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Ecotoxicology and Environmental Safety 59 (2004) 23–30

Ecotoxicology
and
Environmental
Safety

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A nonlinear model of stress hormone levels in rats—the interaction between pollution and parasites

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Received 26 February 2003; received in revised form 27 May 2003; accepted 2 June 2003

Abstract

The impact of an infection with a parasite and a simultaneous cadmium exposure on the stress hormone levels of rats was studied. To this end, we introduce a nonlinear heteroscedastic model, which is able to describe the temporal evolution of cortisol concentrations in groups of rats treated by cadmium or parasite infection. A thorough analysis gives strong evidence that parasitic infection and cadmium exposure affect the stress hormone level of rats in an additive manner. Therefore, the host's response to environmental pollution should be studied in relation to parasite infections.

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Keywords: Nonlinear parametric model; Smoothing spline model; Simultaneous confidence bands; Stress; Pollution; Parasites

1. Introduction

Pollution-induced impacts on the health of organisms are of increasing interest in recent years (e.g., Schüürmann and Markert, 1998). Probably the most intensively studied pollution is the anthropogenic-driven increase of heavy metal levels in the environment (Merian, 1991). Especially toxic metals like Pb, Cd, and Hg have been investigated extensively (Merian, 1991). However, in addition to different forms of pollution, a wide variety of pathogen-induced diseases affect the well-being of plants, animals, and humans. One group of pathogens that threatens the life of other organisms is parasites (protozoans and metazoans), which account for approximately more than 50% of all organisms (Price, 1980). Parasites can significantly reduce the fitness of their hosts (Barnard and Behnke, 1990), often due to interactions with the stress hormone system (e.g., Dunlap and Schall, 1995; Sures et al., 2002). For example, elevation of glucocorticoids like cortisol have deleterious effects in the long run, including inhibition of the reproductive and immune systems (Sapolsky, 1987). On the other hand, decreased

cortisol secretion may also be fatal, as organisms are not able to reestablish their physiological homeostasis after acute stress if their cortisol concentrations are too low.

Under natural conditions, usually a mixture of different forms of stressors, such as endogenous factors, pathogens, pollution, and social interactions, is the cause for deviations from a physiological homeostasis of organisms. The interrelationship among host–parasite associations, and environmental pollution, and the health of the parasitized host has become of interest to ecologists and parasitologists (see reviews by Lafferty, 1997; MacKenzie et al., 1995; Sures, 2001, 2003; Sures et al., 1999). In a recent experiment the impact of a single isolated stressor (cadmium) and a simultaneous infection with a parasite (the acanthocephalan *Moniliformis moniliformis*) on the stress hormone levels of a vertebrate, the laboratory rat, was studied (Sures et al., 2002).

The response to stress was investigated by measuring levels of the stress hormone cortisol by radioimmunoassay. Although corticosterone is the primary glucocorticoid in rats, there is general agreement that both glucocorticoids, corticosterone and cortisol, are regulated in the same way and released in parallel (Saito et al., 1992), which allows cortisol to stand as a general stress measure for adrenocortical function (see, e.g.,

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Milanes et al., 1991). In this experiment, we found some indications that parasite infection reduces cortisol levels in rats. A similar effect was also observed for the oral application of CdCl₂ to rats. Additionally, these effects seem to be synergistic, as rats which were simultaneously infected with the parasite and exposed to Cd showed the most clear reduction in cortisol levels. As it appears from this study that each treatment has an effect on the stress response of rats, it is our goal to find statistical models which describe these responses over time. The models are used subsequently to assess the significance of differences in the stress response between treatment groups, which could not be shown in the study of Sures et al. (2002).

