Environ Sci Pollut Res
DOI 10.1007/s11356-017-9809-4

MOSAIC: a web-interface for statistical analyses in ecotoxicology

Sandrine Charles[1] · **Philippe Veber**[1] · **Marie Laure Delignette-Muller**[1,2]

Received: 27 December 2016 / Accepted: 20 July 2017

Abstract In ecotoxicology, bioassays are standardly conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth, and/or survival of living animals. MOSAIC, standing for *MOdeling and StAtistical tools for ecotoxICology*, is a user-friendly web interface dedicated to the mathematical and statistical modelling of such standard bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists and regulators. Without wasting time on extensive mathematical and statistical technicalities, users are provided with advanced and innovative methods for a valuable quantitative environmental risk assessment. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/.

Keywords Standard bioassay data · Survival statistical analysis · Reprotoxicity statistical analysis · SSD analysis · R software

Introduction

In toxicity assessment bioassays, the effects of chemicals on living animals are usually measured on individual life history traits in the laboratory and according to standards (ISO, OECD). This ensures the control of experimental conditions and thus the reproducibility of the bioassays. These standardized bioassays, in acute or chronic toxicity, generally concern survival, reproduction, and/or growth of laboratory animals. The statistical analysis of data collected through standard bioassays leads to the estimation of critical effect concentrations (CECs) for the measured trait(s). If bioassays have been carried out with a reasonable number of concentrations of the studied chemical substance (typically >5) and if the responses (e.g., mortality) or the effects (e.g., reproduction or growth) are statistically significant, a regression model is recommended to be fitted on experimental data, in order to estimate CECs such as x% lethal concentrations (LC_x) and/or x% effective concentrations (EC_x) (Green et al. 2013). However, to account for the specificity of each type of data (namely, binary, count or continuous data), the most appropriate exposure-response/effect model must be chosen, with both the most appropriate deterministic and stochastic parts. Such a choice is not trivial while it may have a strong impact on the resulting CEC estimates.

To our knowledge, there is today a lack of a turnkey decision tool specifically designed for ecotoxicologists from academia, national agencies and private research, or other risk assessors, in order to perform statistical analyses of standardized bioassay data, in a user-friendly way and with a freely available graphical web interface. Given this statement, we developed the MOSAIC web interface. MOSAIC stands for *MOdeling and StAtistical tools for ecotoxICology*. MOSAIC makes possible the statistical analysis of standard bioassays, without wasting time on extensive mathematical and statistical technicalities and by taking advantage of the latest advanced and innovative methods in the field of ecotoxicology.

Responsible Editor: Philippe Garrigues

✉ Sandrine Charles
sandrine.charles@univ-lyon1.fr

[1] Université de Lyon, Université Lyon 1, UMR CNRS 5558, Laboratoire de Biométrie et Biologie Évolutive, 69100 Villeurbanne, France

[2] Université de Lyon, VetAgro Sup Campus Vetérinaire de Lyon, 69280 Marcy l'Étoiles, France

Published online: 26 August 2017

MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/. It currently offers three operational modules: (i) $\text{MOSAIC}_{\text{surv}}$ allows users to perform a complete statistical analysis of bioassay *survival* data, based on a log-logistic model fitted to the data within a Bayesian framework, thus providing LC_x estimates; (ii) $\text{MOSAIC}_{\text{repro}}$ provides users with a complete statistical analysis of bioassay *reproduction* data simultaneously accounting for mortality all along the bioassay. Concentration-effect models are fitted within a Bayesian framework to provide EC_x estimates; (iii) $\text{MOSAIC}_{\text{SSD}}$ is dedicated to the *Species Sensitivity Distribution* (SSD) approach aiming at defining safe levels for toxic compounds in a community through the estimation of the so-called hazardous concentration for $p\%$ of the species (HC_p), even when the toxicity values are censored. From example datasets, this paper illustrates how MOSAIC can help ecotoxicologists, regulators, managers, national governmental organizations, and other stakeholders in analyzing standard bioassay data in an easy yet statistically sound way.

Datasets and format

Survival and reproduction datasets

A typical survival or reproduction bioassay consists in exposing a group of animals to a certain concentration of contaminant, and reporting at several time points, the number of survivors (in survival bioassay) or the number of offspring (young animals, clutches or eggs) which are produced by a population of adults and collected at this time point, simultaneously with the number of survivors (in reproduction bioassay). Such a bioassay is usually replicated in order to assess the variability of the measured traits. As in most standard bioassays, the concentration is assumed constant over time during the whole experiment. In the end, there are one (in survival bioassay) or two (in reproduction bioassay) measurements for each triplet (replicate, concentration, time).

