

Box 5.1 | Worked example: crab and burrow density on Christmas Island

Green (1997) studied the ecology of red land crabs on Christmas Island and examined the relationship between the total biomass of red land crabs and the density of their burrows within 25 m² quadrats (sampling units) at five forested sites on the island. We will look at two of these sites: there were ten quadrats at Lower Site (LS) and eight quadrats at Drumsite (DS). Scatterplots and boxplots are presented in Figure 5.3. There was slight negative skewness for biomass and burrow density for LS, and an outlier for burrow density for DS, but no evidence of nonlinearity. Pearson's correlation coefficient was considered appropriate for these data although more robust correlations were calculated for comparison.

Site	Correlation type	Statistic	P value
DS (n = 8)	Pearson	0.392	0.337
	Spearman	0.168	0.691
	Kendall	0.036	0.901
LS (n = 10)	Pearson	0.882	0.001
	Spearman	0.851	0.002
	Kendall	0.719	0.004

The H_0 of no linear relationship between total crab biomass and number of burrows at DS could not be rejected. The same conclusion applies for monotonic relationships measured by Spearman and Kendall's coefficients. So there was no evidence for any linear or more general monotonic relationship between burrow density and total crab biomass at site DS.

The H_0 of no linear relationship between total crab biomass and number of burrows at LS was rejected. The same conclusion applies for monotonic relationships measured by Spearman and Kendall's coefficients. There was strong evidence of a linear and more general monotonic relationship between burrow density and total crab biomass at site LS.

Box 5.3 | Worked example of linear regression analysis: coarse woody debris in lakes

Christensen *et al.* (1996) studied the relationships between coarse woody debris (CWD) and shoreline vegetation and lake development in a sample of 16 lakes in North America. The main variables of interest are the density of cabins (no. km^{-1}), density of riparian trees (trees km^{-1}), the basal area of riparian trees ($\text{m}^2 \text{km}^{-1}$), density of coarse woody debris (no. km^{-1}), basal area of coarse woody debris ($\text{m}^2 \text{km}^{-1}$).

CWD basal area against riparian tree density

A scatterplot of CWD basal area against riparian tree density, with a Loess smoother fitted, showed no evidence of a nonlinear relationship (Figure 5.13(a)). The boxplots of each variable were slightly skewed but the residuals from fitting the linear regression model were evenly spread and there were no obvious outliers (Figure 5.13(b)). One lake (Tenderfoot) had a higher Cook's D_i than the others that was due mainly to a slightly higher leverage value because this lake had the greatest riparian density (X-variable). Omitting this lake from the analysis did not alter the conclusions so it was retained and the variables were not transformed.

The results of the OLS fit of a linear regression model to CWD basal area against riparian tree density were as follows.

	Coefficient	Standard error	Standardized coefficient	t	P
Intercept	-77.099	30.608	0	-2.519	0.025
Slope	0.116	0.023	0.797	4.929	<0.001
Correlation coefficient (r) = 0.797, r^2 = 0.634					
Source	df	MS	F	P	
Regression	1	3.205×10^4	24.303	<0.001	
Residual	14	1318.969			

The t test and the ANOVA F test cause us to reject the H_0 that β_1 equals zero. Note that $F(24.307) = t^2(4.929)$, allowing for rounding errors. We would also reject the H_0 that β_0 equals zero, although this test is of little biological interest. The r^2 value (0.634) indicates that we can explain about 63% of the total variation in CWD basal area by the linear regression with riparian tree density.

We can predict CWD basal area for a new lake with 1500 trees km^{-1} in the riparian zone. Plugging 1500 into our fitted regression model:

$$\text{CWD basal area} = -77.099 + 0.116 \times 1500$$

the predicted basal area of CWD is $96.901 \text{ m}^2 \text{km}^{-1}$. The standard error of this predicted value (from Equation 5.10) is 37.900, resulting in a 95% confidence interval for true mean CWD basal area of lakes with a riparian density of 1500 trees km^{-1} of ± 81.296 .

