



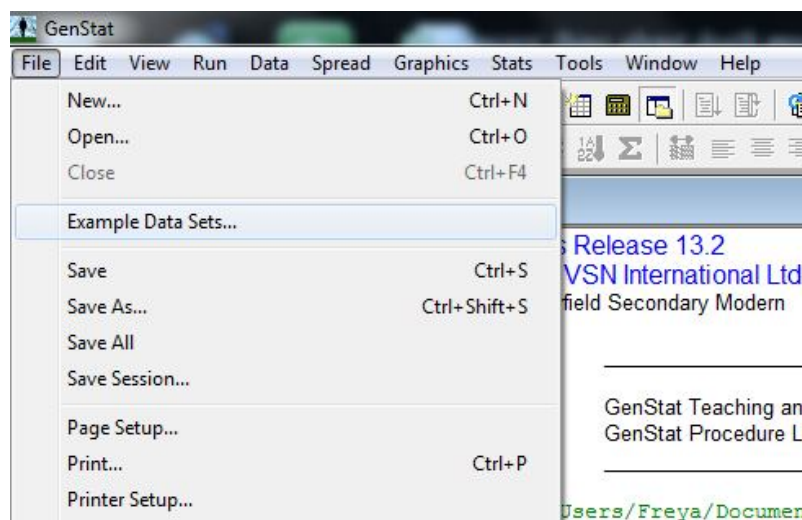
Lesson: Effect of Investigator Disturbance on Hatching Success in Endangered Takahe

The Takahe is a flightless, endangered bird that is endemic to New Zealand, meaning that it is found only in New Zealand. The Takahe used to be widespread over the country, however in the present day the only colony in a natural habitat is in Fiordland, in the lower South Island. The New Zealand Department of Conservation run a management program to monitor and protect the remaining population: some birds were translocated to islands around New Zealand, which are thought of as refuges or safe-havens for the Takahe. The Takahe are monitored on these islands, and as part of the program to help increase their population size, breeding activity is closely monitored in effort to isolate some of the issues that result in the limited breeding success.

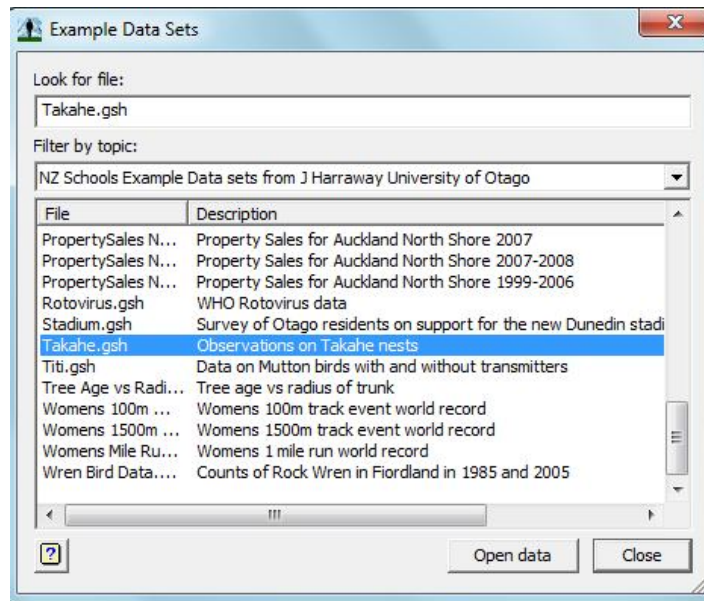
The main hypothesis presented for this data set and the aim of this lesson is to investigate whether human disturbance of the nests (to monitor the breeding) affects breeding success (compared to the breeding success of nests without human disturbance).

Please note that this lesson contains non-parametric tests which are not currently in the New Zealand School Curriculum, however the study design and interpretation of results provide relevant discussion.

