

CONFIDENCE INTERVALS FOR THE OPTIMUM IN THE GAUSSIAN RESPONSE FUNCTION

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Abstract. The optimum of a species on a gradient is an important parameter for ecological interpretation and bioindication. The location of the optimum is easily estimated in the popular Gaussian response model, but it is more difficult to assess the precision of the estimated optima. Methods based on the profile likelihood or quasilielihood function are presented to find confidence intervals for the optimum parameter of the Gaussian response function using generalized linear models. The following four cases are considered: optimum on one gradient; optimum on one gradient when there are additional stratifying variables; optimum on an interesting gradient at a certain level of a stratifying variable when the optimum is dependent on the latter; and simultaneous confidence region for the joint overall optimum on two gradients. The methods are illustrated with two species of testate amoebae (Protozoa: Rhizopoda) in Finnish mires. The first two cases were also analyzed using Fieller's theorem, although it produced generally wider limits.

Key words: community ecology; confidence intervals, estimating; Gaussian response function in gradient analysis; generalized linear models; gradient analysis; overdispersion; profile likelihood; Protozoa; quasilielihood; testate amoebae in mires, ecological optima.

INTRODUCTION

The simplest models predicting species abundances on ecological gradients have at least three characteristics: the location, the size (or height), and the width of the response. The Gaussian response function is a popular alternative (Gauch 1982, Austin et al. 1984, ter Braak and Looman 1986), its parameters being directly interpretable in these terms. The optimum on the gradient gives the location, the maximum abundance attained at the optimum gives the size, and the tolerance parameter gives the width of the response. Some other models may give response curves of the same general form as the Gaussian function but without such an easily interpretable parametrization (Huisman et al. 1993). More parameters are needed if skewness (Huisman et al. 1993) and/or kurtosis (Austin 1976, Minchin 1987) are estimated in addition to location, width, and height of the response. For instance, the proposed beta-function (Austin et al. 1994) fails since it does not have a sufficient number of parameters (Oksanen 1997). The Gaussian function still remains as a good and effective three-parameter model for ecological gradient analysis (Austin et al. 1984, ter Braak and Looman 1986, Oksanen et al. 1988).

The optimum on a gradient is often regarded as the most interesting parameter for ecological interpreta-

tion. Moreover, species ordinations in correspondence analysis, a popular method for ecological ordination, have been justified as estimates of species optima on axes (ter Braak 1985). In addition, weighted averaging, which apparently is based solely on species optima (or some measures possibly related to them), seems to be able to estimate original gradients rather well (ter Braak and Barendregt 1986, Birks et al. 1990).

The Gaussian response function is most easily fitted as a generalized linear model (ter Braak and Looman 1986, Oksanen et al. 1988) defined as a second-degree polynomial with logarithmic link function. In this case, the interesting parameters are not estimated directly, but are derived from the polynomial coefficients of the corresponding log-linear or logistic model. The point estimates of the Gaussian parameters thus obtained are identical with those obtained directly using a nonlinear maximum-likelihood regression for the original Gaussian function. However, in addition to point estimates, confidence intervals for the parameters are also needed for evaluation of statistical precision of the results, and for statistical inference.

Confidence intervals for the optimum demand more involved calculations as the Gaussian optimum is a function of two polynomial parameters, but Fieller's theorem can be used to derive equations to approximate the confidence limits of the optimum (ter Braak and Looman 1986). These equations can be used either for a single gradient, or for several gradients, provided that there are no interactions or mutual dependencies between the gradients.

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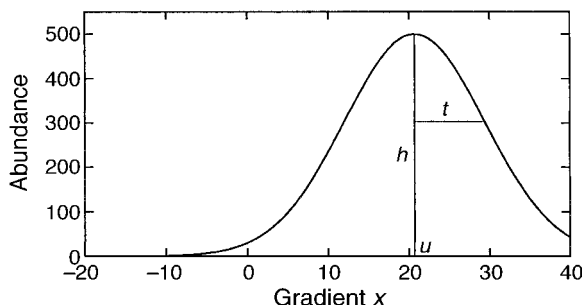


FIG. 1. The Gaussian response function is defined by three parameters: location of the optimum u on the gradient x , the tolerance t , and the maximum height h . The parameterization corresponds to testate amoeba *Trigonopyxis arcuata* on the sample depth gradient discussed in this paper.

