evaluate the log-likelihood values for all model parameters (m, d, h and θ). MCMC simulation in the Bayesian statistical framework was practiced to derive posterior distributions of model parameters, m, d and h. The threshold concentration θ was treated as a fixed parameter, which was determined as the NOEC in the stationary exposure experiment; thus, $\theta = \log(\text{NOEC})$. Preliminary MCMC simulations in which both h and θ were treated as the model parameters to be estimated did not produce results with enough convergence in the posterior distributions (see the next paragraph); therefore, we were not able to make an efficient prediction on the joint distribution of h and θ.

We determined the initial value of h in the MCMC simulations from $h = 1/\{2(\log(EC_{50}) - \log(NOEC))\}$, in which the concentration that gave a half-maximal response, EC_{50}, and the NOEC were determined in the stationary exposure experiment. These values were estimated as NOEC = 74 ng/L and EC_{50} = 240 ng/L (Watanabe et al., 2018), thus we get $\theta = 1.87$ and $h = 0.98$. A uniform distribution was assumed for the prior distributions for h (range: 0.5–5, initial values: 0.9 and 1.1), m (range: 2–7, initial values: 3 and 6) and d (range: 0–2, initial values: 1 and 2), because we did not have any information on the shape of the probability distribution of these parameters. The ranges of prior distributions of m and d were set as biologically plausible values (Matsumoto et al., 2008).

For reproductive inhibition, the MCMC simulations were conducted separately for the two sets of reproductive inhibition experiments (i.e., the constant-exposure and the pulsed-exposure experiments), because the two data sets produced different control fecundities and growth trajectories (i.e., lower fecundity and slower growth in the pulsed-exposure experiment) due to unidentified factors. Parameter estimations were achieved through two steps for each data set. The first step was conducted to get the maximum-likelihood estimates of F_{max} and γ, which characterized the fecundity schedule under the uncontaminated condition, from the control data. We used uniform distributions both for the prior distribution of F_{max} (range: 10–70) and of γ (range: 0.01–1 for the constant-exposure data, and 0.01–0.05 for the pulsed-exposure data).

In the second step, we conducted additional MCMC simulations by using the maximum-likelihood estimates of F_{max} and γ as fixed model parameters for deriving posterior distributions of the hazard coefficients h_D and h_I. The prior distributions were set as uniform distributions (range: 0–1) for both h_D and h_I in either exposure scheme. The second step consisted of three subsets of MCMC simulations, which assumed both the direct and the indirect effects or either effect individually.

The likelihood that the model predicts a brood size by a female is

$$L = \frac{1}{\sqrt{2\pi\sigma}}\exp\left\{-\frac{(D-B)^2}{2\sigma^2}\right\}, \tag{14}$$

in which D is the observed and B is the predicted brood sizes (Eq. (6)). The log-likelihood of the model with a particular set of parameter values is the sum of log-likelihoods of all broods of all parental females in the entire data set.

We used the Metropolis algorithm (Metropolis et al., 1953; McCarthy, 2007) to achieve the MCMC simulations, using a normal likelihood function and a normal proposal function for both the sex change and the reproductive inhibition. To check the convergence of the derived posterior distributions, which indicated the reliability of the results, we conducted a pair of repeated runs of simulations, each of which calculated 12,000 steps of the Metropolis-Hastings algorithm (the first 2000 steps of each run were disregarded). The multivariate scale reduction factor, $\hat{R}^p$ (Gelman and Rubin, 1992; Brooks and Gelman, 1998), which represents the convergence of a set of solutions, was evaluated for the $\overline{f}$- and f_{max}-models of the sex change and for the reproductive inhibition model. When convergence was confirmed, the posterior distributions of the model parameters were redefined and

analyzed further by combining the pair of posterior distributions for the same model parameter.

2.5. Calibration of the model

For testing validity of parameter estimation with the MCMC simulation, the reproductive outputs predicted from the maximum-likelihood estimates of model parameters were compared to the observed reproductive outputs. This process was meant to improve our assumptions with the model and help debug our simulation programs; it is not directly reflected in the parameterization process.

In order to check if the model works well to predict sex ratios, we used the best-fit model with the maximum-likelihood estimates of m, d, and h to get theoretical predictions of sex ratios, and compared them with the observed sex ratios.

As for the reproduction data, the number of neonates produced on a particular date by a particular female (the brood size) was transferred into daily reproductive outputs, because the model directly predicted the daily reproductive outputs rather than brood sizes. This was worked out by dividing the brood size by the number of days passed after the last release (the interval was set to 3 days for the first brood) and by averaging these values across all individuals under the same exposure scheme

2.6. Matrix population model

We assumed that the population-level effect of a change in sex ratio was the result of a loss of females that would have reproduced had they not changed sex. This effect was implemented in the model by assuming that the number of lost females was equal to the number of males produced. The female population size was simulated by using a time-specific, age-structured model because this type of model is most relevant for predicting population numbers when there are temporal changes in age structure or exposure concentration. Denoting the abundance of females of age a at time t as $N_f(a,t)$, the changes in population abundance by reproduction and by change in sex were calculated by using the following recurrence equations:

$$N_{f(a+1,t+1)} = (1-m_a)N_{f(a,t)} \tag{15a}$$

$$N_{f(1,t+1)} = (1-p_t)\sum_{a=\alpha}^{\min(a_{\max},t)} F_{a,t}N_{f(a,t)}, \tag{15b}$$

where m_a is the daily mortality at age a, $F_{a,t}$ is the *per capita* fecundity of females at age a, p_t is the rate of sex change at time t using Eqs. (3) and (13), α is the age at first reproduction, and $a_{\max}$ is maximum age (i.e., longevity). We assumed that daily mortality was constant across ages.

As for the value of $F_{\max}$ and γ, the population simulations used the maximum likelihood (best) estimates of these parameters ($F_{\max} = 17.7$ and $\gamma = 0.168$) obtained from the control data in the constant-exposure experiment. These values resulted in the intrinsic population growth rate to be 0.35, which was in accord with several observed values of the intrinsic population growth rate in *D. magna* and other *Daphnia* species (Andersen, 1997).

The *per capita* age-specific fecundity $F_{a,t}$ was assumed to be proportionate to the reproductive output: $F_{a,t} = F_{\max}w^*_{a,t}$. To quantify the relative importance of the population-level effects of the changes in sex ratio and of the reproductive inhibition, population simulations were repeated using models that included either change in sex ratio or reproductive inhibition or both.

As a measure of demographic effect, we used the intrinsic population growth rate defined from the ratio of total population size TN across all ages at time $t_{\max}$ to that at the initial condition,

$$TN = \sum_{a=1}^{\min[a,t_{\max}]} N_{f(a,t_{\max})} \tag{16}$$

$$r = \frac{1}{t_{\max}-1}ln\left(\frac{TN}{N_1}\right), \tag{17}$$

where N_1 is the initial population size.

To examine how a particular model of sex change affected the predictions of recovery from exposure to pyriproxyfen within a very limited duration (i.e., pulsed exposures), we used two alternative scenarios of exposure, namely, the constant-exposure scenario and the pulsed-exposure scenario. In the constant-exposure scenario, exposure concentrations (range, 70 − 400 ng/L) were fixed during each population simulation. In the pulsed-exposure scenario, each exposure event lasted for a single day only and was followed by a period without exposure, which was set from 1 to 9 days; the first exposure event was set on the tenth day of each population simulation. The mean exposure concentration over time (i.e., the time-weighted mean concentration) was kept constant (200 ng/L), meaning that longer intervals resulted in stronger pulses of exposure.

