Solution to Exercise 7.3 (Version 1, 8/5/15)

from Statistical Methods in Biology: Design & Analysis of Experiments and Regression (2014) S.J. Welham, S.A. Gezan, S.J. Clark & A. Mead. Chapman & Hall/CRC Press, Boca Raton, Florida. ISBN: 978-1-4398-0878-8

© S J Welham, S A Gezan, S J Clark & A Mead, 2014.

Exercise 7.3 (Data: courtesy A. Ferguson, Rothamsted Research)

A two-year field experiment investigated the effects of soil cultivation on activity of beneficial arthropods. Plots of winter oilseed rape were laid out as a RCBD with five blocks of three plots. Three soil cultivation treatments were to be compared: ploughing in both years, minimum tillage in both years, and minimum tillage in year 1 followed by ploughing in year 2. We consider data from the first season, when the latter two treatments were equivalent resulting in two first-year treatments 'plough' ($n_1 = 5$, one plot per block) and 'minimum tillage' ($n_2 = 10$, two plots per block). The accumulated catch of three pitfall traps per plot during a three-month period was recorded for various arthropod species; here we analyse counts of spiders of the taxa *Oedothorax*. The plot-level unit numbers (*ID*), structural factors (Block, Plot), treatments applied (factor Treatment) and total count data (variate *PlotCount*) can be found in file OEDOPLOT.DAT.

- a) Use multi-stratum ANOVA to determine whether these soil cultivation methods affect spider numbers. Obtain the standard errors for each treatment mean and the standard error of the difference between the two means (you will need to take account of the differing replication, as in Section 4.4). Produce and interpret a composite set of diagnostic plots.
- b) The trap-level unit numbers (*ID*), structural factors (Block, Plot, Trap), treatments applied (factor Treatment) and individual counts from the three pitfall traps in each plot (variate *TrapCount*) can be found in file OEDOTRAP.DAT. Obtain the multi-stratum ANOVA table and residual plots for these data. Compare and contrast your results here with those obtained in part (a) and discuss any differences.

Data 7.3a (OEDOPLOT.DAT = plot totals) and **Data 7.3b** (OEDOTRAP.DAT = trap catches)

Treatment allocation (MinTill or Plough) and summer catch of *Oedothorax* spiders from three traps per plot in an experiment to investigate the effect of soil cultivation on the activity of beneficial arthropods. The form of the table shows the RBCD layout in the field with 5 blocks each containing 3 plots.

	Block 1	Block 2	Block 3	Block 4	Block 5
Plot 1	MinTill	Plough	MinTill	Plough	MinTill
	1,1,0	1,5,0	0,2,4	4,4,2	1,1,3
Plot 2	Plough	MinTill	Plough	MinTill	Plough
	3,7,0	1,3,2	1,4,1	0,1,0	3,6,2
Plot 3	MinTill	MinTill	MinTill	MinTill	MinTill
	3,5,1	1,1,4	1,1,1	4,1,7	1,1,0

Solution 7.3

a) A model for the total plot catches takes the standard form for a RCBD and can be written in symbolic form (using the obvious factor names) as

Response variable:	PlotCount
Explanatory component:	[1] + Treatment
Structural component:	Block / Plot

However, after one year there are only two treatments present, with unequal replication, so the structure does not conform to a standard RCBD as described in Chapter 7. Minor modifications to the formulae given in Section 7.3 are required, and these are implemented automatically by statistical software. If we label the observations y_{ij} by the block (index i=1...5) and treatment at the end of the trial (index j=1,2,3 with 1=plough, 2 = min till in both years and 3 = min till then plough), then the block effects and the block sum of squares, BlkSS, can be calculated exactly as stated in Sections 7.2 and 7.3. The effect of the ploughing in year 1 is calculated as the deviation between the sample mean of the plough treatment and the overall mean, as usual, and the effect of minimum tillage in year 1 is calculated as the deviation of the pooled sample mean of the two minimum tillage treatments from the overall mean, as

$$\hat{\tau}_2 = \frac{1}{2}(\overline{y}_{\bullet 2} + \overline{y}_{\bullet 3}) - \overline{y}.$$

The treatment SS accumulates the squares of the estimates over all of the units (as in Section 7.3, page 135). Since there are 5 units with plough treatment (with estimate τ_1) and 10 with min till in year 1 (with estimate τ_2), this becomes

$$TrtSS = 5\hat{\tau}_1^2 + 10\hat{\tau}_2^2.$$

With only 2 treatments, the treatment SS has only 1 df. The remainder of the table is calculated in the usual manner. The multi-stratum ANOVA table is shown in Table S7.3.1. The observed significance level for the treatment variance ratio is P = 0.118 and so we cannot reject the null hypothesis, indicating that there is no evidence in this experiment of any difference between the two treatments in population mean number of spiders per plot.

