

# Kurs Bio144:

# Datenanalyse in der Biologie

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Lecture 9: Modelling count data

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# Overview (todo: check)

- Non-continuous outcomes: counts
- Generalized linear models
- Poisson regression
- Link function
- Residual analysis / model checking / deviances
- Interpretation of the results
- (Overdispersion, zero-inflation)

## Course material covered today

- Chapter 7 of GSWR (Beckerman et al.)

# Introduction

- We have seen: Covariates in regression models can be **continuous**, **categorical** (binary or multi-level) or **counted numbers** (integers).
- However, the **response variable** ( $y$ ) was so far **always continuous**, and the assumption was that the residuals  $e_i \sim N(0, \sigma_e^2)$ .
- **In reality**, the response variable can also be a count (an integer  $\geq 0$ ) or a categorical variable (e.g., presence/absence data).
- Here, we will look at the case where the response variable is a **count**, that is,  $y_i = 0, 1, 2, \dots$ .

# Count data

In biological or medical data, the outcome of interest is quite often a count:

- Counting items in time or space (animals, plants, species)
- Parasites in animals or humans
- Number of offspring in animals or humans
- Number of adverse health events (e.g., exacerbations) or health-related numbers (e.g., polyps)

The research question then is:

**How do certain covariates influence the count outcome?**

## Illustrative/working example: Soay sheep

Hirta, a small island of Scotland, is inhabited by an unmanaged and feral population of Soay sheep.

Ecologists were interested in the **question** whether body mass of female animals influences their fitness, measured as their **lifetime reproductive success** (the number of offspring over their lifespan).

“Are heavier females fitter than lighter females?”

```
> soay <- read.table("../data_examples/GSWR_datasets/SoaySheepFitness.csv",header=T, sep=",")  
> glimpse(soay)
```

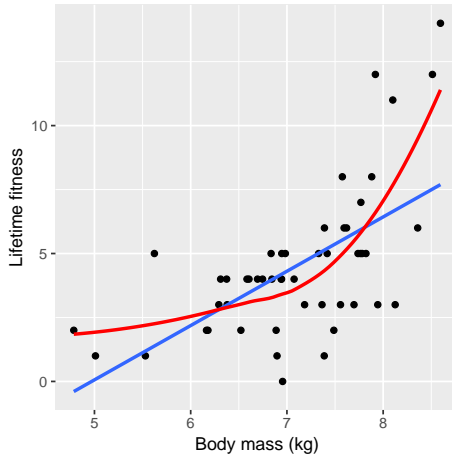
```
Observations: 50
```

```
Variables: 2
```

```
$ fitness <int> 4, 3, 2, 14, 5, 2, 2, 5, 8, 4, 12, 6, 3, 2, 3, 0, 5, 3, 5...
```

```
$ body.size <dbl> 6.373546, 7.183643, 6.164371, 8.595281, 7.329508, 6.17953...
```

As always, start with a graph (see GSWR book for code to produce the figure):



## What can we see from the plot?

- Reproductive success seems to increase with female body weight (not surprising, right?).
- The linear line seems unreasonable, the red seems better. Maybe a quadratic term would be useful?
- However, the problem with these data is more subtle.

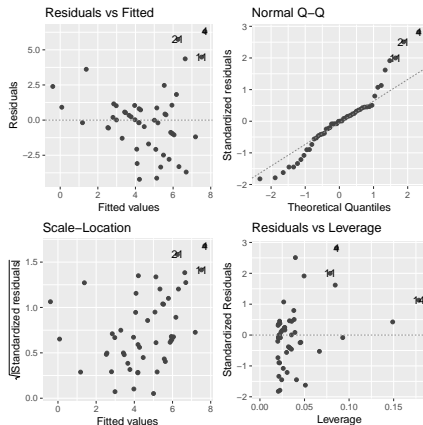
## How does one analyze these data correctly?

- So far, we always used linear regression.
- This is **not** the correct approach here.
- We nevertheless start doing the 'wrong' analysis!