3. Results

3.1. Posterior distributions of the model parameters

The median of the period of sensitivity (m) was estimated as 5 for the $\overline{f}$-model and as 6 for the $f_{\max}$-model without any uncertainties (i.e., there was no variability in the posterior distributions), and the width of the period of sensitivity (d) was determined as 1 for both models, indicating that the embryos were sensitive to pyriproxyfen for a period of 3 days. Estimates of the starting point of the period of sensitivity slightly differed between the versions of the model, although the length of the period did not differ between the models.

The mean value of the hazard coefficient (h) was estimated as 1.21 for the $\overline{f}$-model and as 0.927 for the $f_{\max}$-model (Fig. 1), which were both close to unity, implying that the response curves were so steep that the entire response was complete within one order of magnitude of toxicant concentration. The posterior distributions of h were unimodal with the 95-percent range located within 10% of the mean (Fig. 1), suggesting that the estimations were reasonably narrow. The 95-percent confidence intervals of h lay between 0.774 and 0.976 for the $\overline{f}$-model and between 0.808 and 0.973 for the $f_{\max}$-model.

The maximum log-likelihood values were comparable between the $\overline{f}$-model (−400.8) and the $f_{\max}$-model (−413.7), implying that both models exhibited nearly equivalent performance in fitting the data. Multivariate scale reduction factor was determined as $\hat{R}^p = 1.18$ for the $\overline{f}$-model and $\hat{R}^p = 1.003$ for the $f_{\max}$-model, indicating that the simulations based on the two models had both reached convergence in their solutions. The parameter adoption rates among candidate values were greater than 24% in both models.

From the fecundity data we obtained the maximum-likelihood estimates for $F_{\max}$ of 17.7 and 41.1, and for γ of 0.168 and 0.035, from the first step of the MCMC simulation, according to the control data in the constant-exposure and pulsed-exposure experiments, respectively. The bivariate posterior distributions showed highly convergent results ($\hat{R}^p = 1.00$ and 1.03 for each data set).

The second step of the MCMC simulation produced convergent results of posterior distributions of h_D and h_I except for the case when both h_D and h_I were included in the analysis for the constant-exposure experiment and when only h_D was included in the analysis for the pulsed-exposure experiment (Fig. 2a,b); in the former case, the distribution of h_D was extremely skewed to the lower bound and the mean value was much lower than h_I, and in the latter case, the posterior distribution of h_D was bimodal, and the convergence criterion was insufficiently met, $\hat{R}^p = 1.24$.

For the pulsed-exposure scheme, the joint posterior distribution of h_D and h_I was well estimated with sufficient convergence ($\hat{R}^p = 1.00$), although some colinearity between the two parameters occurred (the correlation coefficient: −0.45). The expected (mean) value of h_I

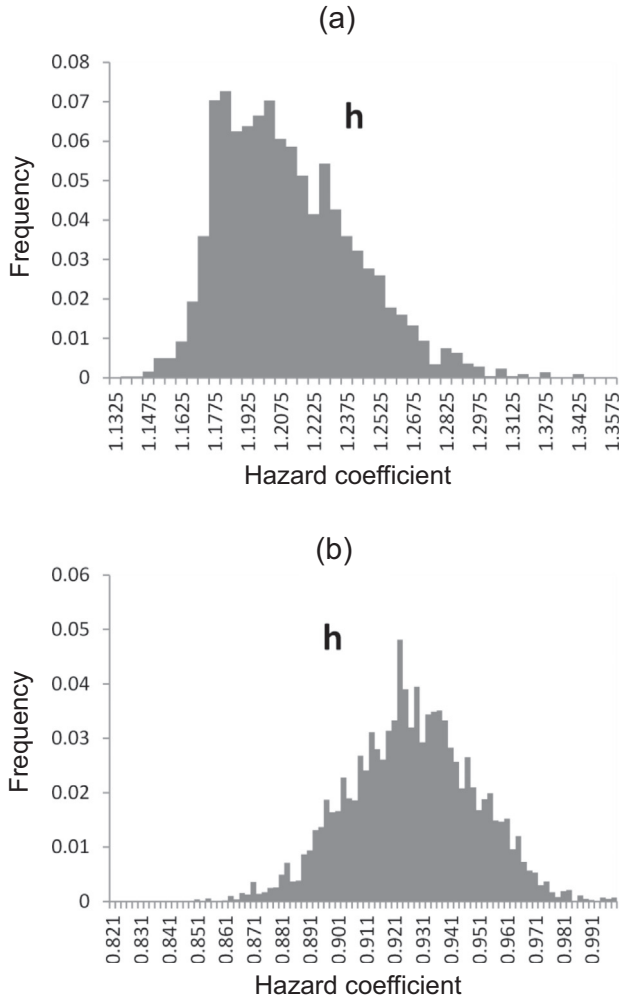

Fig. 1. Posterior distribution of the hazard coefficient in the $\overline{f}$-model (a) and in the f_{max}-model (b) on sex change estimated of *Daphnia magna* under time-varying exposures of pyriproxyfen.

(0.0083) was considerably larger than the expected value of h_D (0.0014), thus suggesting the greater importance of the indirect effect in predicting the toxicant's effect under pulsed exposures on the reproduction of *D. magna* (Table 1).

3.2. Test of the model prediction

The model predictions reproduced well the observed responses, especially with regard to the timing and duration of the changes in sex ratio; however, the predicted changes in sex ratio were generally underestimated for the pulsed-exposure regime (Fig. 3). Because there were no cases where intermediate responses in sex ratio were observed in the pulsed-exposure regime, h was not reliably estimated without including the data obtained for the constant-exposure regime, which showed an incomplete response at 240 ng/L. The present dataset might not have enough information to elucidate the shape of the change in sex ratio in relation to pyriproxyfen concentration in the pulsed-exposure regime.

The predicted reproductive outputs were also in good accordance with the age-specific pattern of the reproduction data observed under the constant-exposure scheme (Fig. 4a). Under the pulsed-exposure scheme, the response function based on the indirect effect produced comparable or slightly better fits to data in comparison to the response function based on the direct effect (Fig. 4b). We disregarded the model that included both the direct and the indirect effects, because the inclusion of the direct effect resulted in no better fits of the model to the data than did the case where the indirect effect alone was included in the model.

In addition, fitting the reproduction data under the pulsed-exposure scheme was more difficult, because the real reproductive outputs fluctuated irregularly over time. Nonetheless, the models that included either the indirect effect only or both effects simulated the pattern of reproductive outputs better than did the model that included only the direct effect. The real *Daphnia* reproduction data did not observe any clear reduction during the exposure periods nor sudden recovery from the reduction like the model that included only the direct effect would have predicted (P1–3, P1–4 and P4; Fig. 4b).