CWD basal area against cabin density

A scatterplot of CWD basal area against cabin density, with a Loess smoother fitted, showed some evidence of a nonlinear relationship (Figure 5.14(a)). The boxplot of

cabin density was highly skewed, with a number of zero values. The residuals from fitting the linear regression model to untransformed data suggested increasing spread of residuals with an unusual value (Arrowhead Lake) with a low (negative) predicted value and a much higher Cook's D_i than the others (Figure 5.14(b)). Following Christensen *et al.* (1996), we transformed cabin density to $\log_{10}$ and refitted the linear model. The scatterplot of CWD basal area against $\log_{10}$ cabin density suggested a much better linear relationship (Figure 5.15(a)). The boxplot of $\log_{10}$ cabin density was less skewed but the residuals from fitting the linear regression model still showed increasing spread with increasing predicted values. Lake Arrowhead was no longer influential but Lake Bergner was an outlier with a moderate Cook's D_i . Finally, we fitted a linear model when both variables were $\log_{10}$ transformed. The scatterplot of $\log_{10}$ CWD basal area against $\log_{10}$ cabin density suggested a slightly less linear relationship (Figure 5.16(a)) and the boxplot of $\log_{10}$ CWD basal area was now negatively skewed. The residuals from fitting the linear regression model were much improved with constant spread and no observations were particularly influential.

Overall, transforming both variables seems to result in a linear model that fits best to these data, although we will present the analysis with just cabin density transformed as per Christensen *et al.* (1996). The results of the OLS fit of a linear regression model to CWD basal area against $\log_{10}$ cabin density were as follows.

	Coefficient	Standard error	Standardized coefficient	<i>t</i>	<i>P</i>
Intercept	121.969	13.969	0	8.732	<0.001
Slope	-93.301	18.296	-0.806	-5.099	<0.001
Correlation coefficient (<i>r</i>) = -0.806, <i>r</i> ² = 0.650					
Source	df	MS	<i>F</i>		<i>P</i>
Regression	1	3.284 × 10 ⁴	26.004		<0.001
Residual	14	1262.870			

The *t* test and the ANOVA *F* test cause us to reject the H_0 that β_1 equals zero. We would also reject the H_0 that β_0 equals zero, although this test is of little biological interest, especially as the slope of the relationship is negative.

Figure 5.13 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of CWD basal area against riparian tree density. (b) Scatterplot of residuals against predicted CWD basal area from linear regression of CWD basal area against riparian tree density.

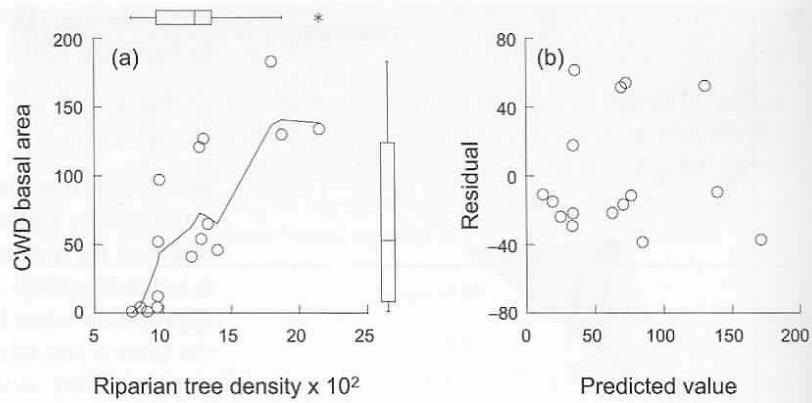
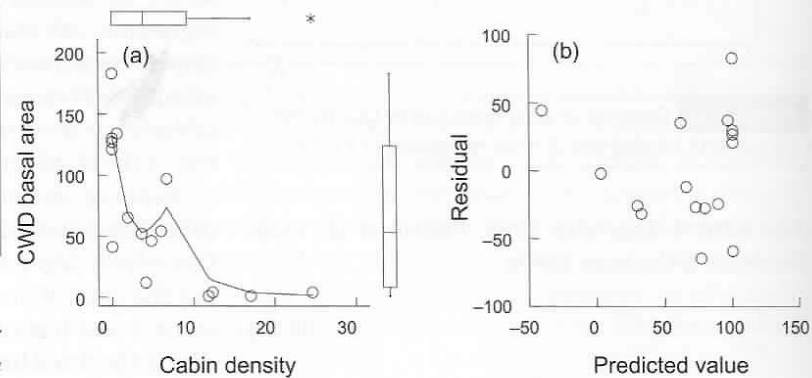