1. To open the data we click on **File>Example Data Sets:**



This brings up the Example Data Sets dialog shown below. Click on the **Filter by topic** drop down menu and select the **NZ Schools Example Data sets from J Harraway University of Otago** option. Choose the file **Takahe.gsh** and click on **Open data**:



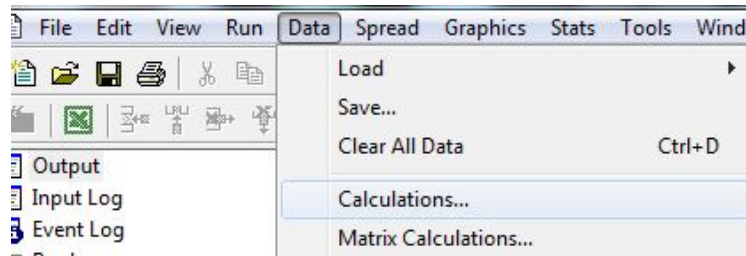
This opens a spreadsheet (shown below) containing data from a study investigating the success of different stages of the breeding cycle of Takahe in non-disturbed and disturbed habitats:

Row	Females	Year	Treatment	No_Eggs	Missing_Eggs	Fertile_Eggs	Infertile_Eggs	Dead_Embryos	No_Hatched	No_Fledged	Notes
1	Bunchy	2000	D	1	0	0	1	0	0	0	
2	Puffin	2000	D	2	0	2	0	2	0	0	
3	Terri	2000	D	1	0	1	0	0	1	1	
4	Toni&Tilly	2000	ND	3	0	2	1	2	0	0	Two females in same nest
5	Toru	2000	ND	1	0	1	0	0	1	1	

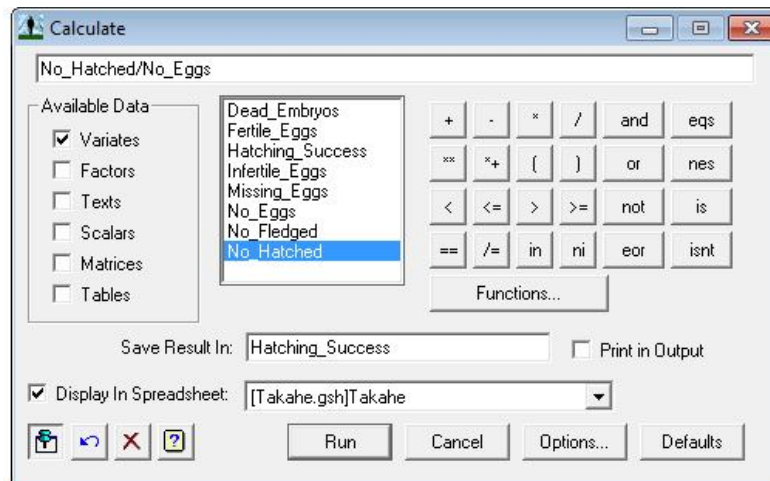
In the spreadsheet the data recorded are **Females** (the names of the female Takahe in each pair), **Year**, **Treatment** (D = disturbed, ND = not disturbed), **No_Eggs** (number of eggs observed), **Missing_Eggs** (the number of eggs that were missed in the count), **Fertile_Eggs**, **Infertile_Eggs**, **Dead_Embryos**, **No_Hatched** (number of eggs that hatched), **No_Fledged** (number of eggs that fledged) and **Notes** (other information). An asterisk (*) is in place of any missing data.

In this study 8 pairs of Takahe (male-female) were monitored and different stages of their breeding success were monitored. As the sample size was so small it was unrealistic to allocate half the pairs to the non-disturbed group and the other half to the disturbed group. Because of this a matched-pairs design was employed. This means that the breeding pairs alternated between treatment (disturbed nests) and control (non-disturbed nests) over four seasons (i.e. each year the treatments swap over for each pair).

- Calculate the average hatching success and fertility percentages for the non-disturbed and disturbed nests. To do this we must first calculate the individual proportions for each pair, and then calculate the averages. Click on **Data>Calculations:**

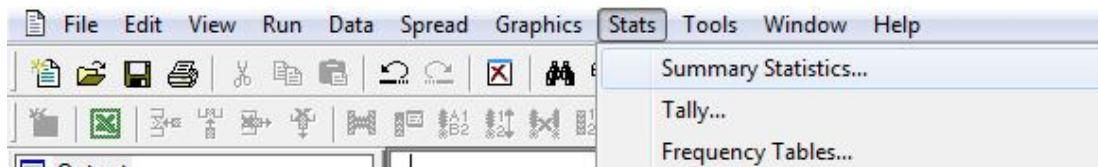


To calculate Hatching Success click onto the empty dialog box at the top of the window and enter **No_Hatched/No_Eggs**. Save the result as an appropriate named variable, such as Hatching_Success, then click **Run:**

