Supporting Information:

How to quantify factors degrading DNA in the environment and predict degradation for effective sampling design

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About the supporting information

This supporting information contains support for the credibility of our model estimates presenting important model information and diagnostics as proposed by Zuur et al. (2016) showing that model assumptions were sufficiently fulfilled and can be found in the model overviews of **Supp_01, 05, 06, 09, 17**, **18** and **20**.

Further, we present the global model estimates (**Supp_09**) with their standard errors before creating all subset models from it for multi model inference and averaging to show that effects were strong before and just did not arise through the multi model inference and selection process following the suggestions of Forstmeier and Schielzeth (2011).

We also included additional overviews of used environmental variables (**Supp_12**) and our response variable the Quality index (QI, **Supp_11 2)** to show environmental variability as well as in extra-organismal DNA (eoDNA) degradation in the system and between sites (roosts and roost-visits RV). We further demonstrate the benefits of using QI in multi-tube approaches over PCR success rate when quantifying eoDNA degradation (Figure **Supp_11 1**, **Supp_20**).

Furthermore, we demonstrate the relation of manual mismatch correction of consensus genotypes and QI and why skipping it is justifiable, when the only goal of the study is to quantify eoDNA degradation and predict from it (**Supp_01**, **Supp_02**).

We also included an in-depth evaluation of the proposed drying effect to explain the positive effect of “HottestDay” found with our modeling pipeline (**Supp_16, 17, 18**).

Lastly, we demonstrate how to use the quantified environmental effects degrading eoDNA to find site specific ideal sampling durations solely based on these environmental effects using the best predictive model (**Supp_14**, **Supp_15**).

To maximize reproducibility, all figures and statistics (except Figure **Supp_16 3, 4**) of this supporting information and the manuscript can be recreated with the three numbered R-Scripts and all necessary input data provided within a portable, package version-controlled R project. The well commented R-pipeline with its 18 custom functions facilitate and speed up the application of our pipeline to other systems. The code and data are accessible on dryad (Naef et al., 2023, https://doi.org/10.5061/dryad.79cnp5hxn). Note that plots might slightly differ due to final polishing outside of R for the publication purposes.

For ease of navigation this supporting information includes interactive bookmarks and clickable links to relevant supplementary information indicated in bold (e.g., **Supp_01**).

Supp_01 Information about the manual mismatch correction model

Model diagnostics and information for the beta binomial model showing that lower QI is associated more likely with changed alleles (ChangedA). All output was created with the mdiag() function included in the version controlled R-Project. Not all information from the 15-page pdf report is shown but can easily be created by running the available R-Script "03_MismatchEffect.R". Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved. This model investigates the influence of the manual mismatch correction step on QI which was skipped in the manuscript. The dataset of this model consists of the combined model building and test data (2616 samples) with the subset of 2322 usable samples consisting of at least eight valid loci (see Figure **Supp_02 1**) as used when fully following the automated pipeline described in (Zarzoso-Lacoste et al., 2018; Zarzoso-Lacoste et al., 2020) and applied in (Jan et al., 2019).

Model overview

name: mod_MMvsQIbeta

family: betabinomial

link: logit

response variable form: matrix

sample size: 2322

N (sample size) / k (terms) : 2322

formula: ChangedA ~ QI_without_RHC108

ziformula: ~0

dispformula: ~1

Figure Supp_01 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the beta-binomial based on 10 000 simulations. A) Dispersion test shows significant deviation from the observed (red line) variance being 0.75 lower than the mean simulated variance (black lines). This will lower statistical power due to inflated standard errors and confidence interval detected effects might be missed but weren’t (see Supp_01 3-4). B) Zero-inflation test shows that the number of observed zeroes (red line) does not differ from simulations with 1.00 times observed zeroes than simulated zeroes (black lines). C) The outlier test based on 100 bootstrap replicates shows no significant outliers (no red bars and red line). D) Quantile-Quantile plot of observed and expected residuals shows no deviation of uniform distribution (red line).

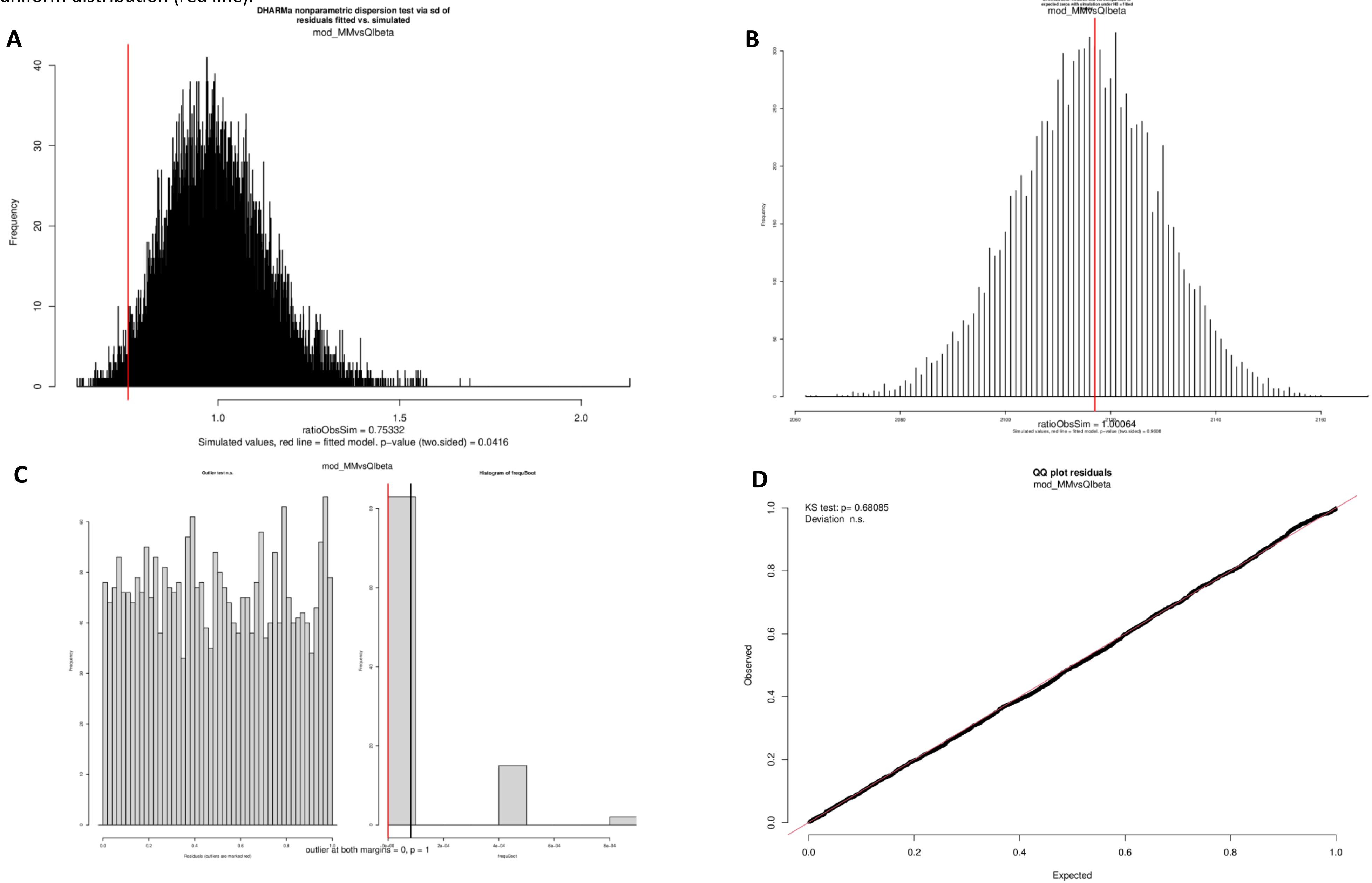


Figure Supp_01 2 Model diagnostics based on simulated residuals using the "DHARMa" package based on 10 000 simulations. Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate weak unproblematic signs of heteroscedasticity but only in the higher two quantiles. An inflation test for 1`s (meaning 16 or 18 alleles were changed) resulted in NA since in no case all alleles were changed with only a maximum changed alleles of 4.

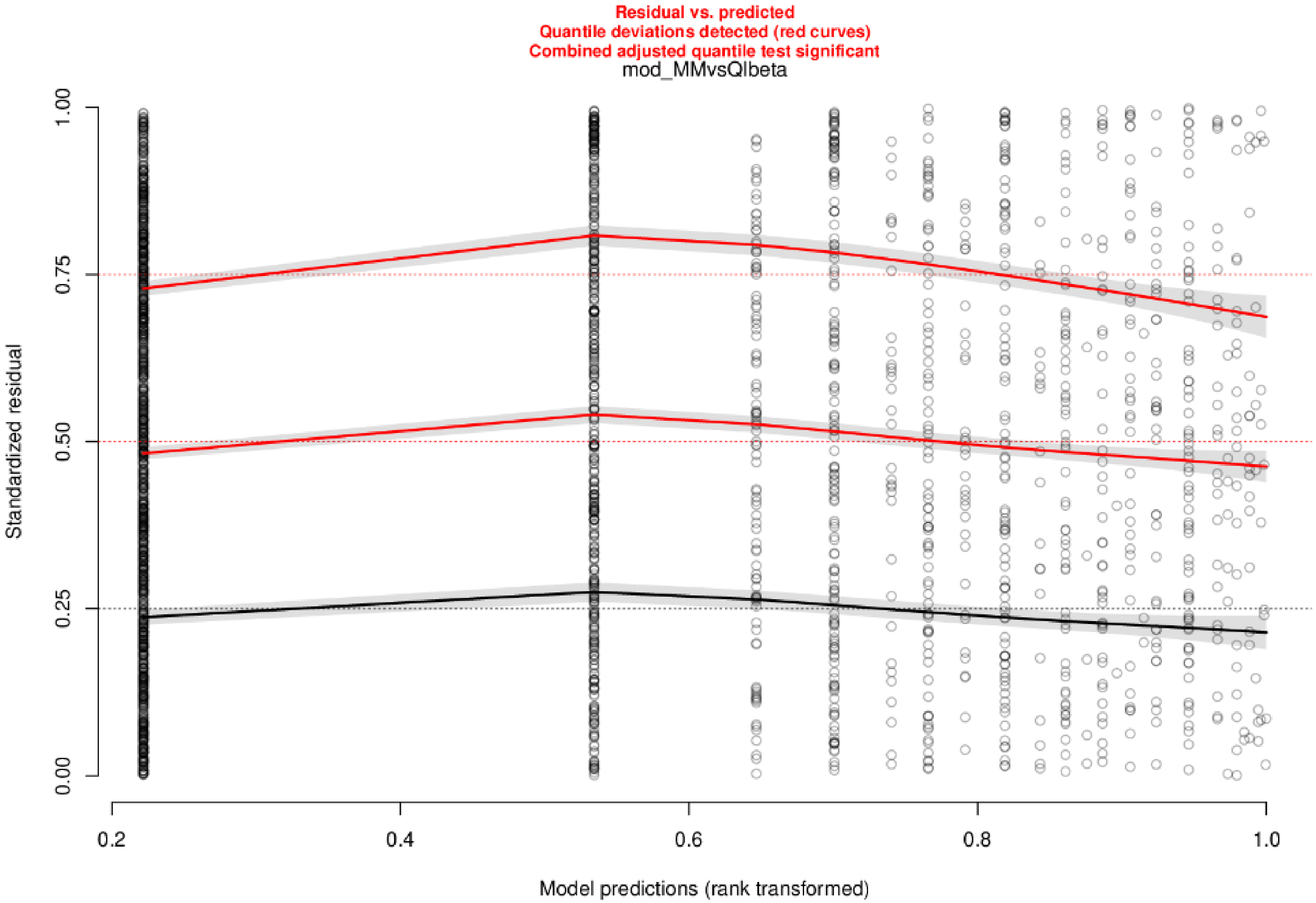
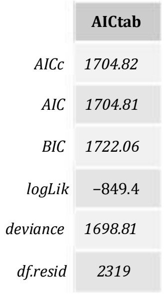


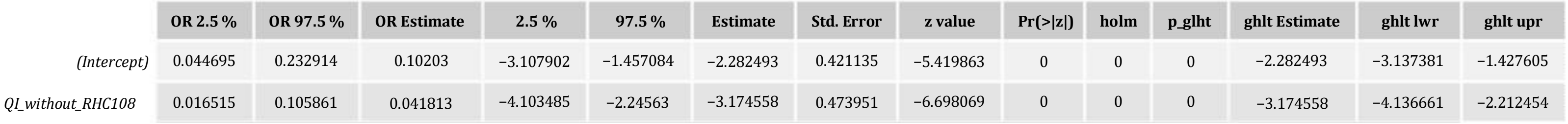
Figure Supp_01 3 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the "multcomp" package and the "glht" function.

A



	AICtab
AICc	*1704.82*
AIC	*1704.81*
BIC	*1722.06*
logLik	−849.4
deviance	*1698.81*
df.resid	*2319*

B



	OR 2.5 %	**OR 97.5 %**	**OR Estimate**	**2.5 %**	**97.5 %**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**holm**	**p_glht**	**ghlt Estimate**	**ghlt lwr**	**ghlt upr**
(Intercept)	0.044695	0.232914	0.10203	−3.107902	−1.457084	−2.282493	0.421135	−5.419863	0	0	0	−2.282493	−3.137381	−1.427605
QI_without_RHC108	0.016515	0.105861	0.041813	−4.103485	−2.24563	−3.174558	0.473951	−6.698069	0	0	0	−3.174558	−4.136661	−2.212454

Figure Supp_01 4 Graphical representation of model estimates (colored dots) with corresponding value above using the “sjPlot” package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. Model estimates are indicated by the circles and the value above. Red indicates a negative effect

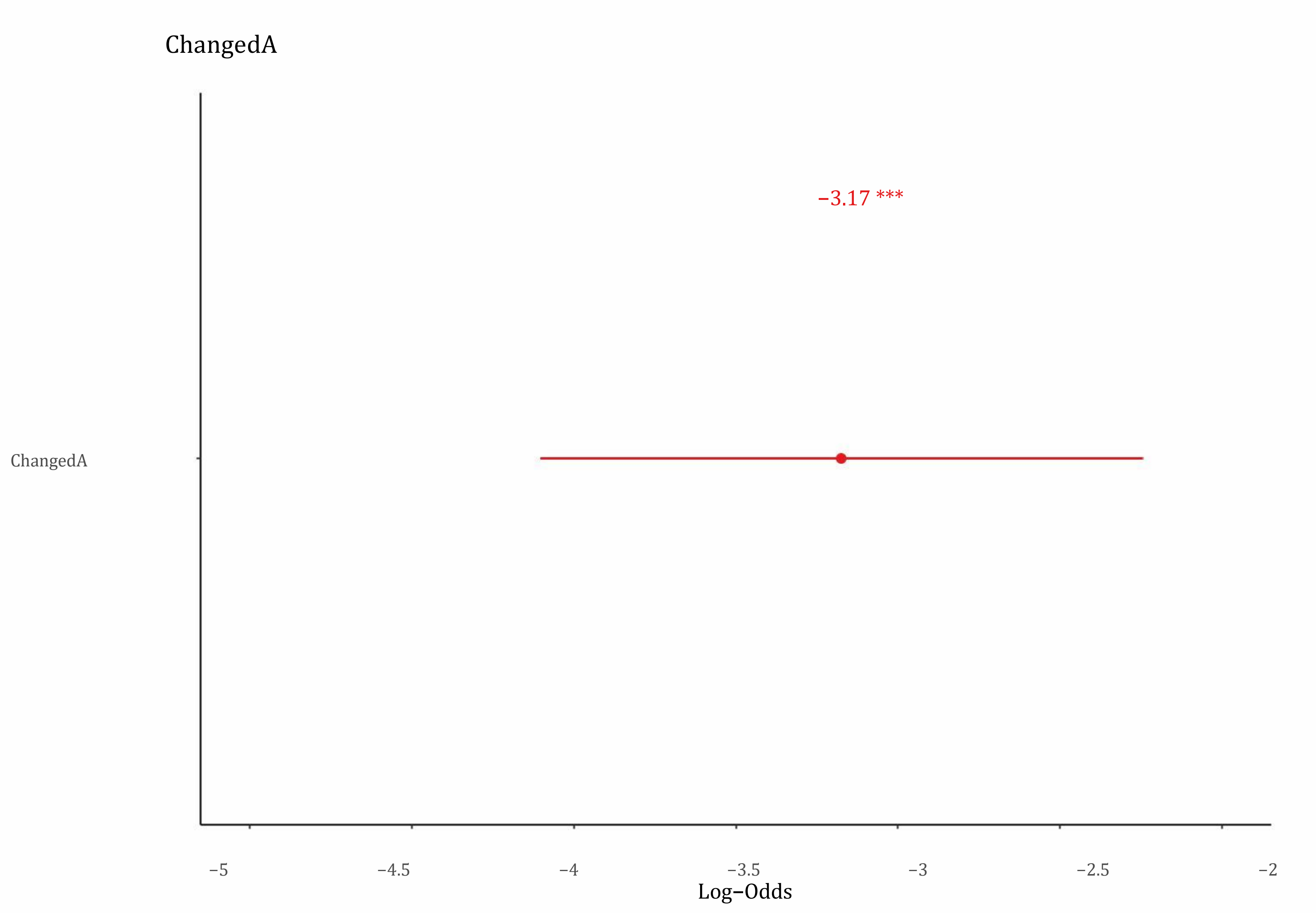
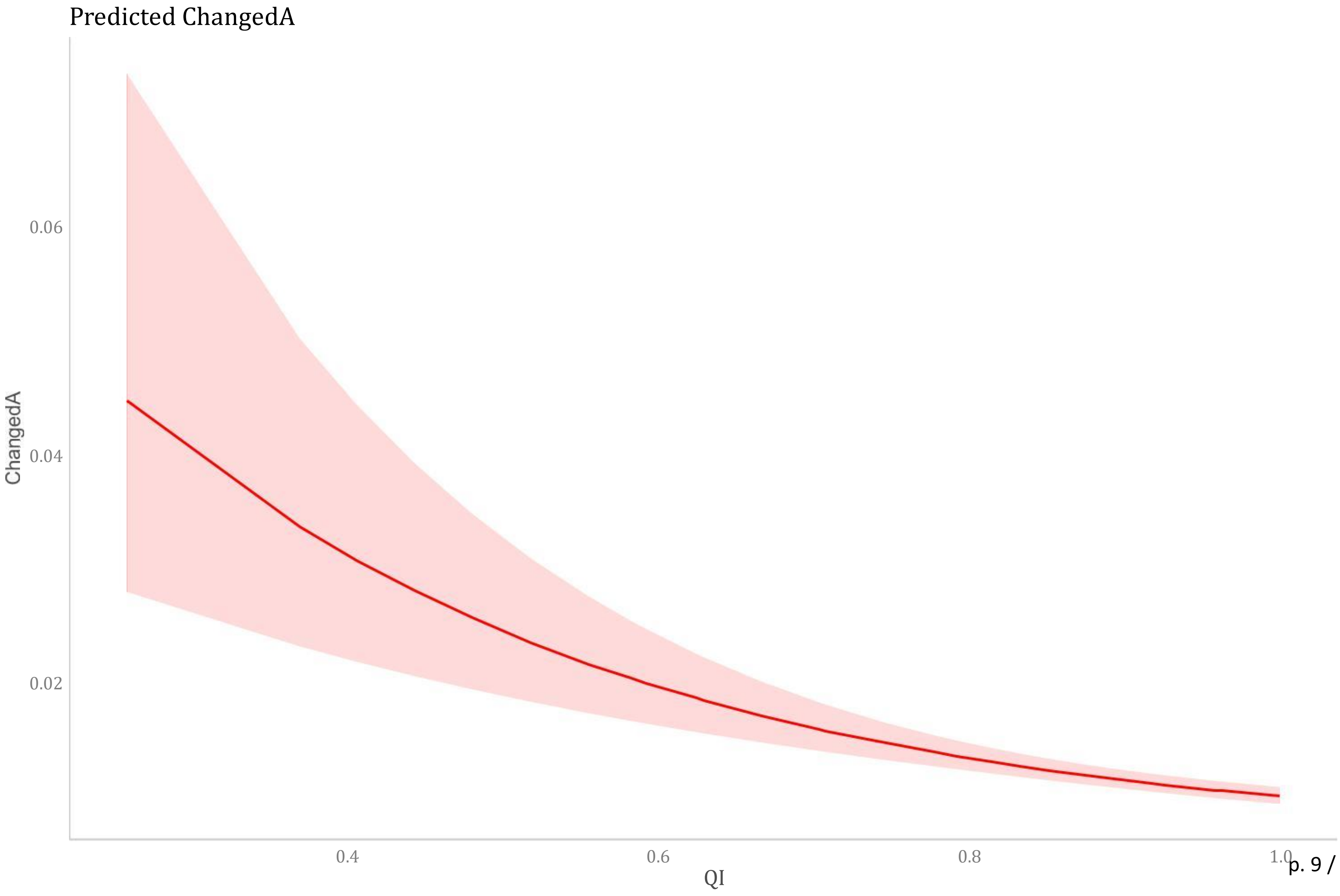


Figure Supp_01 5 Predicted marginal fixed effects (solid lines) of QI on changed alleles (ChangedA) using ggpredict() from the “ggeffects”. 95 % confidence intervals are indicated by the lighter shape. Red indicates negative effects. X-axis represent the observed unstandardized predictor here as proportion of alleles changed during manual correction step compared to the maximum possible number of alleles to be changed. A decrease of QI of 1 to the lowest QI of 0.26 led to an increase of 0% adjusted alleles to 4.2% (2.4% - 7.5% 95% confidence interval) of the alleles in a consensus genotype. This means on average a samples with lowest QI were changed at 0.69 alleles for eight loci or 0.77 allele for 9 loci. Lower sample QI is therefore associated with the need for additional manual mismatch correction and harbours lower quality consensus genotypes whereas a manual correction over the replicates might improve the QI and therefore weaken the signal.



Supp_02 Changes due to manual correction and filtering

Figure Supp_02 1 Sample filtering and manual corrections of consensus genotypes per roost-visit (RV) if the pipeline described in (Zarzoso-Lacoste et al., 2018; Zarzoso-Lacoste et al., 2020) and applied in Jan et al. (2019) would be followed completely. The RVs are depicted in the form “roost/year/session”) where “1” refers to June and “2” to August. Removed samples refer to all samples which were removed due to lack of enough valid loci meaning that at least 8 loci had to show a valid consensus single locus genotype and not NA. Corrected samples describe samples which were altered during the manual mismatch correction step where all consensus genotypes which differed at one or two loci only were altered compared to the consensus from the automated consensus forming pipeline. Between 58% to 96.8% of all initial samples per RV remained uncorrected during this process.

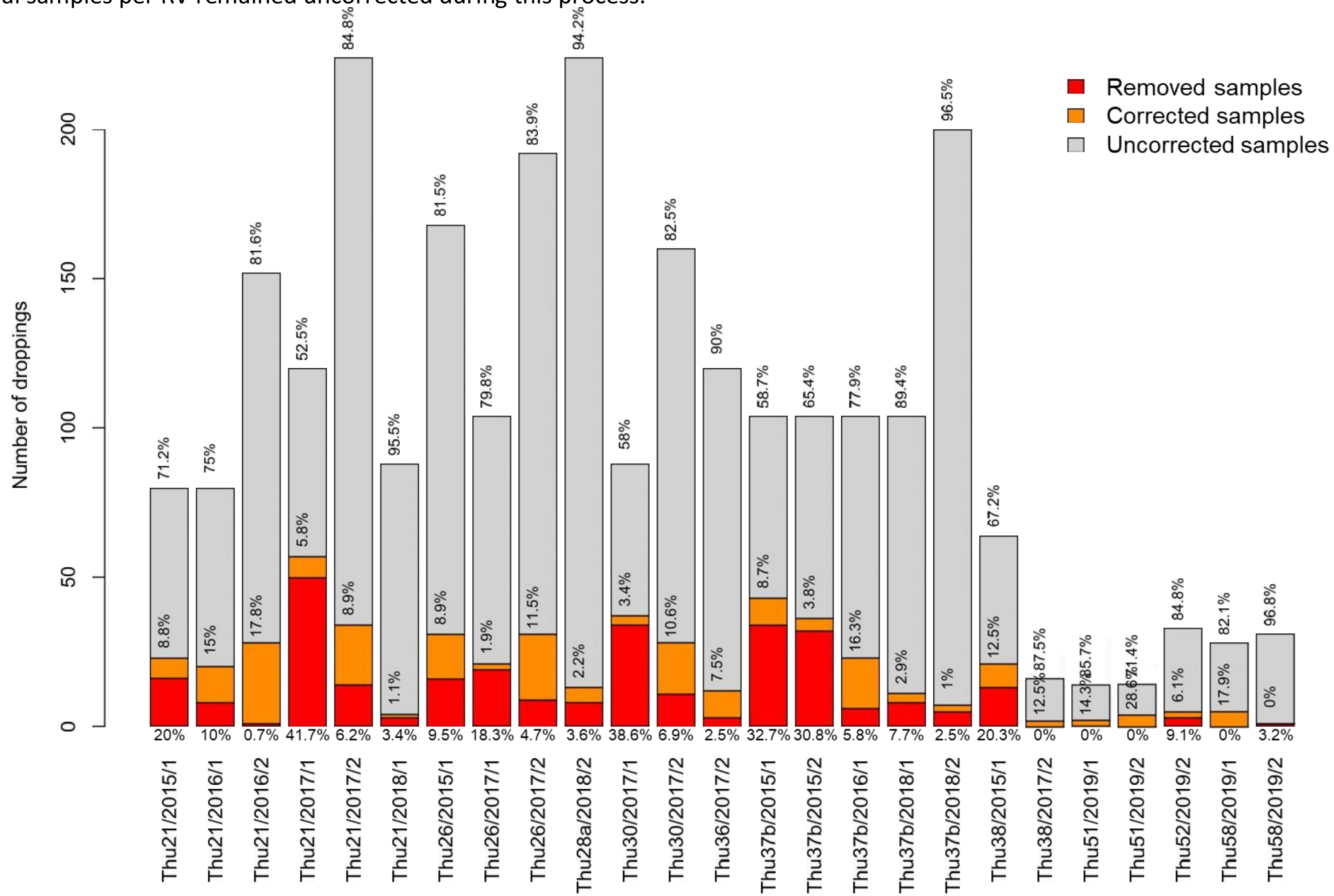
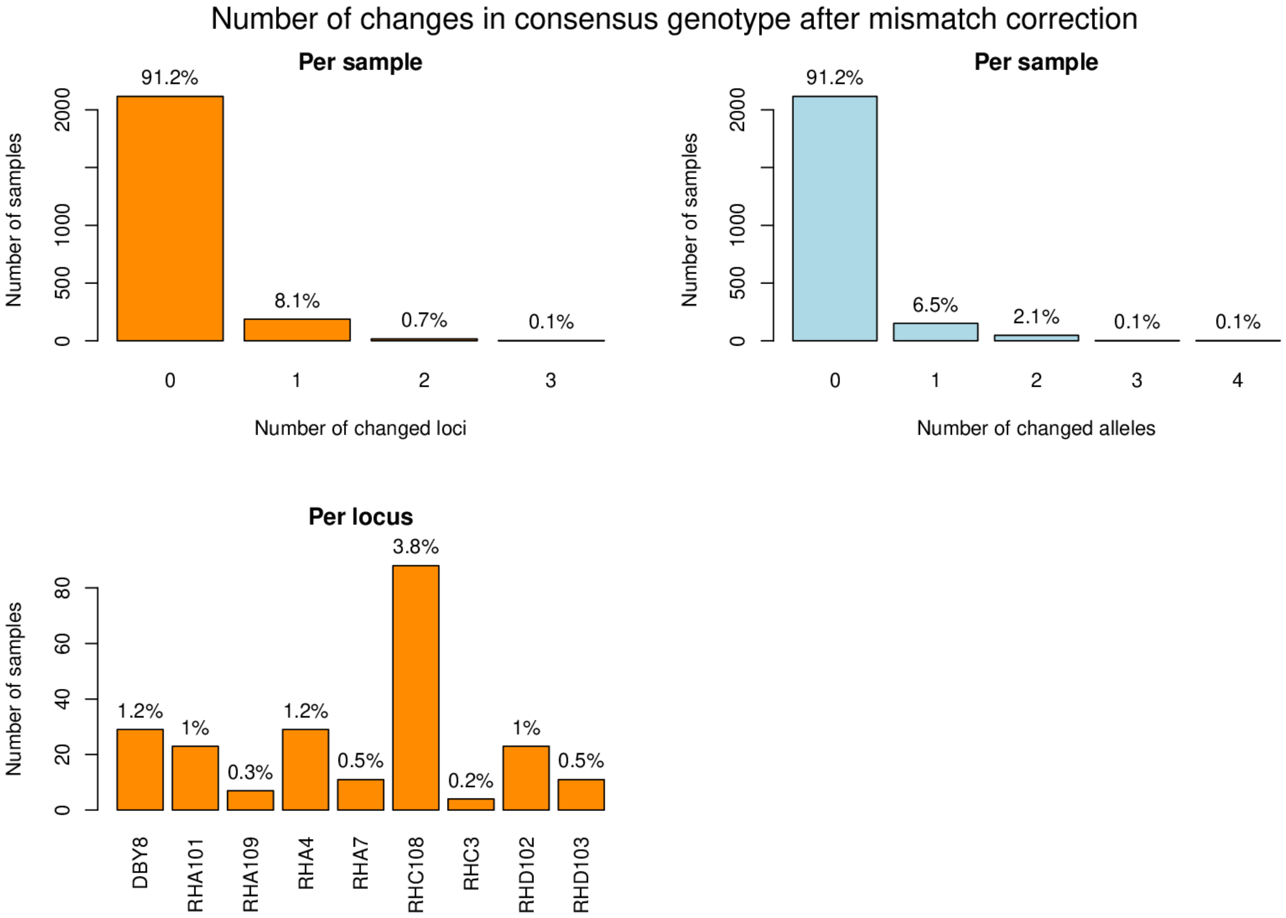
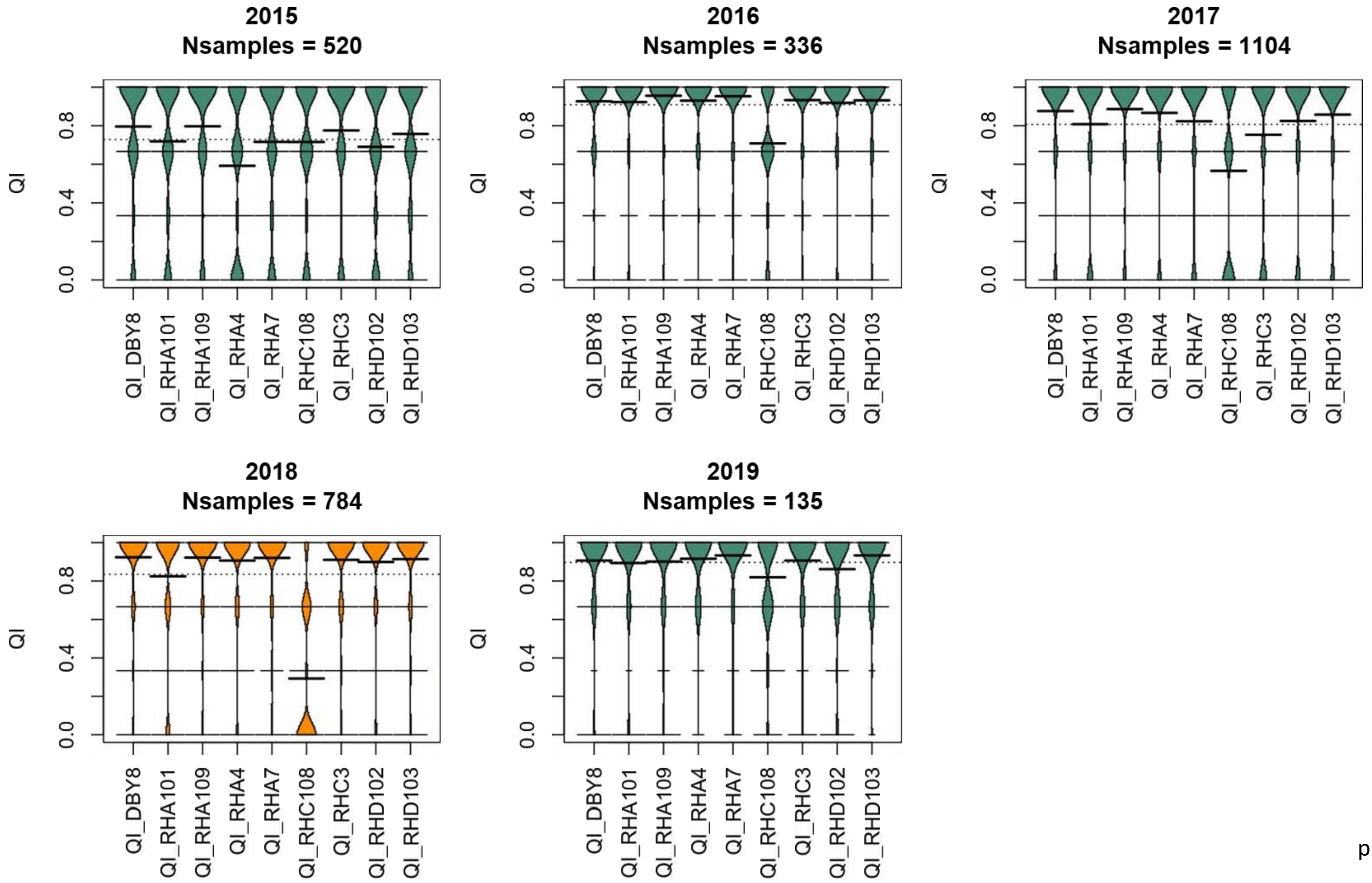


Figure Supp_02 2 Changed loci and alleles due to manual mismatch corrections according to the pipeline described in (Zarzoso-Lacoste et al., 2018; Zarzoso-Lacoste et al., 2020) and applied in Jan et al. (2019) for all consensus loci which differed at one or two loci compared to the consensus genotypes generated by the automated pipeline only. The total sample number refers to the reduced number of all samples of model and test data combined minus the samples with less than eight valid consensus loci (not scored as NA), resulting in 2322 samples (see Figure **Supp_02** 1). It shows that only 8.83% of those samples were altered and most of those only at one locus (8.1%, 91.7% of the 8.83%). This means the potential maximum deviation from QI for those 8.1% samples would be 0.11 for nine loci or 0.13 for eight loci (due to removal of locus RHC108 in 2018) for three replicates. Also note the general issue with marker RHC108 even after removing it in 2018. It needed most manual correction with 3.8% of all samples being changed during manual mismatch correction and is also reflected in lower QI for that marker in most years (**Supp_03**)



Supp_03 Marker quality per year

Locus QI per year as beanplots. Horizontal ticks depict means. Note the bad quality of RHC108 in 2018 due to lab effects. The number of samples is before removing strong other lab effects due to a bad extraction plates. That is why the total sample number is higher here then the 2616 samples in the combined model building and test data used to quantify and predict eoDNA degradation in the publication.



Supp_04 Test data overview

Table Supp_04 1 Test data overview used to test how well the best predictive model and the combination of all 19 top models within ΔAICc<6 could predict mean QI for four different roost-visits (RV) from two completely new roosts. “Z-scored” indicates standardization of variable by two standard deviations and centring to 0 based on the mean and standard deviation of the model building data. All continuous fixed, zero-inflation and dispersion effects were measured per roost-visit.

<table>
<tr><th rowspan="2"></th><th rowspan="2">Response and fixed effects</th><th rowspan="2">Random effects</th><th colspan="2">Corrective model</th></tr>
<tr><th>Zero-inflation</th><th>Dispersion</th></tr>
<tr><td>Final global model:</td><td>QI ~ HMax * TMax + Days + HottestDay + MoistestDay + TTrend + HTrend + Sess</td><td>+ (1 | Roost)
+(1|Year/PCRPlate/ExtrPlate/RVLabUnit)</td><td>~ HMin
+ TMin
+ AbsHTrend
+ AbsTTrend</td><td>~ HMax + AbsTTrend</td></tr>
</table>

Model part	Variable (Abbrev.)	Type/transformation	Description and [range of unstandardized variable]	n
Response	Quality Index **(QI)**	Beta-binomial (logit) (Nsuccess/Nfailure)	Proportion of replicate loci identical/non-identical compared to consensus locus built over three replicate multi-locus genotypes with 8-9 loci each **[3x][0-1]**	307 droppings
Fixed effects	Max. Humidity (**HMax**)	Continuous/Z-scored	Maximum relative humidity [**62.2 – 80.6 %]**	4 values
	Max. Temperature (**TMax**)	Continuous/Z-scored	Maximum temperature **[30.6 – 47.1 °C]**	4 values
	Temperature trend (**TTrend**)	Continuous/Z-scored	Trend towards warmer (positive) or colder (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. temperatures. **[1.2 – 2.5 °C]**	4 values
	Humidity trend (**HTrend**)	Continuous/Z-scored	Trend towards moister (positive) or drier (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. humidity. **[-1.1 – 0.6 %]**	4 values
	Hottest day (**HottestDay**)	Continuous/Z-scored	Day with max. daily median temperature as proportion of total exposure days **[0.5 – 1]**	4 values
	Moistest day (**MoistestDay**)	Continuous/Z-scored	Day with max. daily median humidity as proportion of total exposure days **[0.25– 1]**	4 values
	Exposure days (**Days**)	Continuous/Z-scored	Duration of single collection period in a roost in days (maximum possible days a dropping could have been exposed to the environment) **[9 – 11]**	4 values
	Session (**Sess**)	Factor/Reference June	June/August (before and after offspring is born)	2 levels
Random effects	Roost (**Roost**)	Factor	Different maternity roosts (totally sampled 4 times in 2017 and 2019	2 levels
	Year (**Year**)	Factor	2017, 2019	2 levels

	PCR plate (**PCRPlate**)	Factor	Each PCR plate with 384 wells	6 levels
	Extraction plate (**ExtrPlate**)	Factor	Each plate with 96 wells (each well contains extracted DNA of one dropping)	6 levels
	Roost visit sample block (**RVLabUnit**)	Factor	Groups of droppings of the same roost visit origin ending up on the same extraction and/or PCR plates (accounting for blocked sample distribution on PCR and extraction plates).	6 levels
Zero-inflation effects	Min. humidity (**HMin**)	Continuous/Z-scored	Minimum relative humidity **[20.3 – 40.2 %]**	4 values
	Min. temperature (**TMin**)	Continuous/Z-scored	Minimum temperature **[11.6 – 16.6 °C]**	4 values
	Absolute humidity trend (**AbsHTrend**)	Continuous/Z-scored	Measure of absolute variability in max. humidity between days calculated as median of absolute chronological differences of daily maximum humidity **[1.1 – 2.8 %]**	4 values
	Absoulte temperature trend (**AbsTTrend**)	Continuous/Z-scored	Measure of absolute variability in max. temperature between days calculated as median of absolute chronological differences of daily maximum humidity **[1.5 –3.0°C]**	4 values
Dispersion effects	**AbsTTrend**, **HMax**	Continuous/Z-scored	Variables already explained in previous rows	4 values

Supp_05 Information about the initial global model (binomial)

Model diagnostics and information for the first initial global model (step 1 and 2 in the manuscript) created with the mdiag() function included in the version controlled R-Project. Not all information from the 29-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved.

Model overview

name: initial_global_model_01_binomial

family: binomial
link: logit
response variable form: matrix

sample size: 2309

N (sample size) / k (terms) : 256.6
formula: QIPerdropping ~ HMax * TMax + Days + HTrend

+ TTrend + HottestDay + MoistestDay + Sess + (1 | Roost)

+ (1 | Year/PCRPlate/ExtrPlate/RVLabUnit)

ziformula: ~0

dispformula: ~1

Figure Supp_05 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Dispersion test shows clear overdispersion with the observed (red line) variance being 2.95 stronger than the mean simulated variance (black lines). B) Zero-inflation test shows that the number of observed zeroes (red line) is 3515.52 higher than the mean of the simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows significant outliers (red bars and red line). D) Quantile-Quantile plot of observed and expected residuals shows clear deviation of uniform distribution (red line).

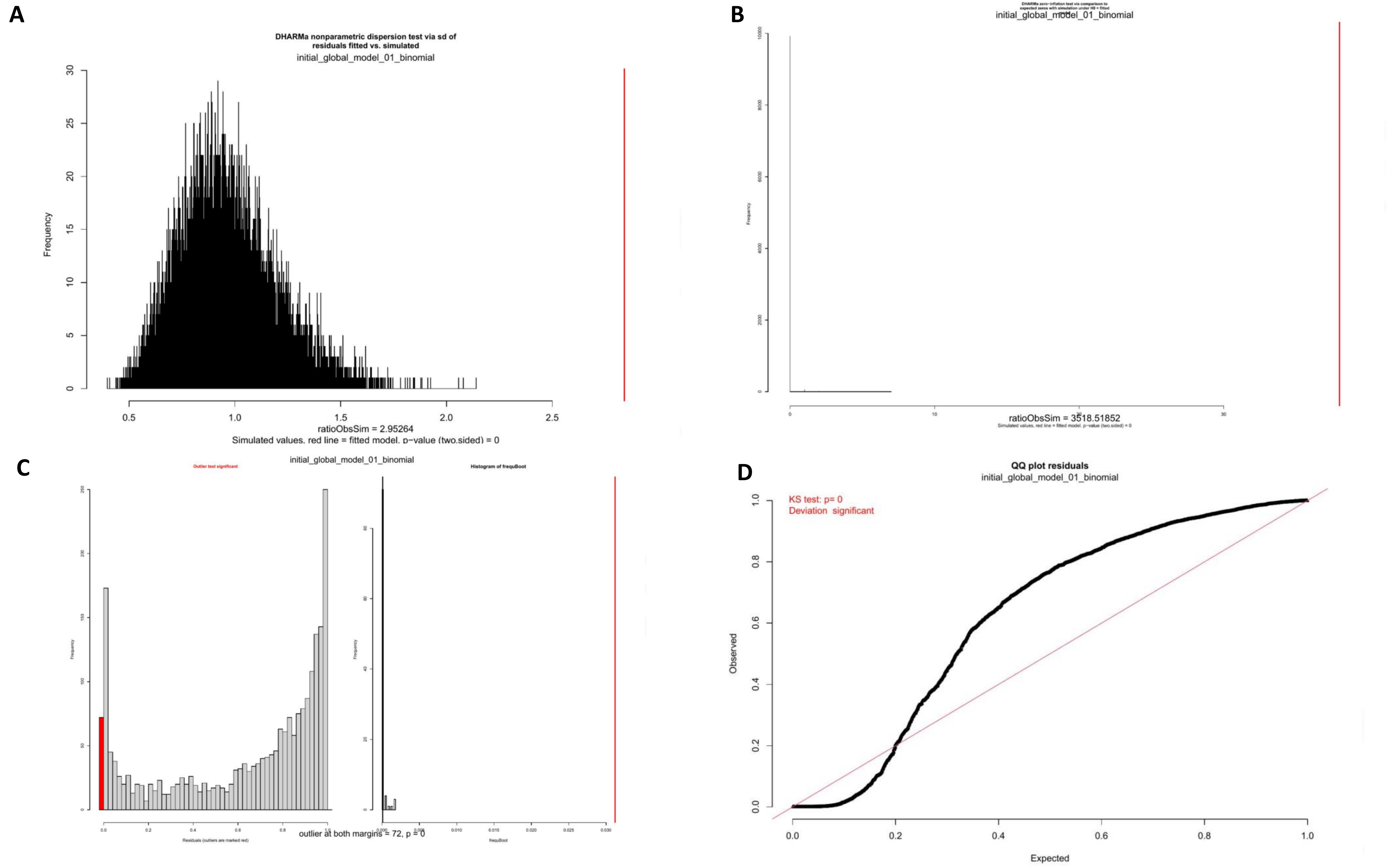


Figure Supp_05 2 Model diagnostics based on simulated residuals using the “DHARMa” package based on 10 000 simulations. A) Testing excess/deficits of QI = 1. Since QI was expressed in matrix form (success/(total number of loci)), a QI of 1 means in Matrix form 27/27. We detected a significant excess of ones with 3.35 times the number of observed ones (red line) compared to the mean number of ones in simulations (black bars).

B) The same as C) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem (see main text). It shows a non-significant excess of ones with 1.15 times more ones than the mean number of simulated ones.

C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate strong and significant signs of heteroscedasticity.

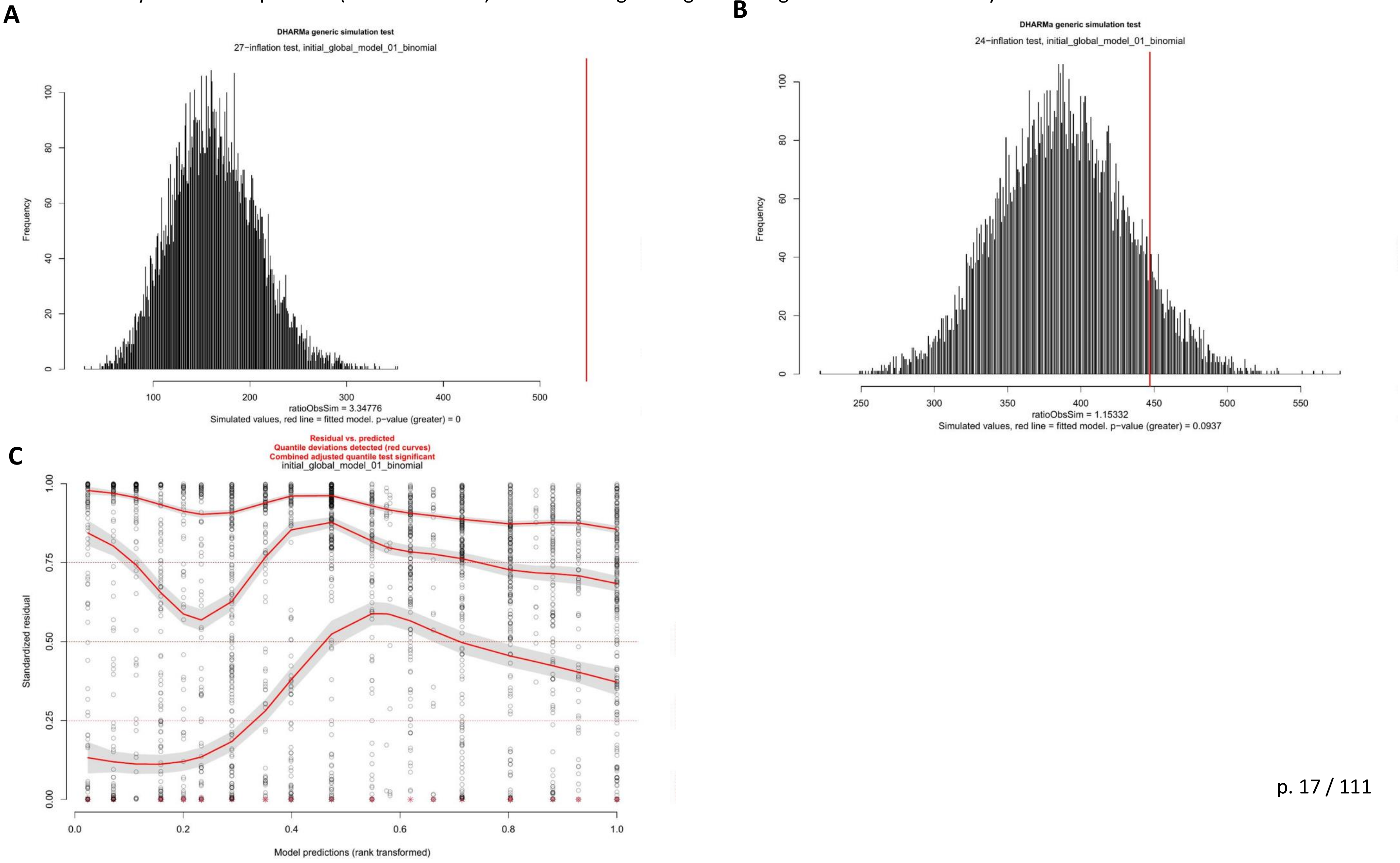


Figure Supp_05 3 Correlations of input variables and multicollinearity. A) Pairwise correlation of all input variables (2309 values per variable) using «Hmisc» and «corrplot» package. Lower triangle indicates Spearman's rank correlation; higher triangle indicates Pearson correlation. Spearman's rank correlation is reported since not all input variables were normally distributed. Lighter colour indicates weaker correlation darker colours stronger correlation. Reddish colours indicate negative correlation, blueish indicate positive correlations. Numbers indicate correlation values. Regardless of the type of chosen correlation statistic, correlation stayed all under 0.6 and are regarded unproblematic in this study. B) shows multicollinearity measured as variance inflation factor (VIF) using the "performance" package. No problematic multicollinearity was detected with all predictors below a VIF of 5.

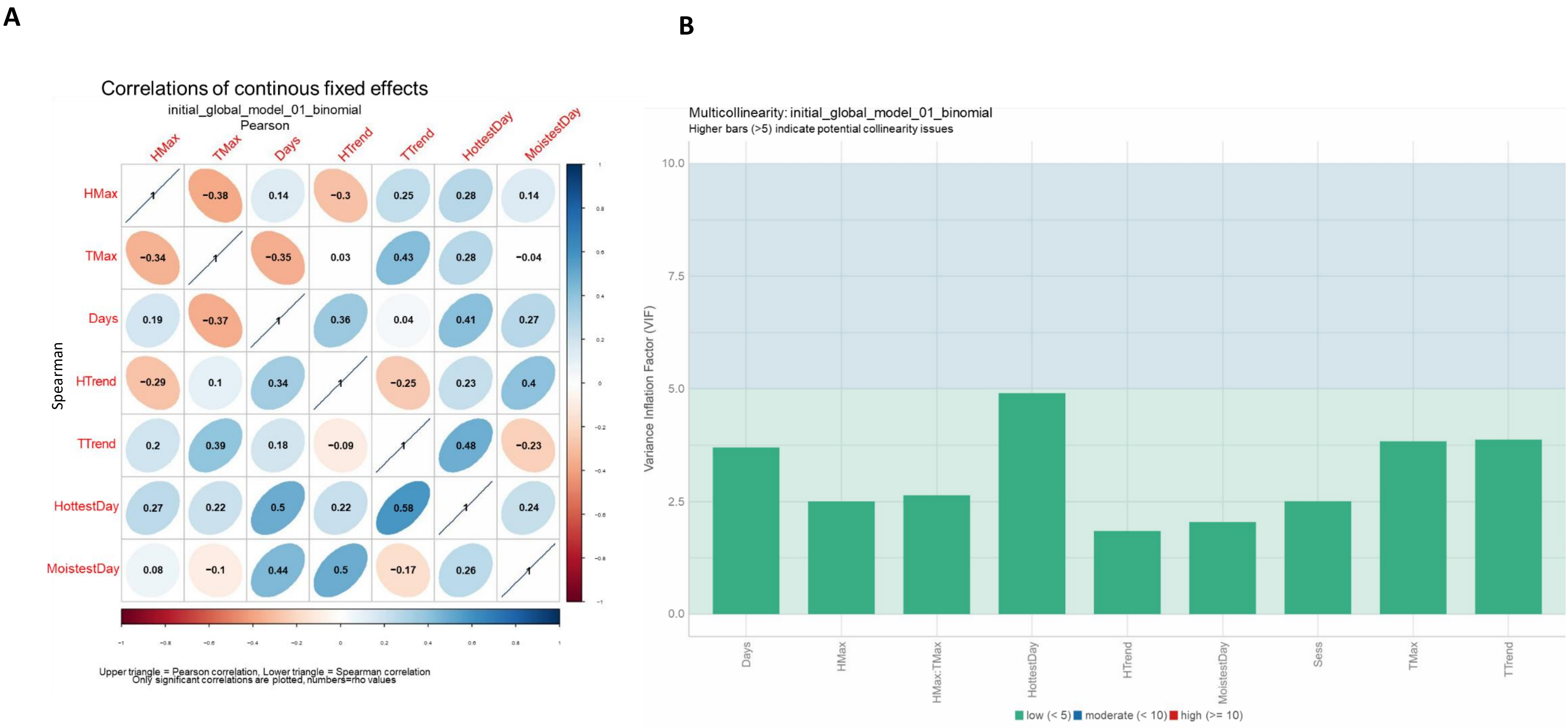


Figure Supp_05 4 Temporal autocorrelation test using DHARMa package based on 10 000 simulations using chronological ordered “Year”/”Sess” info as time variable. No significant temporal autocorrelation was detected using the Durbin-Watson test (p=0.85).