3.3. Simulated population-level effect

The population simulations indicated substantially different risk levels for constant- exposure and pulsed-exposure scenarios, and the relative importance between the two models of sex change was reversed between the exposure scenarios. Under the $\overline{f}$- model (Figs. 5a and 6a), the constant-exposure scenario induced much larger reductions in the intrinsic population growth rate than did the pulsed-exposure scenario. With intervals of 4 days or longer, even an extremely intense exposure concentration (480 ng/L) did not reduce the population growth rate by more than 15% due to sex ratio change (Fig. 6a). The EC_{50} of sex change (male production) under constant exposure was estimated approximately 240 ng/L (Watanabe et al., 2018). The reasoning why the strong pulsed exposure with concentrations much higher than EC_{50} had considerably smaller effect at the level of populations if there were enough intervals between pulses might be given in two ways; (1) in populations consisting of females at various reproductive stages, intermittent exposures affect only a small fraction of all reproductive outputs by females because the sensitive period of sex change is limited, and (2) especially for the $\overline{f}$- model, the probability of sex change is assumed to be proportional to the time-weighted mean concentration during the sensitive period. Thus, the pulsed exposure of high concentration is diluted in its effect on the sex change. However, the latter effect might vanish in the f_{max}-model as described below, in which the strong pulsed exposure would have outweighed the mitigating effect of population structure, resulting in inflated effect by the pulsed exposure.

In contrast, under the f_{max}-model (Figs. 5b and 6b), the pulsed-exposure scenario induced much larger reductions in the intrinsic population growth rate than did the constant-exposure scenario. The population-level effect predicted by the f_{max}-model tended to be smaller than that predicted by the $\overline{f}$-model when the exposure was constant. The effects of pulsed exposure largely depended on the assumption underlying the model of sex change and the interval between the pulses of exposure (Fig. 6a,b). The inflated effect induced by pulsed exposure with an interval of 2 days in the f_{max}-model (Fig. 6b) implied that peak concentrations were more important than the absence of intervals as long as the intervals were not longer than the sensitive period of 3 days.

The above conclusions were made clearer when we compared the population-level effects of sex change with those of the reproductive inhibition (Figs. 5, 6). When change in sex was approximated by the $\overline{f}$-model, the sex change caused approximately the same degree of population-level effects as did the reproductive inhibition, regardless of exposure scenario (Figs. 5a, 6a). However, when change in sex was simulated by the f_{max}-model, the sex change caused a population-level effect that was about half the magnitude of that predicted for the reproductive inhibition under the constant-exposure scenario, whereas it caused a larger population-level effect than that caused by the reproductive inhibition under the pulsed-exposure scenario (Figs. 5b, 6b). Overall, change in sex ratio induced nearly the same amount of population-level effects as did the reproductive inhibition, except for the case where the exposure was pulsed and the change in sex was subject to the f_{max}-model.

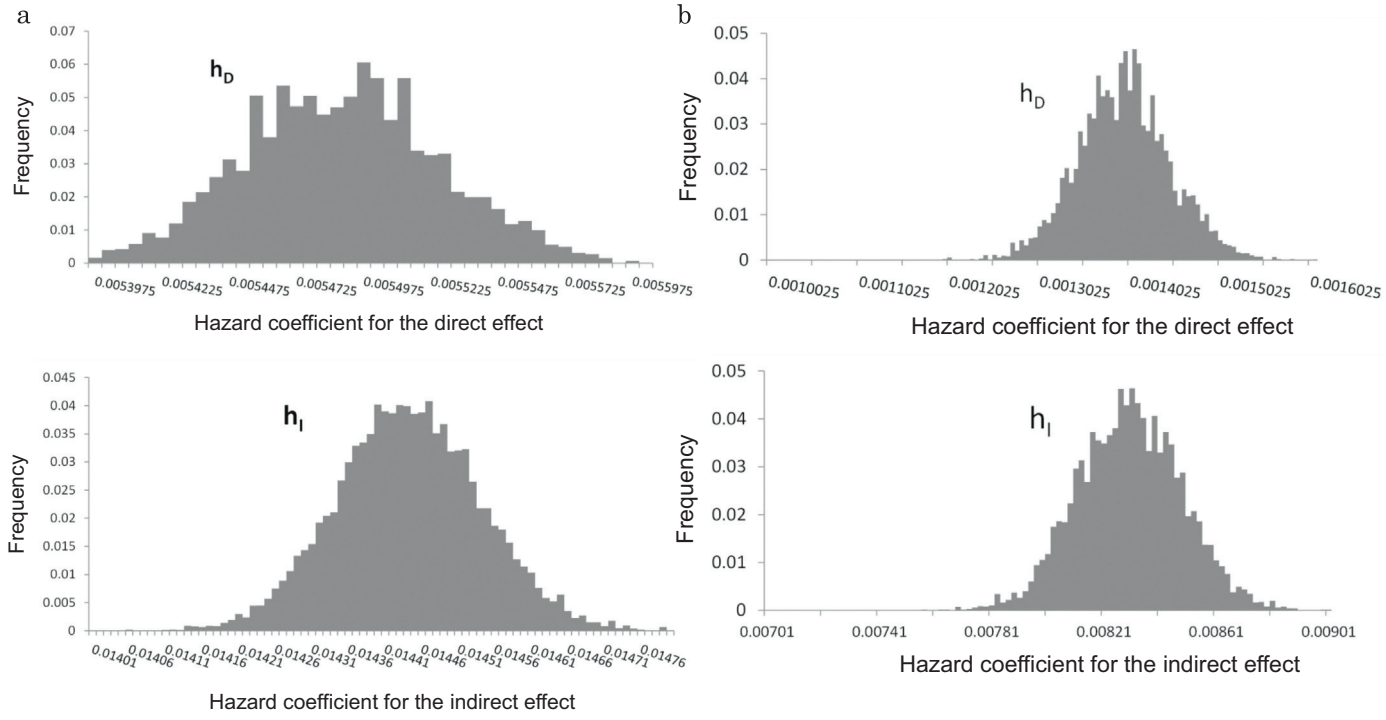

Fig. 2. a Posterior distribution of the hazard coefficient for the direct (upper figure) and the indirect effect (through growth effect; lower figure) on reproduction estimated from *Daphnia magna* reproduction data under constant exposures of pyriproxyfen. The posterior distribution for either parameter was calculated by Markov chain Monte Carlo simulation which disregarded the other parameter. b: Posterior distribution of the hazard coefficient for the direct (upper figure) and the indirect effect (through growth effect; lower figure) on reproduction estimated from *Daphnia magna* reproduction data under pulsed exposures of pyriproxyfen. The marginal distribution was depicted for each parameter. The posterior distributions for the two parameters were calculated by Markov chain Monte Carlo simulation which included the both parameters.

Table 1
Predicted population-level EC_x values under the constant exposure scenario.

Response	pop-EC_5	pop-EC_{10}	pop-EC_{20}
Sex change (TWM)	98.8	126.5	187.3
Sex change (MAX)	108.0	149.1	249.6
Reproductive inhibition	90.0	107.0	149.6
Both effects (TWM)	83.5	93.6	115.7
Both effects (MAX)	84.9	95.8	121.1

The population-level EC_x (pop-EC_x) are indicated in the scale of ng/L. The sex changes were predicted with two alternative assumptions that the sex change was based on time-weighted mean concentrations (TWM) of chemicals during the sensitive period according to the $\bar{f}$-model, and the sex change was based on the maximum concentrations (MAX) of chemicals during the sensitive period according to the f_{max}-model (see the text for explanation).