A more sophisticated method for calculating confidence intervals of the optimum is based on the profile-likelihood method (Nelder and Pregibon 1987, Venzon and Moolgavkar 1988, Aitkin et al. 1989, Hilborn and Mangel 1997), which does not use the stronger approximations needed in Fieller's theorem (ter Braak and Looman 1986), and does not directly rely on the approximate normality of the error distribution of the estimated polynomial coefficients. Moreover, it can be used even when there are interactions between gradients, and to obtain confidence intervals for the location of the overall optimum in two-dimensional gradient space, where the method based on Fieller's theorem does not apply.

In this paper we show how confidence intervals of the Gaussian optimum parameter can be estimated using the profile-likelihood method. Using two species of testate amoebae (Protozoa: Rhizopoda) in mires as an example, we compare the profile-likelihood-based limits with those based on Fieller's theorem. Moreover, we show how the profile method can be used in situations where Fieller's theorem does not apply. We give a method of estimating confidence intervals for an interesting gradient at different levels of another gradient when the optimum on the interesting gradient is dependent on another gradient. We also show how to estimate a region for the location of joint overall optimum in a two-gradient Gaussian response function.

MATHEMATICAL REPRESENTATION OF THE GAUSSIAN RESPONSE FUNCTION

We treat separately the case with a single gradient and the cases with two gradients with and without interaction terms.

The case of single gradient

The Gaussian response function for one gradient estimates the expected abundance μ for a species as a three-parameter function of the value of the gradient (x) (Fig. 1):

$$\mu = h \exp \left[-\frac{(x - u_x)^2}{2t_x^2} \right]. \quad (1)$$

Here the parameters are the maximum expected abundance (h), the optimum on the gradient (u_x), and the tolerance (t_x). The maximum is obtained at the optimum (i.e., when $x = u_x$), and the tolerance describes how fast the expected abundance drops from the maximum as the distance from the optimum increases. The expected abundance at the distance of one tolerance unit from the optimum is 60.7% of the maximum, and at the distance of three tolerance units it is 1.1%.

The Gaussian function (Eq. 1) is nonlinear in its basic form. Its parameters can be estimated using nonlinear regression programs (Gauch and Chase 1974, Oksanen et al. 1988). However, a much more efficient procedure is to fit an equivalent log-linear model that is a special case of generalized linear models (McCullagh and Nelder 1989, Crawley 1993):

$$\mu = \exp(a + b_1x + b_2x^2). \quad (2)$$

This was first applied by Austin et al. (1984). This polynomial form is easily fitted with readily available software for the generalized linear models (Payne 1986, Ihaka and Gentleman 1996, Venables and Ripley 1999), and non-normal error distributions, such as Poisson or binomial models, can be more easily applied than with nonlinear regression.

Eq. 2 is equivalent to a Gaussian response function provided that $b_2 < 0$, being then simply a reparameterization of Eq. 1. The Gaussian parameters can be easily found by the following formulae (ter Braak and Looman 1986, Oksanen et al. 1988):

$$u_x = -\frac{b_1}{2b_2} \quad (3)$$

$$t_x = \sqrt{-\frac{1}{2b_2}} \quad (4)$$

$$h = \exp \left(a - \frac{b_1^2}{4b_2} \right). \quad (5)$$

The case of one gradient and a covariate without interaction

We may have another environmental variable z that perhaps is not of direct interest, but the effect of which we want to adjust for in our analysis concerning x . We first consider a simple extension of Eq. 1 to two gradients, assuming a bivariate Gaussian function without mutual interaction effect (or product term) of x and z :

$$\mu = h \exp \left\{ -\frac{1}{2} \left[\left(\frac{x - u_x}{t_x} \right)^2 + \left(\frac{z - u_z}{t_z} \right)^2 \right] \right\}. \quad (6)$$

An equivalent polynomial form is

$$\mu = \exp(a + b_1x + b_2x^2 + c_1z + c_2z^2) \quad (7)$$

which decomposes into separate factors due to x and z

$$\mu = \exp(a)\exp(b_1x + b_2x^2)\exp[g(z)] \quad (8)$$

where $g(z) = c_1z + c_2z^2$. The location of the optimum and the tolerance (u_x and t_x) are recovered by Eq. 3 and Eq. 4, respectively, and, if desired, u_z and t_z by interchanging the variables and the coefficients. An essential feature of this model is that the response pattern with respect to x , in particular the optimum and the tolerance, is not dependent on the value of the second gradient. The formula, Eq. 8, is easily generalized for any other suitable functional form $g(z)$ for z and can also be extended to include more than one covariate, and even categorical covariates, insofar as product term with x can be avoided.