We also performed a spatial autocorrelation test (Moran’s test for distance-based autocorrelation) with those residuals and could not detect significant spatial autocorrelation (p=0.25). The graph with coordinates is not shown because lesser horseshoe bat *(Rhinolophus hipposideros*) is an endangered and protected species in Germany and roosts location cannot be disclosed.

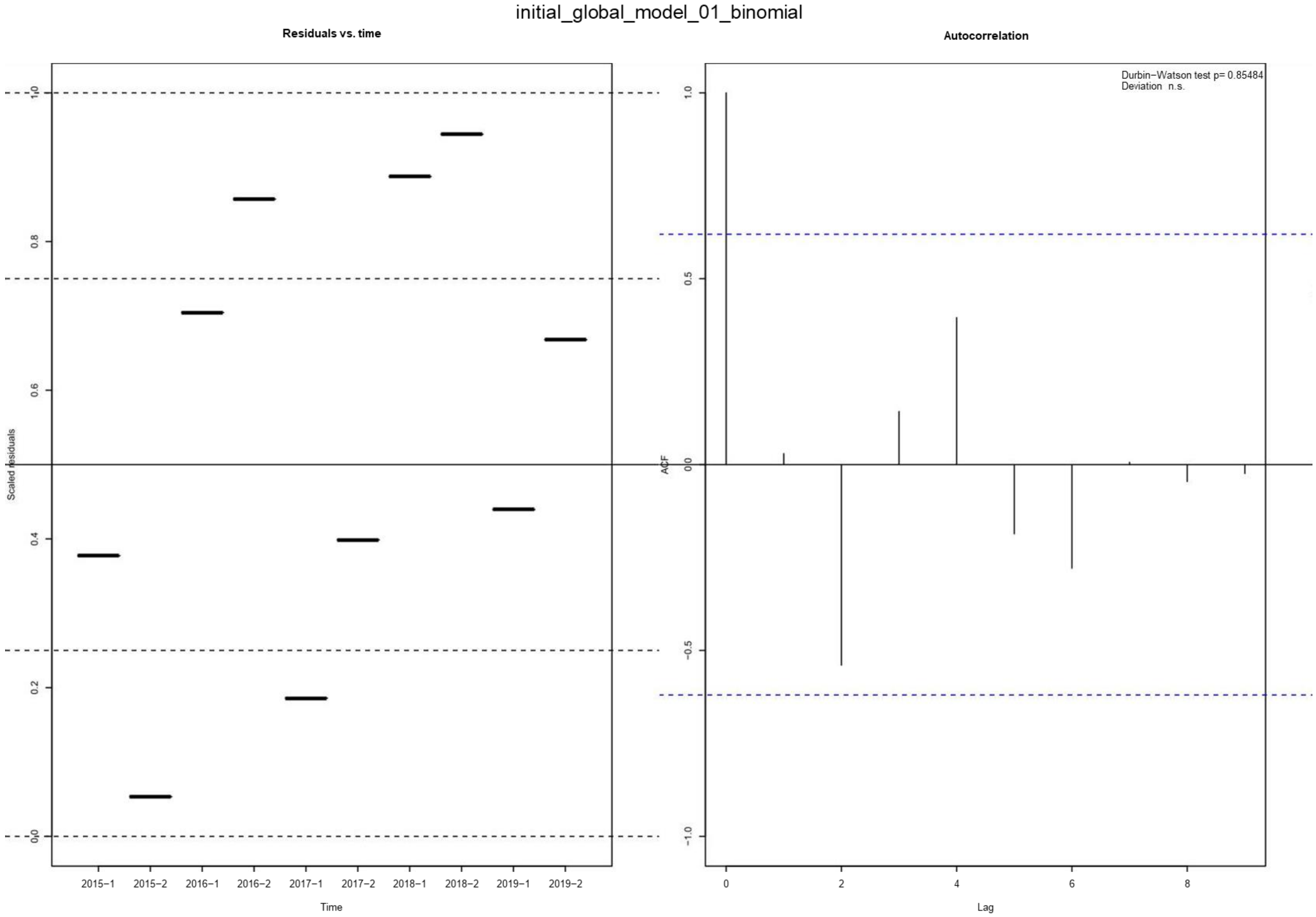


Figure Supp_05 5 Visual check of normality of residuals for random effects using the “performance” and “See” package. No strong deviation was detected.

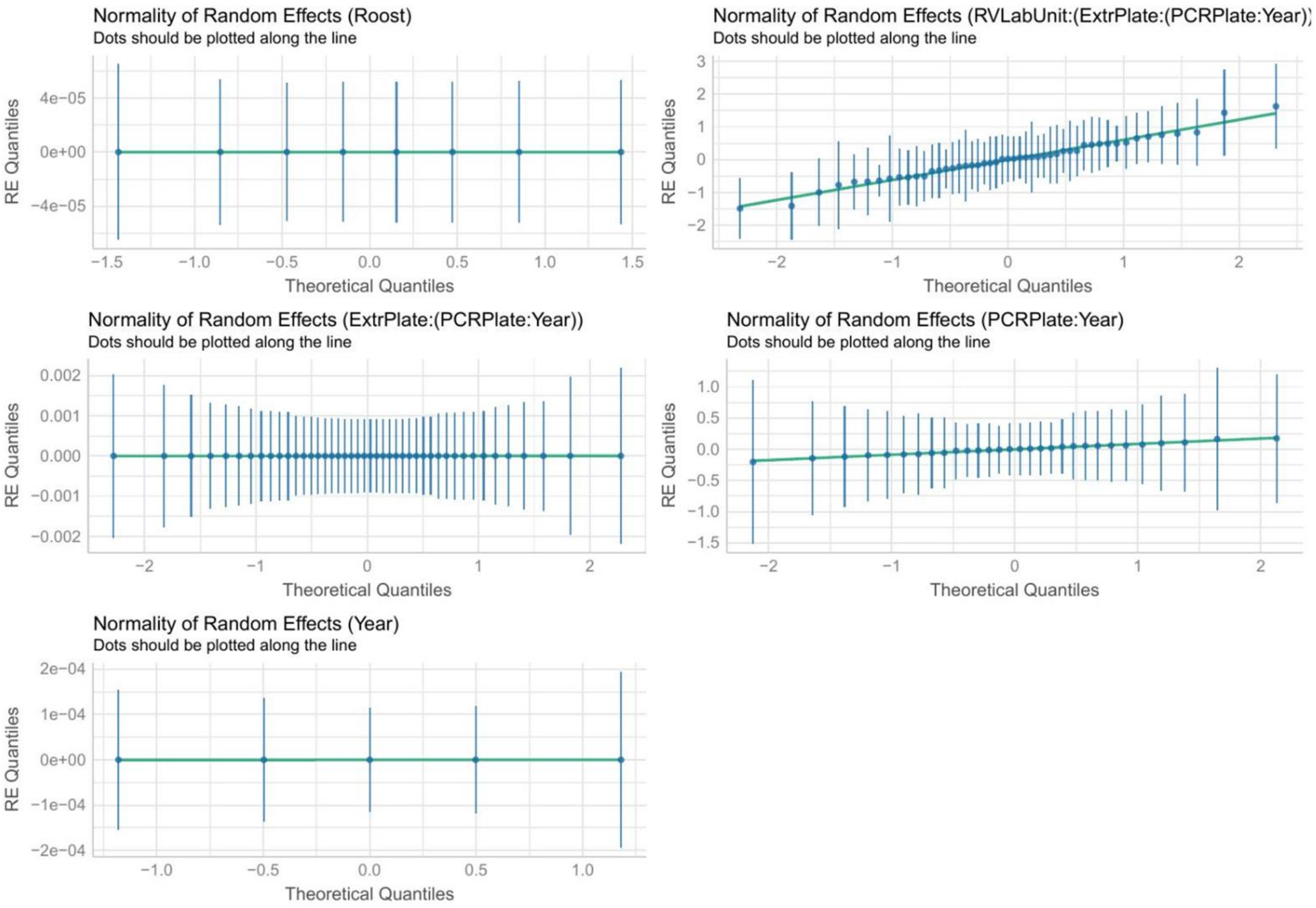
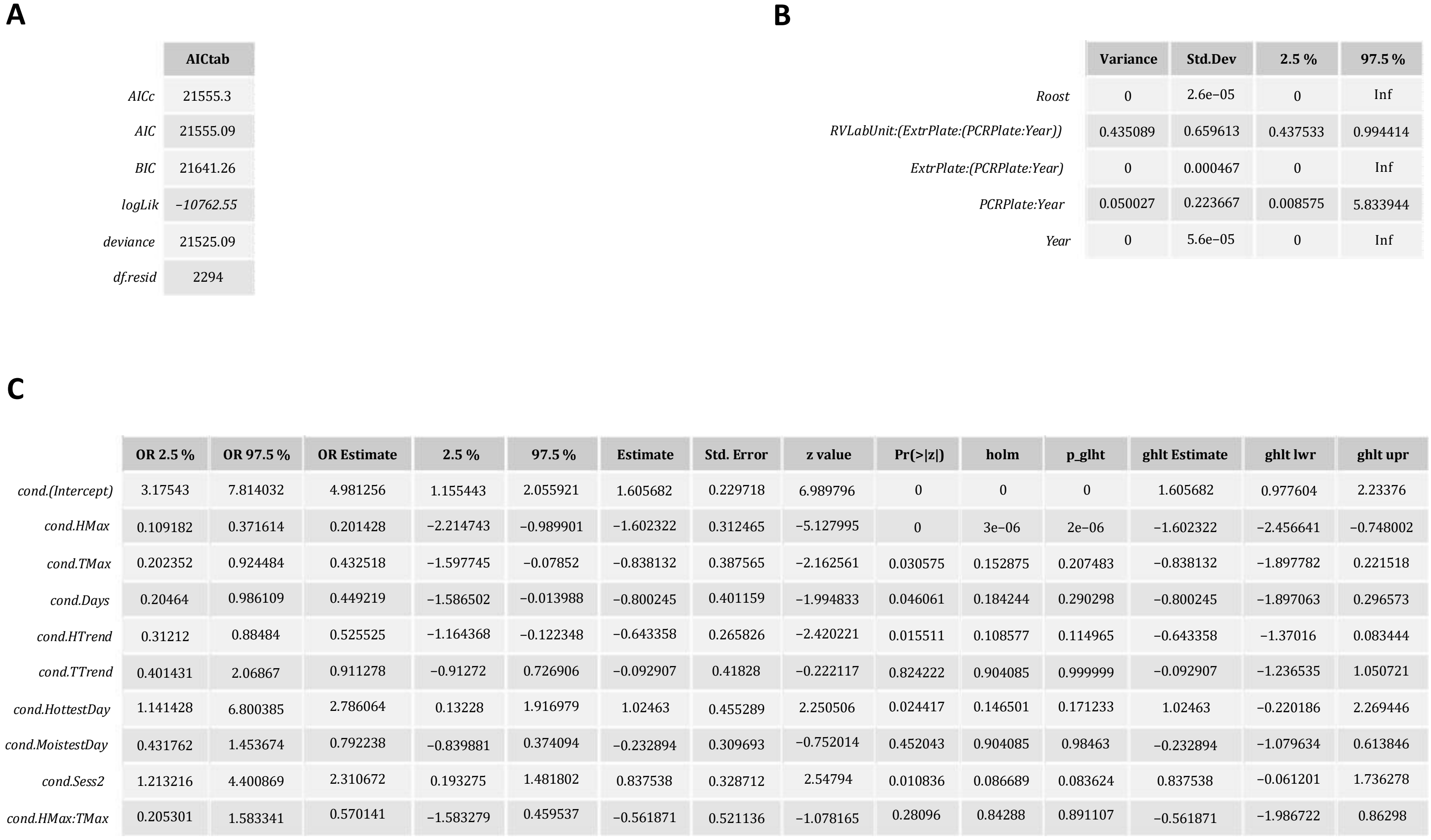


Figure Supp_05 6 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Random effect variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval. C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the “multcomp” package and the “glht” function.



A

	AICtab
AICc	21555.3
AIC	21555.09
BIC	21641.26
logLik	*−10762.55*
deviance	21525.09
df.resid	2294

B

	Variance	Std.Dev	2.5 %	97.5 %
Roost	0	2.6e−05	0	Inf
RVLabUnit:(ExtrPlate:(PCRPlate:Year))	0.435089	0.659613	0.437533	0.994414
ExtrPlate:(PCRPlate:Year)	0	0.000467	0	Inf
PCRPlate:Year	0.050027	0.223667	0.008575	5.833944
Year	0	5.6e−05	0	Inf

C

	OR 2.5 %	OR 97.5 %	OR Estimate	2.5 %	97.5 %	Estimate	Std. Error	z value	Pr(>|z|)	holm	p_glht	ghlt Estimate	ghlt lwr	ghlt upr
cond.(Intercept)	3.17543	7.814032	4.981256	1.155443	2.055921	1.605682	0.229718	6.989796	0	0	0	1.605682	0.977604	2.23376
cond.HMax	0.109182	0.371614	0.201428	−2.214743	−0.989901	−1.602322	0.312465	−5.127995	0	3e−06	2e−06	−1.602322	−2.456641	−0.748002
cond.TMax	0.202352	0.924484	0.432518	−1.597745	−0.07852	−0.838132	0.387565	−2.162561	0.030575	0.152875	0.207483	−0.838132	−1.897782	0.221518
cond.Days	0.20464	0.986109	0.449219	−1.586502	−0.013988	−0.800245	0.401159	−1.994833	0.046061	0.184244	0.290298	−0.800245	−1.897063	0.296573
cond.HTrend	0.31212	0.88484	0.525525	−1.164368	−0.122348	−0.643358	0.265826	−2.420221	0.015511	0.108577	0.114965	−0.643358	−1.37016	0.083444
cond.TTrend	0.401431	2.06867	0.911278	−0.91272	0.726906	−0.092907	0.41828	−0.222117	0.824222	0.904085	0.999999	−0.092907	−1.236535	1.050721
cond.HottestDay	1.141428	6.800385	2.786064	0.13228	1.916979	1.02463	0.455289	2.250506	0.024417	0.146501	0.171233	1.02463	−0.220186	2.269446
cond.MoistestDay	0.431762	1.453674	0.792238	−0.839881	0.374094	−0.232894	0.309693	−0.752014	0.452043	0.904085	0.98463	−0.232894	−1.079634	0.613846
cond.Sess2	1.213216	4.400869	2.310672	0.193275	1.481802	0.837538	0.328712	2.54794	0.010836	0.086689	0.083624	0.837538	−0.061201	1.736278
cond.HMax:TMax	0.205301	1.583341	0.570141	−1.583279	0.459537	−0.561871	0.521136	−1.078165	0.28096	0.84288	0.891107	−0.561871	−1.986722	0.86298

Figure Supp_05 7 Graphical representation of model estimates (coloured dots) with corresponding value above using the “sjPlot” package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. Model estimates are indicated by the circles and the value above.

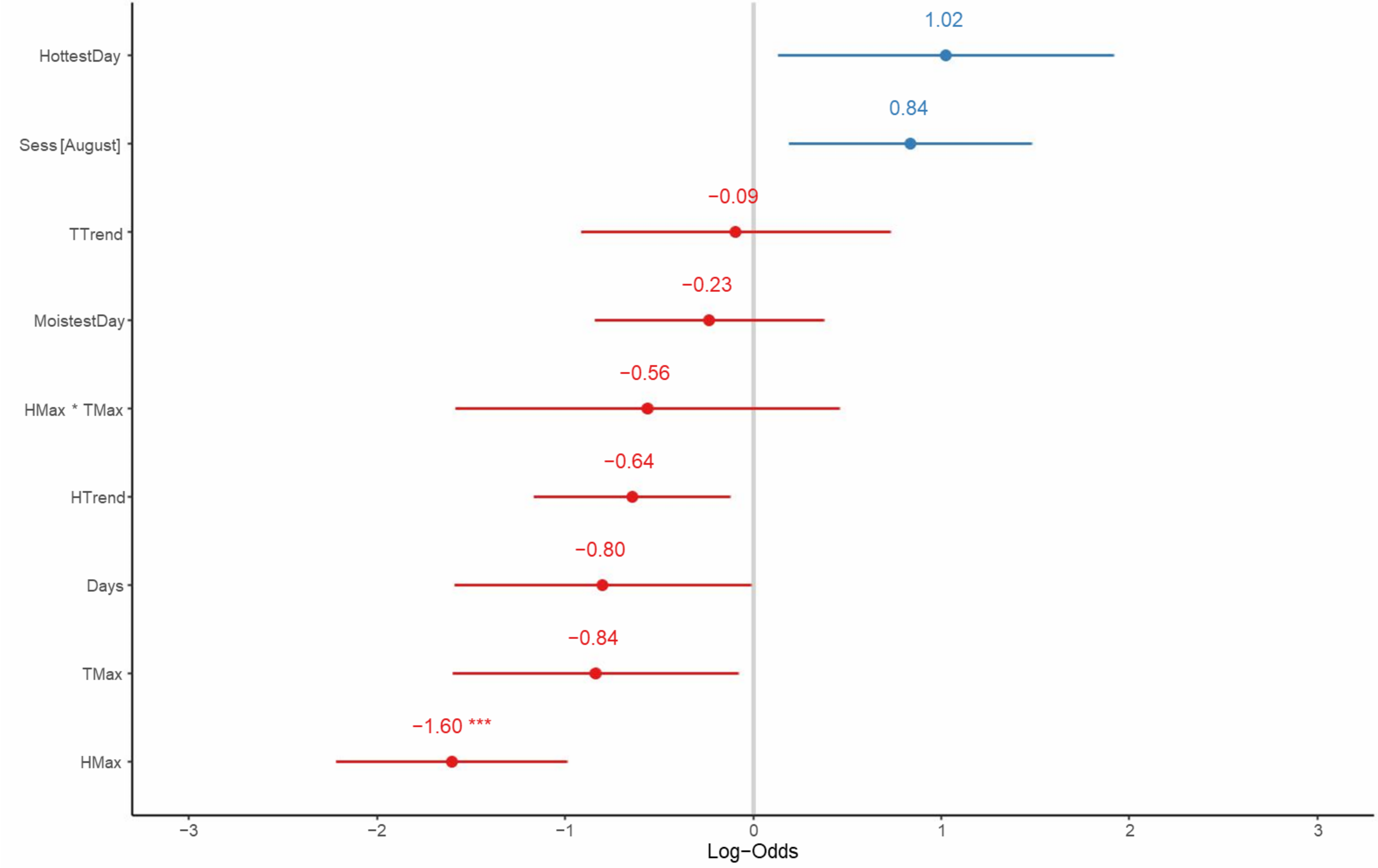
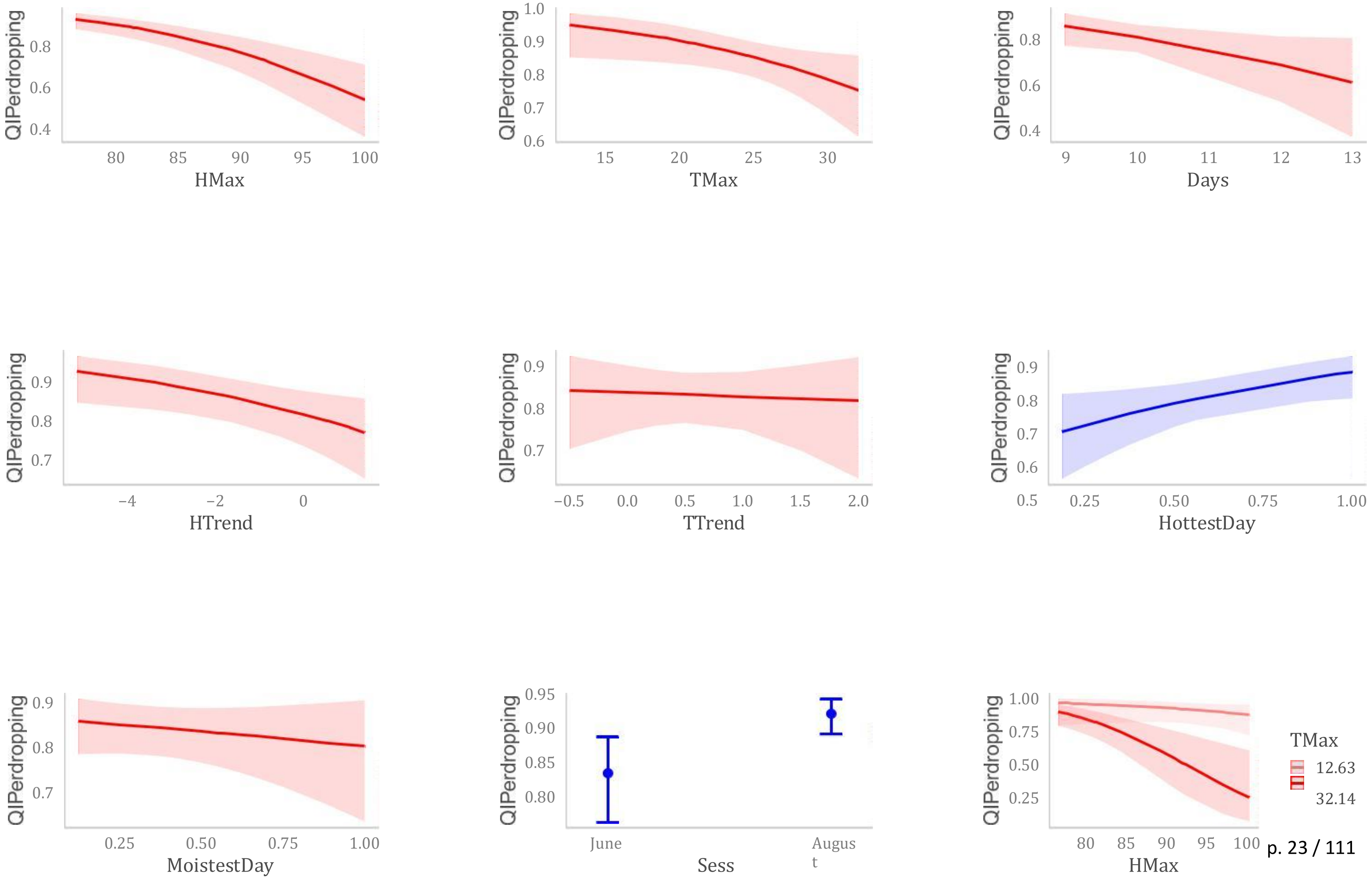


Figure Supp_05 8 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the "ggeffects". 95 % confidence intervals are indicated by the lighter shape. Red indicates negative, blue positive effects. X-axis represent the observed unstandardized predictors. "QIPerdropping" is equal to QI used in the publication and just emphasizes that QI was calculated over the whole sample (across 3 replicates with 9 loci each totaling in 27 loci). For continuous interaction terms the minimum and maximum observed values were used for plotting (e.g.: HMax:TMax, bottom right).



Supp_06 Information about the 2nd global model (beta-binomial)

Model diagnostics and information for the second initial global model (step 1 and 2 in manuscript). created with the mdiag() function included in the version controlled R-Project. Not all information from the 29-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved

Model Overview

name: initial_global_model_02_betabinomial

family: betabinomial

link: logit

response variable form: matrix

sample size: 2309

N (sample size) / k (terms): 256.6

formula: QIPerdropping ~ HMax * TMax + Days + HTrend
+ TTrend + HottestDay + MoistestDay + Sess + (1 | Roost)
+ (1 | Year/PCRPlate/ExtrPlate/RVLabUnit)

ziformula: ~0
dispformula: ~1

Figure Supp_06 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Dispersion test showing no significant overdispersion with the observed (red line) variance being 1.08 stronger than the mean simulated variance (black lines). B) Zero-inflation test shows that the number of observed zeroes (red line) is 5.98 times higher than the mean of the simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows no significant outliers (red bars and line). D) Quantile-Quantile plot of observed and expected residuals shows significant but weak deviation of uniform distribution (red line).

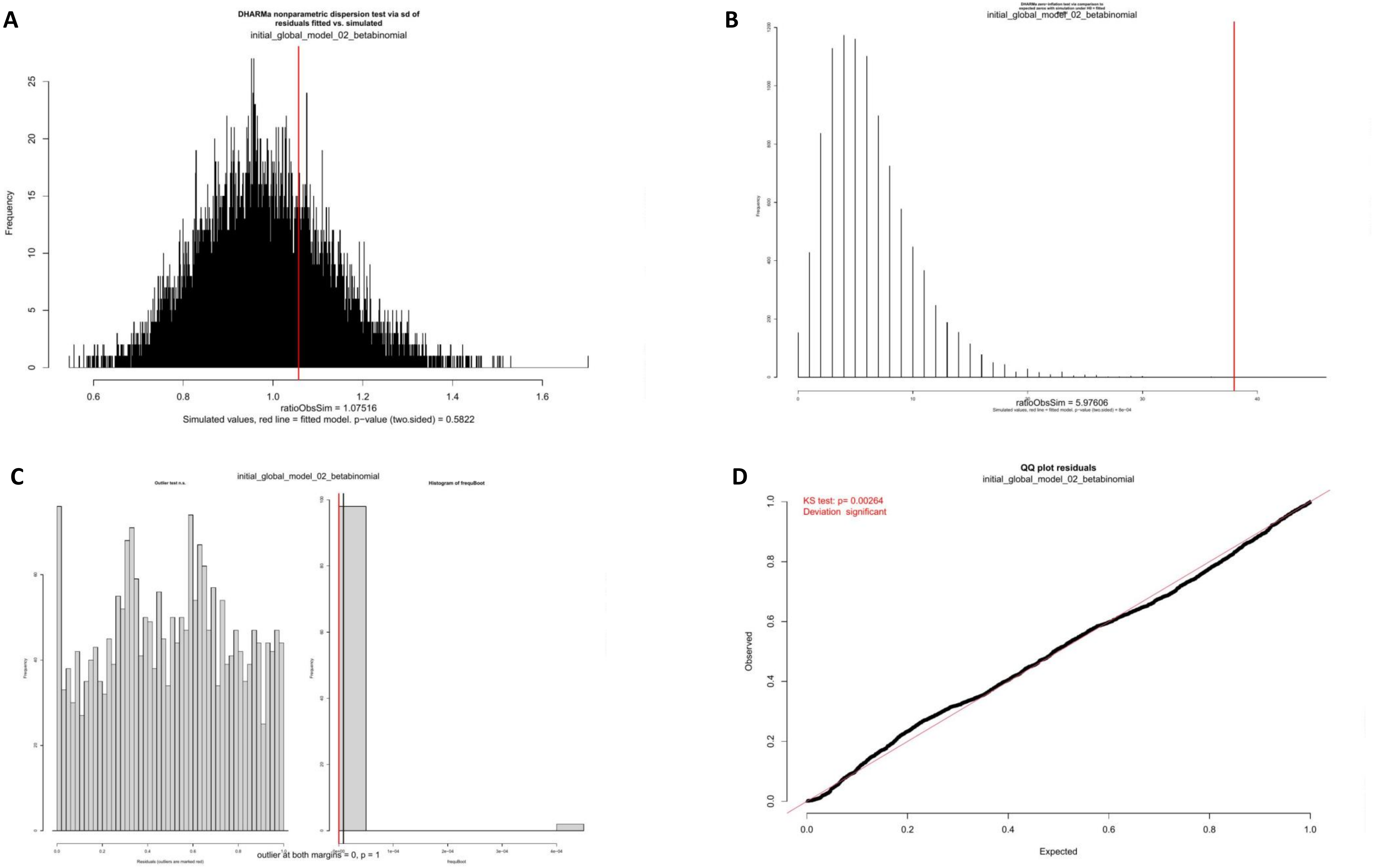


Figure Supp_06 2 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Testing excess/deficits of QI = 1. Since QI was expressed in matrix form (success/(total number of loci) A QI of 1 means in Matrix form 27/27. We didn`t detect a significant excess/deficit of ones with 0.81 times the number of observed ones (red line) compared to the mean simulated number of ones (black bars). B) Shows the same as A) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem (see main text). It shows no significant excess/deficit of ones with 0.96 times observed ones compared to the simulated mean number of ones C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate signs of heteroscedasticity.

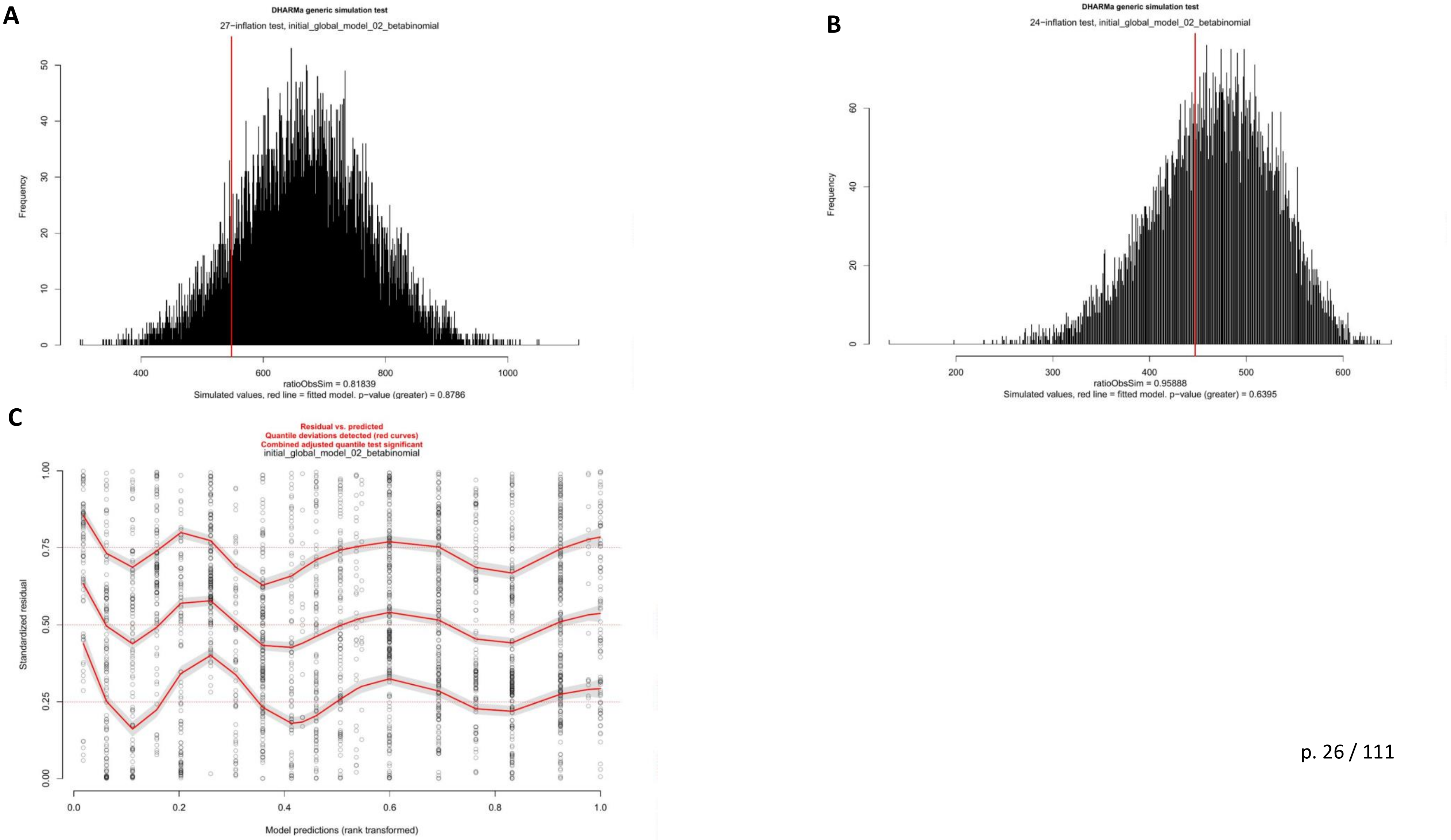


Figure Supp_06 3 Correlations of input variables and multicollinearity. A) Pairwise correlation of all input variables (2309 values per variable) using «Hmisc» and «corrplot» package. Lower triangle indicates Spearman's rank correlation; higher triangle indicates Pearson correlation. Spearman's rank correlation is reported since not all input variables were normally distributed. Lighter colour indicates weaker correlation darker colours stronger correlation. Reddish colours indicate negative correlation, blueish indicate positive correlations. Numbers indicate correlation values. Regardless of the type of chosen correlation statistic, correlation stayed all under 0.6 and are regarded unproblematic in this study. B) shows multicollinearity measured as variance inflation factor (VIF) using the "performance" package. All predictors are below the problematic value of 5. High multicollinearity was detected in predictors "HMax" and "TMax" and moderate multicollinearity in "Days".

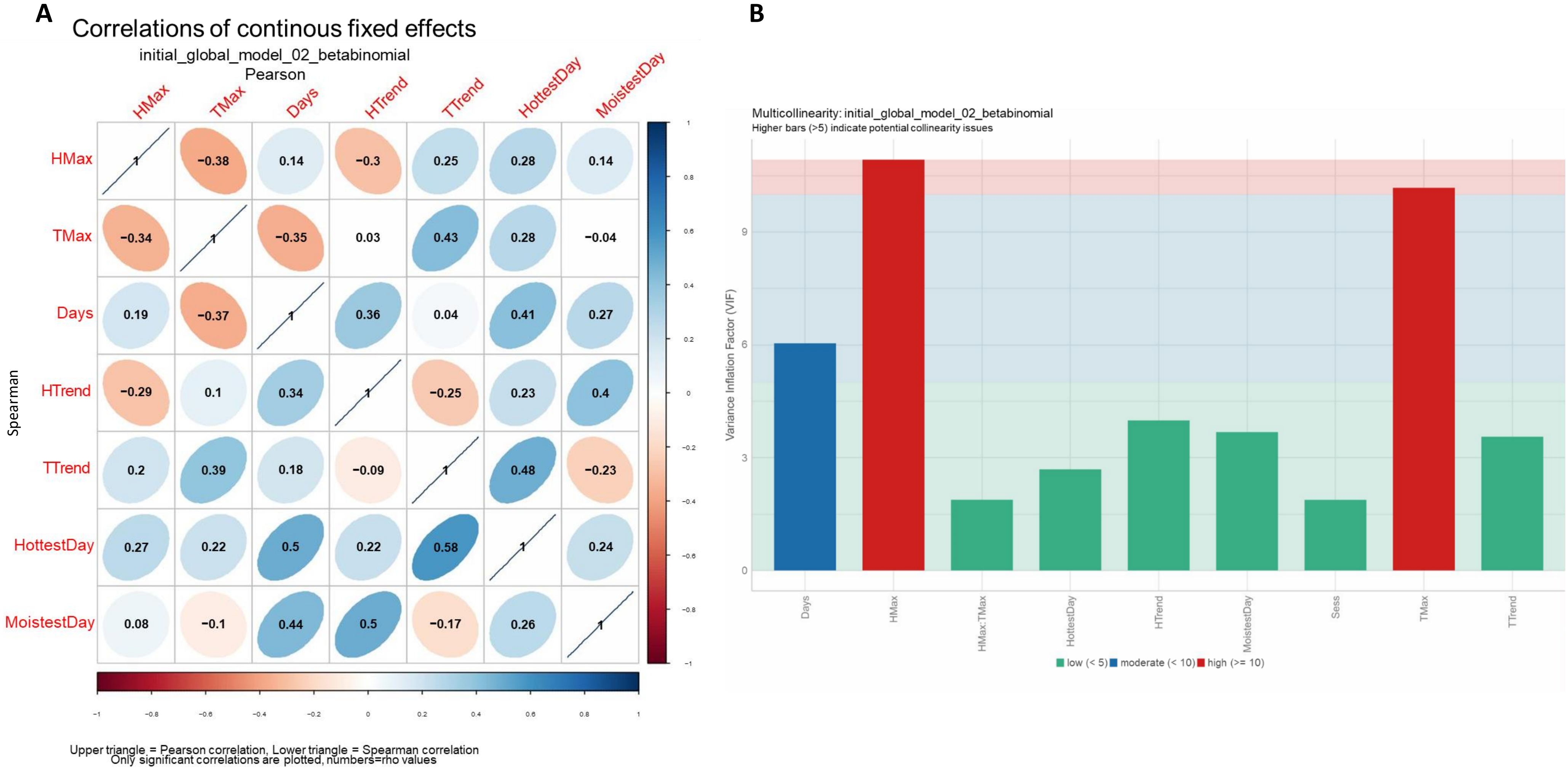


Figure Supp_06 4 Temporal autocorrelation test using "DHARMa" package based on 10 000 simulations using chronological ordered "Year"/"Sess" info as time variable. No significant temporal autocorrelation was detected using the Durbin-Watson test p=0.44.

We also performed a spatial autocorrelation test (Moran's test for distance-based autocorrelation) with those residuals and could not detect significant spatial autocorrelation (p=0.15). The graph with coordinates is not shown because lesser horseshoe bat (*Rhinolophus hipposideros*) is an endangered and protected species in Germany and roosts location cannot be disclosed.

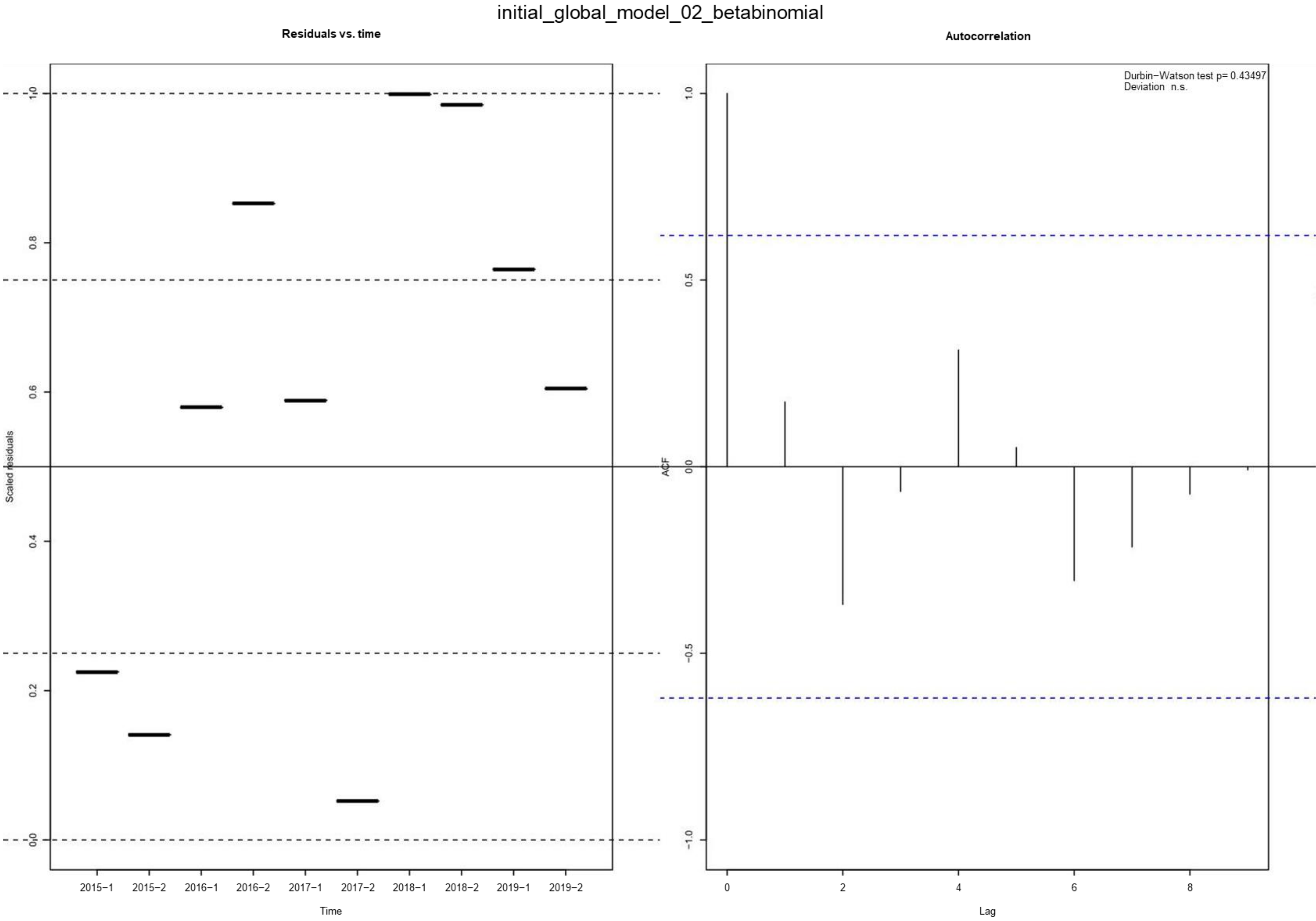


Figure Supp_06 5 Visual check of normality of residuals for random effects using the “performance” and “See” package. No strong deviation was detected.

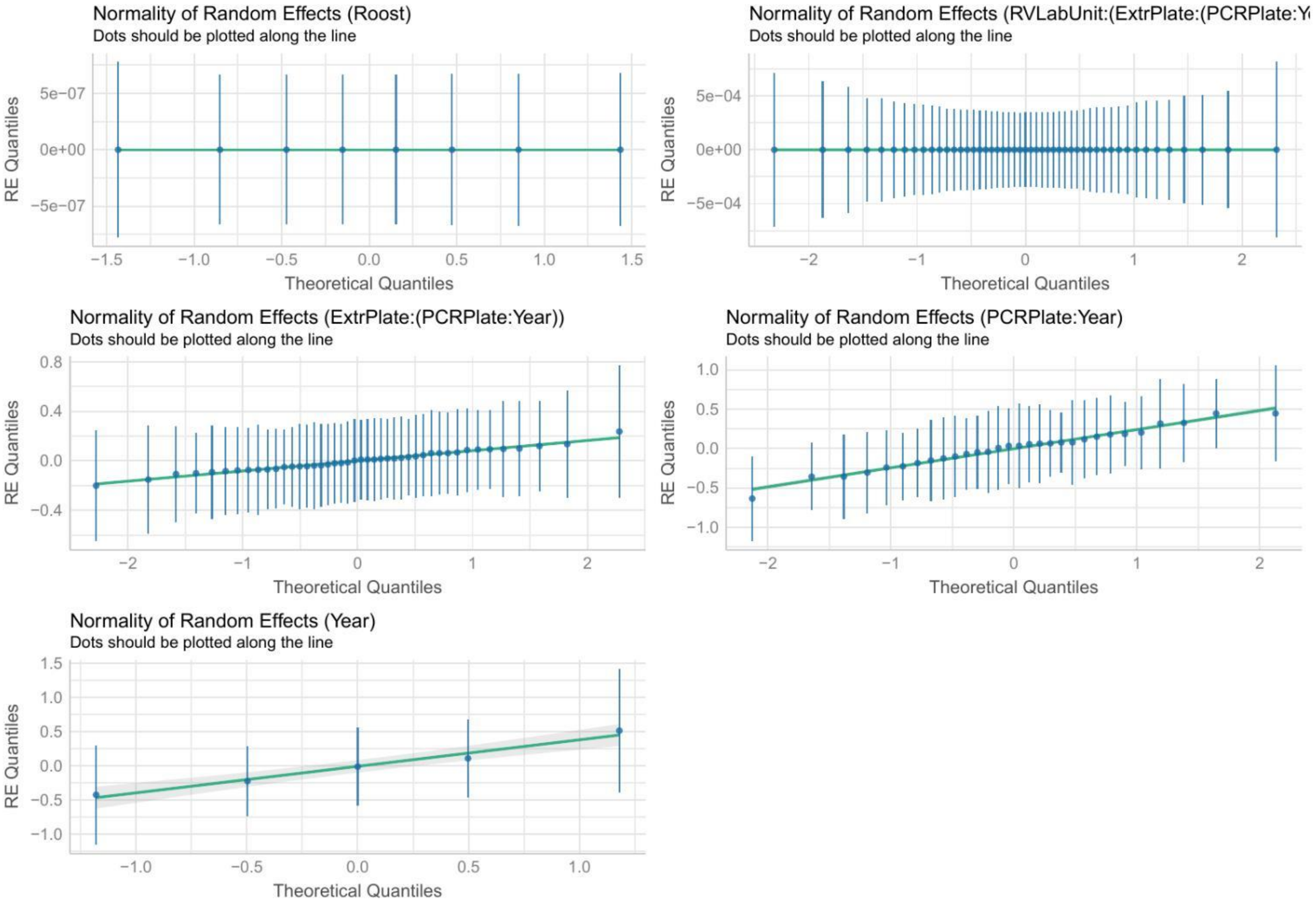
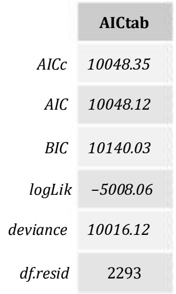


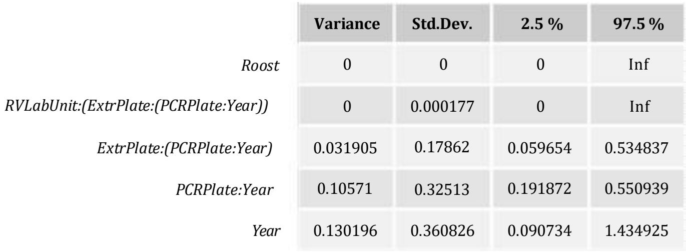
Figure Supp_06 6 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Random effect variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval. C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the "multcomp" package and the "glht" function.

A



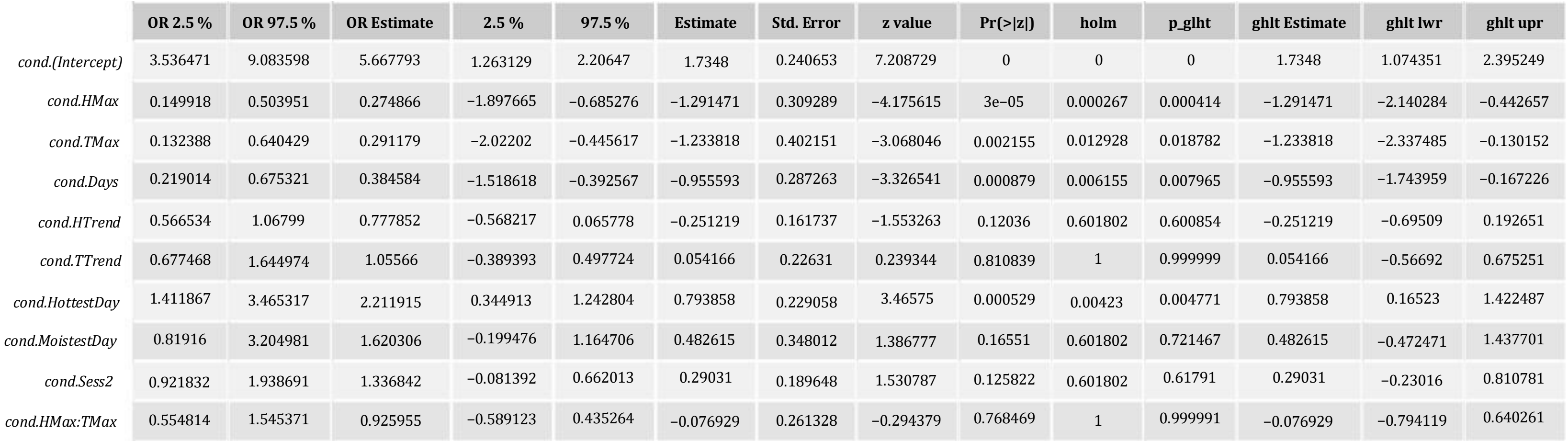
	AICtab
AICc	*10048.35*
AIC	*10048.12*
BIC	*10140.03*
logLik	*−5008.06*
deviance	*10016.12*
df.resid	2293

B



	Variance	**Std.Dev.**	**2.5 %**	**97.5 %**
Roost	0	0	0	Inf
RVLabUnit:(ExtrPlate:(PCRPlate:Year))	0	0.000177	0	Inf
ExtrPlate:(PCRPlate:Year)	0.031905	0.17862	0.059654	0.534837
PCRPlate:Year	0.10571	0.32513	0.191872	0.550939
Year	0.130196	0.360826	0.090734	1.434925

C



	OR 2.5 %	**OR 97.5 %**	**OR Estimate**	**2.5 %**	**97.5 %**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**holm**	**p_glht**	**ghlt Estimate**	**ghlt lwr**	**ghlt upr**
cond.(Intercept)	3.536471	9.083598	5.667793	1.263129	2.20647	1.7348	0.240653	7.208729	0	0	0	1.7348	1.074351	2.395249
cond.HMax	0.149918	0.503951	0.274866	−1.897665	−0.685276	−1.291471	0.309289	−4.175615	3e−05	0.000267	0.000414	−1.291471	−2.140284	−0.442657
cond.TMax	0.132388	0.640429	0.291179	−2.02202	−0.445617	−1.233818	0.402151	−3.068046	0.002155	0.012928	0.018782	−1.233818	−2.337485	−0.130152
cond.Days	0.219014	0.675321	0.384584	−1.518618	−0.392567	−0.955593	0.287263	−3.326541	0.000879	0.006155	0.007965	−0.955593	−1.743959	−0.167226
cond.HTrend	0.566534	1.06799	0.777852	−0.568217	0.065778	−0.251219	0.161737	−1.553263	0.12036	0.601802	0.600854	−0.251219	−0.69509	0.192651
cond.TTrend	0.677468	1.644974	1.05566	−0.389393	0.497724	0.054166	0.22631	0.239344	0.810839	1	0.999999	0.054166	−0.56692	0.675251
cond.HottestDay	1.411867	3.465317	2.211915	0.344913	1.242804	0.793858	0.229058	3.46575	0.000529	0.00423	0.004771	0.793858	0.16523	1.422487
cond.MoistestDay	0.81916	3.204981	1.620306	−0.199476	1.164706	0.482615	0.348012	1.386777	0.16551	0.601802	0.721467	0.482615	−0.472471	1.437701
cond.Sess2	0.921832	1.938691	1.336842	−0.081392	0.662013	0.29031	0.189648	1.530787	0.125822	0.601802	0.61791	0.29031	−0.23016	0.810781
cond.HMax:TMax	0.554814	1.545371	0.925955	−0.589123	0.435264	−0.076929	0.261328	−0.294379	0.768469	1	0.999991	−0.076929	−0.794119	0.640261

Figure Supp_06 7 Graphical representation of model estimates (colored dots) with corresponding value above using the "sjPlot" package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. Model estimates are indicated by the circles and the value above.

