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Ecotoxicology is not normal - A comparison of statistical approaches for analysis of count and proportion data in ecotoxicology

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Abstract Ecotoxicologists often encounter count and proportion data that are rarely normally distributed. To meet the assumptions of the linear model such data are usually transformed or non-parametric methods are used if the transformed data still violate the assumptions. Generalised Linear Models (GLM) allow to directly model such data, without the need for transformation. Here, we compare the performance of two parametric methods i.e. (1) the linear model (assuming normality of transformed data), and (2) GLMs (assuming a Poisson, negative binomial or binomially distibuted response) and (3) non-parametric methods.

We simulated typical data mimicking low replicated ecotoxicological experiments of two common data types (counts and proportions from counts). We compared the performance of the different methods in terms of statistical power and Type I error for detecting a general treatment effect and determining the lowest observed effect concentration (LOEC). In addition, we outlined differences on a real world mesocosm data set.

For count data, we found that the quasi-Poisson model yielded the highest power. The negative binomial GLM resulted in increased Type I errors, which could be fixed using the parametric bootstrap. For proportions, binomial GLMs performed better than the linear model, except to determine LOEC at extremely low sample sizes. The compared non-parametric methods had generally lower power.

We recommend that counts in one-factorial experiments should be analysed using quasi-Poisson models and proportions from counts by binomial GLMs. These methods should become standard in ecotoxicology.

Keywords Generalized Linear Models · Transformations · Simulation · Power · Type I error

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1 Introduction

Ecotoxicologists perform various kinds of experiments yielding different types of data. Examples are animal counts in mesocosm experiments (non-negative, integer-valued data) or proportions of surviving animals (data bounded between 0 and 1, discrete). These data are typically not normally distributed. Nevertheless, such data are often analysed using methods that assume a normal distribution and variance homogeneity (Wang and Riffel 2011). To meet these assumptions data are usually transformed. For example, ecotoxicological textbooks (Newman 2012) and guidelines (EPA 2002; OECD 2006) advise that survival data should be transformed using an arcsine square root transformation. For count data from mesocosm experiments a log(Ay + C) transformation is usually applied, where the constants A and C are either chosen arbitrarily or following general recommendations. For example, van den Brink et al (2000) suggest to set the term Ay to be 2 for the lowest abundance value (y) greater than zero and C to 1. Other transformations, like the square root or fourth root transformation, are also commonly applied in community ecology (Anderson et al 2011). Note that there has been little evaluation and advice for practitioners which transformations to use. If the transformed data still do not meet the assumptions of the linear model, non-parametric tests are usually applied (Wang and Riffel 2011).

Generalised linear models (GLM) provide a method to analyse counts or proportions from counts in a statistically

sound way (Nelder and Wedderburn 1972). GLMs can handle various types of data distributions, e.g. Poisson or negative binomial (for count data) or binomial (for proportions); the normal distribution being a special case of GLMs. Despite GLMs being available for more than 40 years, ecotoxicologists do not regularly make use of them. Recent studies concluded that the linear model should not be applied on transformed data and GLMs be used as they have better statistical properties (O'Hara and Kotze 2010; Warton 2005 (counts), Warton and Hui 2011 (proportions from counts)).

Ecotoxicological experiments often involve small sample sizes due to practical constraints. For example, extremely low samples sizes (n <5) are common in many mesocosm studies (Sanderson 2002; Szöcs et al 2015). Small sample sizes lead to low power in statistical hypothesis testing, on which many ecotoxiological approaches (e.g. risk assessment for pesticides) rely. Such an endpoint are L/NOEC values (Lowest / No observed effect concentration). Although their use has been heavily criticized in the past (Laskowski 1995), they are the predominant endpoint in mesocosm experiments (Brock et al 2015; EFSA PPR 2013).

We explore how GLMs may enhance, when appropriately used, inference in ecotoxicological studies and compared three types of statistical methods (linear model on transformed data, GLM, non-parametric tests). We first illustrate differences between statistical methods using a data set from a mesocosm study. Then we further elaborate differences in detecting a general treatment effect and determining the LOEC using simulations of two common data types in ecotoxicology: counts and proportions from counts.

2 Methods

2.1 Models for count data

2.1.1 Linear model for transformed data

To meet the assumptions of the standard linear model, count data usually needs to be transformed. We followed the recommendations of van den Brink et al (2000) and used a log(Ay + 1) transformation (eqn. 1):

$$Y_{new\ i} = log(AY_i + 1) \tag{1}$$

, where Y_i is the measured and $Y_{new\ i}$ the transformed abundance of the *i*th observation. The factor A was chosen in such way that AY equals 2 for the lowest non-zero abundance value (Y).

Then we fitted the linear model to the transformed abundances (hereafter *LM*):

$$\begin{aligned} Y_{new\ i} &\sim N(\mu_i, \sigma^2) \\ E(Y_{new\ i}) = \mu_i \text{ and } var(Y_{new\ i}) &= \sigma^2 \\ \mu_i &= \beta \times X_i \end{aligned} \tag{2}$$

This model assumes a normal distribution of the transformed abundances. The expected value for each observation i is given by its mean (μ_i) and the variance (σ^2) is constant between treatments. We allow this mean to vary between treatments (X_i codes the treatments) and β are the estimated coefficients related to these changes in transformed abundances between treatments (eqn. 2).

2.1.2 Generalised Linear Models

GLMs extend the linear model to variables that are not normally distributed. Instead of transforming the response variable, the counts could be directly modelled by a Poisson GLM (GLM_p):

$$\begin{aligned} Y_i &\sim P(\mu_i) \\ E(Y_i) = var(Y_i) &= \mu_i \\ log(\mu_i) &= \beta \times X_i \end{aligned} \tag{3}$$

This model assumes Poisson distributed abundances with mean $\mu_i \geq 0$. The expected value for each observation i is given by its mean. Moreover, this model assumes that mean and variance are equal. We are modelling the mean as a function of treatment membership (X_i). However, to avoid negative values of the mean this is done on a log scale. Therefore, β also describes the differences between treatments on a log scale (eqn. 3).

The assumption of equal mean and variance is rarely met with ecological data, which is typically characterized by greater variance than the mean (overdispersion). To overcome this problem a quasi-Poisson model (GLM_{qp}) could be used, which models the variance as a linear function of the mean (eqn. 4):

$$var(Y_i) = \phi \mu_i \tag{4}$$

Here, ϕ is used to account for additional variation and is known as overdispersion parameter. The quasi-Poisson model is a post hoc method, meaning that first a Poisson model is estimated (eqn. 3) and than the standard errors are scaled by the degree of overdispersion (Hilbe 2014).

Another possibility to deal with overdispersion is to model abundances by a negative binomial distribution (GLM_{nb}, eqn. 5):

$$\begin{aligned} Y_i &\sim NB(\mu_i, \kappa) \\ E(Y_i) = \mu_i \text{ and } var(Y_i) &= \mu_i + \mu_i^2/\kappa \\ log(\mu_i) &= \beta \times X_i \end{aligned} \tag{5}$$

This models assumes that abundances are negative binomially distributed, with a mean of $\mu_i \geq 0$ and a variance $\mu_i + \mu_i^2/\kappa$. Similar to the Poisson model we use a log link between mean and treatments. Note, that the quasi-Poisson model assumes a linear mean-variance relationship (eqn. 4), whereas the negative binomial model assumes a quadratic relationship (eqn. 5).

The above described models are most commonly used in ecology (Ver Hoef and Boveng 2007), although other distributions for count data are possible, like the negative binomial model with a linear mean-variance relationship (also known as NB1) or the poisson inverse gaussian model (Hilbe 2014).

2.2 Models for binomial data

A binomial variable counts how often an event x occurs in a fixed number of independent trials N (e.g. *"5 out of 10 fish survived"*), with an equal probability of occurrence π between trials. The number of times an event occurs can also be calculated as proportion x/N.

2.2.1 Linear model for transformed data

To accommodate the assumptions for the standard linear model with such proportions, a special arcsine square root transformation (eqn. 6) is suggested (EPA 2002; Newman 2012):

$$Y_{new\ i} = \begin{cases} arcsin(1) - arcsin(\sqrt{\frac{1}{4n}}) & \text{, if } Y_i = 1 \\ arcsin(\sqrt{\frac{1}{4n}}) & \text{, if } Y_i = 0 \\ arcsin(\sqrt{Y_i}) & \text{, otherwise} \end{cases} \quad (6)$$

, where Y_i are the untransformed proportions, $Y_{new\ i}$ are the transformed proportions and n is the total number of exposed animals per treatment. The transformed proportions are then analysed using the standard linear model (*LM*, eqn. 2). Note, that the coefficients of the linear model are not directly interpretable due to transformation.

2.2.2 Generalised Linear Models

A more natural way to model such data is the binomial distribution with parameters N and π (GLM_{bin}):

$$\begin{aligned} Y_i &\sim Bin(N, \pi_i) \\ E(Y_i) = \pi_i \times N \text{ and } var(Y_i) &= \pi_i(1-\pi_i)/N \\ logit\ (\pi_i) &= \beta \times X_i \end{aligned} \quad (7)$$

This model assumes that the number of occurrences (Y_i) are binomially distributed, where N = number of trials (e.g. exposed animals) and π_i is the probability of occurrences (fish survived), which together give the expected number of occurrences. The variance of the binomial distribution is a quadratic function of the mean. We are modelling the probability of occurrence as function of treatment membership (X_i) and to ensure that $0 < \pi_i < 1$ we do this on a logit scale (eqn. 7). The estimated coefficients (β) of this model are directly interpretable as changes in log odds between treatments.

Non-independent trials (e.g. fish are grouped in aquaria) may lead to overdispersion (Williams 1982). Methods to deal with overdispersed binomial data are for example quasi methods (see above) or Generalized Linear Mixed models (GLMM). However, these are not further investigated in this paper (see Warton and Hui (2011) for a comparison).

2.3 Statistical Inference

After model fitting the next step is statistical inference. Ecotoxicologists are generally interested in two hypotheses: (i) is there any treatment related effect? and (ii) which treatments show a treatment effect (to determine the LOEC)?

Following general recommendations (Bolker et al 2009; Faraway 2006), we used F-tests (*LM* and GLM_{qp}) and Likelihood-Ratio (LR) tests (GLM_p, GLM_{nb} and GLM_{bin}) to test the first hypothesis. However, it is well known that the LR test is unreliable with small sample sizes (Wilks 1938). Therefore, we additionally explored the parametric bootstrap (Faraway 2006) to assess the significance of the LR. Bootstrapping is computationally very intensive and for this reason we applied it only for the LR test of the negative binomial models (using 500 bootstrap samples, denoted as GLM_{npb}).