The case of two gradients with interaction

In many applications involving two gradients, x and z , it is more realistic to extend the one-gradient model Eq. 1 into a more general bivariate Gaussian function:

$$\mu = h \exp \left\{ -\frac{1}{2(1-r^2)} \left[\left(\frac{x-u_x}{t_x} \right)^2 + \left(\frac{z-u_z}{t_z} \right)^2 - 2r \left(\frac{x-u_x}{t_x} \right) \left(\frac{z-u_z}{t_z} \right) \right] \right\} \quad (9)$$

which, in addition to Eq. 6, contains a product term between x and z and the associated interaction parameter r describing the degree of linear association of the joint effects of x and z . The equivalent polynomial form

$$\mu = \exp(a + b_1x + b_2x^2 + c_1z + c_2z^2 + dxz). \quad (10)$$

As indicated by Jongman et al. (1987:57), Eq. 10 defines a two-dimensional Gaussian response function provided that $(4b_2c_2 - d^2) > 0$ and $(b_2 + c_2) < 0$.

We first consider the determination of the joint bivariate optimum point (u_x, u_z), the components of which are related to the polynomial coefficients of (10) by

$$u_x = \frac{b_1}{-2b_2} \left(1 + \frac{p_1d}{q} \right) \quad (11)$$

$$u_z = \frac{c_1}{-2c_2} \left(1 + \frac{p_2d}{q} \right) \quad (12)$$

where $q = 4b_2c_2 - d^2$, $p_1 = d - 2b_2c_1/b_1$ and $p_2 = d - 2c_2b_1/c_1$.

The tolerance parameter in each dimension is obtained from

$$t_x = \sqrt{\frac{1}{-2b_2} \left(1 + \frac{d^2}{q} \right)} \quad (13)$$

$$t_z = \sqrt{\frac{1}{-2c_2} \left(1 + \frac{d^2}{q} \right)} \quad (14)$$

and the interaction parameter is

$$r = \frac{d}{\sqrt{4b_2c_2}}. \quad (15)$$

The expressions for tolerance reduce to those in Eqs.

3 and 4 when there is no interaction ($r = 0$, $d = 0$) and Eqs. 6 and 7 hold.

Second, we may be interested in how the response is related to x at a given value z of the other gradient. We can factor Eq. 9 into a product of the marginal response function for z and the conditional response function for x at a given value of z :

$$\mu = h \exp \left[-\frac{1}{2} \left(\frac{z-u_z}{t_z} \right)^2 \right] \exp \left[-\frac{1}{2} \left(\frac{x-u_{x|z}}{t_{x|z}} \right)^2 \right] \quad (16)$$

in which the conditional optimum and tolerance of x at a given z are in terms of the parameters of Eq. 9:

$$u_{x|z} = u_x + r \left(\frac{t_x}{t_z} \right) (z - u_z) \quad (17)$$

$$t_{x|z} = t_x \sqrt{1 - r^2}. \quad (18)$$

In terms of the polynomial coefficients of Eq. 10 the conditional optimum is

$$u_{x|z} = \frac{b_1 + dz}{-2b_2}. \quad (19)$$

Interchanging the role of x and z , similar expressions are obtained for the conditional optimum on z given x .

POINT ESTIMATION AND CONFIDENCE REGION FOR OPTIMA

Principles of the profile-deviance method

A sophisticated and theoretically sound way of finding confidence intervals for a parameter is based on profile likelihood. In the profile-likelihood method the value of the interesting parameter is temporarily fixed, and with this constraint the remaining parameters are estimated by maximizing the likelihood with respect to them. This is repeated over a grid of conceivable values of the fixed parameter, and the resulting values of the maximized likelihood for the different parameter values give the profile likelihood. The profile-likelihood curve is concave and its highest level is achieved when the interesting parameter has its value at its unconstrained maximum-likelihood estimate (Hilborn and Mangel 1997).