Figure 5.14 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of CWD basal area against cabin density. (b) Scatterplot of residuals against predicted CWD basal area from linear regression of CWD basal area against cabin density.



the standard error of the correlation coefficient (r). Note again the close relationship

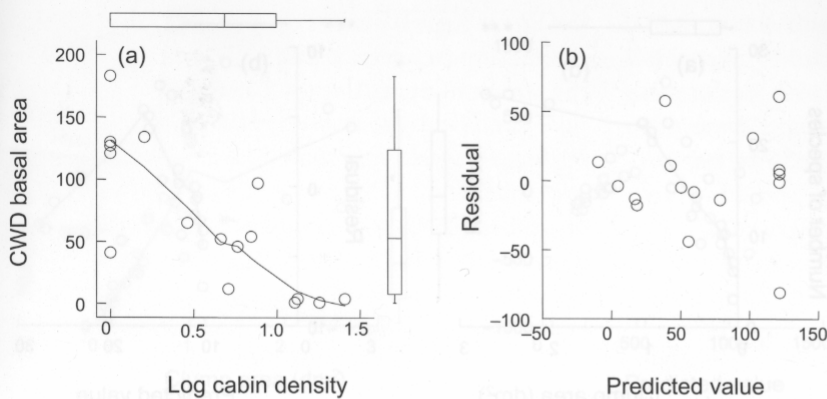


Figure 5.15 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of CWD basal area against $\log_{10}$ cabin density. (b) Scatterplot of residuals against predicted CWD basal area from linear regression of CWD basal area against $\log_{10}$ cabin density.

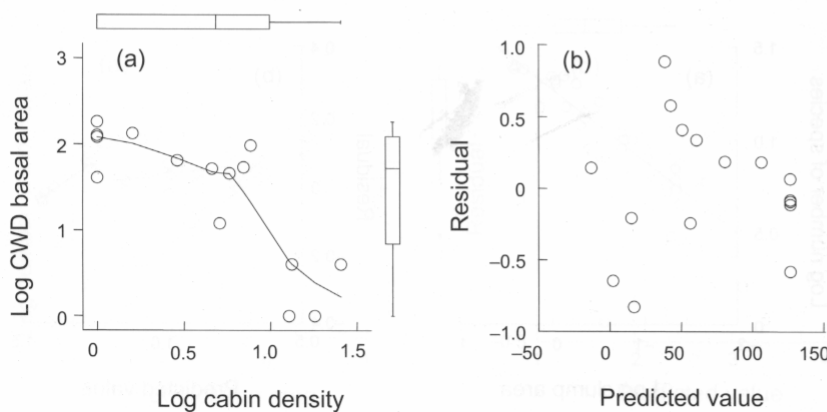


Figure 5.16 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of $\log_{10}$ CWD basal area against $\log_{10}$ cabin density. (b) Scatterplot of residuals against predicted $\log_{10}$ CWD basal area from linear regression of $\log_{10}$ CWD basal area against $\log_{10}$ cabin density.

because the test of the H_0 that the correlation coefficient equals zero is the same test. A more sophisticated decision

Box 5.4 Worked example of linear regression analysis: species richness of macroinvertebrates in mussel clumps

Peake & Quinn (1993) investigated the relationship between the number of species of macroinvertebrates, and the total abundance of macroinvertebrates, and area of clumps of mussels on a rocky shore in southern Australia. The variables of interest are clump area (dm^2), number of species, and number of individuals.

