A1 APPENDICES:

Table A1.1. Description of the parameter set estimated by the asymptotic regression model**.**

Parameter	Description
Asymptote	A value representing the level to which a population declines and then stabilises to form a horizontal asymptote
R0	The parameter representing the initial inoculum, or response (R) value when Time is zero (0)
LRC	Parameter representing the maximum rate of change in the response per unit of time

Table A1.2 Description of the parameter set estimated by the logistic regression model**.**

Parameter	Description
Asymptote	A value representing the level to which a population declines and then stabilises to form a horizontal asymptote
Mid-point	Numeric parameter representing the input at the inflection point of the curve (midway between A and B at mid-point)
Scale Parameter	Numeric parameter representing the maximum rate of change in the response

Table A1.3 Description of parameter set estimated by the four-parameter logistic regression model.

Parameter	Description
Asymptote A	A value representing initial resistance of a population during the early observation period prior to decline
Asymptote B	A value representing the level to which a population declines and then stabilises to form a horizontal asymptote at the later stages of the observation period
Mid-point	Parameter representing a point in time at the inflection point of the curve midway between asymptote A and B at the mid-point
Scale Parameter	Numeric parameter representing the maximum rate of change in the response

Table A1.4 Description of parameter set estimated by the bi-exponential regression model.

Parameter	Description
Asymptote A1	A value representing initial resistance of a population during the early observation period prior to decline
LRC1	Parameter representing the exponential rate of change in the response per unit of time
Asymptote A2	A value representing the level to which a population declines and then stabilises to form a horizontal asymptote at the later stages of the observation period
LRC2	Parameter representing the exponential rate of change in the response per unit of time

Table A1.5 Description of parameter set estimated by the four-parameter Weibull model.

Parameter	Description
Asymptote	A value representing initial resistance of a population during the early observation period prior to decline
Drop	Numeric parameter representing the change from the asymptote to the intercept
LRC	Numeric parameter representing the maximum rate of change in the response per unit of time
Power	Numeric parameter representing the power to which the Time is raised

Table A1.6. Description of parameter set estimated by the log-linear model.

Parameter	Description
Kmax	First order inactivation rate constant
N0	Initial inoculum size

Table A1.7. Description of parameter set estimated by the log-linear model incorporating a shoulder effect.

Parameter	Description
SI	Length of shoulder effect describing initial resistance to challenge
Kmax	First order inactivation rate constant
N0	Initial inoculum size

Table A1.8. Description of parameter set estimated by the log-linear model incorporating an asymptotic function.

Parameter	Description
Kmax	First order inactivation rate constant
Nres	Length of shoulder effect describing initial resistance to challenge
N0	Initial inoculum size

Table A1.9. Description of parameters estimated by the log-linear model incorporating a shoulder effect and asymptotic function.

Parameter	Description
SI	Length of shoulder-effect describing initial resistance to challenge
Kmax	First order inactivation rate constant
Nres	Estimates the point at which an asymptote forms
N0	Initial inoculum size

Table A1.10. Description of parameter set estimated by the Weibull model.

Parameter	Description
δ	The time to the first log-reduction of the first subpopulation
p	Numeric parameter describing the shape of the inactivation curve
N0	Initial inoculum size

Table A1.11. Description of parameter set estimated by the Weibull model incorporating an asymptotic function.

Parameter	Description
Nres	Estimates the point at which an asymptote forms
δ	The time to the first log-reduction of the first subpopulation
p	Numeric parameter describing the shape of the inactivation curve
N0	Initial inoculum size

Table A1.12. Description of parameter set estimated by the mixed Weibull distribution model.

Parameter	Description
α	The fraction of the first subpopulation remaining in the total population
δ1	The time to the first log-reduction of the first subpopulation
p	Numeric parameter describing the shape of the inactivation curve
N0	Initial inoculum size
δ2	The time to the first log-reduction of the second subpopulation

Table A1.13. Description of parameter set estimated by Biphasic model incorporating an asymptotic function.

Parameter	Description
F	Relates to the fraction of the initial subpopulation in a major population
Kmax1	First order inactivation rate constant of the first subpopulation
Kmax2	First order inactivation rate constant of the second subpopulation
N0	Initial inoculum size

Table A1.14. Description of parameter set estimated by the Biphasic model incorporating a shoulder effect and asymptotic function.

Parameter	Description
F	Relates to the fraction of the initial subpopulation in a major population
Kmax1	First order inactivation rate constant of the first subpopulation
Kmax2	First order inactivation rate constant of the second subpopulation
N0	Initial inoculum size
SI	Length of shoulder-effect describing initial resistance to challenge

R Predicted Response Curve Types

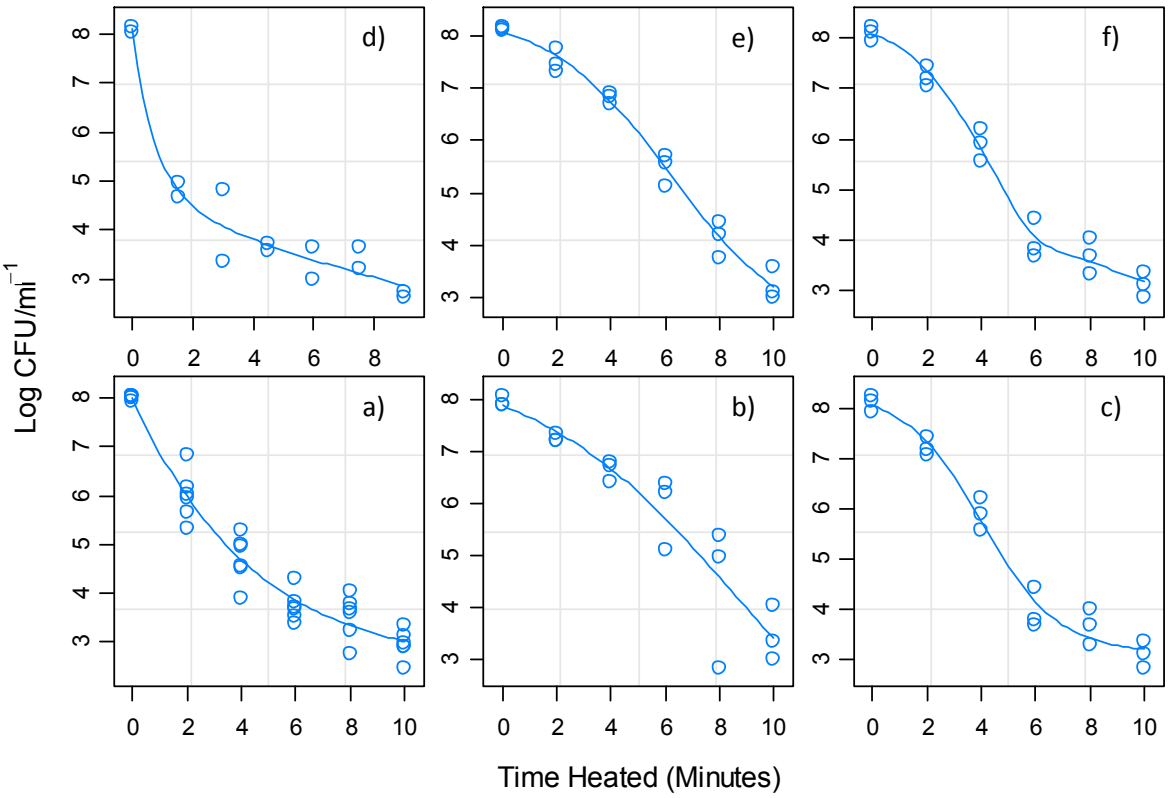

Figure A2. Illustrating the different types of predictive models generated within R that may be used to produce response curves for survival of *Campylobacter*: a) asymptotic regression model, b) logistic regression model, c) four-parameter logistic regression model, d) bi-exponential regression model, e) Four-parameter Weibull regression model and f) Mixed Weibull regression model.

