Appendix 1
Details on the derivation of EC_{50} estimators and their variances

Derivation of point estimators of EC_{50}

For NEL count, the GLM described in the manuscript yielded the following equation:

$$\frac{\widehat{y_J}}{T} = e^{(\hat{\beta}_o+\hat{\tau}_j+\hat{\beta}_1 C+\hat{\beta}_2 t+\hat{\beta}_{3j} t+\hat{\beta}_4 Ct)} \quad (1)$$

where $\frac{\widehat{y_J}}{T}$, is the estimated ratio between NEL count under extract j and the offset T which corresponds to the natural logarithm of total eggs, C and t are extract concentration and time, $\hat{\beta}_o, \hat{\beta}_1, \hat{\beta}_2, \{\hat{\beta}_{3j}\}_{j=1}^{4}, \hat{\beta}_4$ are the maximum likelihood estimates of the intercept, and regression coefficients for concentration, time, extract by time interaction (one coefficient per extract), and concentration by time interaction, $\hat{\tau}_j$ is the maximum likelihood estimate of the effect of extract $j, j = 1,2,3,4$.
Let $p = \frac{\widehat{y_J}}{T}$, from Equation 1, it follows that

$$ln(p) = \hat{\beta}_o + \hat{\tau}_j + \hat{\beta}_1 C + \hat{\beta}_2 t + \hat{\beta}_{3j} t + \hat{\beta}_4 Ct \quad (2)$$

Finding EC_{50} for a given extract and time, say j and t, respectively, amounts to set p=0.5 and solving for C in Equation 2 which yields:

$$\widehat{EC}_{50}(j, t, \hat{\theta}) = \frac{ln(0.5) - (\hat{\beta}_o + \hat{\tau}_j + \hat{\beta}_2 t + \hat{\beta}_{3j} t)}{\hat{\beta}_1 + \hat{\beta}_4 t} \quad (3)$$

Notice that estimated EC_{50} depends on the extract, time and estimated parameters, that is, depends on j, t and estimated model location parameters $\hat{\theta}$, hence the notation $\widehat{EC}_{50}(j, t, \hat{\theta})$.

On the other hand, for the EHT, EC_{50} is obtained according to the following procedure. The GLM used to model these data permits to estimate cumulative probabilities as follows:

$$P(\widehat{Y \leq k}) = \frac{1}{1 + e^{-(\hat{\beta}_{0k}+\hat{\tau}_{jk}+\hat{\beta}_{1jk} C)}} \quad (4)$$

where, $P(\widehat{Y \leq k})$ is the estimated probability of the response variable (the ordinal variable described in the manuscript) being smaller or equal than k, k=1,2, $\hat{\beta}_{ok}$ and $\{\hat{\beta}_{1jk}\}_{j=1}^{4}$, are maximum likelihood estimates of the intercept and regression coefficients for concentration by time interaction for the k^{th} response category, $\hat{\tau}_{jk}$ is the maximum likelihood estimate of the effect extract $j, j = 1,2,3,4$, for the k^{th} response category, and C is the concentration. It is worth mentioning that under this model, although there are three categories, cumulative probabilities are computed only for categories 1 and 2 since $P(Y \leq 3) = 1$.

Let $D \coloneqq (Y = 2) \cup (Y = 3)$, that is, the event of death at ME or EL stages, equivalently, it is the event of not reaching the larvae stage. Thus, by properties of probability measures

$$\widehat{P(D)} = P(\widehat{Y = 2}) + P(\widehat{Y = 3}) = 1 - P(\widehat{Y = 1})$$
$$but, P(\widehat{Y = 1}) = P(\widehat{Y \leq 1}) = \frac{1}{1 + e^{-(\hat{\beta}_{01} + \hat{\tau}_{j1} + \hat{\beta}_{1j1} C)}}$$
$$so, 1 - P(\widehat{Y = 1}) = \widehat{P(D)} = \frac{1}{1 + e^{\hat{\beta}_{01} + \hat{\tau}_{j1} + \hat{\beta}_{1j1} C}}$$

Let $\pi \coloneqq \widehat{P(D)}$, then

$$ln\left(\frac{\pi}{1 - \pi}\right) = -\left(\hat{\beta}_{01} + \hat{\tau}_{j1} + \hat{\beta}_{1j1} C\right) \tag{5}$$

Finding EC_{50} for a given extract, say j, amounts to set $\pi = 0.5$ in Equation 5 and solving for C, thus,

$$ln(1) = -(\hat{\beta}_{01} + \hat{\tau}_{j1} + \hat{\beta}_{1j1} C)$$
$$0 = -(\hat{\beta}_{01} + \hat{\tau}_{j1} + \hat{\beta}_{1j1} C)$$
$$\widehat{EC}_{50}\left(j, \hat{\delta}\right) = -\frac{\hat{\beta}_{01} + \hat{\tau}_{j1}}{\hat{\beta}_{1j1}} \tag{6}$$

Again, notation $\widehat{EC}_{50}\left(j, \hat{\delta}\right)$ is used to remark that estimated EC_{50} depends on extract level and estimated location model parameters $\hat{\delta}$.

