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USING NONLINEAR FIXED and MIXED MODELS with SWITCHING FUNCTIONS to ALLOW for HORMESIS in GROWTH of *ESCHERICHIA Coli*

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ABSTRACT

Individual *Escherichia coli* (*E. coli*) strains can be characterized by measuring growth rate. Strains better adapted to the environment are expected to grow faster. Classic bacterial growth curves display an increase in optical density over time. In this paper, we use the logistic function to model growth in optical density of *E. coli* over time. We examine 16 curves for 8 *E. coli* strains originally isolated from cattle and found many curves have a paradoxical dip at the beginning that is indicative of hormesis (an initial contrarian response showing, stimulation or suppression of growth). We examine several switching functions that allow for the effect of hormesis and compare the ability of nonlinear fixed and mixed models to detect the presence of hormesis.

1. INTRODUCTION

Escherichia coli (*E. coli)* is one type of common bacteria that live in the natural environment. Some *E. coli* bacteria, specifically *E. coli* with the O157:H7 serotype, are important human pathogens that are associated with food and waterborne outbreaks. *E. coli* O157:H7 may cause severe illness and even death in humans. Young children and the elderly are especially susceptible to illness. Cattle are considered a reservoir for *E. coli* O157:H7 and evidence suggests that there may be differences between *E. coli* O157:H7 strains isolated from cattle compared to those isolated from humans (Kim, Nietfeldt, and Benson, 1999). It is important for scientists and physicians to distinguish among the *E. coli* O157:H7 strains, and to understand what factors contribute to the survival and persistence of one strain versus another. One way to characterize different types of bacteria is by measuring bacterial fitness, a measure of reproductive success (Elena and Lenski, 2003).

Fitness can be measured in bacteria by calculating growth rate (Cooper and Lenski, 2000). One way to model fitness of individual bacteria is to study changes in cell density as measured by optical density over time. These changes can be described by logistic or log-logistic regression models. The models provide such estimates as maximum optical density, maximum rate of growth, and time to maximum rate of growth. One drawback to these models is that there is no provision for characterizing the change in optical density if the growth of bacterial strains involves the phenomenon of hormesis. Estimates of individual bacterial fitness (maximum rate of growth) can be affected by the presence of hormesis.

The term hormesis comes from the Greek for "to excite" or 'set into motion" and is often involved in biological, medical and toxicological science (Schabenberger and Pierce, 2002). The term "hormesis" was originally used by C. Southam and his co-worker J.

Erlish in 1943 to indicate that while high concentrations of Oak bark extract inhibited fungal growth, low doses stimulated fungal growth (Bruce, 1987). Mathematically, hormesis represents the failure of a model to be monotonically increasing or decreasing. (Davis and Svendsgaard, 1990; Calabrese, 2004). In terms of growth, hormesis can represent stimulation or suppression in the early stages. This study will focus on detecting the hormesis phenomenon representing suppression of growth in the early stages.

Hormetic models are by definition non-monotonic. A desirable feature of models that allow for hormesis is that they reduce to a standard model in the absence of hormesis. Such models allow for a test of hypothesis to detect the presence of hormesis. Schabenberger and Birch (2001) proposed using switching functions to build such models. For example, Brain and Cousens (1989) proposed a modification to the log-logistic model which is a combination of switching functions and hormetic weights, γX to produce a hormetic model (1.1) that can detect hormesis. That is,

$$Y = \delta + \frac{\alpha - \delta + \gamma X}{1 + \beta \exp[\kappa \ln(X)]} + \varepsilon \qquad (1.1)$$

where δ represents the initial optical density, α represents maximum optical density, κ represents the rate constant, β indicates a scale parameter, γ measures the initial rate of increase at small dosages and is the parameter used to test for the hormesis effect. The hypothesis test for hormesis is $H_0 : \gamma = 0$ vs. $H_a : \gamma \neq 0$. If γ is not significant, one may conclude that the hormesis phenomenon does not exist. Otherwise, the hormesis effect is believed to exist and one should take the hormesis effect into account when making statistical inferences.

