

Use the GLM, Luke!

How the use of proper statistical models can increase statistical power in ecotoxicological experiments.

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September 12, 2014

1 Introduction

In environmental risk assessments statistical tests play an important role to evaluate the effects of pesticides. Ecotoxicologists perform various kinds of experiments yielding to different type of data. Examples are: animal counts in mesocosm experiments (positive and discrete data), proportions of surviving animals (bonded between 0 and 1) or biomass (positive data).

These types of data are inherently not normally distributed. However, in order to use the more familiar and traditional data analysis methods based on normal distribution, ecotoxicologists try to transform their data to met these assumptions. Proportions with binomial distributed data are usually transformed using a arcsin square root transformation (Newman, 2012; OECD, 2006), count data using a $\log(Ax + 1)$ transformation (van den Brink et al., 2000). If the transformed data does not met the normality assumptions, non-parametric tests are usually applied (Wang and Riffel, 2011).

Instead of transforming data appropriate models that fit to the data can be used, namely *Generalized Linear Models* (GLM) (Nelder and Wedderburn, 1972). These type of models can handly various types of data distributions, e.g. poisson or negative binomial (counts), binomial (proportions) or gamma (mass). Despite that GLMs were available since 40 years now, ecotoxicologist do not make use of them. One reason might be that they are not mentioned in standard guidance documents for statistical analysis of ecotoxilogical experiments: GLMs are not discussed in the (OECD, 2006) and (EPA, 2002) guidelines and not include in their schemes for hypothesis testing. Newman (2012) does not cover GLMs in his book. Environment Canada (2005) term GLMS as *useful for toxicological research*, but did not include them in their schemes as *the concept is quite advanced and as yet is not widely used in environmental toxicology*.

Recently studies concluded that data transformations should be avoided and GLMs be used as they have better statistical properties (*Do not log-transform count data*, (O’Hara and Kotze, 2010); *The arcsine is asinine*, (Warton and Hui, 2011)). In this paper we first review what types of analysis are used by ecotoxicologists. Then we demonstrate that GLM provide superior properties compared to data transformation. We used simulated data that mimicked data encountered in ecotoxicology to compare GLMs with two common data types: counts and proportions. Methods were compared in terms of Type I error (maintain a significance level of 0.05 when there is no effect) and power (detect an effect when it is present).

2 Methods

2.1 Review

Literature review of SETAC Journals -¿ NOEC, GLM, log/arcsine transformation? How often, what is applied?

2.2 Simulations

2.2.1 Count data

We simulated count data that mimics count data encountered in mesocosm experiments, with five treatments (T1 - T5) and one control group (C) (e.g. (Brock et al., 2014)). Counts were drawn from a negative binomial distribution with a fixed dispersion parameter for all treatments ($\theta = 3.91$). We simulated datasets with different the number of replicates ($N = \{3, 6, 9, 12\}$) and different abundances in control treatments ($\mu_C = \{2, 4, 8, 16, 32, 128, 512, \}$). For each combination we generated 100 datasets. For power estimation mean abundance in treatments T2 - T5 was reduced to half of the control treatment and T1 ($\mu_{T2} = \dots = \mu_{T5} = 0.5\mu_C = 0.5\mu_{T1}$). For Type I error estimation mean abundance was equal between all groups.

We fitted two parametric models to this data. First a model assuming a normal distribution after a $\ln(Ay + 1)$ transformation the response (eqn 2).

$$y^T = \log(Ay + 1) \tag{1}$$

$$y_i^T \sim N(\mu_i, \sigma^2)$$

$$y_i^T = \alpha + \beta x_i \tag{2}$$

$$var(y_i^T) = \sigma^2$$

where y is the measured abundance and A was selected in such a way that the lowest non-zero abundance of the data set is transformed to 1 (van den Brink et al., 2000). y^T is the transformed abundance and the model assumes a constant variance.

And second GLM assuming that the response follows a negative binomial distribution with a log-link (eqn 3) and the variance is a function of the mean.

$$\begin{aligned} y_i &\sim NB(\mu_i, \theta) \\ \log(\mu_i) &= \alpha + \beta x_i \\ \text{var}(y_i) &= \mu_i + \mu_i^2 / \theta \end{aligned} \tag{3}$$

Additionally we also analysed this data using a non-parametric approach (see below).

2.2.2 Binomial data

We simulated binomial data that mimics count data encountered in survival experiments, with five treatments (T1 - T5) and one control group (C) (e.g. (Newman, 2012), example 5.1 therein). Each group consisted of 10 animals and was sampled from a $\text{bin}(10, p)$ distribution. For power simulation p in the control and T1 was held constant at $p = 0.9$ and varied for T2 - T5 ($p = \{0.5, 0.55, 0.60, \dots, 0.90\}$). For Type I error simulations p was equal between groups ($p_c = p_{T1} = \dots = p_{T6}$; $p = \{0.5, 0.55, 0.60, \dots, 0.90\}$). In both simulations we also varied the number of replicates ($N = \{3, 6, 9, 12\}$). For each combination we generated 100 datasets.

We fitted two parametric models to this data. First a model assuming a normal distribution after arcsin transforming the response (eqn 4 + 5, (EPA, 2002)).

$$y^T = \begin{cases} \arcsin\sqrt{1/4n}, & \text{if } y = 0 \\ \arcsin(1) - \arcsin\sqrt{1/4n}, & \text{if } y = 1 \\ \arcsin\sqrt{y/n}, & \text{otherwise} \end{cases} \tag{4}$$

where y are the number of dead animals, y^T are the transformed proportions and n is the number of animals per replicate ($n = 10$).

$$\begin{aligned} y_i^T &\sim N(\mu_i, \sigma^2) \\ y_i^T &= \alpha + \beta x_i \\ \text{var}(y_i^T) &= \sigma^2 \end{aligned} \tag{5}$$

And second a model assuming a binomial distribution of the response with a logit link (eqn 6).

$$\begin{aligned}y_i &\sim \text{Bin}(\pi_i, 10) \\ \text{logit}(\pi_i) &= \alpha + \beta x_i \\ \text{var}(y_i) &= 10 \times \pi_i \times (1 - \pi_i)\end{aligned}\tag{6}$$

where y is the number of dead animals (out of ten) and π the probability of counting a dead animal.

2.2.3 Comparing methods

A global treatment effect was investigated using Likelihood-Ratio-Tests. Moreover, we applied a non-parametric Kruskal-Wallis-Rank-Sum-Test on the untransformed data.

We also investigated power and Type I error for detecting the LOEC (T1 in the simulations). For the parametric models we used the parametrisation of contrasting to the control group (equivalent to Dunnett contrast). We used a pairwise Wilcoxon-Rank-Sum-Test as a non-parametric method to detect the LOEC. P-values were adjusted for multiple testing using Holm's procedure.

All simulations have been done in R (Version 3.1.1) on a 64-bit Linux machine with 8GB and 2.2GHz. Source code is available...

3 Results

3.1 Simulation scenarios

4 Discussion

5 Conclusion

References

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