The probability of growth of *Listeria monocytogenes* in cooked salmon and tryptic soy broth as affected by salt, smoke compound, and storage temperature

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**A B S T R A C T**

The objectives of this study were to examine and model the probability of growth of *Listeria monocytogenes* in cooked salmon containing salt and smoke (phenol) compound and stored at various temperatures. A growth probability model was developed, and the model was compared to a model developed from tryptic soy broth (TSB) to assess the possibility of using TSB as a substitute for salmon.

A 6-strain mixture of *L. monocytogenes* was inoculated into minced cooked salmon and TSB containing 0–10% NaCl and 0–34 ppm phenol to levels of 10^2–3 cfu/g, and the samples were vacuum-packed and stored at 0–25°C for up to 42 days. A total 32 treatments, each with 16 samples, selected by central composite designs were tested. A logistic regression was used to model the probability of growth of *L. monocytogenes* as a function of concentrations of salt and phenol, and storage temperature. Resulted models showed that the probabilities of growth of *L. monocytogenes* in both salmon and TSB decreased when the salt and/or phenol concentrations increased, and at lower storage temperatures. In general, the growth probabilities of *L. monocytogenes* were affected more profoundly by salt and storage temperature than by phenol. The growth probabilities of *L. monocytogenes* estimated by the TSB model were higher than those by the salmon model at the same salt/phenol concentrations and storage temperatures. The growth probabilities predicted by the salmon and TSB models were comparable at higher storage temperatures, indicating the potential use of TSB as a model system to substitute salmon in studying the growth behavior of *L. monocytogenes* may only be suitable when the temperatures of interest are in higher storage temperatures (e.g., >12°C). The model for salmon demonstrated the effects of salt, phenol, and storage temperature and their interactions on the growth probabilities of *L. monocytogenes*, and may be used to determine the growth probability of *L. monocytogenes* in smoked seafood.

**1. Introduction**

Smoked salmon is a ready-to-eat product commonly sold in vacuum packages, and has a refrigerated shelf life of 3–8 weeks (Rorvik, 2000). If smoked salmon is not processed properly, it could become contaminated with *Listeria monocytogenes*. *L. monocytogenes* was detected in 9.2–13.8% of 165 retail cold-smoked salmon samples collected for a study in 1997 in Italy (Cortesi et al., 1997). A study in Denmark reported that 34–43% of 280 cold-smoked salmon samples collected for a study in 1991 in Italy (Cortesi et al., 1997). A study in Denmark reported that 34–43% of 280 cold-smoked salmon samples collected from various production sites were positive for the presence of *L. monocytogenes*, and the prevalence ranged from <1.4 to 100% in the production sites (Jorgensen and Huss, 1998). A survey in the US showed that *L. monocytogenes* was positive in 4.3% (114/2,644) of smoked seafood samples (Gombas et al., 2003). Risk assessments have indicated that there is an elevated risk of foodborne illnesses caused by consuming smoked salmon contaminated with *L. monocytogenes* (FDA/USDA/CDC, 2003; FAO/WHO, 2004). Because smoked salmon may be contaminated with *L. monocytogenes*, the potential growth of this pathogen in smoked salmon has been the subject of several studies (Dalgaard and Jorgensen, 1998; Ross et al., 2000; Yoon et al., 2004). After reviewing 26 challenge and 13 storage studies examining the growth of *L. monocytogenes* in cold-smoked salmon, Dalgaard and Jorgensen (1998) concluded that the prevailing salt, moisture, and pH of smoked salmon, and storage temperatures supported the growth of *L. monocytogenes* in smoked salmon. Studies have been conducted to evaluate and model the growth kinetics of *L. monocytogenes* as