4. Discussion

4.1. The relevance of population-level effects of sex change

We have two distinct but related motivations for estimating the population-level effects and changes in the intrinsic population growth rate induced by EDs in terms of change in sex ratio in *D. magna*. The first motivation is the ecological relevance of such measures, which are expected to be more directly related to population vulnerability and ecosystem function than are other measures. The second motivation is to evaluate the relative importance of the change in sex ratio due to exposure to EDs compared with other standard endpoints (e.g., acute immobility or reproductive inhibition), because the intrinsic population growth rate can summarize the net effects of various endpoints revealed at the individual level into an ecologically relevant metric (Forbes and Calow, 1999; Tanaka and Nakanishi, 2001; Forbes et al., 2001; Tanaka, 2003; Duquesne, 2006). The latter property is especially important for the ecological risk assessment of EDs, because their unique expression of adverse responses to toxicant chemicals make it more difficult to evaluate the ecological risk than other chemicals.

We supposed *Daphnia* populations as being one of the best test systems for assessing the ecological risk with EDs, because most *Daphnia* in natural populations reproduce by means of cyclical parthenogenesis (Brendonck et al., 1998; Arbačiauskas and Lampert, 2003). This essential part of life history of *Daphnia* is an adaptation to overcome temporal and seasonal environmental stresses (Edmondson, 1955; Allan, 1976; Kleiven et al., 1992; Gyllström and Hansson, 2004; Kato et al., 2011). Thus, disruption of the cyclical parthenogenesis in *Daphnia* by endocrine disruptors may increase the vulnerability of populations to extinction. The production of male neonates may be comprised in such disruption (Tatarazako et al., 2003; Oda et al., 2005a, 2005b; Matsumoto et al., 2008; Ginjupalli and Baldwin, 2013). The sex change from female to male may also affect sexual reproduction in *Daphnia*, in which resting eggs are produced at the end of the breeding season. However, the biological and ecological processes underlying how changes in the sex ratio affect the production of resting eggs, and how the abundance of resting eggs influences the vulnerability of the population in the long term, are not well understood.

In this study, we focused on intrinsic population growth rate in the asexual phase of *Daphnia* as a measure of ecological risk because decreases in population densities of parthenogenetically reproducing females are likely to reduce the potential amount of resting eggs produced at the end of the breeding season. Indeed, it has been suggested that a scarcity of resting eggs in lakes may result in the local extinction of zooplankton populations due to demographic and genetic stresses (e.g., due to fish predation or inbreeding depression; Hairston, 1996; Sarnelle and Knapp, 2004).

The results of the present analysis indicate that different scenarios of ED exposure in *Daphnia* have different consequences at the population level, with repeated pulsed- exposure with intervals as short as a

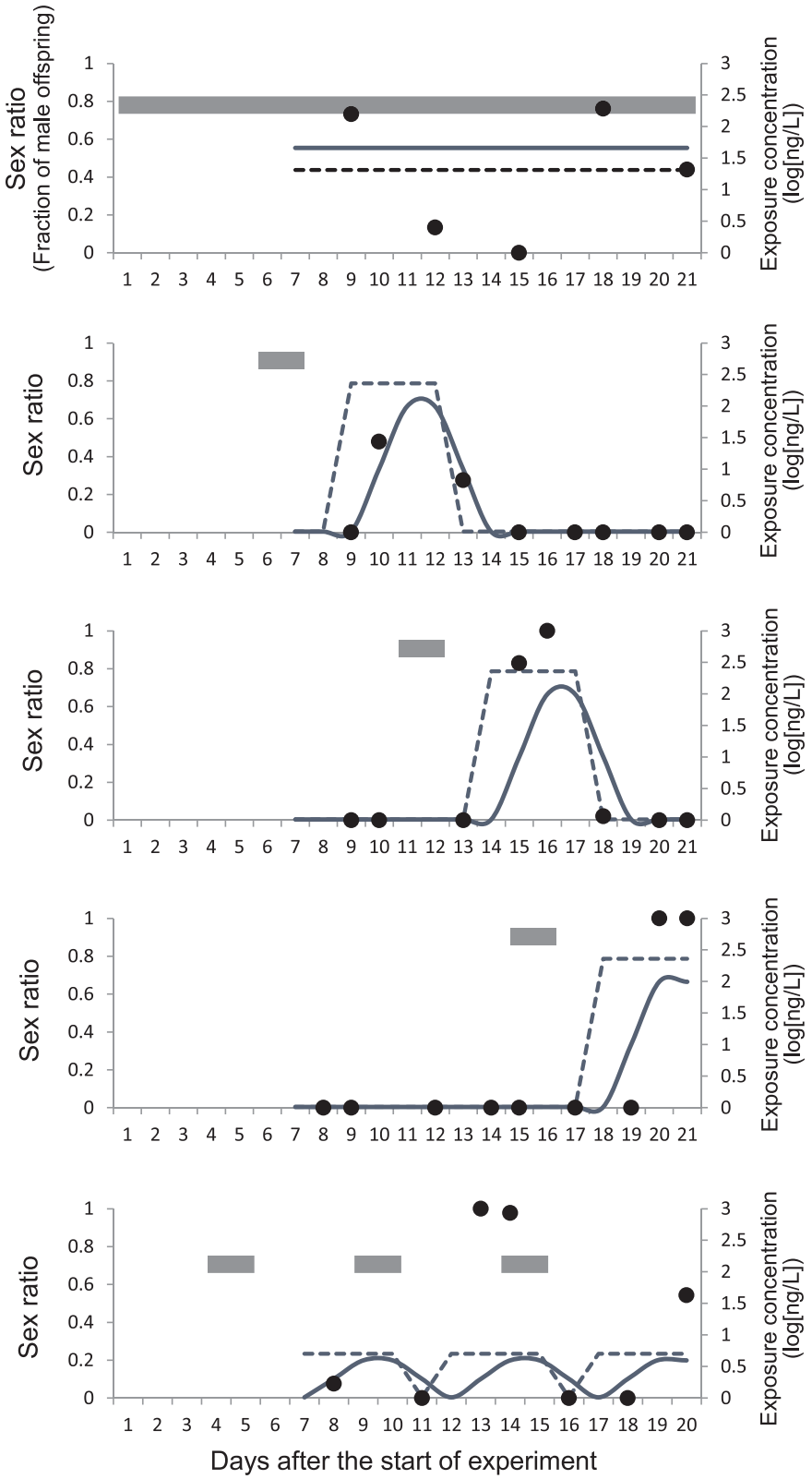

Fig. 3. Fitting data of sex ratio under various exposure regimes with model predictions. The observed fractions of male neonates in all offspring (denotes as dots) were plotted with model predictions. The solid lines represent predicted sex ratios based on the $\overline{f}$-model, whereas the broken lines represent predicted sex ratios based on the f_{max}-model. The multipanels denote different exposure regimes (the grey band in each panel denotes the exposure period in the experiment).

few days being estimated to induce a much weaker impact at the population level than was constant exposure.

