Exercise 26.33 of PSLS3e

Data: Measurement of goldfish ventilation rates for goldfish that had been acclimated to either a cold (12 degrees Celsius) or a warm (22 degrees Celsius) environment, when exposed to one of a range of acute test temperatures (10, 12, 15, 22 and 25 degrees Celsius). Each of the 10 treatment groups included 18 goldfish, for a total of 180 subjects.

The design can be viewed as either a completely randomized design or a randomized block design, whereby the long-term acclimation would be a blocking factor. In the completely randomized design, each of the 180 goldfish would have been randomly allocated to one of the 10 treatment groups. The fish would then have undergone their adaptation to their selected environment, and at the time of acute response measurement each would then have been exposed to its selected acute temperature.

Model: The two-way ANOVA model for these data can be written:

$$\text{vent}_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk},$$

where the errors (ε_{ijk}) are i.i.d. from $\text{N}(0, \sigma)$, and the indices i and j correspond to the row (temperature) and column (acclimation) factors, with $I = 5$, $J = 2$, $n_{ij} = 18$ and $N = 180$.

We start with a descriptive analysis.

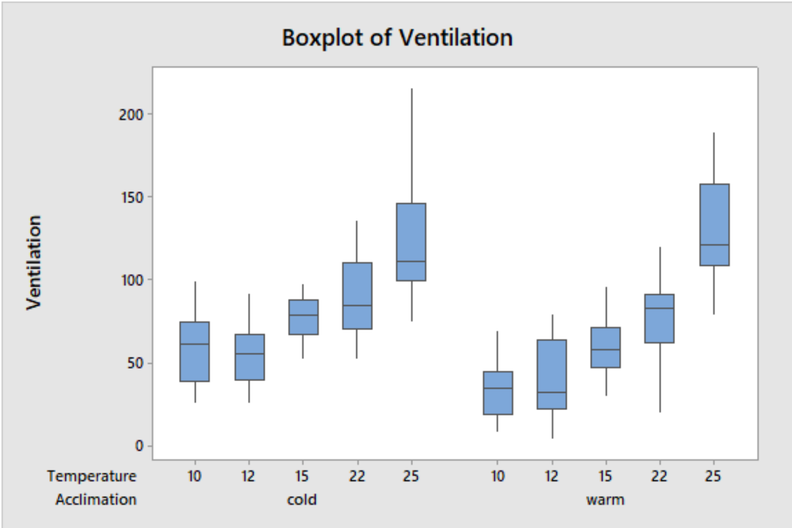
```
MTB > Table 'Acclimation' 'Temperature';
SUBC>   Layout 1 1;
SUBC>   DMissing 'Acclimation' 'Temperature';
SUBC>   Means 'Ventilation';
SUBC>   StDev 'Ventilation';
SUBC>   Counts.
MTB > Boxplot ( 'Ventilation' ) * 'Acclimation';
SUBC>   Group 'Temperature';
SUBC>   IQRBox;
SUBC>   Outlier.
```

Tabulated Statistics: Acclimation, Temperature

Rows: Acclimation   Columns: Temperature

	10	12	15	22	25	All
cold	58.89	55.83	76.83	88.56	124.83	80.99
	21.23	18.44	13.54	24.88	37.22	34.64
	18	18	18	18	18	90
warm	33.94	39.83	59.72	78.28	129.00	68.16
	17.35	24.43	16.74	25.82	30.72	41.42
	18	18	18	18	18	90
All	46.42	47.83	68.28	83.42	126.92	74.57
	22.91	22.82	17.34	25.53	33.70	38.61
	36	36	36	36	36	180

Cell Contents
Ventilation : Mean
Ventilation : Standard deviation
Count



Comments:
The 10 group means vary substantially, from 33.9 (10,warm) to 129.0 (25,warm). The standard deviations are too variable to meet the rule based on the ratio of the largest to the smallest, because $s_{\max}/s_{\min} = 37.2/13.5 = 2.8$. We can also here try the variance tests to examine whether this constitutes enough evidence to reject equal variances, and the answer is yes ($P = 0.027$ and $P = 0.037$; not shown). A visual inspection of the means and standard deviations suggests that the standard deviation increases with the mean. The boxplots look reasonably regular, with no suspected outliers but variable skewness in either direction. The best exploration of the normal distribution assumption will be from the model's residuals, and we start with the simpler menu (and raw residuals).

```
MTB > ANOVA 'Ventilation' = Temperature Acclimation Temperature* &
CONT>       Acclimation;
SUBC>    GFourpack.
MTB > Interact 'Temperature' 'Acclimation';
SUBC>    Response 'Ventilation';
SUBC>    Full.
```