$\text{MOSAIC}_{\text{surv}}$ and $\text{MOSAIC}_{\text{repro}}$ expect to receive bioassay data as a text file with Tabulation-Separated Values (TSV format). Each line of the table corresponds to a time point for a given replicate and a given concentration of the contaminant and provides one or two measurements. The table must contain the four or five following columns:

- `replicate`, a number or a string that is unique for each replicate;
- `conc`, the concentration of the contaminant;
- `time`, the time point of the measurement;
- `Nsurv`, the number of survivors;
- `Nrepro`, the additional number of offspring observed between the previous and the current time point of the measurement.

Please note that the order of columns must be respected and that the first line of the file must contain column headings (Table 1).

$\text{MOSAIC}_{\text{surv}}$ and $\text{MOSAIC}_{\text{repro}}$ are both here illustrated from a typical dataset of a reprotoxicity bioassay where survival and reproduction outputs were simultaneously collected for snails exposed to cadmium (6 concentrations, 6 replicates of 5 animals per concentration) during 56 days (Ducrot et al. 2014; Charles et al. 2016). These example data come from Ducrot et al. (2014). The dataset can be downloaded from http://pbil.univ-lyon1.fr/software/mosaic/survival/dataset/cadmium2.

SSD datasets

The expected data for the fitting of a species sensitivity distribution is a set of CEC estimated for various species and a given contaminant. Data must be uploaded as a tabular text file with one CEC estimate per line corresponding to one species. The exact syntax of the lines differs when dealing with CEC data points or censored CEC data (left and/or right bounded CEC estimates). In any case, only positive values are expected (Table 2).

$\text{MOSAIC}_{\text{SSD}}$ is here illustrated from a typical SSD dataset including censored data, which corresponds to the study of 72-h acute salinity tolerance (LC_{50} estimates)

Table 1 Dataset format to be uploaded within $\text{MOSAIC}_{\text{surv}}$ (columns 1–4) and $\text{MOSAIC}_{\text{repro}}$ (columns 1–5)

Replicate	conc	time	Nsurv	Nrepro
A	0	0	5	0
B	0	0	5	0
C	0	0	5	0
A	78	3	5	279
B	78	3	5	135
C	78	3	5	181
A	124	7	5	190
B	124	7	5	456
C	124	7	5	338
A	232	10	3	46
B	232	10	5	0
C	232	10	2	0
A	284	56	0	0
B	284	56	0	0
C	284	56	0	0
...				

Table 2 Dataset format to be uploaded within $MOSAIC_{SSD}$

Data point	
1.45	
2.31	
0.56	
...	
Censored data	
1.45	1.85
2.31	NA
NA	0.99
1.11	1.11
...	

With censored data, missing bounds must be denoted with NA. If one CEC is only known as a data point, it must be entered twice, both as lower and upper bounds

among riverine macro-invertebrates (Kefford and Nugegoda 2006). This example can be downloaded from http://pbil.univ-lyon1.fr/software/mosaic/data/salinity.txt.

$MOSAIC_{surv}$

$MOSAIC_{surv}$ provides a complete analysis for survival bioassays at final time, whether the number of surviving animals has been followed through time or only measured at the end of the experiment. This analysis includes a descriptive overview of the raw data and automatically provides LC_x estimates without requiring any input besides the survival dataset. All calculations are based on the companion R package `morse` (Delignette-Muller et al. 2016).

Modelling and inference

Within $MOSAIC_{surv}$, the mean survival rate at the end of the experiment is related to the contaminant concentration c with a log-logistic relationship:

$$f(c) = \frac{d}{1 + \left(\frac{c}{e}\right)^b} \quad (1)$$

with the following positive parameters: d corresponds to the survival rate in control condition, e corresponds to the LC_{50} and parameter b is related to the effect intensity of the contaminant.

Assuming that deaths of two animals are two independent events and given an initial number N_{0i} of animals at the ith concentration c_i, the number N_i of surviving animals follows a binomial distribution:

$$N_i \sim \mathcal{B}(N_{0i}, f(c_i)) \quad (2)$$

Model parameters d, e and b are estimated using Bayesian inference, where posterior distributions are computed from the likelihood of the observed data combined with prior distributions on the parameters. All details on priors can be found at https://cran.r-project.org/web/packages/morse/vignettes/modelling.pdf or in the original research paper (Forfait-Dubuc et al. 2012).