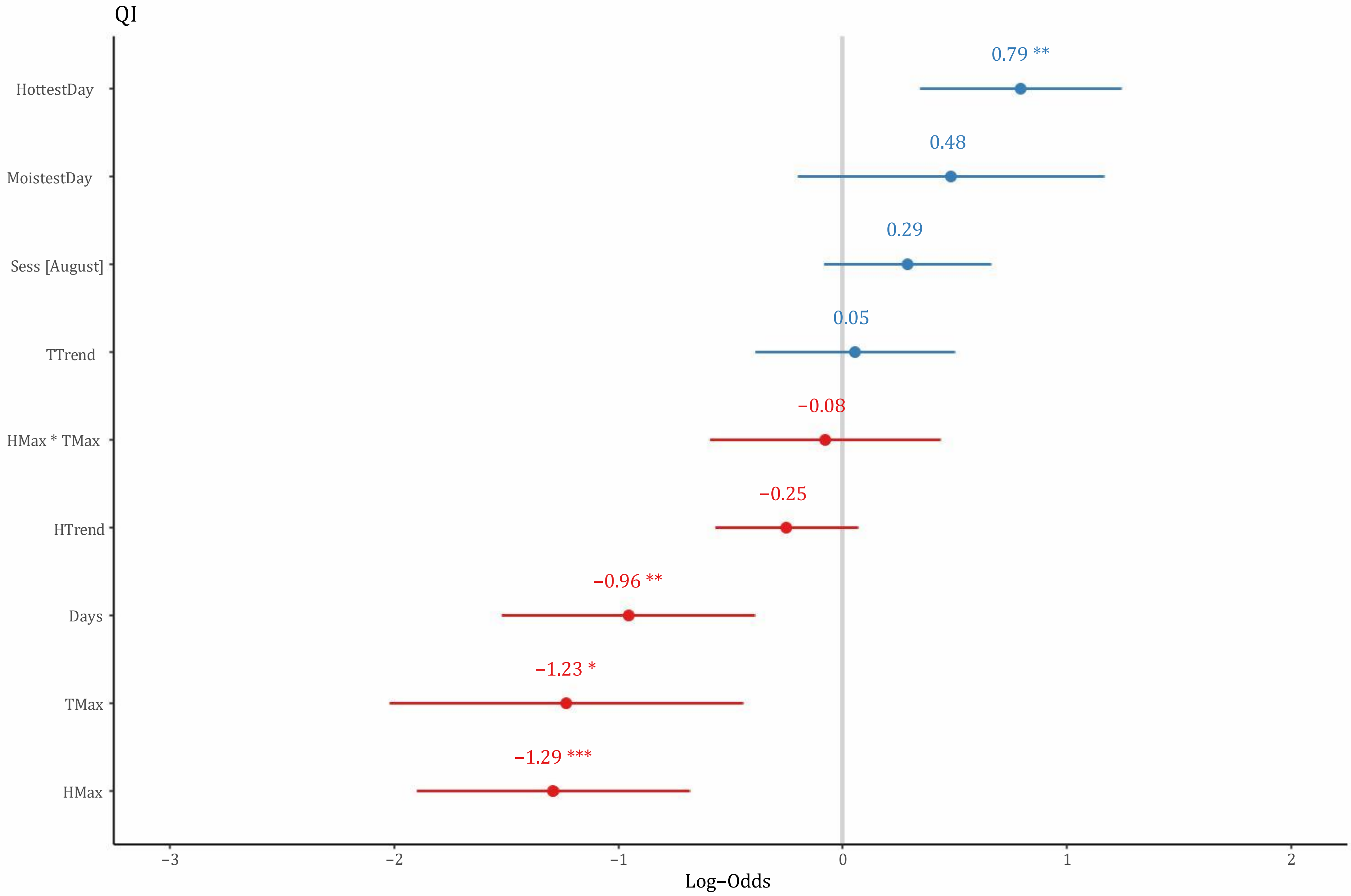
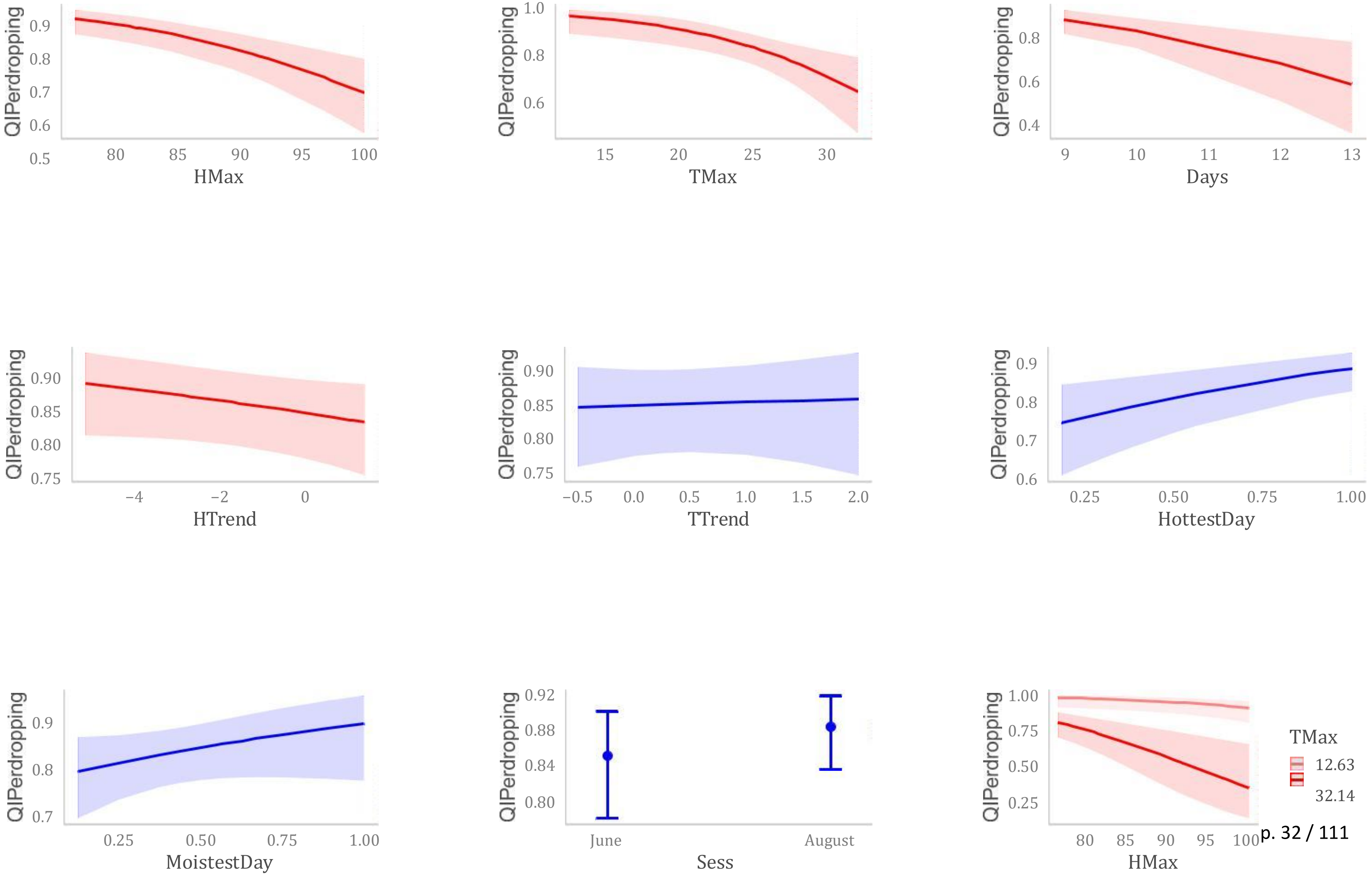


Figure Supp_06 8 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the "ggeffects". 95 % confidence intervals are indicated by the lighter shape. Red indicates negative, blue positive effects. X-axis represent the observed unstandardized predictors. "QIPerdropping" is equal to QI used in the publication and just emphasizes that QI was calculated over the whole sample (across 3 replicates with 9 loci each totaling in 27 loci). For continuous interaction terms the minimum and maximum observed values were used for plotting (e.g. HMax:TMax, bottom right).



Supp_07 Input formulas and variables for the automated correction step

Table Supp_07 1 Potential predictors and their interactions specified in step three of the statistical pipeline to build all possible subsets for the corrective model (zero-inflation and dispersion formula) of the global model. Hereby the model formulas for later calculation where only kept if pairwise correlation between terms was below 0.6 and interactions were accompanied by their main terms resulting in 143 417 possible subset models. The conditional model which was kept unchanged in the process can be seen in the first row. The formula for the corrective model where all possible subsets were built from can be seen in the second row.

Once the models were calculated only those fulfilling basic model assumptions were kept (variance inflation factor (VIF)<5 and no significant (p>0.05 over/under-dispersion or zero-inflation/deflation) ending up in 5892 valid models. Those were then preselected to only contain best fitting models of deltaAICc<6 or deltaBIC<6 ending up in 104 models. From those 104 models the one was selected with lowest mean squared error (MSE) under roost-visit-wise cross-validation. Due to upgrades and compatibility improvements of the pipeline including upgrading the glmmTMB package from version 1.1.2 to 1.1.5 in the process of reviewing this publication the model convergence behaviour slightly changed (see Version_History.txt on Zenodo via Dryad, Naef et al., 2023, https://doi.org/10.5061/dryad.79cnp5hxn). Adding two slightly better potential final corrective models before the old final corrective model. However, they did not always converge. That is why we included an additional convergence stability test to the pipeline for the models with lowest MSE when selecting the final global corrective model to select the most stable lowest MSE model under roost-visit cross validation (We recalculated the top 3 models 100 times and only the 3rd model converged 100% of the time, being identical with the old final corrective model calculated under the older glmmTMB version). This single final corrective model with its final zero-inflation and dispersion formula can be seen in row three and builds together with row one the final global model (table is on the next page). See also **Supp_09** for the final global model.

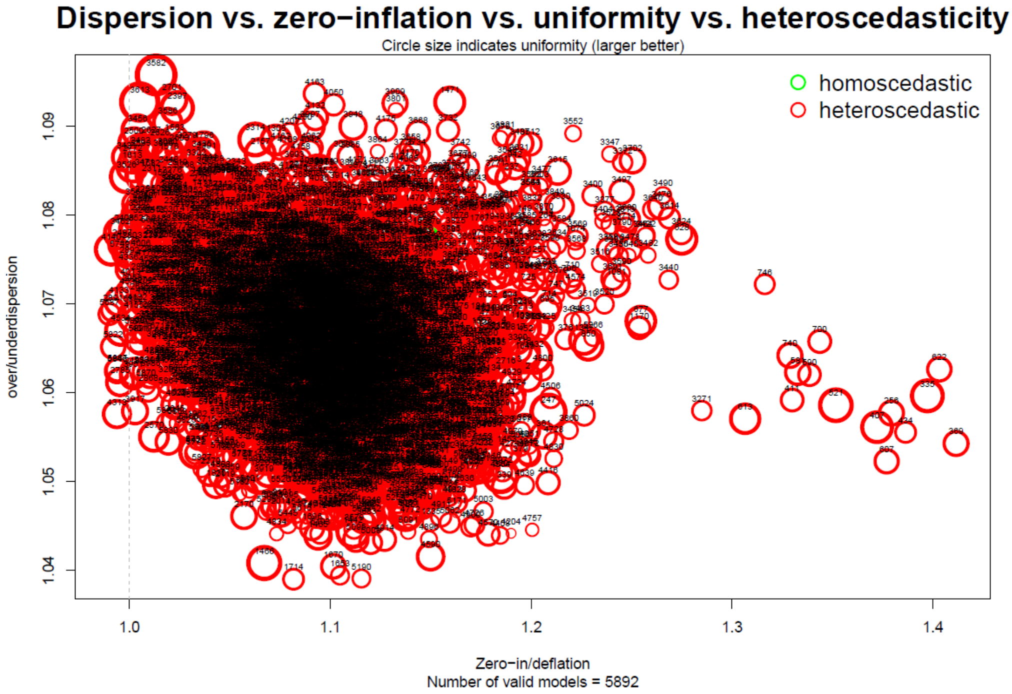
Conditional model (kept unchanged)	**QI ~ HMax * TMax + Days + HottestDay + MoistestDay + TTrend + HTrend + Sess + (1 | Roost) + (1|Year/PCRPlate/ExtrPlate/RVLabUnit)**	
Corrective model formulas to build all subsets	**dispersion formula**	**Zero-inflation formula**
	~ Days*NperRV+ Days:HTrend:HMax:HMin:AbsHTrend + Days:TTrend:TMax:TMin:AbsTTrend + HTrend + HMax + HMin + AbsHTrend + TTrend + TMax + TMin +AbsTTrend	**~ HMin*TMin*AbsHTrend+AbsTTrend+NperRV**
Final corrective model formulas	**~ HMin + TMin + AbsHTrend + AbsTTrend**	**~ HMax + AbsTTrend**

	Variable (Abbrev.)	**Type/**	**Description and [range of unstandardized variable]**	**n**
Predictors used in corrective model formulas to build all subsets	Max. Humidity (**HMax**)	Continuous/Z-scored	Maximum relative humidity **[76.8 – 100 %]**	21 values
	Max. Temperature (**TMax**)	Continuous/Z-scored	Maximum temperature **[12.6 – 32.1 °C]**	21 values
	Temperature trend (**TTrend**)	Continuous/Z-scored	Trend towards warmer (positive) or colder (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. temperatures. **[-0.5 – 2 °C]**	21 values
	Humidity trend (**HTrend**)	Continuous/Z-scored	Trend towards moister (positive) or drier (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. humidity. **[-5.1 – +1.4 %]**	21 values
	Exposure days (**Days**)	Continuous/Z-scored	Duration of single collection period in a roost in days (maximum possible days a dropping could have been exposed to the environment) **[9 – 13]**	21 values
	Min. humidity (**HMin**)	Continuous/Z-scored	Minimum relative humidity **[29.3 – 100 %]**	21 values
	Min. temperature (**TMin**)	Continuous/Z-scored	Minimum temperature **[11.0 – 23.0 °C]**	21 values
	Absolute humidity trend (**AbsHTrend**)	Continuous/Z-scored	Measure of absolute variability in max. humidity between days calculated as median of absolute chronological differences of daily maximum humidity **[0 – 10.9 %]**	21 values
	Absoulte temperature trend (**AbsTTrend**)	Continuous/Z-scored	Measure of absolute variability in max. temperature between days calculated as median of absolute chronological differences of daily maximum humidity **[0 – 2 %]**	21 values
	Number of droppings per roost visit **(NperRV)**	Continuous/Z-scored	Number of droppings genotyped per roost visit used for QI calculations. **[14– 224]**	21 values

Supp_08 Automated corrective model finding selection

Figure Supp_08 1 A) shows the 5892 of 143,417 corrective models fulfilling basic model assumptions (VIF<5, no significant over- or under-dispersion, no significant zero-inflation/deflation) built from the global model. The conditional model was kept unchanged in the process and the all subset models were constructed based on the corrective model structure (Table **Supp_07 1**, second row). The ratio of observed to simulated values using DHARMA (e.g., seen in Figure **Supp_06** 1A, B) based on 250 simulations for zero-in/deflation is shown on the x-axis and for over/under-dispersion on the y axis. The number above the circles indicates model ID (The ID can be higher than 143,417 because we first allowed corrective models with interactions not containing all corresponding main terms in the corrective model. Instead of recalculating we just subset the calculated model pool to only allow interactions if their main terms were included too). B) Shows the same as A but for selected subset of 104 models within either ΔBIC<6 or ΔAICc<6. Heteroscedasticity (red) in both plots is only indicated to investigate potential patterns of homoscedasticity and is defined as significant deviation (p<=0.001) using the quantile regression tests from the "qgam" package within "DHARMa" but was not a selector included in basic model assumptions (VIF<5 and no sign. Over/under dispersion or zero-inflation/deflation) to keep models.

A



B

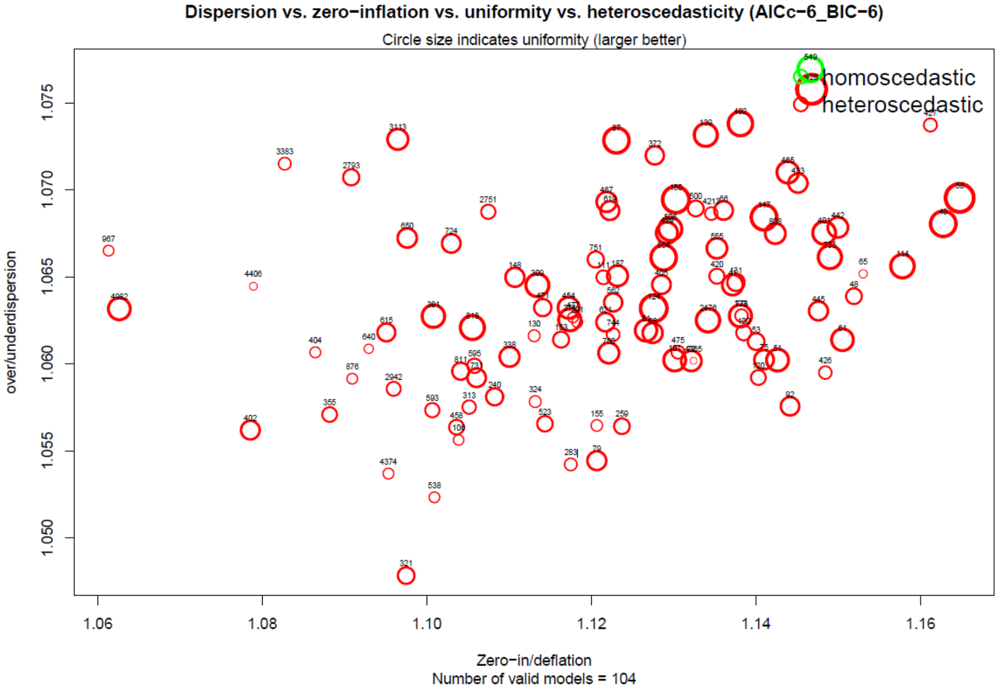
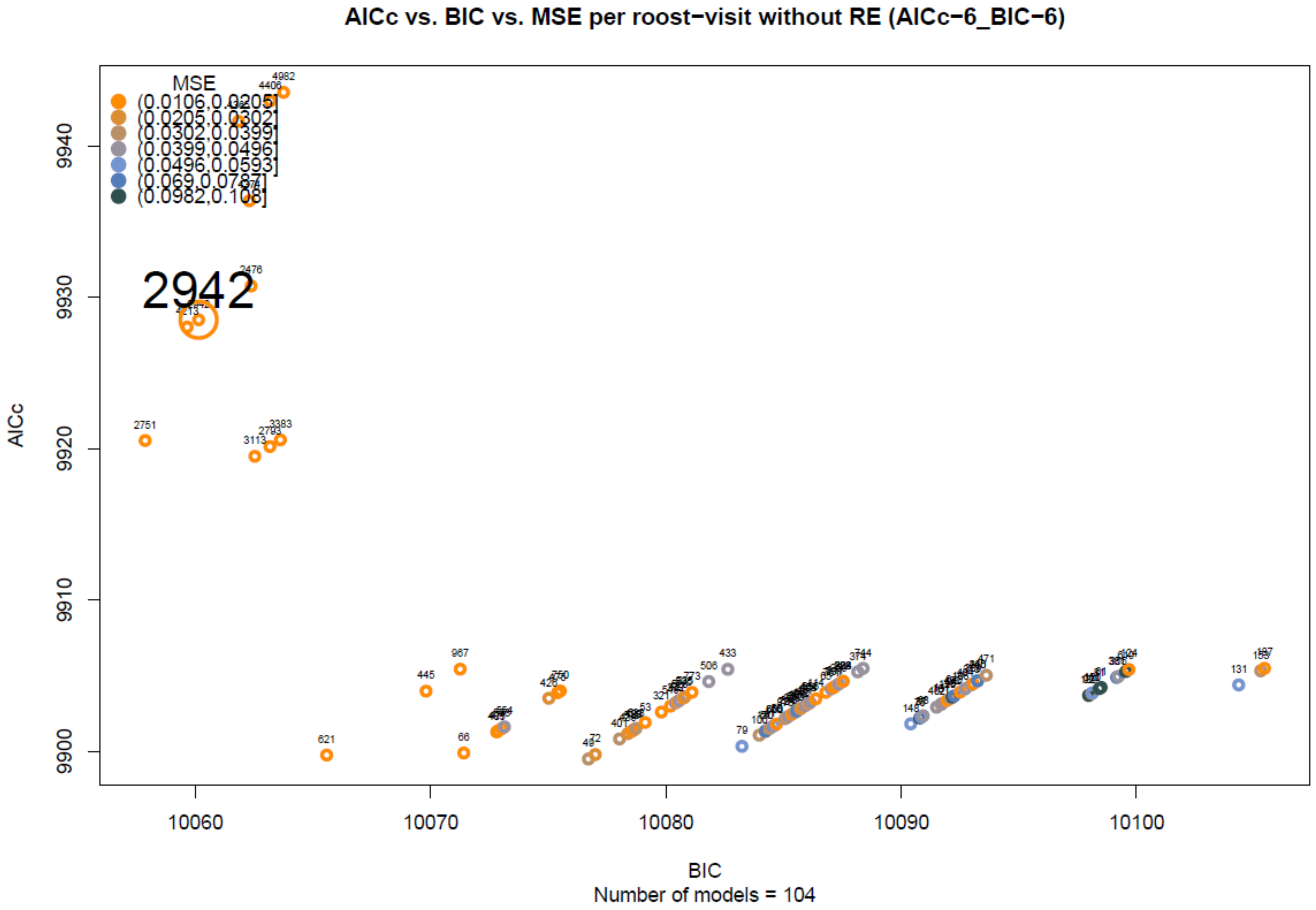


Figure Supp_08 2 Selection of the best fitting corrective model of the 104 models within ΔBIC<6 or ΔAICc<6 using lowest mean squared error (MSE) under roost-visit-wise cross-validation as selection criteria. Circle colour indicates MSE from lowest (orange) to highest (dark blue). The best fitting model is additionally marked by a bigger orange circle and the model number in bold above it and represents the final global model. Note that here BIC seems to be a better choice for model fit without overfitting compared to AICc (orange to blue, dark colours gradient follows BIC from left to right). Note due to "glmmTMB" update to version 1.1.5 the convergence behaviour slightly changed to the previous version 1.1.2. The best model with lowest MSE ("2942") and second best ("4982") here actually did not converge always when recalculating it 100 times instead the third best corrective model was chosen which always converged ("4213", within the bigger orange circle) being identical to the final global model using version 1.1.2 of "glmmTMB".



Supp_09 Information about the final global model (beta-binomial, with corrective model)

Model diagnostics and information for the final global model (step 3 in manuscript) created with the mdiag() function included in the version controlled R-Project. Not all information from the 29-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved.

Model Overview

name: final_global_model

family: betabinomial

link: logit
response variable form: matrix

sample size: 2309
N (sample size) / k (terms) : 256.6

formula: QI~ Days + HMax:TMax + HMax+ HottestDay + HTrend + MoistestDay + Sess + TMax + TTrend + (1 | Roost) + (1 | Year/PCRPlate/ExtrPlate/RVLabUnit)

ziformula: ~AbsHTrend + AbsTTrend + HMin + TMin
dispformula: ~AbsTTrend + HMax

Figure Supp_09 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the final global model based on 10 000 simulations. A) Dispersion test showing no significant overdispersion with the observed (red line) variance being 1.06 times stronger than the mean simulated variance (black lines). B) Zero-inflation test shows that the number of observed zeroes (red line) is not significant with 1.12 times more observed zeroes than simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows no significant outliers (red bars and line). D) Quantile-Quantile plot of observed and expected residuals shows significant but weak deviation of uniform distribution (red line).

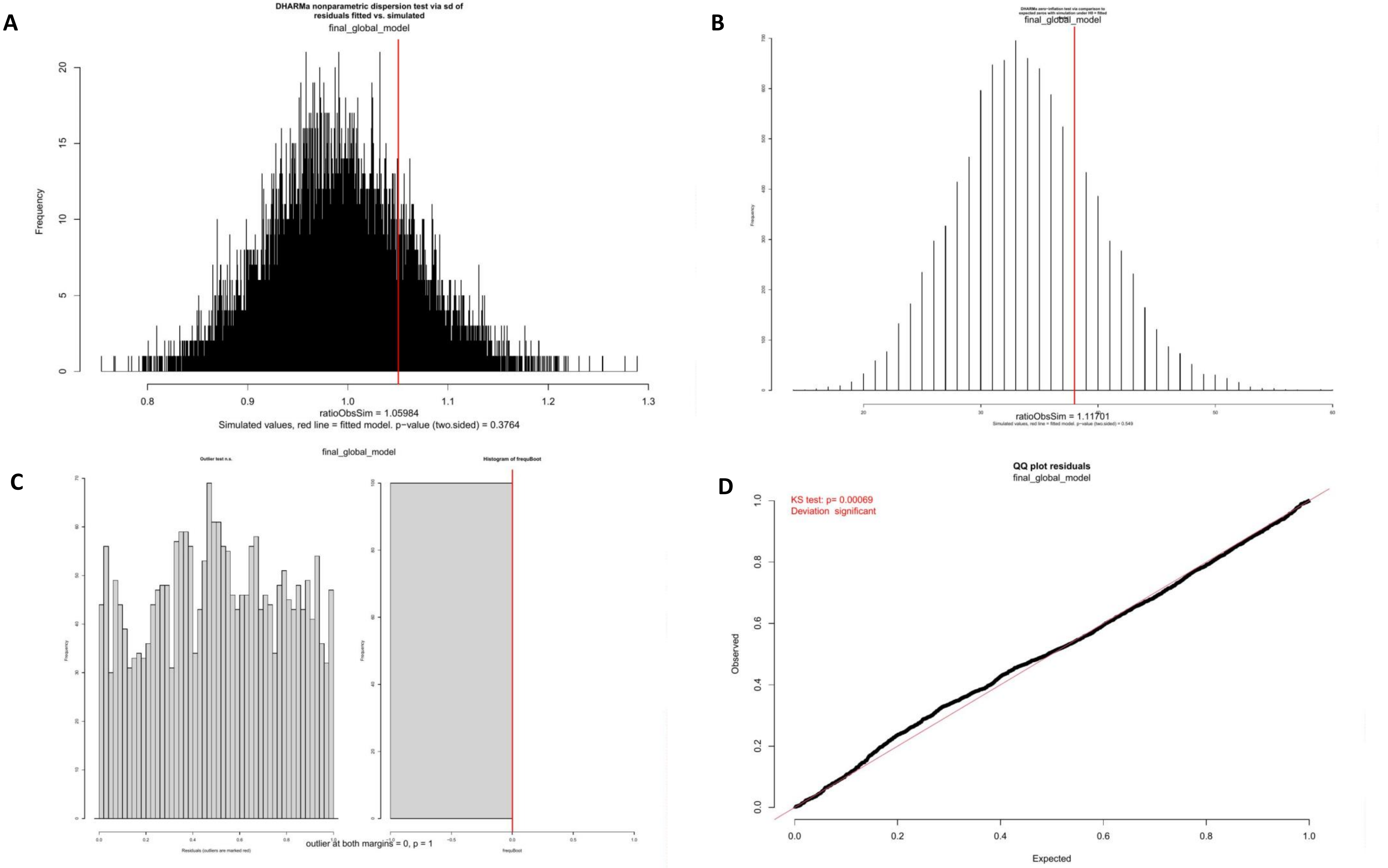


Figure Supp_09 2 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Testing excess/deficits of QI = 1. Since QI was expressed in matrix form (success/(total number of loci) A QI of 1 means in Matrix form 27/27. We did not detect a significant deviation of observed (red line) vs. simulated ones (black lines) with 0.89 times the number of observed ones compared to the mean simulated number of ones (black bars). B) Shows the same as A) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem showing non-significant excess/deficit of ones with 0.95 time less observed ones the mean simulated ones. C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate weak signs of heteroscedasticity but only in the first two quantiles.

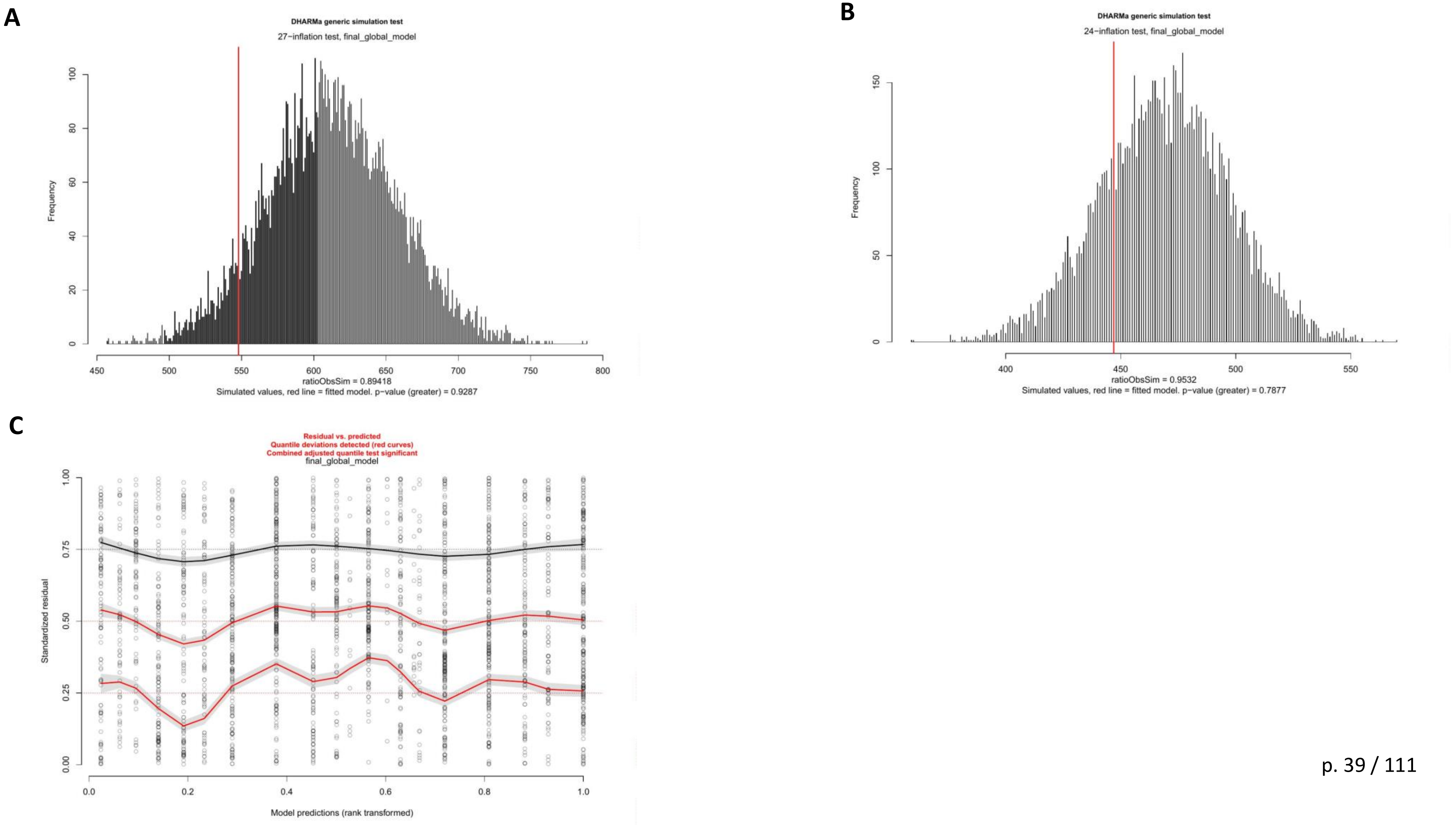


Figure Supp_09 3 Correlations of input variables and multicollinearity. A) Pairwise correlation of all input variables (2309 values per variable) using “Hmisc” and “corrplot” package. Lower triangle indicates Spearman’s rank correlation; higher triangle indicates Pearson correlation. Lighter colour indicates weaker correlation darker colours stronger correlation. Reddish colours indicate negative correlation, blueish indicate positive correlations. Numbers indicate correlations. Correlations under 0.6 are regarded unproblematic in this study. Note that correlations with variables from the corrective model (AbsHTrend, AbsTTrend, HMin, TMin) are also calculated. Correlation containing those predictors were ignored since we were not interested in their estimates but only in modeling zeroes and dispersion to fulfill model assumptions. B) shows multicollinearity measured as variance inflation factor (VIF) using the “performance” package. All VIFs are under five and therefore fulfilling model assumptions

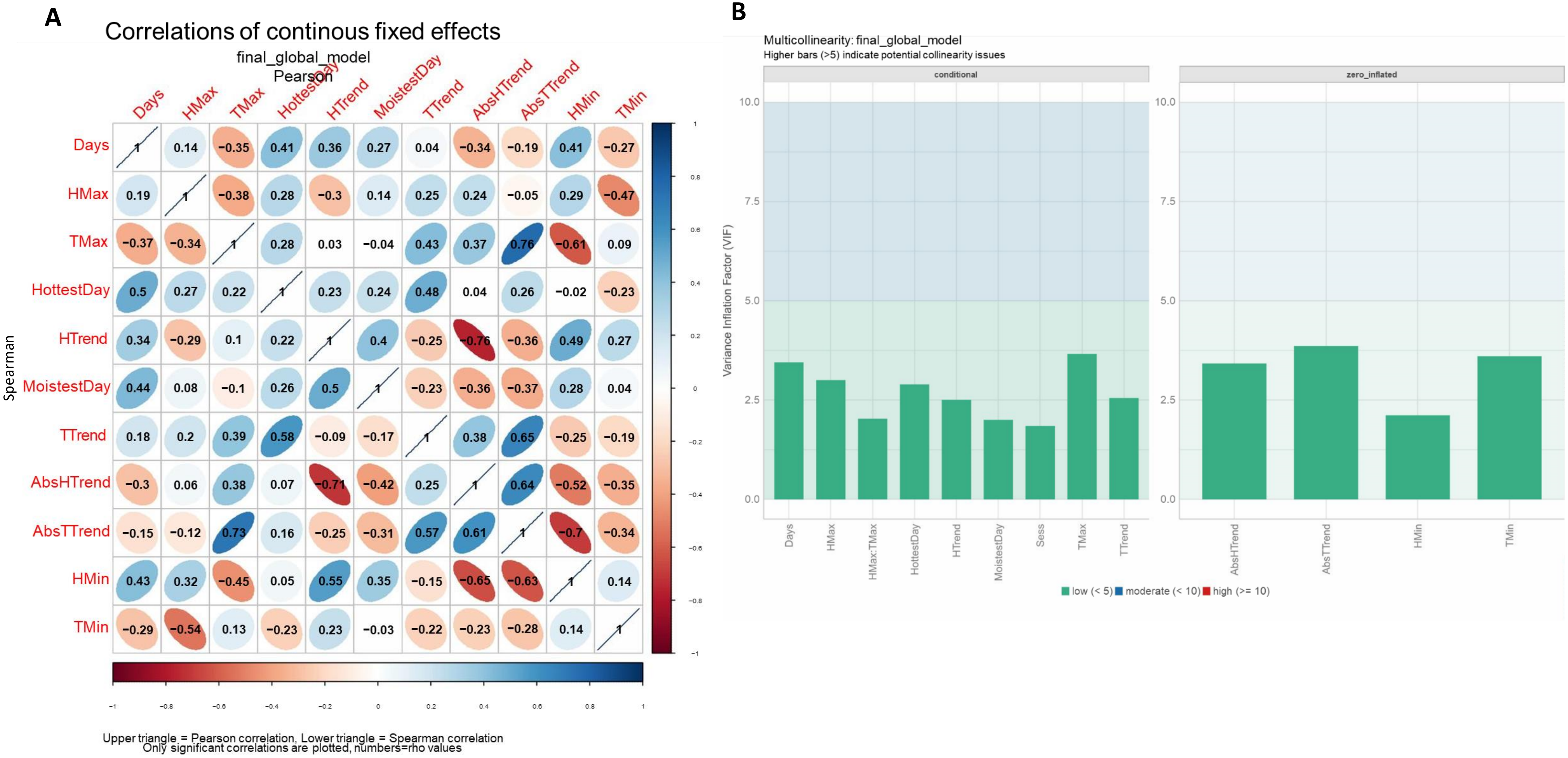


Figure Supp_09 4 Temporal autocorrelation test using DHARMa package based on 10 000 simulations on and sampling timing with year/sess. No significant temporal autocorrelation was detected using the Durbin-Watson test (p=0.84).

We also performed a spatial autocorrelation test (Moran's test for distance-based autocorrelation) with those residuals and could not detect significant spatial autocorrelation (p=0.20). The graph with coordinates is not shown because lesser horseshoe bat (*Rhinolophus hipposideros*) is an endangered and protected species in Germany and roosts location cannot be disclosed.

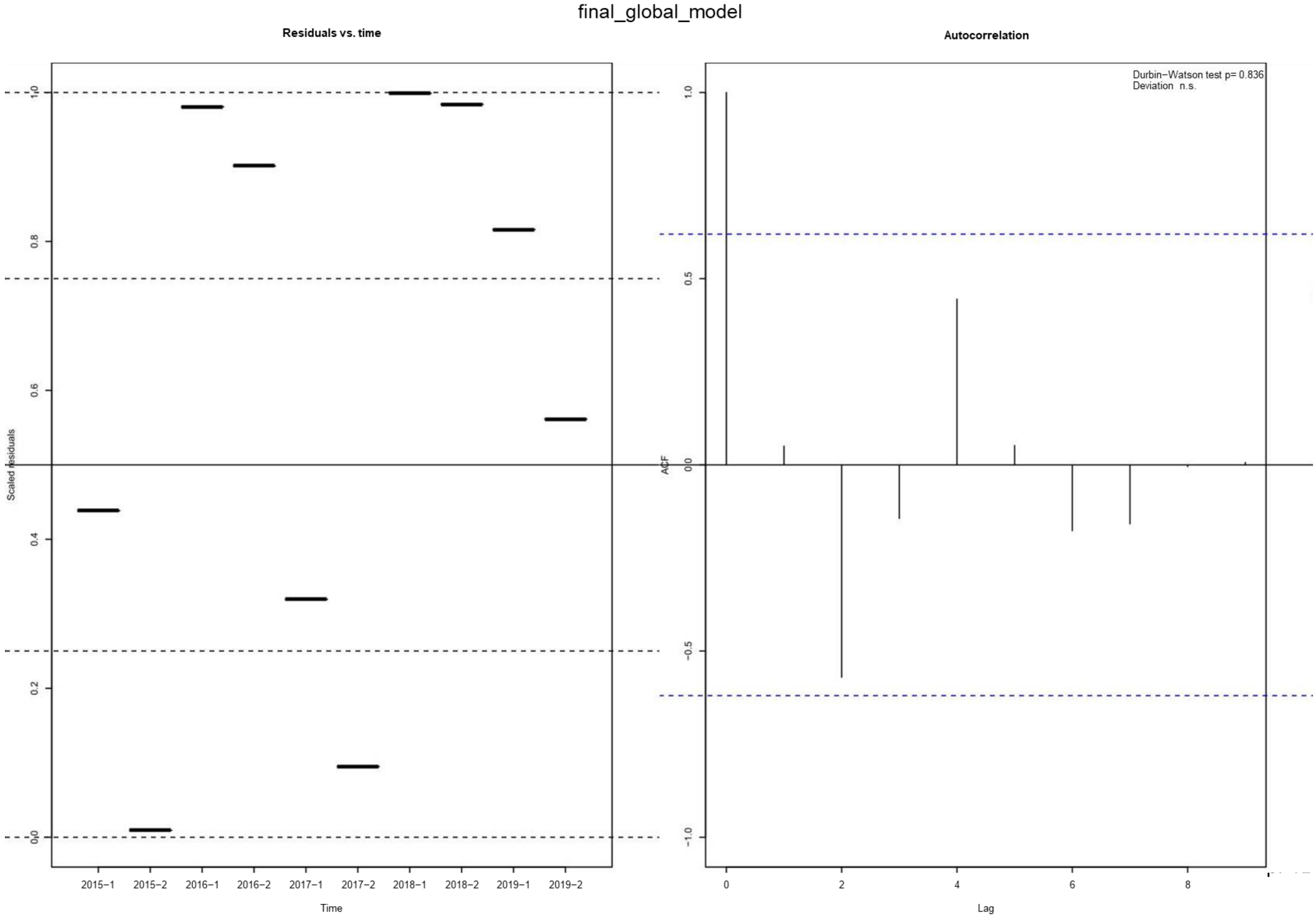


Figure Supp_09 5 Normality of Random Effects using the “performance” and “See” package. No strong deviation was detected.

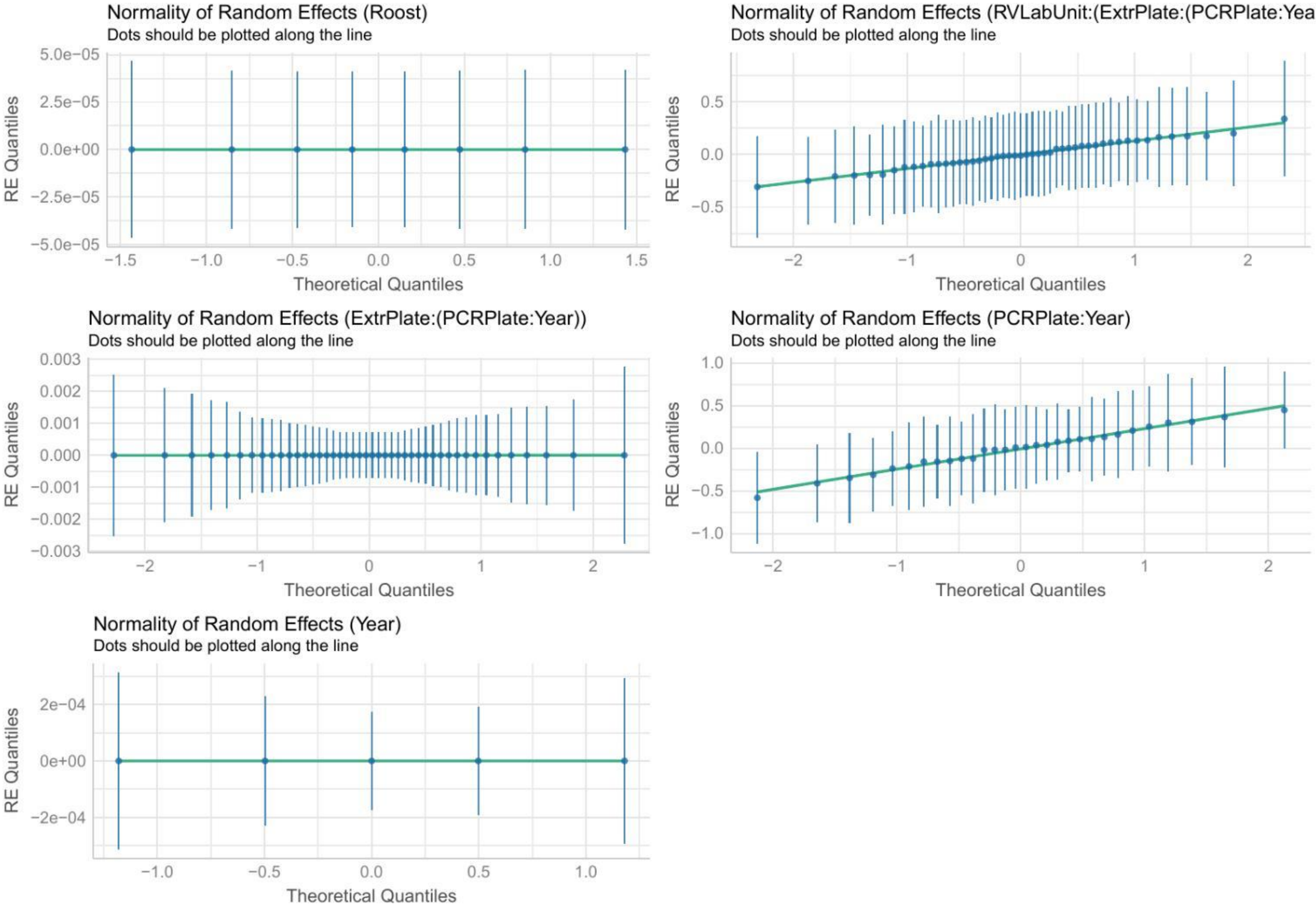
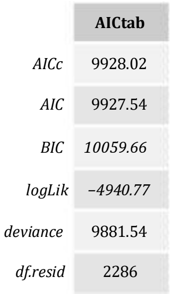


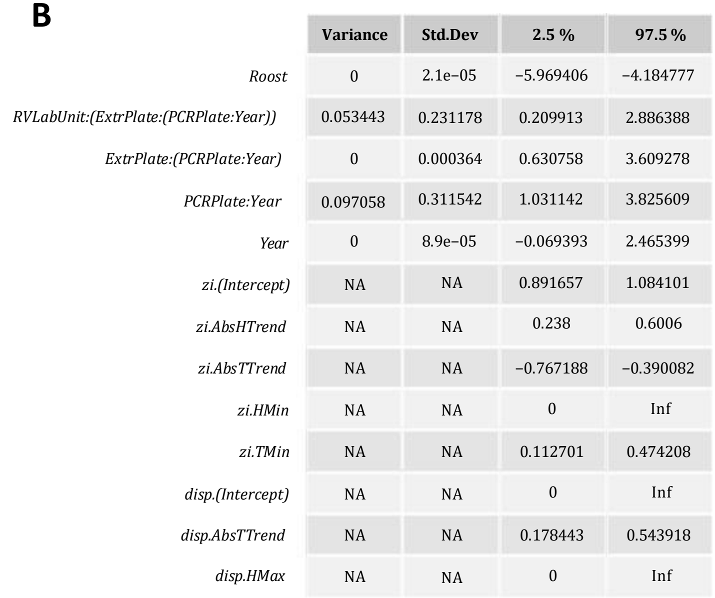
Figure Supp_09 6 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval for the random effects and corrective model (where possible). C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the “multcomp” package and the “glht” function.

A



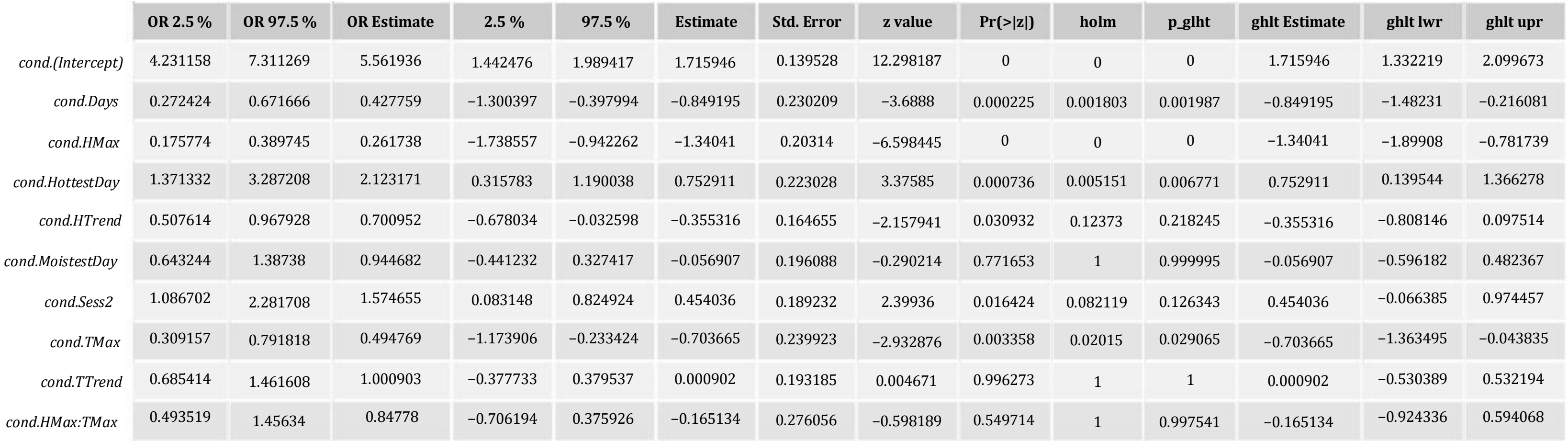
	AICtab
AICc	9928.02
AIC	9927.54
BIC	10059.66
logLik	−4940.77
deviance	9881.54
df.resid	2286

B



	Variance	Std.Dev	2.5 %	97.5 %
Roost	0	2.1e−05	−5.969406	−4.184777
RVLabUnit:(ExtrPlate:(PCRPlate:Year))	0.053443	0.231178	0.209913	2.886388
ExtrPlate:(PCRPlate:Year)	0	0.000364	0.630758	3.609278
PCRPlate:Year	0.097058	0.311542	1.031142	3.825609
Year	0	8.9e−05	−0.069393	2.465399
zi.(Intercept)	NA	NA	0.891657	1.084101
zi.AbsHTrend	NA	NA	0.238	0.6006
zi.AbsTTrend	NA	NA	−0.767188	−0.390082
zi.HMin	NA	NA	0	Inf
zi.TMin	NA	NA	0.112701	0.474208
disp.(Intercept)	NA	NA	0	Inf
disp.AbsTTrend	NA	NA	0.178443	0.543918
disp.HMax	NA	NA	0	Inf

C



	OR 2.5 %	OR 97.5 %	OR Estimate	2.5 %	97.5 %	Estimate	Std. Error	z value	Pr(>|z|)	holm	p_glht	ghlt Estimate	ghlt lwr	ghlt upr
cond.(Intercept)	4.231158	7.311269	5.561936	1.442476	1.989417	1.715946	0.139528	12.298187	0	0	0	1.715946	1.332219	2.099673
cond.Days	0.272424	0.671666	0.427759	−1.300397	−0.397994	−0.849195	0.230209	−3.6888	0.000225	0.001803	0.001987	−0.849195	−1.48231	−0.216081
cond.HMax	0.175774	0.389745	0.261738	−1.738557	−0.942262	−1.34041	0.20314	−6.598445	0	0	0	−1.34041	−1.89908	−0.781739
cond.HottestDay	1.371332	3.287208	2.123171	0.315783	1.190038	0.752911	0.223028	3.37585	0.000736	0.005151	0.006771	0.752911	0.139544	1.366278
cond.HTrend	0.507614	0.967928	0.700952	−0.678034	−0.032598	−0.355316	0.164655	−2.157941	0.030932	0.12373	0.218245	−0.355316	−0.808146	0.097514
cond.MoistestDay	0.643244	1.38738	0.944682	−0.441232	0.327417	−0.056907	0.196088	−0.290214	0.771653	1	0.999995	−0.056907	−0.596182	0.482367
cond.Sess2	1.086702	2.281708	1.574655	0.083148	0.824924	0.454036	0.189232	2.39936	0.016424	0.082119	0.126343	0.454036	−0.066385	0.974457
cond.TMax	0.309157	0.791818	0.494769	−1.173906	−0.233424	−0.703665	0.239923	−2.932876	0.003358	0.02015	0.029065	−0.703665	−1.363495	−0.043835
cond.TTrend	0.685414	1.461608	1.000903	−0.377733	0.379537	0.000902	0.193185	0.004671	0.996273	1	1	0.000902	−0.530389	0.532194
cond.HMax:TMax	0.493519	1.45634	0.84778	−0.706194	0.375926	−0.165134	0.276056	−0.598189	0.549714	1	0.997541	−0.165134	−0.924336	0.594068

Figure Supp_09 7 Graphical representation of model estimates (coloured dots) with corresponding value above using the “sjPlot” package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. The corrective model estimates are also included (dispersion, zero_inflated) but might not be reliable since we detected pairwise correlation above 0.6 in them (Figure Supp_09 3A). Since the goal of the corrective model was to correct model assumption violations by correctly model zeroes and dispersion, the interpretation of the corrective model estimates is therefore neglected in the study.

