

Use the GLM, Luke!

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

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1 Supplement 2 - Examples

1.1 Binomial data

1.1.1 Introduction

Here we will show how to analyse binomial data. Data is provided in Newman (2012) (example 5.1, page 223) and EPA (2002). Ten fathead minnow (*Pimephales promelas*) larvae were exposed to sodium pentachlorophenol (NaPCP) and proportions of the total number alive at the end of the exposure reported.

First we load the data:

```
df <- read.table(header = TRUE, text = 'conc A B C D
0 1 1 0.9 0.9
32 0.8 0.8 1 0.8
64 0.9 1 1 1
128 0.9 0.9 0.8 1
256 0.7 0.9 1 0.5
512 0.4 0.3 0.4 0.2')
df

##   conc    A    B    C    D
## 1     0 1.0 1.0 0.9 0.9
## 2    32 0.8 0.8 1.0 0.8
## 3    64 0.9 1.0 1.0 1.0
## 4   128 0.9 0.9 0.8 1.0
## 5   256 0.7 0.9 1.0 0.5
## 6   512 0.4 0.3 0.4 0.2
```

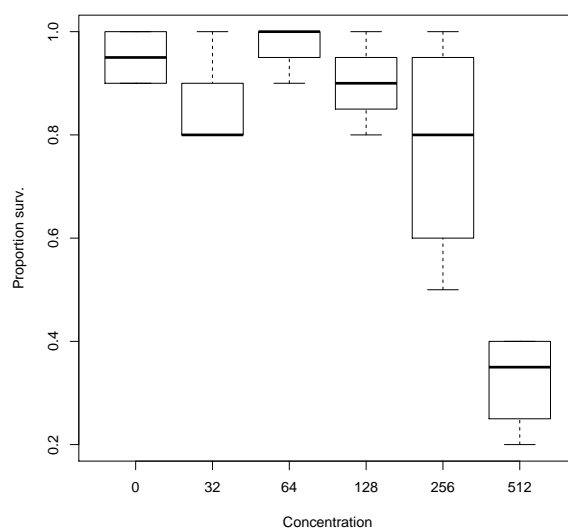
Then we do some house-keeping, reformat the data to the long format and convert concentration to a factor:

```
require(reshape2)
# wide to long
dfm <- melt(df, id.vars = 'conc', value.name = 'y', variable.name = 'tank')
# conc as factor
dfm$conc <- factor(dfm$conc)
head(dfm)
```

```
##   conc tank   y
## 1    0    A 1.0
## 2   32    A 0.8
## 3   64    A 0.9
## 4  128    A 0.9
## 5  256    A 0.7
## 6  512    A 0.4
```

Let's have a first look at the data:

```
boxplot(y ~ conc, data = dfm,
        xlab = 'Concentration', ylab = 'Proportion surv.')
```

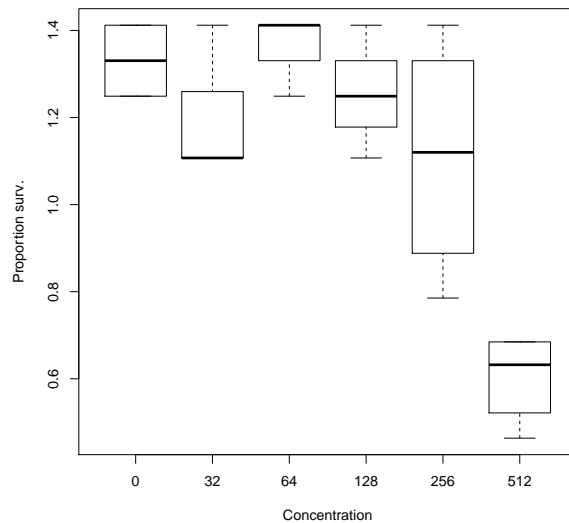


1.1.2 Transforming data

Next we arcsin transform the proportions:

```
dfm$y_asin <- ifelse(dfm$y == 1, asin(1) - asin(sqrt(1/40)),
                    ifelse(dfm$y == 0, asin(sqrt(1/40)),
                          asin(sqrt(dfm$y))
                    )
)
```

```
boxplot(y_asin ~ conc, data = dfm,
        xlab = 'Concentration', ylab = 'Proportion surv.')
```