Source of variation	df	Sum of squares	Mean square	Variance ratio	Р
Block stratum					
Residual	4	12.67	3.17	0.245	0.905
Block.Plot stratum					
Treatment	1	38.53	38.53	2.986	0.118
Residual	9	116.13	12.90		
Total	14	167.33			

Table S7.3.1 Multi-stratum ANOVA table for total plot catches of *Oedothorax* spiders from the soil cultivation experiment.

The treatment sample means are 8.6 and 5.2 spiders per plot for the 'Plough' and 'MinTill' treatments, respectively, with an overall mean of 6.3 spiders per plot. As the replication is unequal here the two predicted treatment means have different precision, and hence different standard errors. The standard error of the difference in population means must also take account of the replication ($n_1 = 5$, $n_2 = 10$). We can use the same formulae presented in Section 4.4 for the CRD and calculate

Plough: SEM₁ =
$$\sqrt{\frac{s^2}{n_1}} = \sqrt{\frac{12.90}{5}} = 1.606$$

MinTill: SEM₂ = $\sqrt{\frac{s^2}{n_2}} = \sqrt{\frac{12.90}{10}} = 1.136$,

SED =
$$\sqrt{s^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)} = \sqrt{12.90 \left(\frac{1}{5} + \frac{1}{10}\right)} = 1.968.$$

The df associated with these SE and the SED are the ResDF from the ANOVA table, here 9 df.

A composite set of residual plots based on standardized residuals are shown in Figure S7.3.1. These show no strong systematic pattern and are reasonably consistent with the patterns expected from normally-distributed data, given the small number of observations. The range of counts is small (1-12) and there is no evidence of variance heterogeneity across this range.



Figure S7.3.1. Composite set of residual plots using standardized residuals from ANOVA of total plot catches from the soil cultivation experiment.

In conclusion, there is no evidence of any difference in the population mean total plot catch of *Oedothorax* spiders from the two soil cultivation treatments implemented in year 1 ($F_{1,9} = 2.99$, P = 0.118), with a predicted population mean of 6.3 spiders caught per plot.

b) A model for the individual trap catches can be written in symbolic form as

Response variable:	TrapCount
Explanatory component:	[1] + Treatment
Structural component:	Block / Plot / Trap

Here, the structural component recognises that traps were nested within plots which in turn were nested in blocks, there being no relationship between traps with the same label in different plots (or between plots with the same label in different blocks). In this case, the traps are pseudo-replicates with respect to the tillage treatments. Plots are the experimental unit for the tillage treatments, and so evaluation of the tillage treatments should be made against plot-to-plot variability, i.e. in the Block.Plot stratum.

The multi-stratum ANOVA table for the individual trap catches is given in Table S7.3.2. Variation in the lowest stratum (Block.Plot.Trap) arises from differences between trap catches within the same plot. The treatment effects are estimated in the Block.Plot stratum and the variance ratios for the Block and Treatment terms are the same as those in Table S7.3.1. The conclusions of this analysis with respect to the treatments would thus be unchanged, as we would hope. However, residual plots (shown in Figure S7.3.2) now clearly suggest variance heterogeneity, and that the trap catch data does not conform to the assumptions underlying the analysis. There are several possible reasons for this apparent discrepancy between trap and plot scales. First, the scale of trap catch values is (obviously) smaller (0-7) than plot totals (1-12), with many counts of zero and one, and it is at this lower end of the range that variance heterogeneity in counts is often most marked. This is also apparent in the fitted values plot. Second, there are 45 trap catches as opposed to just 15 plot totals, so it may be easier to detect variance heterogeneity in this larger sample, i.e. it may be present but undetected in the smaller sample. Third, the act of creating plot totals may smooth over some spatial heterogeneity that is present between individual traps within plots; this may be an example of the tendency of means (or, equivalently, totals with equal numbers of contributing elements) to approximate a normal distribution. We might decide to retain our analysis of plot totals (and this would be legitimate), but we cannot proceed with analysis of individual trap catches without accounting for variance heterogeneity. Alternative approaches would include use of a log transformation with an offset (which we leave as a further exercise for the reader) or the assumption of a Poisson distribution for the counts using a GLM (see Chapter 18). In the latter case, the presence of pseudo-replication complicates the procedure so analysis of plot totals may again be a better option.

Source of variation	df	Sum of	Mean	Variance	D
Source of variation		squares	square	ratio	1
Block stratum					
Residual	4	4.22	1.06	0.245	0.905
Block.Plot stratum					
Treatment	1	12.84	12.84	2.986	0.118
Residual	9	38.71	4.30	1.257	0.300
Block.Plot.Trap stratum					
Residual	30	102.67	3.42		
Total	44	158.44			

Table S7.3.2 Multi-stratum ANOVA table for individual trap catches of *Oedothorax* spiders from the soil cultivation experiment.



Figure S7.3.2. Composite set of residual plots using standardized residuals from ANOVA of individual trap catches from the soil cultivation experiment.