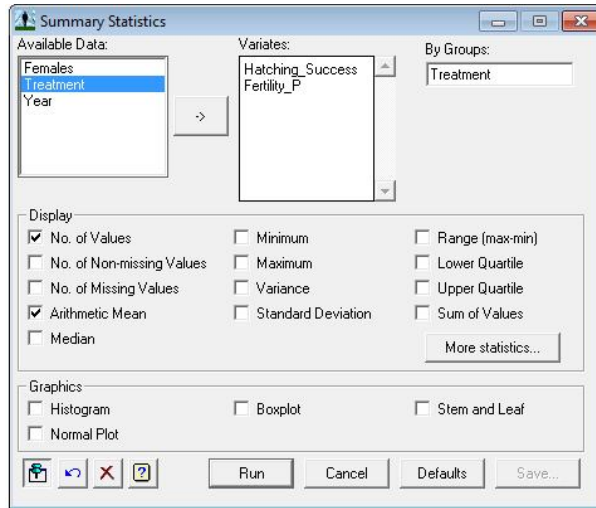


A new column named Hatching_Success with the raw proportions of hatching success for each Takahe pair will appear in your spread sheet. Repeat this process for fertility, using **Fertile_Eggs/No_Eggs** as the formula and saving the result as **Fertility_P**.

Now we want to calculate the average proportions for hatching success and fertility for the non-disturbed and disturbed nests. Click on **Stats>Summary Statistics:**



In the **Variates** box enter **Hatching_Success** and **Fertility_P**, and in the **By Groups** box enter **Treatment**. Under Display tick **No. of Values** and **Arithmetic Mean** then click **Run:**



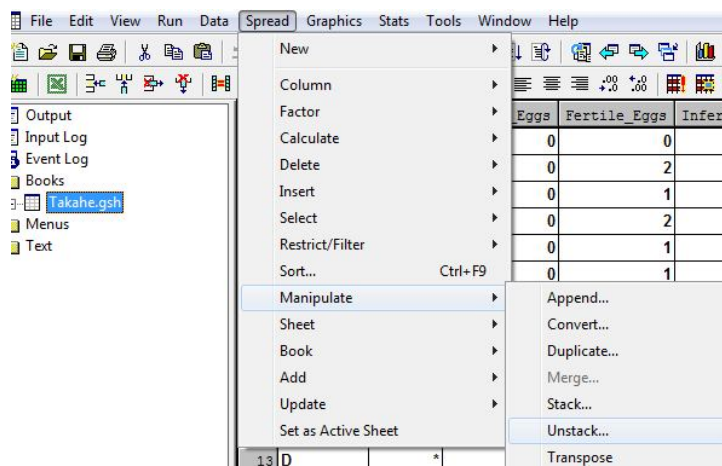
Switch to your GenStat Output Window, where you will find the number of samples and the mean proportions of hatching success and fertility for both the non-disturbed and disturbed nests. An example of part of this output is below:

Summary statistics for Hatching_Success: Treatment D	
Number of values	= 14
Mean	= 0.451

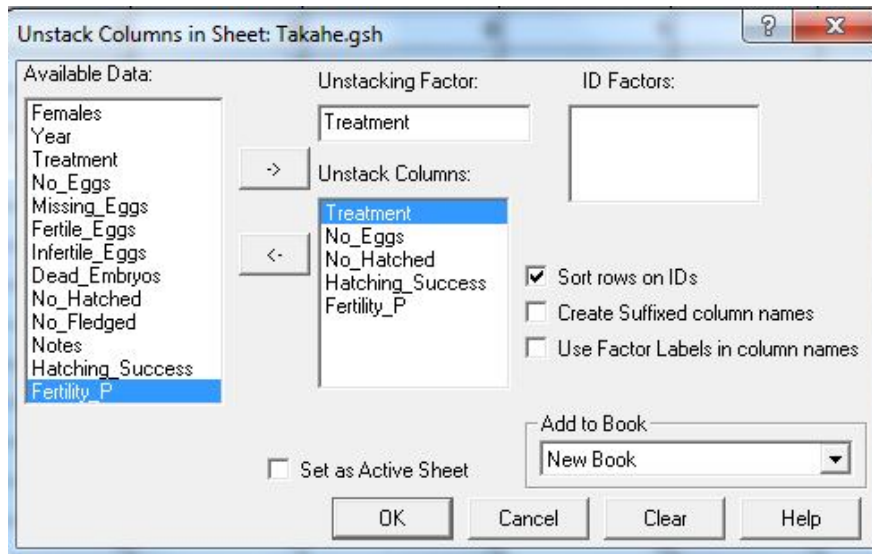
Calculate the differences between these proportions for hatching success and fertility and report any tentative conclusions that you make.

