Supplementary Information for: Temperature-dependent changes to host-parasite interactions alter the thermal performance of a bacterial host.

Author Affiliations

Daniel Padfield[1], Meaghan Castledine[1] & Angus Buckling[1]

[1] College of Life and Environmental Sciences, Environment and Sustainability Institute, University of Exeter, Penryn, Cornwall, TR10 9EZ, U.K.

Corresponding author: Daniel Padfield (d.padfield@exeter.ac.uk)

Author contributions: D.P and A.B conceived the study and designed the experimental work. D.P and M.C conducted the experiments. D.P analysed the data. All authors contributed significantly to the first draft of the manuscript and to revisions.

Data accessibility statement: All data and R code used in the analysis will be made available on GitHub and archived on Zenodo.

Supplementary methods

Data cleaning and model selection process

When processing the data on bacterial growth from the optical density reader, we first corrected the raw OD_{600} by the blank control (OD_{600} corrected = OD_{600} observed – OD_{600} blank). As the inoculum of the bacteria was too small to be accurately measured by the OD reader, if OD_{600} corrected was less than the smallest value the OD reader could measure (0.001), the value was replaced with 0.001. This meant that the estimate of lag time estimates the time at which the bacteria could first be measured by the OD reader, but does not impact any of the estimates of exponential growth.

In the presence of phage, to ensure that the best possible estimate of exponential growth was obtained, we implemented data cleaning after visualising the data. This is because during the bacterial growth curve, phage infections occur which result in decreases in abundance that are not expected based on the shape of the logistic growth curve. Moreover, where in the logistic growth curve these abundance changes due to phage infection occur alters the effect on the logistic growth curve. If lysis of host cells occurred in the lag phase (as determined by the model; Figure S1-S4), there is little to no impact of these changes in abundance on the model fit. However, at 30 ºC (Figure S5), the lysis of host cells occurred in mid-log growth phase and consequently drastically changes the estimate of exponential growth obtained from the model (Figure S5; red line). Consequently, we removed the points that we were certain were a result of phage infection (Figure S1-S8; red points) and then modelled the data. This resulted in the removal of 3.42 % of all points. Reassuringly, if the analysis of TPCs was run on the estimates of exponential growth of the raw data, similar results were obtained (Figure S17), with the biggest cost difference in fitness occurring at intermediate temperatures.

For susceptible and resistant clones, the higher inoculum (ten-fold higher), and a lack of phage, resulted in an alternative data cleaning procedure being implemented. The higher inoculum resulted in fewer readings being initially beyond the range of the OD reader, and therefore a model without a lag time was favoured in most cases. Instead, we simply removed the first measurement (which was prone to error due to the bubbles present after pipetting the inoculum) and set time zero to the time at which the first optical density measurement was detected for each clone.

Table S1. Logistical growth equations used in the modelling of bacterial growth in the presence and absence of phage.

Model	Equation
Gompertz	$log_{10}OD_{600} = log_{10}n_0 + (log_{10}n_{max} - log_{10}n_0) \times e^{(-e^{1+r \times e^1 \times \left(\frac{lag-t}{(log_{10}n_{max} - log_{10}n_0) \times \ln(10)}\right)})}$
Baranyi	$log_{10}OD_{600} = log_{10}n_{max} + log_{10}\left(\frac{-1+e^{r \times lag}+e^{r \times t}}{e^{r \times t}-1+e^{r \times lag} \times 10^{(log_{10}n_{max} - log_{10}n_0)}}\right)$
Baranyi without lag	$log_{10}OD_{600} = log_{10}n_{max} - log_{10}\left(1 + (10^{(log_{10}n_{max} - log_{10}n_0)} - 1) \times e^{-r \times t}\right)$
Buchanan	$log_{10}OD_{600} = log_{10}n_0$ for when $t \leq lag$ $log_{10}OD_{600} = log_{10}n_0 + r(t - lag)$ for when $lag \leq t \leq t_s$ $log_{10}OD_{600} = log_{10}n_{max}$ for when $t \geq t_s$
Buchanan without lag	$log_{10}OD_{600} = log_{10}n_0 + r(t - lag)$ for when $t \leq t_s$ $log_{10}OD_{600} = log_{10}n_{max}$ for when $t \geq t_s$

Where $log_{10}OD_{600}$ is the log10 of the absorbance measurement, $log_{10}n_0$ is the starting density, $log_{10}n_{max}$ is carrying capacity, r is the exponential growth rate (hr^{-1}), lag is the lag time in hours and t_s is the time to stationary phase in hours. Model equations were copied from the R package '*nlsMicrobio*'. Code for fitting each equation and comparing AIC scores can be found on the GitHub repository for this manuscript.

Table S2. Point estimates and 95% credible intervals (as determined using Bayesian methods) for fitted and derived metabolic traits.

Rate	Parameter	Mean	2.5%	97.5%
phage replication	CT_{max} (ºC)	29.2	29	29.4
	T_{opt} (ºC)	27.0	26.5	27.5
bacteria growth without phage	E (eV)	0.84	0.59	1.16
	E_h (eV)	2.36	2.03	2.79
	T_{opt} (ºC)	28.0	27.1	29.0
	r_{max} (hr^{-1})	0.72	0.68	0.76
bacteria growth with phage	E (eV)	0.33	0.20	0.50
	E_h (eV)	4.25	2.57	6.63
	T_{opt} (ºC)	30.6	29.0	32.1
	r_{max} (hr^{-1})	0.57	0.54	0.62
bacteria growth of susceptible clones	E (eV)	0.49	0.42	0.57
	E_h (eV)	2.32	1.95	2.77
	T_{opt} (ºC)	30.5	30.0	31.0
	r_{max} (hr^{-1})	0.77	0.73	0.81
bacteria growth of resistant clones	E (eV)	0.42	0.33	0.56
	E_h (eV)	1.95	1.47	2.57
	T_{opt} (ºC)	30.2	29.2	31.0
	r_{max} (hr^{-1})	0.66	0.63	0.70
bacteria growth	% change in r_{max} due to presence of phage	-20.6	-13.1	-27.3
	% change in r_{max} due to phage resistance	-13.6	-6.8	-20.2

Parameters include CT_{max}, the critical thermal maximum, T_{opt}, the optimum temperature, E, the activation energy, E_h, the deactivation energy, r_{max}, the maximum growth rate and the % change in maximum growth rate due to phage presence and due to phage resistance. Not all parameters are shown for each rate because they were either outside the range of the data collected or were not biologically meaningful for the data collected.

Table S3. Results of multiple pairwise comparisons between resistance through time at each temperature.

Temperature	Contrast	Odds ratio	SE	z ratio	p value
15	12 hours vs. 24 hours	1	0.43	0	1
	12 hours vs. 48 hours	1.26	0.54	0.54	0.85
	24 hours vs. 48 hours	1.26	0.54	0.54	0.85
20	12 hours vs. 24 hours	1.04	0.45	0.08	0.99
	12 hours vs. 48 hours	1.01	0.44	0.03	0.99
	24 hours vs. 48 hours	0.98	0.43	-0.05	0.99
25	12 hours vs. 24 hours	1.16	0.62	0.89	0.65
	12 hours vs. 48 hours	5.61	2.05	4.71	<0.001
	24 hours vs. 48 hours	3.85	1.29	4.02	<0.001
28	12 hours vs. 24 hours	1.62	0.66	1.17	0.47
	12 hours vs. 48 hours	13.9	4.96	7.36	<0.001
	24 hours vs. 48 hours	8.60	2.81	6.59	<0.001
30	12 hours vs. 24 hours	0.02	0.01	-10.46	<0.001
	12 hours vs. 48 hours	0.37	0.16	-2.35	0.049
	24 hours vs. 48 hours	16.8	5.48	8.68	<0.001
33	12 hours vs. 24 hours	0.92	0.41	-0.19	0.98
	12 hours vs. 48 hours	1	0.43	0	1
	24 hours vs. 48 hours	1.1	0.49	0.194	0.98
35	12 hours vs. 24 hours	0.85	0.35	-0.40	0.92
	12 hours vs. 48 hours	0.99	0.43	-0.03	0.99
	24 hours vs. 48 hours	1.12	0.49	0.37	0.93
37	12 hours vs. 24 hours	1	0.433	0	1
	12 hours vs. 48 hours	-	-	-	-
	24 hours vs. 48 hours	-	-	-	-

At temperatures where growth was highest, resistance changed significantly through time. P values were adjusted using the Tukey method for comparing a family of 3 estimates and tests were performed on the log odds ratio scale. An odds ratio of 1 would indicate that resistance was the same in both groups, with a higher odds ratio indicating that resistance was higher in the first group, and a lower odds ratio would indicate that resistance was higher in the second group.