Within generalized linear models (McCullagh and Nelder 1989) an equivalent approach is to use the deviance statistic, which is derived from the log-likelihood function, and thus a profile deviance can be formed (Aitkin et al. 1989). The deviance is always positive, and it is minimized when the profile log-likelihood is maximized. When a given model is correct, the deviance follows asymptotically the chi-square distribution (with 1 degree of freedom), and thus the significance of the difference in deviance can be directly evaluated at the different fixed values of the variable. Even when the variance is higher than assumed in the correct model, a related statistic called "quasideviance" can be used instead. Consequently, if at a fixed point the increase in deviance from its minimum value

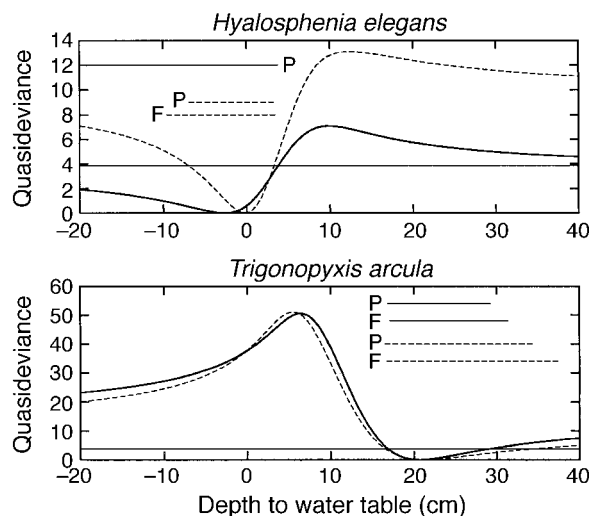


FIG. 2. Principle of the profile-quasideviance method: the optimum is fixed at different values of the gradient, a model is fitted, and the quasideviance is recorded. Those values for which the quasideviance is less than 3.84 larger than that for the maximum-likelihood estimate belong to the 95% asymptotic confidence interval for the optimum. The labeled horizontal lines show the estimated 95% confidence intervals with the profile-quasideviance method (P) and Fieller's approximate method (F). The solid lines and curves show the case for a single gradient, and the broken lines and curves the case with pH as an additional background variable.

is smaller than the selected critical value, the point belongs to the corresponding confidence interval. A commonly used confidence level is 95%, which corresponds to the critical value of 3.84 in chi-square distribution with 1 df, and so all the points whose deviance is less than 3.84 higher than the minimum deviance at the point estimate belong to a confidence interval (Fig. 2).

In ecological data, residual variances are often larger than assumed by the strict likelihood model. This phenomenon is called "overdispersion" (McCullagh and Nelder 1989). Overdispersion can be generated in several alternative and complementary ways. First, we can hardly ever assume that the expected species abundances could be determined from one or two gradients only. Many other gradients, measured and unmeasured, also have an effect on the abundances. Therefore the expected value can be viewed as a random variable with its own error distribution, which is another component of dispersion in addition to the postulated error about the expected value. Moreover, although the expected value could be determined exactly, there are biotic factors that cause some additional variation. Misspecification of the response may also produce overdispersion.

With overdispersed data, the chi-square distribution is no longer a valid approximation for the deviance statistic but tends to give too narrow confidence intervals. Instead a quasilielihood approach (McCullagh

and Nelder 1989) may be used, so that all deviances (and their differences) are divided by the estimated scale parameter to obtain the quasideviance. Inference is based on the assumption that quasideviance follows approximately the chi-square distribution (or, more accurately, the F distribution, as advocated by Anderson [1988]).

Single gradient

For a single gradient, Eq. 2 can be used and the optimum (u_x) can be found by Eq. 3. However, the optimum u_x is not a parameter of Eq. 2 and therefore the profile-deviance method cannot be used directly to estimate its confidence intervals. Therefore a transformed variable x_m may be used, defined by

$$x_m = x - m. \quad (20)$$

If $m = u_x$ this only transforms the origin of x to the optimum u_x without affecting the gradient in other ways, and Eq. 2 reduces to

$$\mu = \exp(a + b_2 x_m^2) \quad (21)$$

where $a = \ln(h)$ and $b_2 = -1/(2t_x^2)$.