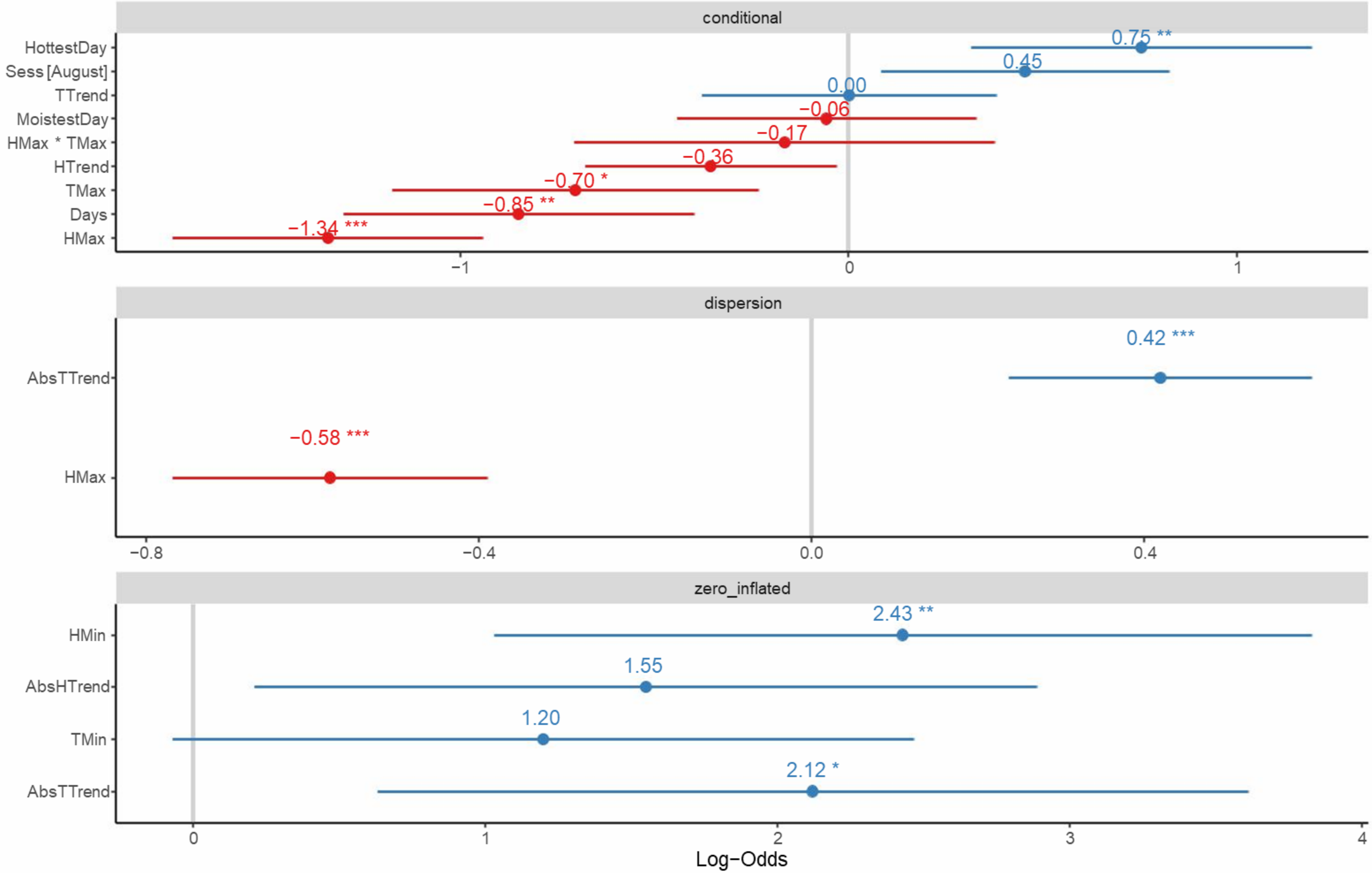
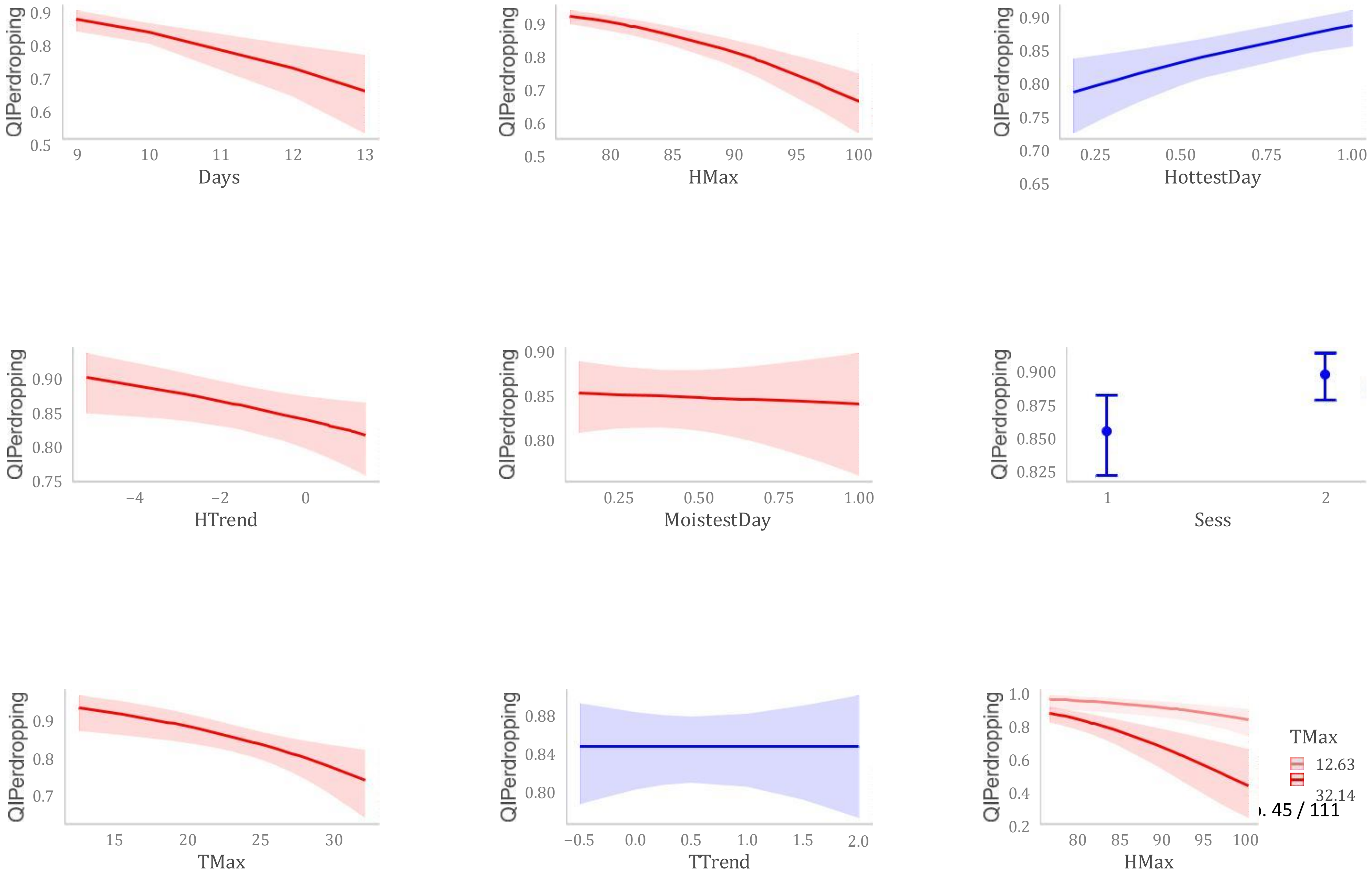


Figure Supp_09 8 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the “ggeffects”. 95 % confidence intervals are indicated by the lighter shape. Red indicates negative, blue positive effects. X-axis represent the observed unstandardized predictors. “QIPerdropping” is equal to QI used in the publication and just emphasizes that QI was calculated over the whole sample (across 3 replicates with 9 loci each totaling in 27 loci). For continuous interaction terms the minimum and maximum observed values were used for plotting (e.g.: HMax:TMax, bottom right).



Supp_10 Top model and best predictive model selection

Figure Supp_10 1 A) shows the 283 of 321 models converged and fulfilling basic model assumptions (VIF<5, no significant over- or under-dispersion, no significant zero-inflation/deflation) built from all possible subsets of the final global model. The corrective model structure (dispersion and zero-inflation model structure) was maintained during the process. The ratio of observed to simulated values using DHARMA (as seen in Figure **Supp_09 1**A, B) based on 250 simulations for zero-in/deflation is shown on the x-axis and for over/under-dispersion on the y axis. B) Shows the same as A but for the 19 top models within ΔAICc<6. Heteroscedasticity (red) in both plots is only indicated to investigate potential patterns of homoscedasticity and is defined as significant deviation (p<=0.001) of the quantile regression tests from the "qgam" package within "DHARMa" but was not a selector included in basic model assumptions (VIF<5 and no sign. Over/under dispersion or zero-inflation/deflation) to keep models.

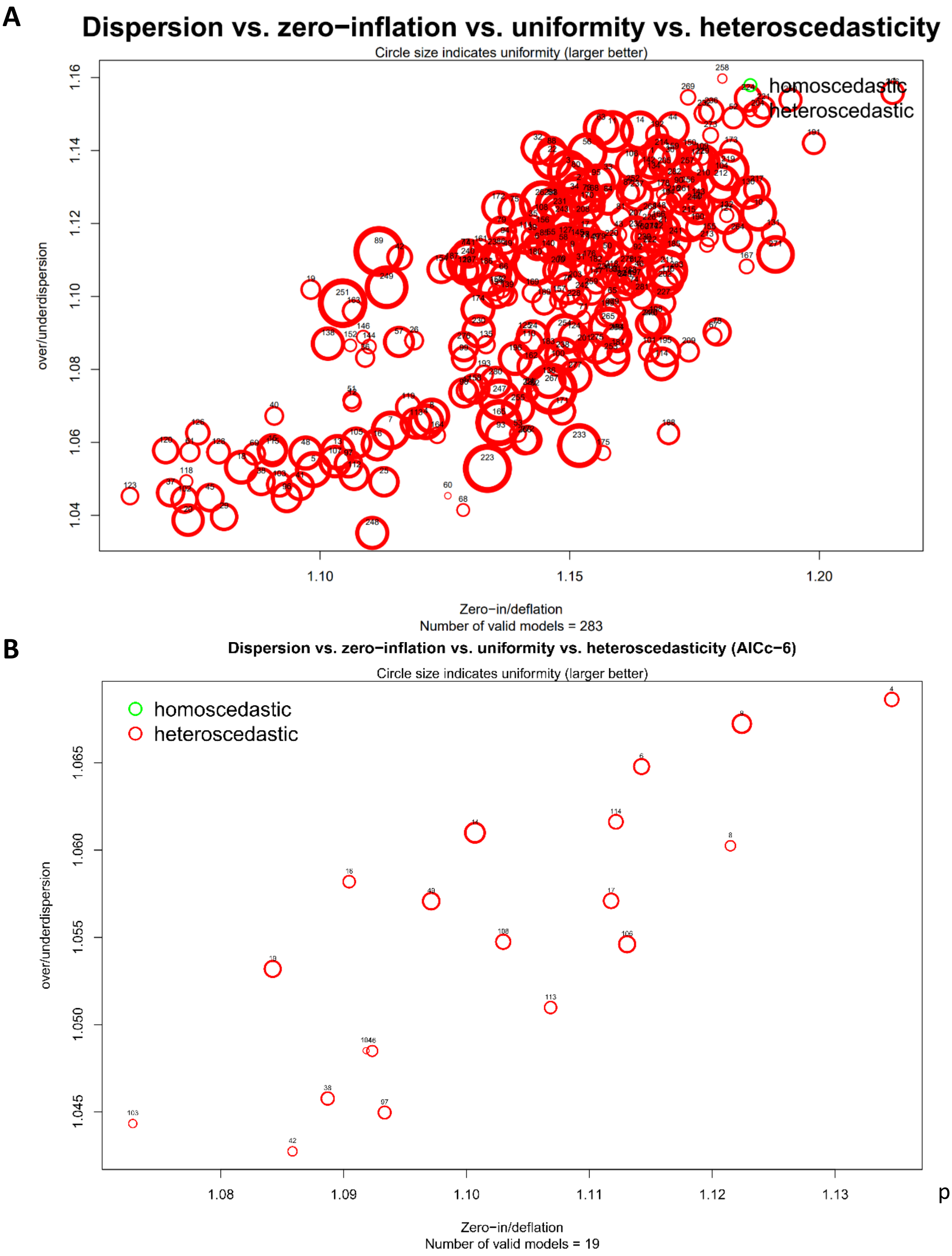
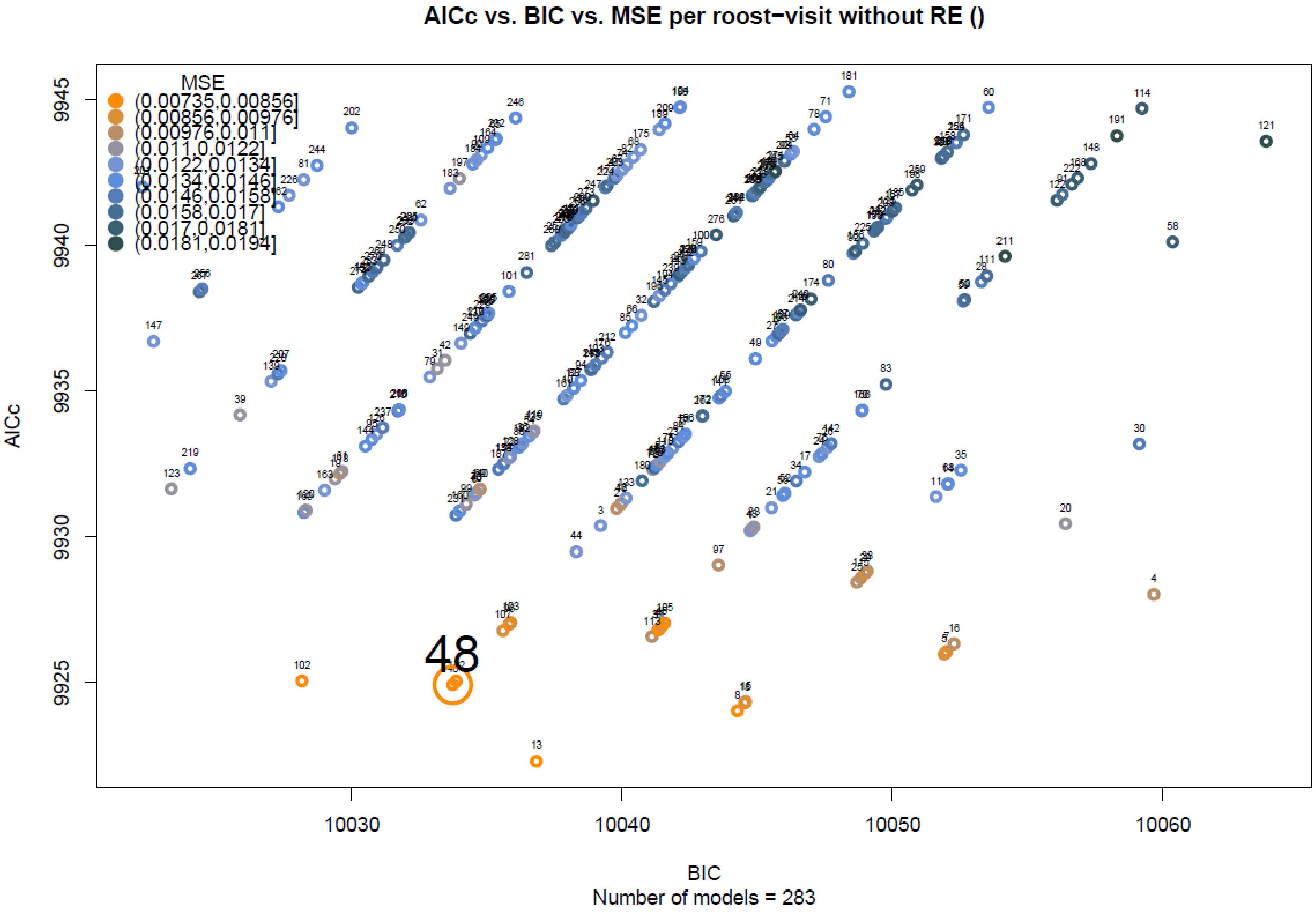


Figure Supp_10 2 Selection of the best predictive models from all 283 subset models fulfilling basic model assumptions (VIF<5, no significant over- or under-dispersion, no significant zero-inflation/deflation) using lowest mean squared error (MSE) under roost-visit-wise cross-validation as selection criteria. Circle colour indicates MSE from lowest (orange) to highest (dark blue). The best fitting model is additionally marked by a bigger orange circle and the model number in bold above it and represents the best predictive model. Note that here AICc seems to be a better choice for model fit without overfitting compared to BIC (orange to blue, dark colours gradient follows AICc from left to right).



Supp_11 QI and PCR success rate overview

Figure Supp_11 1 Overview of QI values per sample and its corresponding PCR success rate over model building and test dataset combined with a total of 2616 samples. Blue shapes are beanplots and indicate the distribution of PCR Success Rate for every discrete QI value. The beanplots are cut at there minimum and maximum values to show the true range of PCR success rate for each sample QI value. Note how the PCR success rate always exceeds its corresponding QI value . The distance of the mean PCR success rate to its corresponding QI value indicated as triangle changes with its corresponding QI value. The distance of mean PCR success rate and corresponding QI value therefore indicates the share of PCR Success rate on the QI value. The closer they are to each other the higher the share of PCR amplification failure on QI and the lower the share of genotype errors measured as disagreement between replicates and consensus genotypes. A QI of one can only be achieved when all loci amplified therefore QI and PCR success rate are identical here. Sample numbers for each QI are depicted as barplot.

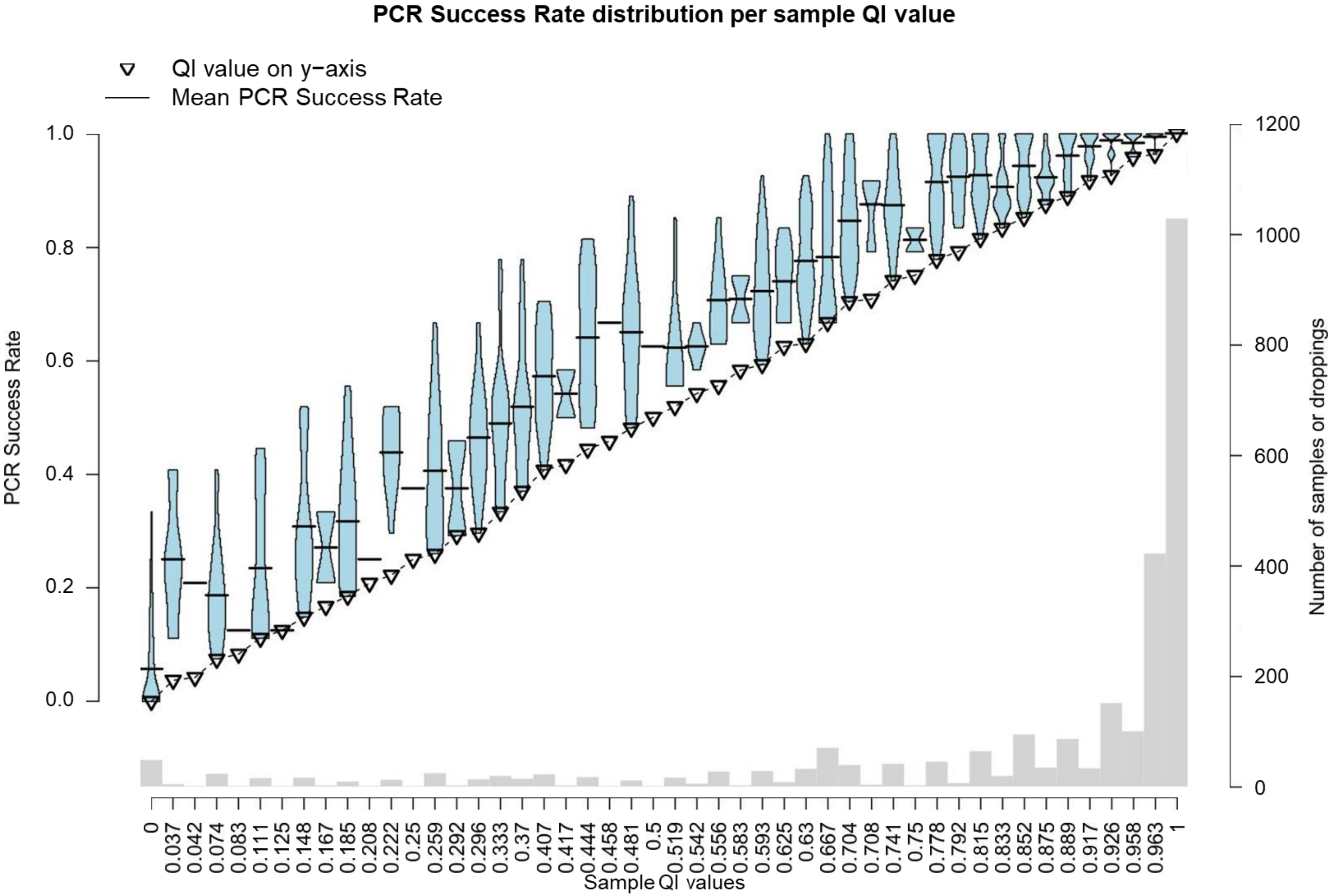
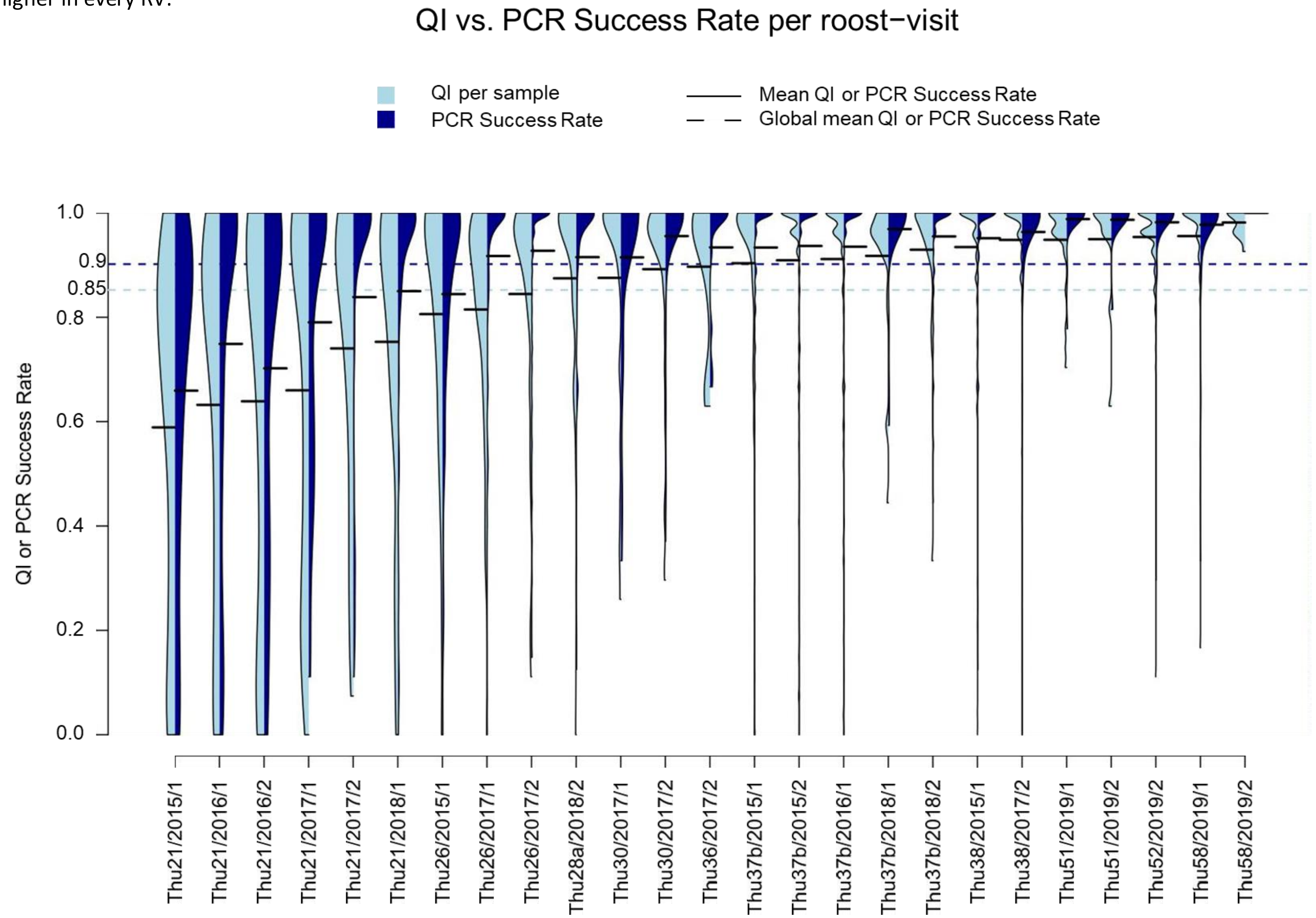
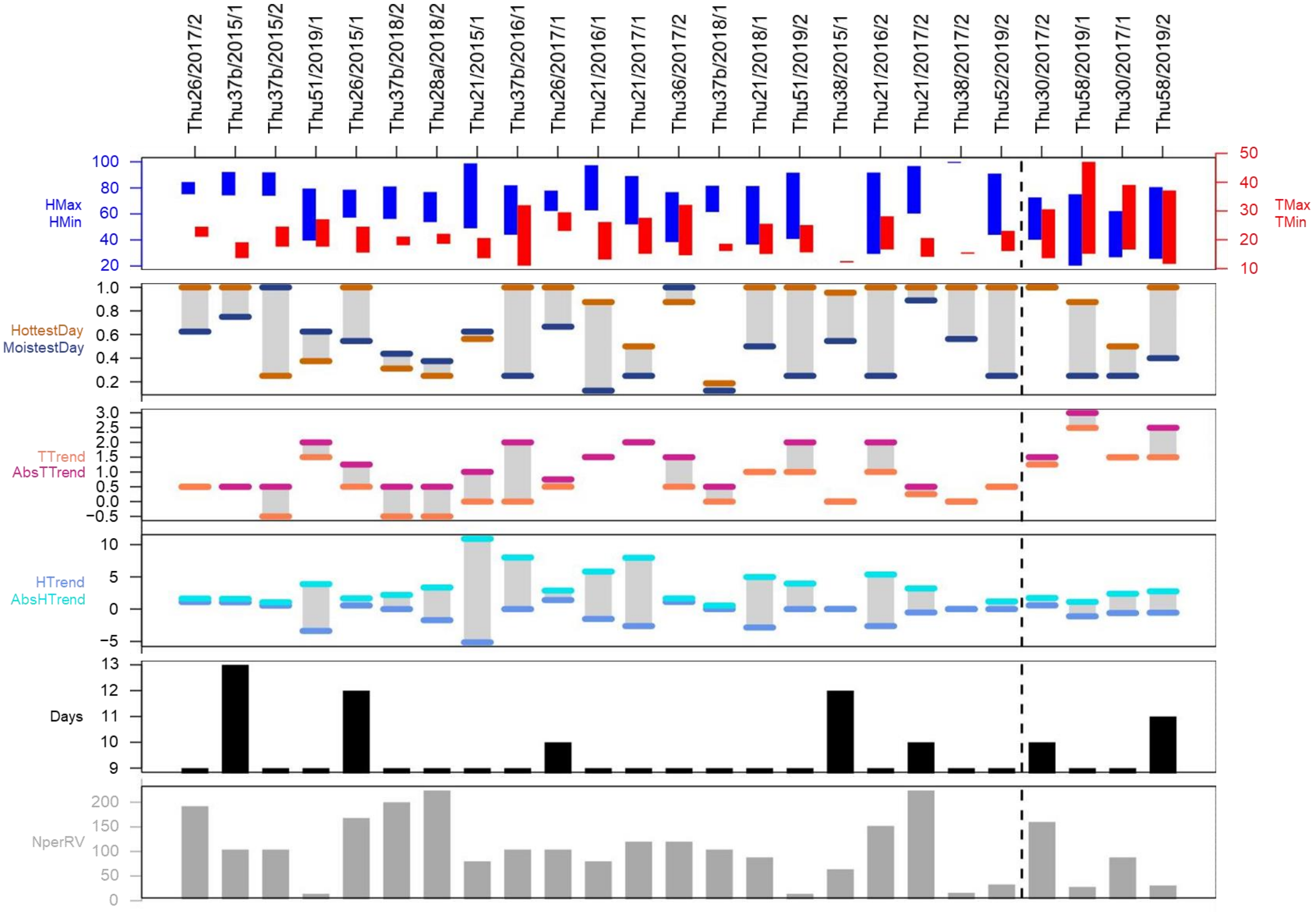


Figure Supp_11 2 Overview of QI values per sample and its corresponding PCR success rate per roost- visit (RV). Model building and test data are combined with a total of 2616 samples distributed over the 25 RV`s. Blue shapes are beanplots and indicate the distribution of QI or PCR success rate per sample. The beanplots are cut at there minimum and maximum values to show the true range for each RV. The average PCR success rate of 0.9 is higher than the average QI value of 0.85 and also higher in every RV.



Supp_12 Environmental overview

Figure Supp_12 1 Overview over all involved unstandardized predictor variables for modelling eoDNA degradation as reduction in QI per sample per roost-visit (RV) following the modelling pipeline of the publication. The dashed black line separates model building data on the left and test data on the right. The RVs are ordered in increasing order according to their mean observed QI as seen in Figure 4 of the publication. See **Supp_07** and Table 2 in the manuscript for the description of all predictors.



Supp_13 Multi-model effect estimates

Figure Supp_13 1 A) Individual effect estimates (darkest shapes and black ticks) and 95% confidence intervals (CI) (lighter shapes and ticks), and their corrected CI for family-wise multiple testing (lightest shapes and ticks) using the “glht” function of the “multcomp” package of the 19 top models (ΔAICc<6). Effect estimates are sorted from negative (red) to positive (blue) by their AICc-weighted average effect estimates (plus symbol, value above). The triangle indicates weighted average estimates according to MSE weights. Circles, solid, and dotted horizontal lines indicate the estimates, CIs, and corrected CIs of the final global model also contained within ΔAICc<6. Best MSE weights (asterisk) corresponds to the best predictive model and is the model with lowest mean squared error (MSE) under roost-visit-wise cross-validation without using random effects variance for predictions. The diamond indicates the model estimates with the lowest AICc. Effect estimates are directly comparable within and between models due to standardized input variables (2 SDs) and their predictors (partial SD) in A. B-C) show corresponding effect estimates and 95% CI according to the dispersion model and the zero-inflation model. Correction for CI’s of the corrective models could not be calculated and that not partial standardization could be performed on the dispersion estimates. D-F) shows the same as A-C but coefficients are not standardized by their partial SD (input variables are still standardized by 2 SDs) to account for differing levels of multicollinearity between the single models depending on the included predictors. Because pairwise correlations of input variables in the corrective model exceeded the absolute value of 0.6 (Figure **Supp_09** 3A, the four bottom right variables) we didn`t interpret those corrective estimates (B,C,E,F) in the study and only used them to model zeroes and dispersion. However, these estimates and their CIs seem to be quite stable over all 19 models.

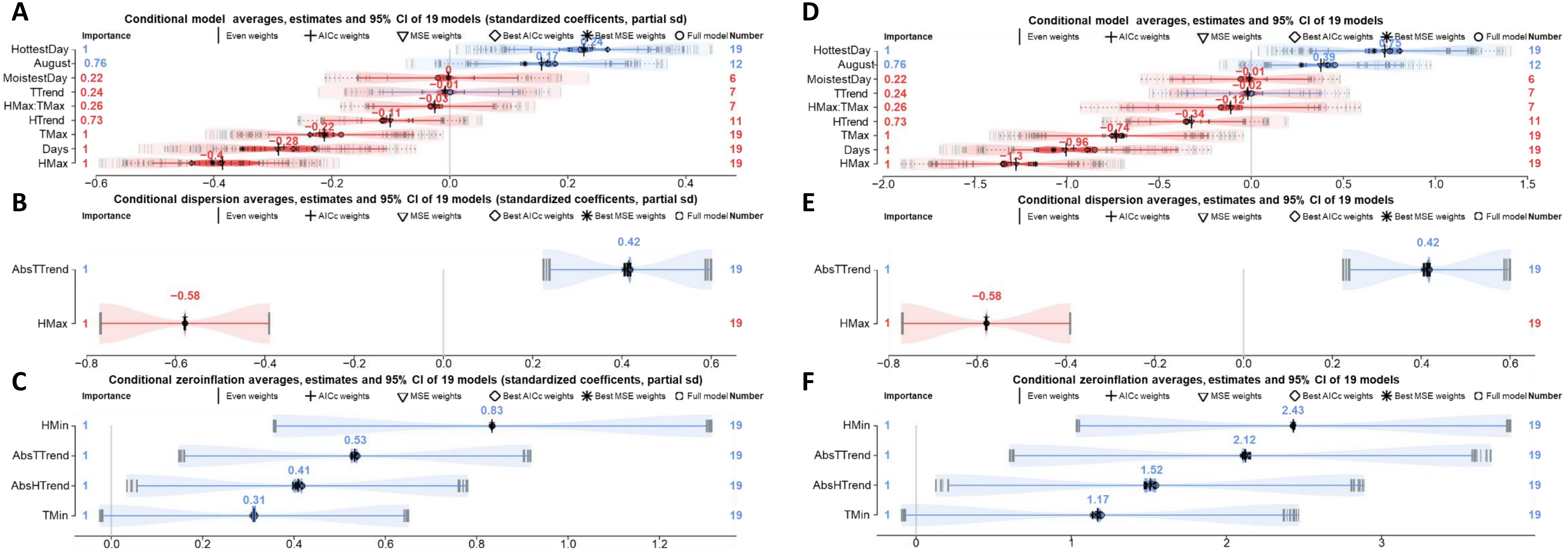
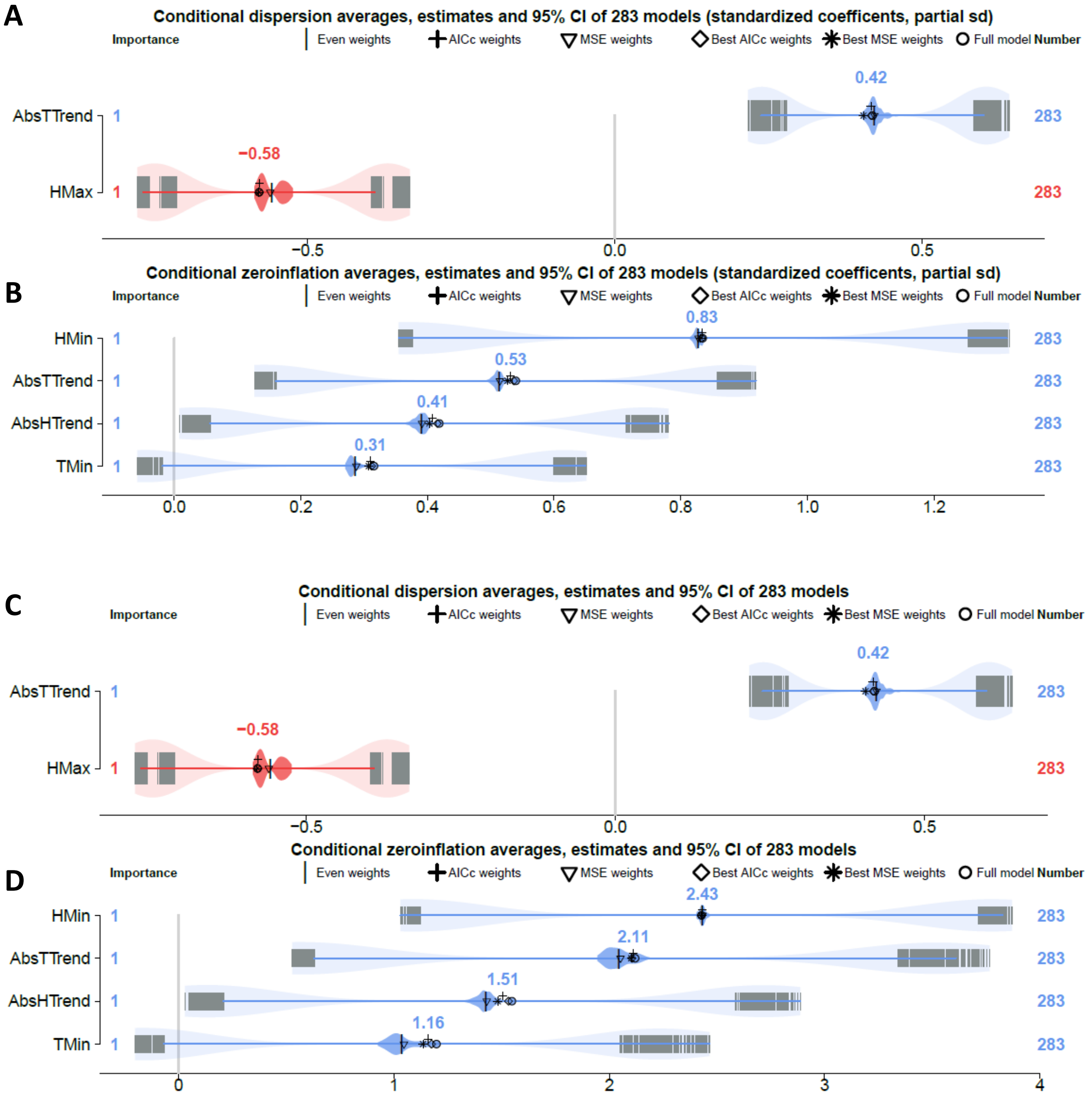


Figure Supp_13 2 Stability of individual corrective model (dispersion A,C) and zero-inflation B,D)) effect estimates (darkest shapes and black ticks) and 95% confidence intervals (CI) (lighter shapes and ticks), and their corrected CI for family-wise multiple testing (lightest shapes and ticks) using the “glht” function of the “multcomp” package for all 283 models fulfilling basic model assumptions. Effect estimates are sorted from negative (red) to positive (blue) by their AICc-weighted average effect estimates (plus symbol, value above). The triangle indicates weighted average estimates according to MSE weights. Circles, solid, and dotted horizontal lines indicate the estimates, CIs, and corrected CIs of the final global model also contained within ΔAICc<6. Best MSE weights (asterisk) corresponds to the best predictive model and is the model with lowest mean squared error (MSE) under roost-visit-wise cross-validation without using random effects variance for predictions. The diamond indicates the model estimates with the lowest AICc. Best predictive model is the model with lowest mean squared error (MSE) under roost-visit-wise cross-validation without using random effects variance for predictions. C-D) shows the same as A-B but coefficients are not standardized by their partial SD anymore (input variables are still standardized by 2 SDs) to account for differing levels of multicollinearity between the single models depending on the included predictors. Because pairwise correlations of input variables in the corrective model exceeded the absolute value of 0.6 (Figure **Supp_09** 3A, the four bottom right variables) we didn`t interpret those corrective estimates (A-D) in the study and only used them to model zeroes and dispersion. However, these estimates and their CIs seem to be quite stable over all 283 models fulfilling basic model assumptions.



Conditional dispersion averages, estimates and 95% CI of 283 models (standardized coefficents, partial sd)

Conditional zeroinflation averages, estimates and 95% CI of 283 models (standardized coefficents, partial sd)

Conditional dispersion averages, estimates and 95% CI of 283 models

Conditional zeroinflation averages, estimates and 95% CI of 283 models

Supp_14 Finding ideal sampling duration to reach a QI of 0.95

Figure Supp_14 1 Simulations of “what-if” scenario to find ideal sampling durations (green) using the best predictive model. We reduced the predictor “Days” stepwise by 1 day forming sliding windows of this length to predict specific QI for every window until the median of same sized windows reached or exceeded a QI of 0.95 (step 7 in manuscript). Since the distribution and collection dates were not measured by the logger, the actual exposure duration “Days” is always one day longer than the measured. A graphical representation of this sliding window approach for every RV is created as pdf in the R script in step 7. (A-B). RV are labeled on the x-axis following the format “Roost”/”Year”/”Sess”. The ideal sampling duration per roost was then defined as minimum ideal sampling duration of all corresponding RVs (C). D-F show the same but on test data instead of model building data. The dashed light green line indicates minimum, the dark green dashed line indicates median ideal sampling duration for the datasets). We detected an unexpectedly high proportion of droppings with zero QI (11.6%) in RV “Thu21/2017/1”, likely due to lab effects (Figure 4A in publication, gap between full vs. hollow circle). Therefore, we excluded this one roost visit in the calculations for ideal exposure duration for Thu21.

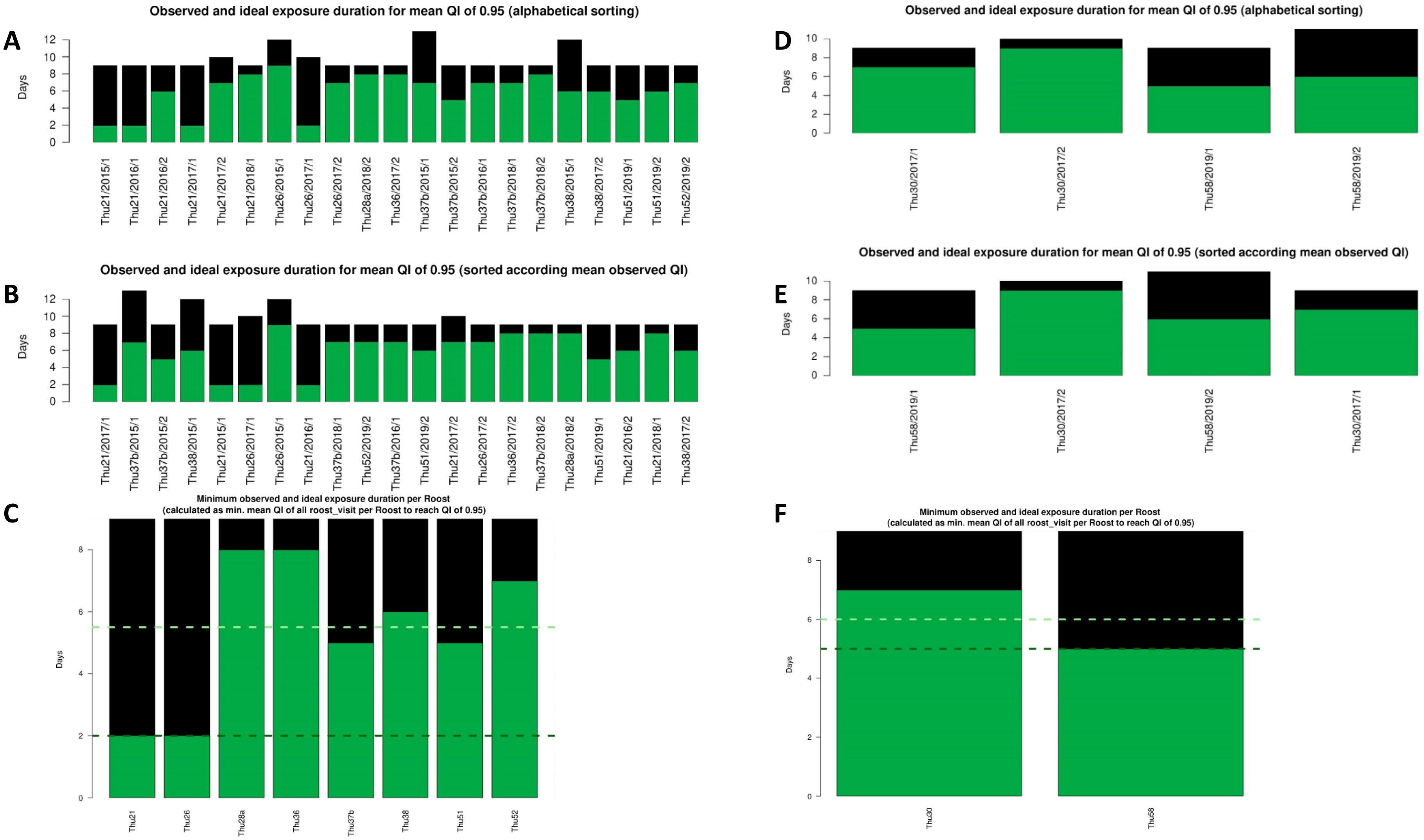
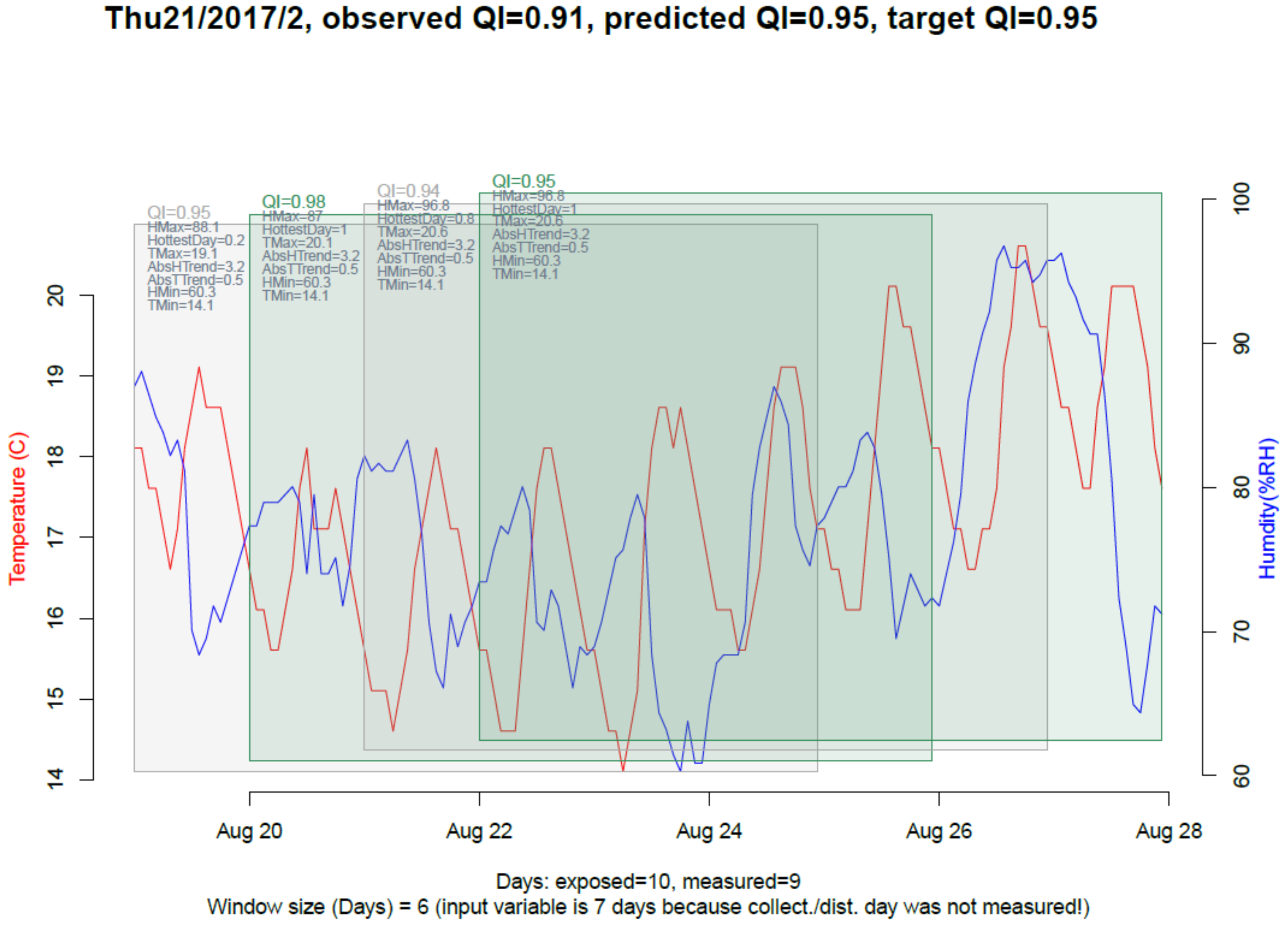


Figure Supp_14 2 Visual representation of the last simulation step to find the ideal sampling duration on the example of roost-visit “Thu21/2017/2” when the sliding window length in days was reduced sufficiently enough to reach a target predicted median QI of 0.95 over all sliding time windows. Green transparent boxes depict windows with predicted QI reaching the target QI whereas grey ones are below the target QI. The unstandardized environmental summary statistics used as predictors in the best predictive model to predict QI for each window as well as the predicted QI are written at the top of every window. The length of the windows reflect the number of days exposed and are visible at the bottom. In this example roost-visit a reduction of exposure duration to 6 days (plus twice a half day for the distribution of newspaper and collection of samples) was enough to reach a target QI of 0.95. All simulation steps for all roost-visits and both target QIs (0.95,0.99) can be produced as pdfs with the published R pipeline in step 7 (“7. Simulate "what if" scenarios to inform sampling“) and will be created inside the corresponding output folder.



Supp_15 Finding ideal sampling duration to reach a QI of 0.99

Figure Supp_15 1 Simulations of “what-if” scenario to find ideal sampling durations (green) using the best predictive model. We reduced the predictor “Days” stepwise by 1 day forming sliding windows of this length to predict specific QI for every window within the observed duration until the median of same sized windows reached or exceeded a QI of 0.99 (step 7 in manuscript). Since the distribution and collection dates were not measured by the logger, the actual exposure duration “Days” is always one day longer than the measured. A graphical representation of this sliding window approach for every RV is created as pdf in the R script in step 7. (A-B). RV are labeled on the x-axis following the format “Roost”/”Year”/”Sess”. The ideal sampling duration per roost was then defined as minimum ideal sampling duration of all corresponding RVs (C). D-F show the same but on test data instead of model building data. The dashed light green line indicates median, the dark green dashed line indicates mean ideal sampling duration for the datasets.

