### **Model Based Statistics in Biology.**

#### Part V. The Generalized Linear Model.

# **Chapter 17.3 Single Categorical Explanatory Variable (Sensitivity Analysis)**

ReCap. Part I (Chapters 1,2,3,4), Part II (Ch 5, 6, 7)

ReCap Part III (Ch 9, 10, 11), Part IV (Ch 13, 14)

- 17 Poisson Response Variables
- 17.1 Poisson Regression
- 17.2 Single Categorical Explanatory Variable (Log-linear Model)

17.3 Single Categorical Explanatory Variable (Sensitivity Analysis)

- 17.4 Two or More Categorical Explanatory Variables
- 17.5 Poisson ANCOVA
- 17.6 Model Revision

on chalk board

Ch17.xls

**ReCap** Part I (Chapters 1,2,3,4) Quantitative reasoning

**ReCap** Part II (Chapters 5,6,7) Hypothesis testing and estimation

**ReCap** (Ch 9, 10,11) The General Linear Model with a single explanatory variable.

ReCap (Ch 12,13,14) GLM with more than one explanatory variable

ReCap (Ch 15) GLM review

**ReCap** (Ch 16)The generalized linear model.

ReCap (Ch 17) Regression and one-way ANOVA with Poisson errors.

Today: Power of a test. Poisson response variable with single categorical explanatory variable.

# Wrap-up.

G-statistics measures goodness of fit of data to model, for any design.

We looked at two way classification (two nominal scale explanatory variables)

The power  $(1-\beta)=(1-\text{Type II error})$  is not worth calculation after analysis.

It is of interest to compute

- -the difference that could have been detected, given the error and n or,
- -the sample size require to detect a specified difference.

# Introduction. Sensitivity analysis of Poisson variable, Single Categorical Explanatory Variable

Standard practice in biology has been to report Type I error in relation to a fixed tolerance at 5%, with no consideration of Type II error. One reason for this is that many applications are for nominal scale  $H_a$  for which Type II error (sensitivity) cannot be calculated easily, if at all. Generally one needs a specific  $H_a$  in order to calculate Type II error  $\beta$  and state sensitivity.

Some rules of thumb about Type II error.

- -Reducing  $\alpha$  the tolerance for type I error will increase Type II error  $\beta$  if the number of observations n is fixed.
- -Tolerance of type I error  $\alpha$  often fixed at 5% not lower, for this reason.
- -An increase in number of measurements n decreases Type II error  $\beta$
- -This increase in number of measurements n increases the sensitivity of test, allowing small contrasts can be detected.

Some tests have lower Type II error  $\beta$  than others. For example, rescaling the quantity of interest to ranks typically reduces the sensitivity and raises type II error.

If the null hypothesis cannot be rejected, we do <u>not</u> undertake power analysis by calculating Type II error (Hoenig). Instead we undertake a sensitivity analysis.

- -Report detectable difference
- -Calculate n required for specified difference

Example: Polymorphic clams, Donax variabilis.

Analyses of poisson frequencies in biology are often made in a two-

Donax data can be analyzed as poisson
 (Prey selection, as here)

It could also be binomial
 (case-control estimate of selection intensity)

Because each animal could either be eaten
 or not, with probability depending on colour.

way classification of the response variable. In a two-way classification we are typically interested in the interaction term, rather than main effects. For example in the case of colour patterns in tiger beetles in four seasons (Sokal and Rohlf 1995 Box 17.8) we are not particularly interested in whether a different number of beetles were collected in each of the four seasons. We have no idea of whether equal effort was made to collect beetles in the four seasons, so it is of little interest to test whether the four frequencies differ from a 1:1:1:1 ratio. Similarly, we are not particularly interested in whether the proportion of Bright red and Not bright red differ from a 1:1 ratio in this particular case. Instead, we are interested in whether the proportions of bright red and not bright red beetles change with season. This is measured by an interaction term.

Another example. Do visual predators (birds) form search images and selectively remove common colour morphs from polymorphic prey populations. Predated and unpredated clams are collected from the beach, and sorted into colour categories.

The first explanatory variable is shell colour (dark, ray, tinge, white). The second explanatory variable is predation (yes or no).

This results in a 2 by 4 table.

The response variable is frequency of 488 Donax in these 8 cells.

We are interested in selective predation--in whether the relative frequency of shell colours taken by birds differs from that in the environment.

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### **Poisson frequencies** (Donax)

The model is:

relative frequency of shell colours depends on whether or not we are looking at a predated or unpredated collection.

$$f = e^{\beta prd*clr}N + residual$$

 $e^{\beta prd^*clr}$  is the expected proportion of Donax in each of 4 colour categories and 2 states, predated by birds or not predated. This parameter will be estimated from the marginal totals. That is, we will use information about the number of Donax in the 4 categories, and about the total number predated or not predate, to estimate  $e^{\exists prd^*clr}$  for each of the 8 observed frequencies that we have.