To assess the LOEC we used Dunnett contrasts (Dunnett 1955) with one-sided Wald t tests (normal and quasi-Poisson models) and one-sided Wald Z tests (Poisson, negative binomial and binomial models). Beside these parametric methods we also applied two, in ecotoxicology commonly used, non-parametric methods: The Kruskal-Wallis test (*KW*) to test for a general treatment effect and a pairwise Wilcoxon test (*WT*) to determine the LOEC. We adjusted for multiple testing using the method of Holm (1979).

2.4 Case study

Brock et al (2015) presents a typical example of data from mesocosm studies, which we use to demonstrate differences between methods. The data are mayfly larvae counts on artificial substrate samplers at one sampling date. A total of 18 mesocosms have been sampled from 6 treatments (Control (n = 4), 0.1, 0.3, 1, 3 mg/L (n = 3) and 10 mg/L (n = 2)) (Figure 1).

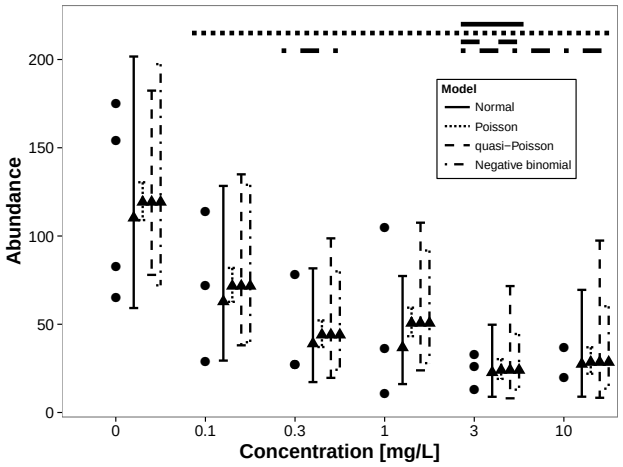

Fig. 1 Data from Brock et al (2015) (dots). Predicted values (triangles) and 95% Wald Z or t confidence intervals from the fitted models (vertical lines) are given beside. Horizontal bars above indicate treatments statistically significant different from the control group (Dunnett contrasts). The data showed considerable overdispersion ($\kappa = 3.91, \phi = 22.41$) and therefore, the Poisson model underestimates the width of confidence intervals.

2.5 Simulations

2.5.1 Count data

To further scrutinise the differences between methods we simulated data sets with known properties. We simulated count data that mimics the data of the case study with five treatments (T1 - T5) and one control group (C). Counts were drawn from a negative binomial distribution with overdispersion at all treatments ($\kappa = 4$, eqn. 5). We simulated data sets with different number of replicates (N = $\{3, 6, 9\}$) and different abundances in control treatments (μ_C = $\{2, 4, 8, 16, 32, 64, 128\}$). For Type I error estimation mean abundance was equal between treatments. For power estimation, mean abundance in treatments T2 - T5 was reduced to half of control and T1 ($\mu_{T2} = ... = \mu_{T5} = 0.5\ \mu_C = 0.5\ \mu_{T1}$), resulting in a theoretical LOEC at T2. We generated 1000 data sets for each combination of N and μ_C and analysed these using the models outlined in section 2.1.

2.5.2 Binomial data

We simulated data from a commonly used design as described in Weber et al (1989), with 5 treated (T1 - T5) and one control group (C). Proportions were drawn from a Bin(10, π) distribution, with varying probability of survival (π = $\{0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95\}$) and varying number of replicates (N = $\{3, 6, 9\}$). For Type I error estimation, π was equal between treatments. For power estimation π was fixed at 0.95 in C and T1 and varied only in treatments T2 - T5. For each combination we simulated 1000 data sets and analysed these using the models outlined in section 2.2.

2.6 Data Analysis

We analysed the case study and the simulated data using the outlined methods. We compared the methods and models in terms of Type I error (detection of an effect when there is none) and power (ability to detect an effect when it is present) at a significance level of $\alpha = 0.05$.

All simulations were done in R (Version 3.1.2) (R Core Team 2014) on an Amazon EC2 virtual Linux server (64bit, 15GB RAM, 8 cores, 2.8 GHz). Source code to reproduce the simulations and paper is available online at `https://github.com/EDiLD/usetheglm`. Moreover, Supplement 2 provides worked examples of the data of Brock et al (2015) and Weber et al (1989).

3 Results

3.1 Case study

The data set showed considerably higher variance then expected by the Poisson model ($\phi = 22.41$ (eqn. 4), $\kappa = 3.91$ (eqn. 5)). Therefore, the Poisson model did not fit to this data and led to underestimated standard errors and confidence intervals, as well as overestimated statistical significance (Figure 1). In this case, inferences on the Poisson model are not valid and we do not further discuss its results. The normal (F = 2.57, p = 0.084) and quasi-Poisson model (F = 2.90, p = 0.061), as well as the Kruskal test (p = 0.145) did not show a statistically significant treatment effects. By contrast, the LR test and parametric bootstrap of the negative binomial model indicated a treatment-related effect (LR = 13.99, p = 0.016, bootstrap: p = 0.042).

All methods predicted similar values, except the normal model predicting always lower abundances (Figure 1). 95% confidence intervals (CI) where most narrow for the negative binomial model and widest for the quasi-Poisson model - especially at lower estimated abundances. Consequently, the LOECs differed (Normal and quasi-Poisson: 3 mg/L, negative binomial: 0.3 mg/L). The pairwise Wilcoxon test did not detect any treatment different from control.

3.2 Simulations

3.2.1 Count data

For detecting a general treatment effect, GLM_{nb} and GLM_p showed inflated Type I error rates, whereas *KW* was conservative at low sample sizes. However, using the parametric bootstrap for the negative binomial model (GLM_{npb}), as

well as *LM* and GLM_{qp} resulted in appropriate Type I error rates. For detecting a treatment effect,GLM_{qp} had the highest power, followed by GLM_{npb}, *LM* and *KW*, the latter having least power (Figure 2). For our simulation design (reduction in abundance by 50%) a sample size per treatment of n = 9 was needed to achieve a power greater than 80%. At small sample sizes (n = 3, 6) and low abundances (μ_C = 2, 4) many of the negative binomial models (GLM_{nb} and GLM_{npb}) did not converge to a solution (convergence rate <85% of the simulations, Supplement 1).

For LOEC determination GLM_{nb} and GLM_p showed an increased Type I error and all other methods were slightly conservative. The inferences on LOEC generally showed less power. *LM* showed a mean reduction of 20.7% and GLM_{qp} of 24.3 %. Power to detect the LOEC was highest for GLM_{qp}. *LM* and *WT* showed less power, with *WT* having no power to detect the LOEC at low sample sizes (Figure 3).

3.2.2 Binomial data

GLM_{bin} showed slightly increased Type I error rates at low sample sizes and small effect sizes. *KW* was more conservative than *LM* and GLM_{bin}. In addition, GLM_{bin} exhibited the greatest power for testing the treatment effect. This was especially apparent at low sample sizes (n = 3), with up to 27% higher power compared to LM. However, the differences between methods quickly vanished with increasing samples sizes (Figure 4).

For inference on LOEC we found that all methods were slightly conservative. *WT* was generally more conservative and GLM_{bin} especially at low effect sizes ($p_E > 0.7$). Inference on LOEC was not as powerful as inference on the general treatment effect. Contrary to the general treatment effect, *LM* showed the higher power than GLM_{bin} at small sample sizes (n = 3, 6). *WT* had no power for n = 3 and showed less power in the other simulation runs (Figure 5).

4 Discussion

4.1 Case study

The outlined case study demonstrates that the choice of the statistical model and procedure can have substantial impact on ecotoxicological inferences and endpoints like the LOEC. Therefore, ecotoxicologists should not base their inferences solely on statistical significance tests, but also on model estimates, their uncertainty and importance (Gelman and Stern 2006). O'Hara and Kotze (2010) showed that the linear model on log transformed data gave unreliable and biased estimates, whereas GLMs performed well with little bias. Bias occurs also when back-transforming fitted means to the original scale, which explains the lower predicted means by *LM* in Figure 1 (Rothery 1988) and should be corrected for (Newman 1993). When applied to non-transformed data, the linear model would predict identical treatment means as GLMs, because for a categorical predictor the predicted means of the LM and GLM are identical. When applied to non-transformed data, the linear model would result in identical predicted treatment means as GLMs. However, predictions would differ with continuous predictors and GLMs are particularly advantageous in this case.

This is further highlighted by the fact that for the same model (linear model applied to transformed data), Brock et al (2015) reported a 10-fold lower LOEC (0.3 mg/L) then found in our study (3 mg/L, Figure 1). The reasons are manifold: (i) Brock et al (2015) used a $log(2\ y+1)$ transformation, whereas we used a $log(A\ y+1)$ transformation, where A = 2 / 11 = 0.182 (van den Brink et al 2000). (ii) We adjusted for multiple testing using Holm's (1979) method. (iii) Brock et al (2015) used a one-sided Williams test (Williams 1972), whereas we used one-sided comparisons to the control (Dunnett contrasts). The choice of transformation contributed only little to the differences. If the assumptions of Williams test are met it has strictly greater power than Dunnett contrasts (Jaki and Hothorn 2013), which explains the differences in the case study. A generalisation of the Williams test as multiple contrast test (MCT) can be used in a GLM framework (Hothorn et al 2008). Nevertheless, such a Williams-type MCT is not a panacea (Hothorn 2014) and our simulated semi-concave dose-response relationship is a situation where it fails and likely underestimates the LOEC (Kuiper et al 2014).

Overdispersion is common for ecological datasets (Warton 2005) and the case study illustrates the potential effects of overdispersion that is not accounted for: standard errors will be underestimated and significance overestimated (Figures 1). This is also shown by our simulations (Figures 2, 3) where GLM_p showed increased Type I error rates because of overdispersed simulated data. However, in factorial designs the mean-variance relationship can be easily checked by plotting mean versus variance of the treatment groups or by inspecting residual versus fitted values plots (see Supplement 2). Our simulations revealed that the LR test for GLM_{nb} is invalid because of increased Type I errors. This explains why it had the lowest p-value in the case study.