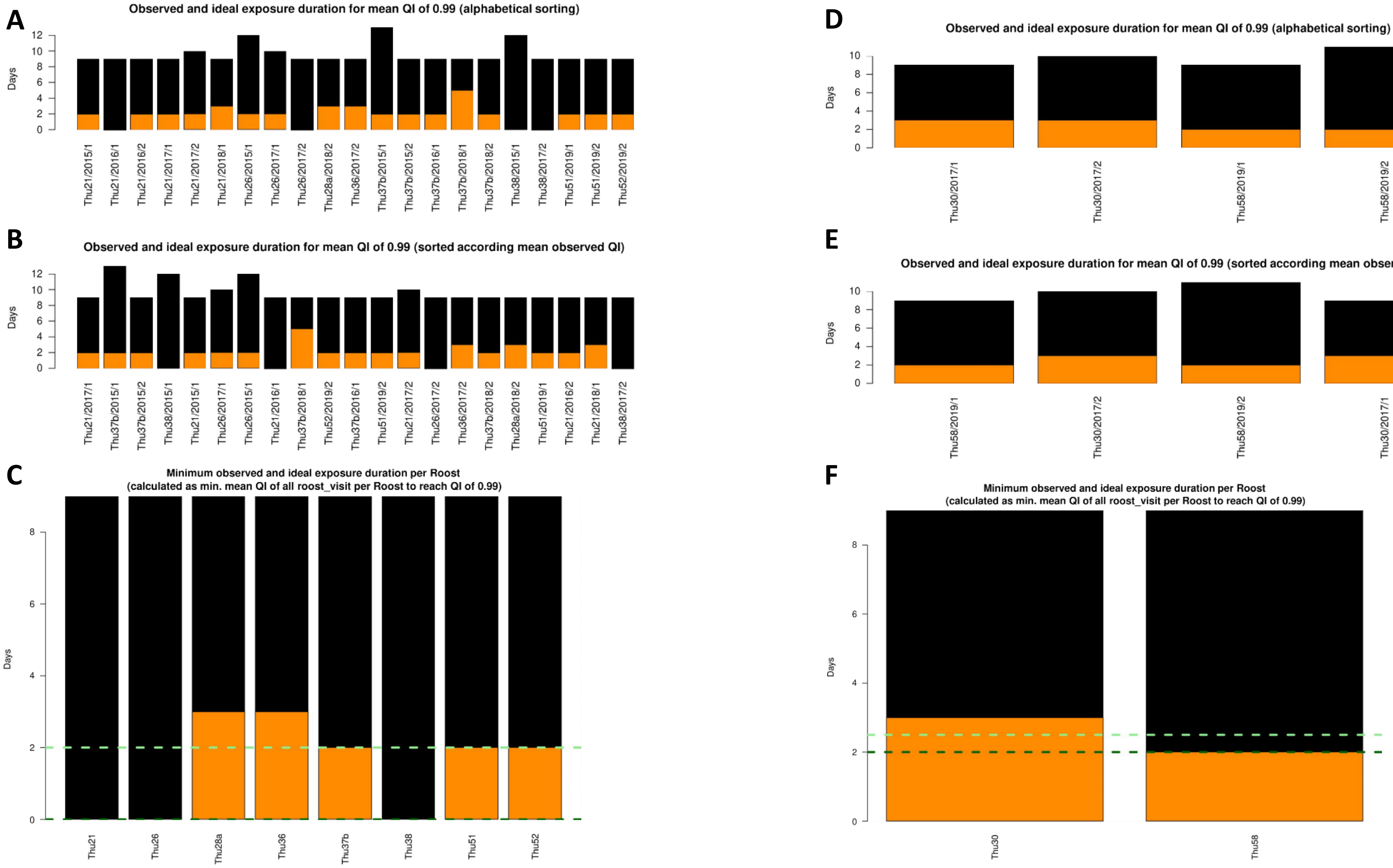
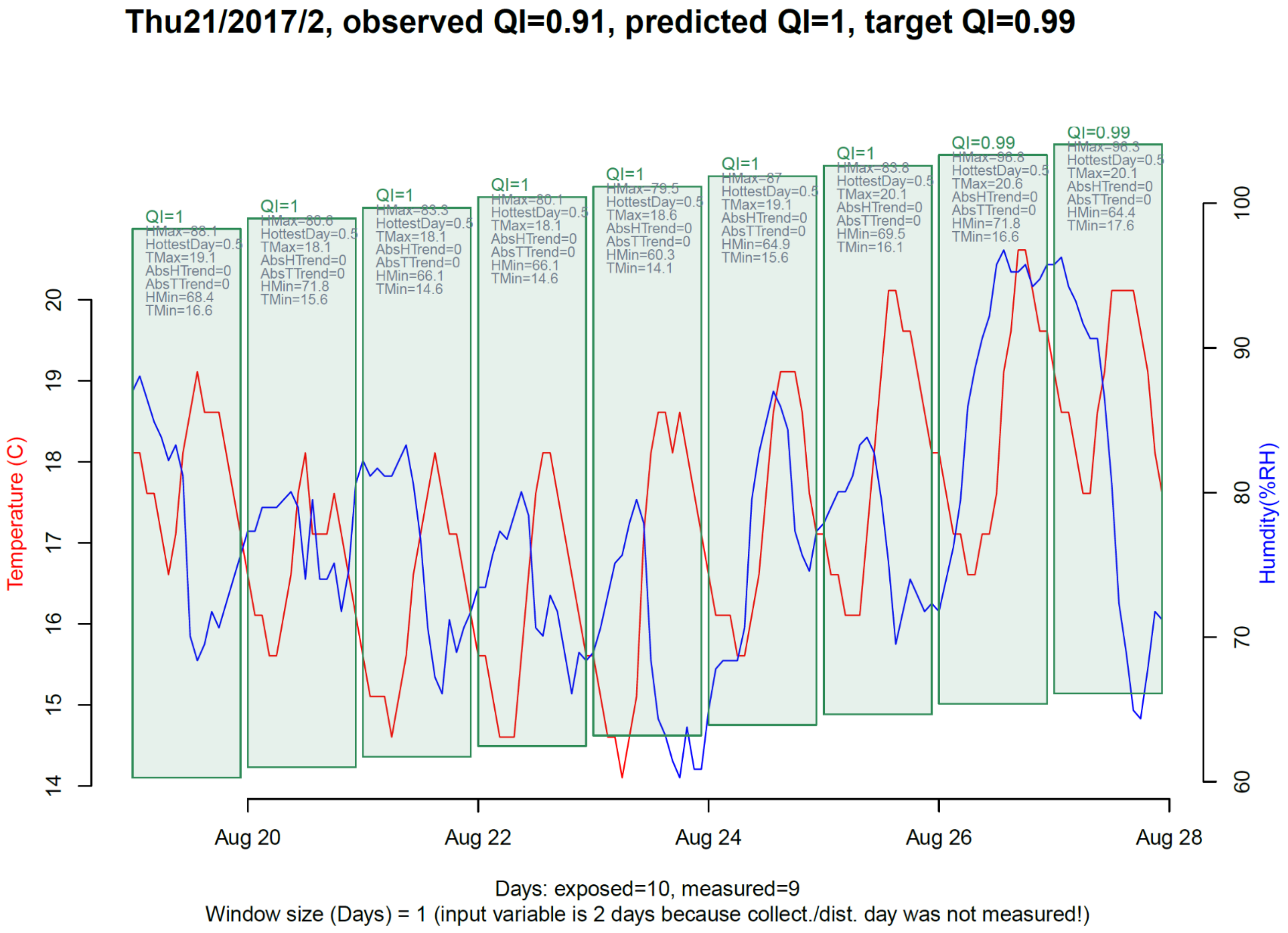


Figure Supp_15 2 Visual representation of the last simulation step to find the ideal sampling duration on the example of roost-visit “Thu21/2017/2” when the sliding window length in days was reduced sufficiently enough to reach a target predicted median QI of 0.99 over all sliding time windows. Green transparent boxes depict windows with predicted QI reaching the target QI whereas grey ones are below the target QI. The unstandardized environmental summary statistics used as predictors in the best predictive model to predict QI for each window as well as the predicted QI are written at the top of every window. The length of the windows reflect the number of days exposed and are visible at the bottom. In this example roost-visit a reduction of exposure duration to 1 day (plus twice a half day for the distribution of newspaper and collection of samples) was enough to reach a target QI of 0.99. All simulation steps for all roost-visits and both target QIs (0.95,0.99) can be produced as pdfs with the published R pipeline in step 7 (“7. Simulate "what if" scenarios to inform sampling“) and will be created inside the corresponding output folder.



Supp_16 The drying effect of “HottestDay”

INTRODUCTION

In the following we investigate the detected positive effect of “HottestDay” from our case study, meaning late hottest days within a roost-visit (RV) were associated with higher eoDNA quality compared to earlier hottest days. We formulated two hypothesis driven auxiliary models and collect further evidence by investigating temporal separation of hot and humid conditions to validate our proposed drying effect on moist droppings.

MATERIALS AND METHODS

We used the same random effect structure, corrective model structure and model building data as described in the manuscript and used to build the final global model. However, to investigate the “HottestDay” we changed the fixed effects and created two separate models (Table Supp_16 1).

We formulated the “Hottest Info model” to check whether the way “HottestDay” is calculated (the day with max median temperature, Table Supp_16 1) could produce a positive effect because of low information content when picking one day of many very similar days (similar median temperatures) within a roost visit. To capture such varying information content of picking a “HottestDay” we introduced the predictor “HottestInfo”. It expresses the outlier character of the “HottestDay” regarding median temperature and chronological directionality. It takes values between 0 and 1 and is the product of the measure of dissimilarity of the “HottestDay” to the other days and the temporal distance between similar days. For example, if the median temperatures of two days within a RV are very similar to each other, picking one or another as a “HottestDay” does not matter that much if both days are following each other (e.g. a few hot days in the middle of the RV) and will result in a similar “HottestDay” value. However, if we consider the two days are in the beginning and in the end of a RV picking one or another as “HottestDay” dramatically changes the predictor of hottest day being close to 0 or 1 although the difference of median temperature between those days might be only minimal. The information content to choose one “HottestDay” is therefore way lower. “HottestInfo” accounts for that and assigns a lower value to the second case. The highest values would be achieved by a clear hottest day outlier and all other days are clearly colder.

Dissimilarity of “HottestDay” to other days can be expressed in R as:

$$MedianDissimilarity2HottestDay = median(1 - \frac{MedianTperDay}{\max(MedianTperDay)}\left[-which.\min\left(\frac{MedianTperDay}{\max(MedianTperDay)}\right)\right]) \tag{i}$$

With “MedianTperDay” describing the median temperature of all days within a RV.

Chronological closeness of similar warm days can be expressed as deviation of ideal increasing or decreasing order in daily median temperatures:

$$Hottestdaydirectionality = \frac{1}{mean(abs(diff(order(MedianDissimilarity2HottestDay, decreasing\ =\ F))))} \tag{ii}$$

We can then express information content “HottestInfo” by multiplying (i) and (ii):

$$HottestInfo = median(1 - \frac{MedianTperDay}{\max(MedianTperDay)}\left[-which.\min\left(\frac{MedianTperDay}{\max(MedianTperDay)}\right)\right]) * \frac{1}{mean(abs(diff(order(MedianDissimilarity2HottestDay, decreasing\ =\ F))))} \tag{iii}$$

We fitted the “Drying effect” model” to test that the effect of “HottestDay” should only be present if conditions tended to be humid towards the end and conditions were hot days would actually be able to dry droppings with the three-way interaction of interest “HottestDay:TMax:HTrend” (Table Supp_16 1).

For both models we checked that no model assumptions were violated as in the publication for the final global model (**Supp_17**, **Supp_18**). Beside neglectable weak heteroscedasticity and deviation from uniformity all model assumptions were fulfilled except for a pairwise correlation in the “HottestInfo” model. Spearman’s rank correlation between input variable “TMax” and “HottestInfo” was slightly above the threshold of 0.6 with 0.65 (Figure **Supp_17** 3A). Both input variables showed deviation of normal distribution (visually and according to Shapiro-Wilcox test for normality) rendering the spearman correlation as the fitting correlation statistics.

Since the violation of pairwise correlation was rather weak and still below 0.7 (Dormann et al., 2013) and multicollinearity with variance inflation factors (VIFs) being well below five (Figure **Supp_17** 3A) we continued with this model. The “HottestInfo” model also showed weak but insignificant signs of outliers based on DHARMa residual simulations (Figure **Supp_17** 1C).

Additionally, we looked into the temporal separation of hottest and moistest hour on “HottestDay” in the raw logger data.

Table Supp_16 1

Model and data overview. “Z-scored” indicates standardization of variable by two standard deviations and centering to 0. All continuous fixed, zero-inflation and dispersion effects were measured per RV. The “Hottest Info” and the “Drying” models are auxiliary models based on the same data as all others to investigate and test hypothesis to identify “HottestDay” as potential drying effect. All models were built with the same model building data and used the same random effects and corrective model as in the publication.

	Response and fixed effects	**Random effects**	**Corrective model**	
			Zero-inflation	**Dispersion**
Global model:	**QI ~ HMax * TMax + Days + HottestDay + MoistestDay + TTrend + HTrend + Sess**	**+ (1 | Roost) +(1|Year/PCRPlate/ExtrPlate/RVLabUnit)**	**~ HMin + TMin + AbsHTrend + AbsTTrend**	**~ HMax + AbsTTrend**
Hottest Info model	**QI ~ HottestDay * HottestInfo + MoistestDay + HTrend*MoistestInfo + HMax + TMax + Days + Sess**			
Drying model	**QI ~ HottestDay * TMax * HTrend + HMax + Sess**			

Model part	**Variable (Abbrev.)**	**Type/transformation**	**Description and [range of unstandardized variable]**	**n**
Response	Quality Index **(QI)**	Beta-binomial (logit) (Nsuccess/Nfailure)	Proportion of replicate loci identical/non-identical compared to consensus locus built over three replicate multi-locus genotypes with 8-9 loci each **[3x]**	2309 droppings
	Max. Humidity (**HMax**)	Continuous/Z-scored	Maximum relative humidity **[76.8 – 100 %]**	21 values
	Max. Temperature (**TMax**)	Continuous/Z-scored	Maximum temperature **[12.6 – 32.1 °C]**	21 values

Fixed effects	Temperature trend (**TTrend**)	Continuous/Z-scored	Trend towards warmer (positive) or colder (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. temperatures. **[-0.5 – 2 °C]**	21 values
	Humidity trend (**HTrend**)	Continuous/Z-scored	Trend towards moister (positive) or drier (negative) days within a single collection period calculated as median of differences between chronologically ordered daily max. humidity. **[-5.1 – +1.4 %]**	21 values
	Hottest day (**HottestDay**)	Continuous/Z-scored	Day with max. daily median temperature as proportion of total exposure days **[0.18 – 1]**	21 values
	Moistest day (**MoistestDay**)	Continuous/Z-scored	Day with max. daily median humidity as proportion of total exposure days **[0.13 – 1]**	21 values
	Exposure days (**Days**)	Continuous/Z-scored	Duration of single collection period in a roost in days (maximum possible days a dropping could have been exposed to the environment) **[9 – 13]**	21 values
	Session (**Sess**)	Factor/Reference June	June/August (before and after offspring is born)	2 levels
	Hottest info (**HottestInfo)**	Continuous/Z-scored	Product of (i) the median similarity of median temperature of all other days to the one “HottestDay” as proportion (1 meaning identical), and (ii) proportion of directional order of median daily temperature of similar days as proportion of deviation of ideal sorting order (warmer to colder or vice versa). **[0.0125 -0.214]**	21 values
Random effects	Roost (**Roost**)	Factor	Different maternity roosts (totally sampled 21 times from 2015 to 2019)	8 levels
	Year (**Year**)	Factor	2015-2019	5 levels
	PCR plate (**PCRPlate**)	Factor	Each PCR plate with 384 wells	30 levels
	Extraction plate (**ExtrPlate**)	Factor	Each plate with 96 wells (each well contains extracted DNA of one dropping)	44 levels
	Roost visit sample block (**RVLabUnit**)	Factor	Groups of droppings of the same roost visit origin ending up on the same extraction and/or PCR plates (accounting for blocked sample distribution on PCR and extraction plates).	49 levels
Zero-inflation effects	Min. humidity (**HMin**)	Continuous/Z-scored	Minimum relative humidity **[29.3 – 100 %]**	21 values
	Min. temperature (**TMin**)	Continuous/Z-scored	Minimum temperature **[11.0 – 23.0 °C]**	21 values
	Absolute humidity trend (**AbsHTrend**)	Continuous/Z-scored	Measure of absolute variability in max. humidity between days calculated as median of absolute chronological differences of daily maximum humidity **[0 – 10.9 %]**	21 values
	Absoulte temperature trend (**AbsTTrend**)	Continuous/Z-scored	Measure of absolute variability in max. temperature between days calculated as median of absolute chronological differences of daily maximum humidity **[0 – 2 %]**	21 values
Dispersion effects	**AbsTTrend**, **HMax**	Continuous/Z-scored	Variables already explained in previous rows	21 values

RESULTS

The two auxiliary models investigating the positive effect of “HottestDay” resulted in low AICc with a ΔAICc of 6.1 for the “HottestInfo” and 4.4 for the “Drying Effect” model compared to the lowest AICc of the 19 top models in step 4 fit on the same data. Both fulfilled almost all model assumptions (Figures **Supp_17** 1-5, **Supp_18** 1-5). The “HottestInfo” model however showed weak signs of outliers.

In the “Hottest Info” model the interaction of interest “HottestDay:HottestInfo” was insignificant after Holm-Bonferroni correction for multiple testing but showed a positive tendency (Figures **Supp_17** 6C, **Supp_17** 7), meaning the positive effect of “HottestDay” was stronger with more information content (Figure Supp_16 1 B,D), steeper slope) and very weak inexistent with little information content (Figure Supp_16 1 A,C, weak slope). This effect was stronger with higher temperatures (Figure Supp_16 1, steeper red lines) and more pronounced when days during exposure tended to become more humid towards the end (Figure Supp_16 1 C,D, more clearly separated confidence intervals).

In the “Drying Effect model”, where we investigated whether the positive effect of “HottestDay” was only present when it was also humid towards the end of the exposure duration and temperature were also high, the interaction of interest “HottestDay:TMax:HTrend” was strongly positive and still significant after Holm-Bonferoni correction for multiple testing (Figures **Supp_18** 6C, **Supp_18** 7). Late hot “HottestDay” (32.1 °C) resulted in a higher QI when days tended to get more humid at the same time during the exposure duration (Figure Supp_16 2 B,D, red line). Cold late “HottestDay” (12.6 °C) led to lower QI instead under the same humidity conditions (Figure Supp_16 2 B,D, orange

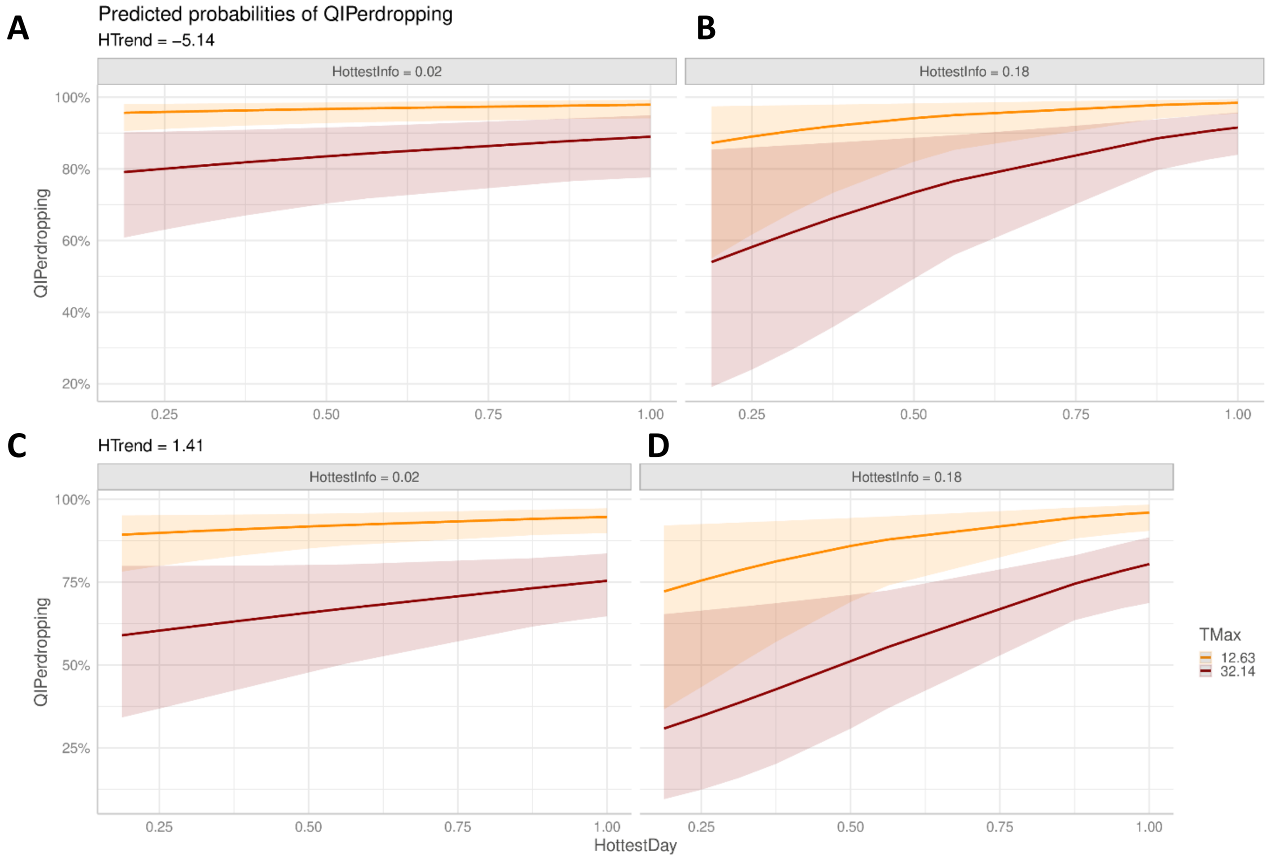


Figure Supp_16 1 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the “ggeffects” on the two way interaction (“HottestDay:HottestInfo”) visualizing the positive effect of “HottestInfo” on “HottestDay” on QI. 95 % confidence intervals are indicated by the lighter shape. QI is expressed in percent on the y-axis. Plots are additionally separated by “TMax” observed in the model building data exploring lowest (orange) and highest (red) ”TMax” observed in RVs. Additionally the predictor “HTrend” separates differences between RVs with trends towards drier (A,B) or more humid (C,D) days during the course of the exposure duration “Days” within a RV.

line) . The pattern was reversed when days tended to be dryer over the course of the exposure duration with hot late “HottestDay” reducing QI while cold late “HottestDay” resulted in higher QI (Figure Supp_16 2 A,B, orange line).

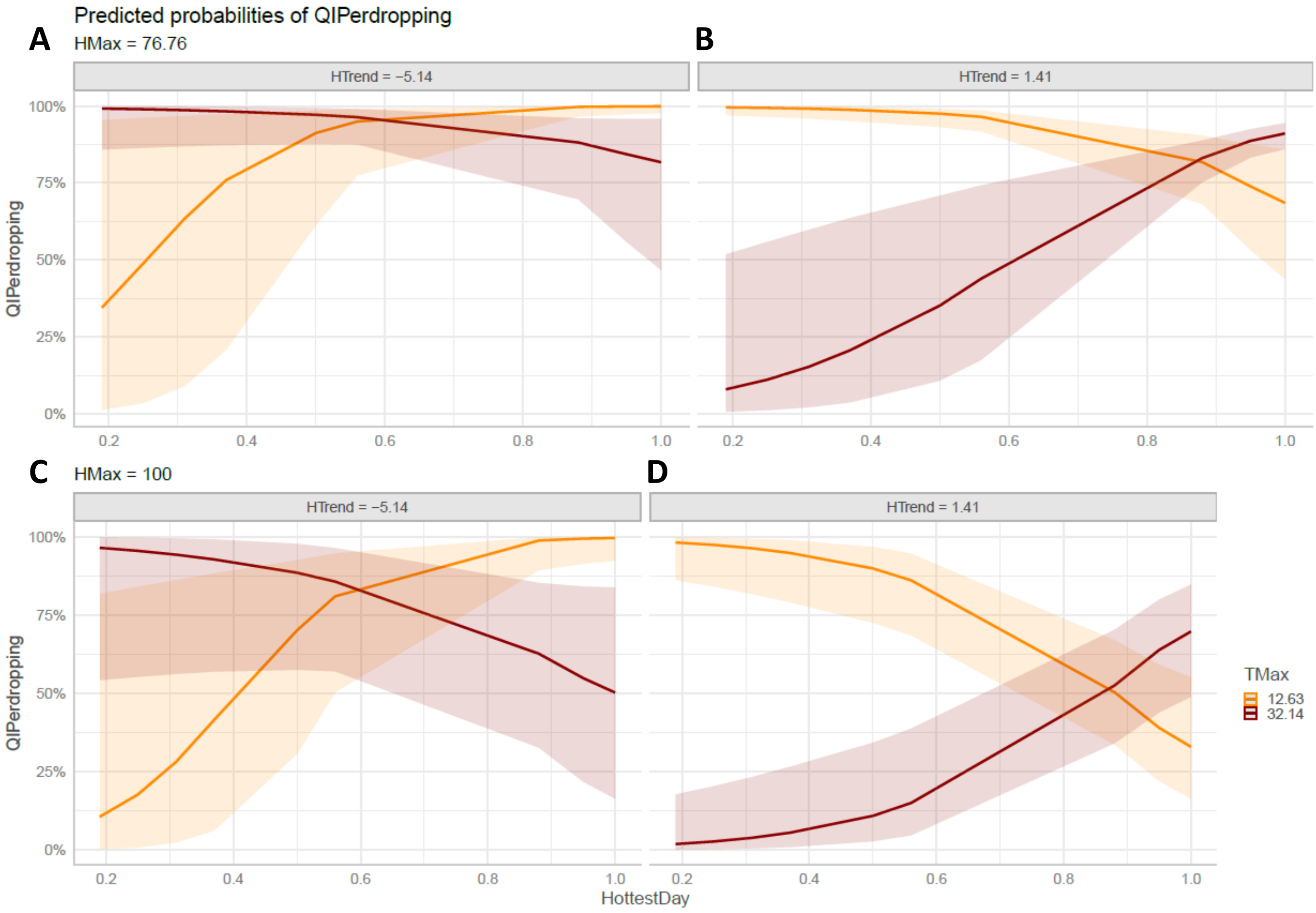


Figure Supp_16 2 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the “ggeffects” on the three way interaction (“HottestDay:TMax:HTrend”) visualizing the positive effect of “HottestDay” on QI when RVs reached high temperatures and tended to get humid towards the end. 95 % confidence intervals are indicated by the lighter shapes. The interactions within a plot are separated by minimum (orange) and maximum (red) observed “TMax”. Additionally the predictor “HTrend” separates differences between RVs with trends towards drier (A,B) or more humid (C,D) days during the course of the exposure duration “Days” within a RV.

Lastly, we detected a significant separation (two-sided pairwise t-test, p=0.0084) of hottest and moistest hour on “HottestDay” by 4.6 hours with the median moistest hour in the morning at 09:00 followed by the hottest hour in the afternoon at 15:00 (Figure **Supp_19** 3).

DISCUSSION

The auxiliary “HottestInfo” model demonstrated that the positive effect of “HottestDay” was not an artefact of its calculation when information content of the selection of one single “HottestDay” was low, and must be truly positive despite the negative effect of high temperature itself (“TMax” in Figure 2 and 3 of the publication).
The “Drying Effect” model revealed that the positive effect of “HottestDay” was only present if days also tended to be more humid towards the end, but negative if trends led to drier days.
However, this is counterintuitive since simultaneously hot and humid conditions should degrade eDNA more strongly (Vili et al., 2013; Walker et al., 2019). Especially because those conditions happen later during the exposure duration, meaning most of the droppings are deposited and experiencing such conditions, the effect on QI per RV should be even stronger and negative (Figure Supp_16 3).

This contradiction originates from the temporal resolution of days instead of hours. On “HottestDay” the hottest and moistest hours are not simultaneous in a RV, they are separated with the median moistest hour in the morning at 09:00 followed by the hottest hour in the afternoon at 15:00. This separation also partially explains, why we did not capture a strong signal of the interaction effect “HMax:TMax” (Figure 2 and Figure 3 in the publication). However,

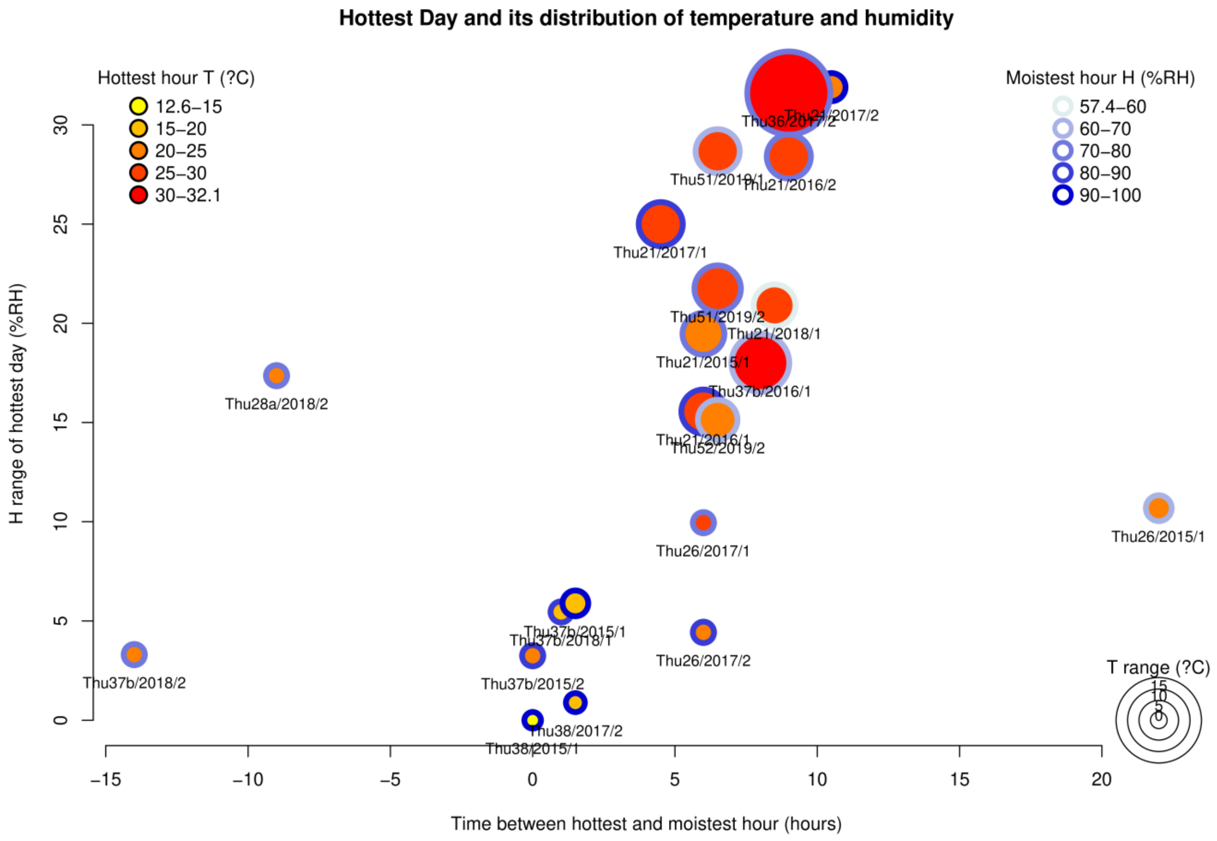


Figure Supp_16 3 Temporal separation of hottest hour and moistest hour on the hottest day (day with max median temperature) per roost-visit (RV) labeled in the format “Roost”/”Year”/”Sess”. The circle size indicates the range of temperatures measured on the hottest day and reflect daily stability in temperature. The range of relative humidity measured on the hottest day is visible on the y-axis and reflects daily stability in humidity. Fill color indicates temperature of hottest hour and border colour indicates the relative humidity of the moistest hour. It shows that RVs with hot hottest hours and larger variability in daily temperature experience the moistest hour of the day before the hottest hour by more than 4 hours. The few cases where the hottest hour occurred before or simultaneous to the moistest hour on the hottest day were only observed in RVs with high daily temperature stability and rather low temperatures and therefore less information content similar to the explanation of “HottestInfo”.

the few RVs were moistest and hottest hour occurred simultaneous or happened after the hottest hour were rather stable roosts regarding humidity and temperature and therefore the selection of two hours from many very similar hours had a rather low information content (Figure Supp_16 3, **Supp_19**, e.g., Thu37/2018/2 vs. Thu36/2017/2).

The positive effect of “HottestDay” is also especially strong when such days occur later, since most droppings will be already deposited (Figure Supp_16 4), are hot and cooccur with more humid days towards the end (Figure Supp_16 2, B,D). If the observed positive effect of “HottestDay” would be solely due to the increasing proportional effect of late bad conditions like high “TMax” the effect of late hot “HottestDay” should be negative and not positive instead. Therefore, the positive effect of late hot “Hottestday” must capture mostly an effect acting on single droppings instead on the entirety of a higher proportion of droppings within a RV being affected by bad conditions if they happen later (Figure Supp_16 4).

Based on the findings of our two auxiliary models, the temporal separation of moistest hour and hottest hour on the hottest day and the logical conclusion of capturing mostly an effect acting on single droppings we strongly support that the positive effect of "HottestDay" is mostly based on a drying effect. Moist droppings from previous humid days and/or mornings dry on hot afternoons. When days tend to become drier towards the end of the exposure duration and temperatures are high this effect turns negative since droppings are already dry and will instead suffer from the negative effects of high temperatures on eoDNA (Figure Supp_16 2 A,C red lines). On the other hand, if days tend to become more humid towards the end and late "HottestDay" are rather cool they will not be enough to dry the humid droppings leading to a negative effect on eoDNA (Figure Supp_16 2 B,D, orange lines).
While we assume evenly distributed dropping deposition over the whole exposure duration "Days" of a RV (Figure Supp_16 4), the real deposit distribution might be less even. Bad weather conditions (e.g., rain, wind) could lead to lower food availability or hunting success and energy saving responses like torpor could lead to a un uneven deposition of droppings through the days. To disentangle the drying effect from the proportional deposit effect (Figure Supp_16 4), final causality needs to be assessed under varying succession of high and low temperature and humidity exposure over multiple days with fresh droppings of the same age (not older than a day) directly in roosts or in experimental setups like climate chambers.

The discovered drying effect was also proposed in non-invasive DNA in bear hair by Sawaya et al. (2015), where moist samples dried faster in direct sunlight opposed to shaded ones and therefore improved eDNA quality. Mechanistically such drying should be beneficial, as it removes the water required for activity of free DNases (Regnaut et al., 2005) and is also the reason why removal of water when collecting and storing eoDNA is so important (e.g. silica gel, freeze-drying, oven-drying, ethanol).

Our findings indicate for our system and supposedly for many other terrestrial temperate systems that one can expect higher eoDNA quality due to the drying capacities of late hot days if samples were previously humid and ending in hot days compared to sampling sessions where they do not. In a broader sense, we demonstrate the importance of temporal succession and interactions of environmental factors to quantify and predict environmental degradation of eoDNA.

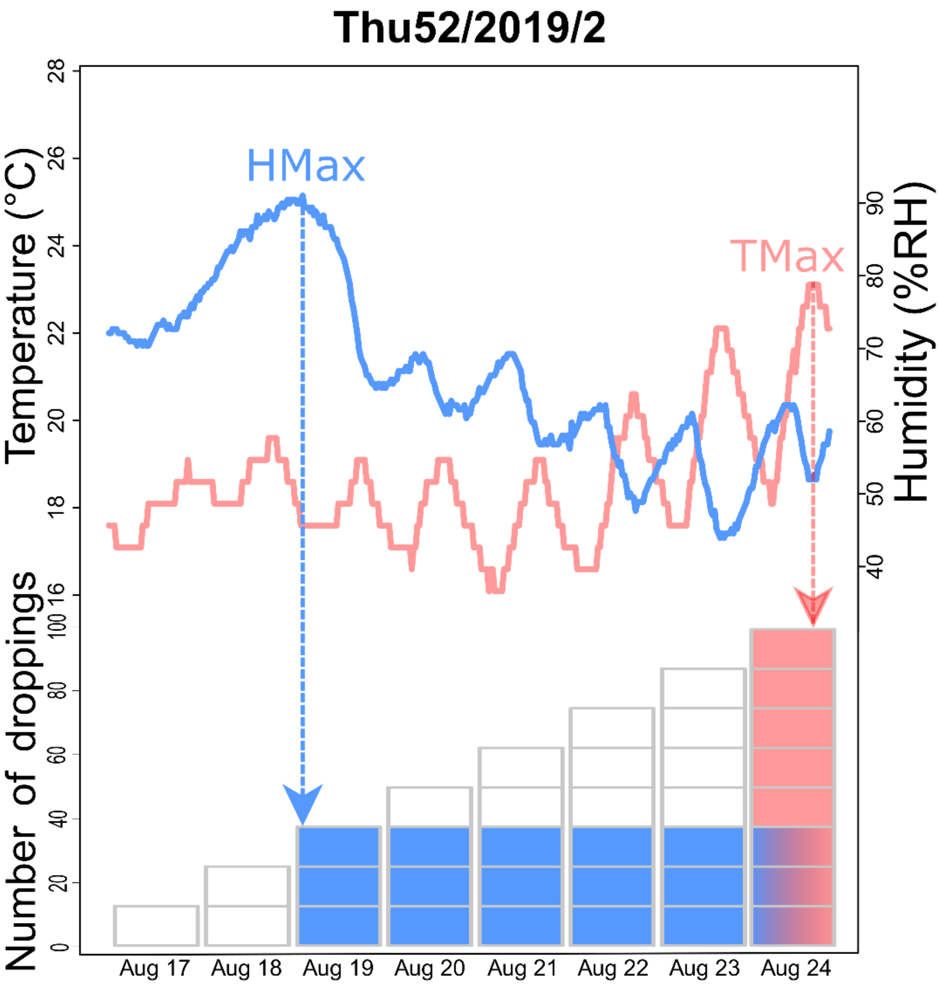


Figure Supp_16 4 Hypothesized role of uncertainty of exact dropping deposit timing within a roost-visit (RV) on real data captured by our environmental predictors. If we assume even distributed deposit of droppings across time this means only 3/8 or 37.5% of all droppings actually experienced the moistest day of "HMax" on day three (August 19) whereas all droppings experienced the hottest day with "TMax" since it happened on the last day of the collection period (day 8, August 24).

Supp_17 Information about the “HottestInfo” model

Model diagnostics and information for the auxiliary model investigating information content influence on “HottestDay” (see **Supp_16**) created with the mdiag() function included in the version controlled R-Project. Not all information from the 29-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved.

Model Overview

name: HottestDay_Info_model

family: betabinomial
link: logit

response variable form: matrix

sample size: 2309

N (sample size) / k (terms) : 209.9

formula: QIPerdropping ~ HottestDay * HottestInfo

+ MoistestDay + HTrend * MoistestInfo + HMax + TMax

+ Days + Sess + (1 | Roost) + (1 | Year/PCRPlate/ExtrPlate/RVLabUnit)

ziformula: ~AbsHTrend + AbsTTrend + HMin + TMin

dispformula: ~AbsTTrend + HMax

Figure Supp_17 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Dispersion test showing no significant overdispersion with the observed (red line) variance being 1.05 times stronger than the mean simulated variance (black lines). B) Zero-inflation test shows no significant zero-inflation with 1.11 more observed zeroes (red line) than the mean number of the simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows no significant outliers (red bars and line). D) Quantile-Quantile plot of observed and expected residuals shows weak significant deviation of uniform distribution (red line).

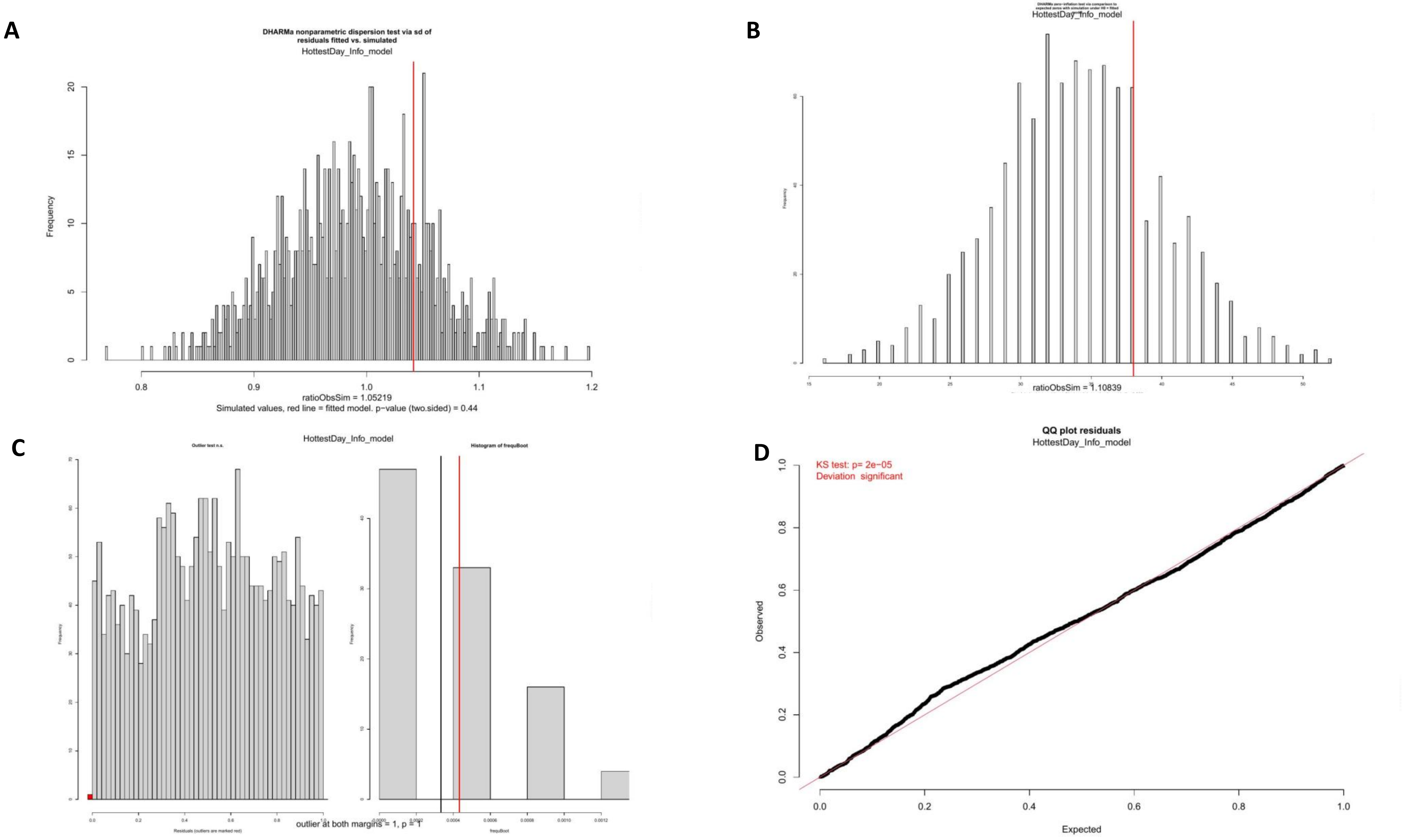


Figure Supp_17 2 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations.
A) Testing excess/deficits of QI = 1. Since QI was expressed in matrix form (success/(total number of loci) A QI of 1 means in Matrix form 27/27. We detected no significant excess/deficit with 0.90 times the number of ones observed compared to the mean number of ones from simulations.
B) The same as A) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem (see main text). It shows no significant excess/deficit of ones with 0.95 times ones observed compared to the mean number of ones from simulations.
C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as (red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate signs of heteroscedasticity but only in the first two quantiles.

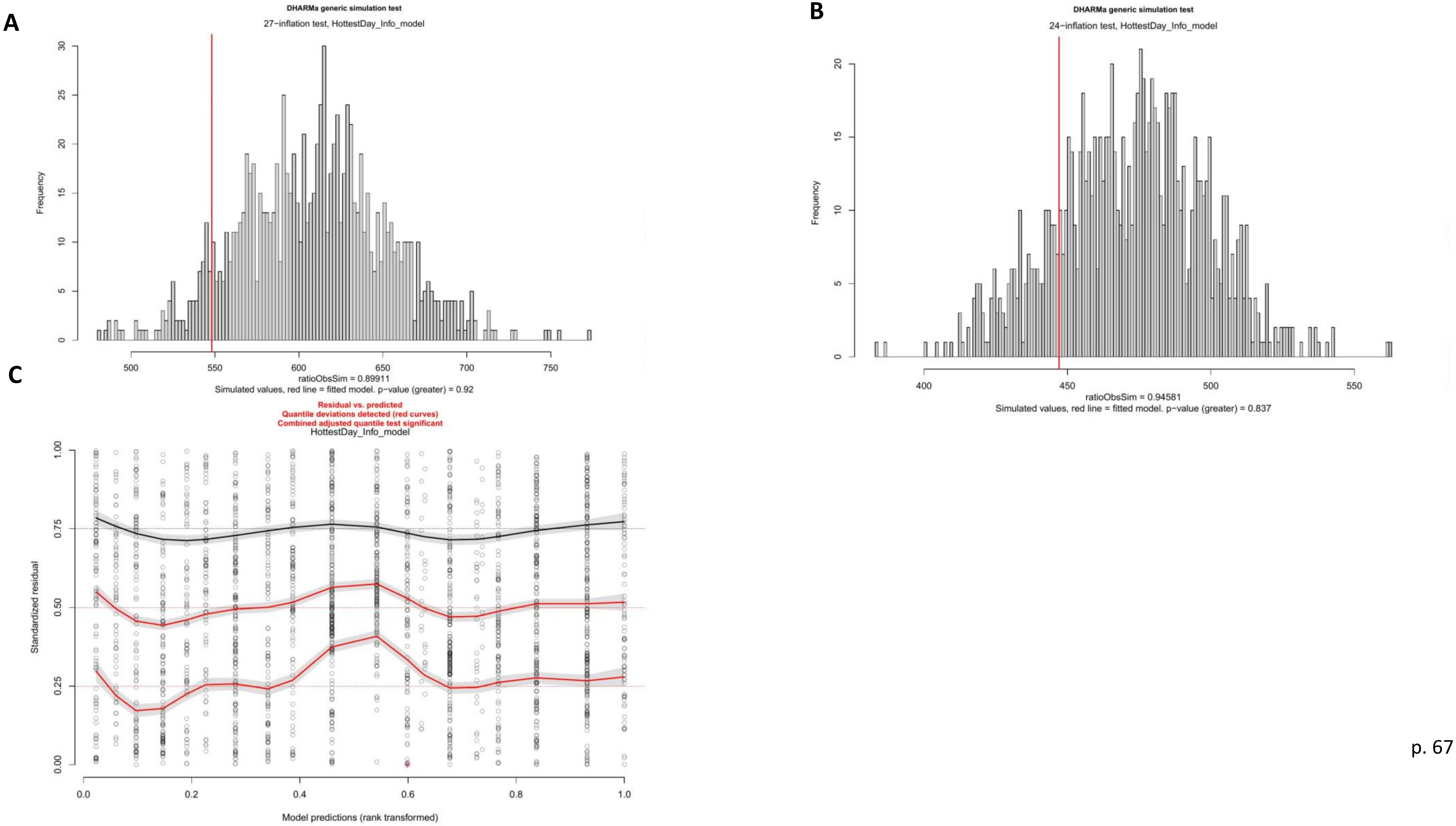
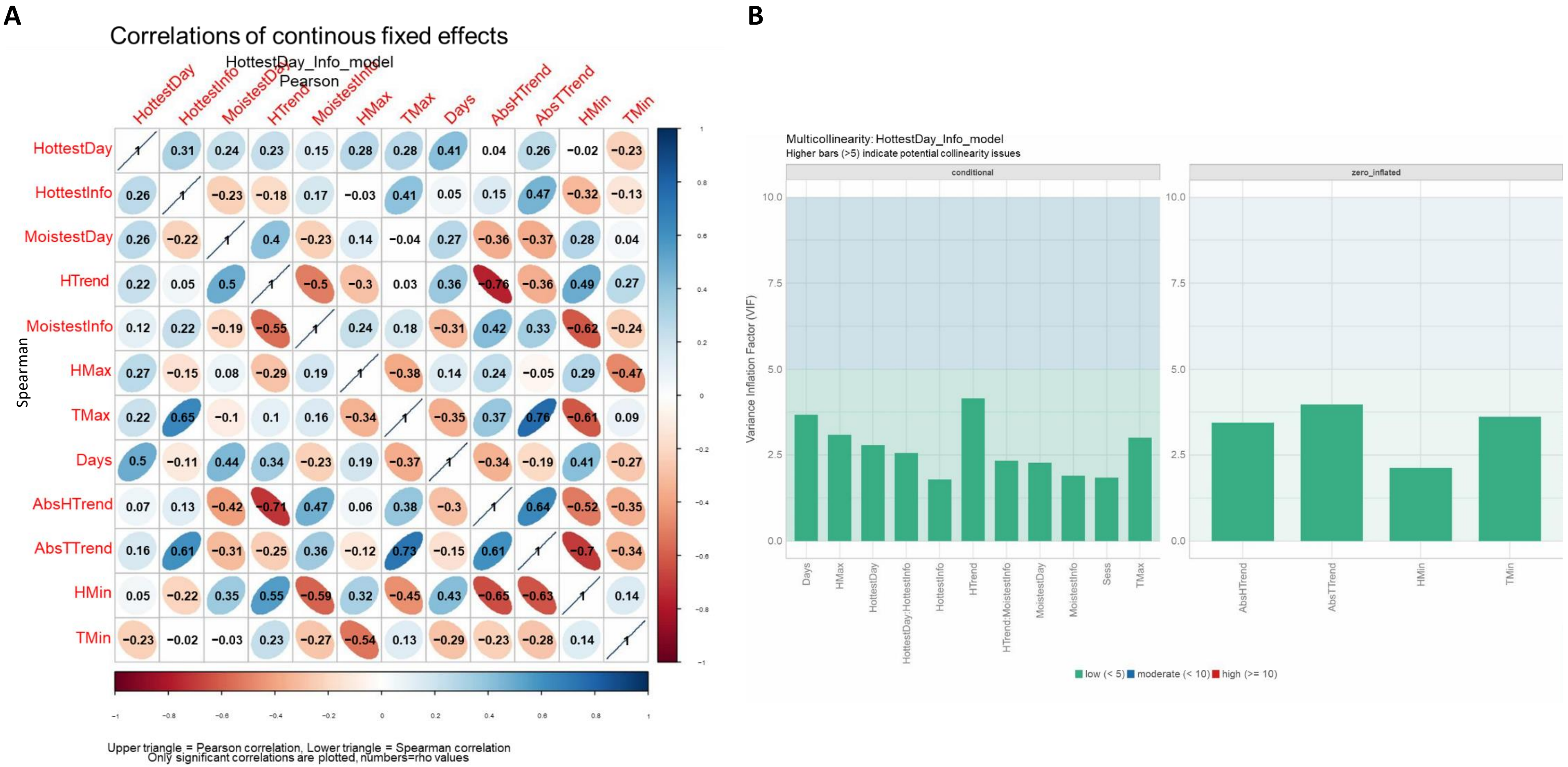


Figure Supp_17 3 Correlations of input variables and multicollinearity. A) Pairwise correlation of all input variables (2309 values per variable) using «Hmisc» and «corrplot» package. Lower triangle indicates Spearman's rank correlation; higher triangle indicates Pearson correlation. Lighter colour indicates weaker correlation darker colours stronger correlation. Reddish colours indicate negative correlation, blueish indicate positive correlations. Numbers indicate correlations. Correlations under 0.6 are regarded unproblematic in this study. Note that correlations with variables from the corrective model (AbsHTrend, AbsTTrend, HMin, TMin) are also calculated. Correlation containing those predictors were ignored since we weren`t interested in their estimates but only in modeling zeroes and dispersion to fulfill model assumptions. B) shows multicollinearity measured as variance inflation factor (VIF) using the "performance" package. All VIFs are under 5 and therefore fulfilling model assumptions.