2. Materials and methods

The experiment was described in detail in Sures et al. (2002). Briefly, male Wistar rats of the CD-M strain free of intestinal helminths as proven by spot checks of fecal matter were kept in cages at 20°C and were fed commercial rat pellets. Rats were randomly divided into four groups (exposed to Cd, infected with *M. moniliformis*, exposed and infected, and unexposed and uninfected controls) containing eight animals each. Each individual rat was kept in a separate cage, all cages were placed in the same room, and the treatments were synchronized. For metal contamination, rats were orally exposed to cadmium twice per week for a period of 5 weeks beginning at day 25 postinfection using a cadmium concentration of 5 µg/g body wt. The cadmium was administered in a cadmium chloride solution prepared from solid CdCl₂. As the rats were conditioned to drink sugar solution from Eppendorf pipettes, these solutions supplemented with CdCl₂ were administered to the exposed animals in the same manner. Untreated control sugar solutions were at the same time administered to the unexposed rats so that all animals were treated in the same manner. Infected rats were inoculated with 10 cystacanths of the acanthocephalan *M. moniliformis* dissected from cockroaches (*Periplaneta americana*) which had been infected 2 months previously by being fed a glucose solution which contained eggs collected from a gravid female *M. moniliformis*. Blood was taken twice a week from each rat (same day and time) by puncturing the lateral vein of the tail and using a syringe and drain tubes without anesthetics. After blood samples were centrifuged, cortisol was extracted with ethanol. Afterward, tritium-labeled cortisol and cortisol antiserum according to Kloas et al. (1994) were added. To isolate the antibody–hormone complex, a dextran-activated carbon suspension was added and centrifuged, and the supernatant containing the antibody–hormone complex was transferred to scintillation vials and filled with scintillation solution. A liquid

scintillation counter (Tri Garb 1900 T; Packard, Dreieich, Germany) was used according to Kloas et al. (1994). Concentrations of cortisol were determined as nanograms per milliliter in the serum. The logarithm of cortisol concentrations was used for all statistical analyses.

3. Results

Fig. 1 shows the course of mean values and standard deviations of the log-cortisol concentrations in the different groups of rats after day 25. There are eight animals in group 1 (controls), seven in group 2 (infected rats, one rat died), and eight in group 3 (Cd-exposed rats); group 4 comprises five infected and Cd-exposed rats (the other three rats were not infected at the time of killing, therefore their cortisol concentrations were not considered in the statistical analysis).

In Section 3.1, we introduce a nonlinear model describing cortisol concentrations over time and examine several ramifications. To emphasize the within-subject dependence, we consider a nonlinear mixed-effect model in Section 3.2. The following section treats the construction of asymptotic confidence intervals and simultaneous confidence bands for the group mean values. By comparing our parametric model with a nonparametric smoothing spline model, we examine in Section 3.4 the appropriateness of the parametric model.

3.1. A nonlinear model describes cortisol concentrations over time

Our aim is to model the logarithm of the cortisol levels after day 25 and to elaborate differences between the different treatment groups. After the beginning of the metal exposure, the mean curves show a sharp increase and a subsequent decline (see Fig. 1A) Hence, we propose the following nonlinear model relating the logarithm of cortisol concentration to time:

$$y_{ijk} = \beta_{1i} + \beta_{2i}(\beta_{3i}(t_{ijk} - 25))^3 \times \exp(-\beta_{3i}(t_{ijk} - 25)) + \varepsilon_{ijk} \quad (t_{ijk} \geq 25) \\ i = 1, \dots, 4, \quad j = 1, \dots, M_i, \quad k = 1, \dots, 9, \quad (1)$$

where $M_1 = 8$, $M_2 = 7$, $M_3 = 8$, $M_4 = 5$, and the error terms ε_{ijk} are assumed to be independently distributed as $\mathcal{N}(0, \sigma^2)$. The value of the parameter vector $\beta_i = (\beta_{1i}, \beta_{2i}, \beta_{3i})$ depends on the treatment group. Note also that there are two levels of grouping in the data: the four treatment groups (i) and the within subject group (j).

Hand and Crowder (1996) used the model $y = \beta_1 + \tilde{\beta}_2 t^3 \exp(-\tilde{\beta}_3 t)$ to model blood glucose levels. The two models differ only in the parametrization.

We fitted the model by the maximum likelihood method, using S-PLUS and the nlme library of Pinheiro