Results

As shown on Fig. 1a, the user of $MOSAIC_{surv}$ either uploads his/her own dataset or may try the platform with an example dataset (`cadmium-2` in Fig. 1a). A click on 'Run' first provides an overview of the raw data which allows the user to check if the data were correctly entered (Fig. 1b). Then, the result page plots the observed fraction of surviving animals associated to its 95% binomial confidence interval (Clopper and Pearson method from function `binom.test` in the R software (R Core Team 2016)) at each tested contaminant concentration (Fig. 1c). At last, Fig. 1d gives the mean survival rate as a function of the contaminant concentration (orange plain line) as well as the 95% credible band around this mean (light gray zone delimited by orange dotted lines). After these graphical results, $MOSAIC_{surv}$ provides parameter estimates (Table 3). Within this table, the estimated parameter e is equal to the LC_{50}; other LC_x values are model outputs obtained from estimated parameters. All parameter estimates are provided as medians (for point estimates) and 2.5 and 97.5 quantiles (for 95% credible intervals) of marginal posterior distributions. These credible intervals quantify the uncertainty on parameter estimates.

$MOSAIC_{repro}$

In a reproduction bioassay, offspring are regularly counted and removed from the medium at each time point, so that the reproducing population cannot increase. However, it can decrease if some animals die during the experiment due to the contaminant concentration. A distinctive feature of $MOSAIC_{repro}$ is its ability to estimate the effect of a contaminant on reproduction even if mortality occurred during the bioassay. In order to properly account for mortality, survival data included in the dataset are used to calculate the effective period of observation during which each animal may have reproduced. The reproduction rate is thus estimated in terms of the number of offspring *per animal-day*.

$MOSAIC_{repro}$ provides a complete analysis for reproduction bioassays at final time. This analysis includes a descriptive overview of the raw data and automatically provides EC_x estimates without requiring any input besides the reproduction dataset. All calculations are based on the