In the introduction we pointed out that there is little advice how to choose between the plenty of possible transformations - how do GLMs simplify this problem? The distribution modelled can be chosen using knowledge about the data (e.g. bounds, integer or continuous data etc). Knowing what type of data is modelled (see Methods section), the model selection process can be completely guided by the data and diagnostic tools. Therefore, choosing an appropri-

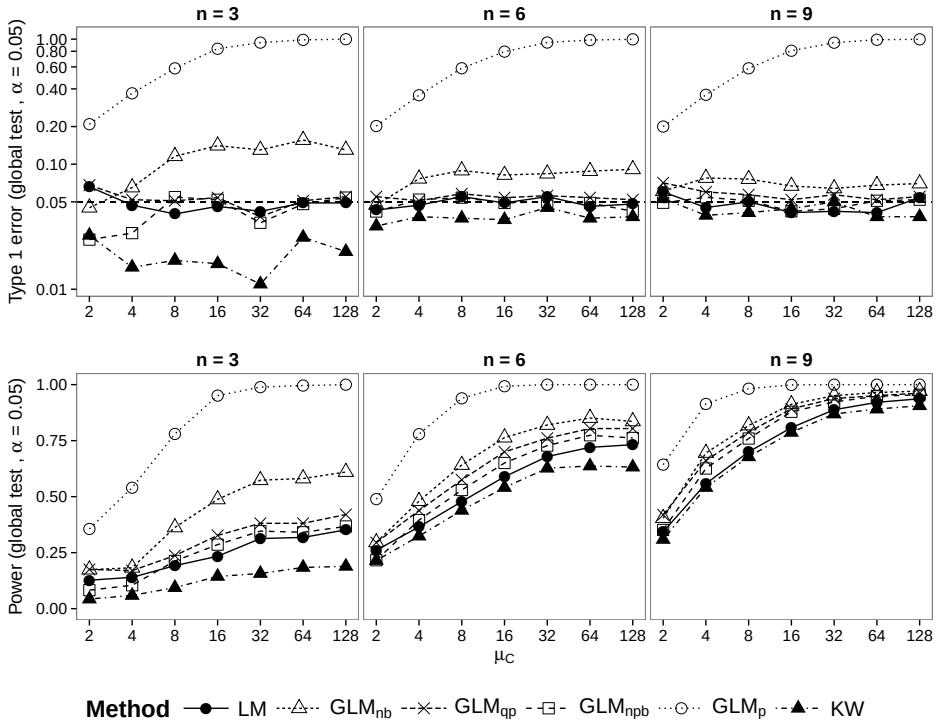

Fig. 2 Count data simulations: Type I error (top) and Power (bottom) for the test of a treatment effect. Type I errors are displayed on a logarithmic scale. Power levels for models with inflated Type I errors (GLM_P and GLM_{qp}) are shown for completeness. For n = {3, 6} and μ_C = {2, 4} less than 85% of GLM_{nb} and GLM_{npb} models did converge. Dashed horizontal line denotes the nominal I error rate at $\alpha = 0.05$.

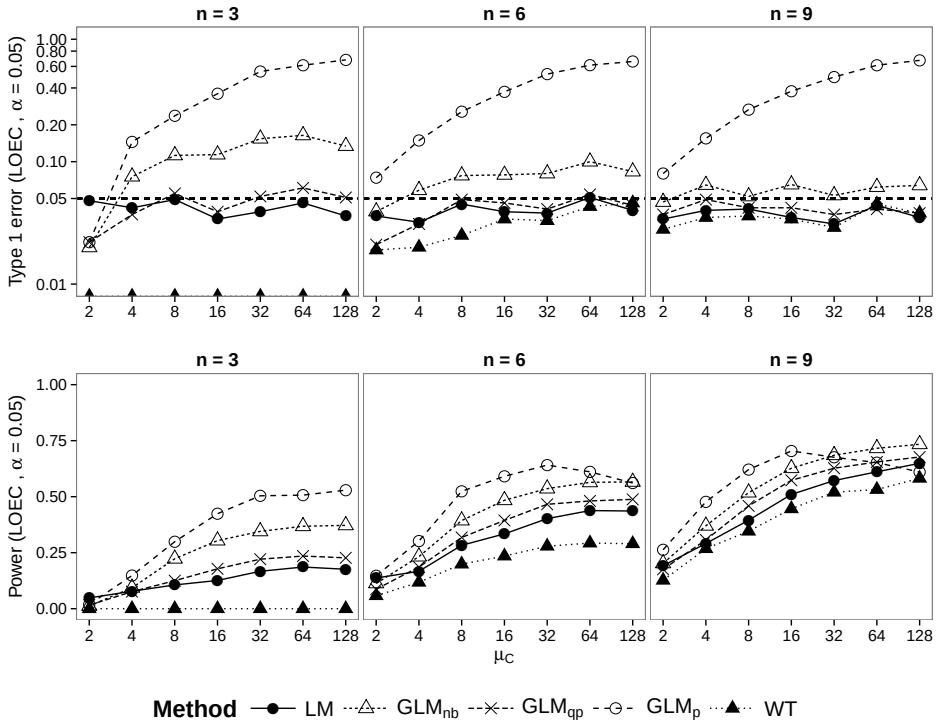

Fig. 3 Count data simulations: Type I error (top) and Power (bottom) for determination of LOEC. Type I errors are displayed one a logarithmic scale. Power levels for models with inflated Type I error are shown for completeness. For n = {3, 6} and μ_C = {2, 4} less than 85% of GLM_{nb} models did converge. Dashed horizontal line denotes the nominal Type I error rate at $\alpha = 0.05$.

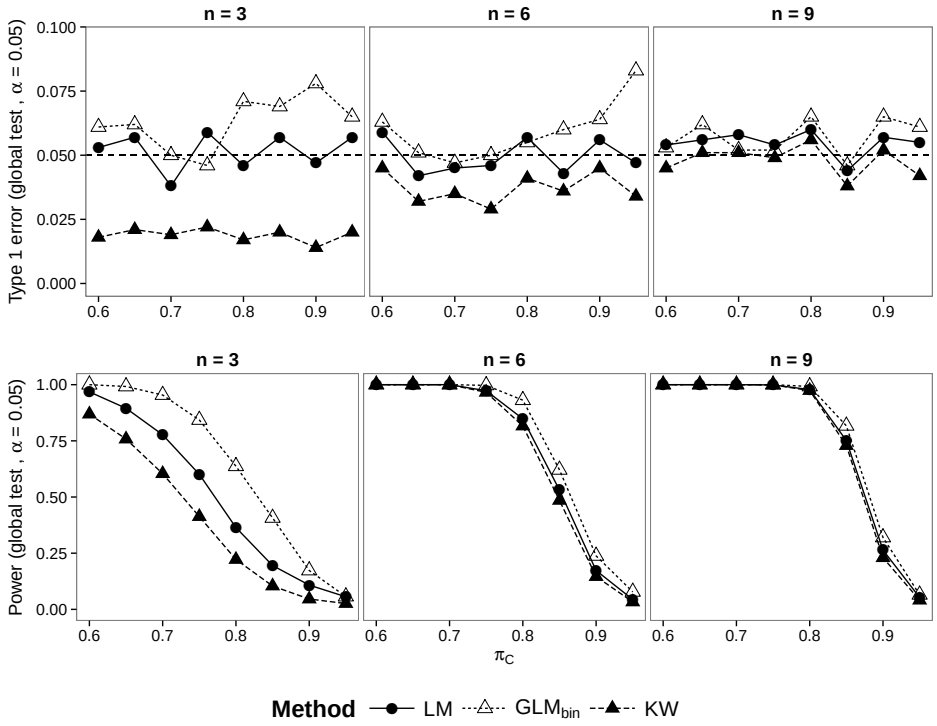

Fig. 4 Binomial data simulations: Type I error (top) and power (bottom) for the test of a treatment effect. Dashed horizontal line denotes the nominal Type I error rate at $\alpha = 0.05$.

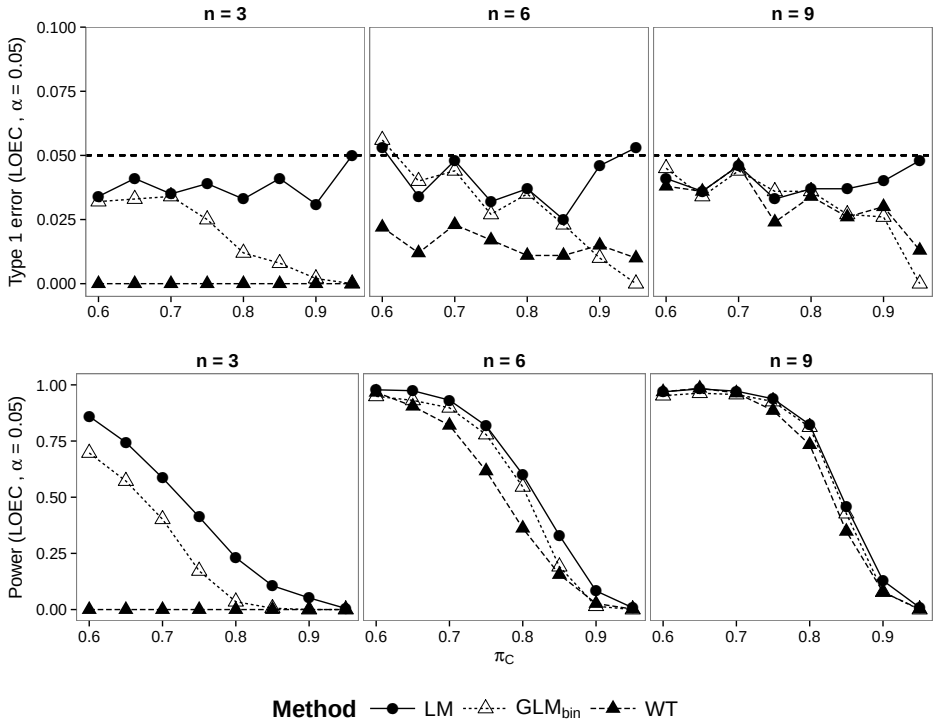

Fig. 5 Binomial data simulations: Type I error (top) and power (bottom) for the test for determination of LOEC. Dashed horizontal line denotes the nominal Type I error rate at $\alpha = 0.05$.

ate model is easier than choosing between possible transformations.

4.2 Simulations

Our simulations showed that GLMs have generally greater power than the linear model applied to transformed data. However, the simulations also suggest that the power at the population level in common mesocosm experiments is low. For common samples sizes ($n \leq 4$) and a reduction in abundance of 50% we found a low power to detect any treatment-related effect (<50% for methods with appropriate Type I error, Figure 2). Statistical power to detect the correct LOEC was even lower (less than 25%), which can be attributed to multiple testing. The low power of all methods to detect significant treatment levels such as the LOEC or NOEC suggests that these endpoints from ecotoxicological studies should be interpreted with caution and underpins their criticism (Laskowski 1995; Landis and Chapman 2011).

