Ecotoxicology is not normal.

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

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2 Supplement 2 - R examples

2.1 Count data example

2.1.1 Introduction

In this example we will analyse data from (Brock et al., 2015). 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.

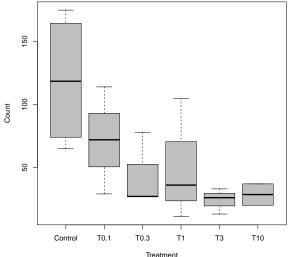
First we load the data and bring it to the long format and remove NA values.

```
df <- read.table(header = TRUE, text = 'Control T0.1 T0.3 T1 T3 T10</pre>
175 29 27 36 26 20
65 114 78 11 13 37
154 72 27 105 33 NA
83 NA NA NA NA
1)
require(reshape2)
dfm <- melt(df, value.name = 'abu', variable.name = 'treatment')</pre>
dfm <- dfm[!is.na(dfm['abu']), ]</pre>
head(dfm)
##
    treatment abu
## 1
      Control 175
## 2
      Control 65
## 3
     Control 154
## 4
      Control 83
## 5
         T0.1 29
         TO.1 114
```

This give a table with two columns - one indicating the treatment and one with the measured abundances.

Let's have a first look at the data:

Raw abundances



We clearly see a treatment related response. Moreover, we may note that variances are increasing with increasing abundances.

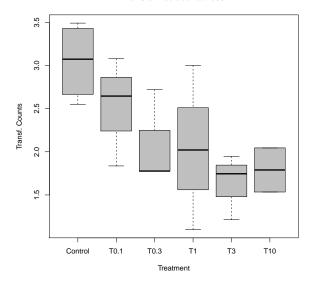
2.1.2 Transforming data

Next we transform the data using a $\ln(Ax+1)$ transformation. A is chosen so that the term Ax equals two for the lowest non-zero abundance. We add these transformed abundances as column to our table.

```
A <- 2 / min(dfm$abu[dfm$abu != 0])
A
## [1] 0.1818182
dfm$abu_t <- log(A * dfm$abu + 1)
```

It looks like the transformation does a good job in equalizing the variances:

Transformed abundances



2.1.3 Assuming a normal distribution of transformed abundances

We start with analysing this data assuming a normal distribution of the transformed abundances with constant variance. This can be easily done using the lm() function:

```
modlm <- lm(abu_t ~ treatment, data = dfm)</pre>
```

The summary() gives the estimated parameters with standard errors and Wald t tests:

```
summary(modlm)
##
## Call:
## lm(formula = abu_t ~ treatment, data = dfm)
##
##
  Residuals:
##
                       Median
        Min
                  1Q
                                     3Q
                                             Max
  -0.94133 -0.31454
                     0.04576 0.31813
                                        0.96033
##
##
##
  Coefficients:
##
                 Estimate Std. Error t value Pr(>|t|)
                                      10.260 2.71e-07 ***
##
  (Intercept)
                   3.0468
                              0.2970
## treatmentT0.1
                 -0.5267
                              0.4536
                                       -1.161
                                               0.26814
## treatmentT0.3
                  -0.9558
                              0.4536
                                       -2.107
                                               0.05682 .
## treatmentT1
                  -1.0069
                              0.4536
                                       -2.220
                                               0.04646 *
## treatmentT3
                  -1.4121
                              0.4536
                                       -3.113
                                               0.00897 **
## treatmentT10
                  -1.2575
                              0.5144
                                       -2.445
                                               0.03089 *
## ---
                   0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## Signif. codes:
##
## Residual standard error: 0.5939 on 12 degrees of freedom
## Multiple R-squared: 0.5167, Adjusted R-squared: 0.3154
## F-statistic: 2.566 on 5 and 12 DF, p-value: 0.08406
```

Or, if you want to have the ANOVA table with an F-test:

```
## Df Sum Sq Mean Sq F value Pr(>F)
## treatment 5 4.526 0.9052 2.566 0.0841 .
## Residuals 12 4.233 0.3528
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

From this output we might infer that we cannot detect any treatment effect (F = 2.566, p = 0.084). Let's move on to the LOEC determination. This can be easily done using the multcomp package (Hothorn et al., 2008): Here we perform a one-sided (alternative='less') Dunnett (mcp(treatment='Dunnett')) test.