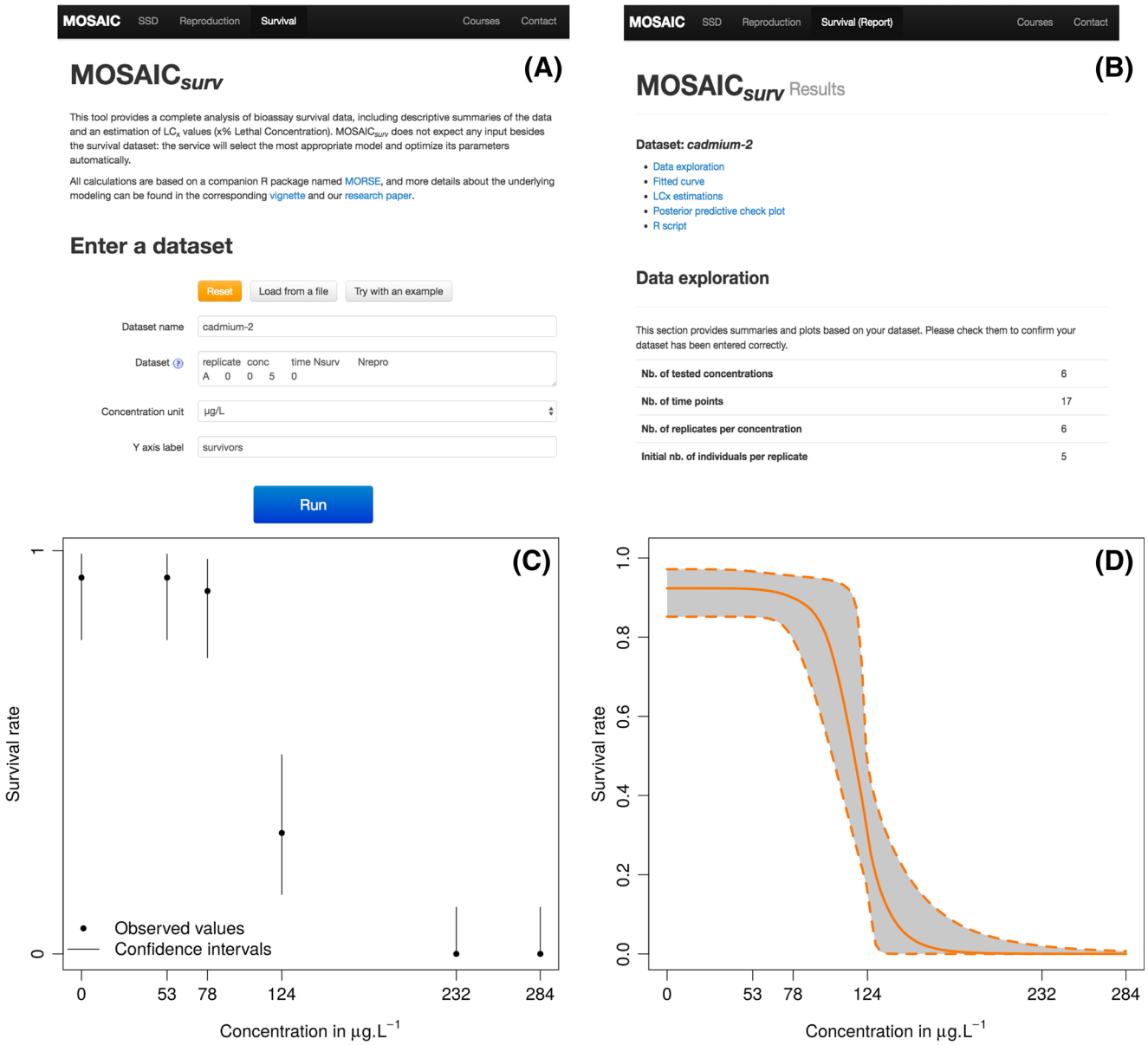

Fig. 1 **a** Home page screenshot of MOSAIC$_{surv}$. **b** First part of the result screenshot after running of MOSAIC$_{surv}$. **c** Observed fraction of surviving animals at the end of the bioassay at each tested contaminant concentration (black dots) with 95% confidence intervals (vertical segments). **d** Mean survival rate as a function of the contaminant concentration (orange plain lines) and 95% credible band around this mean (light gray zone delimited by orange dotted lines)

companion R package `morse` (Delignette-Muller et al. 2016).

Effective period of observation

The effective period of observation is defined as the sum, for all animals, of the time they spent alive during the experiment (Delignette-Muller et al. 2014). This effective period is expressed in individual-days and its value at concentration c_i and replicate j is denoted NID_{ij}. As usual in bioassay, mortality is observed at particular time points only, so that the real life span of an animal is unknown. In practice, we assume that if an animal was alive at time point t_k but dead at time point t_{k+1}, its real life span is approximated as $\frac{t_{k+1}+t_k}{2}$.

Consequently:

$$NID_{ij} = \sum_k n_{ij(k+1)}(t_{k+1}-t_k)+(n_{ijk}-n_{ij(k+1)})\left(\frac{t_{k+1}+t_k}{2}-t_k\right) \quad (3)$$

where n_{ijk} is the observed number of surviving animals at concentration c_i, replicate j and time t_k.

Table 3 Parameter estimates as provided by $MOSAIC_{surv}$: medians (for point estimates) and 2.5 and 97.5 quantiles (for 95% credible intervals) of marginal posterior distributions

Log-logistic model parameters	Point estimate	95% credible interval
d	0.92	[0.85 ; 0.97]
e (μg.L^{-1})	117.76	[104.00 ; 124.57]
b	11.77	[5.29 ; 73.91]
LC_x estimates (μg.L^{-1})	Point estimate	95% credible interval
LC_5	90.92	[62.88 ; 118.00]
LC_{10}	96.97	[71.97 ; 119.23]
LC_{20}	104.15	[83.18 ; 120.62]
LC_{50}	117.76	[104.00 ; 124.57]

Modelling and inference

Within $MOSAIC_{repro}$, the cumulated number of offspring at the end of the experiment is related to the contaminant concentration c with a log-logistic relationship:

$$f(c) = \frac{d}{1 + \left(\frac{c}{e}\right)^b} \tag{4}$$

with the following positive parameters: d corresponds to the reproduction rate in control condition, e corresponds to the EC_{50}, that is to the concentration dividing the average number of offspring by two with respect to the control condition, and parameter b is related to the effect intensity of the contaminant.