Mesocosm studies allow also for inferences on the community level. For community analyses *GLM for multivariate data* (Warton et al 2012) have been proposed as alternative to Principal Response Curves (PRC) and yielded similar inferences, but better indication of responsive taxa (Szöcs et al 2015). However, ter Braak and Šmilauer (2014) argue to use data transformations with community data because of their simplicity and robustness. Although our simulations covered only simple experimental designs at the population level, findings may also extend to more complex situations. Nested or repeated designs with non-normal data could be analysed using Generalised Linear Mixed Models (GLMM) and may have advantages with respect to power (Stroup 2014).

To counteract the problems with low power at the population level Brock et al (2015) proposed to take the Minimum Detectable Difference (MDD), a method to assess statistical power *a posteriori*, for inference into account. However, *a priori* power analyses can be performed easily using simulations, even for complex experimental designs (Johnson et al 2015), and might help to design, interpret and evaluate ecotoxicological studies. Moreover, Brock et al (2015) proposed that statistical power of mesocosm experiments can be increased by reducing sampling variability through improved sampling techniques and quantification methods, though they also caution against depleting populations through more exhaustive sampling. As we showed, using GLMs can enhance the power at no extra costs.

Wang and Riffel (2011) advocated that in the typical case of small sample sizes (n <20) and non-normal data, non-parametric tests perform better than parametric tests assuming normality. In contrast, our results showed that the often applied KW and WT have less power compared to LM. Moreover, $GLMs$ always performed better than non-parametric tests. Though more powerful non-parametric tests may be available (Konietschke et al 2012), these are focused on hypothesis testing and do not provide estimation of effect sizes. Additionally to testing, GLMs allow the estimation and interpretation of effects that might not be statistically significant, but ecologically relevant. Therefore, we advise using GLMs instead of non-parametric tests for non-normal data.

We found an increased Type-I error for GLM_{nb} at low sample sizes. However, it is well known that the LR statistic is not reliable at small sample sizes (Bolker et al 2009; Wilks 1938). Parametric bootstrap (GLM_{npb}) is a valuable alternative in such situations and maintains appropriate levels (Figure 2). Moreover, at small sample sizes and low abundances a significant amount of negative binomial models did not converge. We used an iterative algorithm to fit these models (Venables and Ripley 2002) and other methods assessing the likelihood directly may perform better.

GLM_{qp} showed higher statistical power than GLM_{npb} (Figure 2, bottom). This could be explained by the simpler mean-variance relationship of GLM_{qp} (eqn. 4 and 5), because at small samples sizes, low abundances or few treatment groups it is difficult to determine the mean-variance relationship. Our results are similar to Ives (2015), who compared GLMs to LM applied to transformed data for testing regression coefficients. Because of inflated Type I errors for GLM_{nb} and, in the case of multiple explanatory variables in the model, inflated Type I errors of GLM_{qp} he considered the LM on transformed data as most robust and recommended its preferred use. However, we showed that the parametric bootstrap LR test of GLM_{nb} provides appropriate Type I errors and bootstrapping might be an alternative for testing coefficients. Nevertheless, bootstrapping is computationally very intensive and we found no gains in power compared to GLM_{qp} (Figure 2). Given the higher power, appropriate Type I errors, stable convergence and reduced bias (O'Hara and Kotze 2010) we suggest that count data in one factorial experiments should be analysed using the quasi-Poisson model.

Binomial data are often collected in lab trials, where increasing the sample size may be relatively easy to accomplish. We found notable differences in power to detect a treatment effect for all simulated sample sizes. Similarly, Warton and Hui (2011) also found that GLMs have higher power than arcsine transformed linear models. Though we did not simulate overdispersed binomial data, this should be checked and accounted for. In such situations a GLMM may offer an appealing alternative (Warton and Hui 2011). At low effect sizes GLM_{bin} became conservative with increasing π_C, although this effect lessened as sample size increased (Figure 5). This is because π approaches its boundary and is also known as the *Hauck-Donner effect* (Hauck and Donner 1977). A LR-Test or parametric bootstrap may provide an alternative in such situations (Bolker et al 2009). This can

also explain why *LM* performed better for deriving LOECs at low sample sizes.

GLMs can be fitted with several statistical software packages and many textbooks are available to introduce ecotoxicologists to these models (e.g. Zuur 2013 or Quinn and Keough 2009). We recommend that ecotoxicologists should change their models instead of their data. GLMs should become a standard method in ecotoxicology and incorporated into respective guidelines.

5 Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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Responses to reviewers

Ms. No. ESPR-D-15-00741R1
submitted to
Environmental Science and Pollution Research

Eduard Szöcs and Ralf B. Schäfer

April 20, 2015

Dear editor Dr. Schulz and reviewers,

We are thankful for reviewing our manuscript a second time and the comments that helped to improve the paper. We revised the manuscript accordingly and are re-submitting the manuscript for consideration for publication in *Environmental Science and Pollution Research*.

In the remainder of this document, we describe the changes that we have made to the paper for resubmission. To assist the assessment of our changes we have submitted two versions of the revised manuscript: one with highlighted changes (compared to revision 1) and another without any highlighting. Note, that we did not highlight changes in citations and figures.

Kind regards,
Eduard Szöcs and Ralf B. Schäfer

Response to Reviewer 2

Comment 1: *"One additional point is that Tony Ives has an in press paper at Methods in Ecolgoy and Evolution on a similar topic - arguing that LM more reliably maintains nominal Type I error levels than GLM for count data, and that this is an argument in defence of transform-LM (similar to ter Braak and Smilauer 2014). This should probably get a mention."*

Response: We are thankful for pointing to this recently accepted paper. We picked it up in the discussion. See also comments 17-19.

Comment 2: *"p1 col1 l27 allow one to directly model(?)"*

Response: We fixed this sentence. It now reads :
"Generalised Linear Models (GLM) allow directly model such data, without the need for transformation".

Comment 3: *"p1 col1 l46 extremely; p2 col2 l1 for more than 40; p2 col1 l7 Warton 2005 was about counts not proportions."*

Response: We fixed these typos.

Comment 4: *"p2 col1 l25 may enhance..., when appropriately used [to reflect the change of emphasis requested to caution about misuse, suggested by reviewers 1 and 3]"*

Response: We agree and changed accordingly.

Comment 5: *"equation 1: superscript T is not the best choice, this is standard notation for a matrix transpose. y_new might be worth a shot..."*

Response: We agree and changed accordingly to $Y_{new\ i}$.

Comment 6: *"equation 2-3: β_Treatment_i is awkward notation."*

Response: We agree and changed notation to βX_i.

Comment 7: *"p2 col2 l21 Poisson not poisson"*

Response: We fixed this typo.

Comment 8: *"Section 2.2.2 Reviewer 3 requested a statement of the underlying assump-*

tion that each of the units being counted is iid, which I could not see in this section. This connects to the topic of overdispersion (which arises when not iid)"

Response: We mentioned at the beginning of section 2.2 that trials must be independent. We now also emphasize that non-independent trials may lead to overdispersion.

Comment 9: *"p4 bottom col1: Type I error and power at what significance level."*

Response: We added *"at a significance level of* $\alpha = 0.05$".

Comment 10: *"p4 col2 l18: considerably higher; p4 col2 l21: led to; p4 col2 l50 the parameteric bootstrap"*

Response: We fixed these typos.

Comment 11: *"Fig 2: Type I error off the scale is undesirable, the point that Type I error is poor is harder to see when you can't see it. Maybe use a log-scale for Type I error and power?"*

Response: We agree and, to give focus on alpha = 0.05, displayed Type I errors on a log-scale.

Comment 12: *"p5 col1 l47: Type I not Type 1, happens elsewhere too"*

Response: We fixed this throughout the manuscript.

Comment 13: *"p7 col1 line 60: residual vs fits plots can also be very informative (e.g. Wang et al 2012)"*

Response: We added residual vs. fitted values plot.

Comment 14: *"p8 col 1 line 7 delete "to"; p8 col2 line 1 add space after 2002)"*

Response: We fixed both typos.

Response to Reviewer 3

Comment 15: *"1. take the sentence in the abstract: "Generalised Linear Models (GLM) allow directly model distributions fitting such data." which cannot be understood, neither by an ecologists nor by a statistician (see further under Language) and "*

Response: We agree and rephrased. See also comment 2 and 23.

Comment 16: *"2. eqs 2-5 & 7 where the authors cannot get their math right. The paper need a lot of editing both linguistically and statistically."*

Response: We edited the equations and follow now the notation used in textbooks for ecologists (e.g. Smith et al 2009; Zuur 2013). See also comments 5 and 6.

Comment 17: *"3. Also the paper fails to indicate the trade-off between model and computational complexity, the potential gain in, for example, power and (loss/gain) in control of the type I error. For example, what is the gain of using the npb (where does this abbreviation come from??) over the much simpler qp method, and of the qp method over LM on transformed data? "*

Response: We agree and compared the gains of the different methods. See also comment 1 + 19.

Comment 18: *"4. Some summary measures of gain should be included and "*

Response: See comments 1, 17, 19.

Comment 19: *"5. an overall conclusion in favour of the qp method should be drawn. "*

Response: We agree and after comparison of gains we draw an overall conclusion on GLM_{qp}. However, this is only valid for one-factorial designs - as GLM_{qp} showed increased Type I errors in multiple regression (Ives, 2015). See also comment 1.

Comment 20: *"6. The analysis of LOEC is very inconsistent and should be redone/reconsidered. The reason is that authors claim that the Williams test is easily applied in GLM context (p7,44-48,l), but not used at all. So why is the Williams not used in the simulations? It likely gives a much higher increase in power than any of model comparison performed in the paper."*

Response: We added reference to Hothorn et al (2008) for a Williams-type multiple contrast test in a GLM framework. Moreover, we added justification for the use of Dunnett contrasts. The section now reads:
"The choice of transformation contributed only little to the differences. If the assumptions of Williams test are met it has strictly greater power than Dunnett contrasts (Jaki and Hothorn, 2013), which explains the differences in the case study. A

generalisation of the Williams test as multiple contrast test (MCT) can be used in a GLM framework (Hothorn et al, 2008). Nevertheless, such a Williams-type MCT is not a panacea (Hothorn, 2014) and our simulated semi-concave dose-response relationship is a situation where it fails and underestimates the LOEC (Kuiper et al, 2014). "

Comment 21: *"The real reason why GLMs are great is beyond the scope of experiments analysed in this paper. The real advantage of GLMs is that they allow separate specification of the distribution of the response variable and of the scale on which effects are additive. Because they are just simple means in the models in the paper and nothing what requires additivity or linearity on some scale, this key advantage falls outside the scope of the paper. Please tell something of this sort in the intro or the discussion!"*

Response: We are thankful for this comment and mention continuous predictors in the discussion.