Number of species against clump area

A scatterplot of number of species against clump area, and the plot of residuals against predicted number of species from a linear regression analysis, both suggested a nonlinear relationship (Figure 5.17(a,b)). Although only clump area was positively skewed, Peake & Quinn (1993) transformed both variables because of the nature of the species–area relationships for other seasons in their study plus the convention in species–area studies to transform both variables.

The scatterplot of log number of species against log clump area (Figure 5.18) linearized the relationship effectively except for one of the small clumps. The residual plot also showed no evidence of nonlinearity but that same clump had a larger residual and was relatively influential (Cook's $D_i = 1.02$). Reexamination of the raw data did not indicate any problems with this observation and omitting it did not alter the conclusions from the analysis (b_1 changed from 0.386 to 0.339, r^2 from 0.819 to 0.850, all tests still $P < 0.001$) so it was not excluded from the analysis. In fact, just transforming clump area produced the best linearizing of the relationship with no unusually large residuals or Cook's D_i statistics but, for the reasons outlined above, both variables were transformed.

The results of the OLS fit of a linear regression model to log number of species and log clump area were as follows.

	Coefficient	Standard error	Standardized coefficient	t	P
Intercept	1.270	0.024	0	52.237	<0.001
Slope	0.386	0.038	0.905	10.215	<0.001
Correlation coefficient (r) = 0.905, r^2 = 0.819					
Source	df	MS	F	P	
Regression	1	1.027	104.353	<0.001	
Residual	23	0.010			

The t test and the ANOVA F test cause us to reject the H_0 that β_1 equals zero. We would also reject the H_0 that β_0 equals zero, indicating that the relationship between species number and clump area must be nonlinear for small clump sizes since the model must theoretically go through the origin. The r^2 value (0.819) indicates that we can explain about 82% of the total variation in log number of species by the linear regression with log clump area.

Number of individuals against clump area

A scatterplot of number of individuals against clump area, with a Loess smoother fitted, suggested an approximately linear relationship (Figure 5.19(a)). The plot of residuals against predicted number of individuals from a linear regression model

fitted to number of individuals against clump area (Figure 5.19(b)) showed a clear pattern of increasing spread of residuals against increasing predicted number of individuals (or, equivalently, clump area); the pattern in the residuals was wedge-shaped. The boxplots in Figure 5.19(a) indicated that both variables were positively skewed so we transformed both variables to logs to correct for variance heterogeneity.

The scatterplot of log number of individuals against log clump area (Figure 5.20(a)) showed an apparent reasonable fit of a linear regression model, with symmetrical boxplots for both variables. The residual plot showed a more even spread of residuals with little wedge-shaped pattern (Figure 5.20(b)).

The results of the OLS fit of a linear regression model to log number of individuals and log clump area were as follows.

	Coefficient	Standard error	Standardized coefficient	t	P
Intercept	2.764	0.045	0	60.766	<0.001
Slope	0.835	0.071	0.927	11.816	<0.001
Correlation coefficient (r) = 0.927, r^2 = 0.859					
Source	df	MS	F		P
Regression	1	4.809	139.615		<0.001
Residual	23	0.034			

The t test and the ANOVA F test cause us to reject the H_0 that β_1 equals zero. We would also reject the H_0 that β_0 equals zero, although this test is of little biological interest. The r^2 value (0.859) indicates that we can explain about 86% of the total variation in log number of individuals by the linear regression with log clump area.

Figure 5.17 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of number of species against clump area. (b) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of residuals against predicted number of species from linear regression of number of species against clump area.

