

On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance

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Summary

1. An analysis of variance (ANOVA) or other linear models of the residuals of a simple linear regression is being increasingly used in ecology to compare two or more groups. Such a procedure (hereafter, 'residual index') was used in 8% and 2% of the papers published during 1999 in the *Journal of Animal Ecology* and in *Ecology*, respectively, and has been recently recommended for studying condition.

2. Although the residual index is similar to an analysis of covariance (ANCOVA), it is not identical and is incorrect for at least four reasons:

(i) the regression coefficient used by the residual index differs from the one used in ANCOVA and is not the least-squares estimator of the model.

(ii) in contrast to the ANCOVA, the error d.f. in the residual index are overestimated because of the estimation of the regression coefficient.

(iii) the residual index also assumes the homogeneity of regression coefficients (parallelism assumption), which should be tested with a special ANCOVA design.

(iv) even if the assumptions of the linear model hold for the original variables, they will not hold for the residuals.

3. More importantly, the residual index is an *ad hoc* sequential procedure with no statistical justification, unlike the well-known ANCOVA. For these reasons, I suggest that a *t*-test or an ANOVA of the residuals should never be used in place of an ANCOVA to study condition or any other variable.

Key-words: analysis of variance, ANCOVA, condition factor, regression analysis, statistical analysis.

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Introduction

In ecology and many other disciplines, we often aim to compare a response variable among several treatments or groups after removing the effect of a third, concomitant variable. An example is the comparison of body condition among several animal populations or groups. Body condition is often measured as the weight (mass) relative to the length of the animal. Weight and length are strongly correlated and the concept of body condition emerges as the plumpness, fatness, or wellbeing of the animal after partialling out that correlation, i.e. the size effect. Similar questions in ecology are the comparisons of richness–area relationships among regions,

of root: shoot ratios of plants among several treatments, and of C : N ratios among sites. These types of question were dealt with in about 25% (27 of 99 and 44 of 227) of ecological papers (Table 1). A classical difficulty encountered in these and many other contexts is how to remove the size effect or concomitant variable. Three main statistical procedures have been applied to this end.

The most primitive solution to remove the size effect is to compute a ratio by dividing the response variable (*Y*) by the concomitant variable (*X*) or some power of it (see comprehensive review in Albrecht, Gelvin & Hartman 1993). Among the simplest forms are a 'simple ratio' (*Y/X*) (Albrecht *et al.* 1993) or Fulton's condition factor (*Y/X*³) in the fish literature (García-Berthou & Moreno-Amich 1993). These ratios are then analysed with general linear models, e.g. comparing different groups with a *t*-test or ANOVA. Although this procedure has been criticized unanimously (see e.g. Atchley, Gaskins & Anderson 1976; Packard & Boardman 1988;

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Table 1. Number of articles (and first page of the articles, between parentheses; ‘–’ = not given) using certain statistical tests to study body condition or other variables in volume 68 of the *Journal of Animal Ecology* (1999) and volume 80 of *Ecology* (1999). Total number of papers was 99 and 227, respectively (excluding 4 ‘Forum’ and 3 ‘Comments’ papers, respectively, in which no statistical analyses were given)

| Statistical method used | <i>Journal of Animal Ecology</i> | | <i>Ecology</i> | |
|--|----------------------------------|-------------------------|----------------|--------------------|
| | Condition† | Other variables | Condition† | Other variables |
| Statistical test of a ratio | 0 | 1 (698) | 1 (2793) | 12 (–) |
| ANCOVA‡ | 2 (571, 753) | 20 (–) | 2 (989, 1289) | 25 (–) |
| Statistical test of regression residuals | 4 (73, 571, 595, 753) | 4 (205, 726, 815, 1010) | 2 (989, 1267) | 3 (735, 806, 1522) |
| Any of the above§ | 4 | 24 | 4 | 40 |

†Papers studying the body condition (i.e. mass relative to length) of animals. Of the seven papers studying condition and not using ANCOVA, five used ANOVA and two used simple linear regression. ‡Including multiple linear regression with dummy variables (see text). §It is not the sum of papers because more than one method was used in a few papers. Combining papers studying condition and other variables, the figures for ‘any of the above’ are 27 for the *Journal of Animal Ecology* and 44 for *Ecology*.

Jackson, Harvey & Somers 1990; Albrecht *et al.* 1993), it is still sometimes used in ecology (Table 1).

The analysis of covariance (ANCOVA) has been pointed to repeatedly as a superior alternative to condition factors (see, e.g. Le Cren 1951; García-Berthou & Moreno-Amich 1993) and similar ratios (Packard & Boardman 1988; Jackson *et al.* 1990). ANCOVA is used widely in ecology, appearing in 22% and 12% of the papers published during 1999 in the *Journal of Animal Ecology* and in *Ecology*, respectively (Table 1).