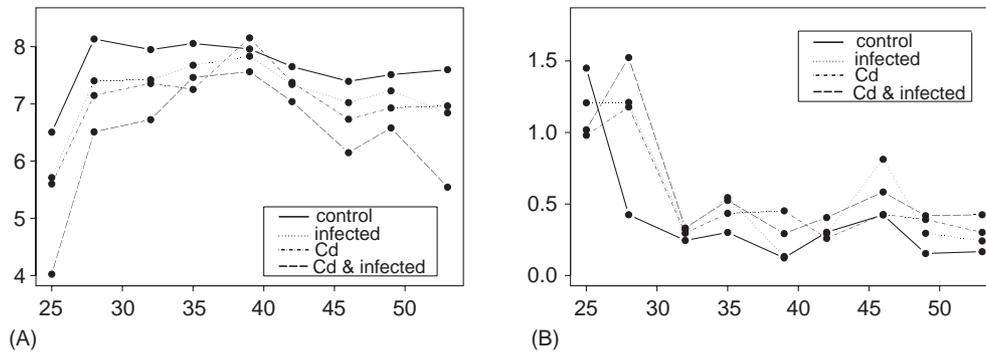


Fig. 1. (A) Mean values and (B) standard deviations of the log-cortisol concentrations in the different treatment groups.

and Bates (2000). An inspection of the standardized residuals and of a quantile–quantile plot of the residuals shows strong deviations from the model assumptions.

3.1.1. A heteroscedastic model

As a first step toward a valid model, we dismiss the assumption of equal variance of the error terms, since Fig. 1B shows a sharp decline of the variance function over time.

To this end, we introduce the variance function

$$\text{Var}(\varepsilon_{ijk}) = \sigma^2(t_{ijk} - 24)^{2\delta_i} \quad (t_{ijk} \geq 25). \quad (2)$$

As for β , the parameter δ may depend on the group. Fitting the model yields the following values:

	Control	Infected	Cadmium	Cd and infected
δ	-0.61	-0.43	-0.42	-0.38

A visual comparison shows that the structure of the empirical standard deviation of the log-cortisol concentrations in the different groups is matched very well by the fitted values of model (2).

The mean curves (estimated again by the maximum likelihood method) are

$$\begin{aligned} E[y_{1jk}] &= 7.50 + 0.46(-0.39(t_{ijk} - 25))^3 \exp(-0.39(t_{ijk} - 25)), \\ E[y_{2jk}] &= 6.76 + 0.73(-0.28(t_{ijk} - 25))^3 \exp(-0.28(t_{ijk} - 25)), \\ E[y_{3jk}] &= 6.59 + 0.80(-0.27(t_{ijk} - 25))^3 \exp(-0.27(t_{ijk} - 25)), \\ E[y_{4jk}] &= 5.32 + 1.66(-0.26(t_{ijk} - 25))^3 \exp(-0.26(t_{ijk} - 25)). \end{aligned} \quad (3)$$

They are drawn together in Fig. 2 to facilitate comparison. Whereas the curves for the infected group and the exposed group are nearly identical, the control group lies above, and the infected-and-exposed group has the lowest values.

Neither a plot of the standardized residuals nor a quantile–quantile plot of the residuals of model (1) with

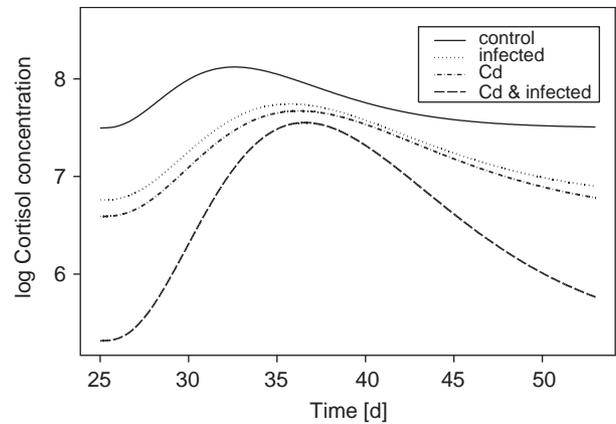


Fig. 2. Estimated mean curves of the log-cortisol concentrations in the different treatment groups.

variance structure (2) shows a noticeable deviation from the model assumptions.

3.1.2. Modeling the correlation structure

The sample autocorrelation function (acf) of the residuals of the heteroscedastic model indicates some dependence between the residuals of the same subject (Fig. 3A). Hence, it is worth considering models with correlated errors.

As the first and simplest correlation structure, we use the model

$$\text{cor}(\varepsilon_{ijk}, \varepsilon_{ijk'}) = \rho, \quad (4)$$

which assumes equal correlation among all errors pertaining to the same subject. The estimated value of ρ is 0.24. Fig. 3B shows the sample acf of the heteroscedastic model with constant correlation structure. There, the sample acf does not show a systematic pattern, but sometimes exceeds the confidence bounds. One should note, however, that these bounds are based on point-wise 95% confidence intervals.