The cumulated number of offspring, denoted by N_{ij} at concentration c_i and replicate j, can be modelled using a Poisson distribution:

$$N_{ij} \sim Poisson\left(f(c_i) \times NID_{ij}\right) \tag{5}$$

A competing model is also fitted to the data, in order to account for a potential between-replicate variability:

$$N_{ij} \sim Poisson\left(F_{ij} \times NID_{ij}\right) \tag{6}$$

where the reproduction rate F_{ij} at concentration c_i and replicate j is a random variable following a gamma distribution:

$$F_{ij} \sim gamma\left(\frac{f(c_i)}{\omega}, \frac{1}{\omega}\right) \tag{7}$$

The greater ω value, the greater the between-replicate variability.

The two competing models are both fitted to data. The Deviance Information Criterion is implemented within $MOSAIC_{repro}$ in order to provide the user with the most appropriate model. Model parameters d, e, b, and ω when required, are estimated using Bayesian inference, where posterior distributions are computed from the likelihood of the observed data combined with prior distributions on the parameters. All details on priors can be found at https://cran.r-project.org/web/packages/morse/vignettes/

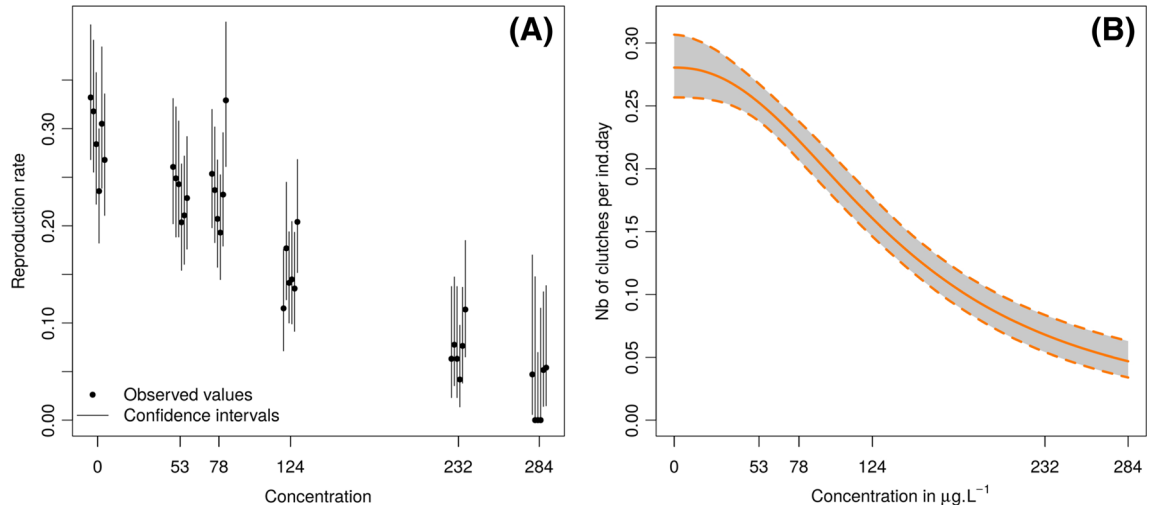

Fig. 2 **a** Observed reproduction rate (expressed as the number of offspring per individual-day) at the end of the bioassay at each tested contaminant concentration (black dots) with 95% confidence intervals (vertical segments). **b** Mean number of offspring per individual-day as a function of the contaminant concentration (orange plain lines) and 95% credible band around this mean (light gray zone delimited by orange dotted lines)

Table 4 Parameter estimates as provided by $MOSAIC_{repro}$: medians (for point estimates) and 2.5 and 97.5 quantiles (for 95% credible intervals) of marginal posterior distributions

Log-logistic model parameters	Point estimate	95% credible interval
d	0.28	[0.26 ; 0.31]
e (μg.L^{-1})	140.59	[122.22 ; 161.36]
b	2.28	[1.78 ; 2.93]
EC_x estimates (μg.L^{-1})	Point estimate	95% credible interval
EC_5	38.60	[24.65 ; 56.71]
EC_{10}	53.58	[37.42 ; 73.57]
EC_{20}	76.50	[58.38 ; 97.49]
EC_{50}	140.59	[122.22 ; 161.36]

modelling.pdf or in the original research paper (Delignette-Muller et al. 2014).

Today, $MOSAIC_{repro}$ is recommended by OECD within the new guideline for the testing of chemicals on *Lymnaea stagnalis* reproduction (OECD 2016).