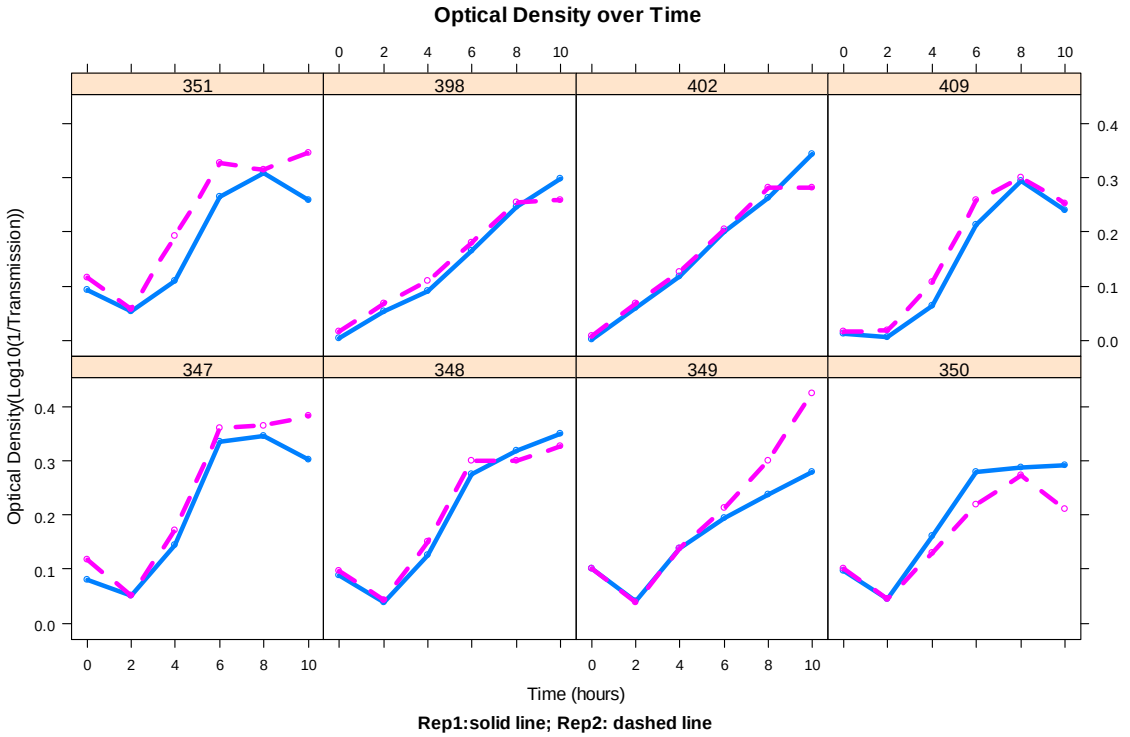
There are three objectives for our study. We plan to use the concepts of switching functions, as exemplified by the Brain-Cousens model, and functions in R (*nls* and *nlme*) to: 1) build models to detect the hormesis effect in *E. coli* O157:H7 strains: 2) identify a suitable nonlinear fixed effect model; and 3) compare results from the nonlinear fixed and mixed models for detecting hormesis. Finally, we will propose a hormetic model for fitting *E. coli* O157:H7 strains which in turn will help researchers detect hormesis, estimate the maximum growth rate, and compare fitness measures of individual bacteria.

2. MATERIALS AND METHODS

2. a. Data

A series of experiments were conducted at the University of Nebraska-Lincoln to study measurements of fitness and competition in *Escherichia coli* (*E. coli)* with a specific focus on *E. coli* O157:H7 strains (Durso, Smith, and Hutkins, 2004). A subset of the individual fitness assays was used in this study. Repeated measures over time were made on eight *E. coli* O157:H7 strains extracted from cattle. There were two replications for each of the eight strains. The response variable, optical density (log10 (1/transmission)) was measured at 0, 2, 4, 6, 8, and 10 hours. Figure 1 contains plots of the optical density over time for each *E. coli* strains: 347, 348, 349, 350, 351, 398, 402, and 409. Six out of eight of the strains have curves with the paradoxical dip at the beginning, suggesting a need to account for the effect of hormesis.

Figure 1. Plots of 8 Cattle *E. coli* Strains with 2 Replications



2. b. Four-parameter Logistic Growth Model

The logistic model is characterized by a sigmoidal growth curve around an inflection point (Ratkowsky, 1990). To describe the increase in optical density over time for *E. coli* strains, we can use the inflection point parameterization of the four parameter logistic model

$$Y = \delta + \frac{\alpha - \delta}{1 + \exp(-\kappa(X - \tau))} + \varepsilon \qquad (2.1)$$

where E[Y | X] is the mean optical density given a particular level of covariate time, where α represents maximum optical density, δ represents the initial optical density, κ represents the rate constant, τ represents the time to maximum rate of change and the errors, $\varepsilon \sim N(0, I\sigma^2)$.

2. c. Switching Functions to Build Non-hormetic and Hormetic Models

Schabenberger and Birch (2001) proposed switching functions which take values between 0 and 1 as the independent covariate X increases. There are two types of a switching function: 1) Switch-on function, S_I (X, θ); and 2) Switch-off function, S_O (X, θ). A switch-on function increases monotonically in X, while a switch-off function decreases monotonically in X. One function is the complement of the other, that is, S_I (X, θ) =1 − S_O (X, θ). They suggest the following techniques for developing non-hormetic and hormetic models.

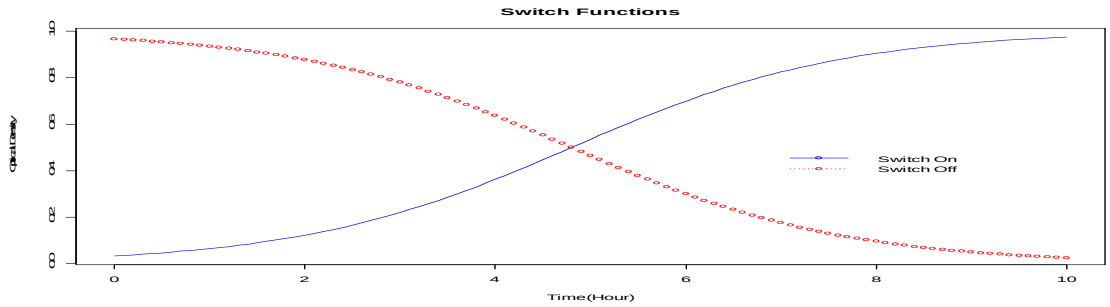
2. c. i. Non-hormetic Growth Model

Since continuous switch-on functions increase monotonically over time, we can choose a cumulative density function (CDF) of a continuous random variable with unimodal density (Seber and Wild, 1989). The CDF distributed over the interval [0, 1] can then be used to model the transition between two asymptotes, $\mu_{\min}$ and $\mu_{\max}$,

$$Y = \mu_{\min} + (\mu_{\max} - \mu_{\min}) S_I(X, \theta) \tag{2.2}$$

The two-parameter logistic model provides a switch-on function when $-\kappa < 0$ and it is a switch-off function when $-\kappa > 0$. See figure 2 for an example of this function.