Table 1 shows the (difference of) parameter values in the different groups for the constant error model.

Since the time points are nearly equidistant, we can also use time-series models for the correlation structure of the errors. An autoregressive-moving average model

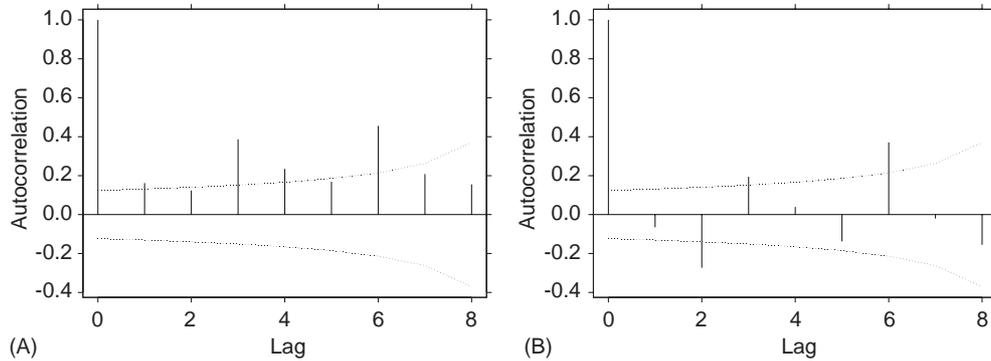


Fig. 3. (A) Sample acf of heteroscedastic model with uncorrelated errors. (B) Sample acf of heteroscedastic model with constant correlation structure.

Table 1
Parameter values in different groups compared to control group for the constant error model

	Coefficient	Value	Standard error	P value ^a
Control	β_{11}	7.51	0.06	<0.001
Infected	$\beta_{12}-\beta_{11}$	-0.71	0.18	<0.001
Cadmium	$\beta_{13}-\beta_{11}$	-0.87	0.19	<0.001
Cd and infected	$\beta_{14}-\beta_{11}$	-2.16	0.27	<0.001
Control	β_{21}	0.48	0.09	<0.001
Infected	$\beta_{22}-\beta_{21}$	0.26	0.17	0.13
Cadmium	$\beta_{23}-\beta_{21}$	0.32	0.17	0.055
Cd and infected	$\beta_{24}-\beta_{21}$	1.18	0.23	<0.001
Control	β_{31}	0.40	0.04	<0.001
Infected	$\beta_{32}-\beta_{31}$	-0.11	0.05	0.033
Cadmium	$\beta_{33}-\beta_{31}$	-0.13	0.05	0.013
Cd and infected	$\beta_{34}-\beta_{31}$	-0.14	0.05	0.004

^a A small P value indicates that the parameter value or the difference in parameter values differs significantly from zero.

of order (p, q) (ARMA(p, q)-model) is defined by

$$\varepsilon_t = \phi_1 \varepsilon_{t-1} + \dots + \phi_p \varepsilon_{t-p} + \theta_1 a_{t-1} + \dots + \theta_q a_{t-q} + a_t,$$

where $\phi = (\phi_1, \dots, \phi_p)$ and $\theta = (\theta_1, \dots, \theta_q)$ are (unknown) parameters, a_t is a centered homoscedastic noise term (independent of the previous observations), and the time points t are assumed to be equidistant. We examined several models; an autoregressive model of order 3 (AR(3)-model), which corresponds to an ARMA(3, 0)-model, clearly yields the best fit. Hence, we choose the model

$$\varepsilon_{ijk} = \phi_1 \varepsilon_{ij(k-1)} + \phi_2 \varepsilon_{ij(k-2)} + \phi_3 \varepsilon_{ij(k-3)} + a_{ijk}, \quad (5)$$

where $a_{ijk} \sim N(0, \sigma^2)$. The estimated values of the coefficients are

$$\phi_1 = 0.113, \quad \phi_2 = 0.016, \quad \phi_3 = 0.468.$$

Looking at the sample acf of the heteroscedastic model with AR(3)-correlation structure, all values are now within the confidence bounds. This seems to be a

clear improvement over the previous model with constant errors; however, the small values of ϕ_1 and ϕ_2 , and the quite large value for ϕ_3 , are conspicuous and not easy to explain.