GlnaFiT Predicted Response Curve Types

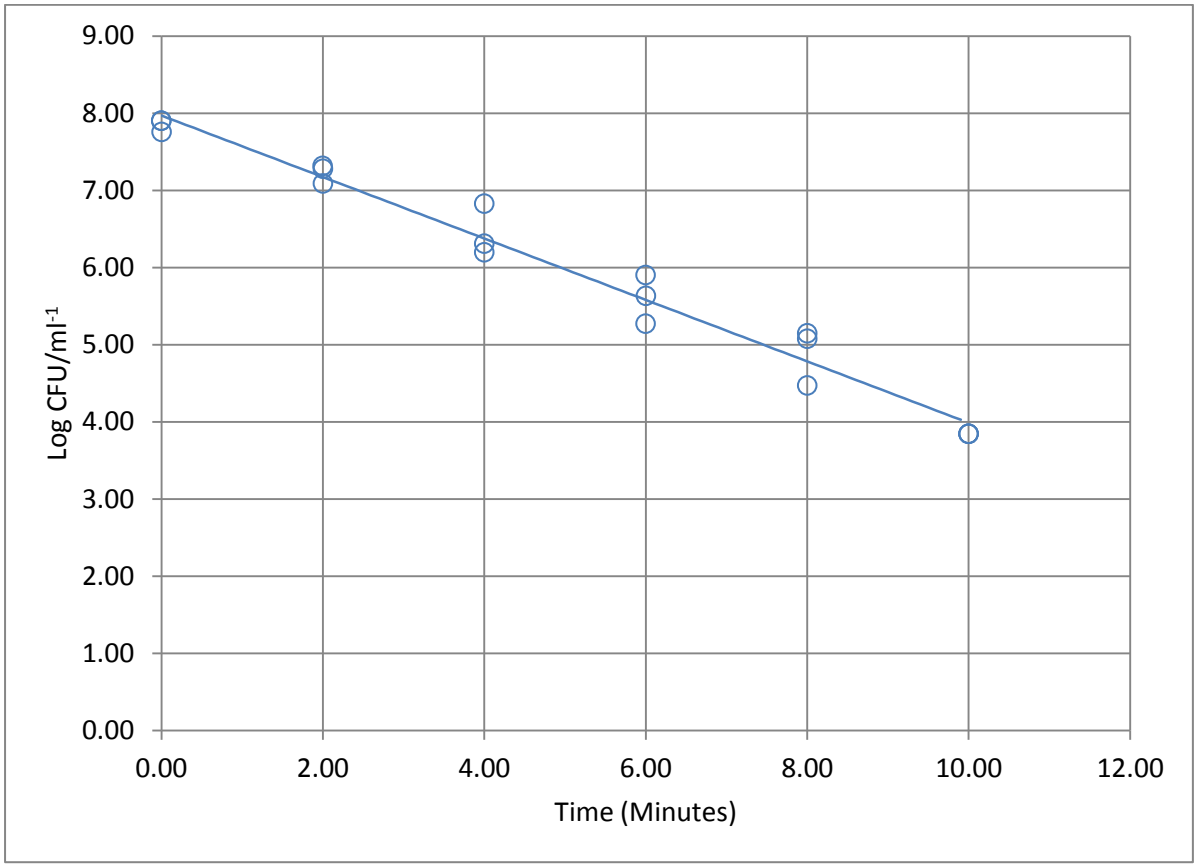

Figure A3.1 Log-linear model (Bigelow and Esty, 1920) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al*., 2005).

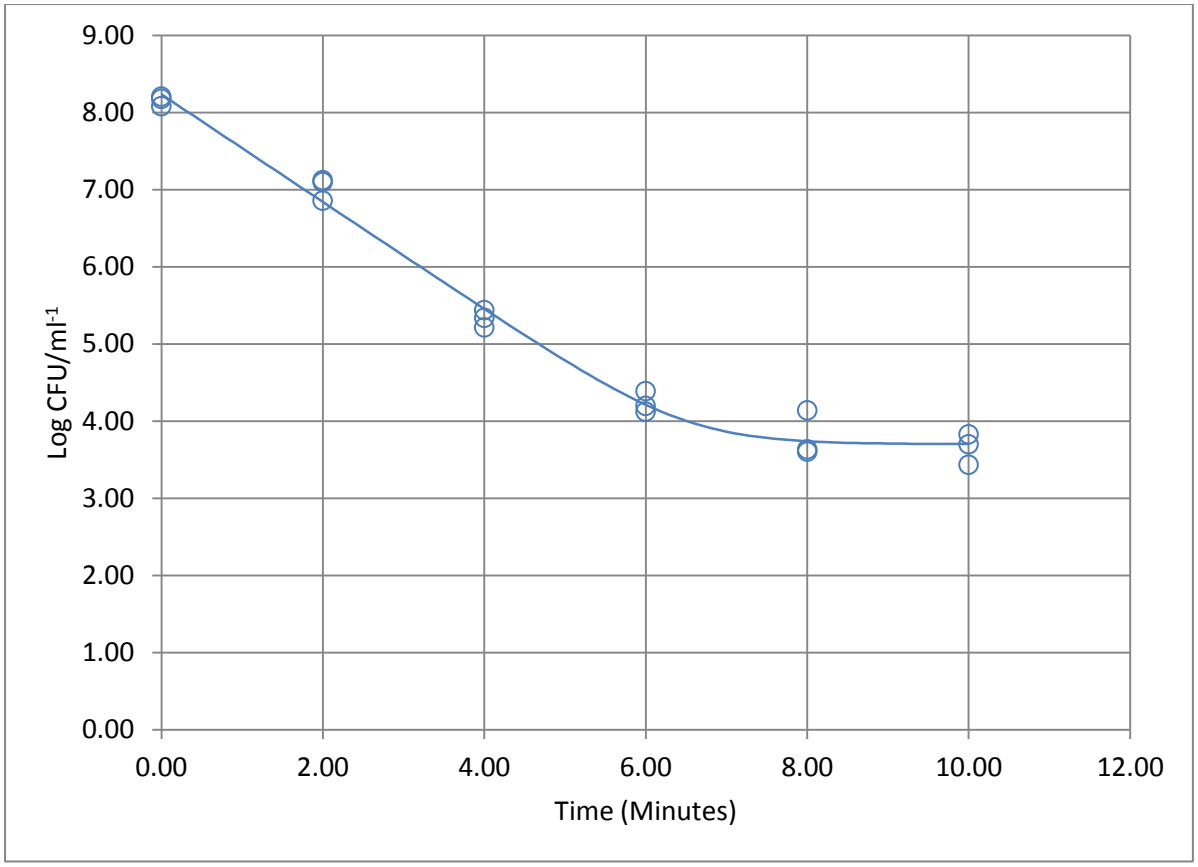

Figure A3.2. Log-linear model incorporating an asymptotic function (Geeraerd *et al*., 2000) predicted response curve generated using GInaFiT (1.6) (Geerared *et al*., 2005).

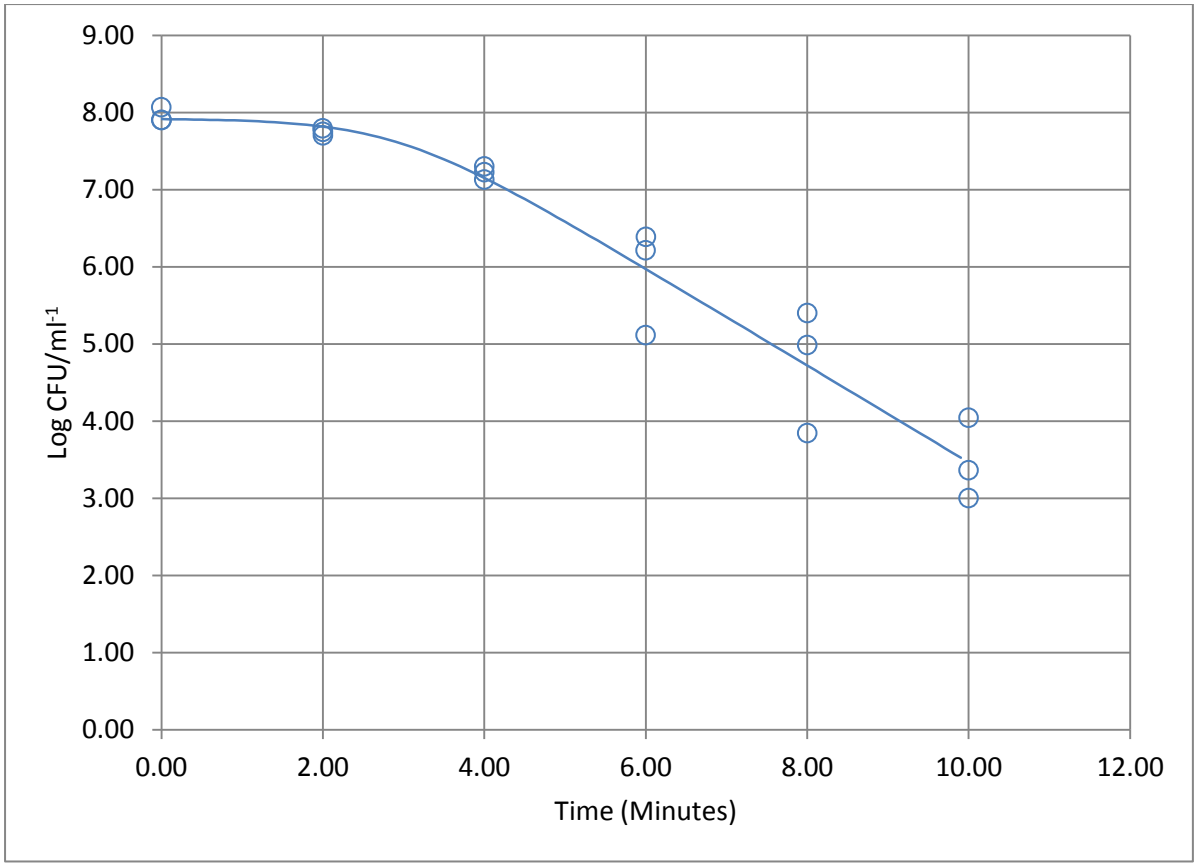

Figure A3.3. Log-linear model incorporating a shoulder-effect (Geeraerd *et al*. 2000) predicted response curve generated using GInaFiT (1.6) (Geerared *et al*., 2005).