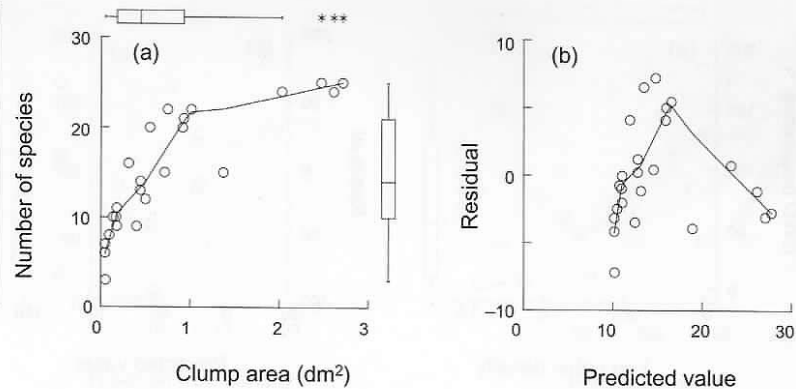
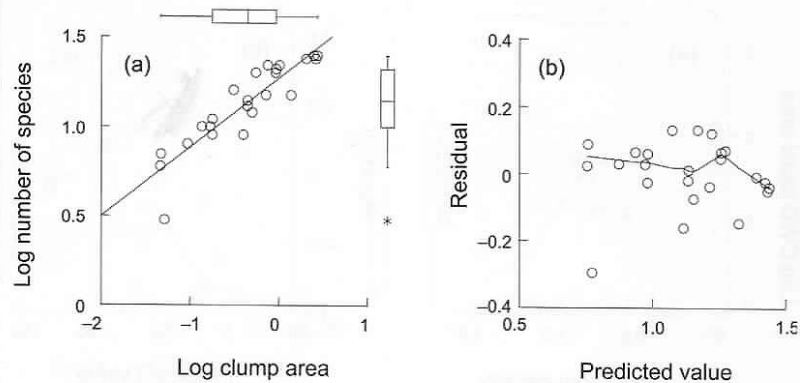


Figure 5.18 (a) Scatterplot (with linear regression line fitted) of $\log_{10}$ number of species against $\log_{10}$ clump area. (b) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of residuals against predicted number of species from linear regression of $\log_{10}$ number of species against $\log_{10}$ clump area.



covariance (see Chapter 12). Both Herrera (1992) and Trussell (1997) tested whether

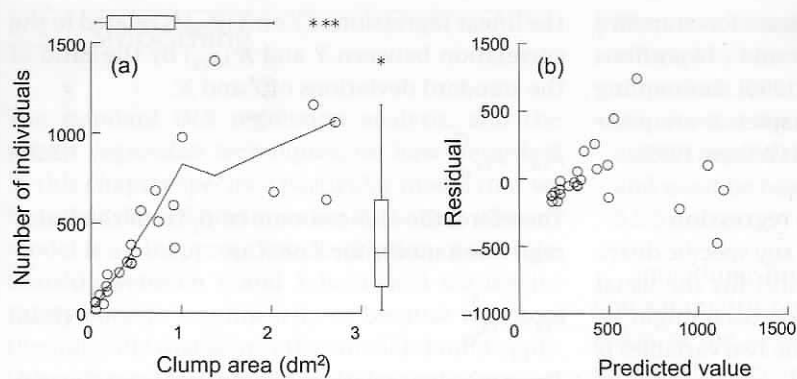


Figure 5.19 (a) Scatterplot (with Loess smoother, smoothing parameter = 0.5) of number of individuals against clump area. (b) Scatterplot of residuals against predicted number of individuals from linear regression of number of individuals against clump area.