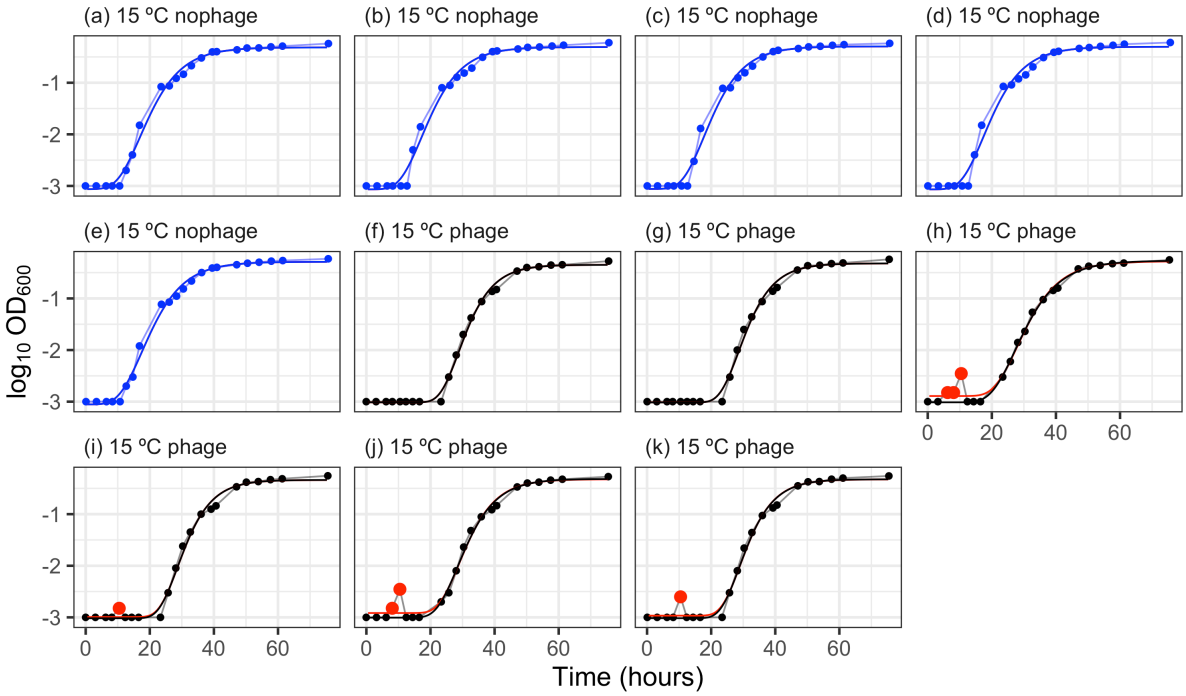


Figure S1. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 15 ºC. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

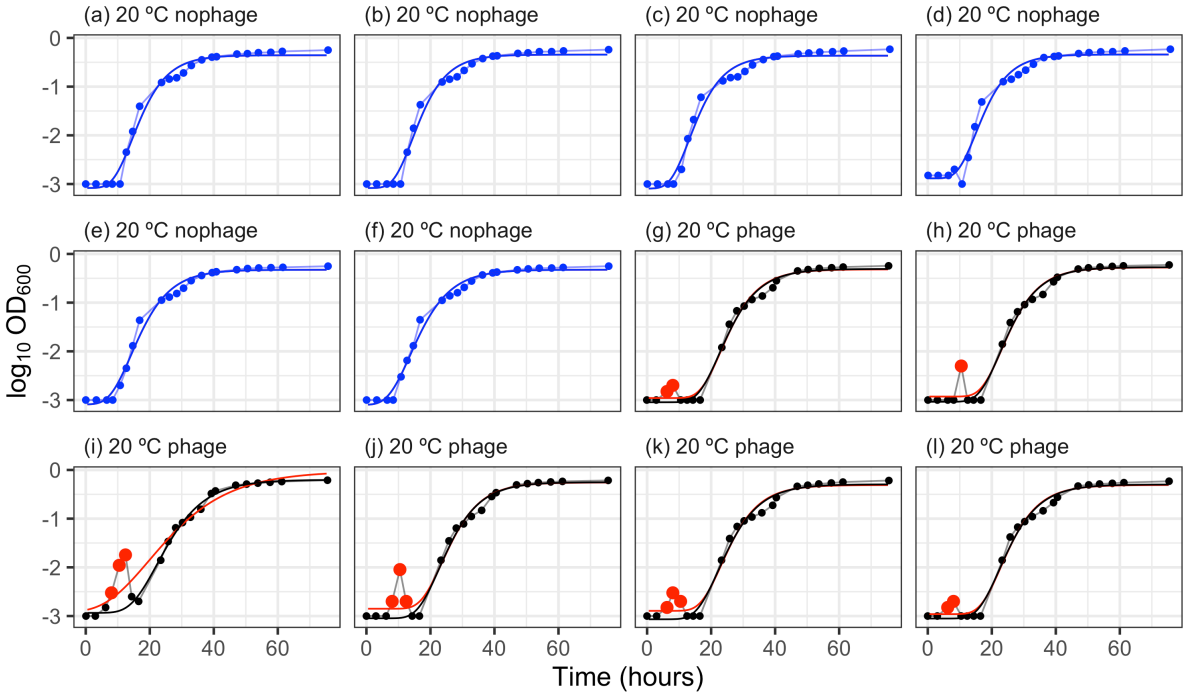


Figure S2. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 20 °C. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

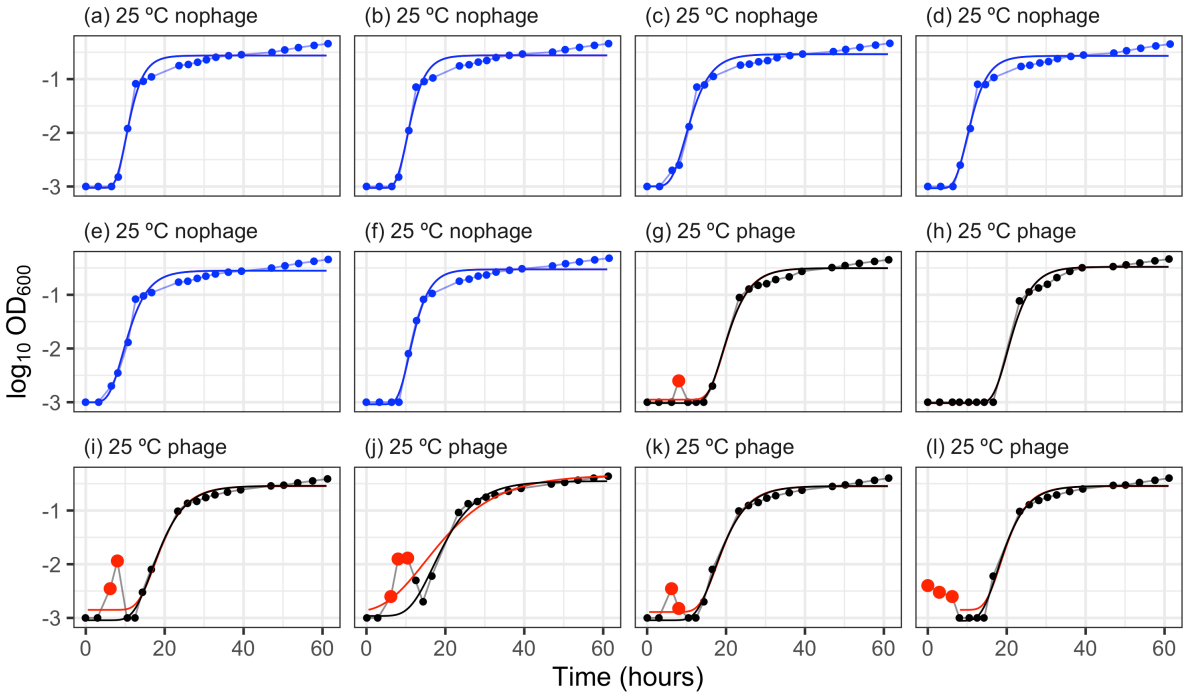


Figure S3. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 25 °C. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