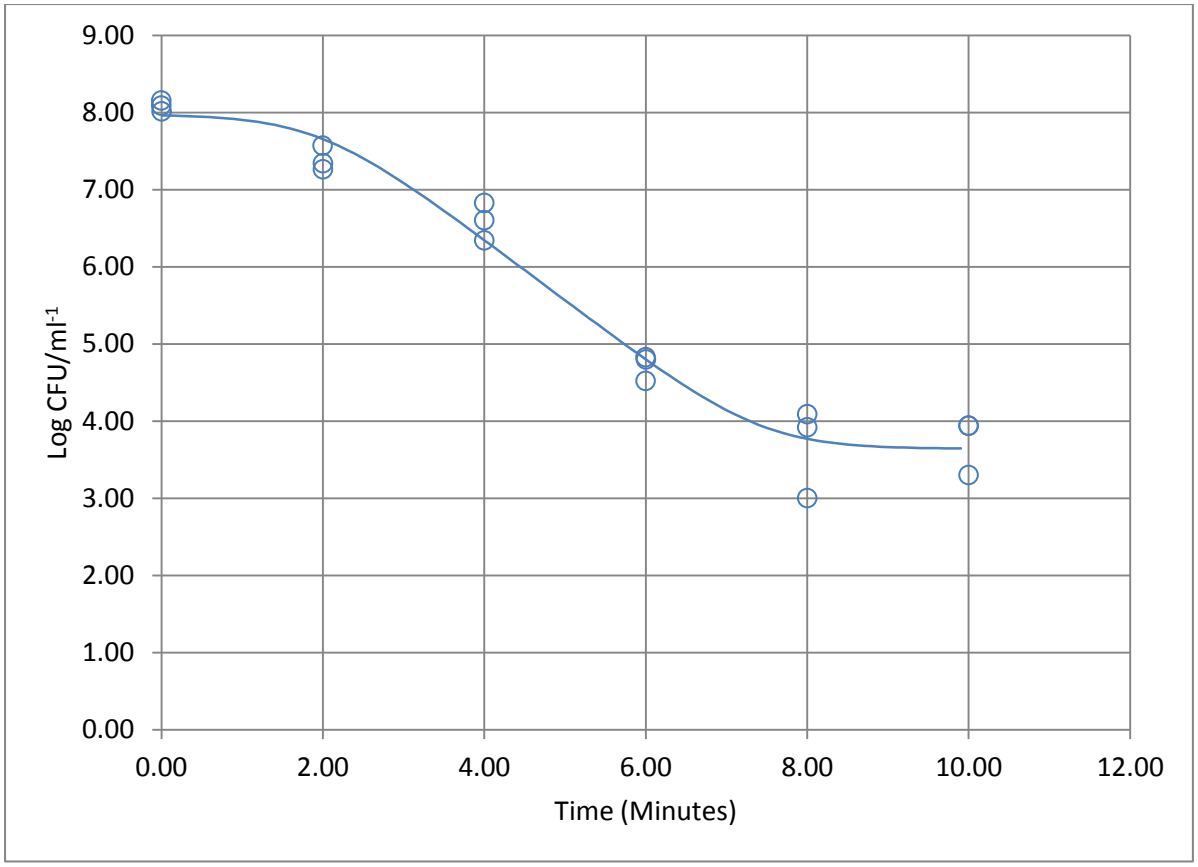

Figure A3.4. Log-linear model incorporating a shoulder-effect and asymptotic function (Geeraerd *et al*. 2000) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al*., 2005).

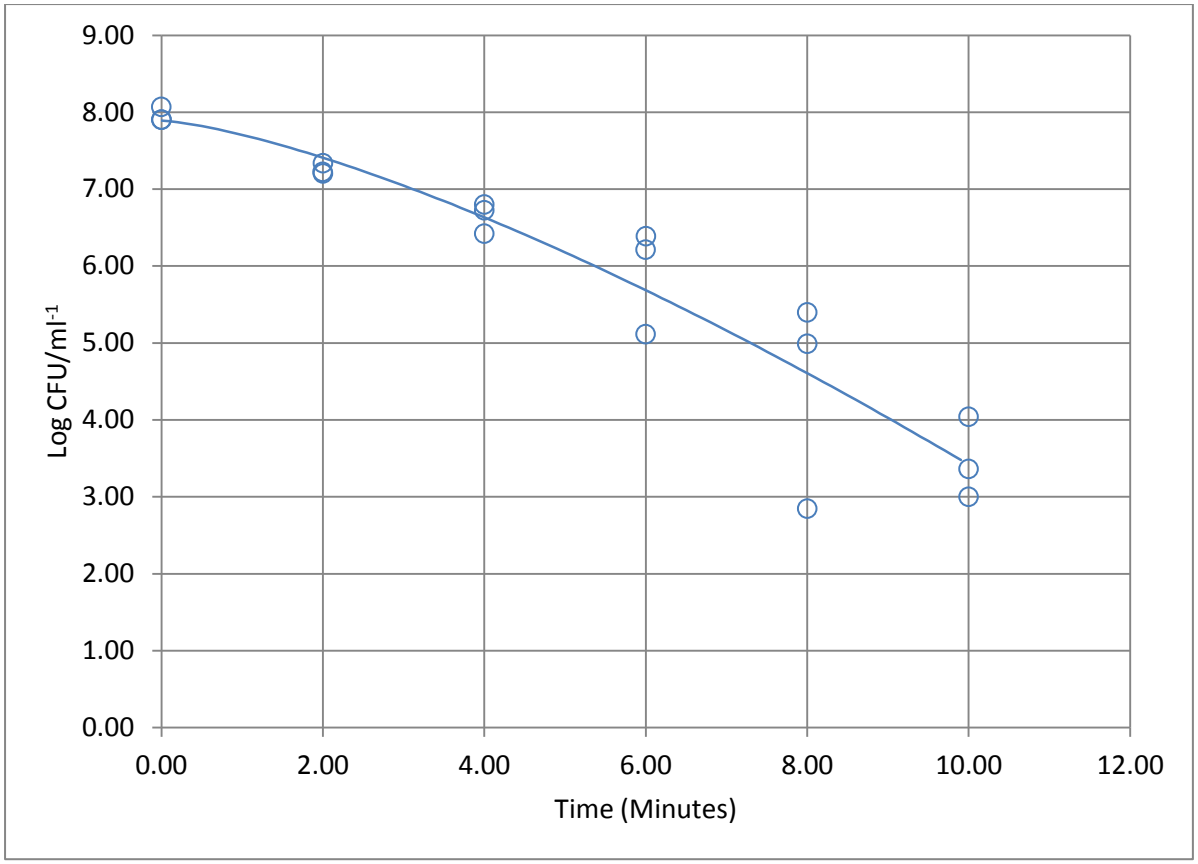

Figure A3.5. Weibull model (Mafart *et al.*, 2002) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al.*, 2005).

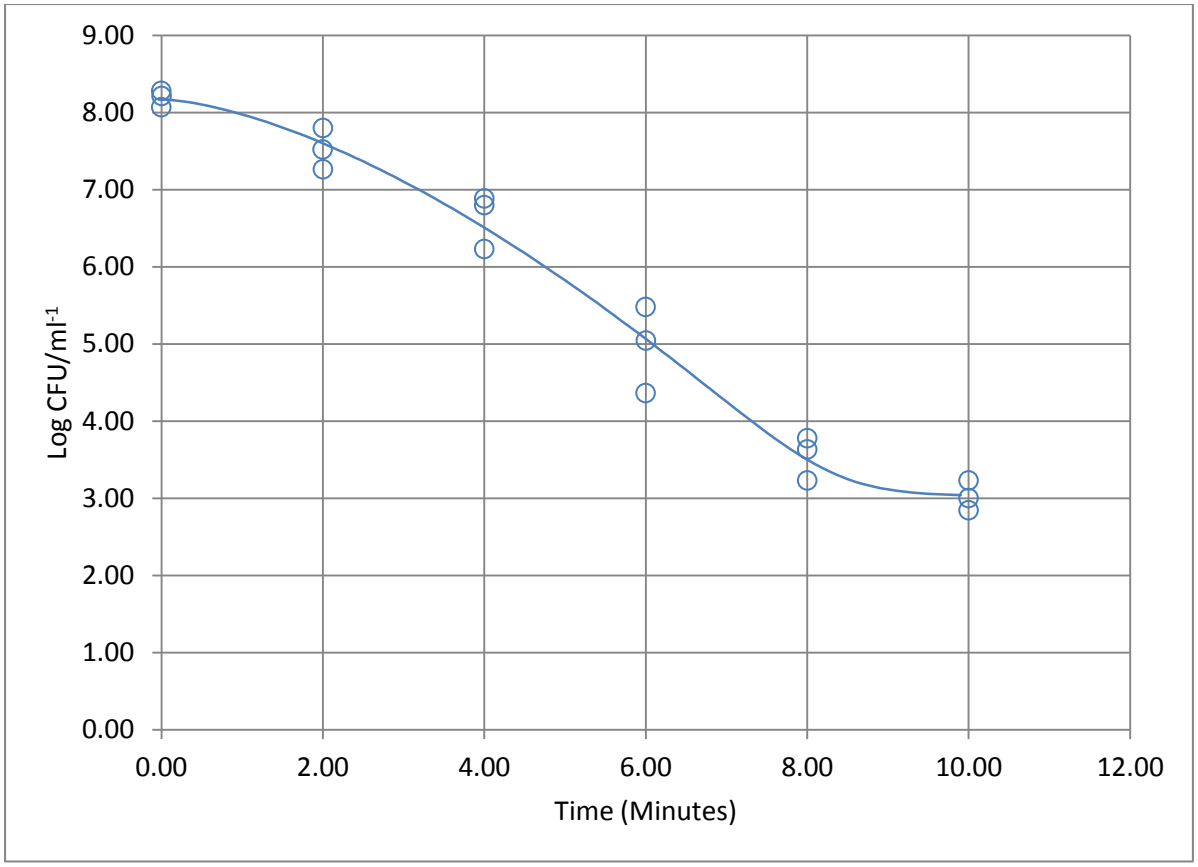

Figure A3.6. Weibull model incorporating an asymptotic function (Albert and Mafart, 2005) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al.*, 2005).