The population-level effects predicted by the present model largely depended on the assumptions of the two versions of the model (i.e., the $\overline{f}$-model and the f_{max}-model). The f_{max}-model, in which the response in sex was determined by using the maximum exposure concentration during the period of sensitivity and postulated an irreversible toxicodynamic or physiological process, predicted a much larger effect on

a

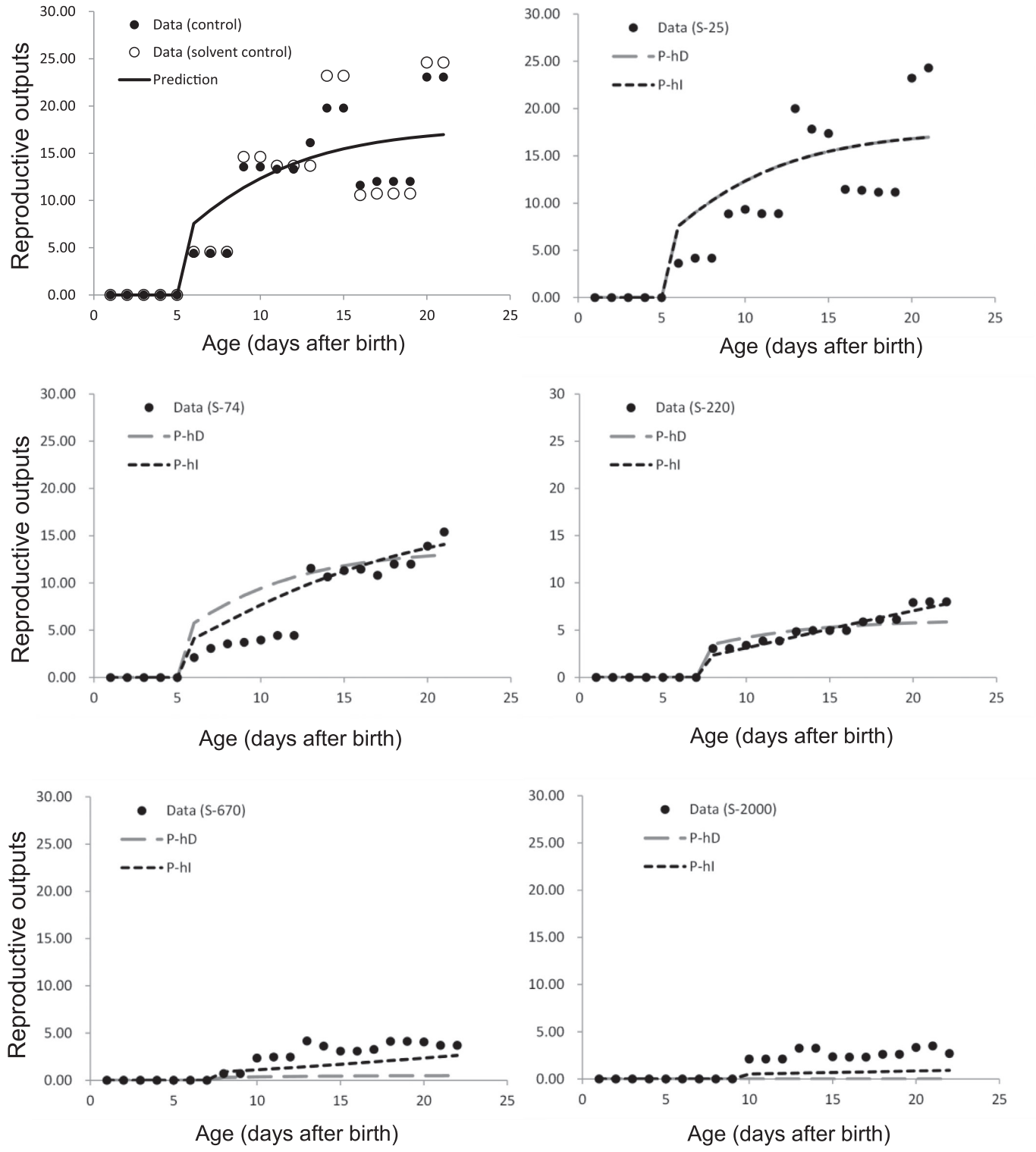

Fig. 4. a Fitting of reproduction data under the constant exposure with the model predictions. The age-specific mean reproductive outputs, which were converted from the brood size data for all individual, were plotted as the empirical data (see text for the explanation). The broken lines represent predictions for reproductive outputs by the two models which respectively assumed the direct effect (P-hD) or the indirect effect (P-hI) of the chemical. The multipanels denote different exposure concentrations (S-25: 25 ng/L, S-74: 74 ng/L, S-220: 220 ng/L, S-670: 670 ng/L and S-2000: 2 μg/L). b: Fitting of reproduction data under the time-varying exposure schemes with the model predictions. The age-specific mean reproductive outputs, which were converted from the brood size data for all individual, were plotted as the empirical data (see text for the explanation). The broken lines represent predictions for reproductive outputs by the two models which respectively assumed the direct effect (P-hD) or the indirect effect (P-hI) of the chemical. The solid lines represent predictions of the model which assumed the both effects. The multipanels denote different exposure regimes (see text for the specific regimes).

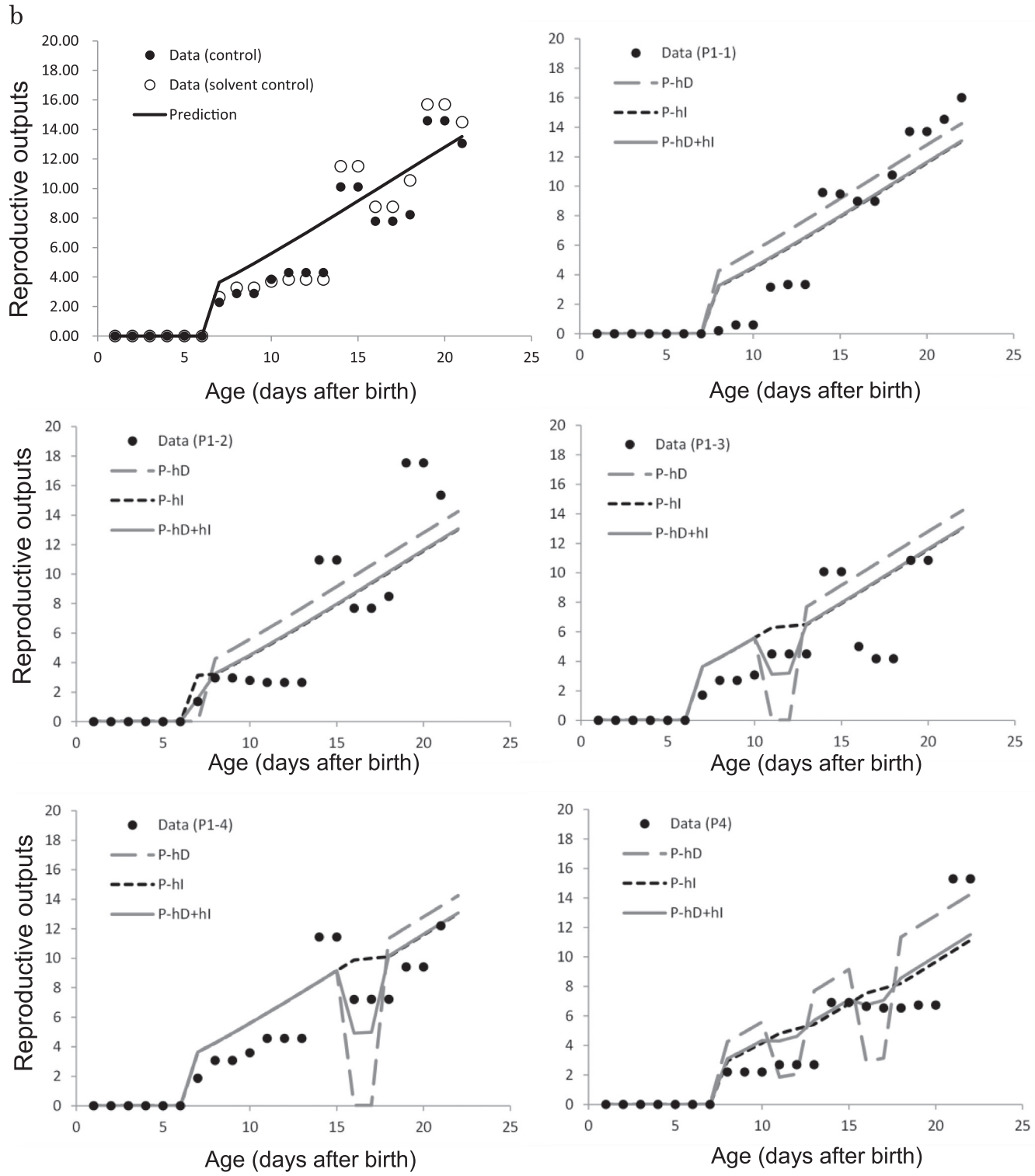

Fig. 4. (*continued*)