- Carry out a non-parametric Wilcoxon matched pairs test on the proportions found above for hatching success and fertility. Also carry out this test for fecundity (the number of eggs). Non-parametric procedures are required for this data set (as opposed to a paired t-test) due to the small sample sizes and type of data recorded.

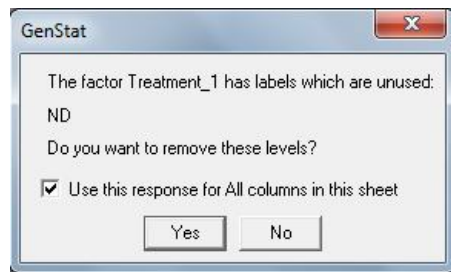
First we want to rearrange our data so that our disturbed nests and non-disturbed nests are separated into two separate columns. Click on **Spread>Manipulate>Unstack**:



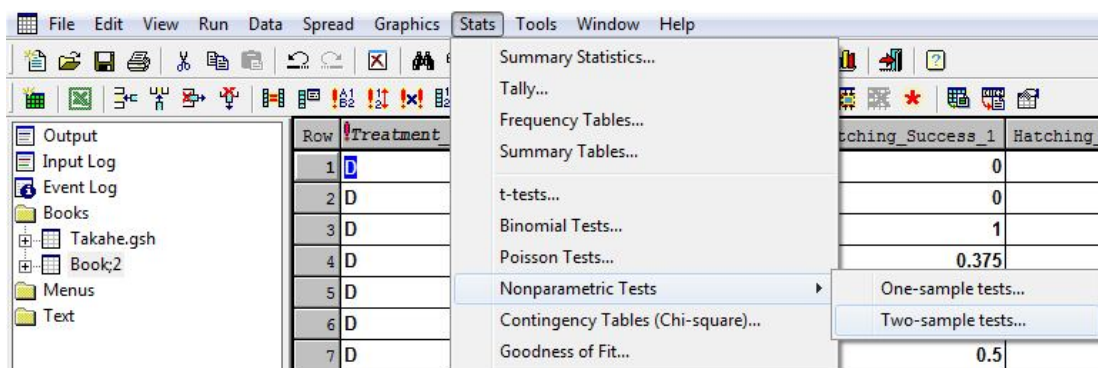
Enter **Treatment** as the **Unstacking Factor**, and in **Unstack Columns** enter **Treatment, No_Eggs, No_Hatched, Hatching_Success** and **Fertility_P**. Click **OK**:



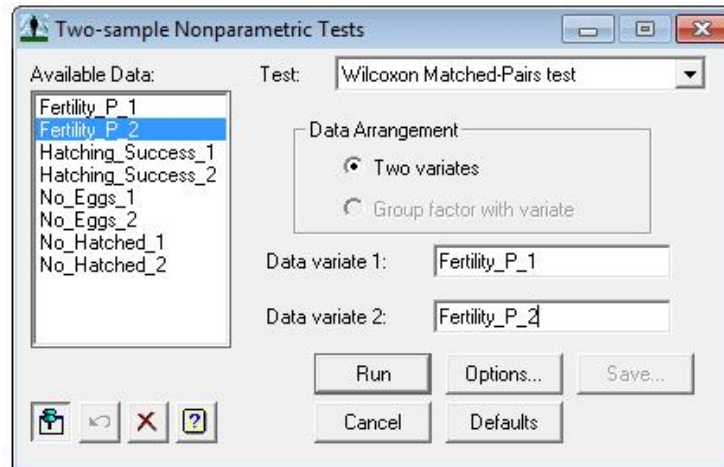
A new spreadsheet will appear, named Book;2 or similar, which contains the relevant variables for the non-parametric Wilcoxon tests. Note that when you navigate away from the spreadsheet, a warning will come up regarding a treatment label that is unused (this is caused from a missing response where the Takahe nest could not be found to allocate a treatment). Tick **Use this response for all columns in this sheet** and click **Yes**:



To carry out the non-parametric Wilcoxon matched-pairs test click on **Stats>Nonparametric Tests>Two-sample tests**:



For the **Test** select **Wilcoxon Matched-Pairs test**. Enter **Fertility_P_1** and **Fertility_P_2** in the boxes **Data variate 1** and **Data variate 2** respectively then click **Run**:



Switch to the GenStat Output Window, where you will find the results of the test:

Wilcoxon Matched-Pairs Test

Variate: Fertility_P_1-Fertility_P_2

Test Statistic: 8.000 (sum of signed ranks is negative)
Sample size: 7 (zero values have been excluded)
Probability: 0.297 (two-sided test)

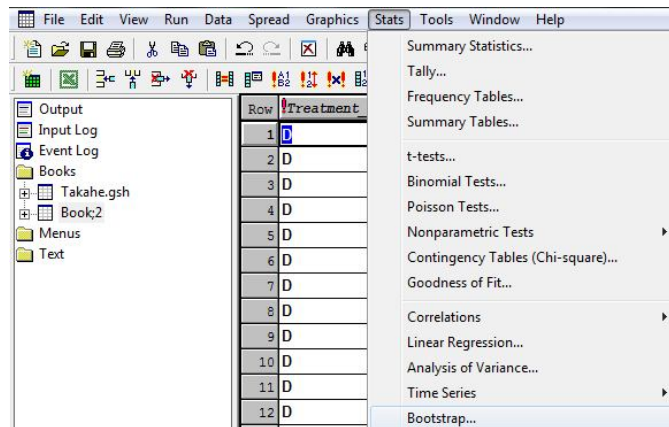
Report your findings and state any conclusions.

Carry out this same Wilcoxon test for the breeding success pairs and the fecundity pairs (number of eggs) and report your findings.

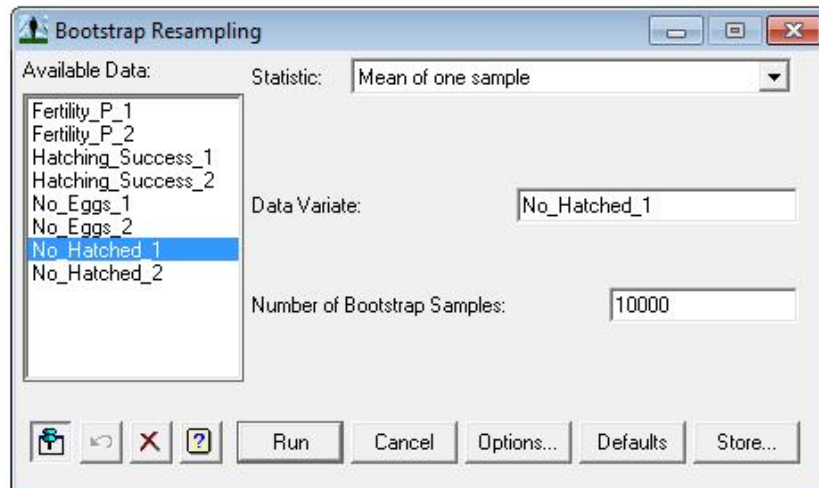
- 4a. Calculate the mean number of hatched eggs for the non-disturbed and disturbed nests and the accompanying 95% confidence interval. To calculate this it is best that we use a bootstrapping procedure.

The bootstrap technique involves randomly sampling a new dataset from the original sampled data. This creates a large number of datasets, that may have been seen if the experiment had been repeated many times (i.e. creates new datasets based on the original dataset). This technique is especially useful for datasets that have a small number of samples, such as the Takahe data set that we are working with now.