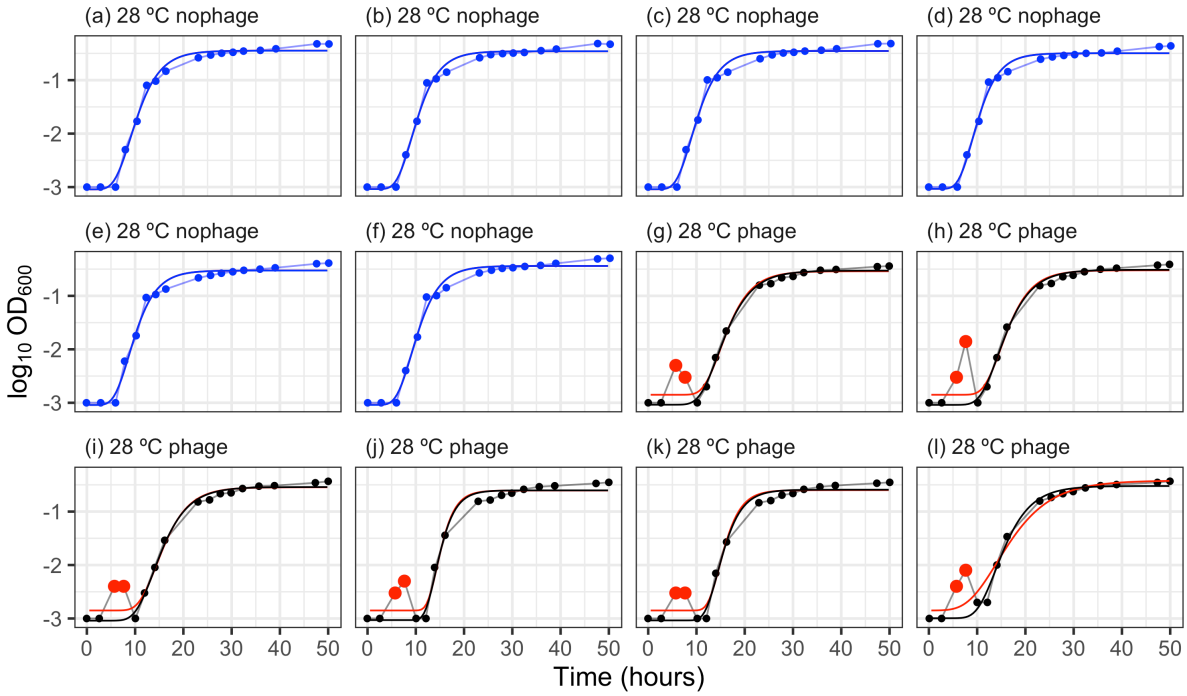


Figure S4. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 28 °C. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

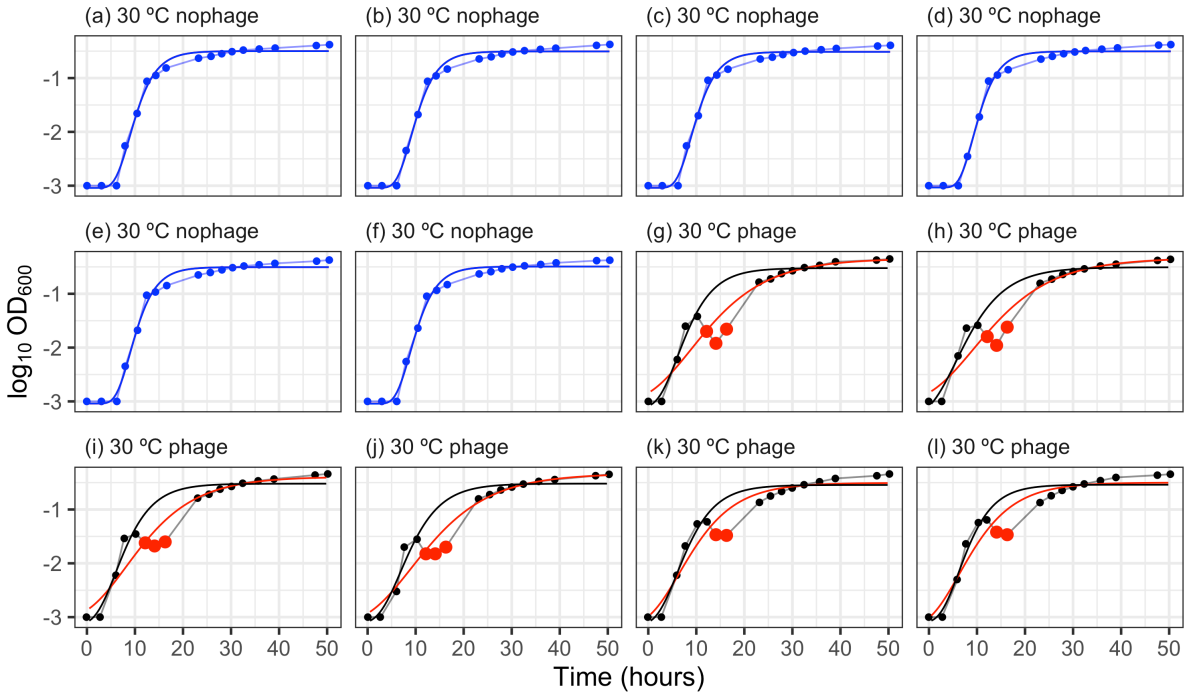


Figure S5. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 30 °C. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