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affected by salt, $\alpha_w$, smoke compound, pH, lactate, lactic acid bacteria, native microflora, or storage temperature in microbiological media and smoked salmon (Membre et al., 1997; Thurette et al., 1998; Augustin and Carlier, 2000a,b; Lebois et al., 2004). Cornu et al. (2006) compared two cardinal models developed by Augustin and Carlier (2000a,b) and two squared root models developed by Ross et al. (2000) and modified by Gimenez and Dalgard (2004). The comparisons were based on the growth rates of L. monocytogenes obtained from predictions by the 4 models and the observed growth rates from 5 smoked salmon samples stored at 4 and 8 °C in a challenge study. They reported that there were significant variations not only in the growth rates of L. monocytogenes among the observed and predicted values but also among the predicted values from the 4 models, and attributed the variations to mainly the differences in products ($\text{salt}/\alpha_w$, smoke compound, pH, food additives), strains of L. monocytogenes, and native microflora in samples used for the model development. Studies for developing growth kinetics models (e.g., growth rate models) often do not examine environmental conditions that are at or near the growth limits of microorganisms (Buchanan and Philpips, 1990; Le Marc et al., 2005). The use of the probability model to model environmental conditions close to the growth/no growth boundaries of microorganisms, which are often of industrial interest, is more appropriate than the kinetics model (Ratkowsky and Ross, 1995; Le Marc et al., 2005). The objectives of this study were to examine and model the growth probability of L. monocytogenes in cooked salmon containing 0–10% salt and 0–34 ppm phenol, and at stored temperatures 0–25 °C. Cooked salmon added with salt and liquid smoke (phenolic compound) to simulate smoked salmon was used to examine a wide range of salt and phenol concentrations. In addition, the growth probability of L. monocytogenes in tryptic soy broth (TSB) containing salt and phenol was also examined. The purpose was to compare the growth probabilities of L. monocytogenes in salmon and TSB to examine the possibility of using TSB as a model system for salmon. The use of a culture broth in place of a food product in developing a model provides significant advantages, such as ease of sample preparation and more homogeneous growth substrates/intrinsic factors (e.g., pH, $\alpha_w$ levels of food additives), and it allows the microbial enumeration to be measured instrumentally, e.g., measuring turbidity or conductivity, which is less labor intensive than the traditional plate count methods.

2. Materials and methods

2.1. L. monocytogenes and inoculum preparation

Six strains of L. monocytogenes (NFP7459; serotype 3b, NFP7533; serotype 4b, NFP7554; serotype 1/2b, NFP7712; serotype 1/2a, NFP7735; serotype 1/2a, and NFP7821; serotype 1/2b) were used in this study. These strains were isolated from smoked seafood in a survey study conducted by the Food Processors Association (Gombas et al., 2003). Each strain was transferred from −80 °C stock culture into 10 ml tryptic soy broth (TSB, Difco Laboratories Inc., Detroit, MI), and incubated at 37 °C for 6 h. A loopful of cell suspension of each strain was then transferred to fresh 10 ml TSB followed by incubation at 37 °C for 24 h. One ml of cell suspension from each strain was combined, and the mixture was further diluted with sterile 0.1% peptone water (PW) to a cell concentration of $10^8$–$10^9$ cfu/ml for use as an inoculum.

2.1.1. Experimental design

Two central composite designs were used to select the levels of salt and liquid smoke (phenol) and the storage temperatures for testing. One design with salt 0–10%, phenol 0–34 ppm, and storage temperatures 0–25 °C, and the other one with 2–10% salt, 0–34 ppm phenol, and temperatures 0–12 °C. The second design was used to select treatments with storage temperatures at refrigerated and mild abuse temperatures, which were more relevant to the typical storage temperatures of smoked salmon. A total of 33 treatments were selected by the experimental designs (Table 1).