1.1.3 Analysing data

We will use the `bbmle`-package to fit the models, as we used this also for the simulations. However, also standard R functions can be use (see below). Let's start with the model assuming a normal distribution of transformed proportions.

```
require(bbmle)
mod_normal <- mle2(y_asin ~ dnorm(mean = mu, sd = s),
  parameters = list(mu ~ conc, s ~ 1),
  data = dfm,
  start = list(mu = 0.5, s = 1)
)
summary(mod_normal)
```

```
## Maximum likelihood estimation
##
## Call:
## mle2(minuslogl = y_asin ~ dnorm(mean = mu, sd = s), start = list(mu = 0.5,
##   s = 1), data = dfm, parameters = list(mu ~ conc, s ~ 1))
##
## Coefficients:
##              Estimate Std. Error z value  Pr(z)
## mu.(Intercept)   1.3305     0.0666  19.97 < 2e-16 ***
## mu.conc32        -0.1472     0.0942  -1.56   0.118
## mu.conc64         0.0407     0.0942   0.43   0.665
## mu.conc128       -0.0762     0.0942  -0.81   0.419
## mu.conc256       -0.2211     0.0942  -2.35   0.019 *
## mu.conc512       -0.7274     0.0942  -7.72 1.2e-14 ***
## s                 0.1333     0.0192   6.93 4.3e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## -2 log L: -28.64
```

This may give some warnings as we did not put boundaries on the parameters (s cannot be 0 or lower), however we can ignore them. The summary gives us the estimated parameters, their standard error and accompanying p-values. Note that this model is parametrised a contrast to the control group so we can directly use the summary-output to determine the LOEC. Note that these p-values are not adjusted for multiple testing (you can use `p.adjust()` for this).

To perform a Likelihood-Ratio-Test we specify a null model and compare both.

```
mod_normal.null <- update(mod_normal,
                          parameters = list(mu ~ 1, sd ~ 1)
                          )
anova(mod_normal, mod_normal.null)

## Likelihood Ratio Tests
## Model 1: mod_normal, y_asin~dnorm(mean=mu,sd=s): mu~conc, s~1
## Model 2: mod_normal.null, y_asin~dnorm(mean=mu,sd=s): mu~1, sd~1
##   Tot Df Deviance Chisq Df Pr(>Chisq)
## 1      7   -28.64
## 2      2    8.49  37.1  5   5.7e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

With base R we could do:

```
mod_normal <- lm(y_asin ~ conc, data = dfm)
drop1(mod_normal, test = 'Chisq')

## Single term deletions
##
## Model:
## y_asin ~ conc
##           Df Sum of Sq  RSS   AIC Pr(>Chi)
## <none>          0.426 -84.7
## conc      5      1.57 2.001 -57.6  5.7e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

or using the `lmtest` package (Zeileis and Hothorn, 2002):

```
require(lmtest)
lrtest(mod_normal)

## Likelihood ratio test
##
## Model 1: y_asin ~ conc
## Model 2: y_asin ~ 1
##   #Df LogLik Df Chisq Pr(>Chisq)
## 1    7  14.32
## 2    2  -4.24 -5  37.1   5.7e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Next we assume that the number of surviving larvae are drawn from a binomial distribution. First we backtransform the proportions reported to counts of surviving and dead larvae:

```
dfm$surv <- dfm$y * 10
dfm$dead <- 10 - dfm$surv
```

And then we specify the logistic model:

```
mod_bin <- mle2(surv ~ dbinom(size = 10, prob = plogis(lp)),
               parameters = list(lp ~ conc),
               data = dfm,
               start = list(lp = 0)
               )
summary(mod_bin)