To calculate the bootstrap mean and confidence interval, click on **Stats>Bootstrap**:



In the **Statistic** drop-down menu select **Mean of one sample**, then in the **Data Variate** box enter **No_Hatched_1** (the number hatched in the disturbed nests). Set the **Number of Bootstrap Samples** to a high value, such as 10000, and click **Run**:



After running the bootstrap sampling, a distribution graph will be produced in your GenStat Graphics Viewer. This graph shows the distribution of the mean of the bootstrap samples. Switching to your GenStat Output Window, you will find the relevant results for the mean number of hatched eggs in the disturbed group, which will be similar to those shown below:

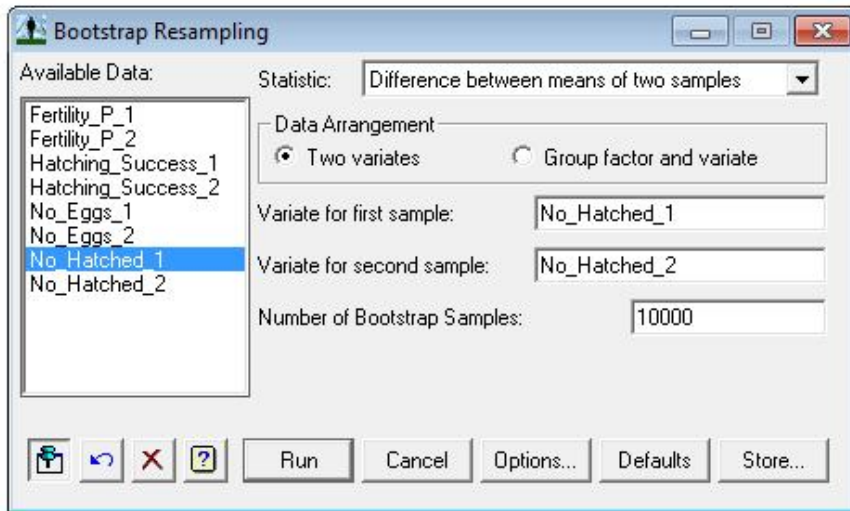
Number of Hatched Eggs in Disturbed Nests:

Bootstrap estimates, from 10000 bootstrap samples				
Label	mean	s.e.	95% confidence interval	
			lower	upper
Mean	1.309	0.253	0.833	1.786

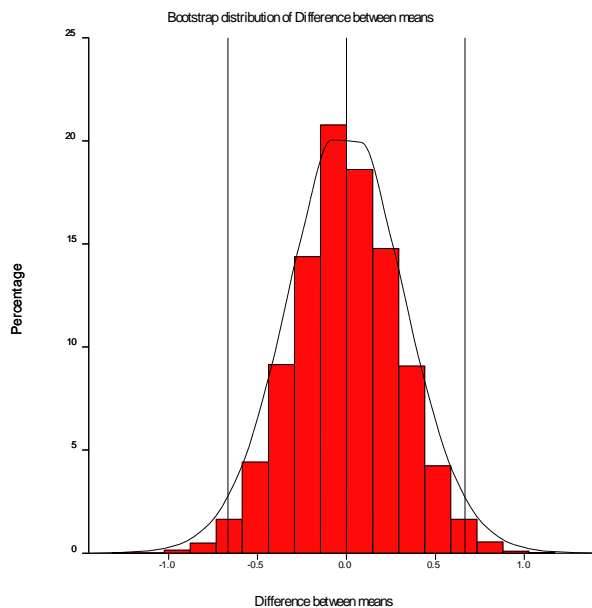
Repeat this bootstrap procedure for the number of hatched eggs in non-disturbed nests and report your findings.

- 4b. Calculate the mean and confidence interval for the difference between the number of hatched eggs in the disturbed and non-disturbed groups, using the bootstrap procedure. Click on **Stats>Bootstrap** as was done in 4a.

In the **Statistic** drop-down menu select **Difference between means of two samples** then enter **No_Hatched_1** and **No_Hatched_2** in the **Variate for first/second sample** boxes. Set the **Number of Bootstrap Samples** to a high value, such as 10000, and click **Run**:



The graph that is in the GenStat Graphics Viewer Window should look similar to below:



What distribution does the bootstrap sampling done above appear to follow? Why do you think this is the case?

Switch to your GenStat Output Window, where your results should be similar to those shown below:

Bootstrap estimates, from 10000 bootstrap samples

Label	mean	s.e.	95% confidence interval	
			lower	upper
Difference between means	0.000	0.341	-0.667	0.667

Report your findings and discuss your conclusions with reference to the previous questions.