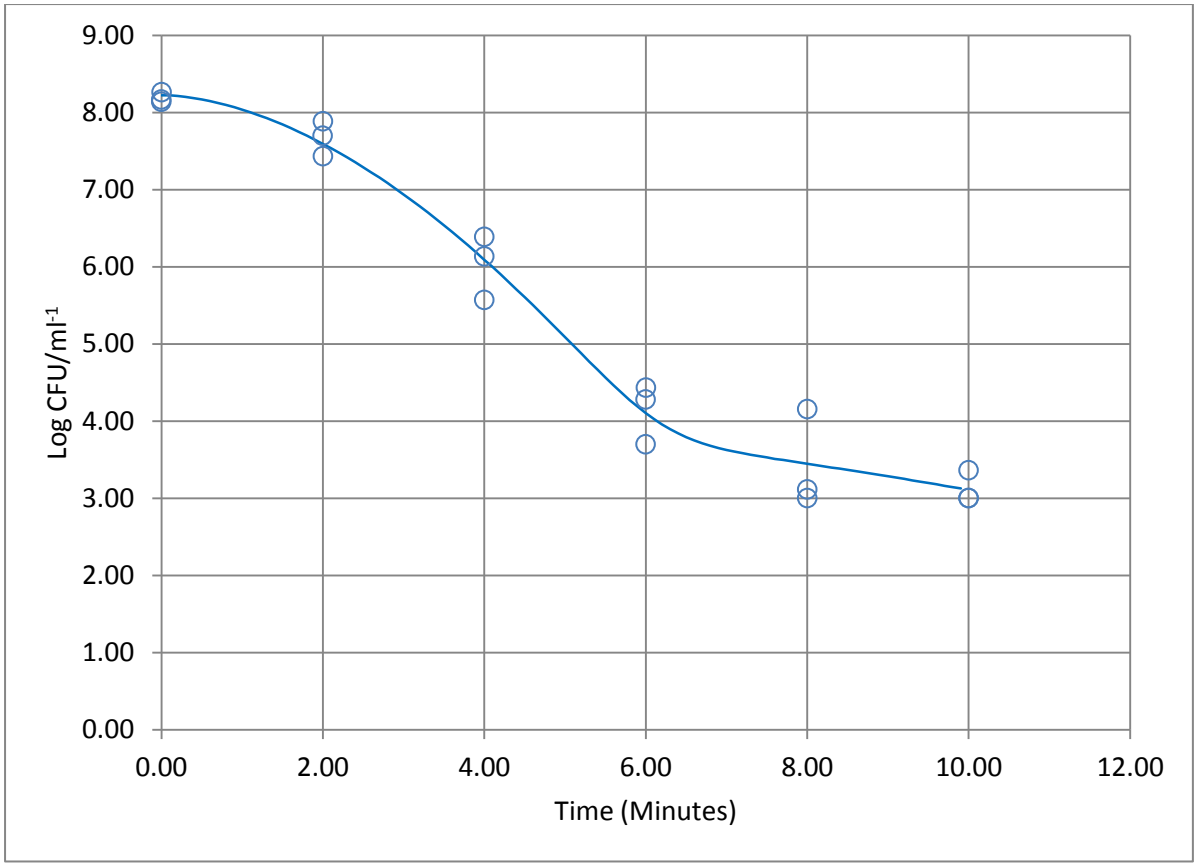

Figure A3.7. Mixed Weibull distribution model (Coroller *et al.*, 2006) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al.*, 2005).

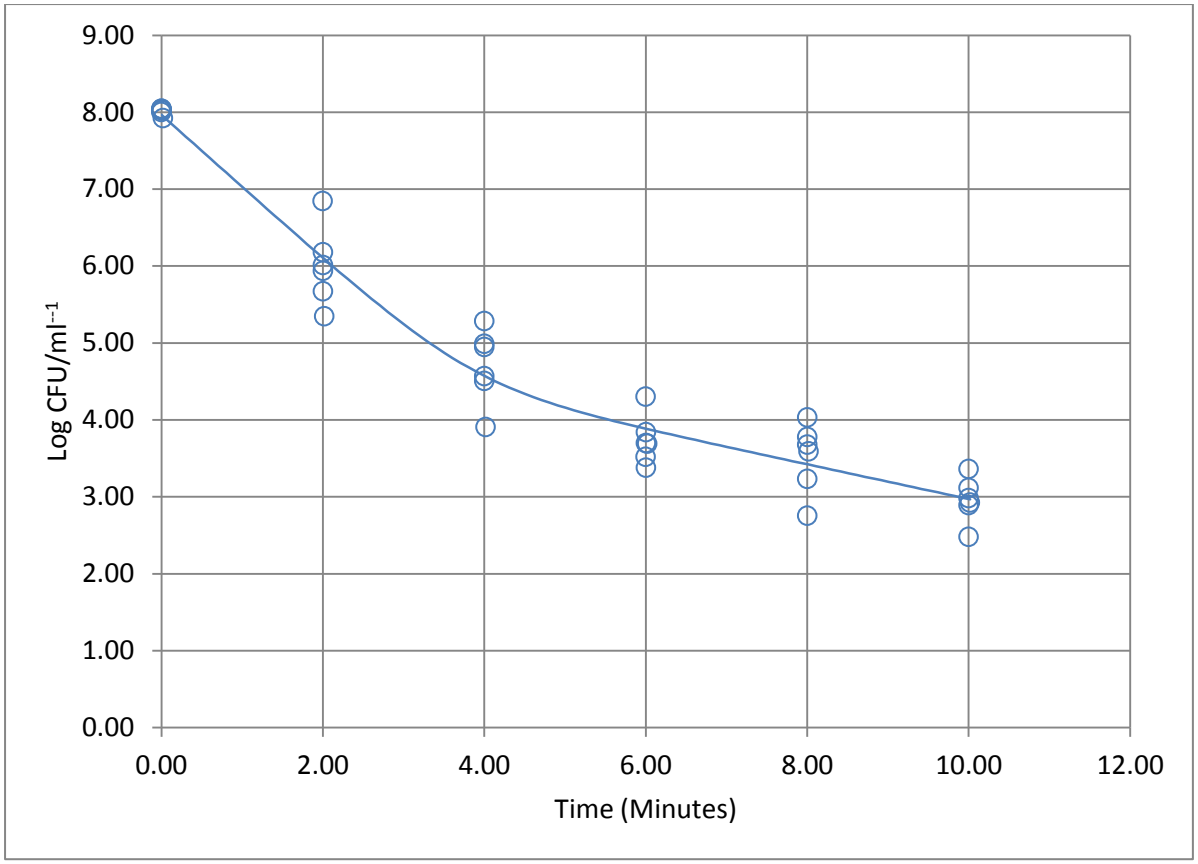

Figure A3.8. Biphasic model (Cerf 1977) predicted response curve generated using GlnaFiT (1.6) (Geerared *et al*., 2005).