Equations 3 and 6 show the estimators of EC_{50} for EHT and LEIT, since model parameters were estimated by maximum likelihood, by the invariance property of this kind of estimators, $\widehat{EC}_{50}(j, t, \hat{\theta})$ and $\widehat{EC}_{50}(j, \hat{\delta})$ are maximum likelihood estimators as well. Moreover, these are ratios of correlated random variables whose variances do not have a closed form. Thus, in order to obtain their standard errors, some approximate method must be used. In this case, the Delta method was employed to derive approximate algebraic expressions for $Var[\,\widehat{EC}_{50}\left(j, \hat{\delta}\right)]$ and $Var[\,\widehat{EC}_{50}\left(j, t, \hat{\theta}\right)]$.

Delta method

Let $\hat{\theta}$ be an estimator of a p-dimensional parameter θ from a sample of size n, such that

$$\sqrt{n}\left(\hat{\theta} - \theta\right) \xrightarrow{D} N_p(0, \Sigma)$$

thus, Σ is the asymptotic covariance matrix of $\hat{\theta}$. Now, let $f\left(t_1, t_2, \dots, t_p\right)$ be a real-valued function with non-zero gradient vector in θ, then, the delta method states that

$$\sqrt{n}\left[f(\hat{\theta}) - f(\theta)\right] \xrightarrow{D} N_p(0, \left(\nabla f(\theta)\right)'\Sigma(\nabla f(\theta)))$$

where $\nabla f(\theta)$ is the gradient vector of $f(\cdot)$ evaluated at θ.

Consequently, approximate standard errors can be computed as the positive square root of the quadratic form $\left(\nabla f(\theta)\right)'\Sigma(\nabla f(\theta))$ evaluated at $\hat{\theta}$ and $\hat{\Sigma}$ and these can be used to build Wald-type confidence intervals.

Thus, in this case, $(1-\alpha)\%$ Walt-type confidence intervals for $EC_{50}(j, \hat{\theta})$and $EC_{50}(j, t, \hat{\theta})$ are given by:

$$\widehat{EC}_{50}(j, \hat{\delta}) \pm z_{\alpha/2}\sqrt{\widehat{Var}\left[\,\widehat{EC}_{50}(j, \hat{\delta})\right]}$$
$$\widehat{EC}_{50}(j, t, \hat{\theta}) \pm z_{\alpha/2}\sqrt{\widehat{Var}\left[\,\widehat{EC}_{50}(j, t, \hat{\theta})\right]}$$

where $z_{\alpha/2}$ is the $1-\alpha$ percentile of the standard normal distribution.

Gradient vectors

The gradient of a scalar-valued differentiable multivariate function is a vector that contains its partial derivatives.

The partial derivatives of $g \coloneqq EC_{50}(j, t, \theta)$ evaluated at $\hat{\theta}$ are

$$\left.\frac{\partial g}{\partial \beta_0}\right|_{\theta = \hat{\theta}} = -\frac{1}{\hat{\beta}_1 + \hat{\beta}_4 t}$$

$$\left.\frac{\partial g}{\partial \tau_i}\right|_{\theta = \hat{\theta}} = -\frac{1}{\hat{\beta}_1 + \hat{\beta}_4 t}$$

$$\left.\frac{\partial g}{\partial \beta_1}\right|_{\theta = \hat{\theta}} = -\frac{ln(0.5) - (\hat{\beta}_0 + \hat{\tau}_j + \hat{\beta}_2 t + \hat{\beta}_{3j} t)}{\left(\hat{\beta}_1 + \hat{\beta}_4 t\right)^2}$$

$$\left.\frac{\partial g}{\partial \beta_2}\right|_{\theta = \hat{\theta}} = -\frac{t}{\hat{\beta}_1 + \hat{\beta}_4 t}$$

$$\left.\frac{\partial g}{\partial \beta_{3j}}\right|_{\theta = \hat{\theta}} = -\frac{t}{\hat{\beta}_1 + \hat{\beta}_4 t}, j = 1,2,3,4$$

$$\left.\frac{\partial g}{\partial \beta_4}\right|_{\theta = \hat{\theta}} = -\frac{t(ln(0.5) - (\hat{\beta}_0 + \hat{\tau}_j + \hat{\beta}_2 t + \hat{\beta}_{3j} t))}{(\hat{\beta}_1 + \hat{\beta}_4 t)^2}$$

Similarly, the partial derivatives of $h \coloneqq EC_{50}(j, \delta)$ evaluated at $\hat{\delta}$ are

$$\left.\frac{\partial h}{\partial \beta_{0k}}\right|_{\delta = \hat{\delta}} = \begin{cases} -\frac{1}{\hat{\beta}_{1k}}, if\ k = 1 \\ 0, otherwise \end{cases}$$

$$\left.\frac{\partial h}{\partial \tau_{jk}}\right|_{\delta = \hat{\delta}} = \begin{cases} -\frac{1}{\hat{\beta}_{1k}}, if\ k = 1 \\ 0, otherwise \end{cases}$$

$$\left.\frac{\partial h}{\partial \beta_{1k}}\right|_{\delta = \hat{\delta}} = \begin{cases} \frac{\hat{\beta}_{0k} + \hat{\tau}_{jk}}{\hat{\beta}_{1k}^2}, if\ k = 1 \\ 0, otherwise \end{cases}$$