2.2. Sample preparation

Raw fresh salmon fillets were obtained from a local grocery store. The fillets were frozen at −80 °C for 3 days, and then thawed at refrigerated temperature. The liquid that came out of the fillet during the thawing was removed. Fillets were cut into pieces, placed in stomacher bags (Spiral Biotech, Inc., Norwood, MA), and cooked in a water bath to product temperature of 63 °C for 30 min to inactivate the native microflora. Cooked salmon pieces were cooled at 4 °C, and then flaked in the bags to near pure. Salmon puree was placed into stomacher bags, mixed with the quantities of salt and liquid smoke (Charsol-PN-9, Red Arrow Products Co., Manitowoc, WI) as indicated in Table 1 to a final sample weight of 50 g. The liquid smoke (pH 5.2, 8.5 mg/ml phenol) was diluted with sterile water 1:1 and filtered through a sterile 0.45 μm tube filter (Corning Inc., Corning, NY). For a concentration of 34 ppm phenol in 50 g sample, 0.4 ml of the diluted liquid smoke was added. The sample was inoculated with a 1-ml cell mixture of L. monocytogenes to achieve an inoculum level of $10^2$–$10^3$ cfu/g. The bag was stomached for 2 min. Eight 5-g inoculated samples were placed into 100-ml stomacher bags (Spiral Biotech, Inc., Norwood, MA), and the bags were vacuum-sealed using a Multivan A300 vacuum sealer (Multivan Inc., Kansas, MS). TSB was prepared by dissolving 30 g of TSB dry media.
(Difco) in 11 d.i. water. The amounts of salt (Table 1) were weighted into screw-capped test tubes, and TSB was added to a total amount of 10 g. The tubes were autoclaved at 121 °C for 15 min. Liquid smoke was then added into TSB to achieve the desired phenol concentrations. The tubes were inoculated with 100 µl mixture of L. monocytogenes to achieve an inoculum level of 10^{2–3} cfu/ml. Eighty 200 µl of the inoculated sample dilution were dispensed into sterile 96-well tissue culture plates (Phoenix Research Products, Hayward, CA). The plates were vacuum-sealed in stomacher bags. The pH and $a_w$ of TSB and salmon were measured before L. monocytogenes inoculation. The pH of TSB were measured directly by using a Daigger 5500 pH meter (A. Daigger and Company Inc., Wernon Hills, IL), while 5 g of salmon were macerated with 15 ml d.i. water in a stomacher bag for 2 min, and the pH was measured. The $a_w$ of TSB and salmon were measured by placing 3 g of the sample in an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA). Salmon ($a_w$ 0.92–0.98, pH 6.4–6.8) and TSB ($a_w$ 0.93–0.99, pH 6.7–7.2) samples were stored at the selected temperatures (Table 1) for up to 42 days. The storage duration was the reported average shelf life of smoked salmon at refrigerated temperature (Rorvik, 2000), and was the length of storage time used in a study by Le Marc et al. (2005) for a L. monocytogenes growth/no growth boundary evaluation. The experiment was performed in two separate trials ($N = 2$) with 8 samples prepared for each trial ($n = 16$).

2.3. Determination of growth/no growth of L. monocytogenes

At the end of storage, levels of L. monocytogenes in samples were enumerated. To salmon samples, 5 ml of sterile 0.1% PW were added into each bag, and the bags were stomached for 2 min. Additional 1:10 dilutions were prepared in sterile 0.1% PW. Duplicate sample dilutions (0.1 ml) were spread-plated onto modified Oxford agar (MOX, Oxoid Ltd., Hampshire, England) plates. To TSB samples, duplicate 50 µl were added into 0.5 ml 0.1% PW, and duplicate dilutions (0.1 ml) were spread-plated onto MOX agar. Initial counts of L. monocytogenes in salmon or TSB were enumerated by spread-plating duplicate 0.1 ml of 1:1 dilution of salmon sample or 50 µl TSB sample. Plates were incubated at 37 °C for 48 h, and black colonies surrounded by black precipitation were counted. A positive growth of L. monocytogenes was recorded for a sample when the L. monocytogenes count after storage was $1.5 \log_{10}$ cfu/g (ml) higher than the initial count. The $1.5 \log_{10}$ criterion was adopted from a study by Le Marc et al. (2005).

2.3.1. Model fitting

The probability of positive growth of L. monocytogenes (numbers of samples with positive growth divided by the numbers of total sample tested) for each treatment as a function of the concentrations of salt and phenol, storage temperature, and their interactions were analyzed using the logistic procedure with stepwise selection of SAS 9.1 for Windows (SAS Institute Inc., Cary, NC) to select variables for fitting the model. The initial model for fitting had the following form: $\logit(p) = \alpha + \beta_1(salt) + \beta_2(phenol) + \beta_3(temperature) + \beta_4(salt \times phenol) + \beta_5(salt \times temperature) + \beta_6(phenol \times temperature) + \beta_7(salt \times phenol \times temperature)$, where $\alpha$ is the intercept, and $\beta_1-\beta_7$ are parameter estimates.