Figure 2. Complementary Switching Functions for Logistic Model
τ =4.8, κ_{on}=0.704, and κ_{off}= -0.704



With an inflection point at τ, the switching function is

$$S_I(X, \theta) = \frac{1}{1 + e^{-\kappa(X - \tau)}} \tag{2.3}$$

Using (2.3) as the switching function in (2.2), results in the four-parameter logistic model (2.1).

2. c. ii. Strategy for Adding Hormetic Behavior

Hormetic behavior can be incorporated into a given model by adding a hormetic component to (2.2).

$$Y = \mu_{\min} + (\mu_{\max} - \mu_{\min}) S_I(X, \theta) + f(X, \gamma) S_O(X, \theta) \tag{2.4}$$

The $S_I(X, \theta)$ and $S_O(X, \theta)$ are switching functions that might be identical $S_I(X, \theta) = S_O(X, \theta) = S(X, \theta)$. The function for the hormetic effect, $f(X, \gamma)$, is a monotonic function as X increases and satisfies two requirements: First, the model reduces to the non-hormetic version in the absence of the hormetic effect, $f(X, \gamma) = 0$ when γ=0; Second, the model has no hormesis at the initial value, $f(X, \gamma) = 0$ when X=0 (Schabenberger and Birch, 2001).

2. c. iii. Proposed Hormetic Models with Linear and Non-monotonic Functions

We construct four models to detect the hormetic effect on the growth curves of the *E. coli* strains using combinations of two switching functions and two hormetic weighting functions. The switching functions are logistic (2.5) and log-logistic (2.6). We re-

parameterized the logistic model in (2.1) so -κ = -1/ψ and re-parameterize the log-logistic switching function used in the Brain-Cousens model (1.1) so that κ = -1/ψ and β=exp (ln(τ)/ψ). The two hormetic weighting functions $f(X,\gamma)$ are linear (2.7), and non-monotonic (2.8).

$$S_{Logistic} = \frac{1}{1+\exp\left[-(X-\tau)/\psi\right]} \tag{2.5}$$

$$S_{Log-Logistic} = \frac{1}{1+\exp\left[-(\ln(X)-\ln(\tau))/\psi\right]} \tag{2.6}$$

$$f_{linear}(X,\gamma) = \gamma X \tag{2.7}$$

$$f_{non-monotonic}(X,\gamma) = \gamma X/\exp(X) \tag{2.8}$$

Using the strategy of Schabenberger and Birch for adding hormetic behavior to a model (2.4) results in four hormetic models, (2.9), (2.10) (2.11), (2.12). Each of these four hormetic model is used to model growth when the potential for hormesis is present;

Brain-Cousens (B-C),

$$Y = \delta + \frac{\alpha-\delta+\gamma X}{1+\exp\left[-(\ln(X)-\ln(\tau))/\psi\right]} \tag{2.9}$$

Modified Brain-Cousens (Modified B-C),

$$Y = \delta + \frac{\alpha-\delta+\gamma X}{1+\exp\left[-(X-\tau)/\psi\right]} \tag{2.10}$$

Non-Monotonic with Logistic (NM-L),

$$Y = \delta + \frac{\alpha-\delta+\gamma X/\exp(X)}{1+\exp\left[-(X-\tau)/\psi\right]} \tag{2.11}$$

Non-Monotonic with Log-Logistic (NM-LL),

$$Y = \delta + \frac{\alpha-\delta+\gamma X/\exp(X)}{1+\exp\left[-(\ln(X)-\ln(\tau))/\psi\right]} \tag{2.12}$$

The B-C model uses a linear (monotonic) hormetic function, $f(X,\gamma) = \gamma X$ to weight the log-logistic switch-off function, as shown in the third term of E[Y|X] given below.

$$E[Y \mid X] = \delta + \frac{\alpha-\delta}{1+\exp\left[-(\ln(X)-\ln(\tau))/\psi\right]} + \frac{\gamma X}{1+\exp\left[-(\ln(X)-\ln(\tau))/\psi\right]}$$

The Modified B-C model is a logistic version of the B-C model.

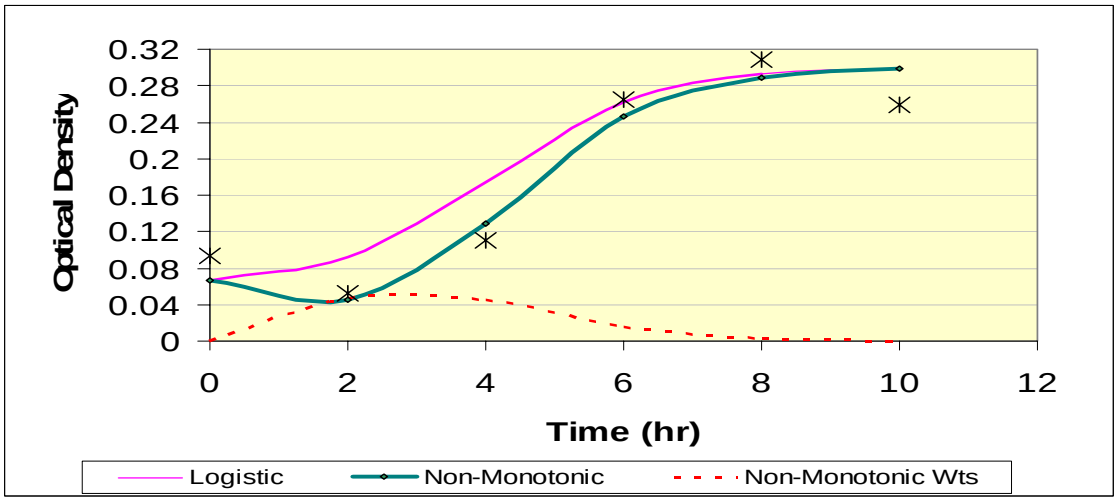
The problem of using a linear hormetic function (2.7) is the hormetic effect never vanishes. We propose using a non-monotonic weighting function to allow the hormesis effect to dominate at the early stage and gradually diminish. The NM-L model uses the

non-monotonic function, (2.8), to weight the logistic switch-off function, as shown in the third term of E[Y|X] given below.