Results

After clicking "Run" from the $MOSAIC_{repro}$ home page, the user gets an overview and a plot of the raw data expressed as the observed reproduction rate (that is the observed number of offspring per animal-day) with its 95% Poisson confidence interval (Fig. 2a); these confidence intervals are calculated using function `pois.exact` from package `epitools` in the R software (R Core Team 2016). Figure 2b gives the mean reproduction rate as a function of the contaminant concentration (orange plain line) as well as the 95% credible band around this mean (light gray zone delimited by orange dotted lines). In addition, $MOSAIC_{repro}$ provides parameter estimates (Table 4).

Posterior predictive check

As illustrated on Figs. 1d and 2b, data are not superimposed to the fitted model. Nevertheless, the fitted model can be further validated using a posterior predictive check plot: the idea is to compare each observed value versus a prediction from the fitted model at the corresponding concentration associated with its 95% credible interval. If the fit is correct, we expect to see 95% of the observed values fall within the credible intervals.

As shown on Fig. 3, the observed values are read on the x-axis, while the y-axis reports the point estimates predicted by the fitted model at the corresponding concentrations (black dots), as well as their 95% credible intervals (vertical segments). The credible interval is coloured in green if it contains the observed value and in red otherwise.

$MOSAIC_{SSD}$

The species sensitivity distribution (SSD) approach is a central tool for environmental risk assessment to define safe levels for contaminants within an ecosystem. It is based on the assumption that species sensitivity to a given contaminant can be described by a probability distribution estimated from previously obtained CECs. $MOSAIC_{SSD}$ is able to estimate the so-called hazardous concentration for p% of the species (HC_p) even if CECs are censored (Kon Kam King et al. 2014). All calculations are based on the companion R package `fitdistrplus` (Delignette-Muller and Dutang 2015).

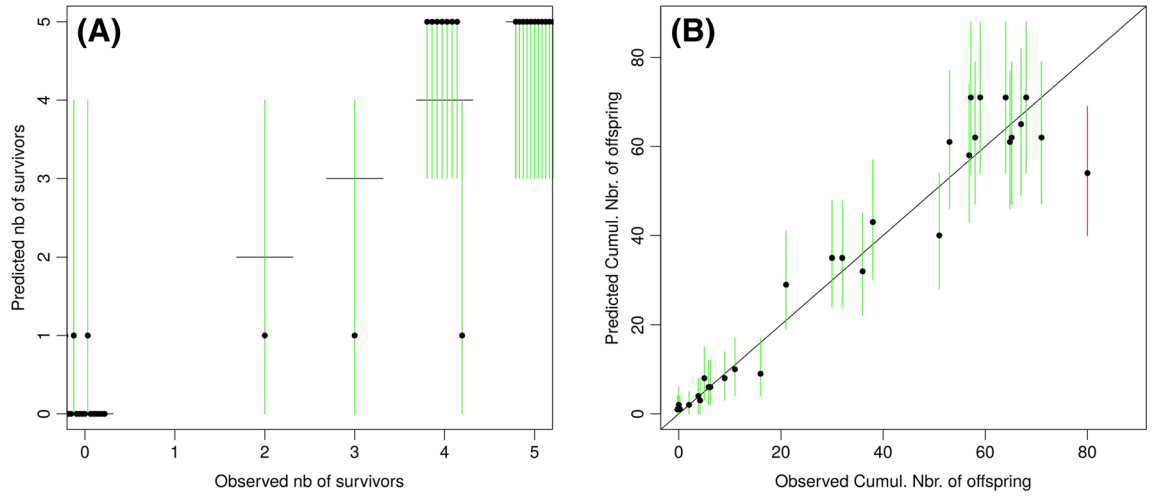

Fig. 3 Posterior predictive check plots for survival (on the left) and reproduction (on the right) provided by $MOSAIC_{surv}$ and $MOSAIC_{repro}$ from the cadmium dataset, respectively