Eq. 21 defines a Gaussian response function with a constraint that the optimum is fixed at m , as defined by Eq. 20. Therefore Eqs. 21 and 20 together can be used to obtain the profile deviance as a function of m and for finding confidence intervals for the optimum. When $m = \hat{u}_x$, i.e., at the unconstrained maximum-likelihood estimate of the optimum, the deviance will be minimized, and for all other values of m the deviance will be larger. Since one parameter (u_x) was fixed, Eq. 21 has only two parameters (a, b_2) to be estimated and thus one residual degree of freedom more than the unconstrained Gaussian Eq. 2 with three free parameters. The quasideviance is distributed asymptotically as chi-square (with 1 df), and hence all values of m that have a deviance difference less than 3.84 compared $m = \hat{u}_x$ belong to the 95% asymptotic confidence interval of the optimum (see Fig. 2).

Expressions based on Fieller's theorem can be used to obtain approximate confidence intervals for the optimum (ter Braak and Looman 1986, Jongman et al. 1987). Naturally, when these are used the overdispersion must also be taken into account. Similarly as above, this can be achieved by multiplying covariances and variances of the parameter estimates of the polynomial expression Eq. 2 by the estimated scale parameter.

One gradient with a covariate

In this case the profile-deviance-based confidence intervals for the optimum can be found using the transformed variable x_m (Eq. 20) in the following equation:

$$\mu = \exp(a + b_2 x_m^2 + c_1 z + c_2 z^2). \quad (22)$$

Only one parameter is fixed, and thus the comparison is based on 1 degree of freedom. Similarly, Fieller's

theorem can be directly used (ter Braak and Looman 1986). Just like the ordinary polynomial expression (Eq. 7), this can be decomposed into separate factors due to x and z (Eq. 8), and so there may be more than one covariate, or the covariate may have other than the polynomial form without influencing the method of estimating confidence intervals for optimum u_x . However, the additional covariates may influence confidence intervals on x .

One gradient with a covariate and interaction

For the profile-deviance method we have to replace x by x_m :

$$\mu = \exp(a + b_2 x_m^2 + c_1 z + c_2 z^2 + dx_m z). \quad (23)$$

Here the only fixed parameter is optimum of x . All coefficients for the variables containing z are still estimated. Therefore the profile-deviance method is again based on 1 degree of freedom comparison. The method of ter Braak and Looman (1986) cannot be used in this case.

Joint optimum on two gradients

We may wish to estimate the confidence region where the joint overall optimum is located. For this purpose we have to fix two points, the coordinates of the optimum both on x and z . We use again transformed variables $x_m = x - m$ and $z_n = z - n$, where n is the fixed trial value for the optimum u_z of z , and fit a model

$$\mu = \exp(a + b_2 x_m^2 + c_2 z_n^2 + dx_m z_n). \quad (24)$$

Now the comparisons are based on 2 degrees of freedom, since two parameters are fixed. Hence the relevant distribution is chi-square with 2 df.

Finding confidence regions on a plane is much more tedious than calculating a one-dimensional interval on a line. Often it is sufficient to find the limits to certain directions only. If the border seems to be reasonably smooth, it is possible to interpolate the limits also to other directions.

This method was essentially used by Oksanen et al. (1990b). The regression model they used was a simple one without the interaction term ($d = 0$) and with circular response contours ($b_2 = c_2$), and thus Eq. 24 was simplified into

$$\mu = \exp[a + b_2(x_m^2 + z_n^2)]. \quad (25)$$

Even in that case the confidence region was rather irregular in shape, and it was difficult to say what was the limit between evaluated directions.

EXAMPLE

Material and methods

The estimation of confidence intervals for the optimum is illustrated here using data on testate amoebae (Protozoa: Rhizopoda) in 89 virgin mires in southern Finland at 61°–62° N, 24°–25°39' E (Tolonen et al.

1992). The species *Hyalosphenia elegans* Leidy and *Trigonopyxis arcuata* Leidy were selected to display different cases of estimating confidence intervals.