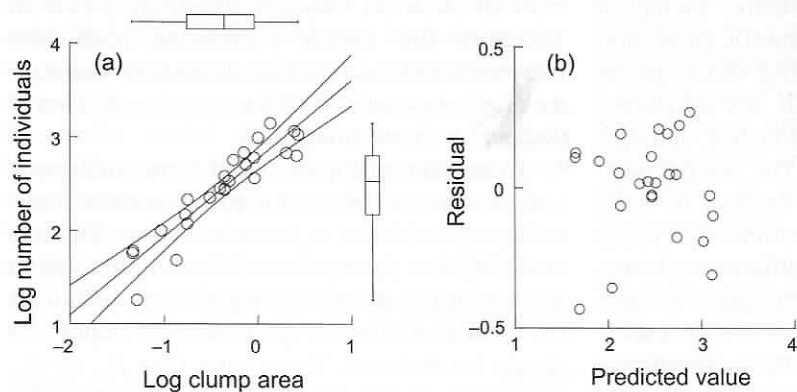


Figure 5.20 (a) Scatterplot (with linear regression line and 95% confidence band fitted) of $\log_{10}$ number of individuals against $\log_{10}$ clump area. (b) Scatterplot of residuals against predicted number of individuals from linear regression of $\log_{10}$ number of individuals against $\log_{10}$ clump area.

cases (Birkes & Dodge 1993). Huber M-estimators, described

Box 3.1 | Fecundity of predatory gastropods

Ward & Quinn (1988) collected 37 egg capsules of the intertidal predatory gastropod *Lepsiella vinosa* from the littorinid zone on a rocky intertidal shore and 42 capsules from the mussel zone. Other data indicated that rates of energy consumption by *L. vinosa* were much greater in the mussel zone so there was interest in differences in fecundity between the zones. The H_0 was that there is no difference between the zones in the mean number of eggs per capsule. This is an independent comparison because individual egg capsules can only be in either of the two zones.

Zone	<i>n</i>	Mean	Median	Rank sum	Standard deviation	SE of mean	95% CI for mean
Littorinid	37	8.70	9	1007	2.03	0.33	8.03–9.38
Mussel	42	11.36	11	2153	2.33	0.36	10.64–12.08

Note that standard deviations (and therefore the variances) are similar and box-plots (Figure 4.4) do not suggest any asymmetry so a parametric *t* test is appropriate.

Pooled variance test:

$$t = -5.39, df = 77, P < 0.001.$$

We would reject the H_0 and conclude there was a statistically significant difference in mean number of eggs per capsule between zones.

Effect size (difference between means) = -2.65 (95% CI: -1.674 to -3.635)

Separate variance test:

$$t = -5.44, df = 77, P < 0.001.$$

Note that the *t* values were almost identical and the degrees of freedom were the same, not surprising since the variances were almost identical.

Although there was little justification for a non-parametric test, we also tested the H_0 that there was no difference in a more general measure of location using the Mann–Whitney–Wilcoxon test.

$$U = 304.00, \chi^2 \text{ approximation} = 21.99 \text{ with } 1 \text{ df}, P < 0.001.$$

Again we would reject the H_0 . In this example, the parametric pooled and separate variance *t* tests and non-parametric test all give *P* values < 0.001 .

A randomization test was done to test the H_0 that there is no difference between the mean number of eggs per capsule so that any possible allocation of observations to the two groups is equally likely.

Mean difference = -2.65 , $P < 0.001$ (significant) for difference as or more extreme than observed based on 10 000 randomizations.

Box 3.3 | Orb spider webs and light intensity

Elgar *et al.* (1996) exposed 17 orb spiders each to dim and light conditions and recorded two aspects of web structure under each condition. The H_0 s are that the two variables (vertical diameter and horizontal diameter of the orb web) were the same in dim and light conditions. Because the same spider spun her web in both light conditions, then this was a paired comparison. Boxplots of paired differences for both variables suggested symmetrical distributions with no outliers, so a parametric paired t test is appropriate.

Horizontal diameter (cm):

Mean difference = 46.18, SE difference = 21.49.

$t = 2.15$, $df = 16$, $P = 0.047$ (significant).

So we would reject the H_0 and conclude that, for the population of female orb spiders, there is a difference in the mean horizontal diameter of spider webs between light and dim conditions.