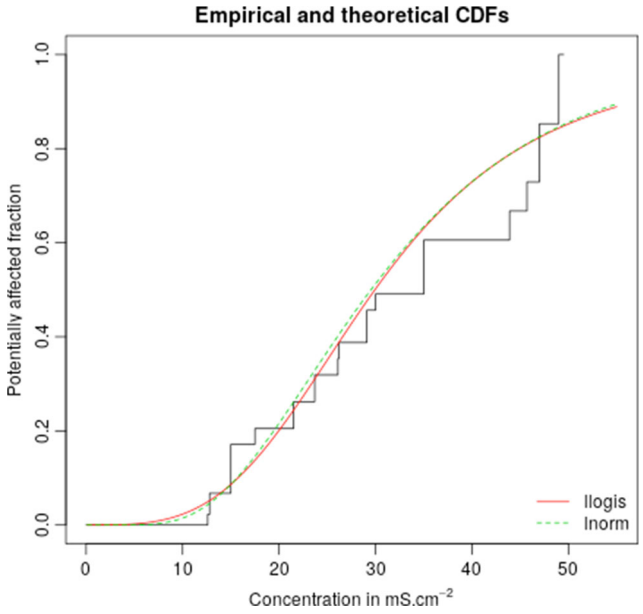

Fig. 4 Screenshot of the result page of MOSAIC$_{SSD}$ on the salinity dataset. Dotted and plain curves correspond to the log-normal and log-logistic fitted distributions, respectively. The stepwise curve corresponds to the Turnbull estimate of the cumulative distribution function (see Kon Kam King et al. 2014 for details)

Basics

MOSAIC$_{SSD}$ enables any user to perform a simple yet statistically sound SSD analysis including censored data without worrying about the conceptually difficult underlying statistical questions. Once the dataset uploaded, the user can choose among the log-normal and log-logistic distribution laws to be fitted. The value of the likelihood function for each distribution is provided on the result page and can be used as a further decision criterion (the highest the likelihood value, the most appropriate the distribution). The log-logistic distribution has heavier tails than the log-normal and is therefore generally more conservative in the determination of the 5% hazardous concentration (HC_5) (Aldenberg and Slob 1993). After clicking "Run," the bootstrap 95% confidence intervals are automatically computed. They yield confidence intervals on the parameters of the distribution and on several computed HC_p. Calculating the confidence intervals using a bootstrap method has the advantage of using a unified framework for every distribution. As the bootstrap procedure does not necessarily converge depending on the size of the dataset, an automatic check of bootstrap convergence is implemented (Kon Kam King et al. 2014).

Results

Figure 4 shows a screenshot of the result page from MOSAIC$_{SSD}$ with a graphical representation of the example censored dataset. This result page also provides estimates of the distribution parameters and HC_p values computed for various interesting values of p associated to their 95% bootstrap confidence intervals (Table 5). Based on Fig. 4, for this particular dataset, we may choose either one of the two distribution laws.

MOSAIC: a gateway to the R software

MOSAIC is developed in OCaml (2016) and is based on a web server Ocsigen (2016) hosted at the Rhône-Alpes Bioinformatics Center PRABI (2016). MOSAIC incorporates an in-car R interpreter and a simple system for

Table 5 Parameter estimates as provided by MOSAIC$_{SSD}$

Log-logistic distribution parameters	Point estimate	95% bootstrap interval
shape	3.4	[2.9 ; 4.3]
scale	30	[26 ; 34]
log-likelihood = −140.1		
Log-normal distribution parameters	**Point estimate**	**95% bootstrap interval**
meanlog	3.4	[3.3 ; 3.5]
sdlog	0.5	[0.41 ; 0.59]
log-likelihood = −139.1		
HC_p in mS.cm^{-2}	Log-logistic estimate	Log-normal estimate
	Point estimate and [95% bootstrap interval]	
HC_5	13[10; 16]	13[11; 16]
HC_{10}	16[13; 19]	16[13; 19]
HC_{20}	20[17; 23]	19[17; 23]
HC_{50}	30[26; 34]	30[26; 33]

distributing calculations. Hence, MOSAIC takes advantage of multi-processor machines and provides a reasonable response time when multiple users work simultaneously on the interface.

Several default choices underlie MOSAIC calculations (only one deterministic part for survival and reproduction, only two distributions for SSD, default colors, ...), thus limiting its use to what we think are the most frequent situations. For example, we do not provide support for modelling hormesis in survival or reproduction analyses, nor for multi-modal SSDs. Another difficult case is when the available data are not appropriate to estimate the CECs (e.g., the LC_{50} is over the highest tested concentration). MOSAIC is designed to detect such situations and warn the user accordingly, but it does not let the user tune options to adapt to its particular case. However, the bottom of the result page provides the user with the R code corresponding to all graphs and calculations from a dataset. This guarantees the transparency and the reproducibility of MOSAIC results. Thanks to a copy and paste operation within the R software, this code can also be used as a stepping stone to change default options and to perform further analyses: it allows the user to modify figures, to automatize the statistical analyses and/or to go further into the modelling process, directly within the R software.