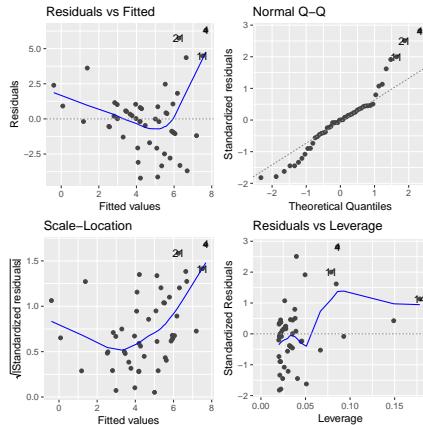
## The 'wrong' analysis

Use the `lm()` function to fit the linear model  $y = \beta_0 + \beta_1 \cdot \text{bodySize} + e$  and look at the diagnostic plots:



→ The diagnostic plots show that the linear regression assumptions are badly violated!

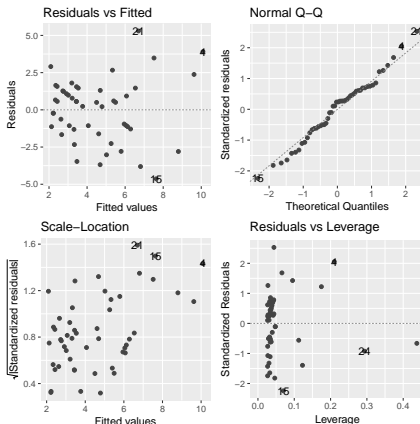
The same plot with smoothed lines (makes the problems even more obvious):



What about the model with a quadratic term?

$$y = \beta_0 + \beta_1 \cdot \text{bodySize} + \beta_2 \cdot \text{bodySize}^2 + e$$

```
> r2.soay <- lm(fitness ~ body.size + I(body.size^2), data=soay)
> autoplot(r2.soay, smooth.colour = NA)
```



→ This looks better. However...

# What is the problem?

Obviously, the **normal distribution** for count data is **violated**. Why?

- The normal distribution is for continuous variables.
- The normal distribution allows values  $< 0$ , count data doesn't.
- The normal distribution is symmetrical, counts are often not!

# A model for count data? I

Do you remember a distribution for count values from Mat183?

The probability distribution of Poisson-distributed random variable  $Y$  with parameter  $\lambda$  is defined as

$$\Pr(Y = k) = \frac{\lambda^k}{k!} e^{-\lambda}, \quad k = 0, 1, 2, \dots$$

In short:

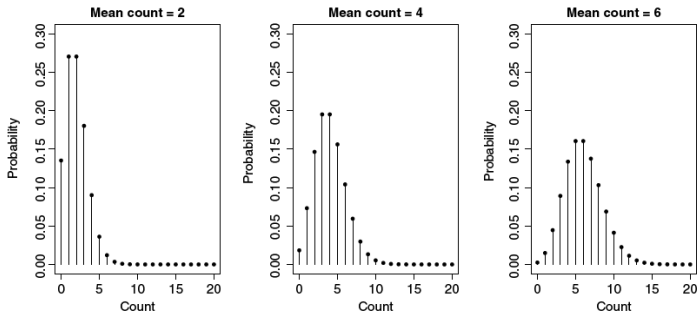
$$Y \sim \text{Po}(\lambda).$$

## Characteristics of the Poisson distribution:

- Suitable to model unbounded counts ( $k = 0, 1, 2, \dots$ ).
- $E(Y) = \text{Var}(Y) = \lambda$ . In words: Mean = variance =  $\lambda$ .
  - The variance of the distribution increases with the mean.

# A model for count data? II

Some examples:



So: How can one use the Poisson distribution for regression modelling?

## Doing it right: The generalized linear model (GLM) for count data

The aim of the GLM approach is that we can still use a **linear predictor**  $\eta_i$  in the form of the linear model:

$$\eta_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \dots + \beta_p x_i^{(p)} .$$

- In **linear regression**,  $\eta_i$  is the **predicted value** for the mean  $E(y_i) = \eta_i$  (Why? Because the residual term  $+e_i$  is missing!).
- However, for counts we cannot simply set  $\eta_i$  equal to  $E(y_i)$ , if  $y_i$  is a **count**!