The numbers of testate amoebae were estimated taking a measured mass of peat to which was added a known number of *Lycopodium* spores as an exotic marker (Stockmarr 1971). The ratio of testate amoebae to exotic markers was used to transform the observed counts into densities per unit volume using the measured bulk density of peat. Models were designed to predict the abundance as counts per cubic centimeter, but the statistical goodness of the model was assessed with the observed counts per sample. This was achieved in generalized linear models using an offset variable, which is an explanatory variable with regression coefficient of 1 (Payne 1986, Venables and Ripley 1999). With logarithmic link function, we used $-\log(k)$ as the offset, where k is the transformation coefficient. The coefficient k varied widely (12.5 to 213) in the data, and therefore the use of offset was essential for the correct statistical assessment.

We used initially the program GLIM (Payne 1986), but these analyses can be performed with several other programs such as the “glm” procedures in R (Ihaka and Gentleman 1996) and S-plus (Venables and Ripley 1999). Confidence intervals were first found by a set of GLIM macros we wrote for the purpose. Later on we fitted the models in R (Ihaka and Gentleman 1996, Venables and Ripley 1999), and the functions for basic analysis in R or S-plus are available in the Appendix. The limits from the profile quasideviance were found iteratively. Confidence intervals based on Fieller's theorem were estimated according to ter Braak and Looman (1986). In both methods, confidence intervals were adapted to overdispersion using deviance divided by the number of residual degrees of freedom as the scale parameter. The overdispersion was usually large: the estimates of the scale parameter were about 8.6 to 10.0 in the cases reported here.

Results

The depth of the sample relative to the water table (being positive if the sample is above the water table) was found in preliminary inspection to be an important gradient to which the testate amoebae in general seemed to have a unimodal response.

Rather similar confidence intervals were produced both by the profile-quasideviance method and Fieller's theorem, but the profile quasideviance gave slightly narrower limits (Fig. 2). Obviously, the approximations and normality assumption used by ter Braak and Looman (1986) worked satisfactorily. No confidence limits were found by Fieller's theorem for *Hyalosphenia*, but the upper limit could still be found with the profile-deviance method (Fig. 2).

Acidity (pH) of free water at the site was used as a secondary gradient, since it also seemed to be a fairly important gradient and independent of depth to the wa-

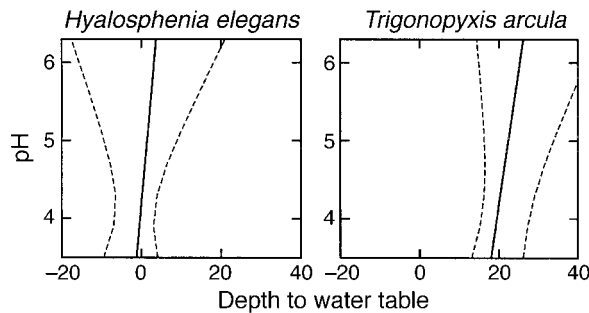


FIG. 3. Optimum at the gradient of depth relative to water table and its 95% asymptotic confidence intervals at different levels of pH when the optimum depth is dependent on pH.

ter table. Its use as a subsidiary gradient did not influence much the confidence limits on sample depth in *Trigonopyxis* (Fig. 2). However, in *Hyalosphenia* the limits became much narrower; they could be estimated with Fieller's theorem, and both limits were found with the profile quasideviance (Fig. 2).

The profile quasideviance was convex close to the estimated optimum, but it started to turn down further away (Fig. 2). Our profile-quasideviance method fixes the maximum of the response curve at a certain position x_m in Eq. 21. Close to the optimum this is indeed at the maximum of the response curve, but further away the second-degree coefficient b_2 may be positive, and so Eq. 21 does not define a Gaussian-type response function, but a response with the minimum at x_m . The change of sign of b_2 seems to happen just at the highest point of the profile deviances in Fig. 2.

When estimating confidence intervals in two-gradient models, we restricted our search to the observed range of the gradients (depth to water table: -20 to 40 cm; pH: 3.5 to 6.3). In some cases the confidence limits were found outside these boundaries. These limits would be unrealistic and have little practical value, and therefore they are not displayed in the graphics.

The width of the confidence limits on the water depth varied with pH, being narrowest at the mid-range of pH (Fig. 3).

In the simultaneous evaluation of the confidence region in two dimension, confidence intervals were evaluated in eight directions, but generally the limits are so regular that the intervals between estimated directions may also be interpolated (Fig. 4). Although the limits are in general fairly narrow, they extend in each case outside the studied range at least in one direction.