$$E[Y \mid X] = \delta + \frac{\alpha - \delta}{1 + \exp\left[-(X - \tau)/\psi\right]} + \frac{\gamma X / \exp(X)}{1 + \exp\left[-(X - \tau)/\psi\right]}$$

Figure 3 shows how a NM-L model over the interval [δ=0,α=1] compares to the sum of two parts: 1) the logistic switch on function (2.3); and 2) the non-monotonic weighted logistic switch-off function. The hormesis effect occurs only in the initial stages then gradually decreases.

Figure 3. Non-Monotonic Hormetic Model (2.11) as a Sum of Logistic Model (2.3), and Non-Monotonic Weighted Switch off Function



The NM-LL model is a log-logistic version of the NM-L model. It is used to make comparisons with the B-C model which also uses the log-logistic.

2. d. Hormetic Nonlinear Mixed Model

A mixed effects version of the hormetic nonlinear fixed model was examined to identify the appropriate random effects. When the optical density, Y, was fit by the Non-Monotonic with Logistic (NM-L) model the following effects were used. That is,

$$Y = \delta + \frac{\alpha - \delta + \gamma X / \exp(X)}{1 + \exp\left[-(X - \tau)/\psi\right]} + \varepsilon \quad \text{where } \varepsilon \sim N(0, I\sigma^2)$$

$$\begin{bmatrix} \alpha \\ \delta \\ \psi \\ \tau \\ \gamma \end{bmatrix} = \begin{bmatrix} A & + {}^{\alpha}b_i & + {}^{\alpha}b_{ij} \\ D & + {}^{\delta}b_i & + {}^{\delta}b_{ij} \\ P & + {}^{\psi}b_i & + {}^{\psi}b_{ij} \\ T & + {}^{\tau}b_i & + {}^{\tau}b_{ij} \\ G & + {}^{\gamma}b_i & + {}^{\gamma}b_{ij} \end{bmatrix}$$

$$ {}^{*}b_i = \begin{bmatrix} {}^{\alpha}b_i \\ {}^{\delta}b_i \\ {}^{\psi}b_i \\ {}^{\tau}b_i \\ {}^{\gamma}b_i \end{bmatrix} \sim N\left(0, \Omega_{strain}\right) \qquad {}^{*}b_{ij} = \begin{bmatrix} {}^{\alpha}b_{ij} \\ {}^{\delta}b_{ij} \\ {}^{\psi}b_{ij} \\ {}^{\tau}b_{ij} \\ {}^{\gamma}b_{ij} \end{bmatrix} \sim N\left(0, \Omega_{rep\,(strain)}\right) $$

where $i = 1,2,..8$ and $j = 1,2$

Fixed and random effects were assigned to all parameters. The fixed effects (α, δ, ψ, τ, γ) represent the population average of the parameters. The level-1 random effects vector, ${}^{*}b_i$, represents the deviation of the i[th] strain from the population average for each parameter. The level-2 random effects, ${}^{*}b_{ij}$, represents the deviation of the j[th] replication of the i[th] strain from the population average for each parameter. The ${}^{*}b_i$ are assumed to be independent for each strain. The ${}^{*}b_{ij}$ are assumed independent for each replication of each strain. The ${}^{*}b_i$, ${}^{*}b_{ij}$, and ε_{ij} are all assumed independent of each other.

2. e. Model Building using *R*

All analyses were performed in ***R*** version 2.5.0. We used the nonlinear least squares function, *nls,* to estimate the fixed effects parameters. Information criteria and log-likelihood functions (*AIC*, *BIC,* and *logLik)* were used to compare results. The nonlinear mixed effects function, *nlme,* was used to estimate the fixed effects with random components. The advantages of using *nlme* include provisions for multilevel random effects, as well as, correlated and heterogeneous errors (Pinheiro and Bates, 2000). Pinheiro, Bates, and Lindstrom (1993), and Pinheiro and Bates (1995) suggest assigning random effects to all parameters and fitting a full covariance structure for the initial analysis. However, this strategy is frequently fraught with convergence problems. The stepwise strategy we used to choose the appropriate random effect structure was to assume a diagonal matrix for all random effects for the initial model. We removed the smallest random effects, one at a time, to create a reduced model and compared the reduced model with the previous one until the log-likelihood ratio test is significant ($p<0.10$). If multiple random effects remain, we then examine the correlated error structure for significance. The following is the code for applying the *nlme* function to the *E. coli* data.