	HottestDay	HottestInfo	MoistestDay	HTrend	MoistestInfo	HMax	TMax	Days	AbsHTrend	AbsTTrend	HMin	TMin
HottestDay	1	0.31	0.24	0.23	0.15	0.28	0.28	0.41	0.04	0.26	−0.02	−0.23
HottestInfo	0.26	1	−0.23	−0.18	0.17	−0.03	0.41	0.05	0.15	0.47	−0.32	−0.13
MoistestDay	0.26	−0.22	1	0.4	−0.23	0.14	−0.04	0.27	−0.36	−0.37	0.28	0.04
HTrend	0.22	0.05	0.5	1	−0.5	−0.3	0.03	0.36	−0.76	−0.36	0.49	0.27
MoistestInfo	0.12	0.22	−0.19	−0.55	1	0.24	0.18	−0.31	0.42	0.33	−0.62	−0.24
HMax	0.27	−0.15	0.08	−0.29	0.19	1	−0.38	0.14	0.24	−0.05	0.29	−0.47
TMax	0.22	0.65	−0.1	0.1	0.16	−0.34	1	−0.35	0.37	0.76	−0.61	0.09
Days	0.5	−0.11	0.44	0.34	−0.23	0.19	−0.37	1	−0.34	−0.19	0.41	−0.27
AbsHTrend	0.07	0.13	−0.42	−0.71	0.47	0.06	0.38	−0.3	1	0.64	−0.52	−0.35
AbsTTrend	0.16	0.61	−0.31	−0.25	0.36	−0.12	0.73	−0.15	0.61	1	−0.7	−0.34
HMin	0.05	−0.22	0.35	0.55	−0.59	0.32	−0.45	0.43	−0.65	−0.63	1	0.14
TMin	−0.23	−0.02	−0.03	0.23	−0.27	−0.54	0.13	−0.29	−0.23	−0.28	0.14	1

Figure Supp_17 4 Temporal autocorrelation test using DHARMa package based on 10 000 simulations on and sampling timing with year/sess. No significant temporal autocorrelation was detected using the Durbin-Watson test p=0.91.

We also performed a spatial autocorrelation test (Moran's test for distance-based autocorrelation) with those residuals and could not detect significant spatial autocorrelation (p=0.14). The graph with coordinates is not shown because lesser horseshoe bat (*Rhinolophus hipposideros*) is an endangered and protected species in Germany and roosts location cannot be disclosed.

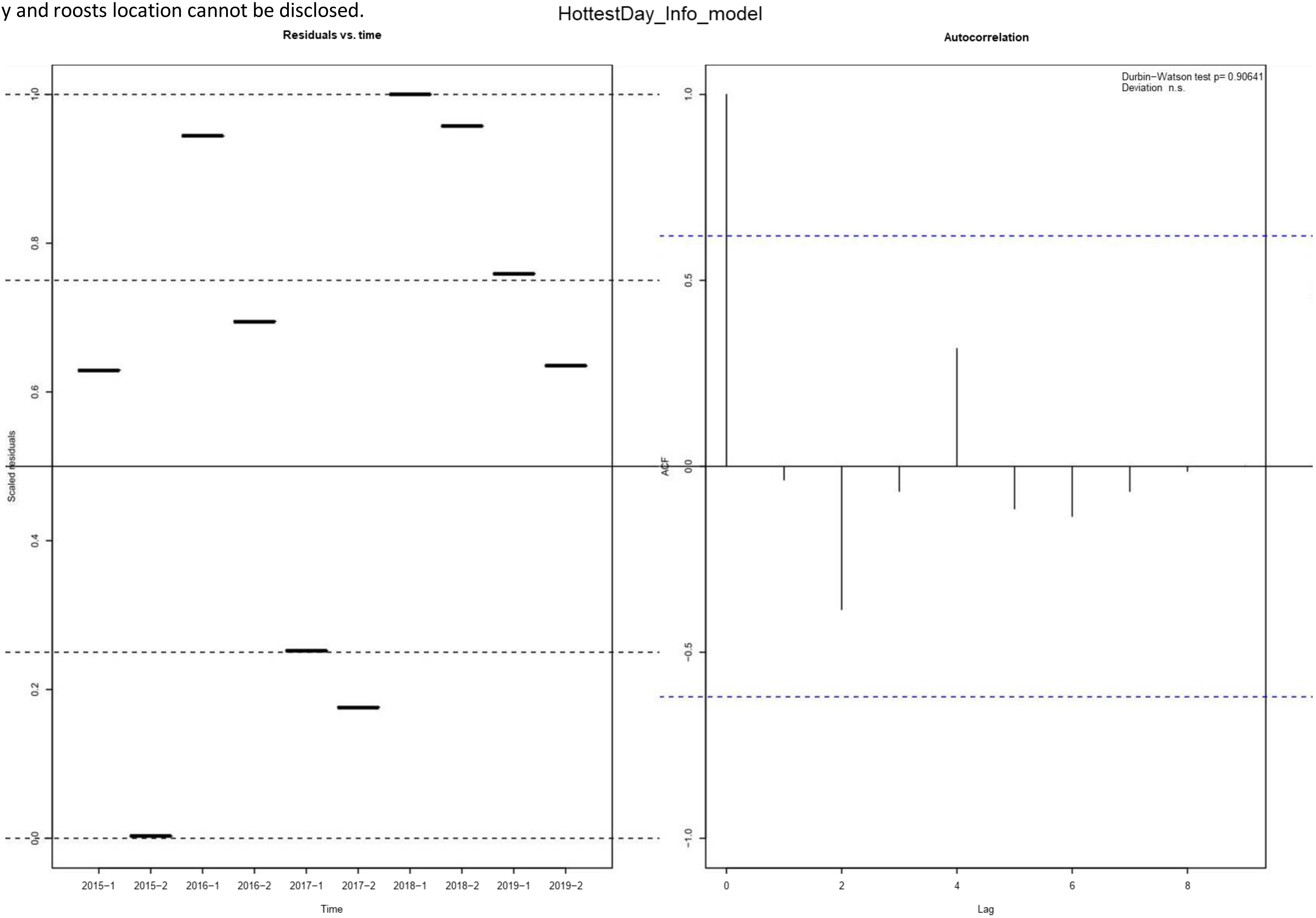


Figure Supp_17 5 Normality of Random Effects using the “performance” and “See” package. No strong deviation was detected.

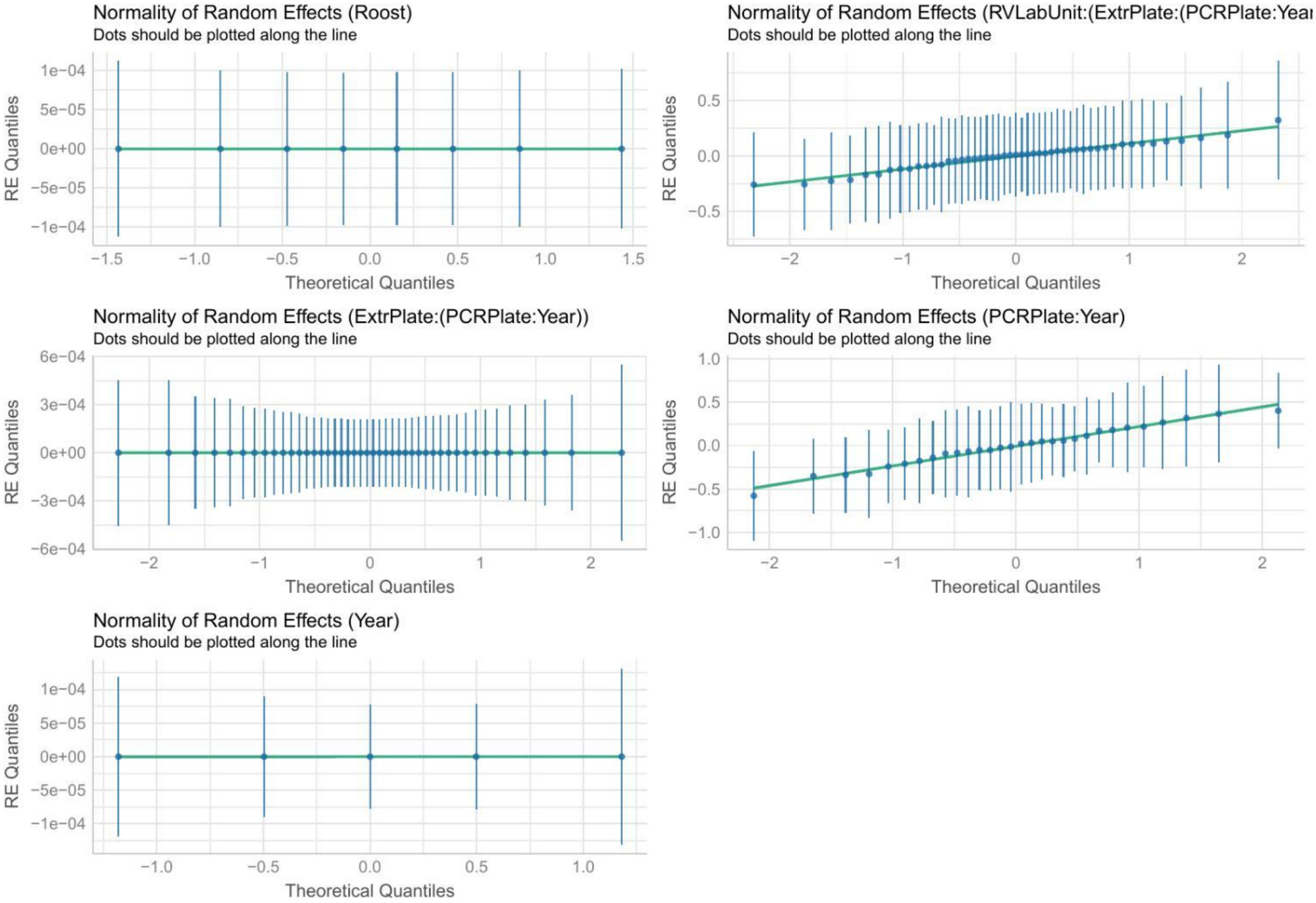
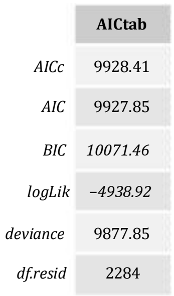


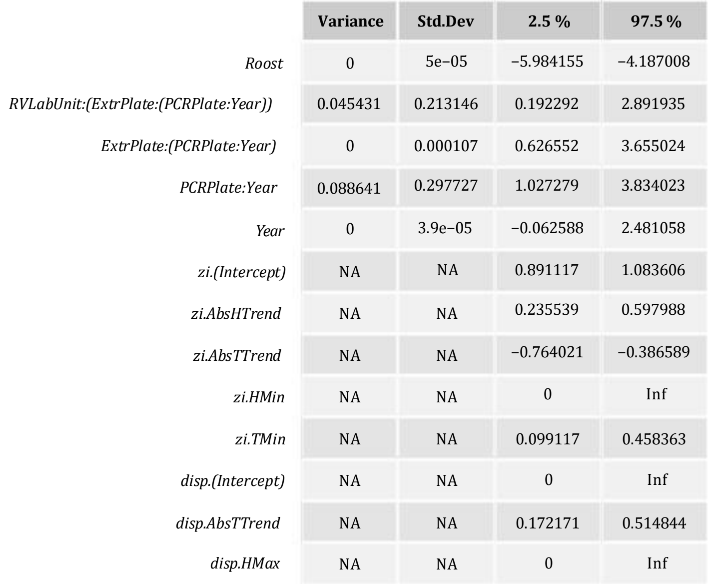
Figure Supp_17 6 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval for the random effects and corrective model (where possible). C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the "multcomp" package and the "glht" function.

A



	AICtab
AICc	9928.41
AIC	9927.85
BIC	*10071.46*
logLik	*−4938.92*
deviance	9877.85
df.resid	2284

B



	Variance	**Std.Dev**	**2.5 %**	**97.5 %**
Roost	0	5e−05	−5.984155	−4.187008
RVLabUnit:(ExtrPlate:(PCRPlate:Year))	0.045431	0.213146	0.192292	2.891935
ExtrPlate:(PCRPlate:Year)	0	0.000107	0.626552	3.655024
PCRPlate:Year	0.088641	0.297727	1.027279	3.834023
Year	0	3.9e−05	−0.062588	2.481058
zi.(Intercept)	NA	NA	0.891117	1.083606
zi.AbsHTrend	NA	NA	0.235539	0.597988
zi.AbsTTrend	NA	NA	−0.764021	−0.386589
zi.HMin	NA	NA	0	Inf
zi.TMin	NA	NA	0.099117	0.458363
disp.(Intercept)	NA	NA	0	Inf
disp.AbsTTrend	NA	NA	0.172171	0.514844
disp.HMax	NA	NA	0	Inf

C

	OR 2.5 %	**OR 97.5 %**	**OR Estimate**	**2.5 %**	**97.5 %**	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**	**holm**	**p_glht**	**ghlt Estimate**	**ghlt lwr**	**ghlt upr**
cond.(Intercept)	4.86828	8.275008	6.347051	1.582741	2.11324	1.84799	0.135334	13.65504	0	0	0	1.84799	1.466661	2.229319
cond.HottestDay	1.751029	3.982422	2.640708	0.560204	1.38189	0.971047	0.209618	4.632466	4e−06	3.3e−05	4.1e−05	0.971047	0.380409	1.561685
cond.HottestInfo	0.687304	1.217892	0.914911	−0.374979	0.197122	−0.088929	0.145947	−0.609322	0.542311	1	0.99922	−0.088929	−0.500161	0.322304
cond.MoistestDay	0.82932	1.822786	1.229501	−0.187149	0.600366	0.206609	0.2009	1.028414	0.303755	0.911265	0.957055	0.206609	−0.359466	0.772683
cond.HTrend	0.420193	0.930242	0.625205	−0.867042	−0.07231	−0.469676	0.202741	−2.316627	0.020524	0.143669	0.183698	−0.469676	−1.040938	0.101586
cond.MoistestInfo	0.711217	1.259157	0.946327	−0.340777	0.230442	−0.055167	0.145722	−0.37858	0.705	1	0.999993	−0.055167	−0.465767	0.355432
cond.HMax	0.189924	0.41419	0.280472	−1.661132	−0.88143	−1.271281	0.198907	−6.391323	0	0	0	−1.271281	−1.83174	−0.710822
cond.TMax	0.301619	0.687072	0.45523	−1.19859	−0.375316	−0.786953	0.210023	−3.746989	0.000179	0.001432	0.002024	−0.786953	−1.378732	−0.195174
cond.Days	0.224044	0.543805	0.349051	−1.495911	−0.609164	−1.052537	0.226215	−4.652818	3e−06	3.3e−05	5.2e−05	−1.052537	−1.689941	−0.415133
cond.Sess2	0.946491	1.924887	1.349774	−0.054994	0.654867	0.299937	0.18109	1.656284	0.097664	0.488322	0.599503	0.299937	−0.21032	0.810193
cond.HottestDay:HottestInfo	0.690107	4.851051	1.829685	−0.370908	1.579195	0.604144	0.497485	1.214397	0.224596	0.898385	0.889612	0.604144	−0.797614	2.005901
cond.HTrend:MoistestInfo	0.923628	3.881458	1.893416	−0.079446	1.356211	0.638382	0.366246	1.743044	0.081326	0.487956	0.533798	0.638382	−0.393585	1.670349

Figure Supp_17 7 Graphical representation of model estimates (coloured dots) with corresponding value above using the "sjPlot" package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. The corrective model estimates are also included (dispersion, zero_inflated) but might not be reliable since we detected pairwise correlation above 0.6 in them (Figure Supp_12 3A). Since the goal of the corrective model was to correct model assumption violations by correctly model zeroes and dispersion, the interpretation of the corrective model estimates is therefore neglected in the study.

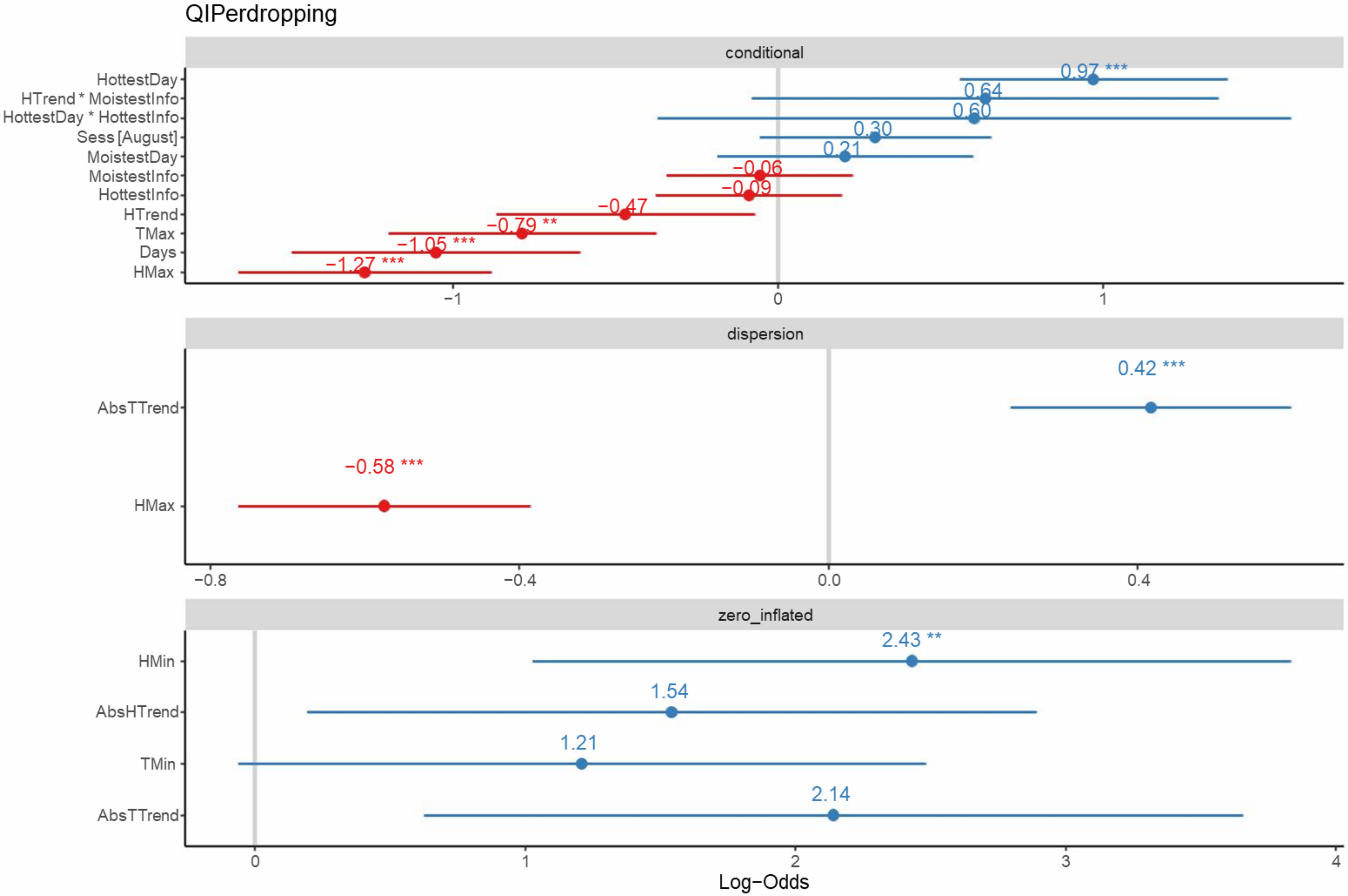
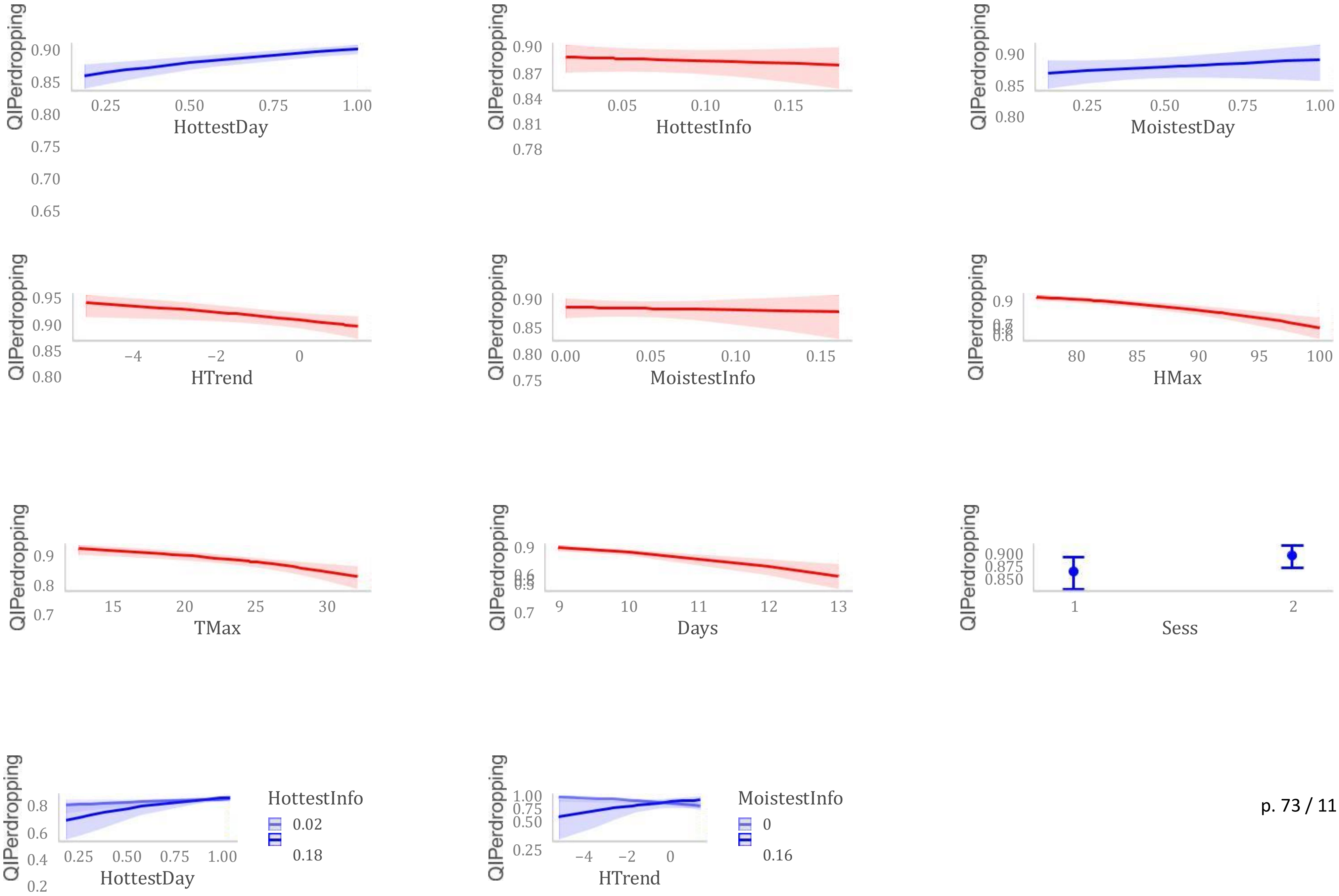


Figure Supp_17 8 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the “ggeffects”. 95 % confidence intervals are indicated by the lighter shape. Red indicates negative, blue positive effects. X-axis represent the observed unstandardized predictors. “QIPerdropping” is equal to QI used in the publication and just emphasizes that QI was calculated over the whole sample (across 3 replicates with 9 loci each totaling in 27 loci). For continuous interaction terms the minimum and maximum observed values were used for plotting (e.g.: HMax:TMax, bottom right).



Supp_18 Information about the "Drying effect" model

Model diagnostics and information for the auxiliary model investigating whether the positive effect of "HottestDay" was only positive if it was also humid and the hottest day was also hot (see **Supp_16**) created with the mdiag() function included in the version controlled R-Project. Not all information from the 29-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved.

Model Overview

name: drying_effect_model

family: betabinomial
link: logit

response variable form: matrix

sample size: 2309

N (sample size) / k (terms) : 256.6

formula: QIPerdropping ~ HottestDay * TMax * HTrend + HMax + Sess + (1 | Roost) + (1 | Year/PCRPlate/ExtrPlate/RVLabUnit)

ziformula: ~AbsHTrend + AbsTTrend + HMin + TMin

dispformula: ~AbsTTrend + HMax

Figure Supp_18 1 Model diagnostics based on simulated residuals using the “DHARMa” package based on 10 000 simulations. A) Dispersion test showing no significant overdispersion with the observed (red line) variance being 1.06 times stronger than the mean simulated variance (black lines). B) Zero-inflation test shows no significant zero-inflation with the number of observed zeroes (red line) is 1.09 higher than the mean of the simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows no significant outliers (red bars and line). D) Quantile-Quantile plot of observed and expected residuals shows significant but weak deviation of uniform distribution (red line).

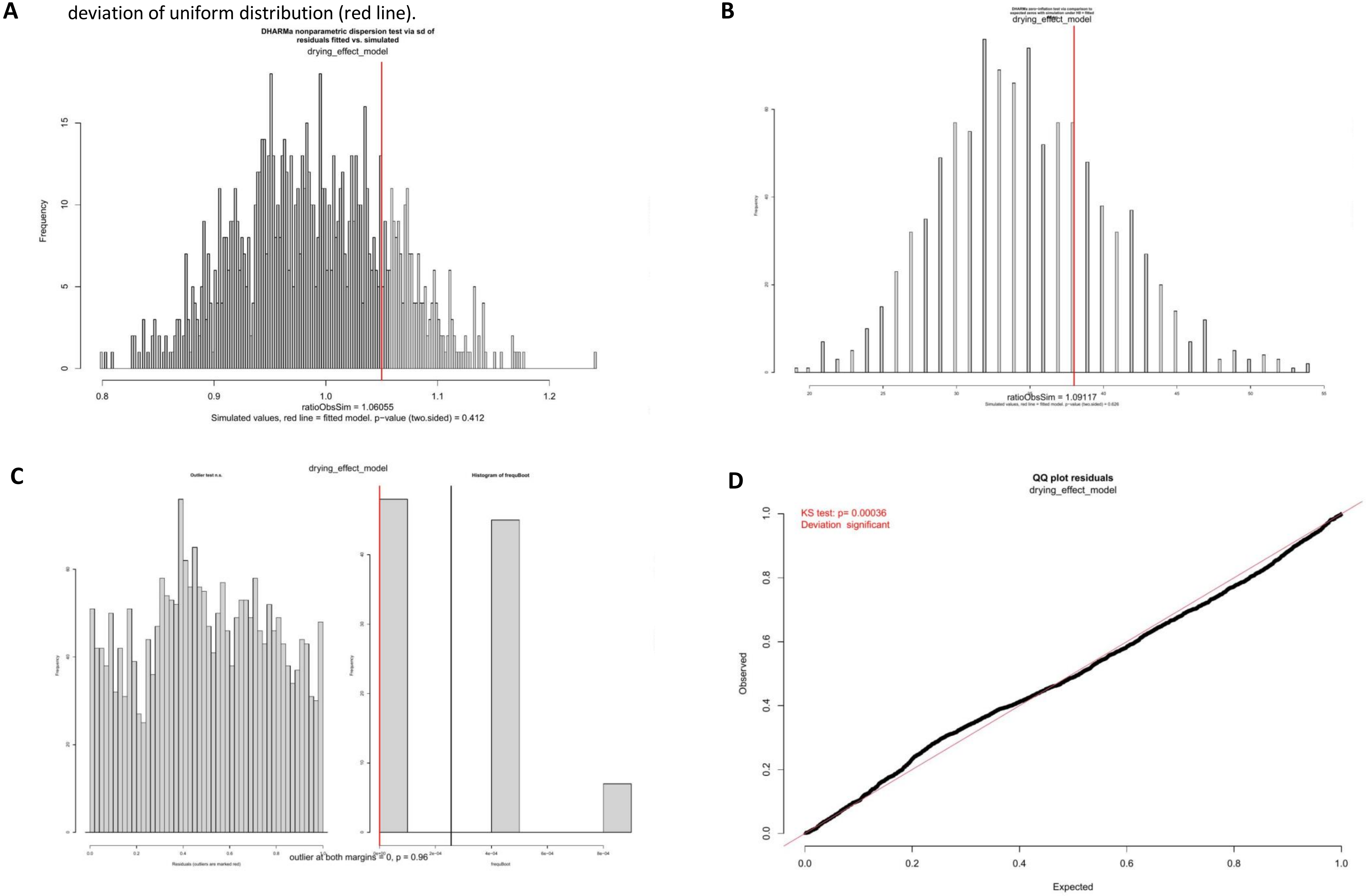


Figure Supp_18 2 Model diagnostics based on simulated residuals using the “DHARMa” package based on 10 000 simulations. A) Testing excess/deficits of QI = 1. Since QI was expressed in matrix form (success/(total number of loci) A QI of 1 means in Matrix form 27/27. We detected no significant excess/deficit with 0.88 times the number of ones observed compared to the mean number of ones from simulations (black bars).

B) The same as A) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem (see main text). It shows no significant excess/deficit of ones with 0.95 times ones observed compared to the mean number of ones from simulations.

C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate weak heteroscedasticity.

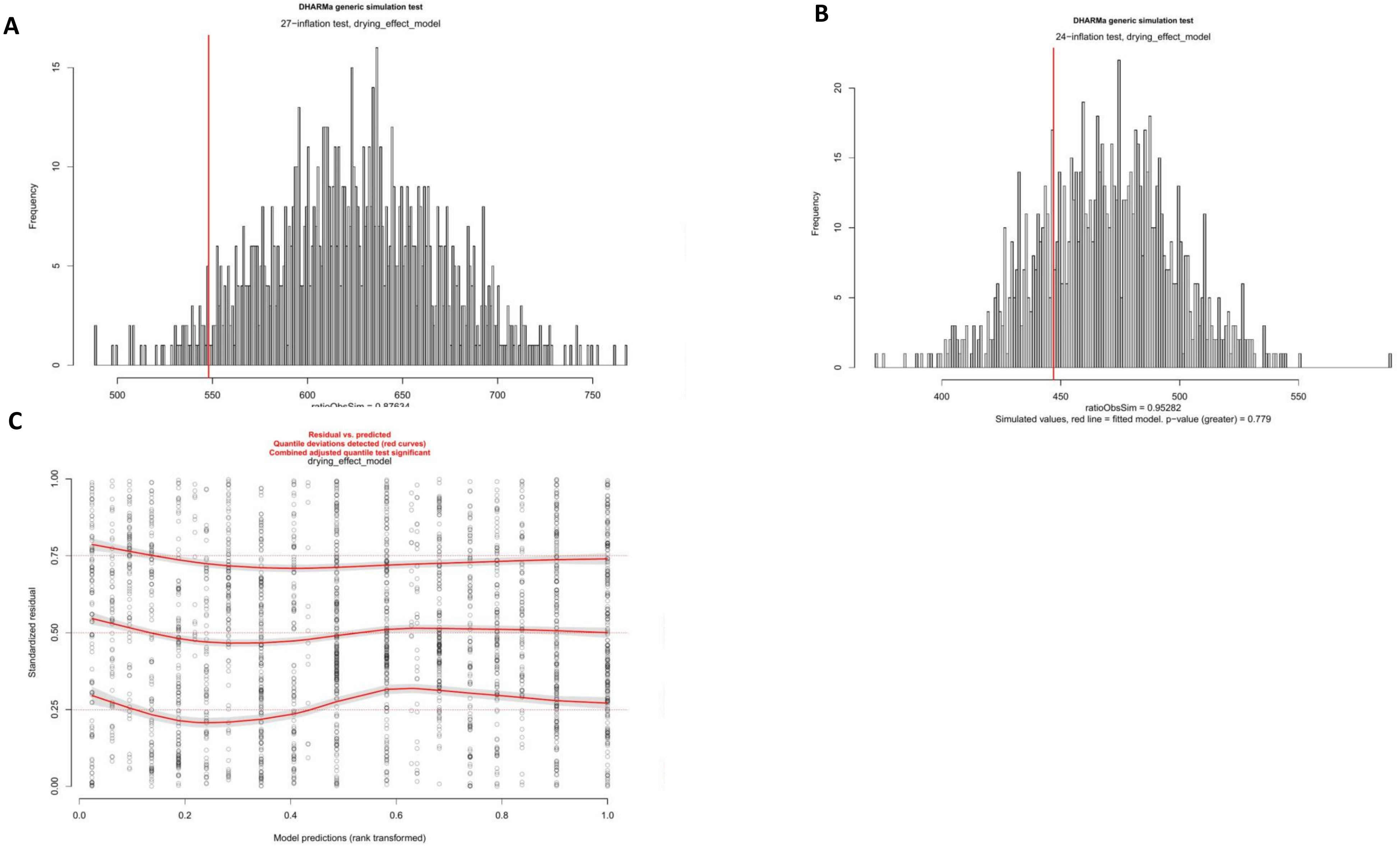


Figure Supp_18 3 Correlations of input variables and multicollinearity. A) Pairwise correlation of all input variables (2309 values per variable) using «Hmisc» and "corrplot" package. Lower triangle indicates Spearman's rank correlation; higher triangle indicates Pearson correlation. Lighter colour indicates weaker correlation darker colours stronger correlation. Reddish colours indicate negative correlation, blueish indicate positive correlations. Numbers indicate correlations. Correlations under 0.6 are regarded unproblematic in this study. Note that correlations with variables from the corrective model (AbsHTrend, AbsTTrend, HMin, TMin) are also calculated. Correlation containing those predictors were ignored since we weren`t interested in their estimates but only in modeling zeroes and dispersion to fulfill model assumptions. B) shows multicollinearity measured as variance inflation factor (VIF) using the "performance" package. All VIFs are under 5 and therefore fulfilling model assumptions.

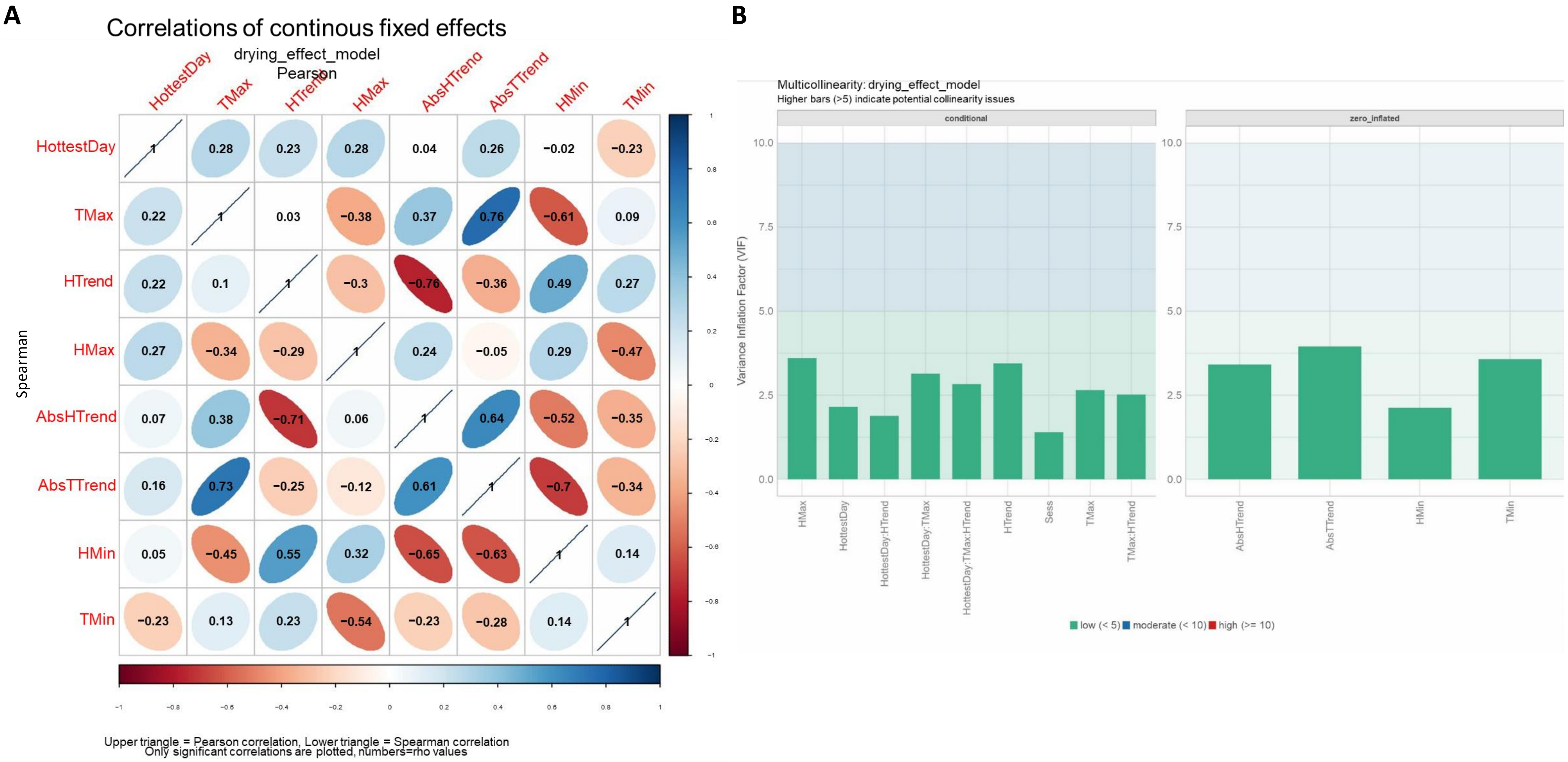


Figure Supp_18 4 Temporal autocorrelation test using DHARMa package based on 10 000 simulations on and sampling timing with year/sess. No significant temporal autocorrelation was detected using the Durbin-Watson test p=0.58.

We also performed a spatial autocorrelation test (Moran's test for distance-based autocorrelation) with those residuals and could not detect significant spatial autocorrelation (p=0.43). The graph with coordinates is not shown because lesser horseshoe bat (*Rhinolophus hipposideros*) is an endangered and protected species in Germany and roosts location cannot be disclosed.

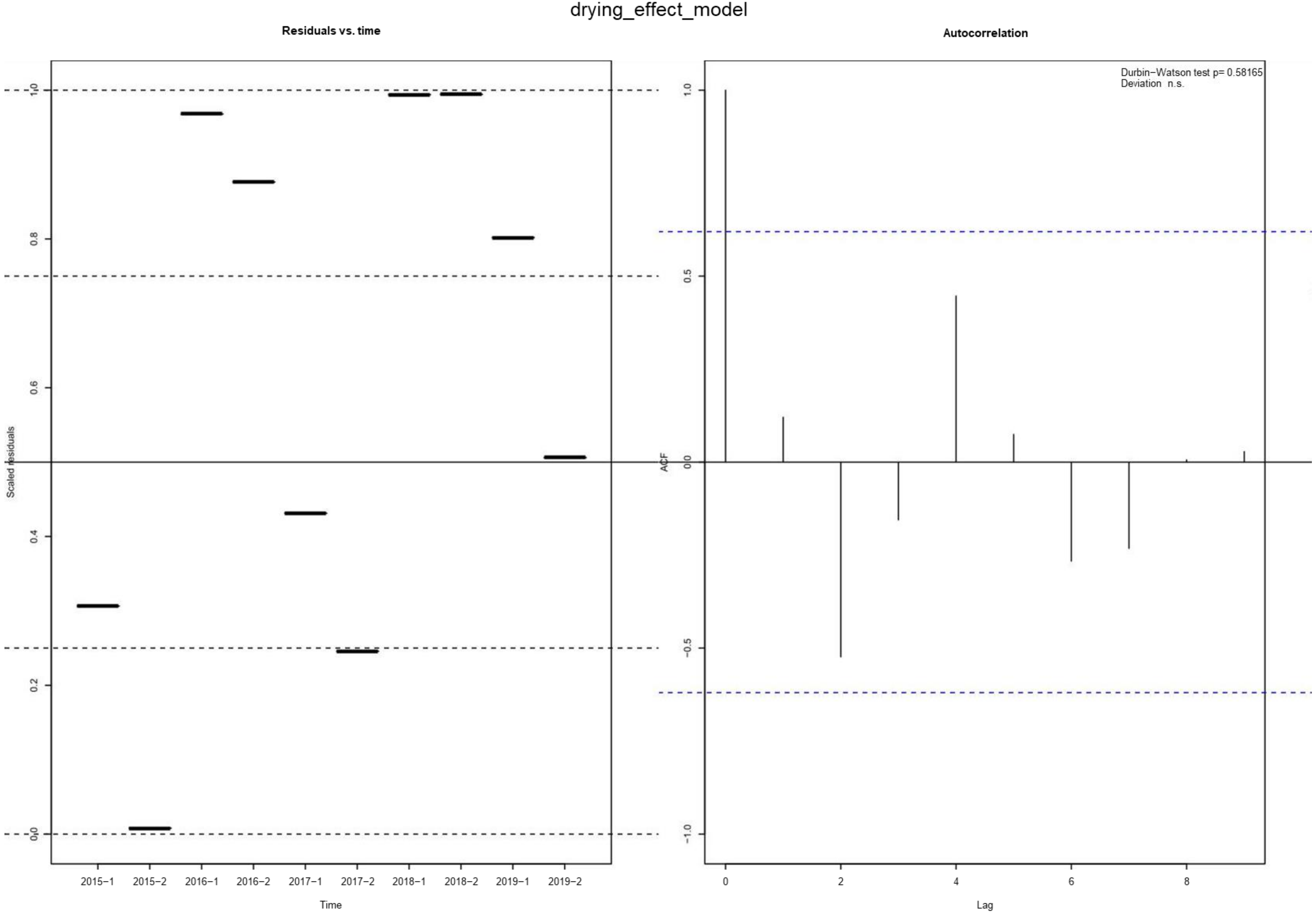


Figure Supp_18 5 Normality of Random Effects using the “performance” and “See” package. No strong deviation was detected.

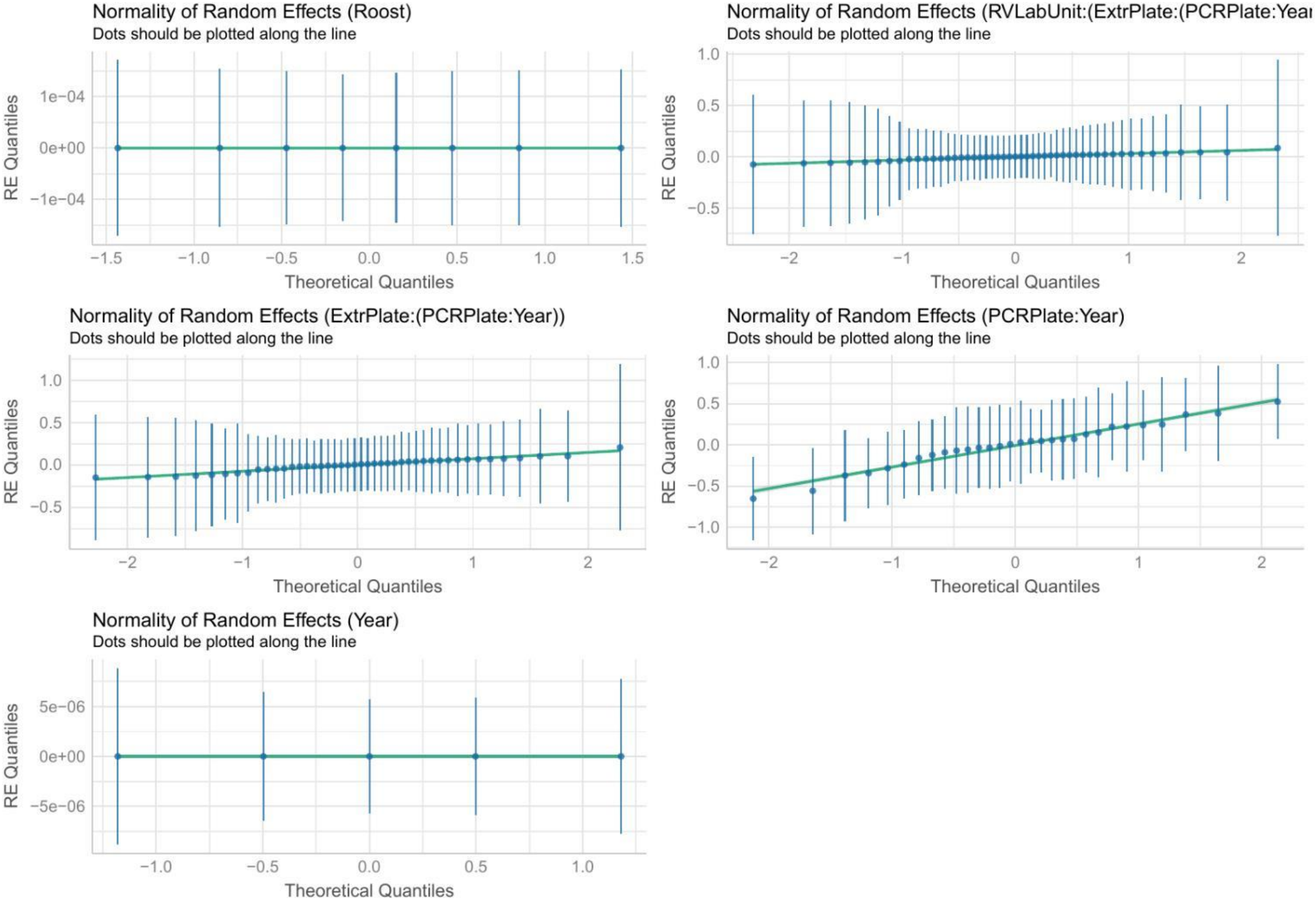
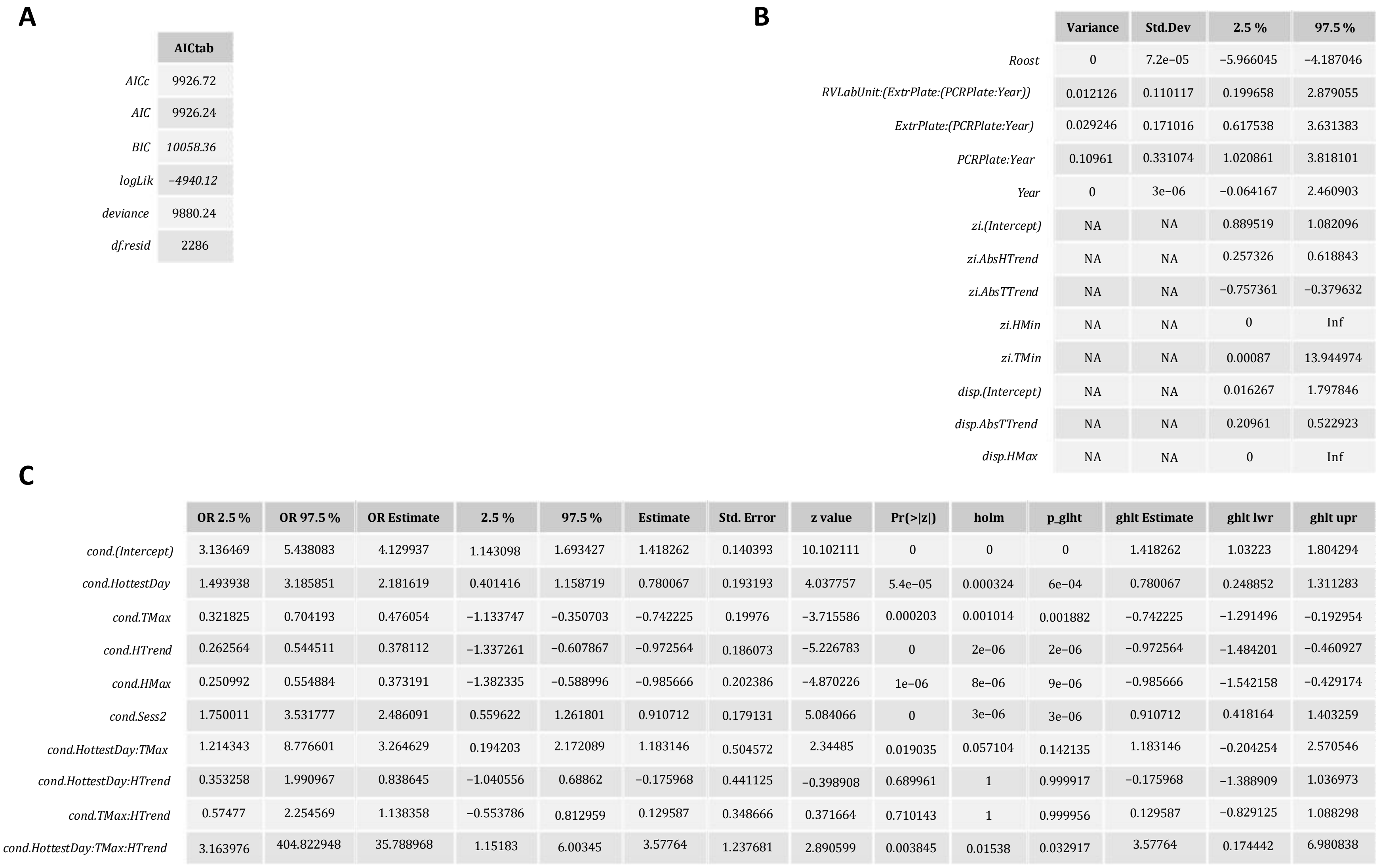


Figure Supp_18 6 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval for the random effects and corrective model (where possible). C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the “multcomp” package and the “glht” function.