ANOVA: Ventilation versus Temperature, Acclimation

Factor Information

Factor	Type	Levels	Values
Temperature	Fixed	5	10, 12, 15, 22, 25
Acclimation	Fixed	2	cold, warm

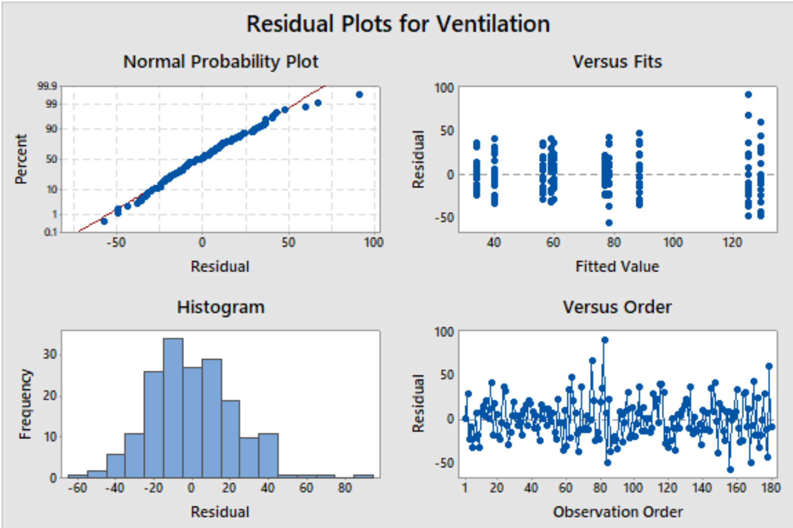
Analysis of Variance for Ventilation

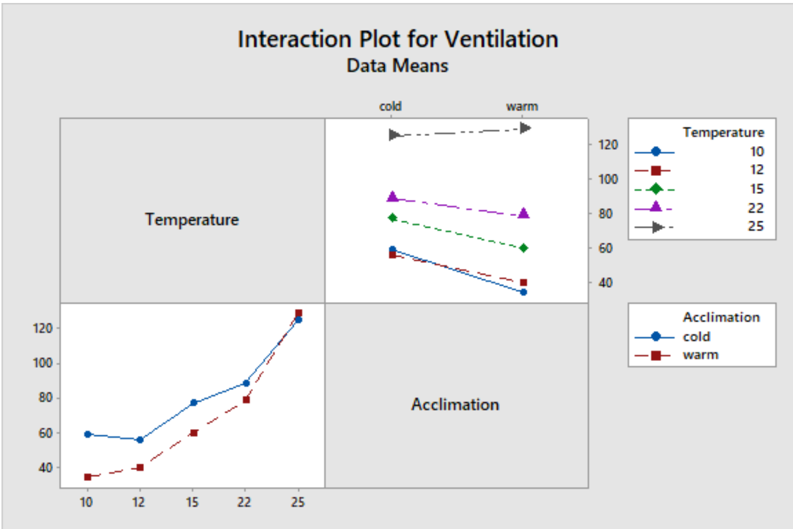
Source	DF	SS	MS	F	P
Temperature	4	157158	39289.4	68.13	0.000
Acclimation	1	7411	7411.2	12.85	0.000
Temperature*Acclimation	4	4235	1058.7	1.84	0.124
Error	170	98036	576.7		
Total	179	266840			

Model Summary

S	R-sq	R-sq(adj)
24.0143	63.26%	61.32%

Residual Plots for Ventilation





Comments:
The ANOVA table shows a non-significant interaction ($P = 0.12$), although the P-value is not too far from significance. The interaction plot shows a clear interaction pattern whereby the difference between the acclimation groups diminishes as the temperature increases. If such an interaction is of biological interest, it may be relevant to base model conclusions on the model with this interaction included, despite its non-significance.