DISCUSSION

We have considered the profile-quasilikelihood or profile-quasideviance approach for estimating a confidence interval only for the optimum using Poisson error. However, the same methodology can be directly applied with presence/absence or frequency data by using a Bernoulli or binomial variance in a generalized linear model (McCullagh and Nelder 1989). Moreover,

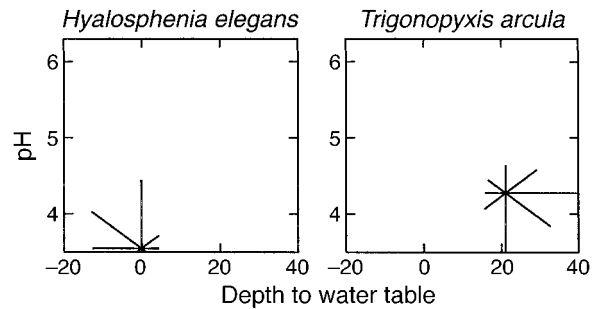


FIG. 4. Location of the overall joint optimum. The lines show the 95% asymptotic confidence intervals to principal directions in the two-gradient Gaussian model with depth relative to water table and pH. The intersection of the lines shows the location of the optimum in the two-gradient space. Both taxa were fitted without the interaction term since it was not significant.

it is possible to estimate confidence intervals for the tolerance parameter as well. Tolerance is a function of only one polynomial coefficient, and its confidence interval is therefore very simple to estimate by profile-quasideviance method, as well as directly from the standard error of the polynomial parameter (ter Braak and Looman 1986, Jongman et al. 1987). It is also in principle possible to estimate confidence limits for the maximum-likelihood prediction of the gradient value in bioindication or calibration (ter Braak and van Dam 1989, Oksanen et al. 1990a), although these limits seem to be too narrow (i.e., to exclude the real gradient value more often than expected). Moreover, the method is general and can be used for many different types of models.

In our example, we based the confidence limits on the assumption of asymptotic chi-square distribution of the quasideviance (McCullagh and Nelder 1989). For the quasilikelihood models based on quasideviance, it would be more accurate for small data sets to use the F distribution (Anderson 1988), which was actually used by Oksanen et al. (1990b). It will give slightly wider limits, but does not change the principles of estimation.

A model with an additional variable can be considered to be more realistic than a single-gradient model. Often the fit improved and confidence intervals became narrower when a second gradient was taken into account. Part of the overdispersion is caused by variables that have an effect on amoebae but are not included in the single-gradient model, and therefore the expected abundances are not completely determined from a single gradient. This overdispersion can be reduced by additional important gradients. However, some problems in regression analysis are bound to appear if the number of variables is too high; e.g., the explanatory variables may become multicollinearly dependent on each other or the fitted response surface may become complicated and sensitive to leverage of deviant observations (Weisberg 1980). Thus there are some prac-

tical limits to the number of explanatory variables in the model. However, the use of background variables does not necessarily mean that confidence intervals for the optimum would be narrower. In particular, if the effect earlier assigned only to one gradient is confounded with the effect of the second gradient or the second gradient is insignificant, much wider confidence intervals can be produced.

The confidence limits for the optimum were generally fairly wide in our tests. Even in single-gradient cases, one or both of the limits were often outside the realistic range. Our tests with other data sets (for instance diatoms) show that in most cases less confidence can be given to the point estimates of optima than most people would wish. Still the relation of the species to the gradient used here seemed to be in general quite good, and jointly they probably could be used to give quite good predictions of the environmental gradients (Oksanen et al. 1990a).