Once the models were selected for salmon and TSB, the growth probabilities ($p$) of L. monocytogenes were calculated using the following formula (SAS Institute Inc., 2004):

$$
\ln\left(\frac{p}{1-p}\right) = \logit(p)
$$

in which the probability of growth is $p = 1/(1 + e^{-\logit(p)})$.

3. Results and discussion

The initial counts of L. monocytogenes were 2.2–2.7 log_{10} cfu/g and 2.4–2.6 log_{10} cfu/ml in salmon and TSB, respectively. Samples that had L. monocytogenes counts greater than 4.2 log_{10} cfu/g (ml) after 42-day storage were recorded as samples with positive growth. The numbers of salmon or TSB samples with positive growth and the growth probability of L. monocytogenes for each treatment are listed in Table 1. No growth of L. monocytogenes was observed in salmon and TSB samples for treatments 1, 2, 4, 5, 6 and 13, which generally contained relatively higher concentrations of salt and phenol, and were stored at lower temperatures ($\leq$ 6 °C). With the same treatments, the growth probabilities of L. monocytogenes in TSB samples were generally higher than those of salmon samples. This indicated that TSB were more supportive to the growth of L. monocytogenes than salmon. As expected, at the same storage temperatures in which L. monocytogenes growth occurred, the growth probabilities were lower in salmon or in TSB samples containing higher salt or phenol concentrations, indicating a growth inhibitory effect of salt and phenol on L. monocytogenes, which has also been observed by other studies (Membre et al., 1997; Leroi and Joffraud, 2000; Lebois et al., 2004).

The parameter estimates for the logistic regression models describing the probabilities of growth of L. monocytogenes in salmon and TSB as a function of the concentrations of salt and phenol and storage temperature are shown in Table 2. The resulting logistic regression models are:

**Salmon**

$$
\logit(p)_{\text{salmon}} = 1.7891 - 0.6064(salt) - 0.0061(phenol) + 0.5295(temperature) - 0.0164(phenol \times temperature)
$$

**TSB**

$$
\logit(p)_{\text{TSB}} = 1.8680 - 0.5273(salt) - 0.0224(phenol) + 0.6021(temperature) - 0.0182(phenol \times temperature)
$$

Measures for assessing the predictive ability of these two models based on the number of pairs of observations with different response values, the number of concordant pairs and the number of discordant pairs (SAS Institute Inc., 2004; Le Marc et al., 2005) are listed in Table 2. The degrees of agreement between the model predictions and observed responses based on the concordance index shows that the agreements were 91 and 90% for the salmon and TSB model, respectively (Table 2). The salt, temperature and the interaction of phenol and temperature were significant ($p < 0.05$) factors that affected the growth/no growth determination of L. monocytogenes in both salmon and TSB (Table 2). Using these two models, the Logit($p$) values for salmon and TSB samples can be obtained, and the probabilities of growth of L. monocytogenes can be estimated using Eq. (1). For example, for samples containing 6% salt, 20 ppm phenol, and stored at 4 °C for 42 days, the estimated probabilities were recorded as samples with positive growth.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Simulated salmon</th>
<th>TSB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Standard error</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.7891</td>
<td>0.0491</td>
</tr>
<tr>
<td>Salt</td>
<td>-0.06064</td>
<td>0.0860</td>
</tr>
<tr>
<td>Phenol</td>
<td>-0.00605</td>
<td>0.0415</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.5295</td>
<td>0.1209</td>
</tr>
<tr>
<td>Pairs</td>
<td>-0.0164</td>
<td>0.00520</td>
</tr>
</tbody>
</table>

Percent concordant 90.8 90.0

Percent discordant 8.4 9.1

Percent tied 0.8 0.9

Table 2 Parameter estimates and associated statistics for the logistic regressions of the growth probability of L. monocytogenes in salmon and TSB.
growth probability of *L. monocytogenes* in salmon is $p_{\text{salmon}} = 1/(1 + \exp(-\text{logit}(p_{\text{salmon}}))) = 0.238$, and in TSB is $p_{\text{TSB}} = 1/(1 + \exp(-\text{logit}(p_{\text{TSB}}))) = 0.312$. The estimated growth probability of *L. monocytogenes* (0.238) in salmon containing 6% salt, 20 ppm phenol, and at temperature of 4 °C was close to a separate test using salmon in which the observed growth probability was 0.25 (4/16).