The fitted values of β_i, σ^2 , and δ_i for both models with correlated errors are nearly identical with the corresponding values in Section 3.1.1. Hence, Fig. 2 remains unchanged when using correlation structure (4) or (5).

3.2. A nonlinear mixed-effects model

As an alternative for modeling the in-subject dependence, we use the following nonlinear mixed-effects model:

$$y_{ijk} = (\beta_{1i} + b_{1ij}) + (\beta_{2i} + b_{2ij})((\beta_{3i} + b_{3ij})(t_{ijk} - 25))^3 \times \exp(-(\beta_{3i} + b_{3ij})(t_{ijk} - 25)) + \varepsilon_{ijk} \quad (t_{ijk} \geq 25),$$

$$i = 1, \dots, 4, \quad j = 1, \dots, M_i, \quad k = 1, \dots, 9,$$

where the random effects $b_{ij} = (b_{1ij}, b_{2ij}, b_{3ij})$ are assumed to be independently distributed as $\mathcal{N}(0, \Psi)$. The statistical analysis showed that a random effect should only be applied to the first coefficient. Hence, we have the model

$$y_{ijk} = (\beta_{1i} + b_{1ij}) + \beta_{2i}(\beta_{3i}(t_{ijk} - 25))^3 \times \exp(-\beta_{3i}(t_{ijk} - 25)) + \varepsilon_{ijk} \quad (t_{ijk} \geq 25), \quad (6)$$

where $b_{1ij} \sim \mathcal{N}(0, \tau^2)$. Fitting the model yields the value $\tau = 0.204$, which is rather small compared to the residual error with value $\sigma = 1.55$.

For the error terms, we used again the variance function (2) and uncorrelated errors, since the sample acf of the residuals of model (6) does not show positive dependence.

A dot-plot of the estimated random effect shows no particular structure (as, for example, between-group differences). Since further diagnostic plots do not indicate violations of the model assumptions, the mixed-effects model seems to be well justified. However, the actual difference of the individual mean curves and the group mean curves is quite small, as can be seen by a

visual inspection of the group predictions vs. the within-subject predictions.

Table 2 shows a statistical comparison of the different heteroscedastic models. Both models with correlated errors and the model (6) with random intercept yield statistically significant improvements over model (1).

The nonlinear mixed-effects model and the model with constant correlation structure have the same number of parameters and comparable AIC (Akaike information criterion) and BIC (Bayesian information criterion) values. The model with AR(3)-error structure has the lowest AIC value and a comparable BIC value.

When looking at these model-selection criteria, it should be noted again that the fitted values of β_i , σ^2 , and δ_i and the corresponding standard errors are nearly identical for the three models. Hence, the statistical findings in this and the following section do not depend on the choice of the correlation structure.

3.3. Asymptotic confidence intervals and bands

Using the usual t -tests, it turns out that there are significant differences between the parameter values of the different groups (Table 1). Whereas such differences are often easy to interpret in linear models, it is more difficult to obtain knowledge about the variability of the mean curve of nonlinear models that way. For this reason, we construct asymptotic confidence intervals and simultaneous confidence bands for the group mean curves of the heteroscedastic model with constant error structure. Since we use maximum likelihood estimation, the estimator $\hat{\beta}_i = (\hat{\beta}_{1i}, \hat{\beta}_{2i}, \hat{\beta}_{3i})'$ has an approximate normal distribution with mean $\beta_i = (\beta_{1i}, \beta_{2i}, \beta_{3i})'$ and covariance matrix Σ_i (see, for example, Pinheiro and Bates, 2000). Σ_i can be consistently estimated by some estimate $\hat{\Sigma}_i$ (which, in the case of maximum likelihood estimation, is the inverse of the information matrix). A Taylor expansion of f around β_i then yields the large sample variance $\nabla_i f'(t) \hat{\Sigma}_i \nabla_i f'(t)$ of $f(t)$, where $\nabla_i f = (\partial f / \partial \beta_{ji})_{1 \leq j \leq 3}$. Hence, approximate point-wise

confidence intervals for the i th group are given by

$$\left(f(t, \hat{\beta}_i) - t_{\alpha/2} \sqrt{\nabla_i f'(t) \hat{\Sigma}_i \nabla_i f'(t)}, \right. \\ \left. f(t, \hat{\beta}_i) + t_{\alpha/2} \sqrt{\nabla_i f'(t) \hat{\Sigma}_i \nabla_i f'(t)} \right),$$

where $t_{\alpha/2}$ is the $(1-\alpha/2)$ quantile of the normal distribution.