## Maximum likelihood estimation
##
## Call:
## mle2(minuslogl = surv ~ dbinom(size = 10, prob = plogis(lp)),
##      start = list(lp = 0), data = dfm, parameters = list(lp ~
##      conc))
##
## Coefficients:
##              Estimate Std. Error z value Pr(z)
## lp.(Intercept)    2.944      0.725   4.06 4.9e-05 ***
## lp.conc32         -1.210      0.850  -1.42  0.155
## lp.conc64          0.719      1.246   0.58  0.564
## lp.conc128        -0.747      0.897  -0.83  0.405
## lp.conc256        -1.708      0.818  -2.09  0.037 *
## lp.conc512        -3.675      0.800  -4.59 4.4e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## -2 log L: 60.86
```

And perform the LRT

```
mod_bin.null <- update(mod_bin,
                      parameters = list(lp ~ 1))
anova(mod_bin, mod_bin.null)

## Likelihood Ratio Tests
## Model 1: mod_bin, surv~dbinom(size=10,prob=plogis(lp)): lp~conc
## Model 2: mod_bin.null, surv~dbinom(size=10,prob=plogis(lp)): lp~1
##   Tot Df Deviance Chisq Df Pr(>Chisq)
## 1      6      60.9
## 2      1     125.6  64.8  5    1.2e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

With base R we could use

```

mod_bin_b <- glm(cbind(surv, dead) ~ conc, data = dfm, family = binomial(link = 'logit'))
summary(mod_bin_b)

##
## Call:
## glm(formula = cbind(surv, dead) ~ conc, family = binomial(link = "logit"),
##      data = dfm)
##
## Deviance Residuals:
##      Min       1Q   Median       3Q      Max
## -1.898  -0.572   0.000   0.787   2.258
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    2.944     0.725    4.06 4.9e-05 ***
## conc32        -1.210     0.850   -1.42  0.155
## conc64         0.719     1.246    0.58  0.564
## conc128        -0.747     0.897   -0.83  0.405
## conc256        -1.708     0.818   -2.09  0.037 *
## conc512        -3.675     0.800   -4.59 4.4e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##      Null deviance: 88.672  on 23  degrees of freedom
## Residual deviance: 23.889  on 18  degrees of freedom
## AIC: 72.86
##
## Number of Fisher Scoring iterations: 5

drop1(mod_bin_b, test = 'Chisq')

## Single term deletions
##
## Model:
## cbind(surv, dead) ~ conc
##      Df Deviance   AIC    LRT Pr(>Chi)
## <none>     23.9   72.9
## conc    5     88.7 127.6 64.8 1.2e-12 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

1.2 Count data

1.2.1 Introduction

In this example we will analyse data from (Brock et al., 2014). The data are count of mayfly larvae in Macroinvertebrate Artificial Substrate Samplers in 18 mesocosms at one sampling day. There are 5 Treatments and one control group.

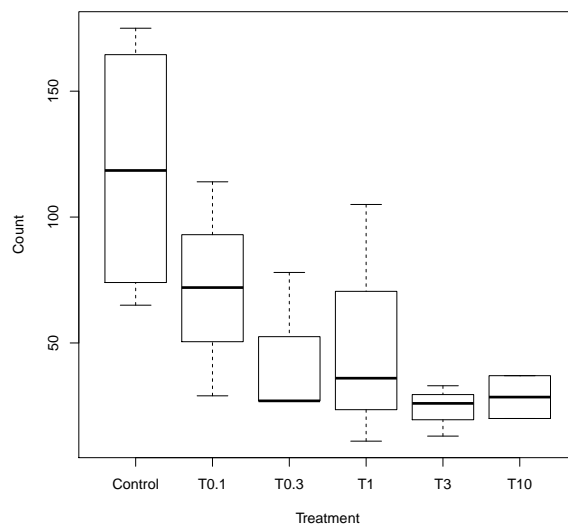
First we load the data and bring it to the long format and remove NA values.