The main effects for acclimation and temperature are both strongly significant ($P < 0.0005$). The ventilation generally increases with temperature and is larger in the cold acclimation group, except at 25 degrees.

The normal probability plot for the residuals shows one rather extreme residual, corresponding to an observation in the (25,cold) group (it would be of interest to compute the corresponding standardized residual, but that would require the model to be refit in the `General Linear Model` menu). Otherwise the distribution of the standardized residuals appears to be close to normal. The residual plot shows a slight fanning to the right. The two 25 degrees groups are more variable than the rest, something that could also be seen in the descriptive statistics and was discussed above. It is not clear how serious this violation of model assumptions is.

Because of the fanning it is tempting to try to improve the compliance with assumptions by transformation, and methods beyond the VHM 801 course (but available as a “Box-Cox transformation” under `Options` in the `General Linear Model` menu) indicate that a square-root transformation might work well. We therefore try this next (as also requested). For simplicity we keep working with the simpler ANOVA menu.

```
MTB > ANOVA 'Ventilation' = Temperature Acclimation Temperature* &
CONT>       Acclimation;
SUBC>    Means  Temperature* Acclimation;
SUBC>    GFourpack.
MTB > Interact 'Temperature' 'Acclimation';
SUBC>    Response 'Ventilation';
SUBC>    Full.
```

ANOVA: rootvent versus Temperature, Acclimation

Factor Information

Factor	Type	Levels	Values
Temperature	Fixed	5	10, 12, 15, 22, 25
Acclimation	Fixed	2	cold, warm

Analysis of Variance for rootvent

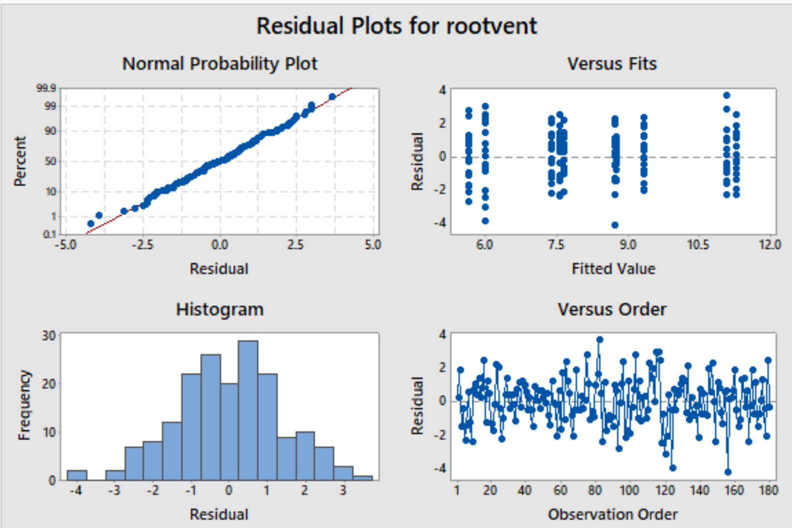
Source	DF	SS	MS	F	P
Temperature	4	516.32	129.079	61.87	0.000
Acclimation	1	41.28	41.281	19.79	0.000
Temperature*Acclimation	4	23.63	5.909	2.83	0.026
Error	170	354.68	2.086		
Total	179	935.91			