the intrinsic population growth rate than did the $\overline{f}$-model, in which the population-level effect faded quickly with intervals between pulses of exposure because the toxicodynamic process to induce sex change decreased at the same rate as the interval between exposures as the period of sensitive was increased. More data are needed to decide which version of the model is the most relevant to predict changes in sex ratio induced by exposure to EDs, although it was suggested that the $\overline{f}$-model performed better than the f_{max}-model with the current analysis.

4.2. Comparison of population-level effects between change in sex ratio and reproductive inhibition

Our results indicated that pyriproxyfen, which induces a change in sex in *Daphnia* neonates, brought about an ecological risk comparable in magnitude with that posed by the adverse effect on reproduction, if the exposure concentration was constant and the effect of the chemical to change sex was linearly associated with the time-weighted average concentrations of the chemical. However, the present analysis also

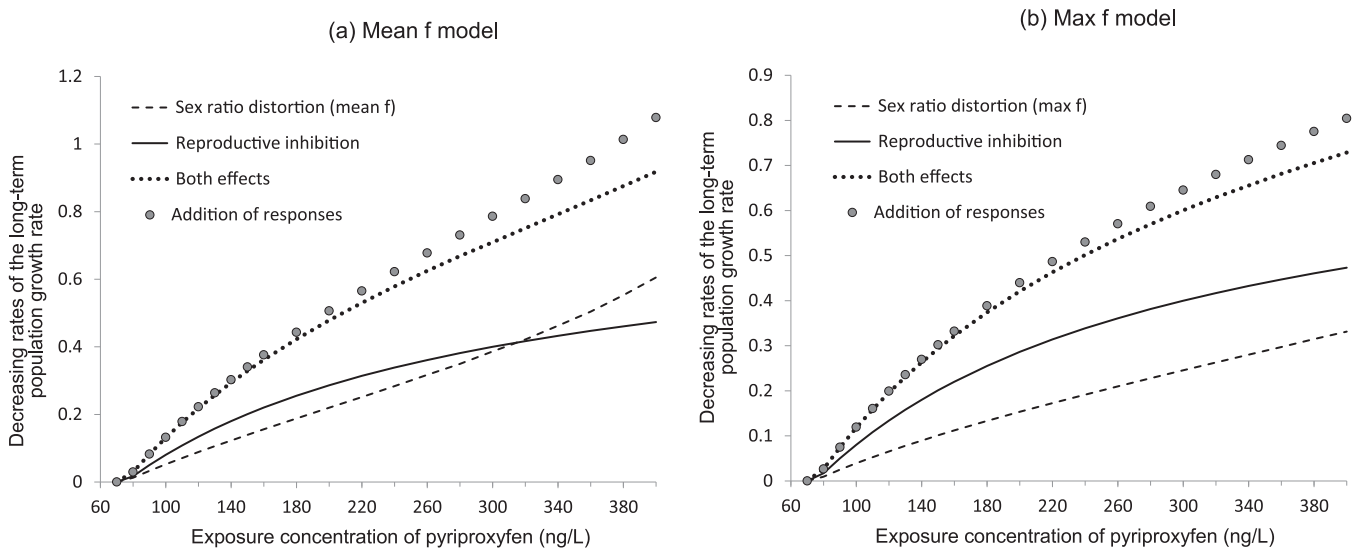

Fig. 5. Population-level effects in terms of reduction of population growth rates by constant exposures of pyriproxyfen. The population growth rates were predicted by population simulations using the age-structured model based on the two alternative assumptions; (a) the $\overline{f}$-model and (b) the f_{max}-model, for sex changes of individuals responding to the exposure. The broken line denotes population-level responses if the response is only due to the sex ratio distortion. The solid line denotes population-level responses if the response is only due to the reproductive inhibition. The dotted line denotes responses by the both effects. The solid circles represent the summations of the two responses.

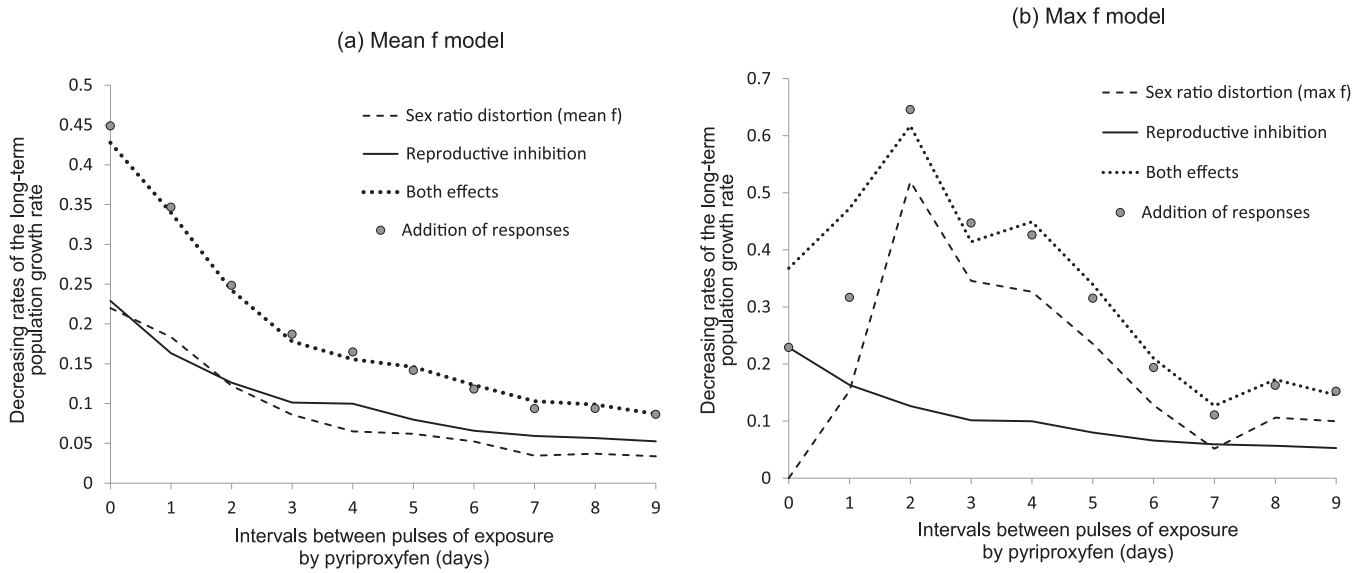

Fig. 6. Population-level effects in terms of reduction of population growth rates by pulsed exposures of pyriproxyfen. The population growth rates were predicted by population simulations using the age-structured population model based on the two alternative assumptions; (a) the $\overline{f}$-model and (b) the f_{max}-model, for sex changes of individuals responding to the exposure. The horizontal axis scales intervals in days between closest pulses of exposure. The time-weighted mean concentrations were fixed as 200 ng/L. The broken line denotes population-level responses if the response is only due to the sex ratio distortion. The solid line denotes population-level responses if the response is only due to the reproductive inhibition. The dotted line denotes responses by the both effects. The solid circles represent the summations of the two responses.

suggested that the assumption of the toxicodynamics for the change in sex at the individual level greatly affected the relative importance of the population-level effect of the sex change and the reproductive inhibition. This finding implies that a more finely tuned analysis of the data using more elaborate mechanistic models of change in sex is required to predict the ecological risk posed by endocrine-disrupting chemicals under time-varying exposure conditions.