A third procedure has appeared recently in the ecological literature. This procedure (hereafter ‘residual index’) was recommended recently by Jakob, Marshall & Uetz (1996) for studying condition. The residual index is computed for each individual (experimental or sampling unit) as the residual from the simple linear regression of volume or mass (appropriately transformed) on the length variable (see below for the formula and Fig. 1 for an illustration). These residuals are then compared among groups with a *t*-test or an analysis of variance (ANOVA). This procedure was used in 8% and 2.2% of the 1999 papers of the *Journal of Animal Ecology* and *Ecology*, respectively, particularly for the study of condition (Table 1). The objectives of this paper are: (1) to point out several pitfalls of the residual index, and (2) to emphasize the use of ANCOVA as the correct alternative.

The residual index vs. the ANCOVA

The procedure of the ‘residual index’ is conceptually similar to the standard design of the one-factor ANCOVA. The model of this ANCOVA design is:

$$Y_{ij} = \mu + \alpha_i + \beta_{within} (X_{ij} - \bar{X}_i) + \varepsilon_{ij},$$

where Y_{ij} are the values of the response (dependent) variable for the j th subject of the i th group, X_{ij} is the covariate (concomitant variable), μ is the grand mean (of the variable), α_i is the Y -intercept (or treatment effect) for the i th group, β_{within} is the slope, \bar{X}_i is the covariate mean for the i th group, and ε_{ij} is the random

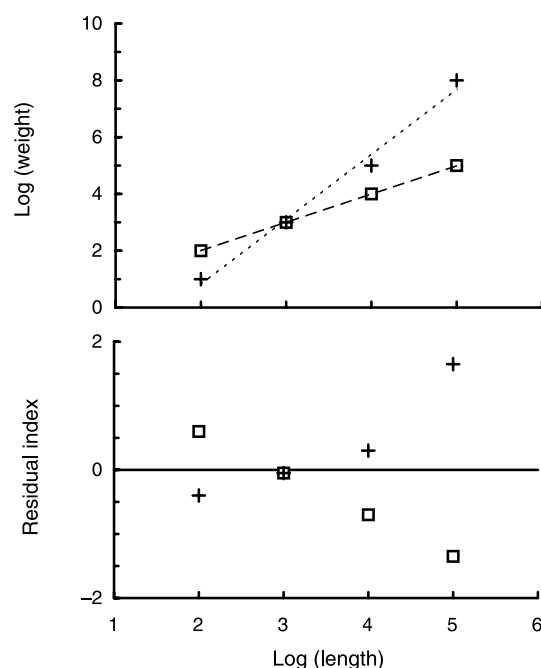


Fig. 1. Hypothetical example of a study of condition with heterogeneity of slopes. Top: weight–length data. The length values are 2, 3, 4, and 5 and the weight values are 2, 3, 4, and 5 for the control and 1, 3, 5 and 8 for the treatment group. The variables are supposed to have been log-transformed to fit the conventional weight–length relationship ($Y = aX^b$). Simple linear regressions for each group are shown. Bottom: the residual index plotted with length. The residual index is computed as the deviation of the weight datum from the overall regression line (pooling the two groups). +, Treatment; □, control.

deviation (see, e.g. Sokal & Rohlf 1995: 503). As suggested by Porter & Raudenbush (1987) and Winer, Brown & Michels (1991), this model can be rewritten as:

$$Y_{ij} - \mu - \beta_{within} (X_{ij} - \bar{X}_i) = \alpha_i + \varepsilon_{ij}.$$

The term on the right of this expression is similar to that of a one-factor ANOVA model ($Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$), whereas the term on the left is similar to the ‘residual index’ (see, e.g. Jakob *et al.* 1996):

residual index = $Y_{ij} - a - bX_{ij} = Y_{ij} - \bar{Y} - b(X_{ij} - \bar{X})$,

since $a = \bar{Y} - b\bar{X}$, where a and b are the intercept and regression coefficient, respectively, of the simple linear regression. Although the ANCOVA is thus quite similar to an ANOVA of the residuals or to an ANOVA of the adjusted values (Winer *et al.* 1991: 747), it is not identical and yields a different statistical result for a number of reasons (see Maxwell, Delaney & Manheimer 1985 for a comprehensive discussion). First, standard ANCOVA uses a pooled regression coefficient within groups (b within) for adjusting the data (Sokal & Rohlf 1995: 507). This ' b within' is a weighted average of the regression coefficients for each group (Keppel 1991: 313) and generally differs from the conventional regression coefficient found on pooling the data, i.e. ignoring the groups, termed ' b overall' by Winer *et al.* (1991: 757). This overall b is the coefficient used by the residual index. The ' b within' is the least-squares estimator in the ANCOVA model, whereas the overall b is the estimator in the linear regression model (ignoring the groups) but should not be used if the objective is to compare the groups (Maxwell *et al.* 1985; Winer *et al.* 1991).