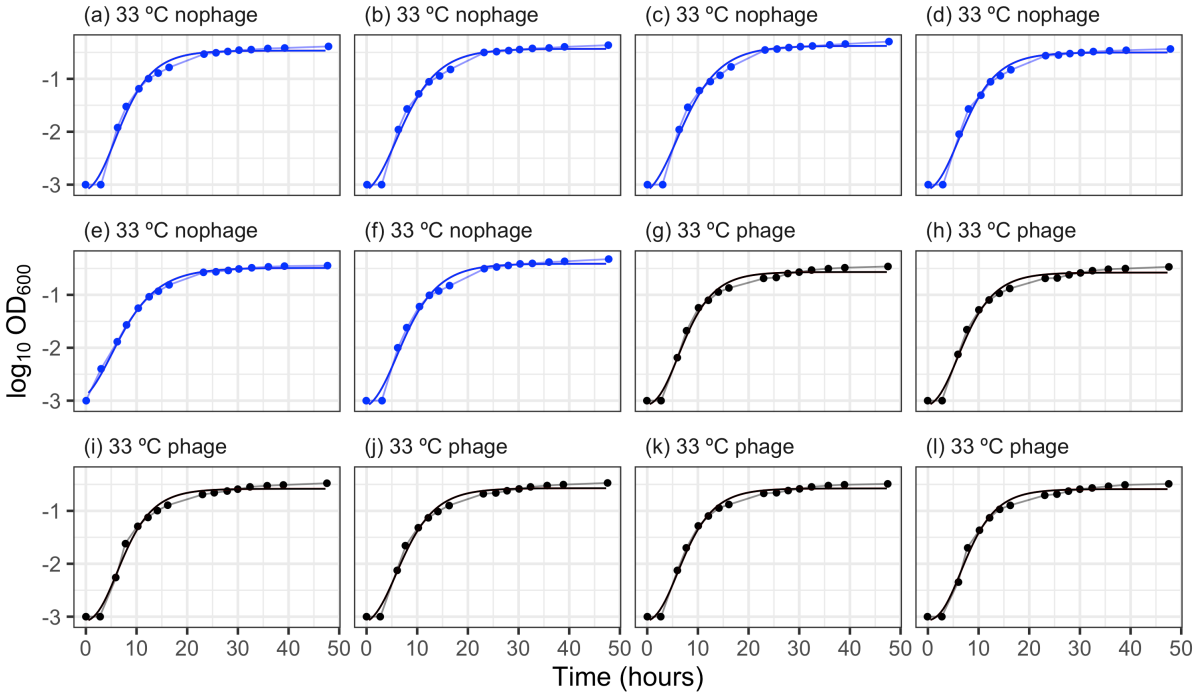


Figure S6. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 33 °C. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

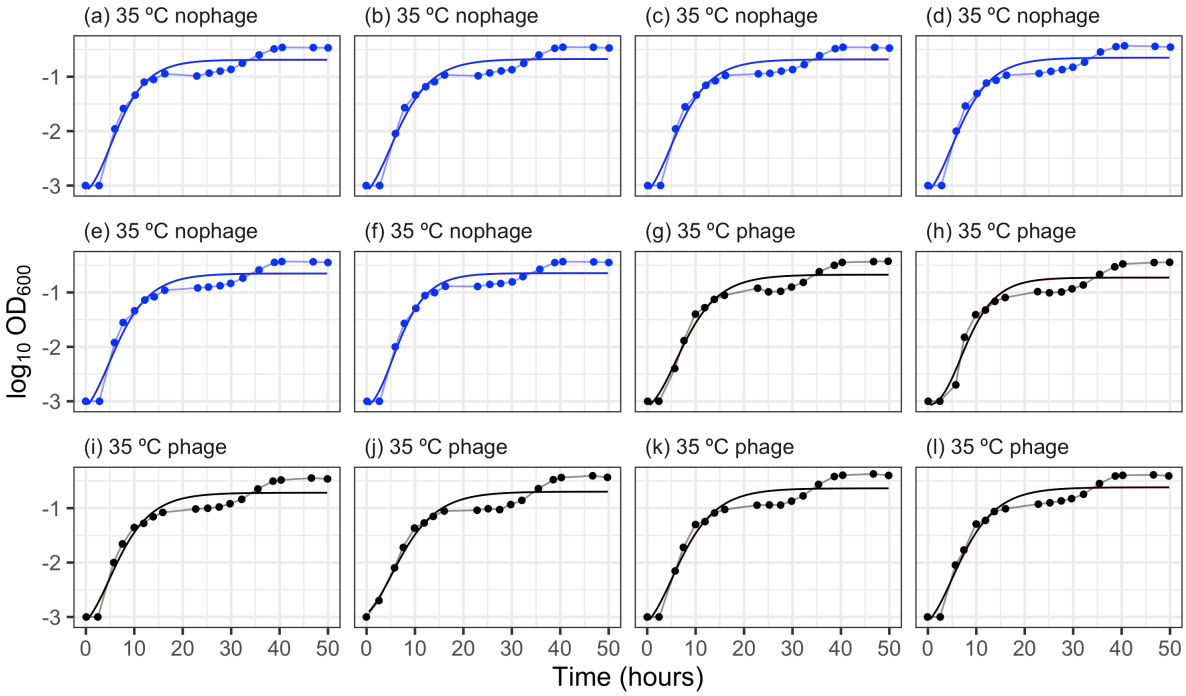


Figure S7. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 35 ºC. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

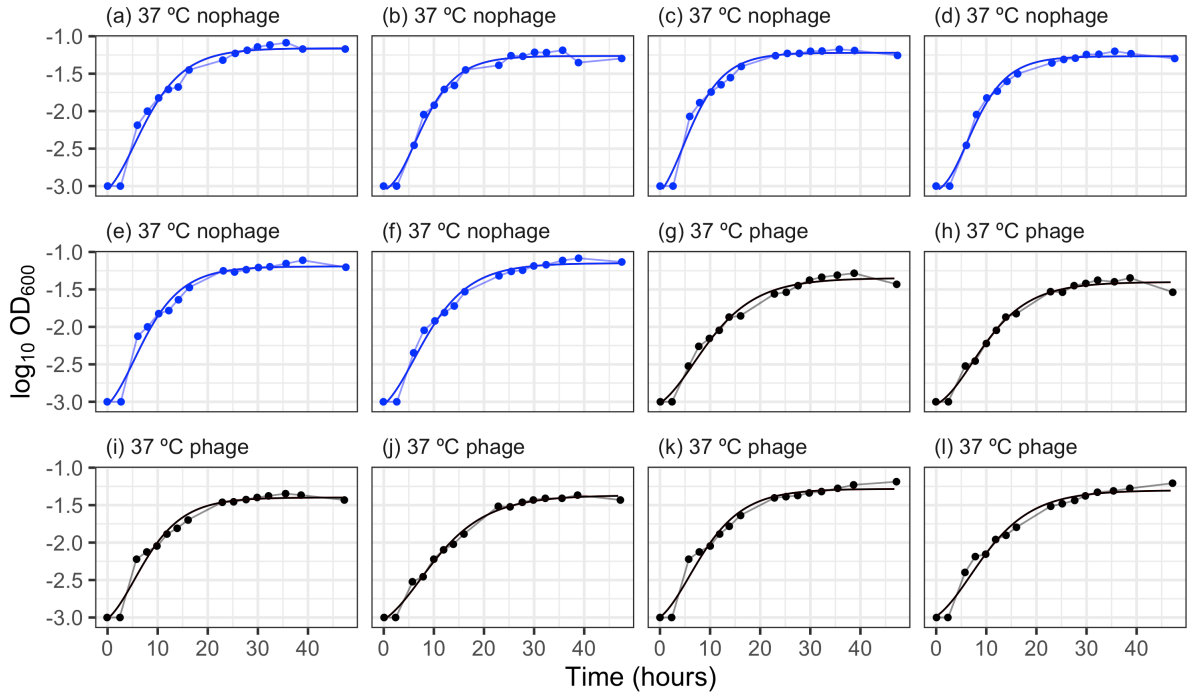


Figure S8. Effect of data cleaning on logistic growth curves for bacterial growth in the presence (black) and absence (blue) of phage at 37 ºC. The Gompertz model for logistic growth was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points that were removed in the final dataset and predictions of the model using the raw dataset are shown in red. A lack of red indicates no points were removed and predictions are equal between the two datasets. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