Comment 22: *"Please also mention that the quasi-likelihood approach to GLMs in which it are not the distribution of the response variable that is key to the method, but the mean-variance relationship (this relates to comments 7 and 26)."*

Response: This is already mentioned in eqn. 4 and accompanying text.

Comment 23: *"Language: There is a tendency of stenography: applying least-squares methods (by the way, a term not used!!) after data transformation is described as data transformation or as transform the data (in the abstract on 44L and 25L). Brevity is nice but it should remain understandable. Another example:"Nevertheless, they are often analysed using methods assuming a normal distribution and variance homogeneity". Who assumes what in this sentence. A method does not assume anything (the user does, and the method is guaranteed to have some properties when the assumptions hold true.) and"They" refers to data which cannot assume anything either. There are many of these misconstructions. "*

Response: We agree and edited the abstract (see also comments 17-19) and the respective sections.

Comment 24: *"My previous comment (in comment 39): "(3) Without the use of a GLM equivalent of the Williams test all the advantage of the use of GLM in terms of power*

are gone. See the example. Discuss this ambiguity. You can perhaps use a bootstrap test based on (GLM?) monotonic regression or similar. I know some cues/leads in this direction." has led to (unverified) statements on the Williams test without implementing the test. See general, point 6."

Response: See response to comment 20.

Comment 25: *"P3,49l. Add (y_i) after number of occurrences, otherwise y_i undefined (or number of occurrences?!)."*

Response: We agree and clarified this section.

Comment 26: *"P3,58L Delete: However. "*

Response: We agree and changed accordingly.

Comment 27: *"P3,58L where can I see the beta is "parameters""*

Response: We fixed this typo and changed to *"coefficients"*.

Comment 28: *"P4,L Rephrase sentences with "kept equal""*

Response: We agree and rephrased these two sentences.

Comment 29: *"P4, 55, R. qp is not mention in remarks onType I error. Why not?"*

Response: We added LM and GLM_{qp} to this section.

Comment 30: *"Legend Fig2. Add inbetween "error are" (GLM_p and GLM_nb)"*

Response: We agree and changed accordingly.

Comment 31: *"Fig.3 Is it explained why npb is not in this figure?"*

Response: As stated in the methods section, we applied the parametric bootstrap only to the LR test.

Comment 32: *"P4,20,R And what is the estimated value of k for the case study. Now it cannot be verified that the simulations loosely mimic the case study."*

Response: We added the value of $\kappa = 3.91$.

Comment 33: *"P4,29R. Say here or in the discussion that this LR test turned out to be invalid as it has inflated Type I error. "*

Response: We agree and added this to the discussion.

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Environmental Science and Pollution Research manuscript No.
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Ecotoxicology is not normal.

A comparison of statistical approaches for analysis of count and proportion data in ecotoxicology.

Eduard Szöcs · **Ralf B. Schäfer**

Received: date / Accepted: date

Abstract ~~Counts and proportions are data types often encountered by ecotoxicologists, which~~ Ecotoxicologists often encounter count and proportion data that are rarely normally distributed. To meet the assumptions of ~~normality and heteroscedasticity, the standard procedure has been to either transform the data or use~~ the linear model such data are usually transformed or non-parametric methods ~~if this fails~~are used if the transformed data still violate the assumptions. Generalised Linear Models (GLM) allow ~~directly model distributions fitting such data~~to directly model such data, without the need for transformation. Here, we compare the performance of ~~parametric methods assuming~~ two parametric methods i.e. (1) the linear model (assuming normality of transformed data~~,~~), and (2) ~~appropriate distributions (Poisson, negativ binomial , binomial~~GLMs (assuming a Poisson, negative binomial or binomially distibuted response) and (3) non-parametric methods.

We simulated typical data mimicking low replicated ecotoxicological experiments of two common data types (counts and proportions from counts). We compared the performance of the different methods in terms of statistical power and ~~type 1~~ Type I error for detecting a general treatment effect and determining the lowest observed effect concentration (LOEC). In addition, we outlined differences ~~and advantages of GLMs~~ on a real world mesocosm data set.

For ~~counts~~count data, we found that the quasi-Poisson model ~~and the negative binomial model in combination with the parametric boostrap had higher statistical power than data transformation~~yielded the highest power. The negative binomial GLM resulted in increased Type I errors, which could be fixed using the parametric bootstrap. For proportions, binomial GLMs performed better than the linear model, except to determine LOEC at ~~extremly~~ extremely low sample sizes. The compared non-parametric methods had generally lower power.

We recommend that counts in one-factorial experiments should be analysed using quasi-Poisson models and proportions from counts ~~should be analysed by making appropriate distributional assumptions and GLMsshould become a standard method~~ by binomial GLMs. These methods should become standard in ecotoxicology.

Keywords Generalized Linear Models · Transformations · Simulation · Power · Type I error

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1 Introduction

Ecotoxicologists perform various kinds of experiments yielding different types of data. Examples are animal counts in mesocosm experiments (non-negative, integer-valued data) or proportions of surviving animals (data bounded between 0 and 1, discrete). These data are typically not normally distributed. Nevertheless, ~~they~~ such data are often analysed using methods ~~assuming~~ that assume a normal distribution and variance homogeneity (Wang and Riffel 2011). To meet these assumptions ~~,~~data are usually transformed. For example, ecotoxicological textbooks (Newman 2012) and guidelines (EPA 2002; OECD 2006) advise that survival data ~~can~~ should be transformed using an arcsine square root transformation. For count data from mesocosm experiments a log(Ay + C) transformation is usually applied, where the constants A and C are either chosen arbitrarily or following general recommendations. For example, van den Brink et al (2000) suggest to set the term Ay to be 2 for the lowest

abundance value (y) greater than zero and C to 1. ~~Moreover, other transformations~~Other transformations, like the square root or fourth root ~~are~~ transformation, are also commonly applied in community ecology (Anderson et al 2011). Note that there has been little evaluation and advice for practitioners ~~,~~ which transformations to use. If the transformed data still do not meet the assumptions ~~(i.e. normality and variance homogeneity)~~of the linear model, non-parametric tests are usually applied (Wang and Riffel 2011).

Generalised linear models (GLM) provide a method to analyse counts or proportions from counts in a statistically sound way (Nelder and Wedderburn 1972). GLMs can handle various types of data distributions, e.g. Poisson or negative binomial (for count data) or binomial (for proportions); the normal distribution being a special case of GLMs. Despite GLMs being available for more than 40 years, ecotoxicologists do not regularly make use of them. Recent studies concluded that ~~data transformations should be avoided~~ the linear model should not be applied on transformed data and GLMs be used as they have better statistical properties (O'Hara and Kotze 2010; Warton 2005 (counts), Warton and Hui 2011 (proportions from counts)).

Ecotoxicological experiments often involve small sample sizes due to practical constraints. For example, extremely low samples sizes (n <5) are common in many mesocosm studies (Sanderson 2002; Szöcs et al 2015). Small sample sizes lead to low power in statistical hypothesis testing, on which many ecotoxiological approaches (e.g. risk assessment for pesticides) rely. Such an endpoint are L/NOEC values (Lowest / No observed effect concentration)~~values~~. Although their use has been heavily criticized in the past (Laskowski 1995), they are the predominant endpoint in mesocosm experiments (Brock et al 2015; EFSA PPR 2013).

We explore how GLMs may enhance, when appropriately used, inference in ecotoxicological studies and compared three types of statistical methods (~~transformation and normality assumption~~linear model on transformed data, GLM, non-parametric tests). We first illustrate differences between statistical methods using a data set from a mesocosm study. Then we further elaborate differences in detecting a general treatment effect and determining the LOEC using simulations of two common data types in ecotoxicology: counts and proportions from counts.

2 Methods

2.1 Models for count data

2.1.1 Linear model for transformed data

To meet the assumptions of the standard linear model, count data usually needs to be transformed. We followed the recommendations of van den Brink et al (2000) and used a log(Ay + 1) transformation (eqn. 1):

$$y_i^T Y_{new\,i} = log(AyAY_i + 1) \quad (1)$$

, where ~~y_i~~ Y_i is the measured and ~~y_i^T~~ $Y_{new\,i}$ the transformed abundance of the ith observation. The factor A was chosen in such way that ~~Ay~~ AY equals 2 for the lowest non-zero abundance value (~~y~~Y).

Then we fitted the linear model to the transformed abundances (hereafter *LM*):

$$y_i^T Y_{new\,i} \sim N(\mu_i, \sigma^2)$$
$$E(y_i^T Y_{new\,i}) = \mu_i \text{ and } var(y_i^T Y_{new\,i}) = \sigma^2 \quad (2)$$
$$\mu_i = \beta Treatment \times X_i$$

This model assumes a normal distribution of the transformed abundances. The expected value for each observation i is given by its mean (μ_i) and the variance (σ^2) is constant between treatments. We allow this mean to vary between treatments (X_i codes the treatments) and β are the estimated coefficients related to these changes in transformed abundances between treatments (eqn. 2).

2.1.2 Generalised Linear Models

GLMs extend the ~~normal model by modelling other distributions~~linear model to variables that are not normally distributed. Instead of transforming the response variable, the counts could be directly modelled by a Poisson GLM (GLM_p):

$$yY_i \sim P(\mu_i)$$
$$E(yY_i) = var(yY_i) = \mu_i \quad (3)$$
$$log(\mu_i) = \beta Treatment \times X_i$$

This model assumes ~~poisson~~ Poisson distributed abundances with mean $\mu_i \geq 0$. The expected value for each observation i is given by its mean. Moreover, this model assumes that mean and variance are equal. We are modelling the mean as a function of treatment membership (X_i). However, to avoid negative values of the mean this is done on a log scale. Therefore, β also describes the differences between treatments on a log scale (eqn. 3).

The assumption of equal mean and variance is rarely met with ecological data, which is typically characterized by greater variance than the mean (overdispersion). To overcome this problem a quasi-Poisson model (GLM_{qp}) could be used, which ~~assumes that variance is~~ models the variance as a linear function of the mean (eqn. 4):

$$var(yY_i) = \Theta\phi\mu_i \tag{4}$$

Here, ~~Θ~~ ϕ is used to account for additional variation and is known as overdispersion parameter. The quasi-Poisson model is a post hoc method, meaning that first a Poisson model is estimated (eqn. 3) and than the standard errors are scaled by the degree of overdispersion (Hilbe 2014).