Wilcoxon signed rank $z = -1.84$, $P = 0.066$ (not significant), do not reject H_0 . Note the less powerful non-parametric test produced a different result.

Vertical diameter (cm):

Mean difference = 20.59, SE difference = 21.32.

$t = 0.97$, $df = 16$, $P = 0.349$ (not significant), do not reject H_0 .

So we would not reject the H_0 and conclude that, for the population of female orb spiders, there is no difference in the mean vertical diameter of spider webs between light and dim conditions.

Wilcoxon signed rank $z = -0.78$, $P = 0.434$ (not significant). In this case, the non-parametric test produced the same conclusion as the t test.

Box 8.1 | Worked example: diatom communities in metal-affected streams

Medley & Clements (1998) sampled a number of stations (between four and seven) on six streams known to be polluted by heavy metals in the Rocky Mountain region of Colorado, USA. They recorded zinc concentration, and species richness and species diversity of the diatom community and proportion of diatom cells that were the early-successional species, *Achanthes minutissima*.

Species diversity versus zinc-level group

The first analysis compares mean diatom species diversity (response variable) across the four zinc-level groups (categorical predictor variable), zinc level treated as a fixed factor. The H_0 was no difference in mean diatom species diversity between zinc-level groups. Boxplots of species diversity against group (Figure 8.1 (a)) showed no obvious skewness; two sites with low species diversity were highlighted in the background and medium zinc groups as possible outliers. The results from an analysis of variance from fitting a linear model with zinc level as the predictor variable were as follows.

Source	SS	df	MS	F	P
Zinc level	2.567	3	0.856	3.939	0.018
Residual	6.516	30	0.217		
Total	9.083	33			

The residual plot from this model (Figure 8.1 (b)) did not reveal any outliers or any unequal spread of the residuals, suggesting the assumptions of the ANOVA were appropriate. Additionally, Levene's test produced no evidence that the H_0 of no differences in variances of species diversity between the zinc-level groups should be rejected (Levene-mean: $F_{3,30} = 0.087$, $P = 0.967$; Levene-median: $F_{3,30} = 0.020$, $P = 0.996$).

Tukey's pairwise comparison of group means: mean differences with Tukey adjusted P values for each pairwise comparison in brackets.

	Background	Low	Medium	High
Background	0.000 (1.000)			
Low	0.235 (0.746)	0.000 (1.000)		
Medium	0.080 (0.985)	0.315 (0.515)	0.000 (1.000)	
High	0.520 (0.122)	0.755 (0.012)	0.440 (0.209)	0.000 (1.000)

The only H_0 to be rejected is that of no difference in diatom diversity between sites with low zinc and sites with high zinc.

We could also analyze these data with more robust methods, especially if we were concerned about underlying non-normality or outliers. To test the H_0 that there is no difference in the location of the distributions of diatom diversity between

zinc levels, irrespective of the shape of these distributions, we would use the Kruskal–Wallis non-parametric test based on ranks sums.

Zinc level	Rank sum
Background	160.0
Low	183.0
Medium	166.5
High	85.5

The Kruskal–Wallis H -statistic equals 8.737. The probability of getting this value of one more extreme when the H_0 is true (testing with a chi-square distribution with 3 df) is 0.033, so we would reject the H_0 .

We might also consider a randomization test, where we reallocate observations to the four groups at random many times to generate a distribution of a suitable test statistic. We used Manly's (1997) program RT, the percentage of total SS attributable to zinc levels (groups) as the statistic and used 1000 randomizations. The percentage of SS_{Total} accounted for by SS_{Groups} was 28.3% and the probability of getting this value or one more extreme if the H_0 of no effects of zinc level on diatom diversity was true was 0.023. Again, we would reject the H_0 at the 0.05 level.