NLME codes for fitting the nonlinear mixed-effects model are as follows:

```
NM.L.nlme<-nlme(density~new(time, alpha, delta, psi, tau,
                  gamma),data=br, fixed=alpha+delta+psi+tau+gamma~1,
     random=list( bh=pdDiag(alpha+delta+psi+tau+gamma~1),
                  rep=pdDiag(alpha+delta+psi+tau+gamma~1)),
                start=c(alpha=0.3, delta=0.06, psi=1.12,
                              tau=4.13,gamma=-1.3))
                summary(NM.L.nlme)
```

3. RESULTS AND DISCUSSION

3.1 Nonlinear Fixed Effect Model

We begin by comparing the parameter estimates and their associated confidence intervals for each model. Table 1 shows both the Non-Monotonic with Logistic (NM-L) and Non-Monotonic with Log-Logistic (NM-LL) models provided statistically significant parameter estimates ($p<0.05$) and confidence intervals of reasonable width. On the other hand, not all parameter estimates are significantly different from zero for either the Brain-Cousens model (B-C) or the Modified Brain-Cousens (Modified B-C) model. The maximum optical density, α, for the B-C model is barely significant and has wide confidence intervals. While the inverse rate constant, ψ, is not significant in the Modified B-C model. More importantly, neither the B-C nor the Modified B-C model were able to detect a significant hormesis effect, γ, ($p=0.8170$, and $p=0.2047$ respectively).

Table 1. Parameter Estimations and 95% Confidence Intervals for the Hormetic Nonlinear Fixed Effect Models

	Logistic Switch Function						Log-Logistic Switch Function					
Model / Parm	NM-L			Modified B-C			NM-LL			B-C		
	Est	L	U	Est	L	U	Est	L	U	Est	L	U
α	.30*	.28	.32	.21*	.06	.35	.31*	.28	.34	.27†	.001	.55
δ	.06*	.04	.08	.05*	.04	.07	.07*	.04	.09	.06*	.04	.07
ψ	1.12*	.56	1.67	.60	-.15	1.36	.27*	.12	.43	.18*	.02	.33
τ	4.13*	3.50	4.76	4.28*	3.53	5.03	3.81*	2.94	4.69	4.58*	3.26	5.9
γ	-1.3*	-2.0	-0.6	.01	-.01	.03	-1.7*	-2.4	-1.0	.003	-.02	.03
R.S.E	0.043			0.044			0.043			0.043		

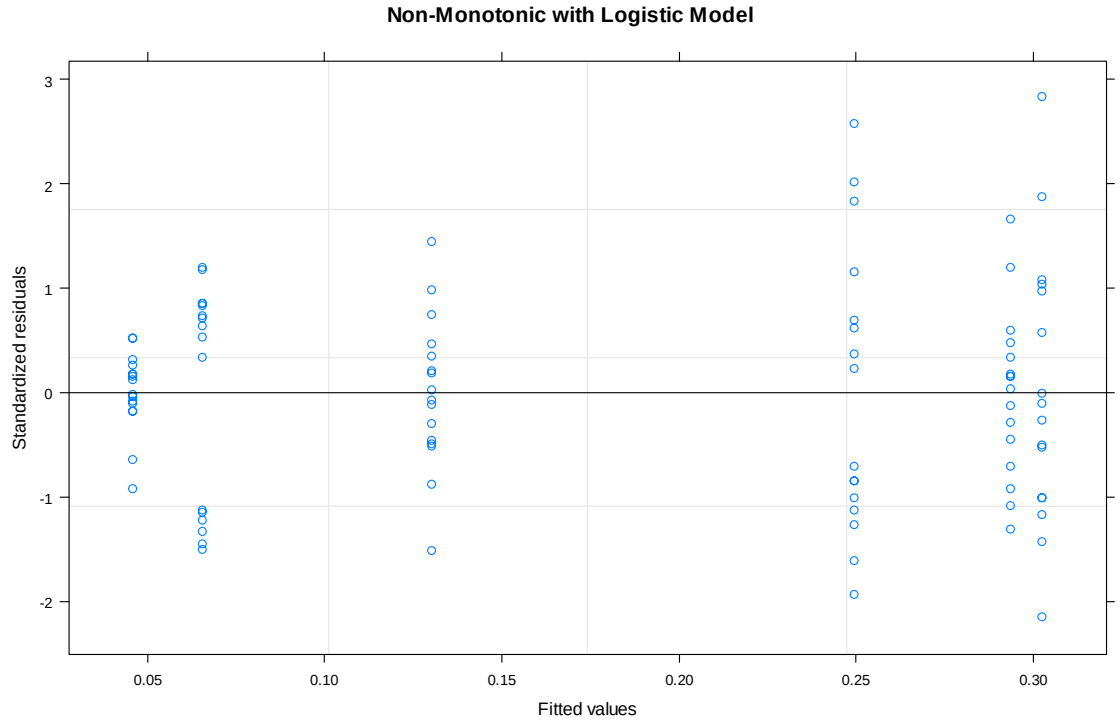
*** P-value < 0.05; † P-value = 0.052**
Est: Estimate; L: Lower 95% Confidence Interval; U: Upper 95% Confidence Interval
Parm: Parameter; R.S.E: Residual Standard Error on 91 degrees freedom

Table 2 shows the models based on the non-monotonic function (2.8) have the smallest AIC, BIC and the largest log likelihood value. The NM-L and NM-LL models appear to be comparable. For both models, the residuals passed the Shapiro-Wilks test for normality (p-value = 0.44 for NM-L and 0.47 for NM-LL); the coefficients of variation are similar (not shown); and, the scatter plots of the standardized residuals vs. fitted values exhibited an undesirable wedge-shaped patterns (Figure 4). (See results of the mixed model analysis below for resolution of this issue). It is not until we consider the nonlinear characteristics that we have a basis to choose between the models.