Approximate simultaneous confidence bands can be constructed in a similar way following the Scheffé method (Cox and Ma, 1995). The resulting bands are

$$\left(f(\cdot, \hat{\beta}_i) - \sqrt{\chi_{p,\alpha}^2 \nabla_i f' \hat{\Sigma}_i \nabla_i f}, \right. \\ \left. f(\cdot, \hat{\beta}_i) + \sqrt{\chi_{p,\alpha}^2 \nabla_i f' \hat{\Sigma}_i \nabla_i f} \right), \tag{7}$$

where p is the number of unknown parameters, i.e., in our case we have $p = 3$. The simultaneous bands and the point-wise confidence intervals are compared for groups 1 and 4 in Fig. 4.

Note that a Bonferroni correction for the nine time points would lead to the same width of the confidence intervals at the different times as the simultaneous bands in Fig. 4 (since, for $\alpha = 0.05$, $(\chi_{3,\alpha}^2)^{1/2} \approx t_{\alpha/18} \approx 2.8$). However, the confidence bands in (7) are simultaneously valid for each t .

Since we are interested in the difference of the cortisol levels between groups, we also constructed (simultaneous) confidence bands for the difference between two treatment models $f(t, \hat{\beta}_{i_1}) - f(t, \hat{\beta}_{i_2})$. Since the estimates $\hat{\beta}_{i_1}$ and $\hat{\beta}_{i_2}$ are asymptotically independent, the large sample variance for the difference is $(\nabla_{i_1} f' \hat{\Sigma}_{i_1} \nabla_{i_1} f)^2 + (\nabla_{i_2} f' \hat{\Sigma}_{i_2} \nabla_{i_2} f)^2$. Whereas the lower confidence bound for the difference between the control group and the Cd and infected group is nearly always positive, this is no longer the case when comparing the control group with the other treatment groups.

Table 2
Statistical comparison of the different heteroscedastic models

No.	Model	np^a	AIC ^b	BIC ^c	log Lik ^d	Test	P value ^e
1	(1)+(2)	17	425.9	485.9	-195.9		
2	(1)+(2)+(4)	18	398.0	461.5	-181.0	1 vs. 2	<0.0001
3	(1)+(2)+(5)	20	391.6	462.2	-175.8	1 vs. 3	<0.0001
4	(6)	18	399.9	463.4	-182.0	1 vs. 4	<0.0001

^aNumber of parameters in the fitted model.

^bAkaike information criterion, $AIC = -2 \log \text{Lik} + 2 \cdot np$.

^cBayesian information criterion, $BIC = -2 \log \text{Lik} + n \cdot np$, where n is the number of observations.

^dValue of log-likelihood.

^eResult of likelihood ratio test of model (1) vs. models (2),(3), and (4), respectively. The small P values indicate that models (2), (3), and (4) are statistically significant improvements over model (1).

3.4. A smoothing spline model

The main aim of this section is to assess the validity of our parametric model. To this end, we fit a (nonparametric) smoothing spline model to the data and compare it to our parametric nonlinear model.

For the general smoothing spline model we use

$$y_{ijk} = g(t_{ijk}) + \varepsilon_{ijk} \quad (t_{ijk} \geq 25),$$

$i = 1, \dots, 4, j = 1, \dots, M_i, k = 1, \dots, 9$. Here, we assume that g has a square integrable second derivative and

$$\varepsilon_{ij} = (\varepsilon_{ij1}, \dots, \varepsilon_{ij9})' \sim N(0, \sigma^2 W_i^{-1}),$$