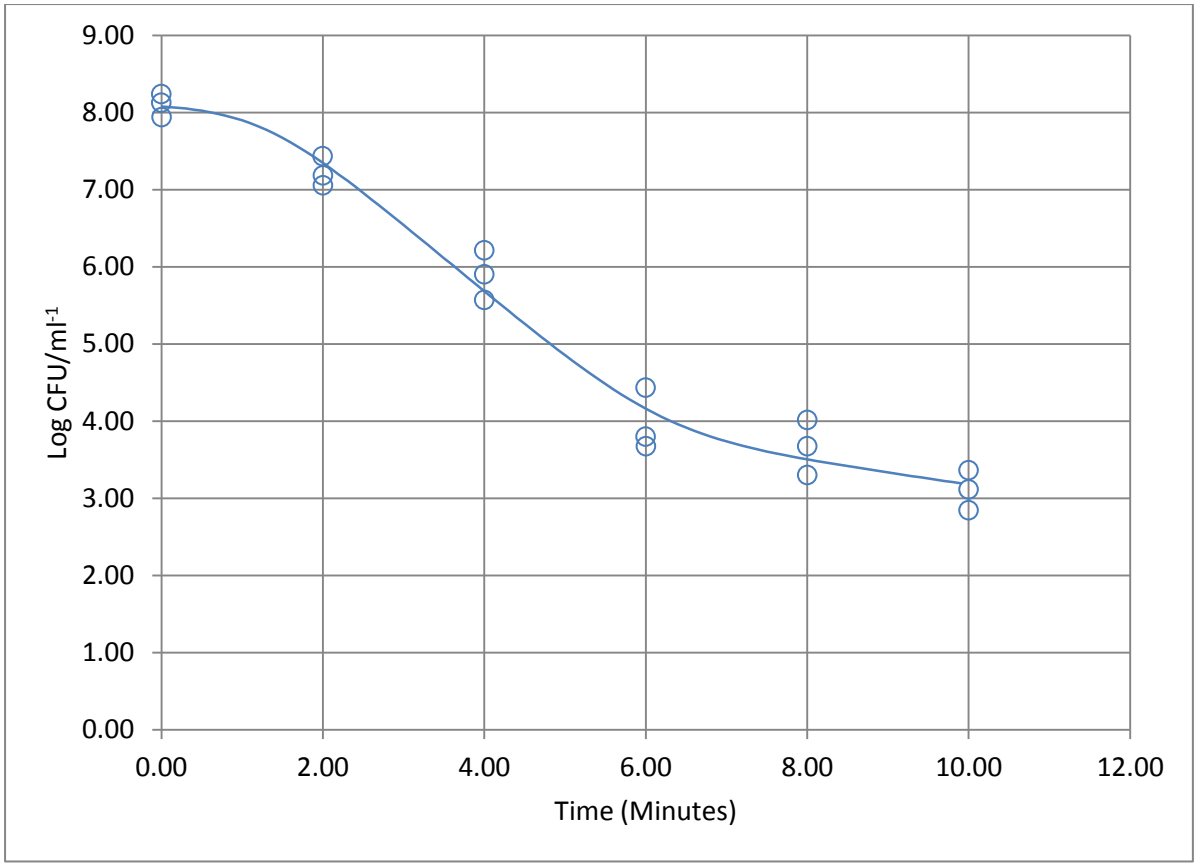

Figure A3.9. Biphasic model incorporating a shoulder-effect (Geeraerd *et al*., 2006) predicted response curve generated using GInaFiT (1.6) (Geerared *et al*., 2005).

A4. EXPERIMENTAL PROTOCOLS

A4.1. *Isolates used in the challenge experiments*

Isolates were derived from poultry meat or human clinical cases (strains kindly provided by Professor Andrew Fox, Public Health England). All isolates were subject to minimal passage before and after receipt at Liverpool and were kept as stock cultures prior to resuscitation for each individual experiment. Isolates marked with an asterisk indicate the smaller panel used in further challenge studies, as it was not possible to perform all experiments on members of the panel. The sub-set of isolates for further study was selected from initial results based upon their apparent differences in response to initial heat challenge experiments, with one *C. coli* isolate used for further experiments, as all four *C. coli* isolates appeared to have a similar response in initial heat challenge experiments.

Table A4.1. The full panel of 14 *C. jejuni* and *C. coli* isolates used in this project, their sequence type and source.

Isolate ID	Sequence Type and Clonal Complex	Species	Source
13126	ST-21,CC-21*	*C. jejuni*	Poultry
13121	ST-45, CC-45*	*C. jejuni*	Poultry
13136	ST-45, CC-45	*C. jejuni*	Poultry
13163	ST-21, CC-21	*C. jejuni*	Poultry
11253	ST-825, CC-828	*C. coli*	Human
11762	ST-829, CC-828	*C. coli*	Human
12628	ST-1773, CC-828*	*C. coli*	Poultry
12610	CC-828	*C. coli*	Poultry
11368	ST-574, CC-574	*C. jejuni*	Human
12645	ST-51,CC-443	*C. jejuni*	Poultry
12662	ST-257, CC-257*	*C. jejuni*	Poultry
12720	ST-51, CC-443	*C. jejuni*	Poultry
12745	ST-257, CC-257	*C. jejuni*	Poultry
12783	ST-574, CC-574	*C. jejuni*	Poultry

Subset of isolates used in all challenge experiments are indicted with *

A4.2. Survival of Campylobacter in pre-heated Campylobacter enrichment broth (CEB) with and without altered pH.

The methods utilised in these experiments were adapted from those described previously by Hughes *et al.* (2009) and Murphy *et al.* (2005).

A4.2.1. Preparation of the Inoculum

A single bead from a culture collection stock strain isolate at -80^{o}C, was streaked on a CAB/FBP plate (Columbia agar base, with 5% defibrinated horse blood and *Campylobacter* growth supplement (Lab M Ltd, Lancashire, UK)) and incubated at 37^{o}C (micro-aerobic conditions) for up to 48 hours. The isolate was sub-cultured onto a fresh CAB-FBP plate, and again incubated at 37^{o}C under micro-aerobic conditions for up to 24 hours. The bacterial inoculum was then prepared by adding a loopful of *Campylobacter* culture to maximum recovery diluent (MRD) and the OD_{600} determined using a 1:10 dilution of suspension in a spectrometer. The inoculum was adjusted to obtain a final reading of OD_{600} = 0.5. A 7ml bijoux containing 5.5ml *Campylobacter* Enrichment Broth (CEB, Bolton formulation, without blood or antibiotic supplement) to minimise headspace, was inoculated with 110µl (approximately 10^{8} colony forming units (CFU)) of the MRD prepared inoculum and incubated at 37^{o}C. Three replicates were prepared for each isolate with an additional negative control broth also incubated. The OD_{600} of *Campylobacter* growth in CEB at late log phase was determined after 18:00-18.50 hours incubation. Nominally, readings were observed to be in the range of 0.12 – 0.22. Serial dilution was undertaken to 10^{-1} to 10^{-6} in CEB and then plated using the Miles-Misra technique (Miles *et al.*, 1938) on CAB-FBP. This provided a pre-experiment CFU/ml^{-1} (approximately 10^{9}). Plates were then incubated using the approach described above.

A4.2.2 Heat and pH Challenge in Broth

For time-temperature survival simulations replicates of 9.0ml of CEB were pre-heated in glass universals in temperature controlled water bath (Starlabs Ltd). The temperature of the broth within the universals was monitored continuously by a digital data temperature recorder (DTR) (ETI Ltd, Worthing, UK) using an additional universal containing CEB without *Campylobacter*. For each experimental replicate, 1ml of *Campylobacter* culture was added to 9ml of pre-heated broth providing an initial *Campylobacter* concentration of ~10^{8} CFU/ml^{-1}. The concentration was then briefly vortexed before returning to the water bath. At appropriate time intervals 100µl of heat

challenged broth was removed and immediately added to 900µl of pre-cooled CEB and stored on ice for enumeration.

For simulations undertaken at 56^{o}C sampling was undertaken at 2 minute intervals from 0:00 – 10:00 minutes (5 observation points). At higher temperatures, the experimental duration was reduced and sampling intervals were shorter; 9 minutes at 60°C and 6 minutes at 64°C. The 10^{-1} dilutions were kept on ice prior to serial dilution and plating, as described previously, with dilutions made to 10^{-5} in pre-cooled CEB at 4^{o}C.

A post-experiment (time zero) serial dilution was prepared for each replicate at the end of the experiment without the *Campylobacter* culture undergoing any heat exposure treatment. This was to ensure that there was no significant change in the numbers of viable non-heated *Campylobacter* due to other factors, such as oxidation and chilling, and that the results obtained in the heat studies reflected accurately the experimental treatment. Colonies were counted at the dilution where the highest number of individual colonies could be counted. The average of 3 replicates was calculated and used to determine the CFU/ml^{-1}.

For the experiments which combined heat challenge with altered pH, adjustments to the pH of CEB were made by the drop-wise addition of 1M HCl (pH 6.5-4.5) and 1M NaOH (pH 8.5), followed by filter sterilisation (0.20µm).

A4.3 Extended Simulations:

Experimental simulations were undertaken to examine potential differences in the survival of sub-lethally damaged cells for two strains of *Campylobacter* 13121 (ST-45, CC-45) and 11168 (ST-43, CC-21). The numbers of cells recovered for each strain were compared using Columbia agar base (5% defibrinated blood) (CAB) plus ferrous sulphate, sodium meta-bisulphite, sodium pyruvate (FBP) or modified charcoal cefoperazone deoxycholate agar (mCCDA) (Figure 15). In addition, the impact of varying initial inocula (6 Log_{10} CFU/ml^{-1} and 8 Log_{10} CFU/ml^{-1}) on the numbers of cells recovered was examined using strains 13121 (ST-45, CC-45) and 13136 (ST-45, CC-45) (Figure 16). During each simulation, *Campylobacter* strains were exposed to 56^{o}C and observations were obtained from 0.0 – 16.0 minutes at 1 minute intervals.

A4.4. The effect of direct (instant) heat and gradual heat on the survival and recovery of Campylobacter attached to meat piece surfaces.