Acknowledgments

This study was supported by the feasibility study of Extended Tasks on Endocrine Disruption 2010 (EXTEND2010) funded by the Ministry of Environment, Japan.

Appendix A

Here we show how Eqs. (1) and (2) in the text are related to the dynamic energy budget model as the underlying theoretical framework. The dynamic energy budget model assumes mass balance of energy within organisms' individuals, and presents a relevant description of tradeoffs

between energy spent for reproduction and body growth per unit time. The basic formula is

$$\kappa v f W^{2/3} = mW + g\frac{dW}{dt}, \tag{A1}$$

in which the parameters denote the proportion of energy invested to respiration κ, the uptake rate of energy per unit body surface v, the relative consumption rate f, the body weight W, the maintenance cost m, and the growth cost g (Kooijman and Metz, 1984). The energy captured by an individual per unit time $vfW^{2/3}$ is assumed to be proportional to the body surface, and is apportioned into reproduction by 1-κ and respiration by κ. And the energy spent for respiration is further decomposed into the maintenance of body mW and the growth of body $g\frac{dW}{dt}$. Thus, a reduction of body growth for any reason has a time-lag effect of decreasing reproduction, because it reduces the future body growth by decreasing the energy uptake rate, and the energy investment to reproduction, the reproductive output, which is defined as $R(t) = (1 - \kappa)vfW^{2/3}$, is determined by the energy uptake rate and then the body weight. In the ecotoxicological sense, this time-lag effect represents the indirect effect to reproduction through growth inhibition, which is persistent and irreversible.

For simplicity, it is assumed that $W=L^3$, in which L is the body length, and $f = 1$, which means that the food level is satiated in standard ecotoxicity tests. The basic formula, denoted as a differential equation for the body length L, has a solution, which is equivalent in form to the von Bertalanffy equation,

$$L(t) = L_{max} - (L_{max} - L_b)e^{-\gamma t}, \tag{A2}$$

in which γ is the growth rate coefficient, L_{max} is the maximum body length (the asymptotic body length at which the energy invested for respiration is completely consumed by the maintenance), and L_b is the initial body length at birth. The parameters in the above body growth model are associated with the parameters in the basic formula as $\gamma = \frac{m}{3g}$ and $L_{max} = \kappa v/m$. Wrighting the body length as relative values to its maximum, $l(t) = L(t)/L_{max}$ and $l_b = L_b/L_{max}$, Eq. (A1) is simplified as

$$l(t) = 1-(1-l_b)e^{-\gamma t}. \tag{A3}$$

To represent time-dependent exposure concentrations, we used numerical solutions for the difference equations of body length,

$$l_{t+1} = l_t + \gamma(1-l_t), \tag{A4}$$

which approximated the differential of Eq. (1),

$$\frac{dl(t)}{dt} = \gamma(1-l(t)). \tag{A5}$$

From the definition of the reproductive output, the reproductive output relative to its maximum is calculated from $w(t) = R(t)/R_{max}$ and $R_{max} = (1 - \kappa)vL_{max}^2$ as,

$$w(t) = l(t)^2. \tag{A6}$$

Thus, the body size and the reproductive output as relative values are simplified such as having only the relative initial body length and the growth rate coefficient as the only model parameters, provided that the asymptotic maximum body length does not change with toxicant's effects.

We assumed that each brood size was determined by the accumulated reproductive output after the previous release or the age of reproductive maturity. Thus, the x-th brood size B_x is

$$B_1 = E^{-1}\sum_{\tau=\alpha}^{t_1-1} R(\tau) \text{ or } B_x = E^{-1}\sum_{\tau=t_{x-1}}^{t_x-1} R(\tau), \tag{A7}$$

in which t_x is the time in day when the x-th brood was released, E is the energy required to produce a neonate on average, and α is the age of reproductive maturity, which was assumed to be three days before the day of first reproduction. The expressions for brood size can be rewritten as follow, using the relative value of reproductive output,

$$B_1 = F_{max}\sum_{\tau=\alpha}^{t_1-1} w(\tau) \text{ and } B_x = F_{max}\sum_{\tau=t_{x-1}}^{t_x-1} w(\tau), \tag{A8}$$

in which F_{max} is the maximum mean fecundity per day $F_{max} = R_{max}/E$.

Appendix B

Estimates of l_b, l_α, γ and F_{max}: According to the experiment by Kooijman and Metz (1984), the body length of *D. magna* at birth and at maturity are 0.8 mm and 2.5 mm respectively. Because the maximum or satiating body length is 6.0 mm (DeRoos et al., 1992), we get $l_b = 0.13$ and $l_\alpha = 0.42$. If we assume the reproductive maturity requires 5 days, we get a guess value for γ as 0.081.

A couple of reports indicated that the maximum brood size (the number of neonates by a release) was approximately 15.0 or 17.5 (van Leeuwen et al., 1985a, 1985b; Van Leeuwen et al., 1986). The mean age of first reproductions was about 8 days, and the average longevity was 20.7 days for a *D.magna* experimental population in which the mean number of releases per female was 6.2 (Fernanedez-Casalderrey et al., 1993). Thus the interval between releases was approximately 2 days. Therefore, the maximum dairy fecundity was estimated as 8 if we took the maximum brood size as 16. We used 8 as the guess value of F_{max} which set the mode of the prior distribution.

References

Abe, R., Watanabe, H., Yamamuro, M., Iguchi, T., Tatarazako, N., 2014. Establishment of a short-term, in vivo screening method for detecting chemicals with juvenile hormone activity using adult *Daphnia magna*. J. Appl. Toxicol. 35, 75–82.

Allan, J.D., 1976. Life history pattern in zooplankton. Am. Natur. 110, 165–180.

Andersen, T., 1997. Pelagic Nutrient Cycles: Herbivores as Sources and Sinks. Springer-Verlag, Berlin.

Arbačiauskas, K., Lampert, W., 2003. Seasonal adaptation of ex-ephippio and parthenogenetic offspring of *Daphnia magna*: differences in life history and physiology. Funct. Ecol. 17, 431–437.

Ashauer, R., Boxall, A.B.A., Brown, C.D., 2007. New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. Environ. Sci. Technol. 41, 1480–1486.