```
df <- read.table(header = TRUE, text = 'Control  T0.1 T0.3 T1 T3 T10
175 29 27 36 26 20
65 114 78 11 13 37
154 72 27 105 33 NA
83 NA NA NA NA NA
')
dfm <- melt(df, value.name = 'n', variable.name = 'treatment')
dfm <- dfm[!is.na(dfm['n']), ]
head(dfm)

##   treatment    n
## 1  Control  175
## 2  Control   65
## 3  Control  154
## 4  Control   83
## 5    T0.1   29
## 6    T0.1  114
```

Next we have a look at the data:

```
boxplot(n ~ treatment, data = dfm, xlab = 'Treatment', ylab = 'Count')
```

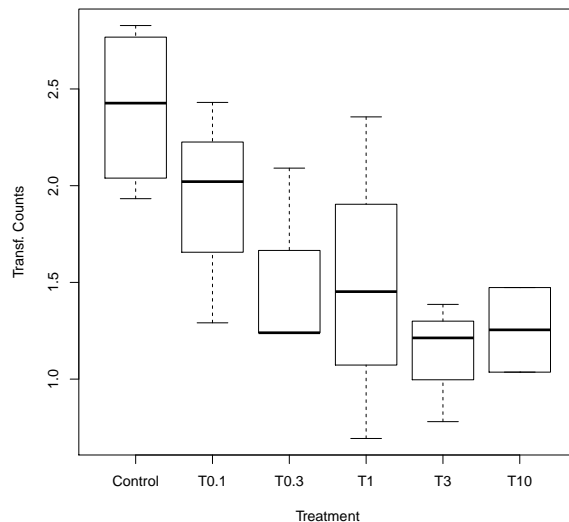


1.2.2 Transforming data

Next we transform the data using a $\ln(Ax + 1)$ transformation:

```
A <- 1 / min(dfm$n[dfm$n != 0])
dfm$nt <- log(A * dfm$n + 1)
```

```
boxplot(nt ~ treatment, data = dfm,
        xlab = 'Treatment', ylab = 'Transf. Counts')
```



1.3 Analysing data

Again we start with the model assuming a normal distribution of transformed counts:

```
mod_normal <- mle2(nt ~ dnorm(mean = mu, sd = s),
                  parameters = list(mu ~ treatment, s ~ 1),
                  data = dfm,
                  start = list(mu = mean(dfm$nt), s = sd(dfm$nt)))
summary(mod_normal)
```

```
## Maximum likelihood estimation
##
## Call:
## mle2(minuslogl = nt ~ dnorm(mean = mu, sd = s), start = list(mu = mean(dfm$nt),
##      s = sd(dfm$nt)), data = dfm, parameters = list(mu ~ treatment,
##      s ~ 1))
##
## Coefficients:
##              Estimate Std. Error z value  Pr(z)
## mu.(Intercept)    2.4035     0.2169  11.08 < 2e-16 ***
## mu.treatmentT0.1 -0.4894     0.3313   -1.48  0.13956
## mu.treatmentT0.3 -0.8801     0.3313   -2.66  0.00789 **
## mu.treatmentT1    -0.9032     0.3313   -2.73  0.00640 **
## mu.treatmentT3    -1.2771     0.3313   -3.86  0.00012 ***
## mu.treatmentT10   -1.1488     0.3756   -3.06  0.00222 **
## s                  0.4337     0.0723    6.00  2e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## -2 log L: 21.01
```



```

mod_normal.null <- update(mod_normal,
                          parameters = list(mu ~ 1, s ~ 1))
anova(mod_normal, mod_normal.null)

## Likelihood Ratio Tests
## Model 1: mod_normal, nt~dnorm(mean=mu,sd=s): mu~treatment, s~1
## Model 2: mod_normal.null, nt~dnorm(mean=mu,sd=s): mu~1, s~1
##   Tot Df Deviance Chisq Df Pr(>Chisq)
## 1      7      21.0
## 2      2      34.3 13.3 5      0.021 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