Why can't we set  $E(y_i) = \eta_i$  for count data?

**Because nothing prevents  $\eta_i$  from being negative!**

Let us try to use the same approach as in linear regression, assuming that  $E(y_i) = \eta_i$  for our soay sheep counts, that is,

$$E(y_i) = \beta_0 + \beta_1 \cdot \text{bodySize}_i ,$$

using a model with  $\beta_0 = -2$  and  $\beta_1 = 1.2$ .

What is the predicted number of offspring for a 1kg sheep? Plug-in:  
 $-2 + 1.2 \cdot 1 = -0.8$ , thus a negative prediction!

This is very unreasonable, right?

A trick is thus needed!



## The trick: Use a link function

Instead of using  $E(y_i) = \eta_i$  as in linear regression, we simply log-transform the expected value.

In order to prevent the expected value  $E(y_i)$  to be negative for count data  $y_i$ , we use

$$\log(E(y_i)) = \eta_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \dots + \beta_p x_i^{(p)} . \quad (1)$$

- The log is called the **link function**.
- The advantage: The predicted fitness  $E(y_i)$  is now **always positive**, because equation (1) is identical to

$$E(y_i) = \exp(\beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \dots + \beta_p x_i^{(p)}) ,$$

which is now **always**  $> 0$ ! (I really hope you remember what the  $\exp()$  function looks like...)

# The probability model

- Finally, we need a reasonable **probability model for the response variable**.
- Remember: we always used the normality assumption  $y_i \sim N(\eta_i, \sigma_e^2)$  in linear regression.
- Given that  $y_i$  are counts, a Poisson model seems reasonable:

$$y_i \sim \text{Po}(E(y_i)) .$$

In words:  $y_i$  is a realization of a Poisson random variable distributed as  $\text{Po}(\lambda_i)$ , where  $\lambda_i = E(y_i)$ .

- We say: The model belongs to the Poisson **family**.

## Key terms for GLMs

In summary, we have introduced three terms related to GLMs:

- **Family:** The family corresponds to the likelihood model that is used for the (transformed) response. Here, the family is Poisson. Another very common distribution is the **binomial** family for **binary** outcome (see next week). Other families are the gamma or the negative binomial. The family is determined by the data type!
- **Linear predictor:** The linear predictor is always given as

$$\eta_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \dots + \beta_p x_i^{(p)} .$$

In the soay shee example, it is  $\eta_i = \beta_0 + \beta_1 \cdot \text{bodySize}_i$  for sheep  $i$ .

- **Link function:** It defines how the linear predictor  $\eta_i$  is **related** to  $E(y_i)$ . In Poisson regression this is typically the log:  $\log(E(y_i)) = \eta_i$ ! We will see another important link function next week (for binary data).

## Doing it right: Fitting a Poisson GLM

Uff... that was hard! But we have all the tools for fitting a Poisson GLM now. A statistician would now say:

“Let's fit a Poisson GLM with a log-link!”

- Basically, the idea is again to perform **maximum-likelihood estimation**, but the details are a bit more complicated (nothing for us).
- Luckily, there is an R-function that works (almost) like `lm()`, namely `glm()`:

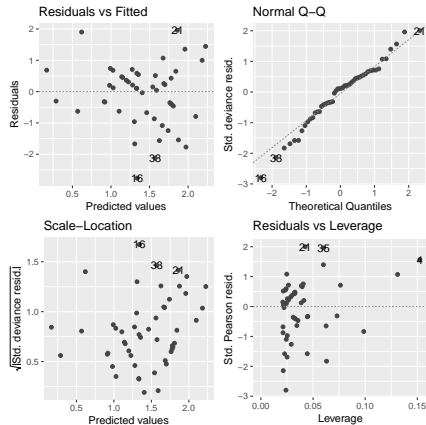
```
> soay.glm <- glm(fitness ~ body.size, data = soay, family = poisson(link=log))
```

You **must** specify the **family**, but you could leave away the `link=log` option here, because R automatically picks the log link for the Poisson family...