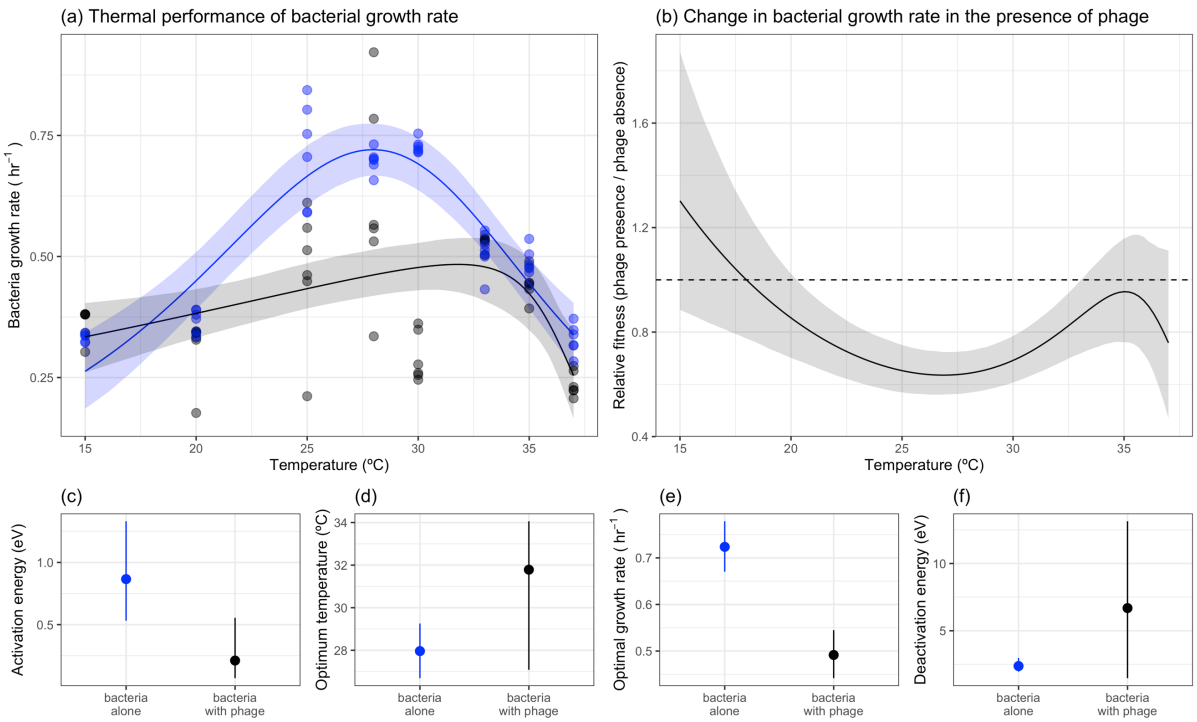
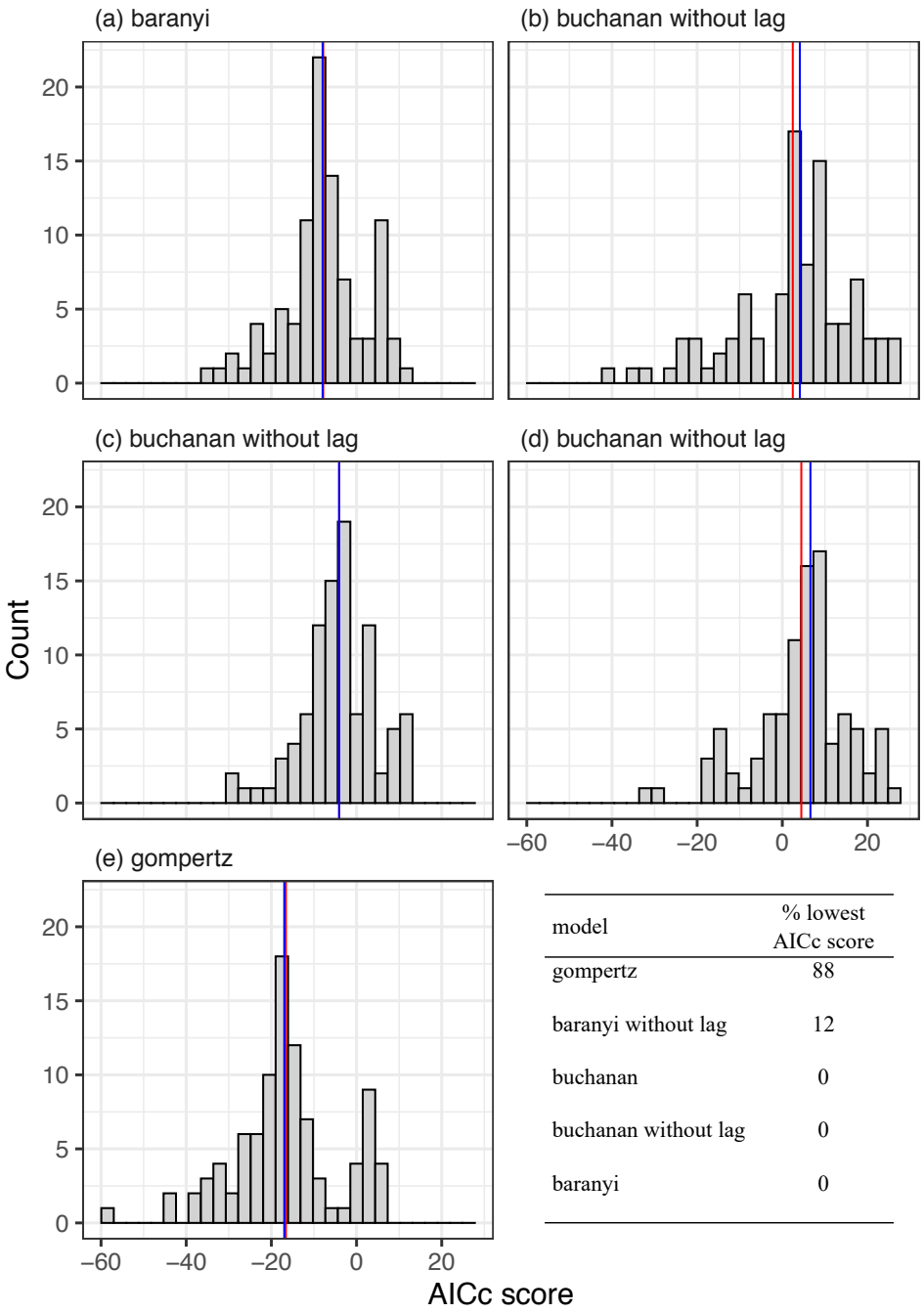
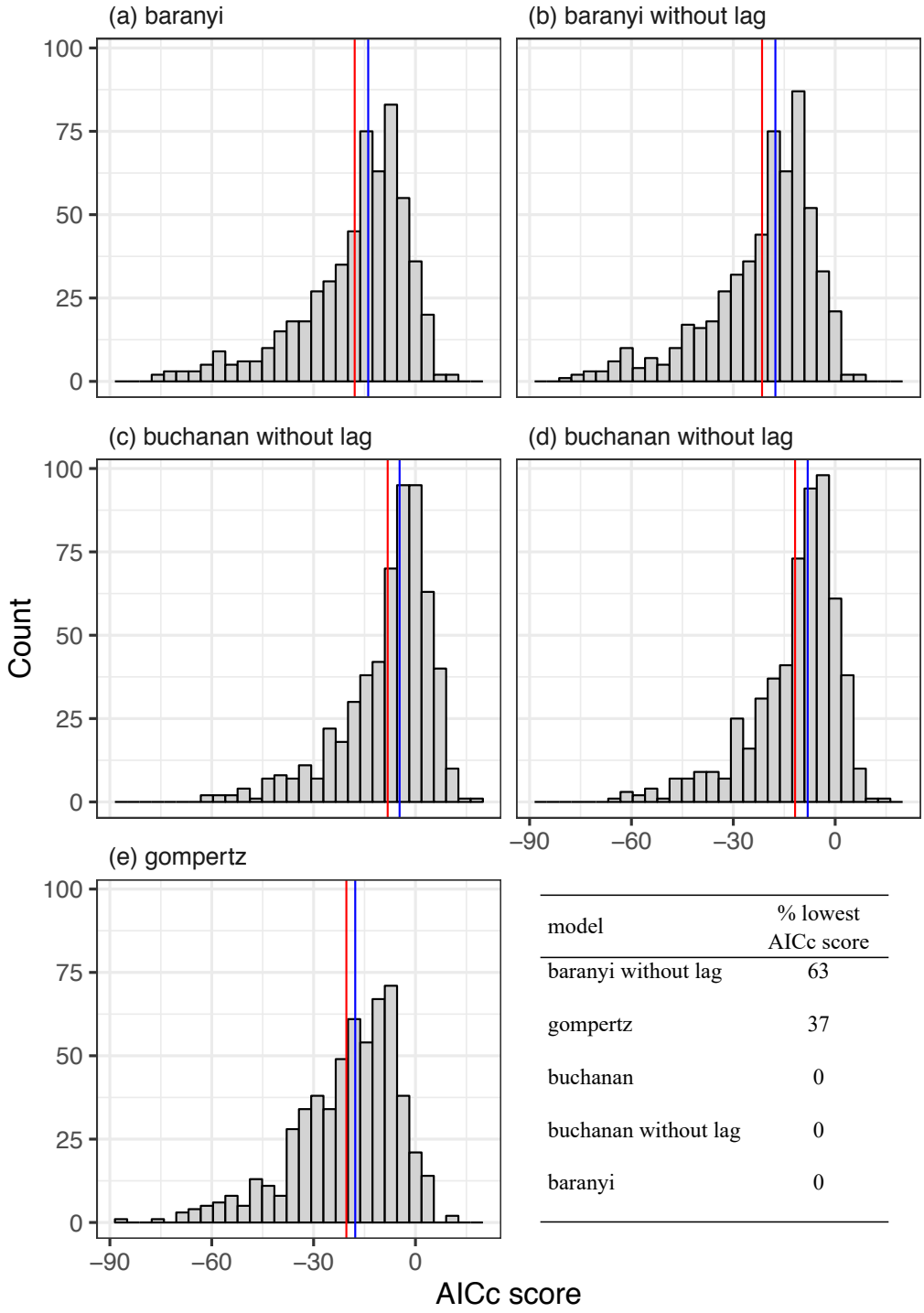