Another possibility to deal with overdispersion is to ~~fit~~ model abundances by a negative binomial distribution (GLM_{nb}, eqn. 5):

$$\begin{aligned} yY_i &\sim NB(\mu_i, \kappa) \\ E(yY_i) = \mu_i \text{ and } var(yY_i) &= \mu_i + \mu_i^2/\kappa \\ log(\mu_i) &= \beta Treatment \times X_i \end{aligned} \tag{5}$$

This models assumes that abundances are negative binomially distributed, with a mean of $\mu_i \geq 0$ and a variance $\mu_i + \mu_i^2/\kappa$. Similar to the Poisson model we use a log link between mean and treatments. Note, that the quasi-Poisson model assumes a linear mean-variance relationship (eqn. 4), whereas the negative binomial model assumes a quadratic relationship (eqn. 5).

The above described models are most commonly used in ecology (Ver Hoef and Boveng 2007), although other distributions for count data are possible, like the negative binomial model with a linear mean-variance relationship (also known as NB1) or the poisson inverse gaussian model (Hilbe 2014).

2.2 Models for binomial data

A binomial variable counts how often an event x occurs in a fixed number of independent trials N (e.g. *"5 out of 10 fish survived"*), with an equal probability of occurrence π between trials. The number of times an event occurs can also be calculated as proportion x/N.

2.2.1 Linear model for transformed data

To accommodate the assumptions for the standard linear model with such proportions, a special arcsine square root transformation (eqn. 6) is suggested (EPA 2002; Newman 2012):

$$y_i^T Y_{new\ i} = \begin{cases} arcsin(1) - arcsin(\sqrt{\frac{1}{4n}}) & \text{, if } yY_i = 1 \\ arcsin(\sqrt{\frac{1}{4n}}) & \text{, if } yY_i = 0 \\ arcsin(\sqrt{yY_i}) & \text{, otherwise} \end{cases} \tag{6}$$

, where ~~y_i^T are the~~ Y_i are the untransformed proportions, $Y_{new\ i}$ are the transformed proportions and n is the total number of exposed animals per treatment. The transformed proportions are then analysed using the standard linear model (LM, eqn. 2). Note, that the ~~parameters~~ coefficients of the linear model are not directly interpretable due to transformation.

2.2.2 Generalised Linear Models

A more natural way to model such data is the binomial distribution with parameters N and π (GLM_{bin}):

$$\begin{aligned} yY_i &\sim Bin(N, \pi_i) \\ E(yY_i) = \pi_i \times N \text{ and } var(yY_i) &= \pi_i(1-\pi_i)/N \\ logit\ (\pi_i) &= \beta Treatment \times X_i \end{aligned} \tag{7}$$

This model assumes that the number of ~~occurences~~ occurrences (Y_i) are binomially distributed, where N = number of trials (e.g. exposed animals) and π_i is the probability of occurrences (fish survived), which together give the expected number of occurrences. The variance of the binomial distribution is a quadratic function of the mean. We are modelling the probability of occurrence as function of treatment membership (X_i) and to ensure that $0 < \pi_i < 1$ we do this on a logit scale (eqn. 7). ~~However, the parameters~~ The estimated coefficients (β) of this model are directly interpretable as changes in log odds between treatments.

~~Similarly to counts, binomial data may also show overdispersion~~ Non-independent trials (e.g. fish are grouped in aquaria) may lead to overdispersion (Williams 1982). Methods to deal with overdispersed binomial data are ~~either~~ for example quasi methods (see above) or Generalized Linear Mixed models (GLMM). However, these are not further investigated in this paper (see Warton and Hui (2011) for a comparison).

2.3 Statistical Inference

After model fitting ~~and parameter estimation~~ the next step is statistical inference. Ecotoxicologists are generally interested in two hypotheses: (i) is there any treatment related effect? and (ii) which treatments show a treatment effect (to determine the LOEC)?

Following general recommendations (Bolker et al 2009; Faraway 2006), we used F-tests (*LM* and GLM_{qp}) and Likelihood-Ratio (LR) tests (GLM_p, GLM_{nb} and GLM_{bin}) to test the first hypothesis. However, it is well known that ~~LR test are~~ the LR test is unreliable with small sample sizes (Wilks 1938). Therefore, we additionally explored the parametric bootstrap (Faraway 2006) to assess the significance of the LR. Bootstrapping is computationally very intensive and for this reason we applied it only for the LR test of the negative binomial models (using 500 bootstrap samples, denoted as GLM_{npb}).

To assess the LOEC we used Dunnett contrasts (Dunnett 1955) with one-sided Wald t tests (normal and quasi-Poisson models) and one-sided Wald Z tests (Poisson, negative binomial and binomial models). Beside these parametric methods we also applied two, in ecotoxicology commonly used, non-parametric methods: The Kruskal-Wallis test (*KW*) to test for a general treatment effect and a pairwise Wilcoxon test (*WT*) to determine the LOEC. We adjusted for multiple testing using the method of Holm (1979).

2.4 Case study

Brock et al (2015) presents a typical example of data from mesocosm studies, which we use to demonstrate differences between methods. The data are mayfly larvae counts on artificial substrate samplers ~~were~~ at one sampling date. A total of 18 ~~mesocosm~~ mesocosms have been sampled from 6 treatments (Control (n = 4), 0.1, 0.3, 1, 3 mg/L (n = 3) and 10 mg/L (n = 2)) (Figure 1).

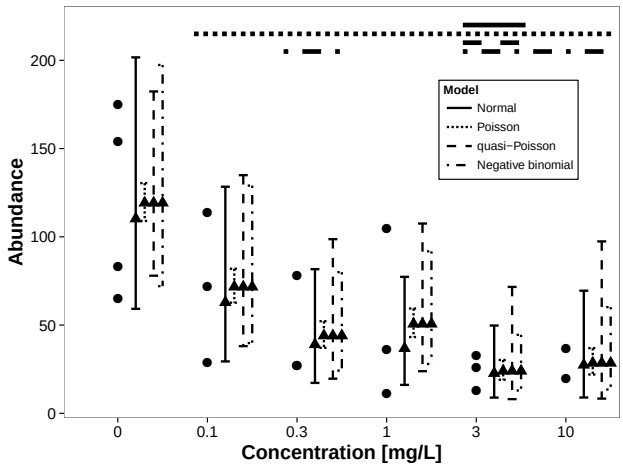

Fig. 1 Data from Brock et al (2015) (dots). Predicted values (triangles) and 95% Wald Z or t confidence intervals from the fitted models (vertical lines) are given beside. Horizontal bars above indicate treatments statistically significant different from the control group (Dunnett contrasts). The data showed considerable overdispersion (~~$\kappa = 4$~~ $\kappa = 3.91$, $\phi = 22.41$) and therefore, the Poisson model underestimates the width of confidence intervals.

2.5 Simulations

2.5.1 Count data

To further scrutinise the differences between methods we simulated data sets with known properties. We simulated count data that mimics the data of the case study with five treatments (T1 - T5) and one control group (C). Counts were drawn from a negative binomial distribution with overdispersion at all treatments ($\kappa = 4$, eqn. 5). We simulated data sets with different number of replicates (N = {3, 6, 9}) and different abundances in control treatments (μ_C = {2, 4, 8, 16, 32, 64, 128}). For Type I error estimation mean abundance was equal between treatments. For power estimation, mean abundance in treatments T2 - T5 was reduced to half of control and T1 ($\mu_{T2} = \ldots = \mu_{T5} = 0.5\ \mu_C = 0.5\ \mu_{T1}$), resulting in a theoretical LOEC at T2. ~~Mean abundance was kept equal between all groups in Type 1 error simulations.~~ We generated 1000 data sets for each combination of N and μ_C and analysed these using the models outlined in section 2.1.

2.5.2 Binomial data

We simulated data from a commonly used design as described in Weber et al (1989), with 5 treated (T1 - T5) and ~~a~~ one control group (C). Proportions were drawn from a Bin(10, π) distribution, with varying probability of survival (~~π_C~~ π = {0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95}) and varying number of replicates (N = {3, 6, 9}). For Type ~~1~~ I error estimation, ~~π_C was held constant between groups~~ π was equal between treatments. For power estimation ~~π_C in C and T1~~ π was fixed at 0.95 ~~and was set to values between 0.6 and 0.95 for the~~ in C and T1 and varied only in treatments T2 - T5. For each combination we simulated 1000 data sets and analysed these using the models outlined in section 2.2.

2.6 Data Analysis

We analysed the case study and the simulated data using the outlined methods. We compared the methods and models in terms of Type ~~1~~ I error (detection of an effect when there is none) and power (ability to detect an effect when it is present) at a significance level of $\alpha = 0.05$.

All simulations were done in R (Version 3.1.2) (R Core Team 2014) on an Amazon EC2 virtual Linux server (64bit, 15GB RAM, 8 cores, 2.8 GHz). Source code to reproduce the simulations and paper is available online at `https://github.com/EDiLD/usetheglm`. Moreover, Supplement 2 provides worked examples of the data of Brock et al (2015) and Weber et al (1989).

3 Results

3.1 Case study

The data set showed ~~considerable~~ considerably higher variance then expected by the Poisson model (~~$\Theta = 22.41$,~~ $\phi = 22.41$ (eqn. 4)~~.~~, $\kappa = 3.91$ (eqn. 5)). Therefore, the Poisson model did not fit to this data and ~~lead~~ led to underestimated standard errors and confidence intervals, as well as overestimated statistical significance (Figure 1). In this case, inferences on the Poisson model are not valid and we do not further discuss its results. The normal (F = 2.57, p = 0.084) and quasi-Poisson model (F = 2.90, p = 0.061), as well as the Kruskal test (p = 0.145) did not show a statistically significant treatment effects. By contrast, the LR test and parametric bootstrap of the negative binomial model indicated a treatment-related effect (LR = 13.99, p = 0.016, bootstrap: p = 0.042).

All methods predicted similar values, except the normal model predicting always lower abundances (Figure 1). 95% confidence intervals (CI) where most narrow for the negative binomial model and widest for the quasi-Poisson model - especially at lower estimated abundances. Consequently, the LOECs differed (Normal and quasi-Poisson: 3 mg/L, negative binomial: 0.3 mg/L). The pairwise Wilcoxon test did not detect any treatment different from control.