# Doing it right: Model diagnostics

Before we look at the output, let's do some model diagnostics (as we did it for linear regression):

```
> autoplot(soay.glm, smooth.colour = NA)
```



- Model diagnostics seem ok (similar to when we used a quadratic term in the linear model, see slide 11).
- Do you find it strange that the same diagnostic plot can be used as in linear regression?
- The definition of a “residuum” is no longer clear when link-functions are used (on which scale should residuals be calculated? On the link scale or on the observed scale?)
- We don't care too much about this aspect, but you should remember:
  - There are **different types of residuals**.
  - `autoplot()` **automatically picks** the residuals that “make sense”.

## Doing it right: Interpreting the coefficients

Let's now look at the `summary()` output (yes! this works also for `glm` objects):

```
> summary(soay.glm)
```

Call:

```
glm(formula = fitness ~ body.size, family = poisson(link = log),  
     data = soay)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.7634	-0.6275	0.1142	0.5370	1.9578

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	-2.42203	0.69432	-3.488	0.000486 ***
body.size	0.54087	0.09316	5.806	6.41e-09 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)

Null deviance: 85.081 on 49 degrees of freedom  
Residual deviance: 48.040 on 48 degrees of freedom  
AIC: 210.85

Number of Fisher Scoring iterations: 4

You see several (familiar and less familiar) components in the output. For the moment, we are interested in the coefficients, which are estimated as

$$\hat{\beta}_0 = -2.422 \quad \text{and} \quad \hat{\beta}_1 = 0.541$$

with respective standard errors and  $p$ -values. In particular,  $p < 0.001$  for  $\hat{\beta}_1$  indicates **very strong evidence for a positive (because  $\hat{\beta}_1 > 0$ !) effect of female weight on reproductive success** (number of offspring)!

**Good to know:** Theory says that the estimated coefficients  $\hat{\beta}_0$  and  $\hat{\beta}_1$  are approximately **normally distributed** around the true values:

$$\hat{\beta} \sim N(\beta, \sigma_{\beta}^2) \tag{2}$$

Thus a 95% CI can be approximated by the usual  $\hat{\beta} \pm 2 \cdot \sigma_{\beta}$  idea.



## What do the coefficients tell us?

Remember our model

$$\log(E(y_i)) = \beta_0 + \beta_1 \cdot \text{bodySize}$$

Thus the  $\exp(\cdot)$  transformation leads to

$$E(y_i) = \exp(\beta_0 + \beta_1 \cdot \text{bodySize})$$

.

Our estimates  $\hat{\beta}_0$  and  $\hat{\beta}_1$  can be plugged into this equation!

For example, a 5 kg female is **expected** to produce  $\exp(-2.422 + 0.541 \cdot 5) = 1.33$  lambs, while a 7 kg female would already be expected to produce  $\exp(-2.422 + 0.541 \cdot 7) = 3.91$  lambs.

Makes sense, right?

## Doing it right: The `anova()` table

Remember: We were sometimes using ANOVA tables, for instance for linear regression output with categorical covariates.

Here, it is useful again, but it gives us an **Analysis of Deviance** table:

```
> anova(soay.glm)
```

```
Analysis of Deviance Table
```

```
Model: poisson, link: log
```

```
Response: fitness
```

```
Terms added sequentially (first to last)
```

		Df	Deviance	Resid. Df	Resid. Dev
NULL				49	85.081
body.size	1	37.041		48	48.040

**Note:** The deviance is essentially a difference of likelihoods.

In brief, the larger the likelihood, the better fits the data to your model...

Here, the total deviance is given by the so-called **NULL** deviance: 85.081. It is the analogon to the total variability of the data in linear regression...

Of this, 37.041 is **explained by bodysize**.

The question is, whether this is “much”! This can be tested by a  $\chi^2$  test, because the value is  $\chi^2$  distributed with 1 degree of freedom:

```
> 1-pchisq(37.041,1)
```

```
[1] 1.156713e-09
```

You would get the same if you directly specify the test you want to carry out in the `anova()` call:

```
> anova(soay.glm, test="Chisq")
```

## Your turn!

Let's look at another data example, taken from Hothorn and Everitt (2014), chapter 7:

A new drug was tested in a clinical trial (Giardiello et al. 1993, Piantodsi 1997), aiming at **reducing the number of polyps** in the colon (Dickdarm). The data is publicly available from the Hothorn/Everitt book package:

```
> library(HSAUR3)
> data("polyps")
```

**Scientific question:** Does the drug influence (reduce) the number of polyps?

Data: Number of polyps (outcome), treatment, age (covariates).

number	treat	age
63	placebo	20
2	drug	16
28	placebo	18
17	drug	22
61	placebo	13
1	drug	23
7	placebo	34
15	placebo	50
44	placebo	19
25	drug	17

number	treat	age
3	drug	23
28	placebo	22
10	placebo	30
40	placebo	27
33	drug	23
46	placebo	22
50	placebo	34
3	drug	23
1	drug	22
4	drug	42

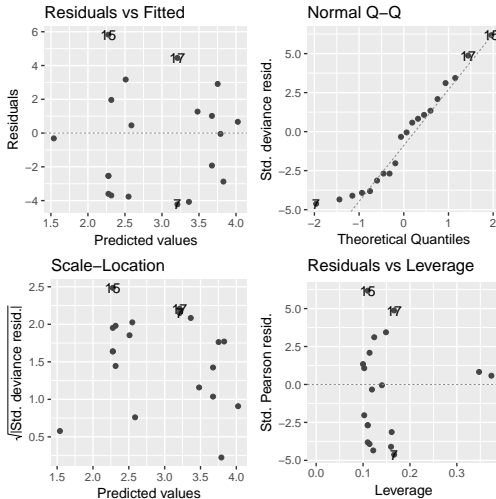
## Your tasks (in teams, if you like):

Look at the analysis done in next three slides, and answer the following questions:

- 1 Are there any problems visible from the diagnostics plots?
- 2 Does the treatment seem to be effective? If yes, can you **quantify the effect**?
- 3 Is age a relevant variable? If yes, what happens when patients grow older?

```
> polyps.glm <- glm(number ~ treat + age,data=polyps, family="poisson")
```

```
> autoplot(polyps.glm,smooth.colour = NA)
```



```
> anova(polyyps.glm,test="Chisq")
```

```
Analysis of Deviance Table
```

```
Model: poisson, link: log
```

```
Response: number
```

```
Terms added sequentially (first to last)
```

		Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
NULL				19	378.66	
treat	1	150.101		18	228.56	< 2.2e-16 ***
age	1	49.018		17	179.54	2.536e-12 ***

```
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



```
> summary(polyps.glm)
```