Table 2. Information Criteria and Log Likelihood for the Nonlinear Fixed Effect Models

Model	# of Iterations	D.F	AIC	BIC	Log Likelihood
Logistic Switch Function					
NM-L	4	6	-325.013	-309.628	168.507
Modified B-C	13	6	-321.683	-306.298	166.842
Log-Logistic Switch Function					
NM-LL	6	6	-324.988	-309.602	168.494
B-C	13	6	-322.735	-307.349	167.368

Figure 4. Scatter Plot of Standardized Residuals vs. Fitted Values for the Nonlinear Fixed-Effect Model Fit



Statistics for assessing nonlinear behavior are presented in Table 3. The NM-LL model has higher parameter-effects curvature, PE (Bates and Watts, 1980, 1988). In addition, the NM-LL model, generally, has higher percent bias and percent excess variance (λ). Finally, there is more correlation among the parameter estimates in the NM-LL model (data not shown). Four pairs of parameters have a $|r| > 0.5$ in the NM-LL model; only 2 pairs have a $|r| > 0.5$ in the NM-L model. The above results, lead us to propose using the NM-L model to analyze the observed growth behavior.

Table 3. Statistics for Curvature and Close-to-Linear Behavior

	NM-L Model			NM-LL Model		
	IC=0.18 PE=0.99			IC= 0.25 PE=98.19		
	Estimate	%Bias	λ	Estimate	%Bias	λ
α	0.30	0.48	6.62	0.31	1.26	2437
δ	0.06	-2.91	8.51	0.07	-0.05	2400
ψ	1.12	1.32	8.90	0.27	4.71	849
τ	4.13	-0.53	9.22	3.81	75.38	6221
γ	-1.30	-0.10	12.4	-1.74	-5.97	2482

IC = Intrinsic Curvature, PE=Parameter effects curvature, λ = % Excess variance, (add 0.01for time=0 in NM-LL Model)

3.2 Nonlinear Mixed Effects Model

The pattern in the residual scatter plot (Figure 4) suggests incorporation of random effects may improve the Non-Monotonic with Logistic (NM-L) model. To identify which parameters, if any, require random effects, we fit the nonlinear mixed model for several sets of diagonal random-effects structures and check for model equivalency. We begin with Model 1 using the full diagonal structure. Then, we removed the smallest random effect, δ, for replications within strains and fit Model 2 (Table 4). We compare the models to see if the random effect we removed is significant. Since the likelihood ratio test is not significant (Table 5), we conclude the random effect is not significantly different from zero and continue the process. Finally, the smallest random effect was removed from Model 8 resulting in Model 9. Comparing Models 8 and 9 results in a significant drop in the log-likelihood (p=0.0059). So we keep Model 8. We then fit Model 10, the full variance-covariance between δ and τ for strains with α for the replication within strains, (not shown) and check for significance. The likelihood test indicated no significant improvement over Model 8 when Model 10 was used (p=0.15).

Table 4. Standard Deviations for Diagonal Random Effect Structures in the Non-Monotonic with Logistic Model, NM-L

Model	Strains					Replications within Strains				
	α	δ	ψ	τ	γ	α	δ	Ψ	τ	γ
1*	0.02	0.02	3e-5	0.78	5e-4	0.03	9e-7	2e-5	1e-3	4e-5
2	0.02	0.02	4e-5	0.78	1e-6	0.03		1e-5	8e-5	2e-5
3	0.02	0.02	4e-5	0.78		0.03		6e-6	7e-5	1e-5
4	0.02	0.02	4e-5	0.78		0.03			9e-5	1e-5
5	0.02	0.02	3e-5	0.78		0.03				5e-5
6	0.02	0.02	4e-5	0.78		0.03				
7	0.02	0.02		0.78		0.03				
8		0.02		0.80		0.03				
9				0.97		0.03				

*Model 1: All random effects are diagonal variance-covariance structure

Table 5. Information Criteria, Log-Likelihoods, and Ratios for Comparing Random Structures in the Non-Monotonic with Logistic Model, NM-L

Model	AIC	BIC	L Like	Test	LRT	P-value
1	-339	-298	185.6.			
2	-341	-302	185.6	1 vs. 2	1e-5	0.99
3	-343	-307	185.6	2 vs.3	2e-7	0.99
4	-345	-311	185.6	3 vs.4	0.01	0.92
5	-347	-316	185.6	4 vs. 5	0.02	0.90
6	-349	-321	185.6	5 vs. 6	0.01	0.92
7	-351	-325	185.6	6 vs. 7	0.01	0.92
8*	-353	-329	185.5	7 vs. 8	0.26	0.61
9	-351	-330	183.7	8 vs. 9	3.55	0.059

***** Model 8: Based on the smallest AIC value and non-significance of LRT (p=0.61), Model 8 is the best model in Table 5.

The fixed effects parameter estimates for the NM-L nonlinear mixed effects model (Model 8) are given in Table 6. The estimates are similar to those from the NM-L nonlinear fixed models; however, the nonlinear mixed effects model provides a smaller residual standard error. The statistics given in Table 7 provide further ways to compare the two versions (fixed and mixed) of the NM-L model. The mixed version has a lower AIC, BIC and a significantly larger log-likelihood. Also, the nonlinear mixed effect model reduces the correlations between the fixed parameters (Table 8), suggesting the pervasive simultaneous confidences may have more legitimacy than usual. In other words, the simultaneous confidence intervals of the nonlinear mixed model are safer to use for making statistical inferences than those from the nonlinear fixed model, because the correlations between fixed parameters of the nonlinear mixed model are less than the correlations in the nonlinear fixed model.