Figure S9. Effect of phage on the thermal performance of bacteria using the raw data. (a) Bacteria growth shows unimodal responses to temperature in the presence (black) and absence of phage (blue). However, phage changed the shape of the thermal response. (b) Phage altered the growth rate of bacteria (calculated as relative fitness) in a non-linear fashion with increasing temperatures. (c-f) The effect of phage on key thermal performance traits. Phage altered the (c) activation energy, (d) optimum temperature, (e) optimal growth rate and (f) deactivation energy. In (a) the solid line represents the mean prediction and shaded band represents the 95% credible interval of predictions. The dashed line at y = 1 would indicate that phage do not alter growth rate. Below 1, phage reduces the growth rate of the bacteria. In (c-f) points and lines represent the mean and 95% credible intervals of the estimated parameters.



model	% lowest AICc score
gompertz	88
baranyi without lag	12
buchanan	0
buchanan without lag	0
baranyi	0

Figure S10. Distribution of AICc scores for different logistical growth models fitted to bacteria growth in the presence and absence of phage. Numerous logistical growth models were fitted to each bacterial growth curve in the presence and absence of phage. The Akaike's Information Criterion score adjusted for small samples (AICc) for each model was calculated and compared across models to select the best, consensus model. The table in the bottom right demonstrates that for 74% of the curves, the Gompertz model returned the lowest AICc score. The red and blue lines per panel represent the mean and median AICc score of that model respectively.



model	% lowest AICc score
baranyi without lag	63
gompertz	37
buchanan	0
buchanan without lag	0
baranyi	0

Figure S11. Distribution of AICc scores for different logistical growth models fitted to bacteria growth of susceptible and resistant clones. Numerous logistical growth models were fitted to each bacterial growth curve in the presence and absence of phage. The Akaike's Information Criterion score adjusted for small samples (AICc) for each model was calculated and compared across models to select the best, consensus model. The table in the bottom right demonstrates that for 63% of the curves, the Baranyi model without a lag phase returned the lowest AICc score. The red and blue lines per panel represent the mean and median AICc score of that model respectively.

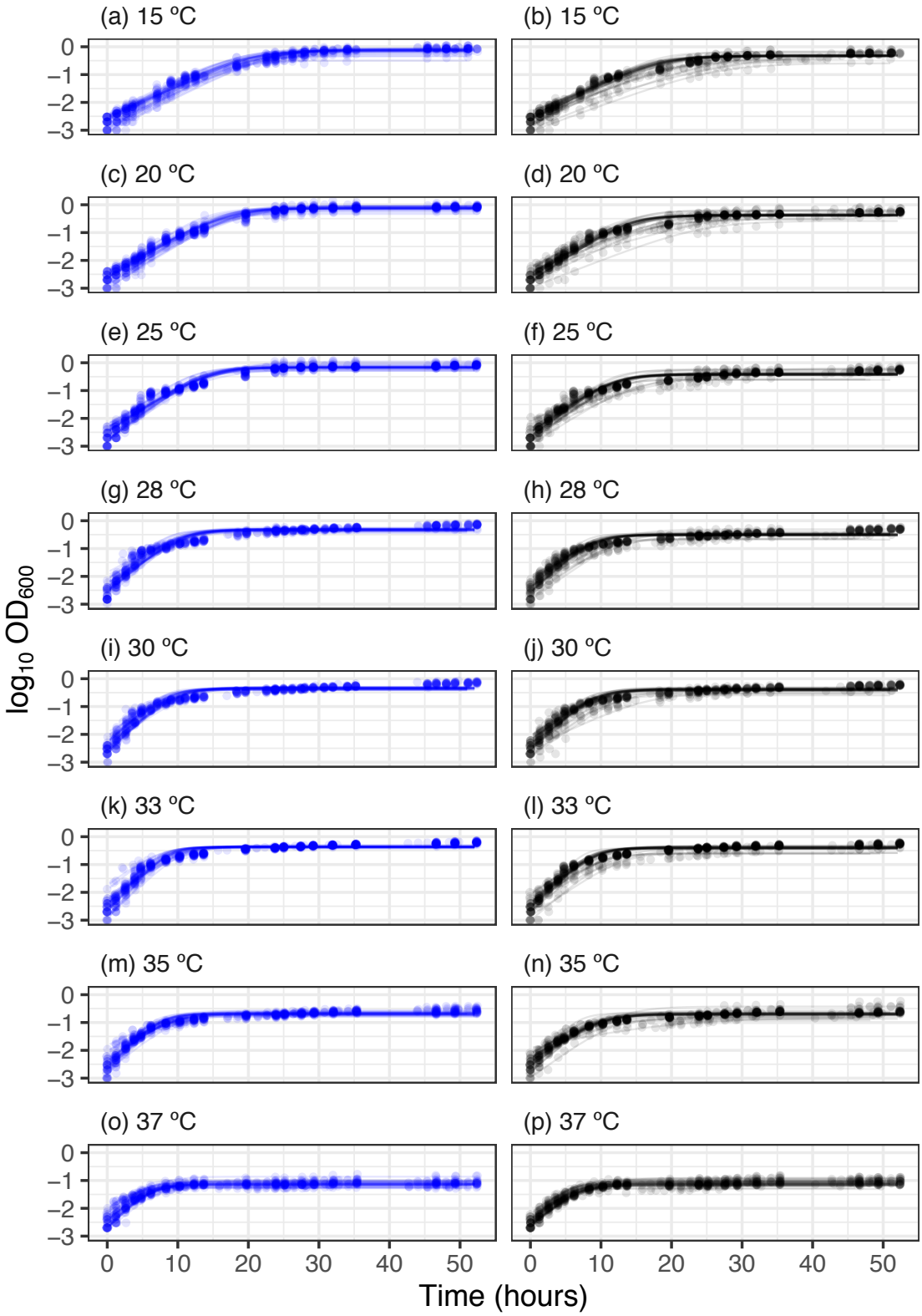


Figure S12. Logistic growth curves for bacterial growth of susceptible (blue) and resistant (black) clones. The Baranyi model without a lag phase was fitted to each independent replicate and the exponential growth parameter was extracted for use in the thermal performance curves. Points represent individual measurements and lines represent predictions of the best fitting model for each replicate at each temperature.