Conclusion

MOSAIC appears particularly useful to estimate critical effect concentrations from standard data collected either from survival or reproduction bioassays. Besides its user-friendliness, MOSAIC is free of use and guarantees the privacy of the uploaded data as well as the reproducibility and the transparency of the results. For now, 3 years, MOSAIC has been intensively used throughout the world, either by users from academia, industry or regulation agencies. Further developments are currently in progress to incorporate in MOSAIC a new module dedicated to toxicokinetic-toxicodynamic (TK-TD) modelling approaches.

Acknowledgements The authors thank the French National Agency for Water and Aquatic Environments (ONEMA, now denominate French Agency for Biodiversity) for its financial support in the development of the MOSAIC web-interface.

References

Aldenberg T, Slob W (1993) Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol Environ Saf 25:48–63

Charles S, Ducrot V, Azam D, Benstead R, Brettschneider D, De Schamphelaere K, Filipe Goncalves S, Green JW, Holbech H, Hutchinson TH, Faber D, Laranjeiro F, Matthiessen P, Norrgren L, Oehlmann J, Reategui-Zirena E, Seeland-Fremer A, Teigeler M, Thome JP, Tobor Kaplon M, Weltje L, Lagadic L (2016) Optimizing the design of a reproduction toxicity test with the pond snail Lymnaea stagnalis. Regul Toxicol Pharmacol 81:47–56

Delignette-Muller ML, Dutang C (2015) `fitdistrplus`: an R package for fitting distributions. J Stat Softw 64(4):1–34. http://www.jstatsoft.org/v64/i04/. [Online; accessed 23-June-2017]

Delignette-Muller ML, Lopes C, Veber P, Charles S (2014) Statistical handling of reproduction data for exposure-response modeling. Environ Sci Technol 48(13):7544–7551

Delignette-Muller ML, Ruiz P, Charles S, Duchemin W, Lopes C, Kon-Kam-king G, Veber P (2016) `morse`: modelling tools for reproduction and survival data in ecotoxicology. https://CRAN.R-project.org/package=morse. R package version 2.2.0 [Online; accessed 23-June-2017]

Ducrot V, Askem C, Azam D, Brettschneider D, Brown R, Charles S, Coke M, Collinet M, Delignette-Muller ML, Forfait-Dubuc C, Holbech H, Hutchinson T, Jach A, Kinnberg KL, Lacoste C, Le Page G, Matthiessen P, Oehlmann J, Rice L, Roberts E, Ruppert K, Davis JE, Veauvy C, Weltje L, Wortham R, Lagadic L (2014) Development and validation of an OECD reproductive toxicity test guideline with the pond snail *Lymnaea stagnalis* (Mollusca, Gastropoda). Regul Toxicol Pharmacol 70:605–614

Forfait-Dubuc C, Charles S, Billoir E, Delignette-Muller M (2012) Survival data analyses in ecotoxicology: critical effect concentrations, methods and models. What should we use? Ecotoxicology 12:1072–1083

Green JW, Springer TA, Staveley JP (2013) The drive to ban the NOEC/LOEC in favor of ECx is misguided and misinformed. Integr Environ Assess Manag 9:12–16

Kefford B, Nugegoda D (2006) Validating species sensitivity distributions using salinity tolerance of riverine macroinvertebrates in the southern Murray-Darling Basin (Victoria, Australia). Can J Fish Aquat Sci 63:1865–1877

Kon Kam King G, Veber P, Charles S, Delignette-Muller ML (2014) MOSAIC_SSD: a new web tool for species sensitivity distribution to include censored data by maximum likelihood. Environ Toxicol Chem/SETAC 33(9):2133–2139

OCaml (2016) http://ocaml.org/. [Online; accessed 23-June-2017]

Ocsigen (2016) http://ocsigen.org/ocsigenserver/. [Online; accessed 23-June-2017]

OECD (2016) Test No. 243: *Lymnaea stagnalis* reproduction test. OECD Guideline (July), 1–31

PRABI (2016) http://www.prabi.fr/. [Online; accessed 23-June-2017]

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org. [Online; accessed 23-June-2017]