Table 6. Fixed Effect Estimates in Nonlinear Fixed and Mixed Model for Non-Monotonic with Logistic Model, NM-L

Parameter	Fixed Model Estimates (STD)	Mixed Model Estimates (STD)
α	0.30** (0.01)	0.31** (0.01)
δ	0.06** (0.01)	0.06** (0.01)
ψ	1.12** (0.28)	1.19** (0.19)
τ	4.13** (0.32)	4.29** (0.35)
γ	-1.30** (0.37)	-1.34** (0.22)
R.S.E	0.043	0.028

** P-value < 0.001

Table 7. Information Criteria, Log-Likelihoods, and Ratios for Comparing Nonlinear Mixed and Fixed Models in the Non-Monotonic with Logistic Model, NM-L

Model	D.F.	AIC	BIC	L Like	Test	LR	P-Value
Mixed	9	-352	-329	185			
Fixed	6	-325	-309	168	Mixed vs. Fixed	34	<.0001

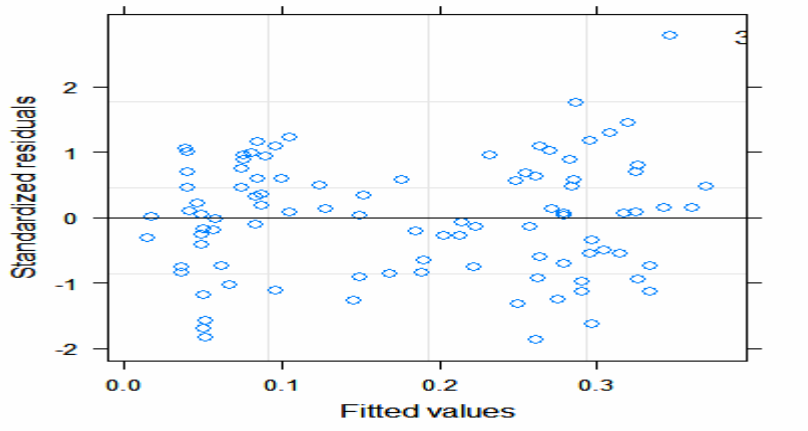
Table 8. Correlation Coefficients between Fixed Parameters for Fixed and Mixed Non-Monotonic with Logistic Model, NM-L

		Mixed				
		α	δ	ψ	τ	γ
Fixed	α	**1**	**-0.126**	**0.416**	**0.182**	**0.112**
	δ	*-0.236*	**1**	**-0.344**	**0.004**	**-0.522**
	ψ	*0.627*	*-0.408*	**1**	**0.083**	**0.474**
	τ	*0.494*	*-0.159*	*0.205*	**1**	**0.075**
	γ	*0.194*	*-0.735*	*0.458*	*0.367*	**1**

3.3. Assessing the Nonlinear Mixed Non-Monotonic with Logistic Model, NM-L

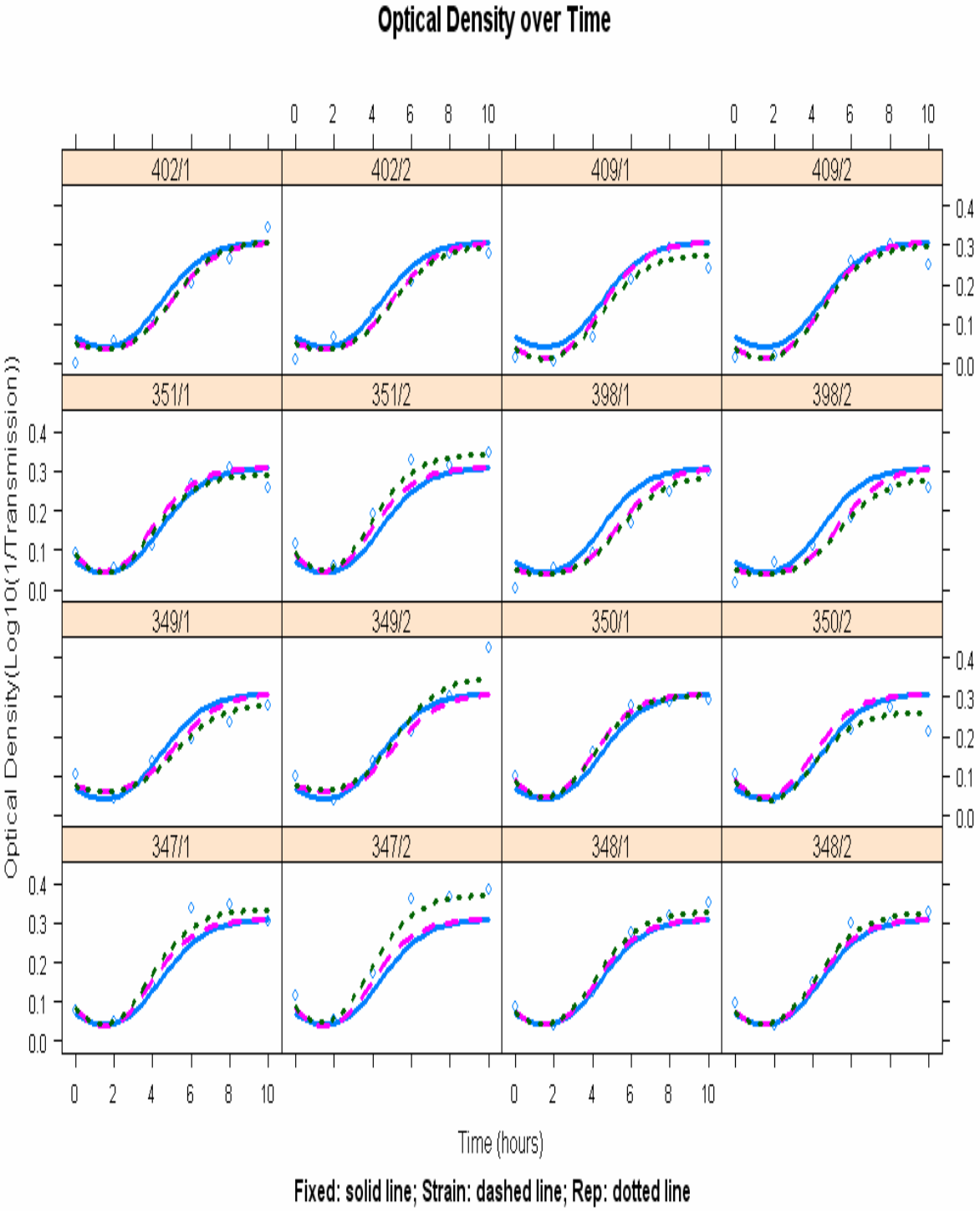
Model diagnostics were performed for the nonlinear mixed version of the Non-Monotonic with Logistic (NM-L) model to examine the validity of the classical assumptions. The NM-L model was fit with provisions for correlated errors and non-constant variance. The results of the information criteria and log-likelihoods were consistent with the assumptions of uncorrelated and constant errors. The scatter plot of the standardized residuals vs. fitted values (Figure 5) indicates the random-effects removed the wedge-shaped pattern displayed by the residuals from the fixed version (Figure 4). The assumption of normality for the within-group residuals was consistent using the Shapiro-Wilkes test (p=0.8872) and the normal probability plot (not shown).