```
require(multcomp)
# one-sided Dunnett test
summary(glht(modlm, linfct = mcp(treatment = 'Dunnett'), alternative = 'less'))
##
##
    Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: lm(formula = abu_t ~ treatment, data = dfm)
## Linear Hypotheses:
                      Estimate Std. Error t value Pr(<t)
##
## T0.1 - Control >= 0 -0.5267
                                   0.4536 -1.161 0.3841
## T0.3 - Control >= 0 -0.9558
                                   0.4536 -2.107 0.1030
## T1 - Control >= 0
                       -1.0069
                                   0.4536
                                           -2.220 0.0859 .
## T3 - Control >= 0
                       -1.4121
                                   0.4536
                                          -3.113 0.0182 *
## T10 - Control >= 0
                       -1.2575
                                   0.5144
                                          -2.445 0.0588 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

This indicates that only treatment 3 shows a statistically significant from control and is the determined LOEC.

2.1.4 Assuming a Poisson distribution of abundances

Instead transforming the data, we could assume a Poisson distribution and fit a GLM to this data. This can be done using the glm() function:

```
modpois <- glm(abu ~ treatment, data = dfm, family = poisson(link = 'log'))</pre>
```

, family = poisson(link = 'log') specifies that we want to fit a poisson model using a log link between response and predictors.

The summary gives the estimated parameters, standard errors and Wald Z tests:

```
summary(modpois)
##
## Call:
## glm(formula = abu ~ treatment, family = poisson(link = "log"),
##
     data = dfm)
##
## Deviance Residuals:
                  Median 3Q
     Min 1Q
                                      Max
## -6.7625 -2.7621 -0.8219 2.7172
                                   6.6602
##
## Coefficients:
##
              Estimate Std. Error z value Pr(>|z|)
## (Intercept)
              4.78122 0.04579 104.423 < 2e-16 ***
## treatmentT1 -0.85595 0.09314 -9.190 < 2e-16 ***
## treatmentT3 -1.60317 0.12643 -12.680 < 2e-16 ***
## treatmentT10 -1.43132 0.14014 -10.213 < 2e-16 ***
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for poisson family taken to be 1)
##
##
      Null deviance: 604.79 on 17 degrees of freedom
## Residual deviance: 273.77 on 12 degrees of freedom
## AIC: 387.63
##
## Number of Fisher Scoring iterations: 5
```

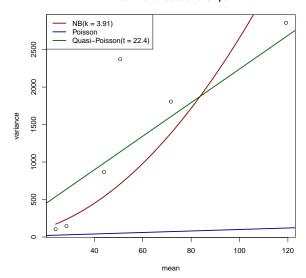
To perform a LR-Test we can used drop1():

which indicates a strong treatment effect.

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:

```
require(plyr)
# mean and variance per treatment
musd <- ddply(dfm, .(treatment), summarise,</pre>
              mu = mean(abu),
              var = var(abu))
musd
##
     treatment
                      mu
                               var
## 1
       Control 119.25000 2857.583
## 2
          T0.1
                71.66667 1806.333
          T0.3
                44.00000
                          867.000
## 3
## 4
            T1
                50.66667 2370.333
## 5
            Т3
                24.00000
                          103.000
## 6
           T10
                28.50000
                          144.500
# plot
plot(var ~ mu, data = musd, xlab = 'mean', ylab = 'variance', main = 'Mean-variance relat
# poisson
abline(a = 0, b = 1, col = 'darkblue', lwd = 2)
# quasi-Poisson
abline(a = 0, b = 22.41, col = 'darkgreen', lwd = 2)
# Negative binomial
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', 'Quasi-Poisson(t = 22.4)'),
       col = c('darkred', 'darkblue', 'darkgreen'),
       lty = c(1,1, 1),
       lwd = c(2,2, 2))
```

Mean-variance relationships



I also added the assumed mean-variance relationships of the Poisson, quasi-Poisson and negative binomial models. We clearly see that the variance increases much more than would be expected under the poisson distribution (the data is overdispersed). Moreover, we could check overdispersion from the summary: If the ratio of residual deviance to degrees of freedom is >1 the data is overdispersed.