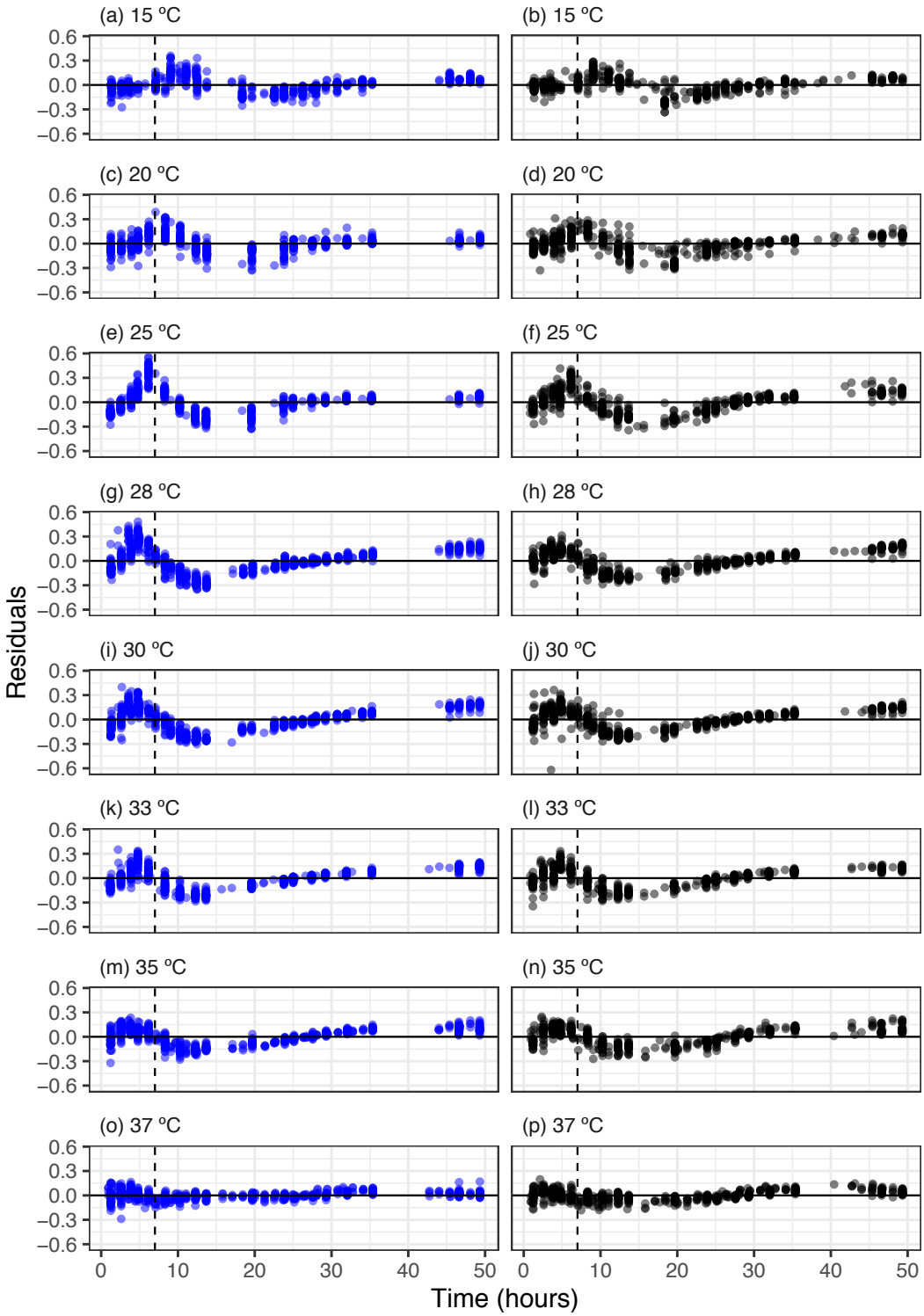


Figure S13. Fit residuals through time of logistic growth curves of susceptible (blue) and resistant (black) bacterial clones. The residuals of the Baranyi model without a lag phase were plotted as a function of time for each clone. There is some systematic variation in the residuals that are similar across most temperatures and resistant and susceptible clones. However, there does appear to be systematic variation in the first 7 hours after growth was first measured which could result in growth being underestimated at some temperatures more than others. The vertical line is drawn after 7 hours after growth was first detected and is a key portion of the curve used to estimate exponential growth rate.

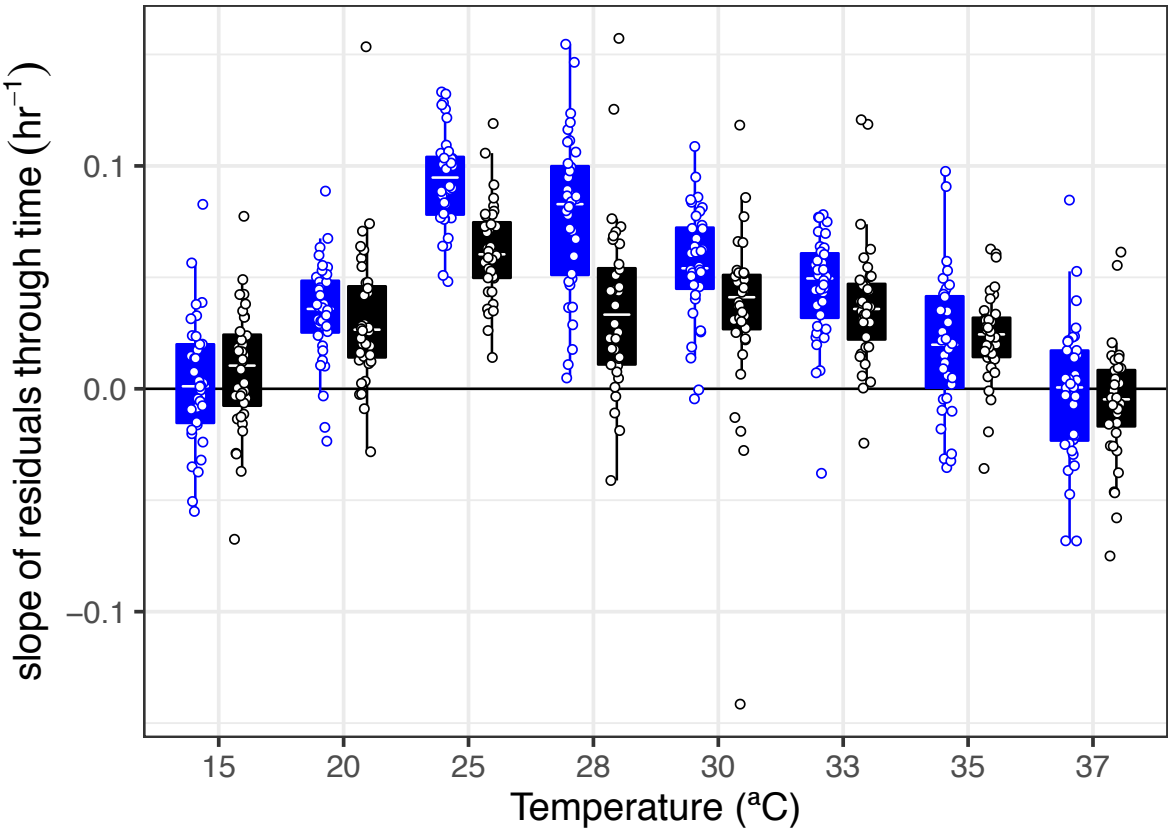


Figure S14. Systematic variation in the residuals during the exponential growth phase of susceptible (blue) and resistant (black) bacterial clones. The slope between the residuals and time over the first 7 hours growth was detected was investigated. A slope of 0 would indicate that the model estimates exponential growth adequately, whereas a slope greater than 1 would indicate that the model underestimates growth rate given the data. Exponential growth rate is underestimated at temperatures where bacteria grew best, and at these temperatures there was a significantly greater underestimation of growth rate in susceptible, rather than resistant bacteria. Points represent the slope of individual fits. Tops and bottoms of the bars represent the 75th and 25th percentiles of the data, the white lines are the medians, and the whiskers extend from their respective hinge to the smallest or largest value no further than 1.5 * interquartile range.