Figure 5. Scatter Plot of Standardized Residuals vs. Fitted Values for the Nonlinear Mixed-effects Non-Monotonic with Logistic Model, NM-L



The quality of fit can be assessed by the augmented predictions plot, Figure 6. The plot shows the population predictions (Fixed: solid line), the within strain predictions and the replication within strain predictions (Rep: dotted line) superimposed on the optical density observations (circles). The Strain and Rep curves show how adjustments to the population fixed predictions from the *Escherichia coli* (*E. coli*) strains help the model get closer to the observed values to more adequately describe the optical density growth in the *E. coli* data.

Figure 6. Augmented Predictions Plot for Nonlinear Mixed-effects Non-Monotonic with Logistic Model, NM-L



4. CONCLUSIONS

Logistic regression can be used to characterize changes in cell density as measured by optical density over time. However, the regression model needs modification to capture the initial dip indicative of hormesis. This paper demonstrates that switching functions provide a useful tool for building models to detect the hormesis effect in *Escherichia coli (E. coli)* O157:H7 strains. The non-monotonic function is superior to the linear function used in the Brain-Cousens (B-C) model, while the logistic switching function was similar to the log-logistic switching function used in the B-C model. Both of these nonlinear fixed models, Non-Monotonic function with Logistic (NM-L) and Non-Monotonic function with Log-Logistic (NM-LL), were able to detect hormesis. However, we recommend the NM-L model. The NM-L model with the logistic switching function tended to perform better than the log-logistic switching in terms of close-to-linear behavior and had fewer significant correlations between parameters.

The nonlinear mixed effects model provides better prediction results than the nonlinear fixed effect model. Random effects for the optical density baseline (lower asymptote, δ) and the time to maximum rate of change (τ) help the model adjust to each strain. Random effects for the maximum optical density (upper asymptote, α) help the model predict each replication within a strain.

The augmented prediction plots show the ability of the NM-L nonlinear mixed model to characterize the growth in optical density of *E. coli* O157:H7 strains isolated form cattle. The NM-L model catches the observed optical density response of each replication within a strain and provides a statistical test for hormesis.

5. SUMMARY

The ability to distinguish among *Escherichia coli (E. coli)* O157:H7 strains is important for understanding the reproductive process of human pathogens that are associated with food and waterborne outbreaks. One measure of fitness in bacteria is growth rate. Growth rate is one of the parameters obtained when a logistic regression model is used to characterize changes in cell density as measured by optical density over time, but the logistic model does not provide a way to detect a potential hormetic effect. Switching functions were used to build hormesis models. The linear hormetic weights and log-logistic switching function of the Brain-Cousens model were compared to combinations of non-monotonic hormetic weights and logistic switching function. Data was gathered from a multilevel repeated measurements design on the optical density responses of eight *E. coli* strains, each strain was repeatedly measured at six time points. There were two replications for each strain. Models fit with the non-monotonic hormetic weights were superior to those with linear hormetic weights. There was little difference between the switching functions except for the correlation between parameters and the nonlinear behavior. The new model (Non-Monotonic with Logistic, NM-L) consisting of the non-monotonic hormetic weights and logistic switching function was selected. The nonlinear mixed effects version of the NM-L model was studied to identify an appropriate random effects structure. The nonlinear mixed effects model shows significant improvement over the fixed effects version. Random effects were identified for within strains (the baseline, δ, and time to maximum rate of change, τ) and for replications within strains (maximum optical density, α). A plot of the augmented predictions shows that the NM-L mixed model characterizes the growth behavior of *E. coli* well. Although both the

fixed and mixed models indicated the presence of hormesis, the mixed model provided smaller standard errors. We recommend using the NM-L nonlinear mixed effects model to allow for hormesis in characterizing growth in optical density of *E. coli* O157:H7 strains. Using NM-L nonlinear mixed effects model, would allow researchers to detect hormesis and better estimate the growth rate dimension of fitness for a strain

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