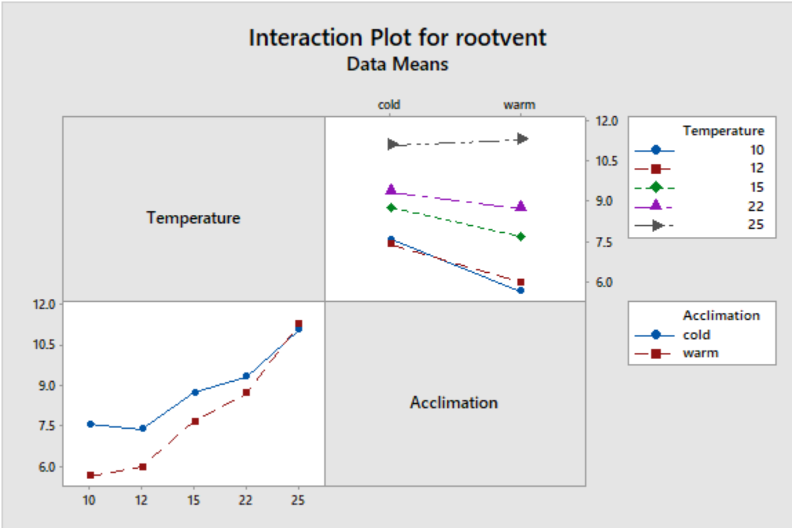
Model Summary

S	R-sq	R-sq(adj)
1.44441	62.10%	60.10%

Means

Temperature*Acclimation	N	rootvent
10 cold	18	7.5471
10 warm	18	5.6287
12 cold	18	7.3740
12 warm	18	5.9772
15 cold	18	8.7318
15 warm	18	7.6559
22 cold	18	9.3219
22 warm	18	8.7079
25 cold	18	11.0648
25 warm	18	11.2811





Comments:
Two major conclusions stand out: the model assumptions seem to be met better after transformation, and the interaction is now significant. In the residual plot, the variation is more evenly distributed across the range of fitted values. The most extreme residuals are now in the left tail, and overall the normal distribution plot looks better. Unless we believe that the observation with the extreme residual should really be dropped (the descriptive statistics don't indicate the observation to be totally outside the range of the others), the analysis on transformed scale may be somewhat better and hence preferable.

The significant interaction ($P = 0.026$) may be surprising, but the P-value in the first analysis was not so far from significance, and more importantly the concept of interaction is scale-dependent. That is, additivity (i.e., no interaction) exists (perfectly) on one scale, but after non-linear transformation the additivity no longer holds (perfectly), the question of statistical significance being another issue. In any case, it is entirely reasonable and not too uncommon to see a significant interaction on one scale and a non-significant interaction on another scale. Our first interpretation of the interaction based on the interaction plot would be that differences between acclimation groups are temperature-dependent and indeed seem to become smaller as the temperature increases. At low temperatures, the cold-acclimated fish seem to have higher ventilation rates.

If we decided to use the analysis on square-root scale, we would base our conclusions on the interaction plot and suitable pairwise comparisons between group means. The listing above included means for the interaction, and we repeat the table here with the backtransformed means (interpretable as median ventilation rates) included as well:

temp	acclimation	rootvent	vent
10	cold	7.5471	56.95
10	warm	5.6287	31.69
12	cold	7.3740	54.38
12	warm	5.9772	35.72
15	cold	8.7318	76.24
15	warm	7.6559	58.61
22	cold	9.3219	86.90
22	warm	8.7079	75.83
25	cold	11.0648	122.43
25	warm	11.2811	127.26

In order to carry out pairwise comparisons, we compute a LSD-value for unadjusted comparisons, using $t^* = t_{.975}(170) = 1.974$ (from Minitab):

$$\text{LSD}(.95) = t^* \cdot s\sqrt{2/18} = 1.974 \cdot 1.444\sqrt{2/18} = 0.95.$$

This means, any two of the rootvent means in the table are significantly different by unadjusted pairwise comparisons, if they differ by more than 0.95.

Within two combined factors it is often most natural to carry out only comparisons with one factor fixed. First, we compare the two acclimations within each temperature: it is seen that significance holds for temperatures 10, 12 and 15, but not for 22 and 25 degrees. At 10, 12 and 15 degrees, the fish from cold acclimation have higher ventilation. Next we compare temperatures within each acclimation:

- acclimation = cold: $10, 12 < 15, 22 < 25$,
- acclimation = warm: $10, 12 < 15, 22 < 25$.

So we have the same pattern in the two acclimation groups.

If one wants to adjust for multiple comparisons, the Bonferroni method can be used with a reduced number of comparisons corresponding to the ones just carried out. The number of comparisons carried out was

$$5 \cdot 1 + 2 \cdot (5 \cdot 4/2) = 25,$$

substantially less than the total number of comparisons among 10 groups ($10 \cdot 9/2 = 45$). The adjusted t^* value therefore corresponds to a tail probability of $0.025/25 = 0.001$, and is found (using software) to be 3.139. Therefore, the adjusted LSD becomes: $3.139 \cdot s\sqrt{2/18} = 1.51$. With this value, substantially less of the comparisons become significant; for example, only the acclimation comparison at temperature 10 degrees is significant.