Another important difference is that in the standard ANCOVA the error degrees of freedom (d.f.) are $N-k-1$, where N is the total sample size and k is the number of groups (Sokal & Rohlf 1995: 509). In contrast, in a t -test or ANOVA of the residual index the error d.f. are $N-k$ although they should be reduced by one, as was performed in a similar procedure by Packard & Boardman (1988), due to the fact that the slope is estimated from the same data (Maxwell *et al.* 1985; Keppel 1991: 312; Winer *et al.* 1991: 749). The different d.f. affect the error mean square and hence the F and P -values. Without considering the different regression coefficients, an overestimated error d.f. implies an underestimated error mean square and hence a higher Type I error rate.

Some numerical examples will illustrate these differences. For instance, Sokal & Rohlf (1995: 512) describe thoroughly an example of the standard design of ANCOVA in which the result for the factor (among groups) is $F_{3,16} = 195.98$, $P < 0.0005$. An ANOVA of the residual index for the same data set (without correcting the degrees of freedom) would result in $F_{3,17} = 39.6$, $P < 0.0005$. Similarly, an ANCOVA reported in Fig. 2c of Packard & Boardman (1988) was $F_{1,13} = 6.10$, $P = 0.028$, whereas the ANOVA of the residual index yields $F_{1,14} = 6.54$, $P = 0.023$. An artificial data set for which the two procedures give opposite conclusions ($P < 0.0005$ and $P = 0.33$, respectively) is (X, Y) : (1, 2) (3, 3) (5, 4) and (7, 4) for one group and (11, 12) (13, 13) (15, 14) and (17, 14) for another. Although the overall conclusion is probably similar for most real data sets, the statistical results differ for the reasons explained above. For instance, for Sokal & Rohlf's example the ' b within' is 21.1 while the overall b is 17.3. The error d.f. are overestimated by one in the ANOVA of the residuals.

Another advantage of ANCOVA is its flexibility in implementing any aspect of the experimental design

(e.g. factorial, nested or repeated-measured designs). For instance, a special design with the factor \times covariate interaction allows us to test the assumption of homogeneity of regression coefficients (parallelism assumption), which is essential to the standard ANCOVA (García-Berthou & Moreno-Amich 1993; Sokal & Rohlf 1995). Jakob *et al.* (1996) criticized the 'slope-adjusted ratio index' because it assumes the homogeneity of slopes, without realizing that their residual index also makes this assumption. A hypothetical example will show that the residual index can lead to incorrect biological conclusions if this assumption is not satisfied. An ANOVA of the residual index of the data in Fig. 1 would not detect significant treatment effects on condition (without correcting the degrees of freedom, $F_{1,6} = 1.49$, $P = 0.27$), because these effects are size-dependent (ANCOVA, $F_{1,4} = 56.3$, $P = 0.002$), i.e. the treatment increases the condition of larger individuals and decreases the condition of smaller individuals. In these situations when the parallelism assumption is not met, Huitema (1980: 104) suggests ignoring the adjusted treatment effects test (i.e. the factor of the standard ANCOVA) and computing and reporting separate regression slopes for all groups and/or employing the Johnson–Neyman procedure.

A final, more technical problem is that even if the assumptions of the linear model hold for the original variables, they will not hold for the residuals. Theil (1965) showed that the residuals will necessarily be intercorrelated and heteroscedastic (see also Hayes & Shonkwiler 1996).

Conclusions

ANCOVA is a well-established statistical procedure that has received an enormous amount of attention and scrutiny in the literature. General linear models such as ANOVA, ANCOVA or simple linear regression involve the computation of various residuals and the graphical analysis of residuals is also essential in order to verify the assumptions of most statistical tests. However, there is no statistical reference to justify the direct ANOVA of the residuals (residual index) as a correct alternative to the ANCOVA. On the contrary, Maxwell *et al.* (1985) already showed in a insightful psychometric paper that ANOVA of residuals is an incorrect procedure. More recently, Hayes & Shonkwiler (1996) thoughtfully pointed out that, although there are some complex equivalencies in the simplest cases, such tests of residuals are unnecessary. Similarly, Smith (1999) has criticized a similar use of the residuals in a comprehensive review of the statistics of sexual size dimorphism. He suggested that multiple linear regression (or other general linear models) should be generally used instead of using residuals as data, which leads to misspecified regression equations. Note that ANCOVA can also be computed via multiple linear regression with dummy variables (see, e.g. Neter, Wasserman & Kutner 1985: 853; Kleinbaum, Kupper & Muller 1988: 299).

In summary, I suggest that the 'residual index' should never be used for statistical analyses of condition or any other variable. The various designs of ANCOVA or other linear models of the original response variable are the reliable, well-known methodology called for. Excellent accounts of ANCOVA are given for instance in Neter *et al.* (1985), Keppel (1991) and Sokal & Rohlf (1995) (see also Winer *et al.* 1991 for a more mathematical treatment). A more comprehensive monograph on ANCOVA and alternative techniques is by Huitema (1980).

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