### Poisson frequencies (Donax continued)

If Two-way Poisson analysis covered (beetles) then skip ahead at this point to G = 4.16 p = 0.26 Cannot reject  $H_{\circ}$ 

### There are 8 observed frequencies

	Live	Predated		
Dark	24	4	28	
Rays	118	35	153	
Tinge	90	38	128	
White	139	40	179	
	371	117	488	

The expected proportion of live donax, in the dark category, out of 488 Donax, is

$$e^{\beta prd^*clr}$$
 =  $\frac{28}{-}$  ·  $\frac{371}{-}$  =  $0.057 \cdot 0.76 = 0.044$ 

In similar fashion, we estimate the other 7 values of the parameter  $e^{\beta prd^*clr}$  The 8 values of  $e^{prd^*clr}$  are

Predated 0.014 0.075 0.063	Live 0.044 0.238 0.199	Dark Rayed Tinge White
0.088	0.279	White
0.075	0.238	Rayed

We then use these to compute the fitted values  $e^{\exists}N$ , which appear here in 8 data equations.

equation					
f	=	e·N	+ 1	residual	$2\ln L = 2 f \ln(f/e^{\beta}N)$
24	=	$0.044 \cdot 488$	+	2.71	5.76
118	=	$0.238 \cdot 488$	+	1.68	3.39
90	=	$0.199 \cdot 488$	_	7.73	-14.06
139	=	$0.279 \cdot 488$	+	•	5.89
4	=	$0.032 \cdot 488$		•	
35	=	$0.026 \cdot 488$		•	
38	=	$0.106 \cdot 488$		•	
40	=	$0.084 \cdot 488$			
G = 2	$\sum_{i} \sum_{j=1}^{n} \frac{1}{2}$	$f \ln(f/e^{\beta}N)$			4.16

While these computations can be done in any statistical package, they are also easily completed in a spreadsheet.

The G-statistic is 4.16

The likelihood ratio is  $LR = \exp(4.16/2) = 8$ 

The model with the interaction term 8 times more likely than the model with no interaction term. We have insufficient evidence for an interactive effect.

The p-value for this G-statistic is computed from a Chi-square distribution with three degrees of freedom. The degrees of freedom are computed exactly the same as was the case with the interaction term in a two-way ANOVA. The degrees of freedom for the interaction term are calculated as the product of the degrees of freedom for the main effects:

$$df_{prd*clr} = df_{prd} * df_{clr} = (2-1)*(4-1) = 3.$$
  
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The Minitab command to compute the p-value is

More than 75.53% of the G-statistics obtained by chance will be less than our observed G = 4.16. And 24.47% will be greater. So we cannot reject the null hypothesis that relative

```
MTB > cdf 4.16;
SUBC> chisquare 3.
4.16 0.7553
```

proportions of Donax colour morphs taken by birds matches that for the available prey. The symbol  $p_{prd|clr}$  is read "the proportion predated, given the colour."

```
H_o: p_{prd|dark} = p_{prd|ray} = p_{prd|tinge} = p_{prd|white}
```

We reject the alternative hypothesis

H<sub>A</sub>: proportions are not equal.

### **Sensitivity Analysis**

Skip ahead to here, if 2-way analysis (beetle example) covered in previous lecture.

Because we cannot reject the null hypothesis, Type II error becomes of interest.

```
HoTrue HoFalse
Cannot reject Ho ok II
Reject Ho I ok
```

Zar (1996) gives many examples of computations of Type II error. However, these example are usually for comparing two groups. Ie comparing 2 means, or 2 proportions. Power (which is 1 - Type II error) is difficult to calculate if we have multiple groups. However, once we have done the analysis it is too late to do a power analysis (Hoenig)

In these cases, it is of interest to look at the difference that could have been detected. It is also of interest to calculate the sample size needed to detect at specified difference.

In fact these questions are of more interest than calculating the Type II error rate. The questions of detectable difference and adequate sample size arise when the null hypotheses (it is just due to chance) cannot be rejected.

To demonstrate this approach we will look at the detectable difference for the Donax analysis, using an iterative method.

The difference between expected (no selective predation) and observed (weak effects if any) was 10 clams too many for Donax tinged with slight pink.

	Live	Predated	Expected	Deviation	Dev+	"Predated"
Dark	24	4	8	<b>-4</b>	-6	2
Rays	118	35	37	-2	-3	34
Tinge	90	38	28	+10	+10	43
White	139	40	44	<b>-4</b>	-6	38
	371	117	117	0	0	117
			•	-		· ·

This number was successively increased until the G-statistic reached the criterion value where  $p < \alpha$ .

Dev	0 Dev1	Dev2	Dev3	Dev4	Dev5
_4	-5	-5	-6	-6	-6
-2	-2	-2	-2	-2	-3
+10	+11	+12	+13	+14	+15
_4	<b>-4</b>	-5	-5	-6	-6

G-statistic rises as the deviation is increased.

When deviation reached +15,  

$$G = 10.37$$
 df = 3 p = 0.0157

It would have been possible to detect a deviation of 15 Donax "too many" in the tinged category.

The observed deviation was 10 out of 117 Donax or 9%

The detectable deviation was 15 out of 117 Donax of 13% We can be sure that selection was less than 13%, given the sample size we were able to obtain.