Prior to inoculation, isolates of *Campylobacter* were prepared as described above and placed onto the surface of chicken meat (1.2.1). Small pieces of meat were cut from whole chicken breast fillets using a stainless steel tissue core borer (3.8mm diameter and approximately 0.1-0.2g). Pieces where then sterilised in 70% ethanol. Individual pieces were placed in 60mm x 60mm re-sealable bags, flattened and rinsed using 0.5ml sterile MRD prior to inoculation with *Campylobacter*. The original overnight culture broth (~10^9 CFU/ml-^{1}) was diluted 1:10 (0.5ml to 4.5ml) in CEB to give ~10^8 CFU/ml^{-1}, with each meat piece inoculated directly on its surface with 10µl of the 1:10 dilution (~10^6 CFU/ml^{-1}). As for previous experiments, 3 culture broth replicates were used for each isolate. The meat pieces were kept in the re-sealable bags and prior to heat challenge were incubated at 37°C for 30 minutes to allow for attachment of *Campylobacter* (normal challenge). Following incubation, a subset of *Campylobacter* inoculated meat pieces were stored overnight at 4°C (pre-chill challenge) prior to heating. The pH of the chicken breast meat was tested for each experiment and was found to be in the range of pH 5.9-6.5.

For direct (instant) heating, a Star-lab mini water bath containing glass universals containing 10ml water was pre-heated to the required challenge temperature (56°C, 60°C, 64°C, 68°C and 70°C). A pre-heat challenge sample was obtained for each replicate at time zero (0 minutes). Individual bags containing a single meat piece was placed into the glass universals and at specified time points were removed and cooled rapidly on ice prior to serial dilution. Each meat piece was transferred to 900µl CEB, mixed using a 200µl tip to break up the piece and then vortexed vigorously for 20 seconds prior to serial dilution in CEB. Meat pieces which had been stored at 4°C after *Campylobacter* inoculation were allowed to reach room temperature prior to heating, to ensure comparability with those without chilling. For each set of experiments, a 0.1 – 0.2g piece of un-inoculated chicken meat was also tested to ensure there was no natural contamination of meat by *Campylobacter*.

For the gradual heating experiments, the water bath was set at 25°C and allowed to reach equilibrium with the glass universals containing the bagged inoculated meat pieces prior to heating to specified temperatures (56-70°C), with the removal of individual meat pieces at timed intervals up to 22 minutes following the water bath reaching the required temperature. Serial dilutions were plated on CAB/FBP and incubated under micro-aerobic conditions as described previously.

A4.5. The survival and recovery of Campylobacter from poultry meat interiors after gradual and direct (instant) heat exposure.

Chicken breast fillets were preserved at -20°C for approximately 3 hours in order to semi-freeze the tissue permitting easier removal of individual ~2g pieces using a tissue borer. Meat pieces were placed individually in re-sealable bags (60mm x 60mm) before storing at 4°C overnight. Appropriate sized un-inoculated meat piece (~2g) were also tested to ensure there was no internal contamination by *Campylobacter*.

Preparation of *Campylobacter* culture broths were prepared as described above (1.2.1) with growth to ~10^9 CFU/ml with 3 replicate culture broths tested for each isolate. Meat pieces were injected with 100µl of *Campylobacter* broth into the centre of each piece. The individual pieces were removed from their bag and placed in glass universals containing 10ml CEB. For gradual heating, the water bath was allowed to reach 25°C prior to heating, whereas simulations undertaken using direct heating, meat pieces were placed into universals containing 10ml pre-heated CEB. The internal temperature of the meat pieces was monitored by the insertion of a DTR probe into the centre of a non-inoculated meat piece. For both procedures, universals were removed at timed intervals with the meat pieces and CEB transferred to a stomacher bag for treatment (90 seconds) prior to serial dilution and plating as described previously. Where specified, meat pieces underwent a gradual cooling process by switching off the water bath heat source and allowing the universals to cool at the same rate as the water bath.

A4.6. The Survival and Recovery of Campylobacter from Poultry Meat Interiors following Direct (Instant) Heating by Sous Vide Method.

Preparation of both *Campylobacter* broths and ~2g meat pieces were as described previously, with 100µl of broth inoculated into each piece. Individual pieces were vacuum sealed prior to heating in a commercial sous vide water bath (Sous Vide Supreme 9L Demi Water Oven). Three replicates for each isolate were prepared, with time intervals of 5, 10, 15 and 20 minutes used at both 52°C and 56°C. After heating, each piece underwent stomaching in 10ml CEB, before serial dilution and plating on CAB/FBP as described previously.

A4.7. The Survival and recovery of Campylobacter from within whole chicken fillets after heating by sous vide method.

Chicken breast fillets were kept at -20°C for 3h approximately to semi-freeze them before scoring a number of individual areas with a sterile tissue borer (15mm). Each marked area within a fillet was injected with 100µl of *Campylobacter* culture broth, prepared as described previously, into the mid-

point. Three replicate broths of each isolate were used per fillet, with each fillet representing a sampling time. Whole fillets were placed individually in a sous vide bag and vacuum sealed, followed by a 1 hour incubation at 37^{o}C prior to overnight storage at 4^{o}C. As before, each batch of chicken fillets was tested to ensure there was no significant contamination of *Campylobacter*.

Experimental simulations were conducted over a range of comparatively lower temperatures (50^{o}C – 56^{o}C). Sampling was undertaken at 20 minute intervals from 0:00 – 60:00 minutes. The duration of heating was extended to 2 hours for simulations undertaken at 50^{o}C and 52^{o}C. Each fillet was placed in the pre-heated water bath directly from the fridge at 4^{o}C, to ensure consistent starting temperature conditions. After removal from the water bath, each fillet was allowed to cool naturally at room temperature prior to removal of the pre-marked meat pieces. Each piece was added to a universal containing 10ml CEB and weighed before transfer to individual stomacher bags and treated for 90 seconds total (60 seconds before mixing and a further 30 second treatment). Serial dilutions were made for each replicate, and plated on CAB-FBP and incubated as described previously.

A4.8. Experiments to determine the upper temperature growth limits of Campylobacter in CEB.

For these experiments, isolates from -80°C stocks were prepared as described previously. Three replicate broths were prepared as for all previous experiments for each *Campylobacter* isolate, at each incubation temperature tested (37°C, 41°C, 44°C, 45°C and 46°C), and were incubated overnight for 18-18.5 hours. The OD_{600} of each broth was recorded, before serial dilution and plating on CAB/FBP as described previously.

A4.9 REFERENCES

Hughes, R.A., Hallett, K., Cogan, T., Enser, M. and Humphrey, T. (2009). The Response of *Campylobacter jejuni* to Low Temperature Differs from that of *Escherichia coli*. *Applied Environmental Microbiology*; **75**(19): 6292-6298.

Miles, A. A., Misra, S.S. and Irwin, J.O. (1938). The Estimation of the Bactericidal Power of the Blood. *The Journal of Hygiene* **38** (6): 732–749.

Murphy, C., Carroll, C. and Jordan, K.N. (2005). The Effect of Different Media on the Survival and Induction of Stress Responses by *Campylobacter jejuni*. *Journal of Microbiological Methods*; **62**(2): 161-166.