3.2 Simulations

3.2.1 Count data

For detecting a general treatment effect, GLM_{nb} and GLM_p showed inflated ~~type 1~~ Type I error rates, whereas KW was conservative at low sample sizes. However, using the parametric bootstrap for the negative binomial model (GLM_{npb}), as well as LM and GLM_{qp} resulted in appropriate ~~type 1~~ Type I error rates. For detecting a treatment effect,~~GLM_{npb} and~~ GLM_{qp} ~~exhibited higher powerthan~~ had the highest power, followed by GLM_{npb}, LM and KW, the latter having least power (Figure 2). For our simulation design (reduction in abundance by 50%) a sample size per treatment of n = 9 was needed to achieve a power greater than 80%. At small sample sizes (n = 3, 6) and low abundances (μ_C = 2, 4) many of the negative binomial models (GLM_{nb} and GLM_{npb}) did not converge to a solution (convergence rate <85% of the simulations, Supplement 1).

For LOEC determination GLM_{nb} and GLM_p showed an increased Type ~~1~~ I error and all other methods were slightly conservative. The inferences on LOEC generally showed less power. LM showed a mean reduction of 20.7% and GLM_{qp} of 24.3 %. Power to detect the LOEC was highest for GLM_{qp}. LM and WT showed less power, with WT having no power to detect the LOEC at low sample sizes (Figure 3).

3.2.2 Binomial data

GLM_{bin} showed slightly increased ~~type 1~~ Type I error rates at low sample sizes and small effect sizes. KW was more conservative than LM and GLM_{bin}. In addition, GLM_{bin} exhibited the greatest power for testing the treatment effect. This was especially apparent at low sample sizes (n = 3), with up to 27% higher power compared to LM. However, the differences between methods quickly vanished with increasing samples sizes (Figure 4).

For inference on LOEC we found that all methods were slightly conservative. WT was generally more conservative and GLM_{bin} especially at low effect sizes ($p_E > 0.7$). Inference on LOEC was not as powerful as inference on the general treatment effect. Contrary to the general treatment effect, LM showed the higher power than GLM_{bin} at small sample sizes (n = 3, 6). WT had no power for n = 3 and showed less power in the other simulation runs (Figure 5).

4 Discussion

4.1 Case study

The outlined case study demonstrates that the choice of the statistical model and procedure can have substantial impact on ecotoxicological inferences and endpoints like the LOEC. Therefore, ecotoxicologists should not base their inferences solely on statistical significance tests, but also on ~~parameter~~ model estimates, their uncertainty and importance (Gelman and Stern 2006). ~~Nevertheless,~~ O'Hara and Kotze (2010) showed that ~~*LM* using a log transformation~~ the linear model on log transformed data gave unreliable and biased ~~parameter~~ estimates, whereas GLMs performed well with little bias. Bias occurs also when back-transforming fitted means to the original scale, which explains the lower predicted means by LM in Figure 1 (Rothery 1988) and should be corrected for (Newman 1993). When applied to non-transformed data, the linear model would predict identical treatment means as GLMs, because for a categorical predictor the predicted means of the LM and GLM are identical. When applied to non-transformed data, the linear model would result in identical predicted treatment means as GLMs. However, predictions would differ with continuous predictors and GLMs are particularly advantageous in this case.

This is further highlighted by the fact that for the same model (linear model ~~of~~ applied to transformed data), Brock et al (2015) reported a 10-fold lower LOEC (0.3 mg/L) then found in our study (3 mg/L, Figure 1). The reasons are manifold: (i) Brock et al (2015) used a $log(2\ y + 1)$

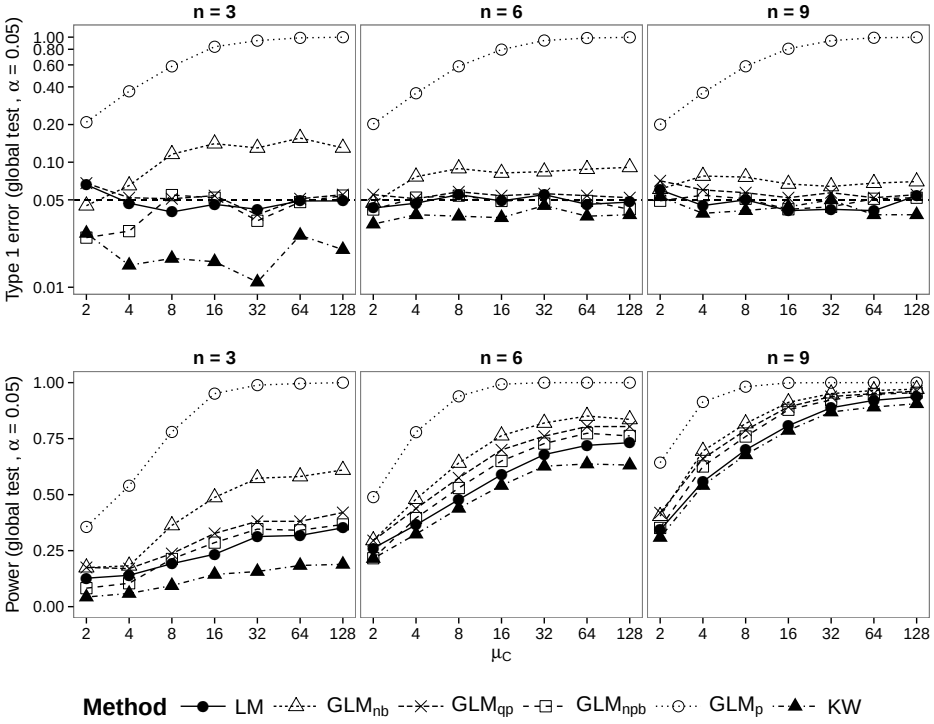

Fig. 2 Count data simulations: Type ~~1~~ I error (top) and Power (bottom) for the test of a treatment effect. ~~Only type 1~~ Type I errors ~~<25%~~ are displayed on a logarithmic scale. ~~*GLM$_p$* showed type 1 errors >20% in all simulation scenarios.~~ Power levels for models with inflated ~~type~~ Type I ~~error~~ errors (*GLM$_P$* and *GLM$_{qp}$*) are shown for completeness. For n = {3, 6} and μ_C = {2, 4} less than 85% of *GLM$_{nb}$* and *GLM$_{npb}$* models did converge. Dashed horizontal line denotes the nominal ~~Type 1~~ I error rate at $\alpha = 0.05$.

transformation, whereas we used a $log(A\ y + 1)$ transformation, where A = 2 / 11 = 0.182 (van den Brink et al 2000). ~~However, this contributed only little to the differences. A much bigger impact had the type of multiple comparison:~~ (ii) We adjusted for multiple testing using Holm's (1979) method. (iii) Brock et al (2015) used a one-sided Williams test (Williams 1972), whereas we used one-sided comparisons to the control (Dunnett contrasts). ~~In contrast to the Williams test, Dunnett contrasts do not assume a monotonic dose-response relationship and allow individual comparisons between treatment groups and the control. However, under monotonicity they have less power,~~ The choice of transformation contributed only little to the differences. If the assumptions of Williams test are met it has strictly greater power than Dunnett contrasts (Jaki and Hothorn 2013), which explains the differences ~~. Both types of multiple comparisons are available~~ in the case study. A generalisation of the Williams test as multiple contrast ~~tests~~ test (MCT) can be used in a GLM framework (Hothorn et al 2008). Nevertheless, such a Williams-type MCT is not a panacea (Hothorn 2014) and our simulated semi-concave dose-response relationship is a situation where it fails and likely underestimates the LOEC (Kuiper et al 2014). ~~Therefore, our comparison of methods should be independent from the choice of contrast, which is determined by assumptions and research questions.~~

Overdispersion is common for ecological datasets (Warton 2005) and the case study illustrates the potential effects of overdispersion that is not accounted for: standard errors will be underestimated and significance overestimated (Figures 1). This is also shown by our simulations (Figures 2, 3) where *GLM$_p$* showed increased ~~type 1~~ Type I error rates because of overdispersed simulated data. However, in factorial designs the mean-variance relationship can be easily checked by plotting mean versus variance of the treatment groups or by inspecting residual versus fitted values plots (see Supplement 2). Our simulations revealed that the LR test for *GLM$_{nb}$* is invalid because of increased Type I errors. This explains why it had the lowest p-value in the case study.

In the introduction we pointed out that there is little advice how to choose between the plenty of possible transformations - how do GLMs simplify this problem? The distribution modelled can be chosen using knowledge about the data (e.g. bounds, integer or continuous data etc). Knowing what type of data is modelled (see Methods section), the model selection process can be completely guided by the data and diagnostic tools. Therefore, choosing an appropri-

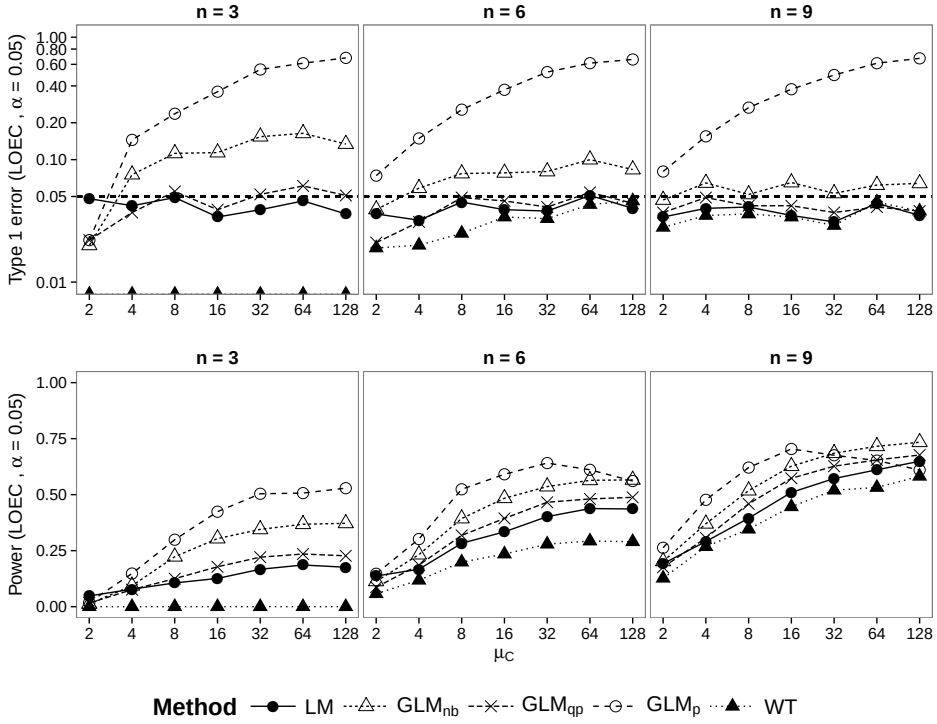

Fig. 3 Count data simulations: Type ~~I~~ I error (top) and Power (bottom) for determination of LOEC. ~~For clarity only type I~~ Type I errors ~~<25%~~ are displayed one a logarithmic scale. Power levels for models with inflated ~~type~~ Type I error are shown for completeness. For n = {3, 6} and μ_C = {2, 4} less than 85% of *GLM*$_{nb}$ models did converge. Dashed horizontal line denotes the nominal Type ~~I~~ I error rate at $\alpha = 0.05$.