With base R you could do:

```

mod_normal_b <- lm(nt ~ treatment, data = dfm)
summary(mod_normal_b)
drop1(mod_normal_b, test = 'Chisq')

```

Next we analyse the raw counts assuming a poisson distribution with a log link:

```

mod_pois <- mle2(n ~ dpois(lambda = exp(logmu)),
                 parameters = list(logmu ~ treatment),
                 data = dfm,
                 start = list(logmu = mean(dfm$nt)))
summary(mod_pois)

## Maximum likelihood estimation
##
## Call:
## mle2(minuslogl = n ~ dpois(lambda = exp(logmu)), start = list(logmu = mean(dfm$nt)),
##      data = dfm, parameters = list(logmu ~ treatment))
##
## Coefficients:
##              Estimate Std. Error z value Pr(z)
## logmu.(Intercept)    4.7812     0.0458  104.42 < 2e-16 ***
## logmu.treatmentT0.1 -0.5093     0.0821   -6.20 5.7e-10 ***
## logmu.treatmentT0.3 -0.9970     0.0983  -10.14 < 2e-16 ***
## logmu.treatmentT1    -0.8560     0.0931   -9.19 < 2e-16 ***
## logmu.treatmentT3    -1.6032     0.1264  -12.68 < 2e-16 ***
## logmu.treatmentT10   -1.4313     0.1401  -10.21 < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## -2 log L: 375.6

```

Or with base R

```

mod_pois <- glm(n ~ treatment, data = dfm, family = poisson(link = 'log'))
summary(mod_pois)

```

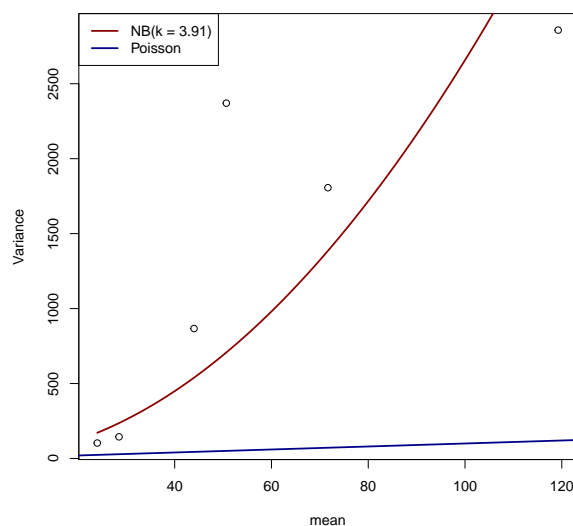
But is a poisson distribution appropriate here? A property of the poisson distribution is that its variance is equal to the mean. A simple diagnostic would be to plot group variances vs group means, other more formal statistics are also available.

```
require(plyr)
musd <- ddply(dfm, .(treatment), summarise,
              mu = mean(n),
              var = var(n))

musd

##   treatment      mu    var
## 1   Control 119.25 2857.6
## 2     T0.1   71.67 1806.3
## 3     T0.3   44.00  867.0
## 4        T1   50.67 2370.3
## 5        T3   24.00  103.0
## 6       T10   28.50  144.5

plot(var ~ mu, data = musd, xlab = 'mean', ylab = 'Variance')
abline(a = 0, b = 1, col = 'darkblue', lwd = 2)
curve(x + (x^2 / 3.91), from = 24, to = 119.25, add = TRUE, col = 'darkred', lwd = 2)
legend('topleft', c('NB(k = 3.91)', 'Poisson'),
      col = c('darkred', 'darkblue'),
      lty = c(1,1),
      lwd = c(2,2))
```