Call:

```
glm(formula = number ~ treat + age, family = "poisson", data = polyps)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-4.2212	-3.0536	-0.1802	1.4459	5.8301

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )
(Intercept)	4.529024	0.146872	30.84	< 2e-16 ***
treatdrug	-1.359083	0.117643	-11.55	< 2e-16 ***
age	-0.038830	0.005955	-6.52	7.02e-11 ***

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for poisson family taken to be 1)

Null deviance: 378.66 on 19 degrees of freedom  
Residual deviance: 179.54 on 17 degrees of freedom  
AIC: 273.88

Number of Fisher Scoring iterations: 5

# Overdispersion

“Overdispersion” means “extra variability”. Why could this be a problem?

**Remember:** The variance of the Poisson distribution increases with the mean, because **mean=variance** (see slide 13).

In Poisson regression it is assumed that, for each observation  $i$ ,

$$y_i \sim \text{Po}(E(y_i)) .$$

However, the **variance is often larger than the mean** in reality, because there are factors that influence the response (“cause variability in it”) that cannot be captured by the covariates!

Why? Maybe you simply cannot observe the variable or it is too expensive to monitor, or...

## Detecting overdispersion

Look at the summary output from your GLM object, check the “Residual deviance” and compare it to the “degrees of freedom”.

**Soay sheep data:** Res. deviance: 48.040, df=48

**Polyps data:** Res. deviance: 178.54, df=17

The residual deviance should be approximately  $\chi^2$  distributed with df degrees of freedom. This means that one should check whether

$$\text{Residual deviance} \approx \text{df}.$$

The sheep data seem fine, but the polyps data have  
Residual deviance  $\gg$  df  $\rightarrow$  overdispersion!

If unclear, use the  $\chi^2$  test with df degrees of freedom.

# What is the problem with overdispersion?

When there is unaccounted overdispersion, the  $p$ -values that are calculated are usually **too small**!

## Possible solutions:

- Use as your regression family **quasipoisson** instead of `family = poisson`.

This allows to estimate the variance parameter (denoted as **dispersion parameter**) separately.

- Use a **negative binomial regression** (there is the `glm.nb()` function from the MASS package in R to fit it, check it out by `?glm.nb()`).

# Reanalyzing the polyps data with quasipoisson

```
> polyps.glm2 <- glm(number ~ treat + age, data=polyps, family="quasipoisson")
> summary(polyps.glm2)
```

Call:

```
glm(formula = number ~ treat + age, family = "quasipoisson",
     data = polyps)
```

Deviance Residuals:

Min	1Q	Median	3Q	Max
-4.2212	-3.0536	-0.1802	1.4459	5.8301

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	4.52902	0.48106	9.415	3.72e-08 ***
treatdrug	-1.35908	0.38533	-3.527	0.00259 **
age	-0.03883	0.01951	-1.991	0.06284 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 10.72805)

Null deviance: 378.66 on 19 degrees of freedom

Residual deviance: 179.54 on 17 degrees of freedom

AIC: NA

Number of Fisher Scoring iterations: 5

- The **dispersion parameter** is now estimated as 10.73, which is calculated as

Residual deviance / df.

- The  $p$ -values for the coefficients are now larger! In particular, there is now only weak evidence ( $p = 0.063$ ) for an effect of age!

→ The poisson family gave too optimistic  $p$ -values!

We say: The  $p$ -values were **anti-conservative** or non-conservative.

(Anti-conservative results are really **the worst** that can happen. Guess why?)

## Underdispersion?

Can it happen that the observations are **less variable** than expected?

Yes: Especially when there are **dependent observations**!

You can **detect it** by checking if  $\text{Residual deviance} < \text{df}$ .

In that case, your  $p$ -values are usually too large, that is, the results are **conservative** (just the opposite of the overdispersion problem).

The quasipoisson regression is a pragmatic solution in that case, too.

## Zero-inflation

A special type of overdispersion may be caused by an **overrepresentation of zeros** in the observations.

**Example:** Numbers of cigarettes smoked

Some people are never-smokers, so they will always produce a zero observation, while smokers may smoke any number of cigarettes.

Please read chapter 7.5.2 of GSWR for some ideas how to handle this case.



## A note on interpretation and model selection

Just as a reminder:

**The same remarks and warnings from the last weeks for linear models also apply to GLMs!**

# Summary

- Poisson regression is useful to model counted outcomes.
- Pretending that counted outcomes are continuous may lead to wrong results.
- The main ingredients of GLMs are
  - The family
  - The linear predictor
  - The link function.
- Model diagnostics are similar as in the linear case (`autoplot()`).
- Interpret the coefficients by back-transforming to the original scale.
- Analysis of deviance is the analogon to ANOVA (`anova()`).
- Over-(under-)dispersion, how to detect it and what do do.

## References:

Hothorn, T. and B. S. Everitt (2014). *A Handbook of Statistical Analyses Using R* (3 ed.). Boca Raton: Chapman & Hall/CRC Press.