Models describing the growth boundary or growth probability of *L. monocytogenes* in lightly preserved seafood (Mejlholm and Dalgaard, 2007), liquid dairy products, cheese, meat and seafood products (Augustin et al., 2005), and tryptic soy broth and agar (Koutsoumanis et al., 2004) have been reported. The comparison of models from this study to the models reported by Augustin et al. (2005) and Koutsoumanis et al. (2004) are limited by the differences in substrates and the parameters used in model development. Mejlholm and Dalgaard (2007) reported that *L. monocytogenes* increased >100-fold in lightly preserved seafood containing 3.03–5.26% water phase salt (wps) and 4.9–20.1 ppm phenol within 40-day of storage at 8 or 15 °C. Models from this study predicted that the growth probability of *L. monocytogenes* were 0.220–0.999 (salmon model) and 0.348–1.000 (TSB model) between the least favorable growth condition (5.26% wps (7.5% w/w), 20.1 ppm phenol, and stored at 8 °C) and the most favorable growth condition (3.03% wps (4.3% w/w), 4.9 ppm phenol and 15 °C). This indicated that the models from this study were accurate in predicting the growth probability of *L. monocytogenes* in smoked seafood. Using these two models, growth probabilities of *L. monocytogenes* in salmon and TSB containing 0–10% salt and 0–34 ppm phenol at storage temperatures of 0–25 °C can be estimated. Fig. 1 shows the estimated growth probabilities in salmon and TSB at storage temperatures of 4 and 12 °C. The figures show that the increases of salt and/or phenol concentrations decrease the probabilities of growth of *L. monocytogenes*, the growth probabilities at same salt/phenol concentrations are lower in salmon than in TSB, and, in both salmon and TSB, the growth probabilities are higher at higher storage temperatures. The figures also show that salt and storage temperature are more profound than phenol in affecting the growth probability of *L. monocytogenes*. To examine the correlation between the growth probabilities of *L. monocytogenes* in salmon and TSB, the boundary of growth/no growth of *L. monocytogenes* in salmon and TSB at growth probabilities of 0.1 and 0.5 ($p = 0.1$ and 0.5) as affected by the concentrations of salt and phenol at refrigerated (4 °C) and abuse temperatures (12 and 20 °C) were compared (Fig. 2). At the three storage temperatures, the salt and phenol concentrations are lower in salmon than in TSB to have

![Fig. 1. Estimated growth probability of L. monocytogenes in salmon and TSB containing 0–10% salt, 0–34 ppm phenol and stored at 4 °C (a, salmon; b, TSB) and 12 °C (c, salmon; d, TSB).](image-url)
the same growth probability of L. monocytogenes, particularly at 4 °C. For example, at 4 °C and \( p = 0.1 \), salmon samples would need 20 ppm phenol/7.3% salt or 10% salt/1 ppm phenol, whereas TSB samples would need 20 ppm phenol/8.5% salt or 10% salt/13 ppm phenol (Fig. 2a). At 4 °C, at higher salt concentrations (e.g., 10%), higher phenol concentrations are needed in TSB (13 ppm) than in salmon (1 ppm) to have the same growth probability for L. monocytogenes, whereas at higher phenol concentrations (e.g., 34 ppm), approximately the same salt concentrations are needed in both salmon (6.0% salt) and TSB (6.2% salt) to have the growth probability of 0.1 (Fig. 2a). The differences between the salt and/or phenol concentrations in salmon and TSB to have same growth probabilities for L. monocytogenes decrease as the storage temperatures increase (Figs. 2b,c). Comparisons show that the growth probabilities of L. monocytogenes estimated by TSB model are higher than those by salmon model, particularly at lower storage temperatures. The estimations by both models are closer at lower temperatures. It is reasonable to assume that, at more favorable growth temperatures, L. monocytogenes could overcome the differences in the intrinsic factors (e.g., \( a_w \), pH, and nutritional properties) between salmon and TSB and exhibit similar growth ability in salmon and TSB. The comparisons between the growth/no growth interface at \( p = 0.1 \) and \( p = 0.5 \) for both salmon and TSB show that the transition width from conditions for which growth are likely (\( p > 0.5 \)) to conditions for which growth are unlikely (\( p < 0.1 \)) decrease as storage temperatures increase, i.e., transitions are wider at higher storage temperatures (Fig. 2). The wider transitions at lower temperatures is mainly contributed by phenol, indicating the increases of phenol concentration at lower storage temperatures are more effective in lowering the growth probabilities of L. monocytogenes than at lower storage temperatures. The transition of conditions observed in this study was similar to that reported by Le Marc et al. (2005). Using a model proposed by Ratkowsky and Ross (1995), Le Marc et al. (2005) modeled the probability of growth of Yersinia enterocolitica and Escherichia coli as a function of temperature, pH, and \( a_w \) with data obtained from published literatures. These authors showed that the transition were wider for both microorganisms at lower temperatures than at higher temperatures.