A

	AICtab
AICc	9926.72
AIC	9926.24
BIC	*10058.36*
logLik	*−4940.12*
deviance	9880.24
df.resid	2286

B

	Variance	Std.Dev	2.5 %	97.5 %
Roost	0	7.2e−05	−5.966045	−4.187046
RVLabUnit:(ExtrPlate:(PCRPlate:Year))	0.012126	0.110117	0.199658	2.879055
ExtrPlate:(PCRPlate:Year)	0.029246	0.171016	0.617538	3.631383
PCRPlate:Year	0.10961	0.331074	1.020861	3.818101
Year	0	3e−06	−0.064167	2.460903
zi.(Intercept)	NA	NA	0.889519	1.082096
zi.AbsHTrend	NA	NA	0.257326	0.618843
zi.AbsTTrend	NA	NA	−0.757361	−0.379632
zi.HMin	NA	NA	0	Inf
zi.TMin	NA	NA	0.00087	13.944974
disp.(Intercept)	NA	NA	0.016267	1.797846
disp.AbsTTrend	NA	NA	0.20961	0.522923
disp.HMax	NA	NA	0	Inf

C

	OR 2.5 %	OR 97.5 %	OR Estimate	2.5 %	97.5 %	Estimate	Std. Error	z value	Pr(>|z|)	holm	p_glht	ghlt Estimate	ghlt lwr	ghlt upr
cond.(Intercept)	3.136469	5.438083	4.129937	1.143098	1.693427	1.418262	0.140393	10.102111	0	0	0	1.418262	1.03223	1.804294
cond.HottestDay	1.493938	3.185851	2.181619	0.401416	1.158719	0.780067	0.193193	4.037757	5.4e−05	0.000324	6e−04	0.780067	0.248852	1.311283
cond.TMax	0.321825	0.704193	0.476054	−1.133747	−0.350703	−0.742225	0.19976	−3.715586	0.000203	0.001014	0.001882	−0.742225	−1.291496	−0.192954
cond.HTrend	0.262564	0.544511	0.378112	−1.337261	−0.607867	−0.972564	0.186073	−5.226783	0	2e−06	2e−06	−0.972564	−1.484201	−0.460927
cond.HMax	0.250992	0.554884	0.373191	−1.382335	−0.588996	−0.985666	0.202386	−4.870226	1e−06	8e−06	9e−06	−0.985666	−1.542158	−0.429174
cond.Sess2	1.750011	3.531777	2.486091	0.559622	1.261801	0.910712	0.179131	5.084066	0	3e−06	3e−06	0.910712	0.418164	1.403259
cond.HottestDay:TMax	1.214343	8.776601	3.264629	0.194203	2.172089	1.183146	0.504572	2.34485	0.019035	0.057104	0.142135	1.183146	−0.204254	2.570546
cond.HottestDay:HTrend	0.353258	1.990967	0.838645	−1.040556	0.68862	−0.175968	0.441125	−0.398908	0.689961	1	0.999917	−0.175968	−1.388909	1.036973
cond.TMax:HTrend	0.57477	2.254569	1.138358	−0.553786	0.812959	0.129587	0.348666	0.371664	0.710143	1	0.999956	0.129587	−0.829125	1.088298
cond.HottestDay:TMax:HTrend	3.163976	404.822948	35.788968	1.15183	6.00345	3.57764	1.237681	2.890599	0.003845	0.01538	0.032917	3.57764	0.174442	6.980838

Figure Supp_18 7 Graphical representation of model estimates (coloured dots) with corresponding value above using the “sjPlot” package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. The corrective model estimates are also included (dispersion, zero_inflated) but might not be reliable since we detected pairwise correlation above 0.6 in them (Figure Supp_13 3A). Since the goal of the corrective model was to correct model assumption violations by correctly model zeroes and dispersion, the interpretation of the corrective model estimates is therefore neglected in the study.

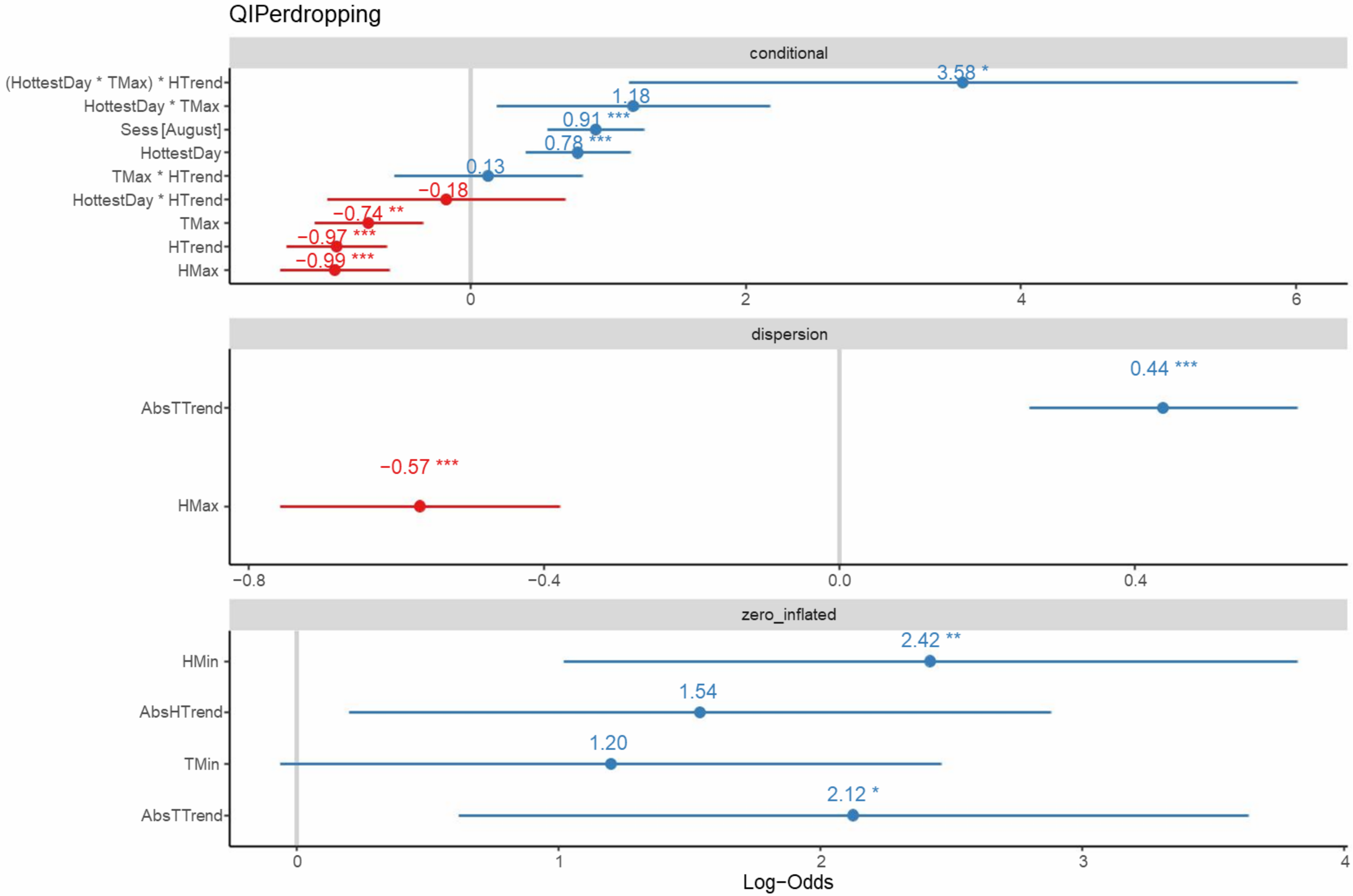
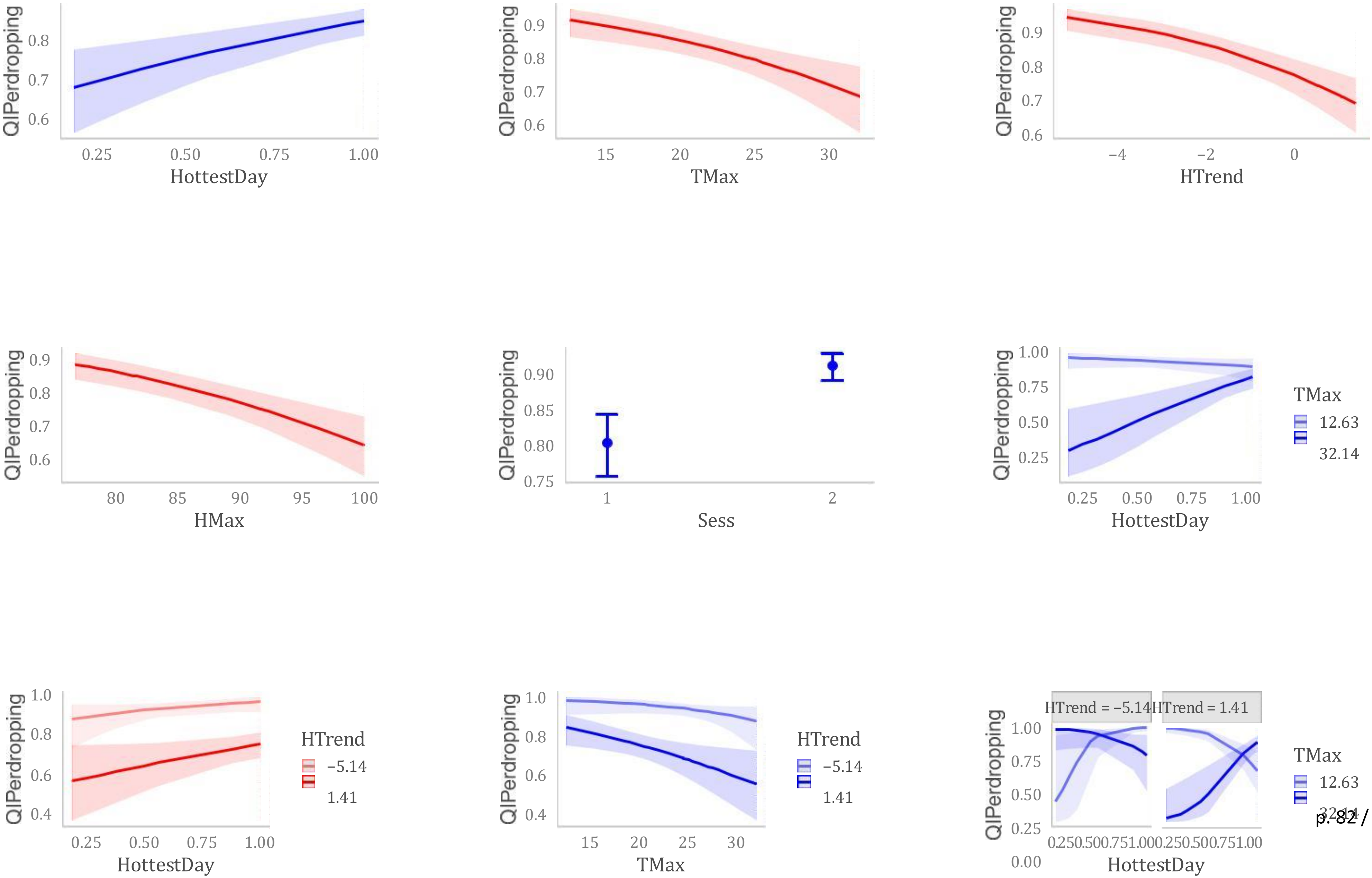


Figure Supp_18 8 Predicted marginal fixed effects (solid lines) on QI using ggpredict() from the “ggeffects”. 95 % confidence intervals are indicated by the lighter shape. Red indicates negative, blue positive effects. X-axis represent the observed unstandardized predictors. “QIPerdropping” is equal to QI used in the publication and just emphasizes that QI was calculated over the whole sample (across 3 replicates with 9 loci each totaling in 27 loci). For continuous interaction terms the minimum and maximum observed values were used for plotting (e.g.: HMax:TMax, bottom right).



Supp_19 Temporal separation of hottest and moistest hours on hottest day

Figure Supp_19 1 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

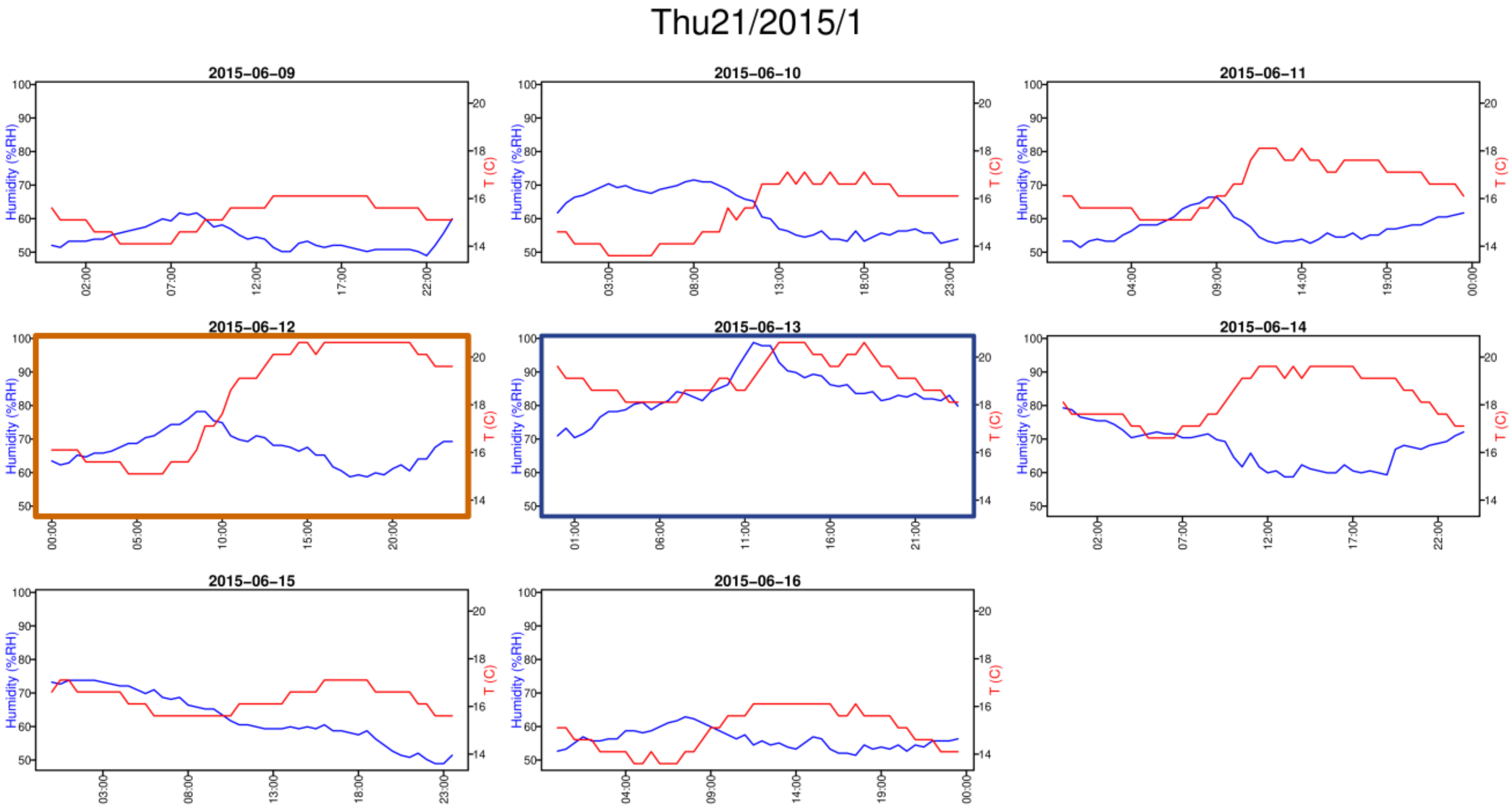


Figure Supp_19 2 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

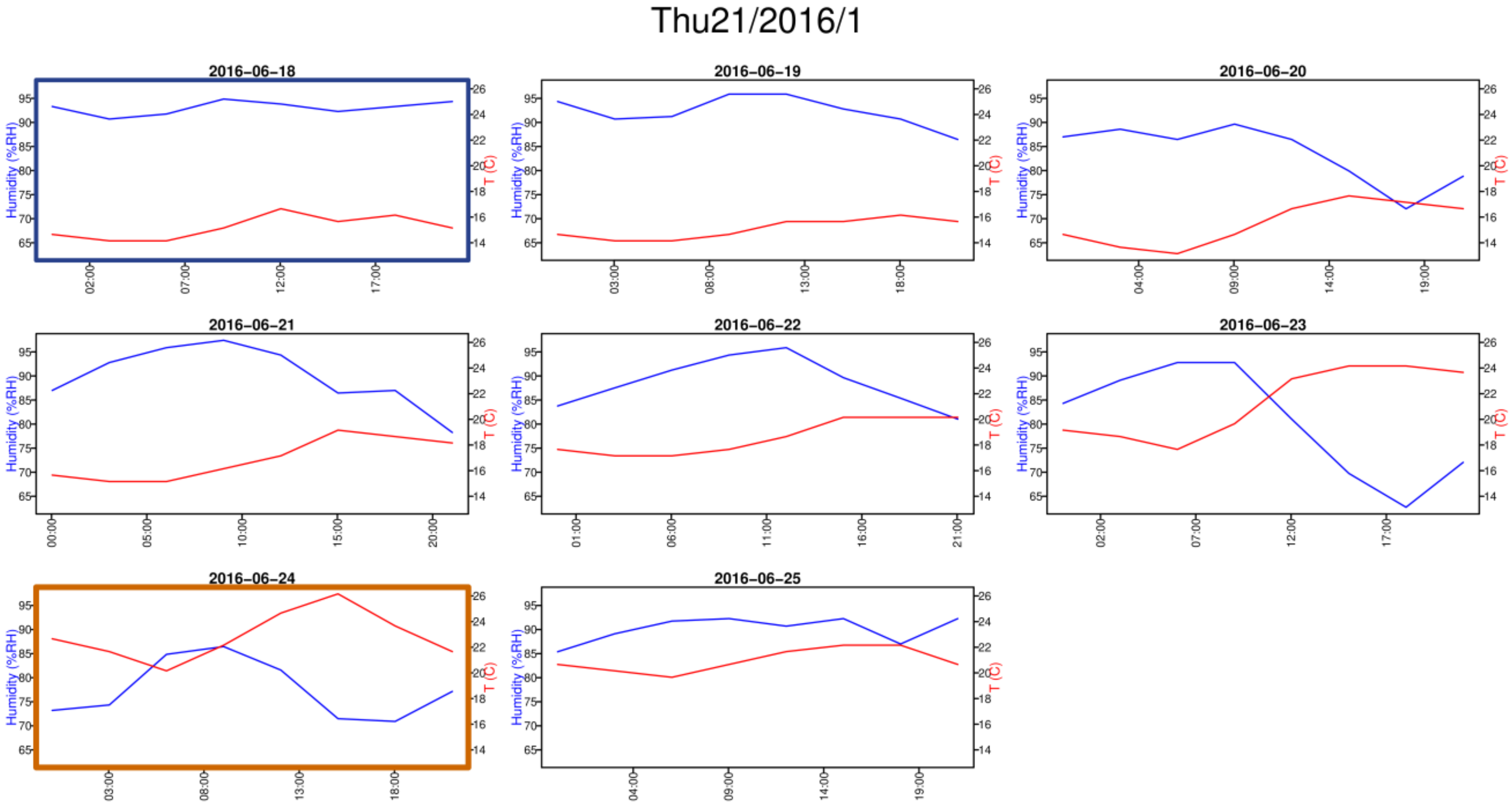
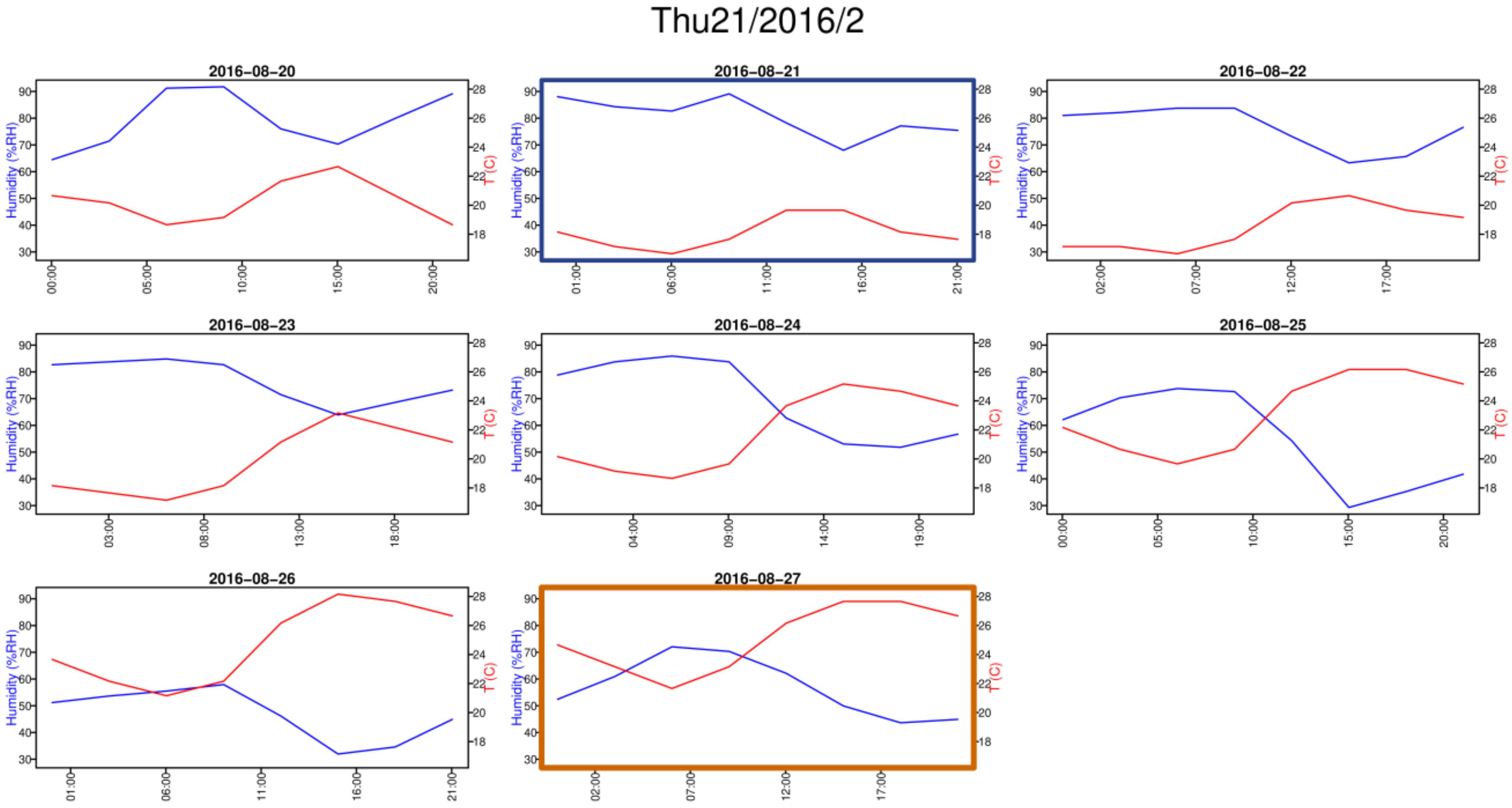


Figure Supp_19 3 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").



Thu21/2016/2

2016–08–20, 2016–08–21, 2016–08–22, 2016–08–23, 2016–08–24, 2016–08–25, 2016–08–26, 2016–08–27

Figure Supp_19 4 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

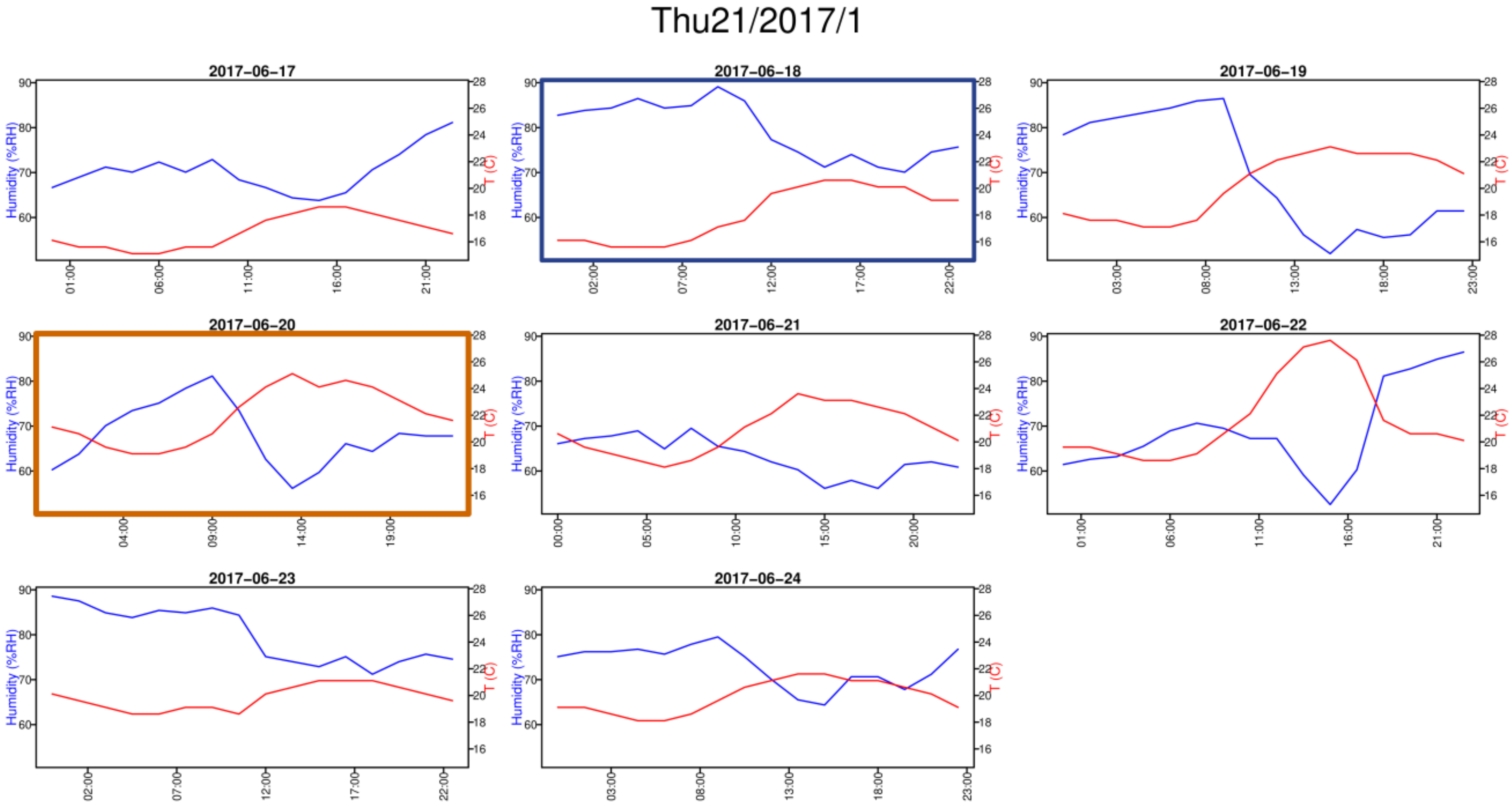


Figure Supp_19 5 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

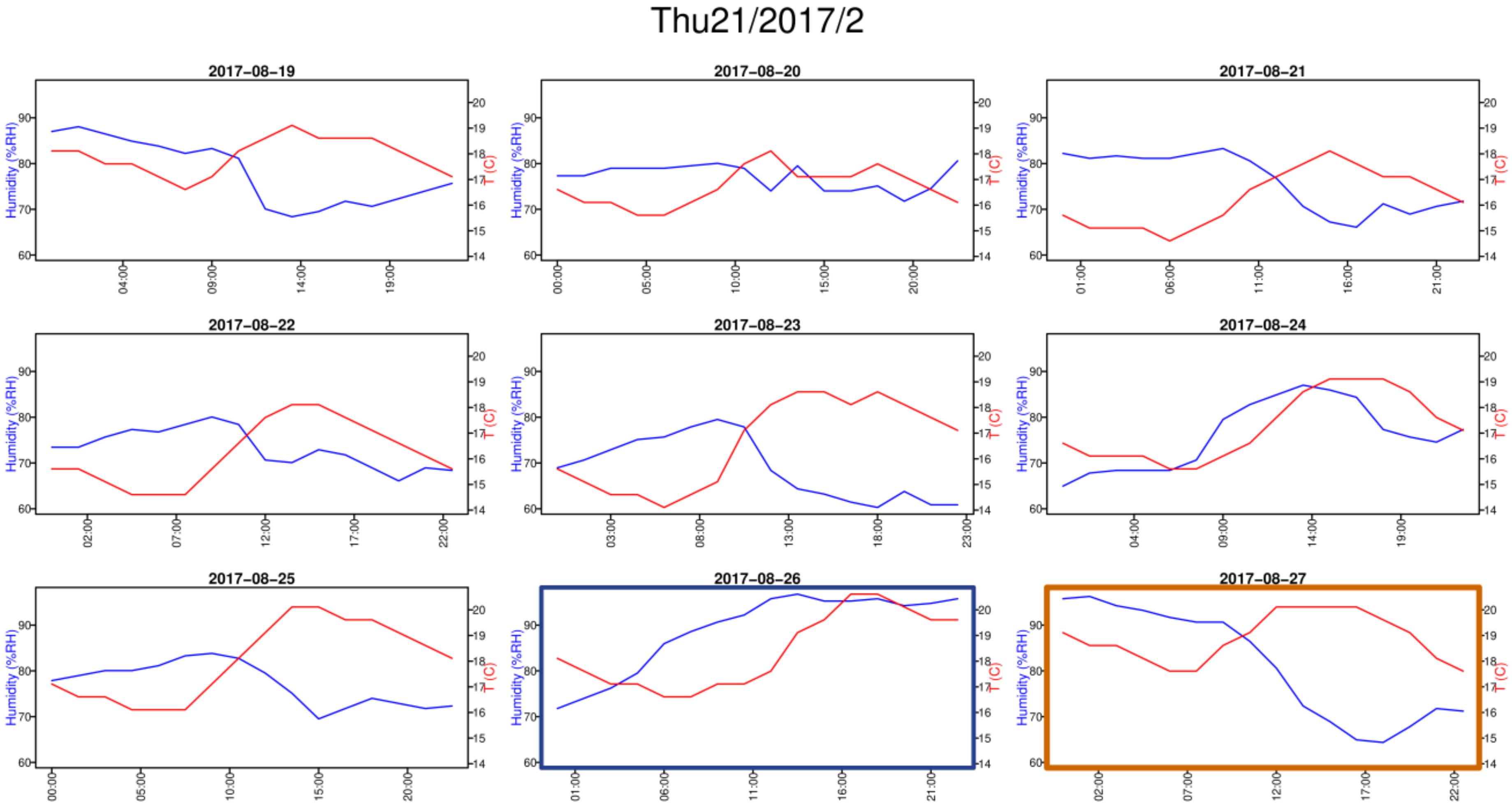


Figure Supp_19 6 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").

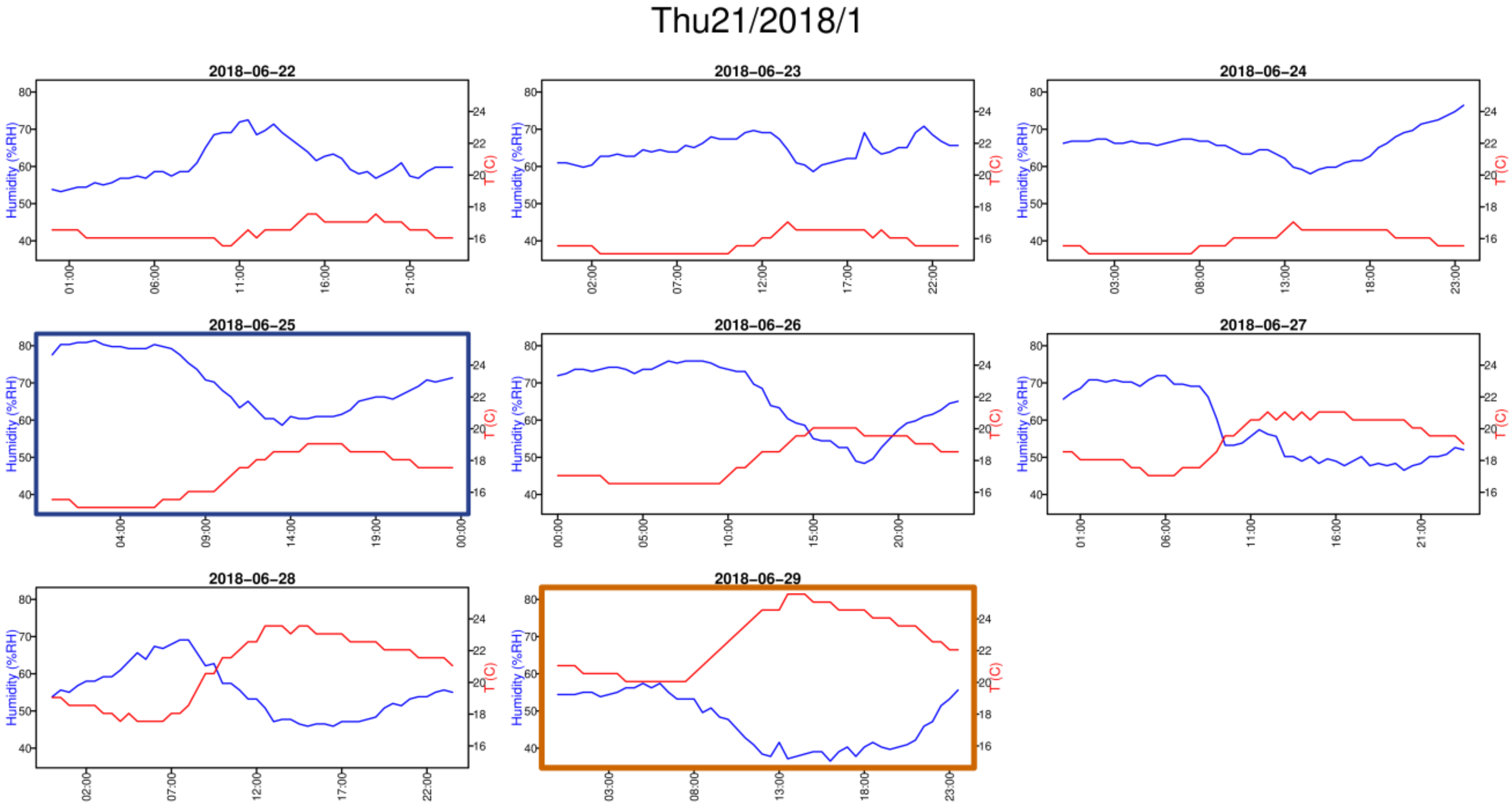


Figure Supp_19 7 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").

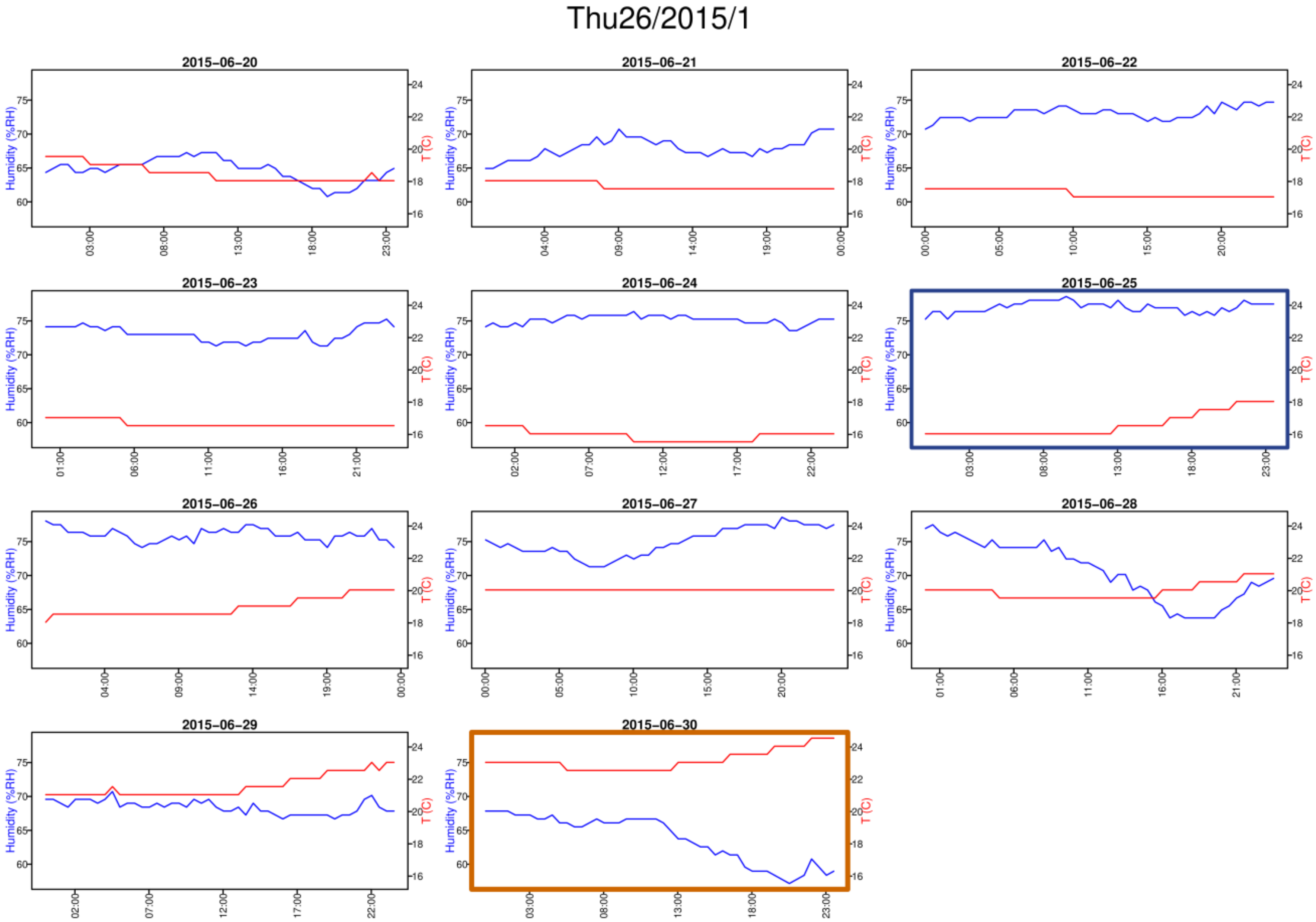


Figure Supp_19 8 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

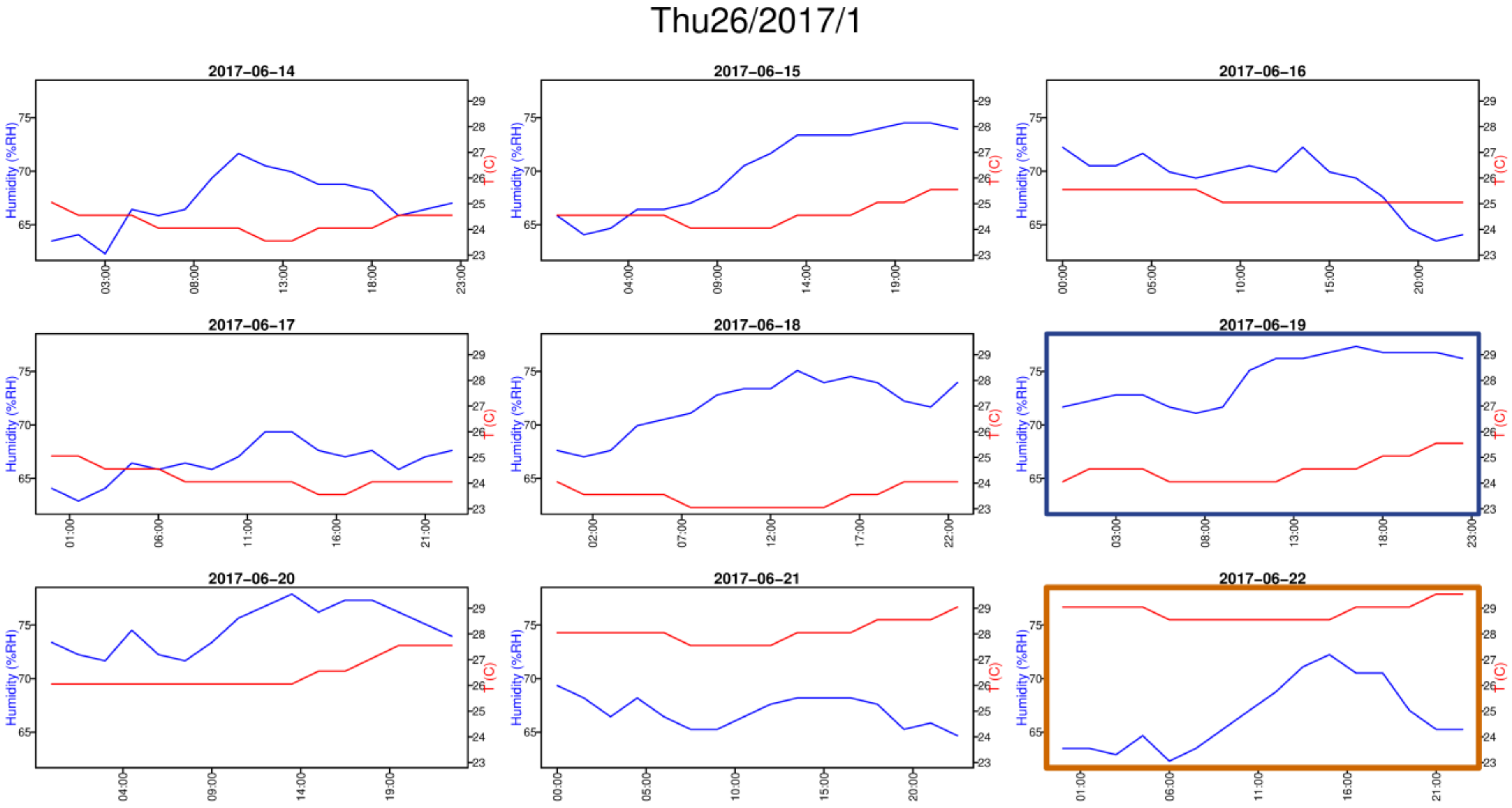


Figure Supp_19 9 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

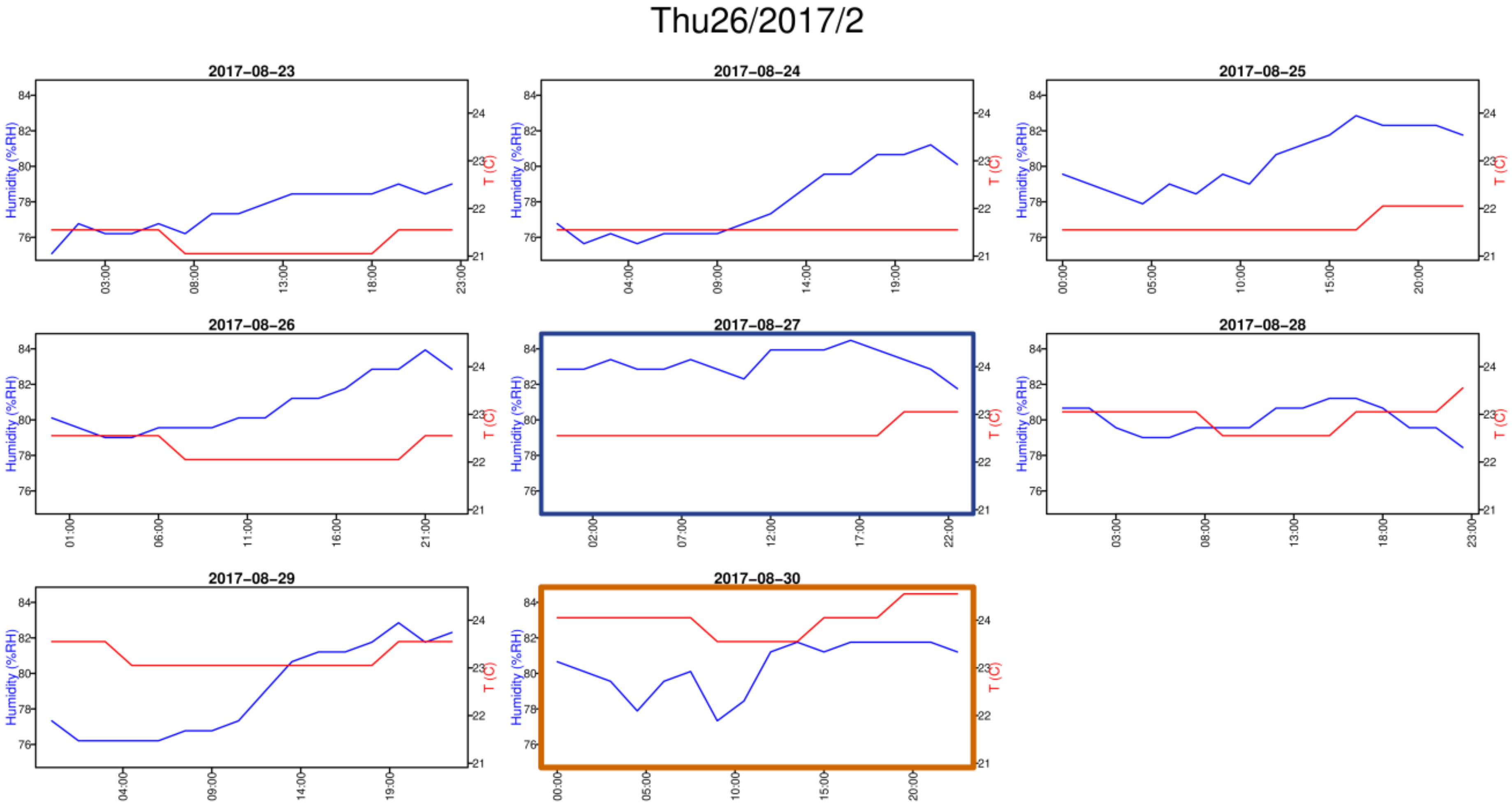


Figure Supp_19 10 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

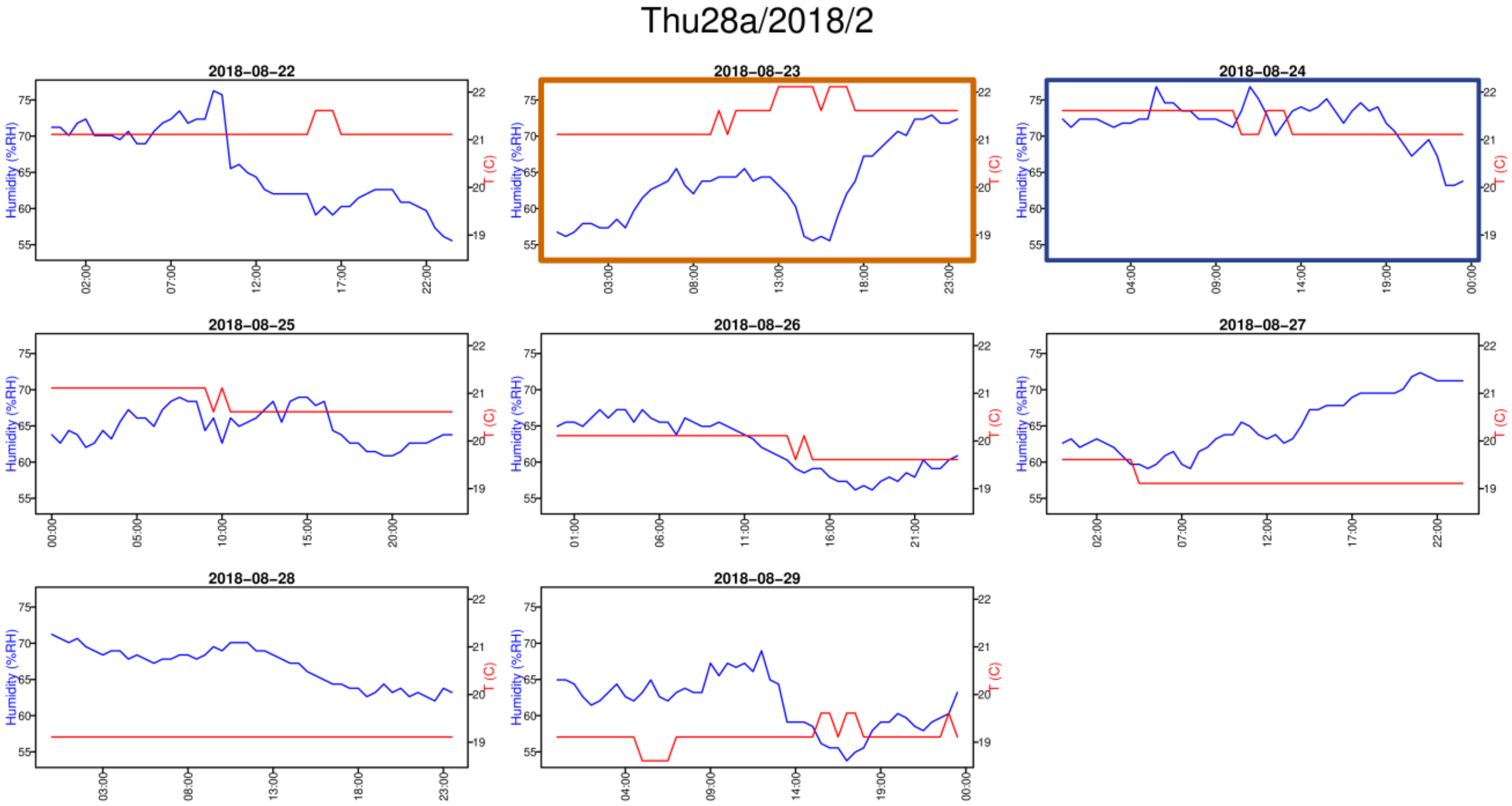


Figure Supp_19 11 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").