A5.0 Water-bath Diagnostic Assessment

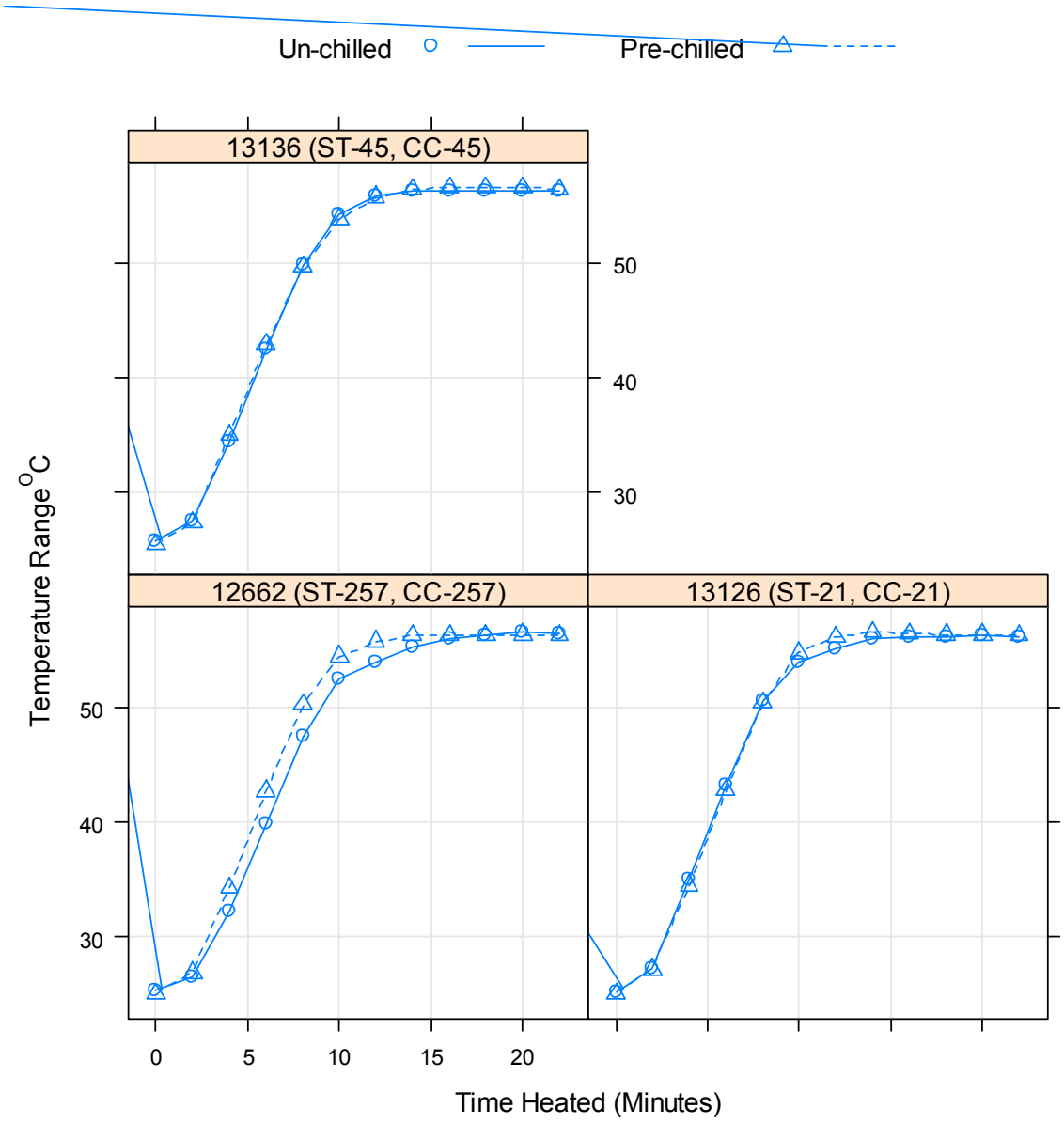

Figure A5.1. Scatter-plot illustrating the duration of gradual of a water-bath to 56°C.

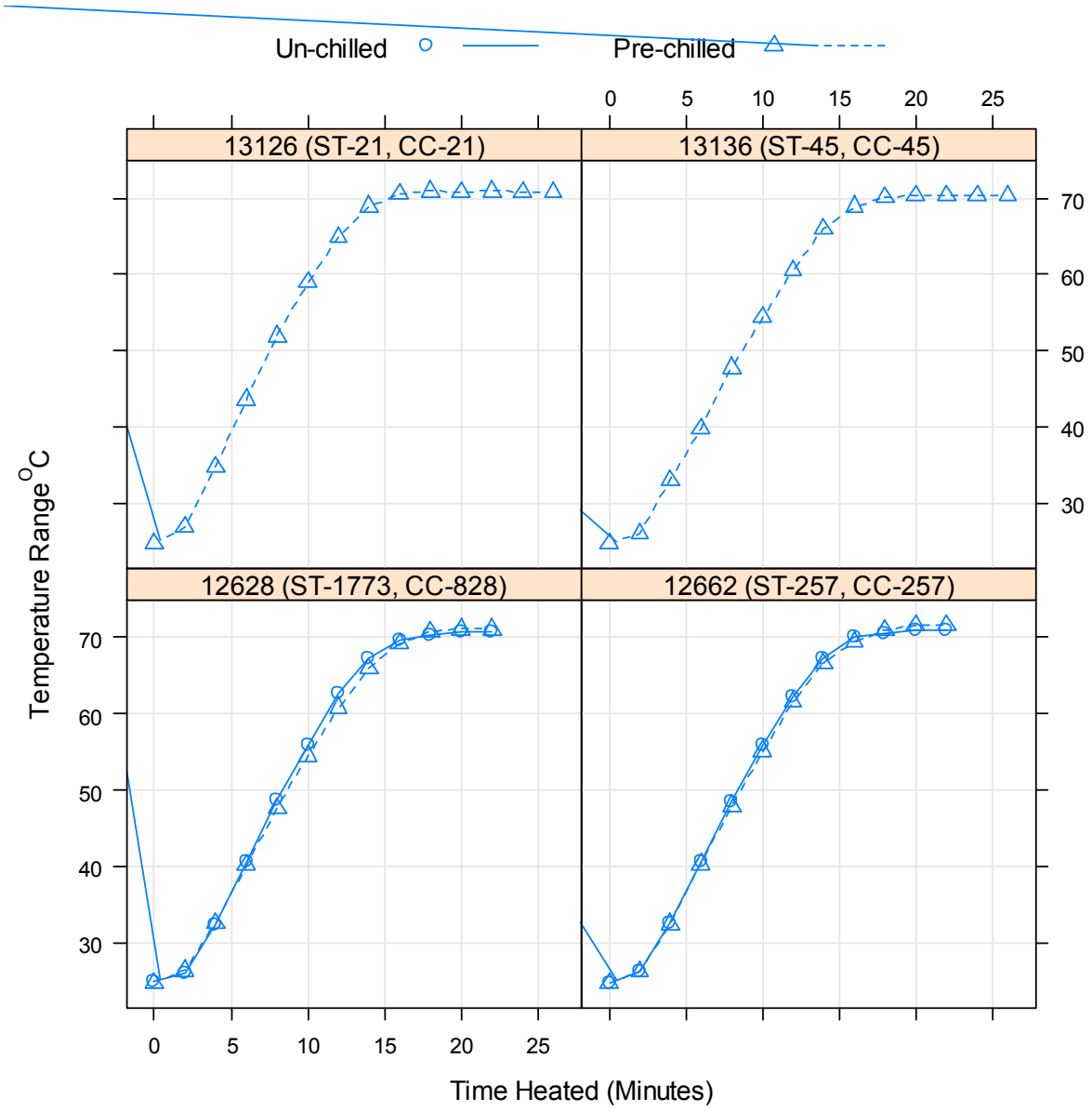

Figure A5.2. Scatter-plot illustrating the duration of gradual of a water-bath to 70°C.

A 6.0 Comparison of D-values from Statistical Modelling Packages

A 6.1 Introduction

Each modelling package used during the course of this study employed a unique set of non-linear models to generate survival curves for *Campylobacter* in response bio-physical and bio-chemical stress. Overall, 14 non-linear models were used to generate these survival curves.

In some instances, more than one type of model was found to be suited to this task, where models used to describe the response of a given strain to a specific challenge differed in their mathematical properties. For instance, a sigmoidal survival curve may be analysed within R using the four-parameter logistic regression model (Table A1.3) or the four-parameter Weibull model (Table A1.5). Within GlnaFiT, first-order kinetics models can be used to describe sigmoidal response curves. The log-linear model incorporating a shoulder effect and asymptotic function uses four parameters to generate predicted response curves (Table A1.9). Correspondingly, the biphasic model comprising a shoulder effect and asymptotic function uses five parameters to generate a predicted response curve (Table A1.14).

Models generated using R were principally semi-mechanistic regression models that can also be used in the testing of hypotheses. Models generated using GlnaFiT are also semi-mechanistic insofar as they are used to describe survival curves for particular classes of response (Geeraerd et al. 2005, 2013).

An important difference between each statistical package is the means by which the fit of different models are compared to one another. Within the R framework, Information theoretic approaches (Burnham and Anderson, 2002) such as AIC were used to compare the relative fit of competing models. By contrast, AIC is not provided by models within GlnaFiT and Geeraerd et al. (2005) suggest that RMSE is a suitable means of assessing model fit to data. As such, there was no direct means with which to adjudge model efficacy across modelling frameworks.

However, the time to first log-reduction (D-value) was calculated for models generated within both R and GlnaFiT. We used this metric as a general means of comparing model predictions between statistical packages. We used Generalised Linear Models (GLM) to compare the ranges of D-values generated by predictive models for each statistical framework and for all experimental simulations (Figures A6.1 – A6.2).

Should significant differences between packages be observed, this may potentially indicate fundamental differences between packages regarding how models are fit to data, and reliability of such models in describing the underlying of *Campylobacter* in response to bio-physical and bio-chemical stress.