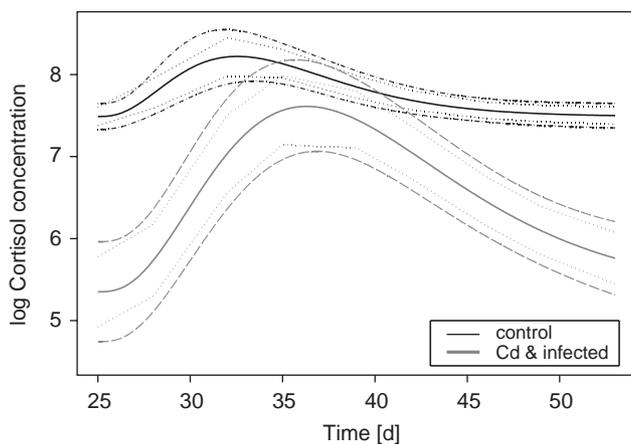


Fig. 4. Simultaneous 95% confidence bands (dashed) compared to point-wise 95% confidence intervals (dotted) for the control and Cd and infected groups.

where $\sigma^2 W_i^{-1}$ denotes the covariance matrix resulting from variance function (2) together with an AR(1)-correlation structure. The nonparametric estimate \hat{g}_λ is the solution of

$$\min_g \left\{ \frac{1}{n} (y - g)' W (y - g) + \lambda \int_{t_1}^{t_2} g''(t) dt \right\}. \quad (8)$$

The second term in (8) is a penalty to the roughness of g .

We fitted the smoothing spline model using the ASSIST package of Wang and Ke (2002). A crucial step in the fitting procedure is the choice of the smoothing parameter λ (which may also depend on the treatment group). In ASSIST, several data-adaptive methods are available. We used the generalized maximum likelihood method (Wahba, 1990) to estimate the unknown parameters (δ, λ) . Fig. 5 shows the smoothing spline models of the four groups with the observed mean values. The estimated curves follow the mean values very closely. The dashed lines in Fig. 5 are Bayesian confidence intervals, which also have good frequentist properties (Wang and Wahba, 1995).

Instead of assessing the goodness of fit of the parametric model with a formal test, Fig. 6 visualizes the parametric mean curve together with the nonparametric fits for groups 1 and 4. Apparently, parametric and nonparametric curves are quite similar, and the parametric curve lies within the confidence bands, indicating the suitability of the parametric model. However, for all four groups, the parametric curves are smoother than the nonparametric models.

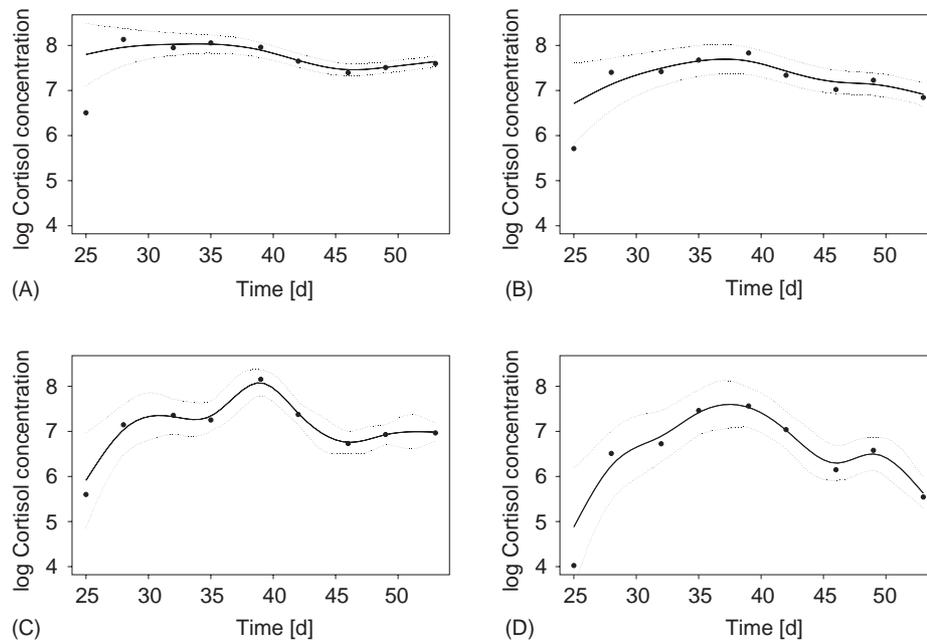


Fig. 5. Smoothing spline models of the four groups [(A) control, (B) infected (C) cadmium, (D) Cd and infected] with 95% confidence intervals and observed mean values.