2.1.5 Assuming a quasi-Poisson distribution of abundances

The plot suggests that the variance may increasing stronger then the mean and quasi-Poisson or negative binomial models might be more appropriate for this data. Fitting a quasi-Poisson GLM is straight forward:

```
modqpois <- glm(abu ~ treatment, data = dfm, family = quasipoisson)</pre>
```

The summary gives the estimated parameters:

```
summary(modqpois)
##
## Call:
## glm(formula = abu ~ treatment, family = quasipoisson, data = dfm)
## Deviance Residuals:
##
      Min
            1Q
                    Median
                                  3Q
                                           Max
## -6.7625 -2.7621 -0.8219 2.7172
                                        6.6602
##
## Coefficients:
##
                Estimate Std. Error t value Pr(>|t|)
## (Intercept) 4.7812 0.2168 22.058 4.43e-11 ***
## treatmentT0.1 -0.5092
                                             0.2149
                             0.3889 -1.309
## treatmentT0.3 -0.9970 0.4656 -2.142 0.0534 .
## treatmentT1 -0.8560 0.4409 -1.941 0.0761 .
## treatmentT3 -1.6032 0.5985 -2.679 0.0201 *
## treatmentT10 -1.4313
                            0.6634 -2.157 0.0519 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for quasipoisson family taken to be 22.41055)
##
      Null deviance: 604.79 on 17 degrees of freedom
## Residual deviance: 273.77 on 12 degrees of freedom
## AIC: NA
##
## Number of Fisher Scoring iterations: 5
```

with the dispersion parameter $\Theta=22.41055$. Note, that the parameter estimates are the same as from the Poisson model, only the standard errors are scaled/wider.

An F-test can be performed using drop1():

```
drop1(modqpois, test = 'F')

## Single term deletions
##

## Model:
## abu ~ treatment
## Df Deviance F value Pr(>F)
## <none> 273.77
```

```
## treatment 5 604.79 2.9019 0.06059 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

, which also indicates no treatment effect.

The LOEC can be determined with multcomp:

```
summary(glht(modqpois, linfct = mcp(treatment = 'Dunnett'), alternative = 'less'))
##
##
    Simultaneous Tests for General Linear Hypotheses
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: glm(formula = abu ~ treatment, family = quasipoisson, data = dfm)
##
## Linear Hypotheses:
##
                    Estimate Std. Error z value Pr(<z)
## T10 - Control >= 0 -1.4313
                              0.6634 -2.157 0.0710 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

2.1.6 Assuming a negative binomial distribution of abundances

To fit a negative binomial GLM we could use glm.nb() from the MASS package (Venables and Ripley, 2002):

```
require(MASS)
modnb <- glm.nb(abu ~ treatment, data = dfm)</pre>
```

The estimated parameters:

```
##
## Call:
## glm.nb(formula = abu ~ treatment, data = dfm, init.theta = 3.905898474,
## link = log)
##
## Deviance Residuals:
## Min 1Q Median 3Q Max
## -2.2554 -0.8488 -0.3020 0.5954 1.5899
##
## Coefficients:
```

```
Estimate Std. Error z value Pr(>|z|)
##
                 4.7812 0.2571 18.596 < 2e-16 ***
## (Intercept)
## treatmentT0.1 -0.5092
                           0.3951 -1.289 0.19746
## treatmentT0.3 -0.9970
                            0.3988 -2.500 0.01241 *
## treatmentT1
                -0.8560
                            0.3975 -2.153 0.03130 *
## treatmentT3
                -1.6032
                            0.4066 -3.943 8.05e-05 ***
## treatmentT10
                 -1.4313
                            0.4601 -3.111 0.00186 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for Negative Binomial(3.9059) family taken to be 1)
      Null deviance: 39.057 on 17 degrees of freedom
##
## Residual deviance: 18.611 on 12 degrees of freedom
## AIC: 181.24
##
## Number of Fisher Scoring iterations: 1
##
##
                Theta: 3.91
##
##
            Std. Err.: 1.37
##
   2 x log-likelihood: -167.238
##
```

, with $\kappa=1/3.905898$ (glm.nb uses a slightly other parametrisation). For an LR-Test we need to first fit a reduced model:

```
modnb.null <- glm.nb(abu ~ 1, data = dfm)</pre>
```