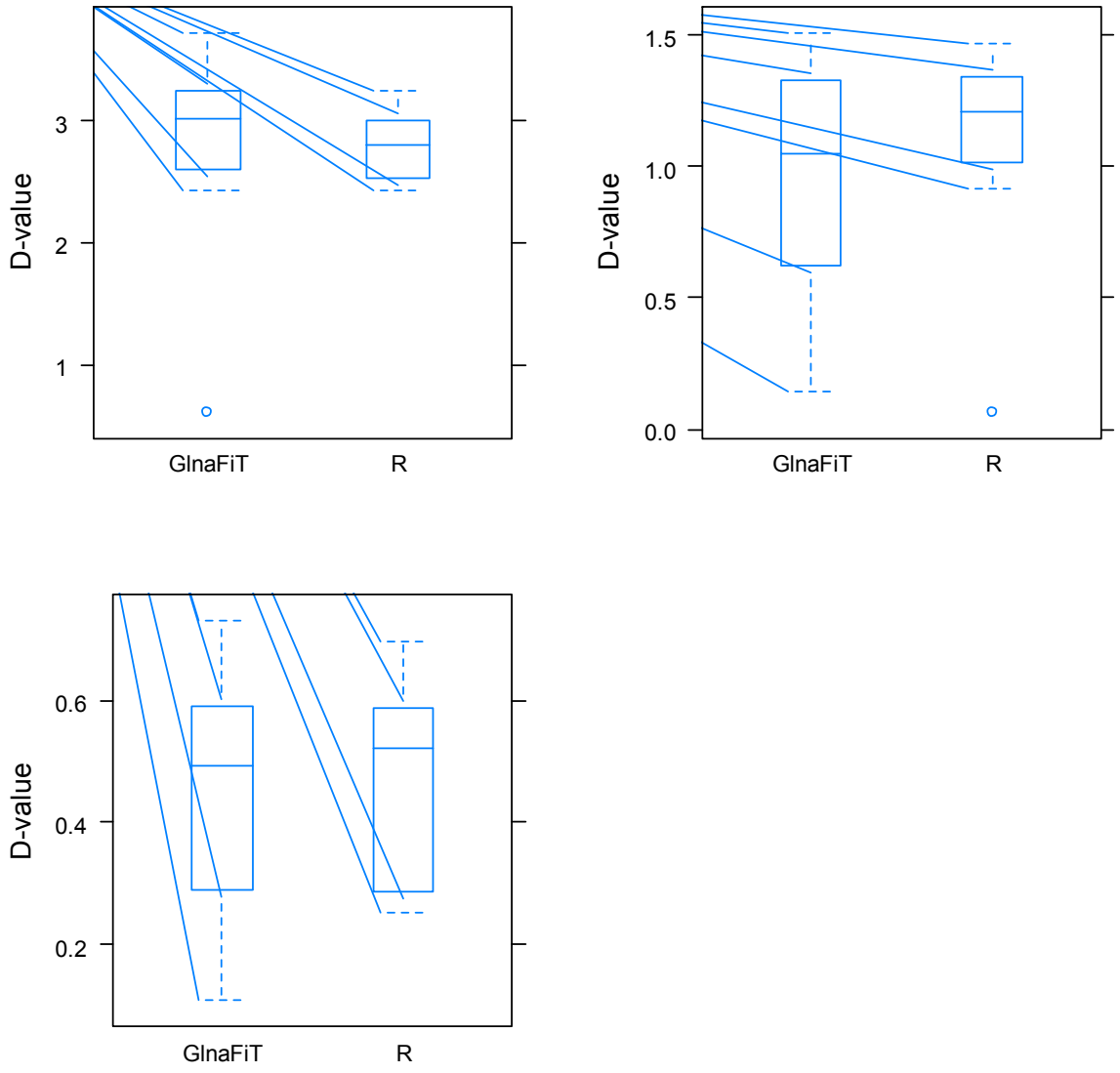

Figure A6.1. Box-plots comparing the range and distribution of D-values generated by models of time-temperature simulations from each statistical modelling package.

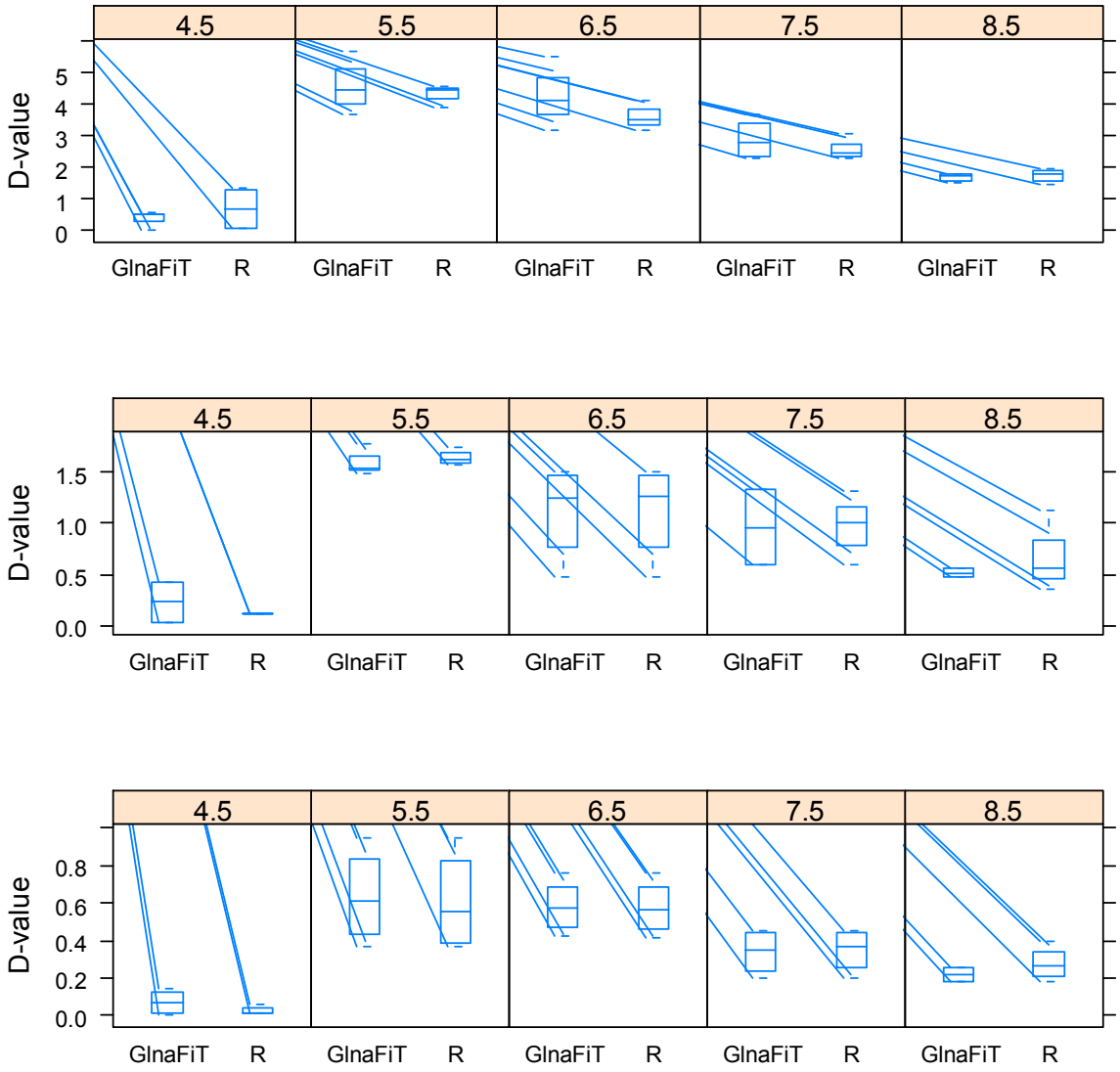

Figure A6.2. Box-plots comparing the range and distribution of D-values generated by models of combined pH and time-temperature simulations from each statistical modelling package.

A6.2 Results

A6.2.1 Time-temperature Simulations

There were no statistical differences observed between statistical packages with regard to the ranges of D-values generated by predictive models across each time-temperature simulations (Tables A6.2.1 – A6.2.3).

Table A6.2.1. Generalized linear model comparing D-values generated by models of time-temperature simulations undertaken at 56^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	2.808	0.366	7.657	8.15E-19
Package R	-0.383	0.525	-0.729	0.471

Table A6.2.2. Generalized linear model comparing D-values generated by models of time-temperature simulations undertaken at 60^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	0.927	0.121	7.644	0.000
Package R	0.153	0.194	0.788	0.440

Table A6.2.3. Generalized linear model comparing D-values generated by models of time-temperature simulations undertaken at 64^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	0.427	0.072	5.934	4.95E-05
Package R	0.505	0.114	0.443	0.665

A 6.3. pH and Time-temperature Simulations

There were no statistical differences observed between statistical packages with regard to the ranges of D-values generated by predictive models across each combined pH and time-temperature simulation (Table A6.3.1 – A6.3.3).

Table A6.3.1. Generalized linear model comparing D-values generated by models of combined pH and time-temperature simulations undertaken at 56^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	0.631	0.248	2.548	0.016
pH 5.5	3.885	0.313	12.429	3.81E-13
pH 6.5	3.386	0.324	10.441	2.46E-11
pH 7.5	2.191	0.313	7.01	1.04E-07
pH 8.5	1.158	0.302	3.838	0.001
Package R	-0.153	0.198	-0.774	0.445

Table A6.3.2. Generalized linear model comparing D-values generated by models of combined pH and time-temperature simulations undertaken at 60^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	0.158	0.164	0.962	0.016
pH 5.5	1.436	0.189	7.616	5.69E-08
pH 6.5	0.944	0.189	5.005	3.68E-05
pH 7.5	0.792	0.199	3.965	0.001
pH 8.5	0.433	0.207	2.095	0.047
Package R	0.033	0.112	0.296	0.770

Table A6.3.3. Generalized linear model comparing D-values generated by models of combined pH and time-temperature simulations undertaken at 64^{o}C from each statistical modelling package.

	Estimate	Standard Error	t-value	P-value
Intercept	0.048	0.055	0.884	0.383
pH 5.5	0.574	0.071	8.097	1.93E-09
pH 6.5	0.529	0.071	7.461	1.18E-08
pH 7.5	0.295	0.07	4.159	0.002
pH 8.5	0.198	0.071	2.797	0.008
Package R	-0.003	0.112	-0.064	0.95

A 6.4. Conclusions

Generalised linear modelling was used to compare the ranges of D-values between statistical packages. No statistically significant differences between packages were observed and predictive models generated between packages across simulations can be considered to be similar.