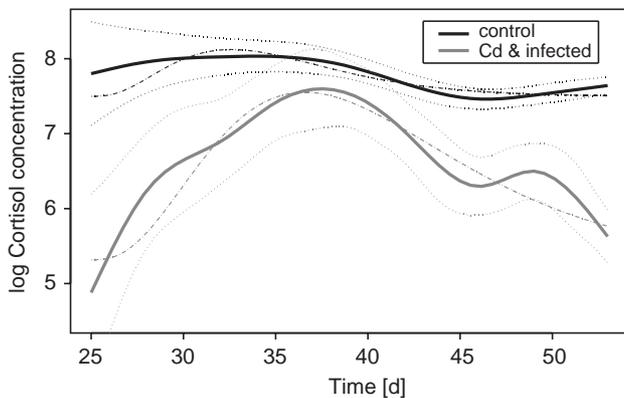


Fig. 6. Smoothing spline models of the control and Cd and infected (solid lines) groups together with 95% confidence intervals (dotted) and mean curve of parametric model (dashed).

4. Discussion

In this article, we have introduced a nonlinear heteroscedastic model which is able to describe well the temporal evolution of cortisol concentrations in groups of rats treated by cadmium exposure and/or parasite infection. The model reproduces the sharp increase with the beginning of cadmium application and the subsequent decline in all groups.

In our model, the mean curve is given by $y = \beta_1 + \beta_2(\beta_3(t - 25))^3 \exp(-\beta_3(t - 25))$ for $t \geq 25$. This choice of parametrization has the advantage that β_3 can be interpreted as time scale. Looking at the fitted values in Table 1, one finds that β_3 is largest in the control group and smaller in the other groups. Similarly, β_1 is largest in the control group, smaller in the infected group and the exposed group, and smallest in the Cd and infected group. This indicates that the reaction to stress is weaker and slower for the infected and/or exposed groups compared to the controls.

Besides the mean values, it is necessary to specify a correlation structure for the errors. We examined different heteroscedastic models and compared them in Table 2. Both models with correlated errors and the model (6) with random intercept yield statistically significant improvements over model (1).

Model (6) and the model with constant correlation structure have the same number of parameters and comparable AIC and BIC values. Thus, one may question whether the additional complexity of model (6) compared to the model with constant correlation is justified; we tend to favor the latter.

The model with AR(3)-error structure has the lowest AIC value and a comparable BIC value. However, in spite of the results of Table 2, we prefer the two other models, since, as mentioned in Section 3.1.2, the estimated values of the autoregressive parameters look rather artificial.

The fitted model separates the mean curves of the control group and the infected-and-exposed group from the curves of the other two groups. The latter have nearly identical mean curves. The control group lies significantly higher than the infected-and-exposed group; the significance bands in Section 5 show nearly no overlap. The other two groups lie in between, giving strong evidence that the effect of parasite infection and of cadmium exposure is to increase the stress hormone level of rats in an additive manner.

Recent studies have demonstrated that parasites may affect the stress response of their hosts (e.g., Barnard et al., 1998; Dunlap and Schall, 1995; Sures et al., 2001). This effect appears to be enhanced if the parasitized organisms are simultaneously confronted with environmental pollution (Sures et al., 2002). Such deviations from a physiological homeostasis, measured, e.g., as an increased or decreased stress hormone concentration, are often the first step in a series of physiological reactions, such as a depressed immune response (reviewed, e.g., by Besedovsky and Del Ray, 1996; Weigent and Blalock, 1995) or other fundamental physiological reactions (Chrousos and Gold, 1992; Hart et al., 1989). There are, for example, also cases where a biomarker reaction, i.e., the expression of heat-shock proteins in cells, is affected by stress hormones (Iwama et al., 1999; Sathiyaa et al., 2001). The similarity and the additive effects of exposure and infection on basic physiological processes is of great importance in terms of ecotoxicological research. Especially, in studies aiming at the question of whether exposure to certain chemicals affects the physiological status of a test organism, it is important to also use infected organisms, as the vast majority of free-living animals used for biomarker studies is believed to be infected with a variety of parasites (Price, 1980).

Accordingly, it would be inappropriate to ignore possible infections in organisms used for ecotoxicological and toxicological tests, as the extrapolation of results obtained from healthy uninfected animals in the laboratory is insufficient to determine the effects on infected hosts under natural conditions. However, the impact of and the exact ways how parasites may affect the physiology of their hosts must be determined in future studies.

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