Baldwin, W.S., Bailey, R., Long, K.E., Klaine, S., 2001. Incomplete ecdysis is an indicator

of ecdysteroid exposure in *Daphnia magna*. Environ. Toxicol. Chem. 20, 1564–1569.
Billoir, E., Delignette-Muller, M.L., Péry, A.R.R., Charles, S., 2008. A Bayesian approach to analyzing ecotoxicological data. Environ. Sci. Technol. 42, 8978–8984.
Brendonck, L., De Meester Jr., L., Hairston, N.G., 1998. Evolutionary and ecological aspects of crustacean diapause. Arch. Hydrobiol. Spec. Issue.: Adv. Limnol. 52.
Brooks, S.P., Gelman, A., 1998. General methods for monitoring convergence of iterative simulations. J. Comput. Graph. Stat. 7, 434–455.
Dang, Z., Cheng, Y., Chen, H.M., Cui, Y., Yin, H.H., Traas, T., Montforts, M., Vermeire, T., 2012. Evaluation of the *Daphnia magna* reproduction test for detecting endocrine disruptors. Chemosphere 88, 514–523.
DeRoos, A.M., Diekmann, O., Metz, J.A.J., 1992. Studying the dynamics of structured population models: a versatile technique and its application to *Daphnia*. Am. Nat. 139, 123–147.
Duquesne, S., 2006. Effects of an organophosphate on *Daphnia magna* at suborganismal and organismal levels: implications for population dynamics. Ecotoxicol. Environ. Saf. 65, 145–150.
Edmondson, W.T., 1955. Seasonal life history of *Daphnia* in an arctic lake. Ecology 36, 439–455.
Fernanedez-Casalderrey, A., Ferrnando, M.D., Andreu-Moliner, E., 1993. Effects of endosulfan on survival, growth and reproduction of Daphnia magna. Comp. Biochem. Physiol. 106, 437–441.
Forbes, V.E., Calow, P., 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environ. Toxicol. Chem. 18, 1544–1556.
Forbes, V.E., Sibly, R.M., Calow, P., 2001. Toxicant impacts on density-limited populations: a critical review of theory, practice, and results. Ecol. Appl. 11, 1249–1257.
Gelman, A., Rubin, D., 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7, 457–511.
Ginjupalli, G.K., Baldwin, W.S., 2013. The time- and age-dependent effects of the juvenile hormone analog pesticides, pyriproxyfen on *Daphnia magna* reproduction. Chemosphere 92, 1260–1266.
Gyllström, M., Hansson, L.A., 2004. Dormancy in freshwater zooplankton: induction, termination and the importance of benthicpelagic coupling. Aquat. Sci. 66, 274–295.
Hairston, N.G.J., 1996. Zooplankton egg banks as biotic reservoirs in changing environments. Limnol. Oceanogr. 41, 1087–1092.
Hanson, N., Stark, J.D., 2011. A comparison of simple and complex population models to reduce uncertainty in ecological risk assessment of chemicals: example with three species of *Daphnia*. Ecotoxicology 20, 1268–1276.
Ishaaya, I., Horowitz, A.R., 1995. Pyriproxyfen, a novel insect growth regulator for controlling whiteflies: mechanisms and resistance management. Pestic. Sci. 43, 227–232.
Kato, Y., Kobayashi, K., Watanabe, H., Iguchi, T., 2011. Environmental sex determination in the branchipod crustacean *Daphnia magna*: deep conservation of a doublesex gene in the sex-determining pathway. PLoS Genet. 7, e1001345.
Kleiven, O.T., Larsson, P., Hobæk, A., 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. Oikos 65, 197–206.
Kooijman, S.A.L.M., Metz, A.J., 1984. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. Ecotoxicol. Environ. Saf. 8, 254–274.
Kooijman, S.A.L.M., Bedaux, J.J.M., 1996. The Analysis of Aquatic Toxicity Data. VU University Press, Amsterdam.
Liess, M., Pieters, B., Duquesne, S., 2006. Long-term signal of population disturbance after pulse exposure to an insecticide: rapid recovery of abundance, persistent alteration of structure. Environ. Toxicol. Chem. 25, 326–331.
McCarthy, M.A., 2007. Bayesian Methods for Ecology. Cambridge University Press, Cambridge.
Matsumoto, T., Ikuno, E., Itoi, S., Sugita, H., 2008. Chemical sensitivity of the male daphnid, *Daphnia magna*, induced by exposure to juvenile hormone and its analog. Chemosphere 72, 451–456.
Metropolis, N., Rosenbluth, A.W., Rosenbluth, M.N., Teller, A.H., 1953. Equation of state calculations by fast computing machines. J. Chem. Phys. 21, 963–964.
Oda, S., Tatarazako, N., Watanabe, H., Morita, M., Iguchi, T., 2005a. Production of male neonates in four cladoceran species exposed to a juvenile hormone analog, fenoxycarb. Chemosphere 60, 74–78.
Oda, S., Tatarazako, N., Watanabe, H., Morita, M., Iguchi, T., 2005b. Production of male neonates in *Daphnia magna* (Cladocera, Crustacea) exposed to juvenile hormones and their analogs. Chemosphere 61, 1168–1174.
OECD, 2012. *Daphnia magna* Reproduction Test (No. 211 Extended version), OECD Guidelines for the Testing of Chemicals. OECD Publishing.
Olmstead, A.W., LeBlanc, G.A., 2002. Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean Daphnia magna. J. Exp. Zool. 293, 736–739.
Olmstead, A.W., LeBlanc, G.A., 2003. Insecticidal juvenile hormone analogs stimulate the production of male offspring in the crustacean *Daphnia magna*. Environ. Health Perspect. 111, 919–924.
Sarnelle, O., Knapp, R.A., 2004. Zooplankton recovery after fish removal: limitation of the egg bank. Limnol. Oceanogr. 49, 1382–1392.
Tanaka, Y., 2003. Ecological risk assessment of pollutant chemicals: extinction risk based on population-level effects. Chemosphere 53, 421–425.
Tanaka, Y., Nakanishi, J., 2000. Mean extinction time of populations under toxicant stress and ecological risk assessment. Environ. Toxicol. Chem. 19, 2856–2862.
Tanaka, Y., Nakanishi, J., 2001. Model selection and parameterization of the concentration-response functions for population-level effects. Environ. Toxicol. Chem. 20, 1857–1865.
Tatarazako, N., Oda, S., 2007. The water flea *Daphnia magna* (Crustacea, Cladocera) as a test species for screening and evaluation of chemicals with endocrine disrupting effects on crustaceans. Ecotoxicology 16, 197–203.
Tatarazako, N., Oda, S., Watanabe, H., Morita, M., Iguchi, T., 2003. Juvenile hormone agonists affect the occurrence of male *Daphnia*. Chemosphere 53, 827–833.
van Leeuwen, C.J., Luttmer, W.J., Griffioen, P.S., 1985a. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. Ecotoxicol. Environ. Saf. 9, 26–39.
Van Leeuwen, C.J., Moberts, F., Niebeek, G., 1985b. Aquatic toxicological aspects of dithiocarbamates and related compounds. II. Effects on survival, reproduction and growth of *Daphnia magna*. Aquat. Toxicol. 7, 165–175.
Van Leeuwen, C.J., Rijkeboer, M., Niebeek, G., 1986. Population dynamics of *Daphnia magna* as modified by chronic bromide stress. Hydrobiologia 133, 277–285.
Wang, H.Y., Olmstead, A.W., Li, H., LeBlanc, G.A., 2005. The screening of chemicals for juvenoid-related endocrine activity using the water flea *Daphnia magna*. Aquat. Toxicol. 74, 193–204.
Watanabe, H., Oda, S., Abe, R., Tanaka, Y., Tatarazako, N., 2018. Comparison of the effects of constant and pulsed exposure with equivalent time-weighted average concentrations of the juvenile hormone analog pyriproxyfen on the reproduction of *Daphnia magna*. Chemosphere 195, 810–816.