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LITERATURE CITED

- Aitkin, M., D. Anderson, B. Francis, and J. Hinde. 1989. Statistical modelling in GLIM. Oxford University Press, Oxford, UK.
- Anderson, D. A. 1988. Some models for overdispersed binomial data. *Australian Journal of Statistics* **30**:125–148.
- Austin, M. P. 1976. On non-linear species response models in ordination. *Vegetatio* **33**:33–41.
- Austin, M. P., R. B. Cunningham, and P. M. Fleming. 1984. New approach to direct gradient analysis using environmental scalars and statistical curve-fitting. *Vegetatio* **55**:11–27.
- Austin, M. P., A. O. Nicholls, M. D. Doherty, and J. A. Meyers. 1994. Determining species response functions to an environmental gradient by means of β -function. *Journal of Vegetation Science* **5**:215–228.
- Birks, H. J. B., J. M. Line, S. Juggins, A. C. Stevenson, and C. J. F. ter Braak. 1990. Diatoms and pH reconstruction. *Philosophical Transactions of the Royal Society of London B* **327**:263–278.
- Crawley, M. J. 1993. GLIM for ecologists. Blackwell, Oxford, UK.
- Gauch, H. G., Jr. 1982. Multivariate analysis in community ecology. Cambridge University Press, Cambridge, UK.
- Gauch, H. G., Jr., and G. B. Chase. 1974. Fitting the Gaussian curve to ecological data. *Ecology* **55**:1377–1381.
- Hilborn, R., and M. Mangel. 1997. The ecological detective: confronting models with data. Princeton University Press, Princeton, New Jersey, USA.
- Huisman, J., H. Olff, and L. F. M. Fresco. 1993. A hierarchical set of models for species response analysis. *Journal of Vegetation Science* **4**:37–46.
- Ihaka, R., and R. Gentleman. 1996. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics* **5**:299–314.
- Jongman, R. H., C. J. F. ter Braak, and O. F. R. van Tongeren. 1987. Data analysis in community and landscape ecology. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
- McCullagh, P., and J. A. Nelder. 1989. Generalized linear models. Chapman & Hall, London, UK.
- Minchin, P. R. 1987. An evaluation of relative robustness of techniques for ecological ordinations. *Vegetatio* **69**:89–107.
- Nelder, J., and D. Pregibon. 1987. An extended quasi-likelihood function. *Biometrika* **74**:221–232.
- Oksanen, J. 1997. Why the beta-function cannot be used to estimate skewness of species responses. *Journal of Vegetation Science* **8**:147–152.
- Oksanen, J., E. Läärä, P. Huttunen, and J. Meriläinen. 1988. Estimation of pH optima and tolerances of diatoms in lake sediments by the methods of weighted averaging, least squares and maximum likelihood, and their use for the prediction of lake acidity. *Journal of Paleolimnology* **1**:39–49.
- Oksanen, J., E. Läärä, P. Huttunen, and J. Meriläinen. 1990a. Maximum likelihood prediction of lake acidity based on sedimented diatoms. *Journal of Vegetation Science* **1**:49–59.
- Oksanen, J., S. Tynnyrinen, and L. Kärenlampi. 1990b. Testing for increased abundance of epiphytic lichens on a local pollution gradient. *Annales Botanici Fennici* **27**:301–307.
- Payne, C. D. 1986. The GLIM system release 3.77 manual. Numerical Algorithms Group, Oxford, UK.
- Stockmarr, J. 1971. Tablets and spores used in absolute pollen analysis. *Pollen et Spores* **13**:615–621.
- ter Braak, C. J. F. 1985. Correspondence analysis of incidence and abundance data: properties in terms of a unimodal response model. *Biometrics* **41**:859–873.
- ter Braak, C. J. F., and L. G. Barendregt. 1986. Weighted averaging of species indicator values: its efficiency in environmental calibration. *Mathematical Biosciences* **78**:57–72.
- ter Braak, C. J. F., and C. W. N. Looman. 1986. Weighted averaging, logistic regression and the Gaussian response model. *Vegetatio* **65**:3–11.
- ter Braak, C. J. F., and H. van Dam. 1989. Inferring pH from diatoms: a comparison of old and new calibration methods. *Hydrobiologia* **178**:209–233.
- Tolonen, K., B. G. Warner, and H. Vasander. 1992. Ecology of testaceans (Protozoa: Rhizopoda) in mires in southern Finland. I. Autecology. *Archiv für Protistenkunde* **142**:119–138.
- Venables, W. M., and B. D. Ripley. 1999. Modern applied statistics with S-plus. Third edition. Springer-Verlag, Heidelberg, Germany.
- Venzon, D. J., and S. H. Moolgavkar. 1988. A method for computing profile-likelihood-based confidence intervals. *Applied Statistics* **37**:87–94.
- Weisberg, S. 1980. Applied linear regression. John Wiley & Sons, New York, New York, USA.

APPENDIX

The R and S-plus functions for the analyses presented in this paper are available in ESA's. Electronic Data Archive: *Ecological Archives* E082-015.