Species diversity versus stream

The second analysis compared diatom species diversity across the streams. Streams are treated as a random factor, assuming these streams represent a random sample of all possible streams in this part of the Rocky Mountains. The H_0 then is that there is no added variance (above the variation between stations) due to differences in diatom species diversity between streams in this part of the Rocky Mountains.

Source	SS	df	MS	F	P
Stream	1.828	5	0.366	1.411	0.251
Residual	7.255	28	0.259		
Total	9.083	33			

The residual plot (Figure 8.2) indicates no variance heterogeneity, although the sample sizes within each stream are too small for useful boxplots. We used the ANOVA, ML and REML methods to estimate the two variance components (σ_ϵ^2 and σ_α^2). ML and REML estimates are tedious to calculate by hand so we used SPSS (Ver 9.0) to obtain these estimates. Confidence intervals (95%) are provided for σ_ϵ^2 only; unequal sample sizes preclude reliable confidence intervals for σ_α^2 .

Method	Estimate of σ_ϵ^2	Estimate of σ_α^2
ANOVA	0.259 (0.159–0.452)	0.0189
ML	0.257	0.0099
REML	0.258	0.0205

Note that there is little difference in the estimates of σ_ϵ^2 , although both ML and REML estimates will be biased. The estimates of σ_α^2 differ considerably between estimation methods, however. Based on Section 8.2.1, the REML estimate of 0.0205 is probably the most reliable. Most of the variance is due to differences between stations within streams rather than due to differences between all possible streams.

Box 9.4 Worked example of two factor fixed effects ANOVA

Quinn (1988) examined the effects of season (winter/spring and summer/autumn) and adult density (8, 15, 30 and 45 animals per 225 cm² enclosure) on the production of egg masses by intertidal pulmonate limpets (*Siphonaria diemenensis*). There were three replicate enclosures per treatment combination and the response variable was the number of egg masses per limpet in each enclosure.

The null hypotheses were as follows.

No difference between mean number of egg masses laid in each season, pooling densities.

No difference in mean number of egg masses laid at each density, pooling seasons.

No interaction between season and density, i.e. the effect of density on mean numbers of egg masses laid is independent of season and vice versa.

Source	df	MS	F	P
Density	3	1.76	9.67	0.001
Linear	1	5.02	27.58	<0.001
Quadratic	1	0.24	1.29	0.272
Season	1	3.25	17.84	0.001
Density × season	3	0.06	0.30	0.824
Residual	16	0.18		

There were no outliers and the residual plot (Figure 9.4(a)) did not suggest problems with assumptions. There was no evidence of an interaction ($P = 0.824$, see Figure 9.5(a)). There were significant effects of season (more egg masses in winter/spring than summer/autumn) and density. The main effect of density was further analyzed with orthogonal polynomials (see Chapter 8 and Section 9.2.10). There was a significant negative linear trend in egg mass production with density but no quadratic trend.

Quinn (1988) did a similar experiment at a lower level of the same shore where the limpets were larger. Different densities were used (6, 12, 24) but the same two seasons with three replicate enclosures per treatment combination. The null hypotheses were the same as above, except that there were only three densities. Again, the residual plot did not suggest any problem with variance heterogeneity (Figure 9.4(b)).

Source	df	MS	F	P
Density	2	2.00	13.98	0.001
Season	1	17.15	119.85	<0.001
Density × season	2	0.85	5.91	0.016
Density 6 vs 12 & 24 × season	1	1.53	10.66	0.007
Linear density × season	1	1.44	10.07	0.008
Residual	12	0.14		

There was a significant interaction between density and season ($P = 0.016$, Figure 9.5(b)). Treatment–contrast interaction tests showed that the comparison between control density and increased density varied between seasons and the linear trend in density was also significantly different between seasons. We also tested simple main effects of density separately for each season.

Source	df	MS	F	P
Winter density	2	0.17	1.21	0.331
Summer density	2	2.67	18.69	<0.001
Residual	12	0.14		

The effect of density was only significant in summer, not in winter. Note that the original MS_{Residual} was used for both tests.