, so that the dispersion parameter κ is re-estimated for the reduced model. Then we can compare these two models with a LR-Test:

```
anova(modnb, modnb.null, test = 'Chisq')
## Likelihood ratio tests of Negative Binomial Models
##
## Response: abu
                theta Resid. df
                                    2 x log-lik.
##
        Model
                                                   Test df LR stat.
## 1
            1 1.861577
                             17
                                       -181.2281
## 2 treatment 3.905898
                             12
                                       -167.2383 1 vs 2
                                                           5 13.98985
     Pr(Chi)
##
## 1
## 2 0.015674
```

, which suggests a treatment related effect on abundances. Similar for LOEC:

```
summary(glht(modnb, linfct = mcp(treatment = 'Dunnett'), alternative = 'less'))
##
## Simultaneous Tests for General Linear Hypotheses
```

```
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: glm.nb(formula = abu ~ treatment, data = dfm, init.theta = 3.905898474,
##
      link = log)
##
## Linear Hypotheses:
                      Estimate Std. Error z value Pr(<z)
## T0.1 - Control >= 0 -0.5092 0.3951 -1.289 0.31520
## T0.3 - Control >= 0 -0.9970
                                  0.3988 -2.500 0.02743 *
## T1 - Control >= 0
                       -0.8560
                                   0.3975 -2.153 0.06496 .
## T3 - Control >= 0
                       -1.6032
                                   0.4066 -3.943 < 0.001 ***
## T10 - Control >= 0
                       -1.4313
                                   0.4601 -3.111 0.00454 **
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

which suggests a LOEC at the 0.3 treatment.

2.2 Binomial data example

2.2.1 Introduction

Here we will show how to analyse binomial data $(x \ out \ of \ n)$. Data is provided in Newman (2012) (example 5.1, page 223) and EPA (2002). Ten fathead minnow (*Pimephales promelas*) larvals 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:

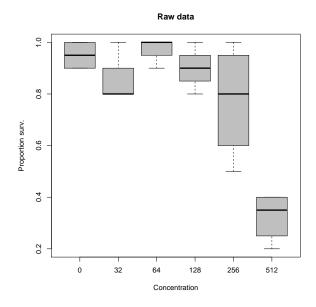
```
df <- read.table(header = TRUE, text = 'conc A B C D</pre>
0 1 1 0.9 0.9
32 0.8 0.8 1 0.8
64 0.9 1 1 1
128 0.9 0.9 0.8 1
256 0.7 0.9 1 0.5
512 0.4 0.3 0.4 0.2')
df
##
     conc
            Α
                В
                    C
       0 1.0 1.0 0.9 0.9
## 1
## 2
       32 0.8 0.8 1.0 0.8
      64 0.9 1.0 1.0 1.0
     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
```

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

```
require(reshape2)
# wide to long
```

```
dfm <- melt(df, id.vars = 'conc', value.name = 'y', variable.name = 'tank')</pre>
# conc as factor
dfm$conc <- factor(dfm$conc)</pre>
head(dfm)
##
     conc tank
                  У
## 1
        0
              A 1.0
## 2
       32
              A 0.8
## 3
       64
              A 0.9
## 4
     128
              A 0.9
## 5
              A 0.7
      256
## 6 512
              A 0.4
```

Let's have a first look at the data:



This plot indicates a strong effect at the highest concentration.

2.2.2 Transforming data

Next we arcsine transform the proportions:

2.2.3 Assuming a normal distribution of transformed proportions

Like in the count data example we fit the model using lm():

```
modlm <- lm(y_asin ~ conc, data = dfm)</pre>
```

The summary gives the estimated parameters:

```
summary(modlm)
##
## Call:
## lm(formula = y_asin ~ conc, data = dfm)
##
## Residuals:
      Min
                 1Q
                      Median
                                   3Q
## -0.32401 -0.08149 -0.00527 0.08150 0.30261
##
## Coefficients:
##
             Estimate Std. Error t value Pr(>|t|)
                         0.07693 17.295 1.16e-12 ***
## (Intercept) 1.33053
## conc32
             -0.14717
                          0.10880 -1.353
                                          0.1929
              0.04074
                          0.10880
                                   0.374
                                            0.7124
## conc64
                          0.10880 -0.701
              -0.07622
                                            0.4925
## conc128
              -0.22113
                          0.10880 -2.032
## conc256
                                            0.0571 .
## conc512
              -0.72735
                       0.10880 -6.685 2.86e-06 ***
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Residual standard error: 0.1539 on 18 degrees of freedom
## Multiple R-squared: 0.7871,Adjusted R-squared: 0.7279
## F-statistic: 13.31 on 5 and 18 DF, p-value: 1.612e-05
```

The F-test suggests a treatment related effect:

```
drop1(modlm, test = 'F')

## Single term deletions

##

## Model:

## y_asin ~ conc

## Df Sum of Sq RSS AIC F value Pr(>F)

## <none> 0.42613 -84.746

## conc 5 1.5753 2.00142 -57.621 13.308 1.612e-05 ***

## ---

## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

And the LOEC is at the highest concentration:

```
summary(glht(modlm, linfct = mcp(conc = 'Dunnett'), alternative = 'less'))
##
##
    Simultaneous Tests for General Linear Hypotheses
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: lm(formula = y_asin ~ conc, data = dfm)
##
## Linear Hypotheses:
            Estimate Std. Error t value Pr(<t)
## 64 - 0 >= 0 0.04074 0.10880 0.374 0.9202
0.10880 -2.032 0.0986 .
## 256 - 0 >= 0 -0.22113
## 512 - 0 >= 0 -0.72735
                       0.10880 -6.685 <0.001 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

2.2.4 Assuming a binomial distribution

The binomial model with a logit link between predictors and response can be fitted using the glm() function:

Here the weights arguments, indicates how many fish where exposed in each treatment (=10). The summary gives the estimated parameters:

```
summary(modglm)
##
## Call:
## glm(formula = y ~ conc, family = binomial(link = "logit"), data = dfm,
      weights = rep(10, nrow(dfm)))
##
## Deviance Residuals:
     Min 1Q Median
                               3Q
                                        Max
## -1.8980 -0.5723 0.0000 0.7869
                                     2.2578
##
## Coefficients:
             Estimate Std. Error z value Pr(>|z|)
##
## (Intercept) 2.9444 0.7255 4.059 4.94e-05 ***
             -1.2098
                         0.8499 -1.423 0.1546
## conc32
              0.7191
## conc64
                        1.2458 0.577 0.5638
## conc128
              -0.7472
                        0.8967 -0.833 0.4047
              -1.7077 0.8183 -2.087 0.0369 *
## conc256
              -3.6753
                        0.8002 -4.593 4.37e-06 ***
## conc512
## ---
## Signif. codes: 0 '***' 0.001 '**' 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: 72.862
##
## Number of Fisher Scoring iterations: 5
```

To perform a LR-test we can used the drop1() function:

```
drop1(modglm, test = 'Chisq')

## Single term deletions

##

## Model:

## y ~ conc

## Df Deviance AIC LRT Pr(>Chi)

## <none> 23.889 72.862

## conc 5 88.672 127.645 64.783 1.243e-12 ***

## ---

## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

Also with the binomial model the LOEC is at the highest concentration:

```
summary(glht(modglm, linfct = mcp(conc = 'Dunnett'), alternative = 'less'))
##
## Simultaneous Tests for General Linear Hypotheses
##
```

```
## Multiple Comparisons of Means: Dunnett Contrasts
##
##
## Fit: glm(formula = y ~ conc, family = binomial(link = "logit"), data = dfm,
       weights = rep(10, nrow(dfm)))
##
##
## Linear Hypotheses:
##
               Estimate Std. Error z value Pr(<z)
               -1.2098
## 32 - 0 >= 0
                           0.8499 -1.423 0.203
## 64 - 0 >= 0
                 0.7191
                            1.2458
                                     0.577 0.923
## 128 - 0 >= 0 -0.7472
                            0.8967
                                    -0.833 0.432
## 256 - 0 >= 0 -1.7077
                                    -2.087 0.059 .
                            0.8183
## 512 - 0 >= 0 -3.6753
                            0.8002
                                    -4.593 <0.001 ***
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

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. (2015). The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environmental Science and Pollution Research*, 22(2):1160–1174.

EPA (2002). Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. U.S. Environmental Protection Agency.

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal*, 50(3):346–363.

Newman, M. C. (2012). Quantitative ecotoxicology. Taylor & Francis, Boca Raton, FL.

Venables, W. N. and Ripley, B. D. (2002). *Modern Applied Statistics with S.* Springer, New York, fourth edition.