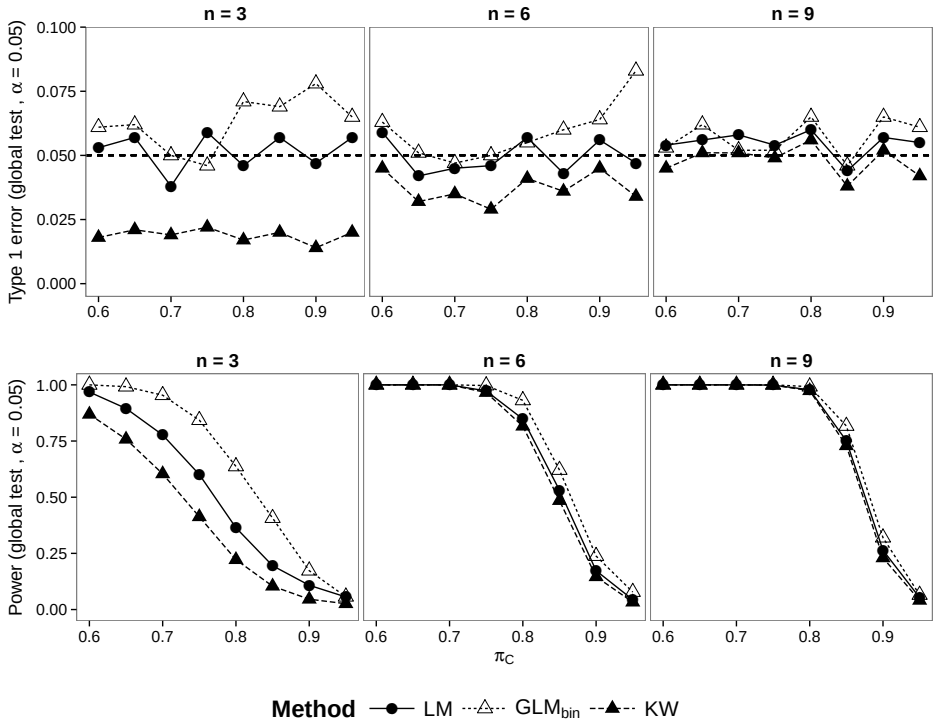

Fig. 4 Binomial data simulations: Type ~~I~~ I error (top) and power (bottom) for the test of a treatment effect. Dashed horizontal line denotes the nominal Type ~~I~~ I error rate at $\alpha = 0.05$.

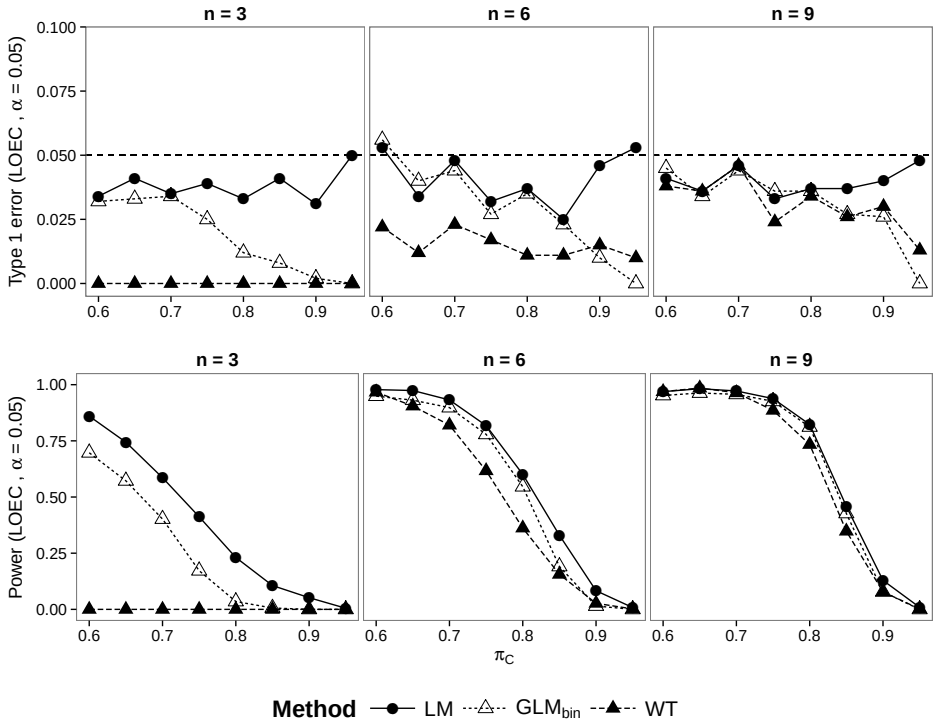

Fig. 5 Binomial data simulations: Type ~~I~~ I error (top) and power (bottom) for the test for determination of LOEC. Dashed horizontal line denotes the nominal Type ~~I~~ I error rate at $\alpha = 0.05$.

ate model is easier than choosing between possible transformations.

4.2 Simulations

Our simulations showed that ~~generally GLMs have~~ GLMs have generally greater power than ~~data transformations~~the linear model applied to transformed data. However, the simulations also suggest that the power at the population level in common mesocosm experiments is low. For common samples sizes ($n \leq 4$) and a reduction in abundance of 50% we found a low power to detect any treatment-related effect (<50% for methods with appropriate Type ~~I~~ I error, Figure 2). Statistical power to detect the correct LOEC was even lower (less than 25%), which can be attributed to multiple testing. The low power of all methods to detect significant treatment levels such as the LOEC or NOEC suggests that these endpoints from ecotoxicological studies should be interpreted with caution and underpins their criticism (Laskowski 1995; Landis and Chapman 2011).

Mesocosm studies allow also ~~inferences on~~ for inferences on the community level. For community analyses *GLM for multivariate data* (Warton et al 2012) have been proposed as alternative to Principal Response Curves (PRC) and yielded ~~to~~ similar inferences, but better indication of responsive taxa (Szöcs et al 2015). However, ter Braak and Šmilauer (2014) argue to use data transformations with community data because of their simplicity and robustness. Although our simulations covered only simple experimental designs at the population level, findings may also extend to more complex situations. Nested or repeated designs with non-normal data could be analysed using Generalised Linear Mixed Models (GLMM) and may have advantages with respect to power (Stroup 2014).

To counteract the problems with low power at the population level Brock et al (2015) proposed to take the Minimum Detectable Difference (MDD), a method to assess statistical power *a posteriori*, for inference into account. However, *a priori* power analyses can be performed easily using simulations, even for complex experimental designs (Johnson et al 2015), and might help to design, interpret and evaluate ecotoxicological studies. Moreover, Brock et al (2015) proposed that statistical power of mesocosm experiments can be increased by reducing sampling variability through improved sampling techniques and quantification methods, though they also caution against depleting populations through more exhaustive sampling. As we showed, using ~~appropriate statistical methods (like GLMs)~~ GLMs can enhance the power at no extra costs.

Wang and Riffel (2011) advocated that in the typical case of small sample sizes (n <20) and non-normal data, non-parametric tests perform better than parametric tests as-

suming normality. In contrast, our results showed that the often applied *KW* and *WT* have less power compared to *LM*. Moreover, *GLMs* always performed better than non-parametric tests. Though more powerful non-parametric tests may be available (Konietschke et al 2012), these are focused on hypothesis testing and do not provide estimation of effect sizes. Additionally to testing, GLMs allow the estimation and interpretation of effects that might not be statistically significant, but ecologically relevant. Therefore, we advise using GLMs instead of non-parametric tests for non-normal data.

We found an increased Type-I error for GLM_{nb} at low sample sizes. However, it is well known that the LR statistic is not reliable at small sample sizes (Bolker et al 2009; Wilks 1938). Parametric bootstrap (GLM_{npb}) is a valuable alternative in such situations and maintains appropriate levels (Figure 2). Moreover, at small sample sizes and low abundances a significant amount of negative binomial models did not converge. We used an iterative algorithm to fit these models (Venables and Ripley 2002) and other methods assessing the likelihood directly may perform better.

GLM_{qp} showed higher statistical power than GLM_{npb} (Figure 2, bottom). This could be explained by the simpler mean-variance relationship of GLM_{qp} (eqn. 4 and 5), because at small samples sizes, low abundances or few treatment groups it is difficult to determine the mean-variance relationship. Our results are similar to Ives (2015), who compared GLMs to LM applied to transformed data for testing regression coefficients. Because of inflated Type I errors for GLM_{nb} and, in the case of multiple explanatory variables in the model, inflated Type I errors of GLM_{qp} he considered the LM on transformed data as most robust and recommended its preferred use. However, we showed that the parametric bootstrap LR test of GLM_{nb} provides appropriate Type I errors and bootstrapping might be an alternative for testing coefficients. Nevertheless, bootstrapping is computationally very intensive and we found no gains in power compared to GLM_{qp} (Figure 2). Given the higher power, appropriate Type I errors, stable convergence and reduced bias (O'Hara and Kotze 2010) we suggest that count data in one factorial experiments should be analysed using the quasi-Poisson model.

Binomial data are often collected in lab trials, where increasing the sample size may be relatively easy to accomplish. We found notable differences in power to detect a treatment effect for all simulated sample sizes. Similarly, Warton and Hui (2011) also found that GLMs have higher power than arcsine transformed linear models. Though we did not simulate overdispersed binomial data, this should be checked and accounted for. In such situations a GLMM may offer an appealing alternative (Warton and Hui 2011). At low effect sizes GLM_{bin} became conservative with increasing π_C, although this effect lessened as sample size increased (Figure 5). This is because π approaches its boundary and is also known as the *Hauck-Donner effect* (Hauck and Donner 1977). A LR-Test or parametric bootstrap may provide an alternative in such situations (Bolker et al 2009). This can also explain why *LM* performed better for deriving LOECs at low sample sizes.

GLMs can be fitted with several statistical software packages and many textbooks are available to introduce ecotoxicologists to these models (e.g. Zuur 2013 or Quinn and Keough 2009). We recommend that ecotoxicologists should change their models instead of their data. GLMs should become a standard method in ecotoxicology and incorporated into respective guidelines.

5 Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

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