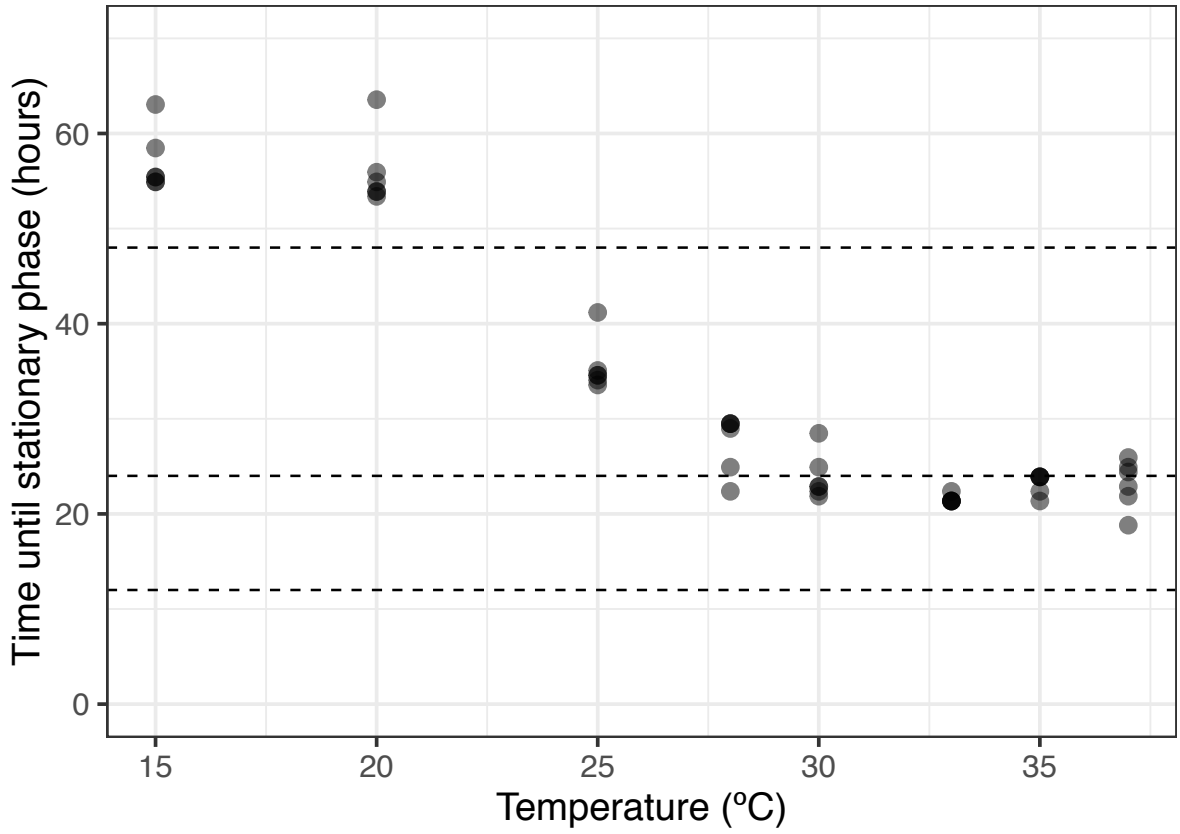


Figure S15. Time to stationary phase of bacteria growth in the presence of phage across temperatures. Time to stationary phase was estimated as the time at which the predictions of the model were 90% of the estimated carrying capacity, $log_{10}n_{max}$. Temperatures above 20 ºC are all in stationary phase before the final sampling point of 48 hours, indicating nutrient limitation between 24 and 48 hours at these temperatures. Points represent the time to stationary phase of independent replicates. Dashed lines indicate the times at which samples were taken to test for resistance in equivalent trials.

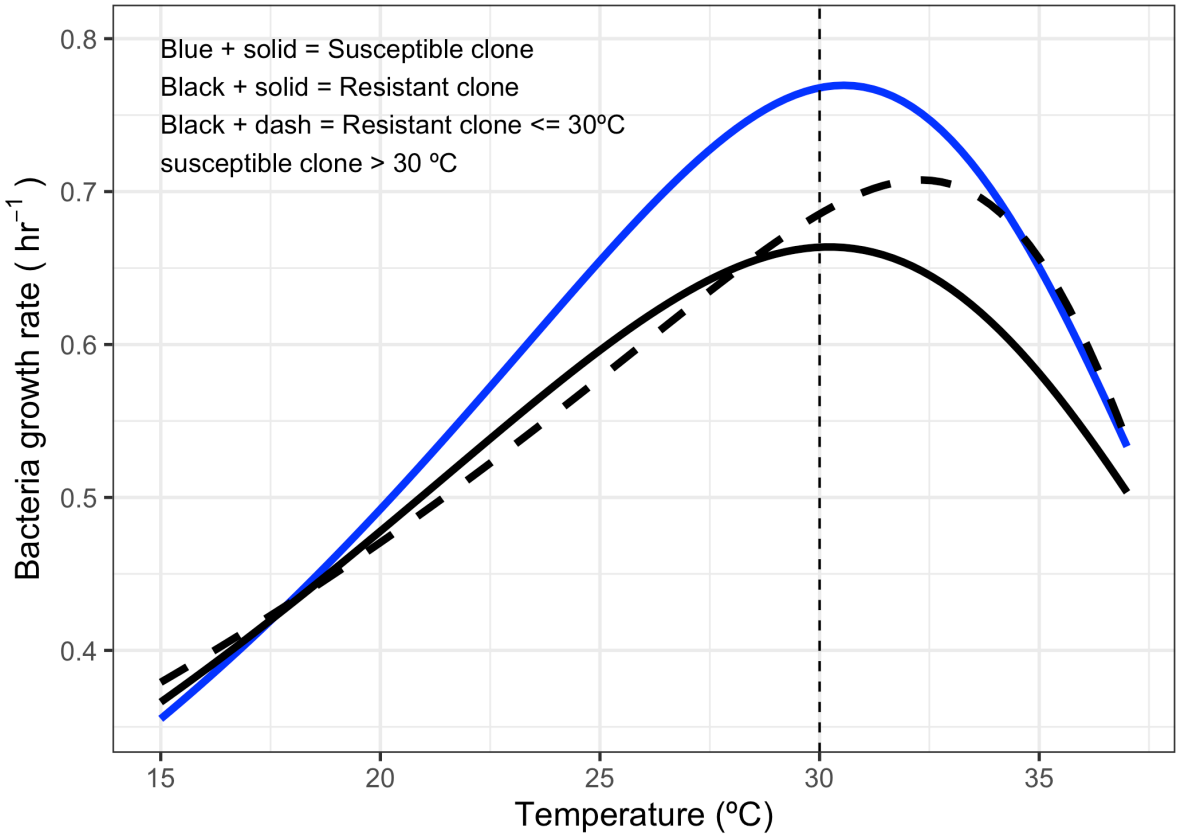


Figure S16. Temperature dependent evolution and cost of resistance in *Pseudomonas fluorescens*. The thermal performance of the average susceptible clone (blue, solid line) and resistant clone (blue, solid line) represent the same curve as in Figure 4. However, there is very little phage infection beyond 30 ºC (Figure 3), so to emphasise the effect of ecological (differences in CT_{max}) and evolutionary (evolution of resistance) mechanisms, we plotted the modelled thermal performance curve of the average resistant clone at temperatures <= 30 ºC and the average susceptible clone at temperatures > 30 ºC. The shift in T_{opt} observed in Figure 1 is only visible by combining ecology and evolutionary mechanisms. Lines represent predictions based on the model fit to the mean rate values for each curve in (Figure 4). Dashed, vertical line represents the CT_{max} of the phage, beyond which little phage infection occurred.