Results from this study showed that the growth probabilities of L. monocytogenes in salmon and TSB were affected differently by the concentrations of salt and phenol and storage temperature, and higher salt and phenol concentrations were generally needed in TSB than in salmon to have the same growth probabilities for L. monocytogenes. The differences in the estimated growth probabilities between TSB and salmon models were smaller for phenol concentrations, at higher storage temperatures, and lower salt concentrations, indicating the growth probabilities of L. monocytogenes were similar in salmon and TSB at temperatures and salt levels that were more favorable for the growth of L. monocytogenes. Therefore, the use of TSB to substitute salmon in studying the growth behavior of L. monocytogenes may only be suitable for higher storage temperatures (e.g., >12 °C), and in examining the effect of phenol concentration on L. monocytogenes at lower salt concentrations (e.g., <6% at 4 °C).

The comparisons of the growth probabilities (Fig. 1) and boundary of growth/no growth of L. monocytogenes (Fig. 2) showed that the correlation between the growth probabilities of L. monocytogenes in salmon and TSB were salt, phenol and storage temperature dependent. Since \( \ln(p/(1 - p_{salmon})) = \text{logit}(p)_{salmon} \) and \( \ln(p/(1 - p_{TSB})) = \text{logit}(p)_{TSB} \), therefore \( \ln(p/(1 - p_{salmon})) = \text{logit}(p)_{salmon} - \text{logit}(p)_{TSB} + \ln(p_{TSB}/(1 - p_{TSB})) \), and \( \text{logit}(p)_{TSB}/(1 - p_{TSB}) - 0.0789 - 0.0791(\text{salt}) - 0.0164(\text{phenol}) + 0.0726(\text{temperature}) + 0.0018(\text{phenol} \times \text{temperature}) \), in which the parameter estimates are the differences between the estimates for \( \text{logit}(p)_{salmon} \) and \( \text{logit}(p)_{TSB} \). Therefore, \( p_{salmon} \) and \( p_{TSB} \) have the following correlation:

\[
p_{salmon} = 1/1 + \exp\left[-(0.2824 - 0.2742(\text{salt}) + 0.0423(\text{phenol}) + 0.0888(\text{temperature}) - 0.0073(\text{phenol} \times \text{temperature}) + \ln\left(\frac{p_{TSB}}{1 - p_{TSB}}\right)\right]
\]

The results of this study demonstrated the growth probabilities of L. monocytogenes in salmon and TSB as affected by salt, phenol, and storage temperature. The models may be used to estimate the growth probabilities of L. monocytogenes at selected concentrations of salt and phenol and storage temperatures or to estimate the concentrations of salt and phenol and storage temperature that may provide an acceptable probability of growth for L. monocytogenes in smoked seafood containing both additives. The models indicate that high concentrations of salt and phenol and lower storage temperatures are generally needed for L. monocytogenes to have a probability of unlikely to growth (e.g., \( p < 0.1 \)), in which the conditions may not be practical or obtainable for food products. Therefore, the application of the models is more useful in understanding the effect of salt, phenol, storage temperature, and their interactions on the growth probabilities of L. monocytogenes than finding the conditions that are capable of preventing the growth of L. monocytogenes.
Acknowledgments

The author would like to thank Mrs Benne Marmer and Ms Stacy Raleigh for their technical assistance with performing part of the laboratory component of this study, and Dr John Phillips for providing consultation with the regression analysis.

References