We clearly see that the variance increases much more than would be expected under the poisson distribution (the data is overdispersed). One possibility to deal with overdispersion is using a negative binomial distribution:

```
mod_negbin <- mle2(n ~ dnbinom(mu = exp(logmu), size = k),
                  parameters = list(logmu ~ treatment, k ~ 1),
                  data = dfm,
                  start = list(logmu = log(mean(dfm$n)), k = 1))
```

```
summary(mod_negbin)

## Maximum likelihood estimation
##
## Call:
## mle2(minuslogl = n ~ dnbinom(mu = exp(logmu), size = k), start = list(logmu = log(mean
##     k = 1), data = dfm, parameters = list(logmu ~ treatment,
##     k ~ 1))
##
## Coefficients:
##              Estimate Std. Error z value Pr(z)
## logmu.(Intercept)    4.781      0.257  18.60 < 2e-16 ***
## logmu.treatmentT0.1 -0.509      0.395  -1.29  0.1974
## logmu.treatmentT0.3 -0.997      0.399  -2.50  0.0124 *
## logmu.treatmentT1    -0.856      0.398  -2.15  0.0313 *
## logmu.treatmentT3    -1.603      0.407  -3.94 8.1e-05 ***
## logmu.treatmentT10   -1.431      0.460  -3.11  0.0019 **
## k                    3.906      1.366   2.86  0.0042 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## -2 log L: 167.2

mod_negbin.null <- update(mod_negbin,
                          parameters = list(logmu ~ 1))
anova(mod_negbin, mod_negbin.null)

## Likelihood Ratio Tests
## Model 1: mod_negbin, n~dnbinom(mu=exp(logmu),size=k): logmu~treatment, k~1
## Model 2: mod_negbin.null, n~dnbinom(mu=exp(logmu),size=k): logmu~1
##   Tot Df Deviance Chisq Df Pr(>Chisq)
## 1      7      167
## 2      2      181    14 5      0.016 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Or using the MASS package (Venables and Ripley, 2002):

```
require(MASS)
mod_negbin_m <- glm.nb(n ~ treatment, data = dfm)
summary(mod_negbin_m)

##
## Call:
## glm.nb(formula = n ~ treatment, data = dfm, init.theta = 3.905898474,
##     link = log)
##
## Deviance Residuals:
##      Min       1Q   Median       3Q      Max
## -2.255  -0.849  -0.302   0.595   1.590
##
```

```
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept)    4.781      0.257   18.60 < 2e-16 ***
## treatmentT0.1 -0.509      0.395   -1.29  0.1975
## treatmentT0.3 -0.997      0.399   -2.50  0.0124 *
## treatmentT1    -0.856      0.398   -2.15  0.0313 *
## treatmentT3    -1.603      0.407   -3.94  8.1e-05 ***
## treatmentT10   -1.431      0.460   -3.11  0.0019 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Negative Binomial(3.906) family taken to be 1)
##
##      Null deviance: 39.057  on 17  degrees of freedom
## Residual deviance: 18.611  on 12  degrees of freedom
## AIC: 181.2
##
## Number of Fisher Scoring iterations: 1
##
##              Theta:  3.91
##             Std. Err.:  1.37
##
## 2 x log-likelihood: -167.24

lrtest(mod_negbin_m)

## Likelihood ratio test
##
## Model 1: n ~ treatment
## Model 2: n ~ 1
##   #Df LogLik Df Chisq Pr(>Chisq)
## 1    7  -83.6
## 2    2  -90.6 -5    14    0.016 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Note, that we cannot use `drop1()` with `glm.nb()`!

References

- Brock, T. C. M., Hammers-Wirtz, M., Hommen, U., Preuss, T. G., Ratte, H.-T., Roessink, I., Strauss, T., and Van den Brink, P. J. (2014). The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environmental Science and Pollution Research*.
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- Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S*. Springer, New York, fourth edition. ISBN 0-387-95457-0.
- Zeileis, A. and Hothorn, T. (2002). Diagnostic checking in regression relationships. *R News*, 2(3):7–10.