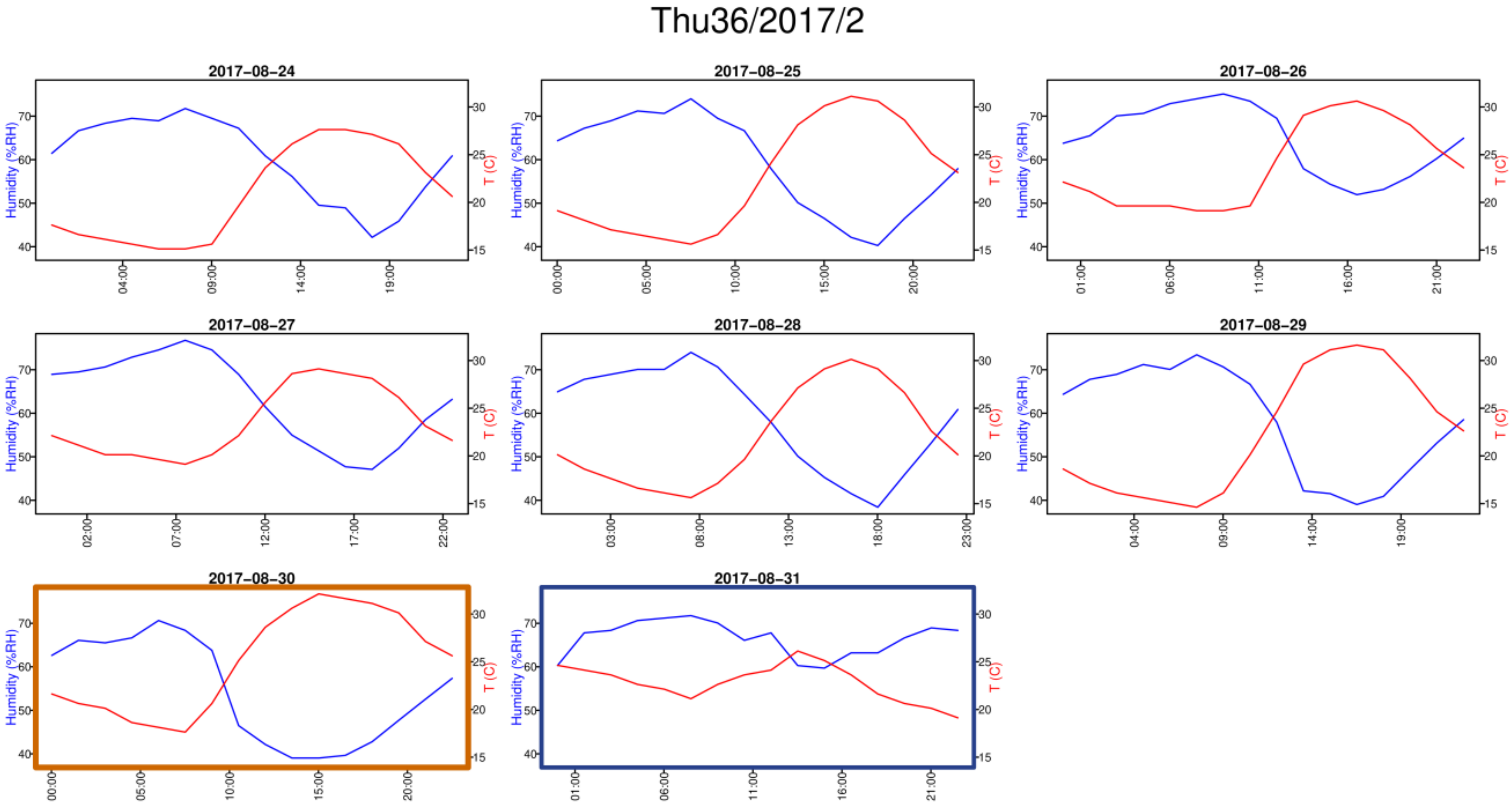


Figure Supp_19 12 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

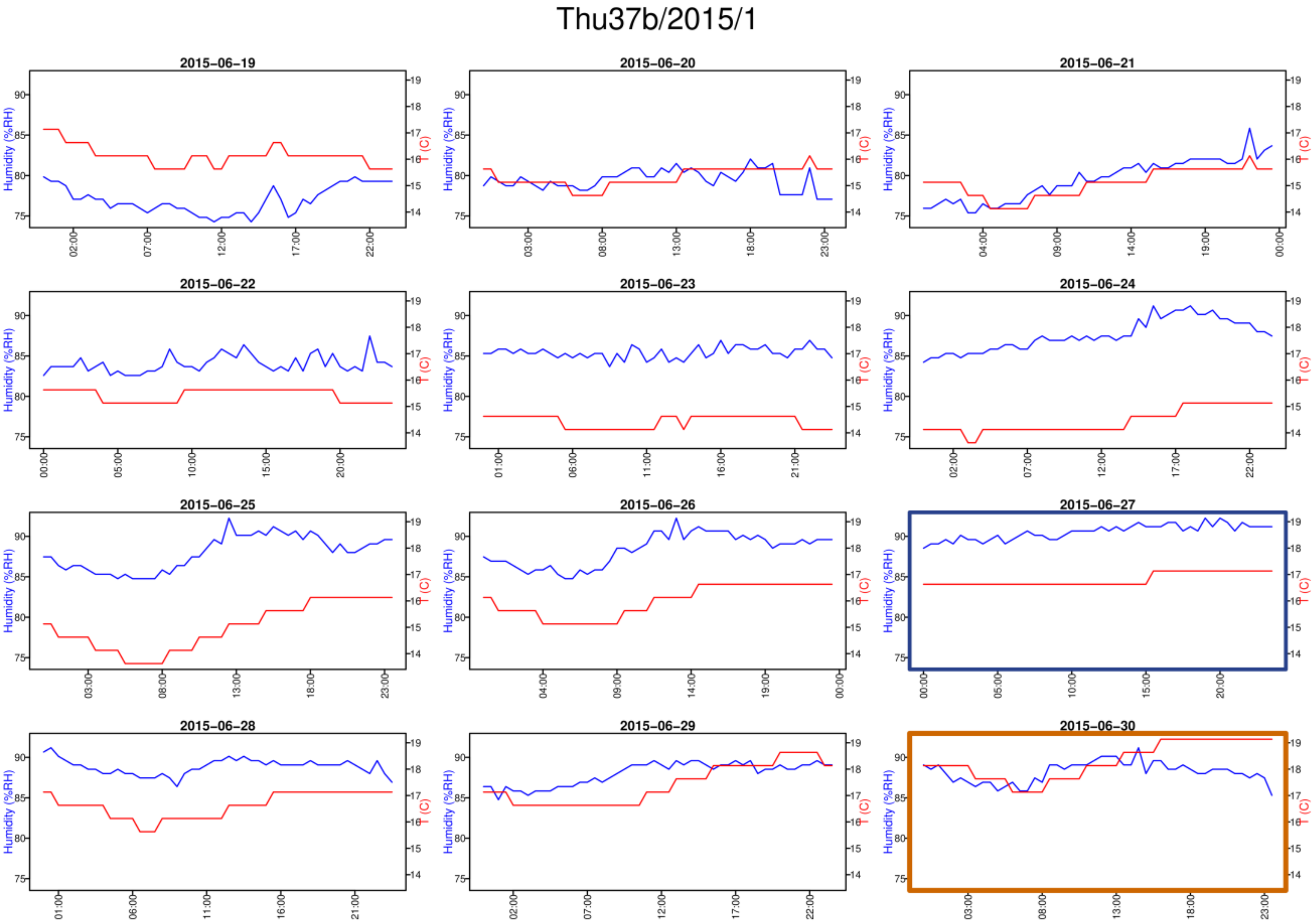


Figure Supp_19 13 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").

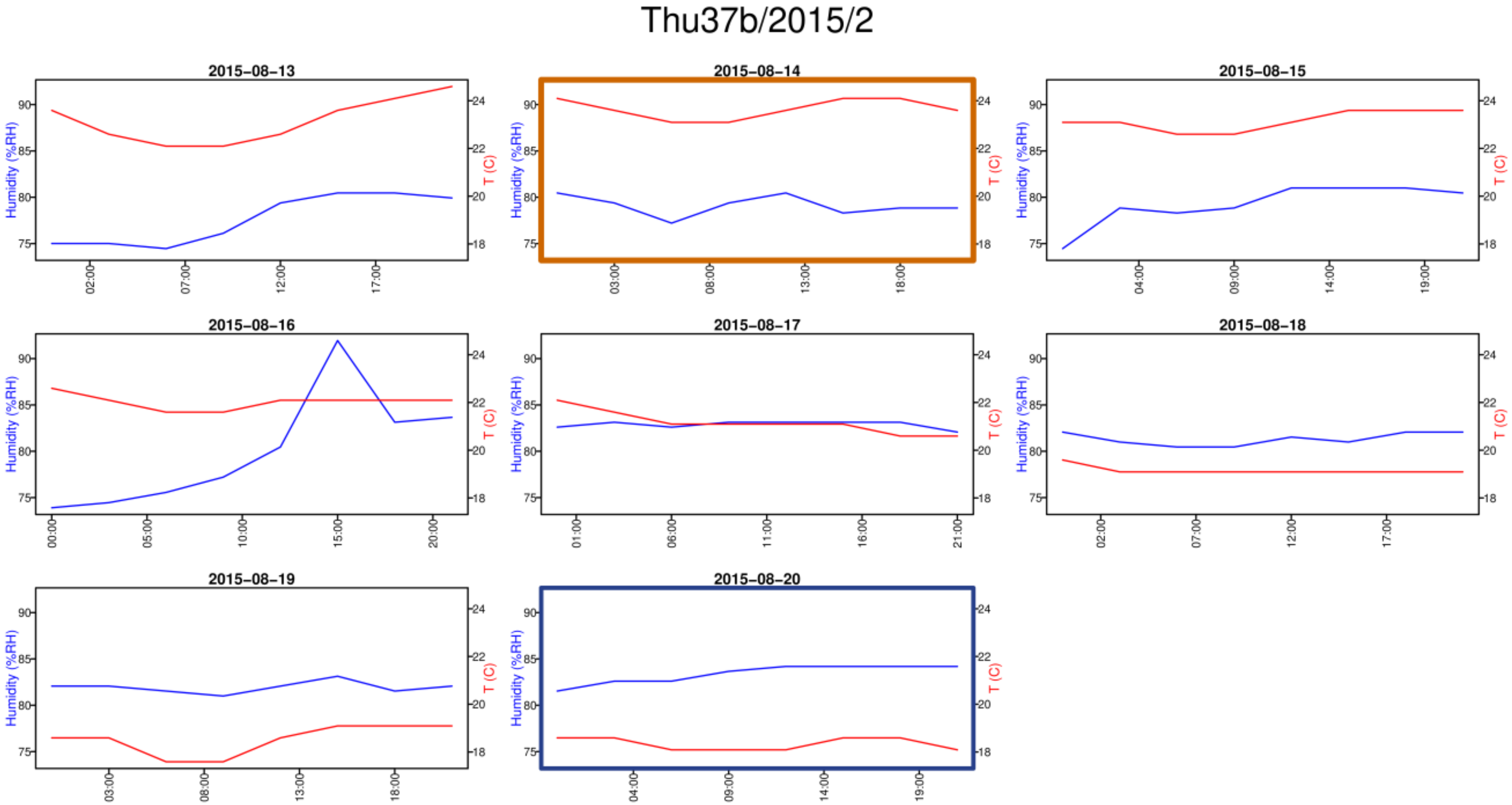


Figure Supp_19 14 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

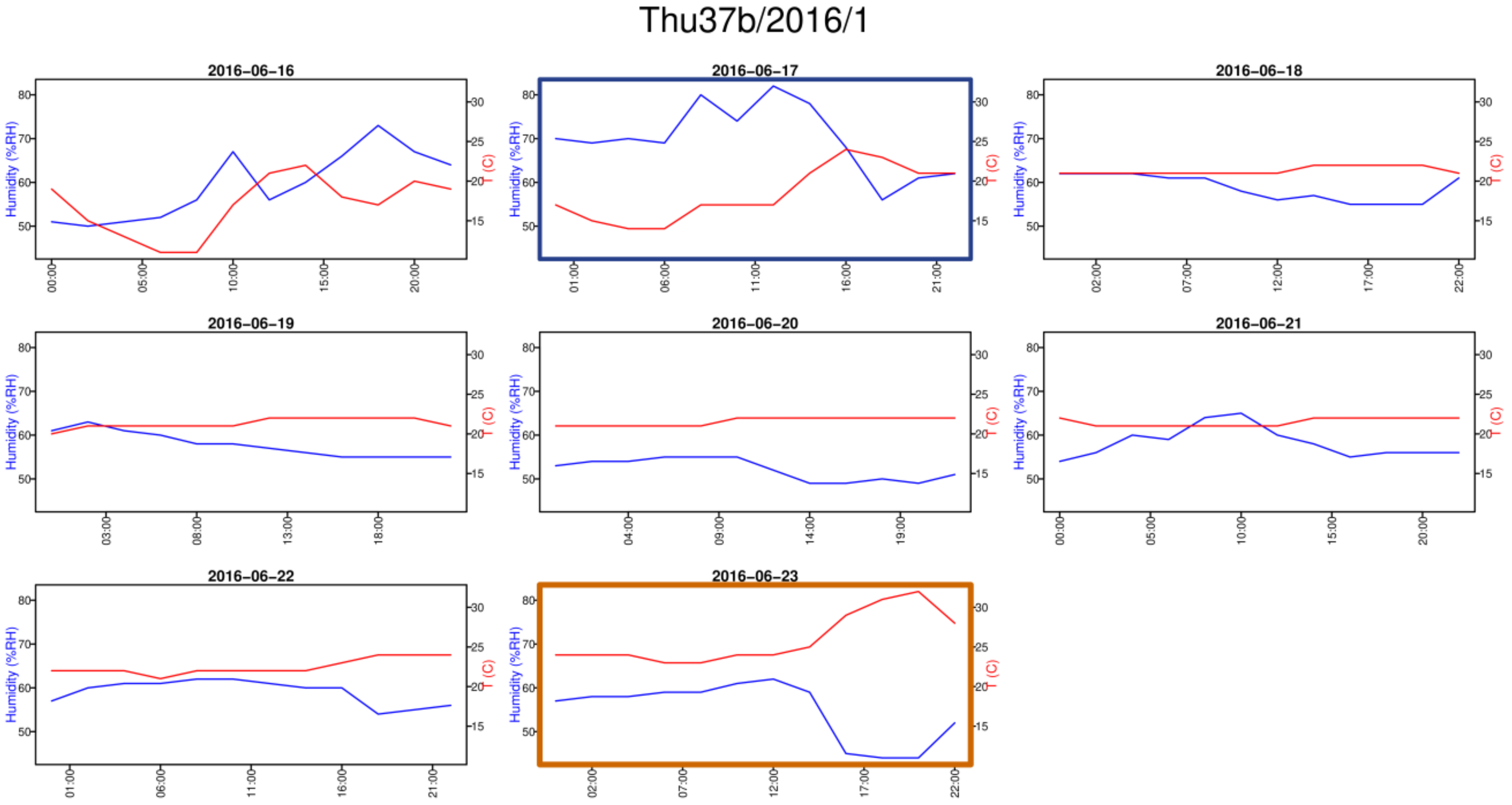


Figure Supp_19 15 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

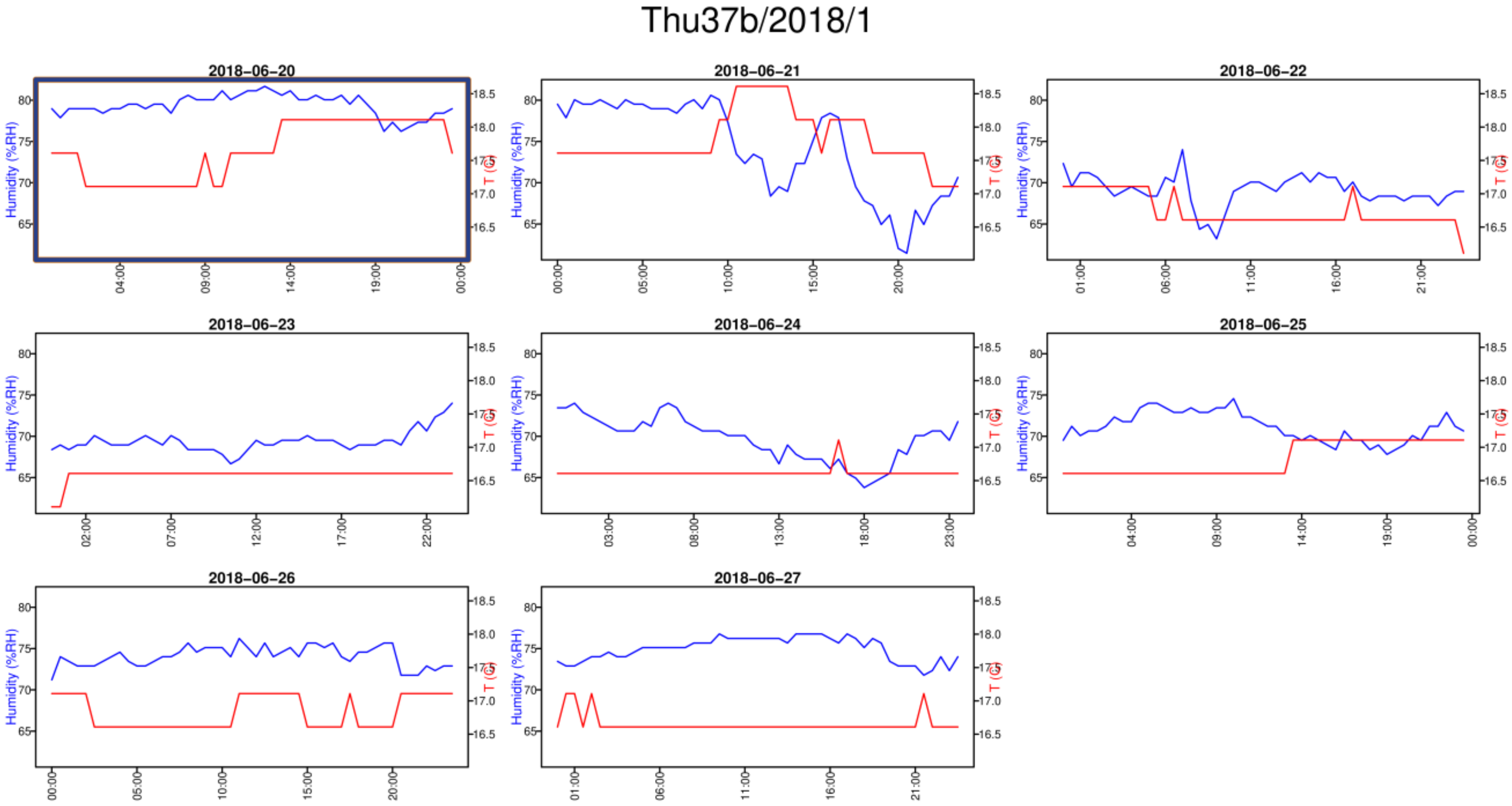


Figure Supp_19 16 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

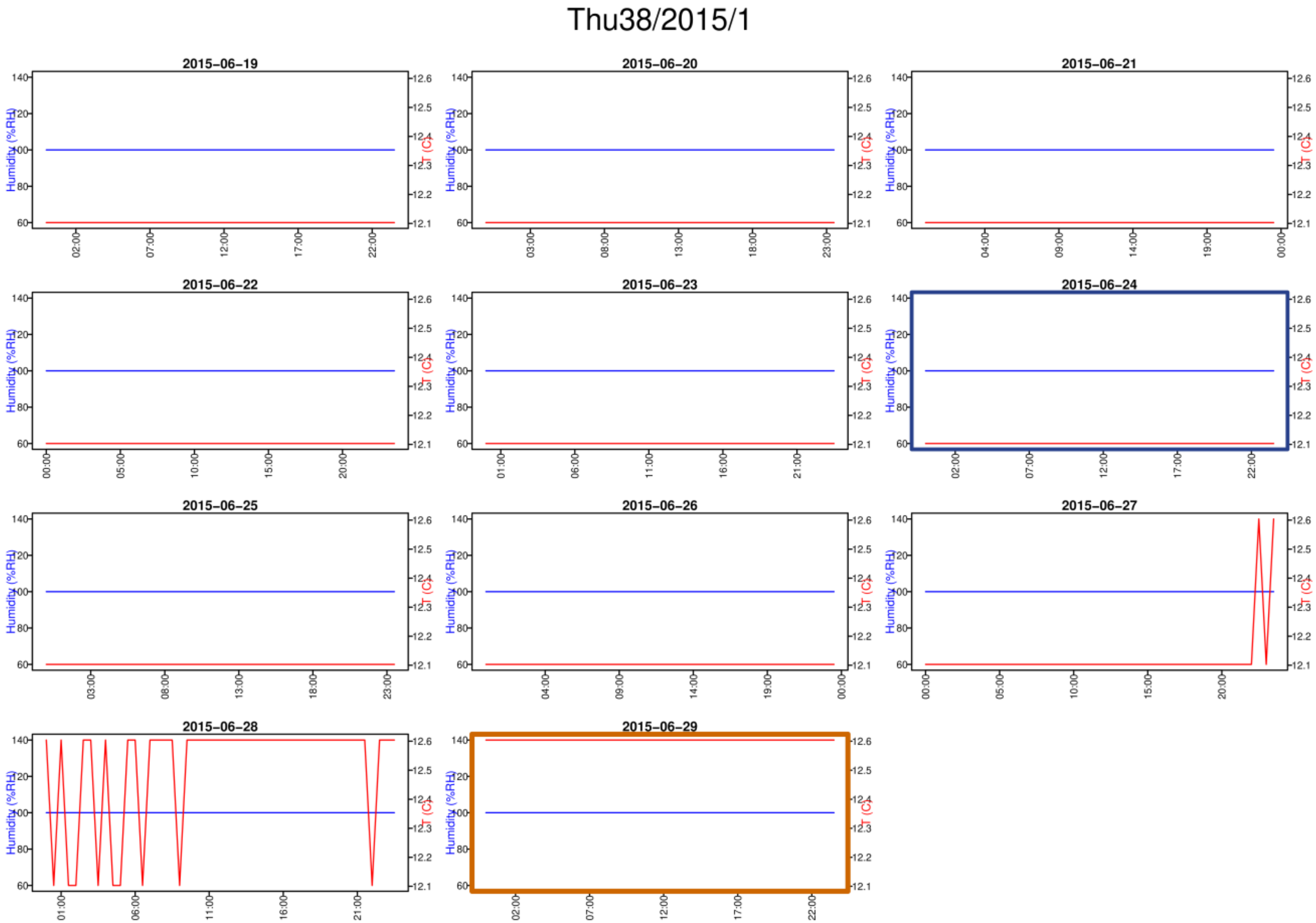


Figure Supp_19 17 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

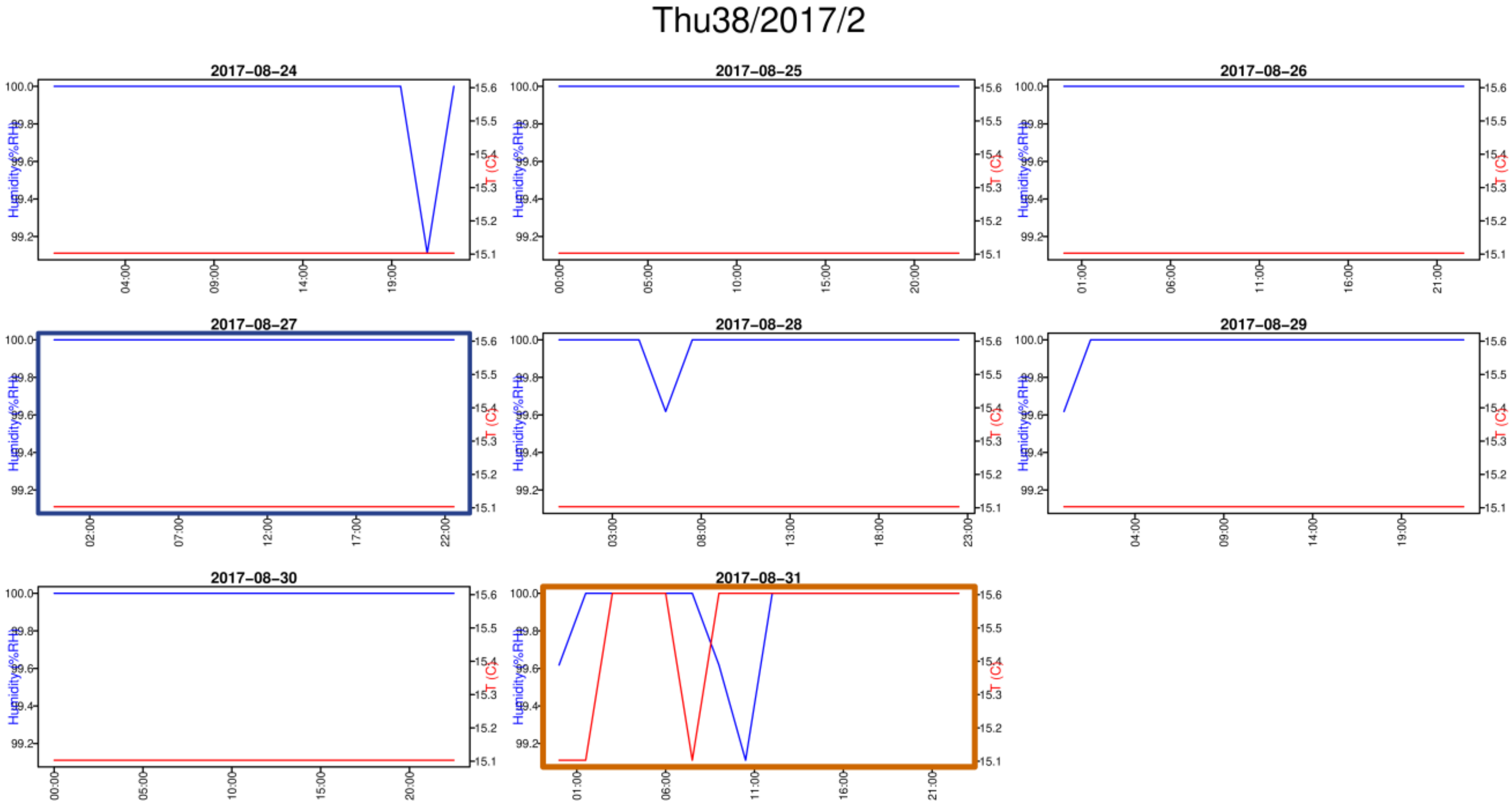


Figure Supp_19 18 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format "Roost"/"Year"/"Sess". Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The "HottestDay" is marked with an orange border and the "MoistestDay" is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration ("Days").

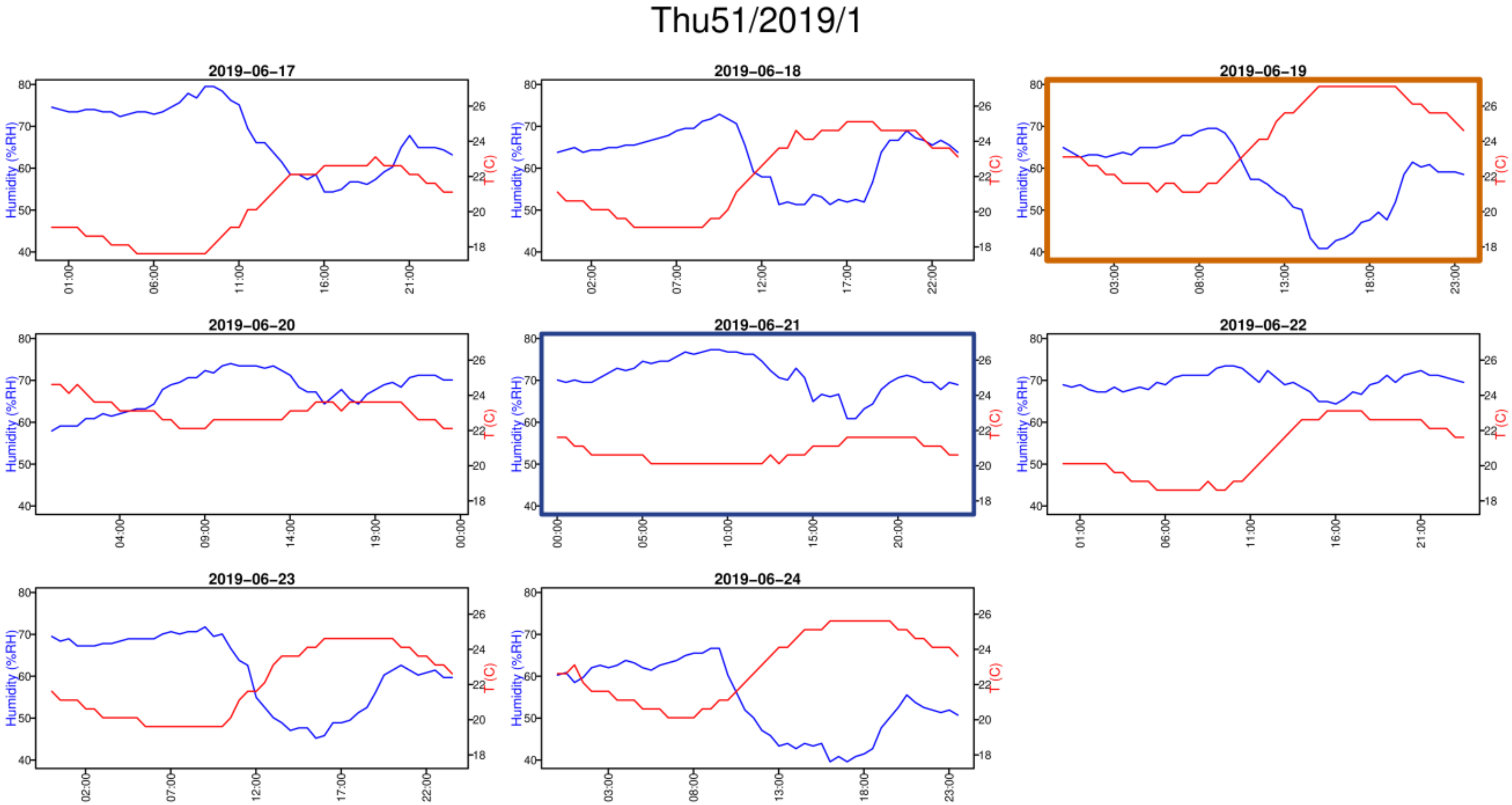


Figure Supp_19 19 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

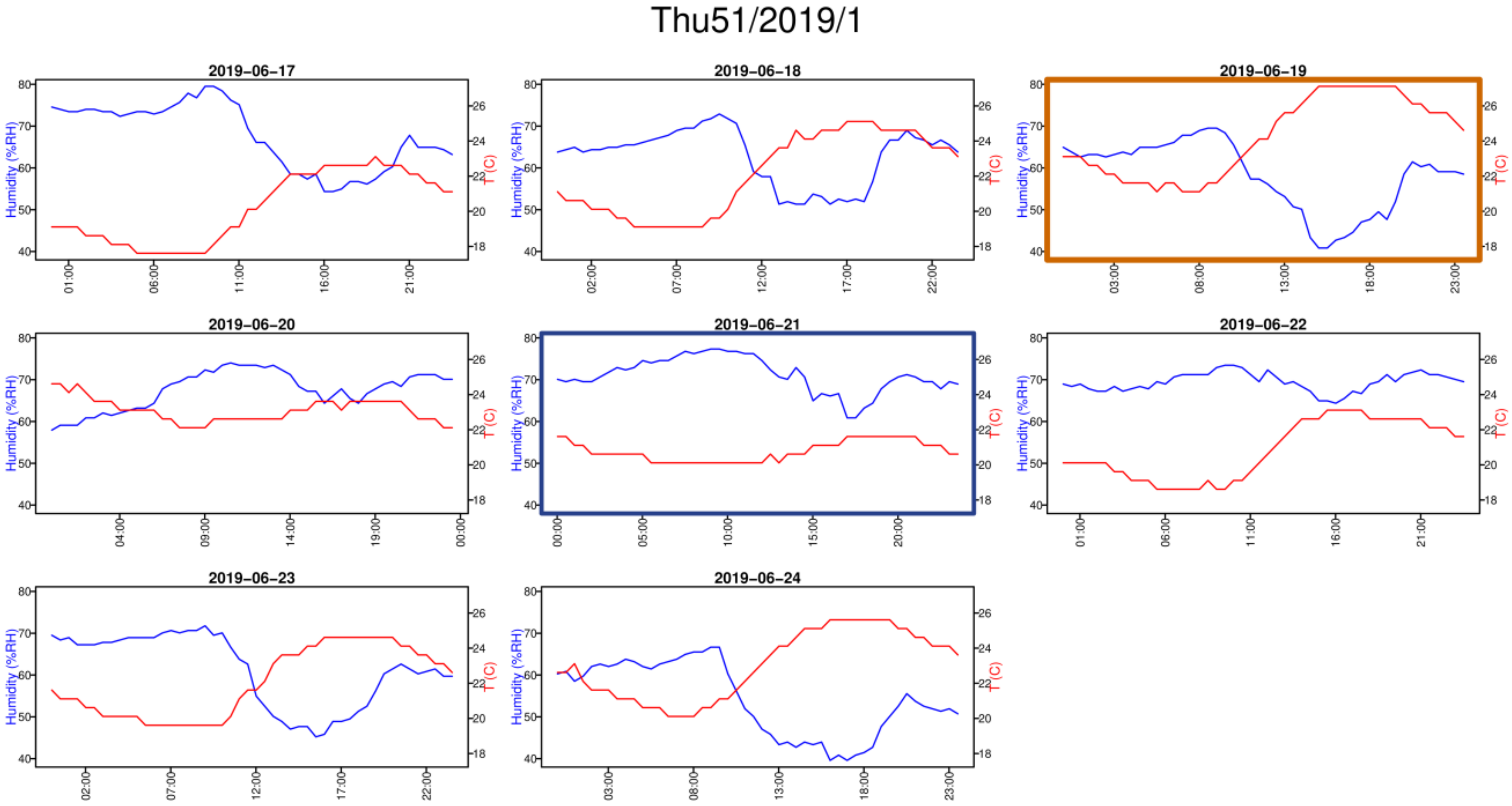


Figure Supp_19 20 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).

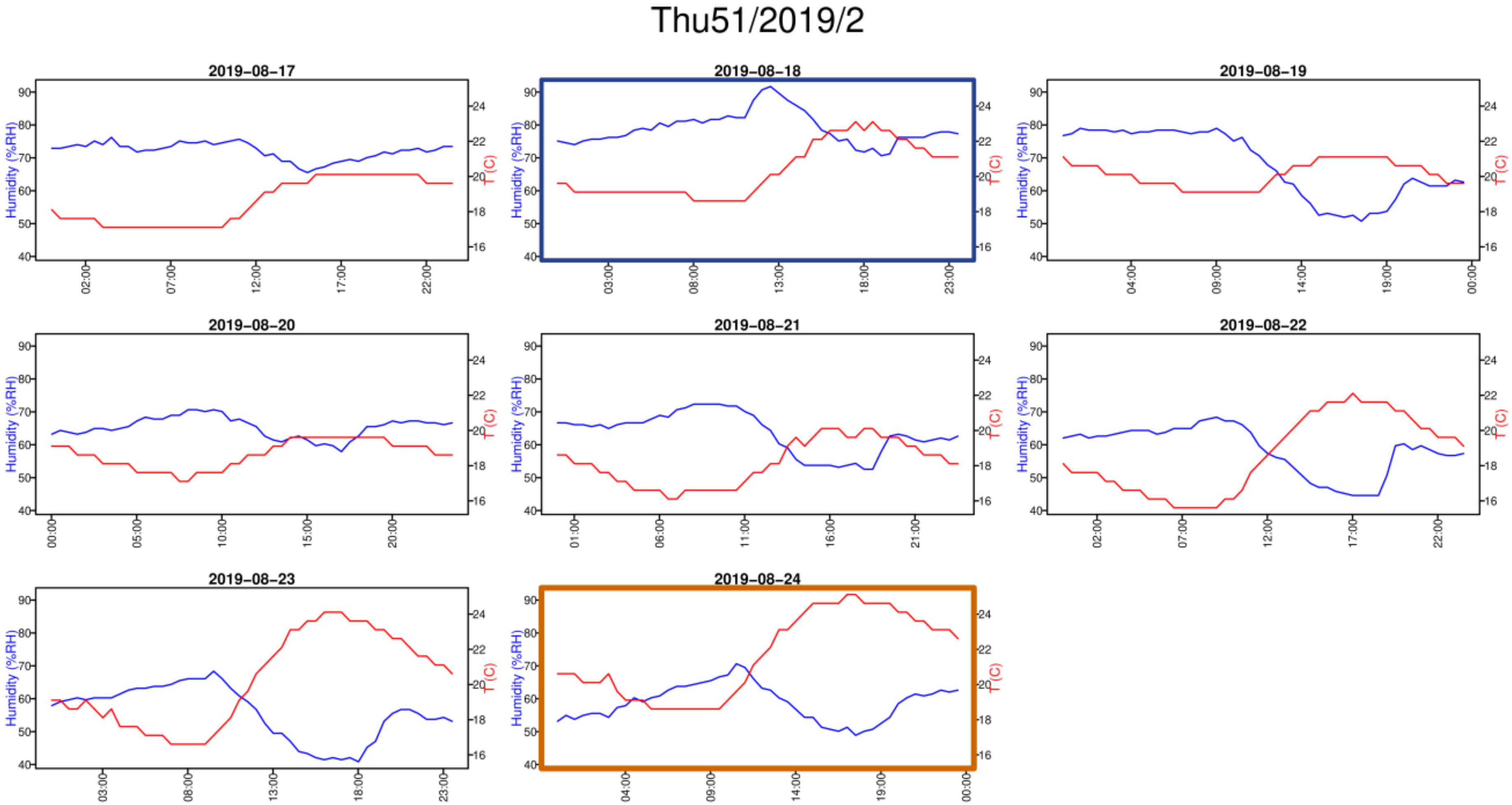
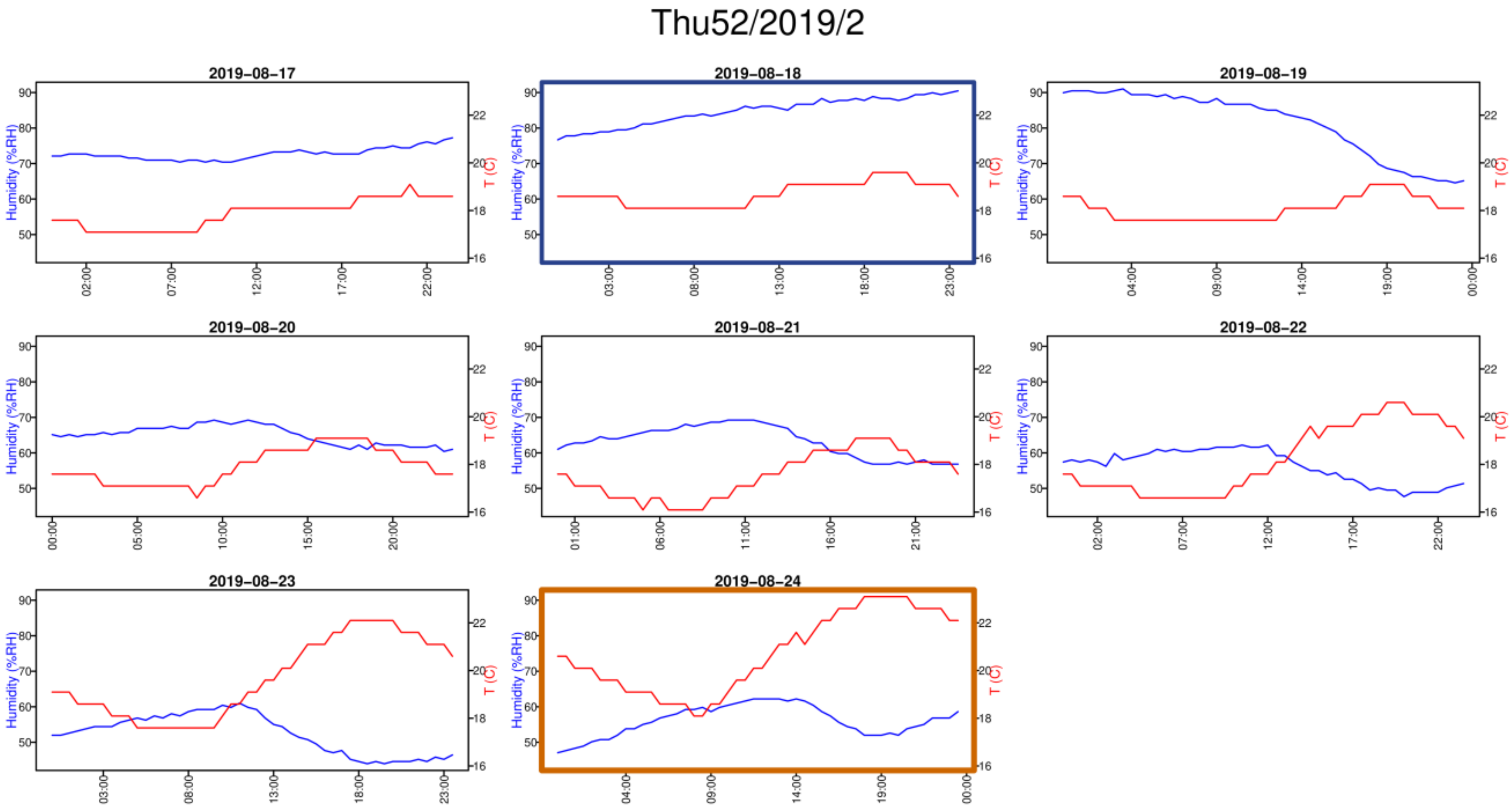


Figure Supp_19 21 Daily relative humidity (blue) and temperature (red) measured by the iButton loggers in the roosts for the model building data in all 21 roost-visits (RVs) labeled on top in the format “Roost”/”Year”/”Sess”. Within a RV all daily plots share the same y-axis to facilitate comparison of temperature and humidity between multiple days. The “HottestDay” is marked with an orange border and the “MoistestDay” is marked with a dark blue border. Dates are formatted in the format year-month-day. Note that the date when newspaper was deployed to collect fresh dropping and the date of dropping collection was always removed from the logging data because exact deployment time of the loggers were not always recorded meaning that the plots are one day short of actual exposure duration (“Days”).



Supp_20 Information about the PCR success rate model

Model diagnostics and information for the PCR success rate model investigating the effect of PCR success rate on QI created with the mdiag() function included in the version controlled R-Project. Not all information from the 15-page pdf report is shown but can easily be created by running the available R-Script. Graphs were slightly altered from the pdf output to ensure consistent naming with the publication and facilitate reading. Rerunning the R code on a different machine, the DHARMa based diagnostics might look different and its values might slightly differ because it is a simulation approach. However, after running it once all other reruns on the same machine should look the same since the random state is saved. To account for detected zero-inflation and overdispersion in the initial binomal model we modelled zeroes and dispersion as part of QI since PCR success rate is tightly linked to QI. We also addressed some heteroscedasticity by accounting for the lab effects of every year since they certainly influenced PCR success rate since samples were processed every year and the environmental conditions for the system changed every year too. This model contains all samples from model building data and test data combined. However regardless of these adjustments the QI had a highly significant positive effect on PCR success rate in each model and the marginal predicted effects were very similar.

Model overview

name: mod_PCRvsQIbetdisp

family: betabinomial
link: logit

response variable form: matrix

sample size: 2616

N (sample size) / k (terms) : 2616

formula: PCR_SuccR ~ QI_without_RHC108 + (1 | Year)

ziformula: ~QI_without_RHC108

dispformula: ~QI_without_RHC108

Figure Supp_20 1 Model diagnostics based on simulated residuals using the “DHARMa” package of the final global model based on 10 000 simulations. A) Dispersion test shows no significant deviation with the observed (red line) variance being 0.90 times weaker than the mean simulated variance (black lines). B) Zero-inflation test shows that the number of observed zeroes (red line) is not significantly different with 1.27 times more observed zeroes than mean simulated zeroes (black lines). C) Outlier test based on 100 bootstrap replicates shows no significant outliers (red bars and line). D) Quantile-Quantile plot of observed and expected residuals shows significant but weak deviation of uniform distribution (red line).

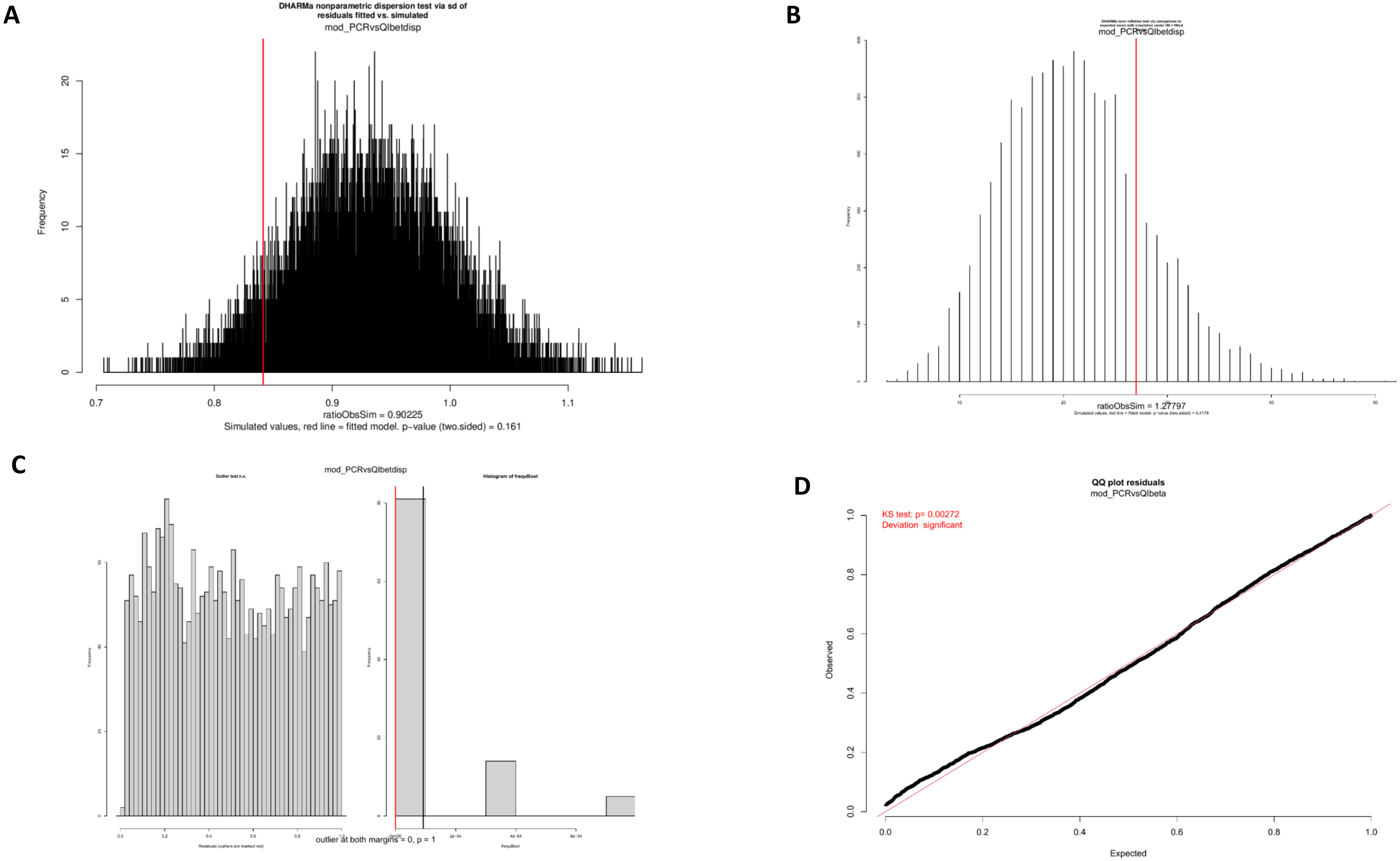


Figure Supp_20 2 Model diagnostics based on simulated residuals using the “DHARMa” package of the initial binomial global model test based on 10 000 simulations. A) Testing excess/deficits of PCR success rate = 1. Since PCR success rate was expressed in matrix form (success/(total number of loci) a PCR success rate of 1 means in Matrix form 27/27. We did not detect a significant deviation of observed (red line) vs. simulated ones (black lines) with 1.05 times the number of observed ones compared to the mean simulated number of ones (black bars). B) Shows the same as A) but for only 24 since we had to remove locus RHC108 in 2018 because of a marker problem showing non-significant excess/deficit of ones with 1.03 times more observed ones the mean simulated ones. C) Standardized simulated residuals on y-axis plotted against predicted response on x-axis to detect deviations from homoscedasticity. Single residuals are summarized as red solid quantile splines. Expected uniformity quantiles are shown as red dashed lines. Simulation outliers are indicated by red asterisks. Deviations of quantile splines from uniformly distributed quantiles (red dotted lines) indicate signs of heteroscedasticity in all quantiles.

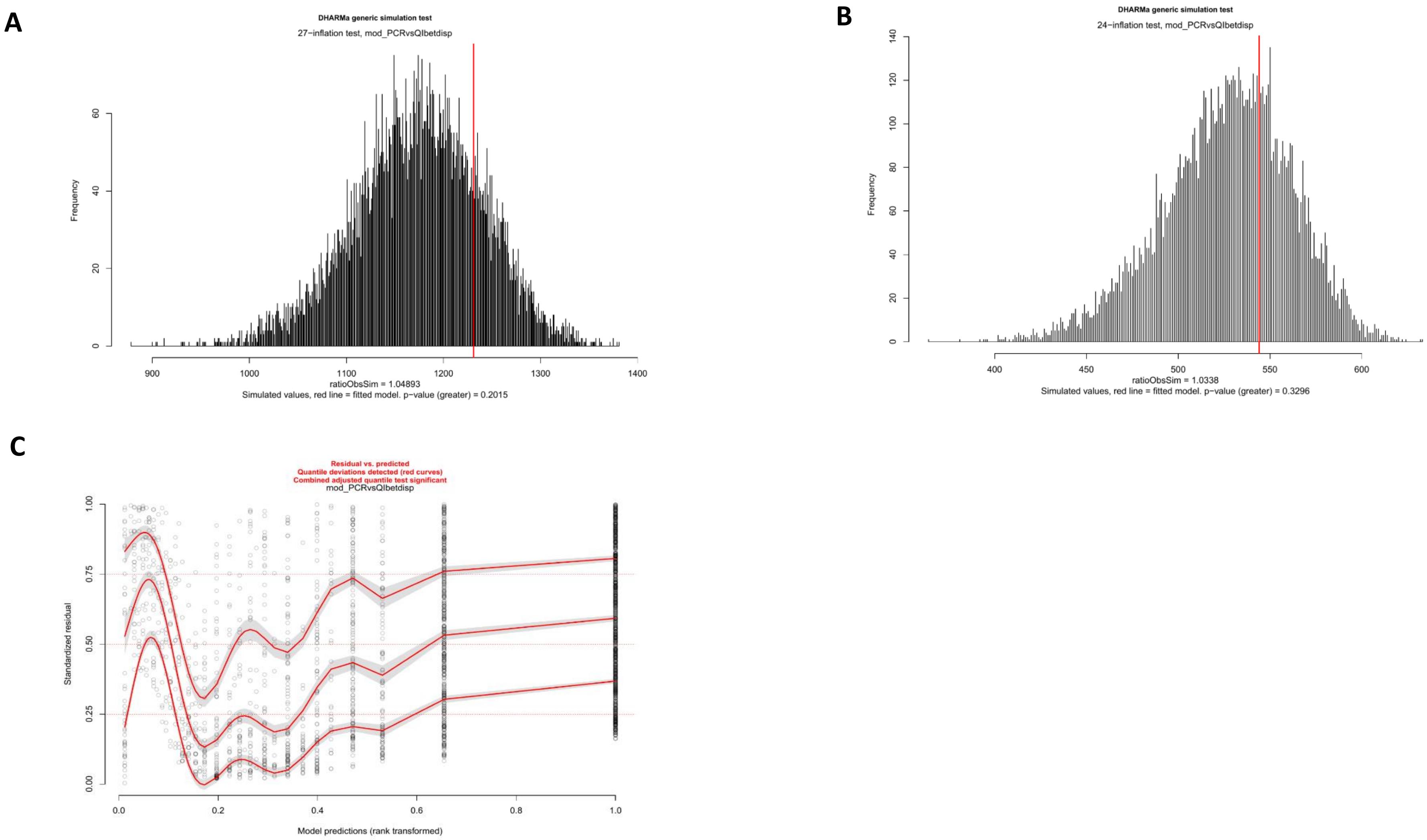


Figure Supp_20 3 Visual check of normality of residuals for random effects using the “performance” and “See” package. No strong deviation was detected.

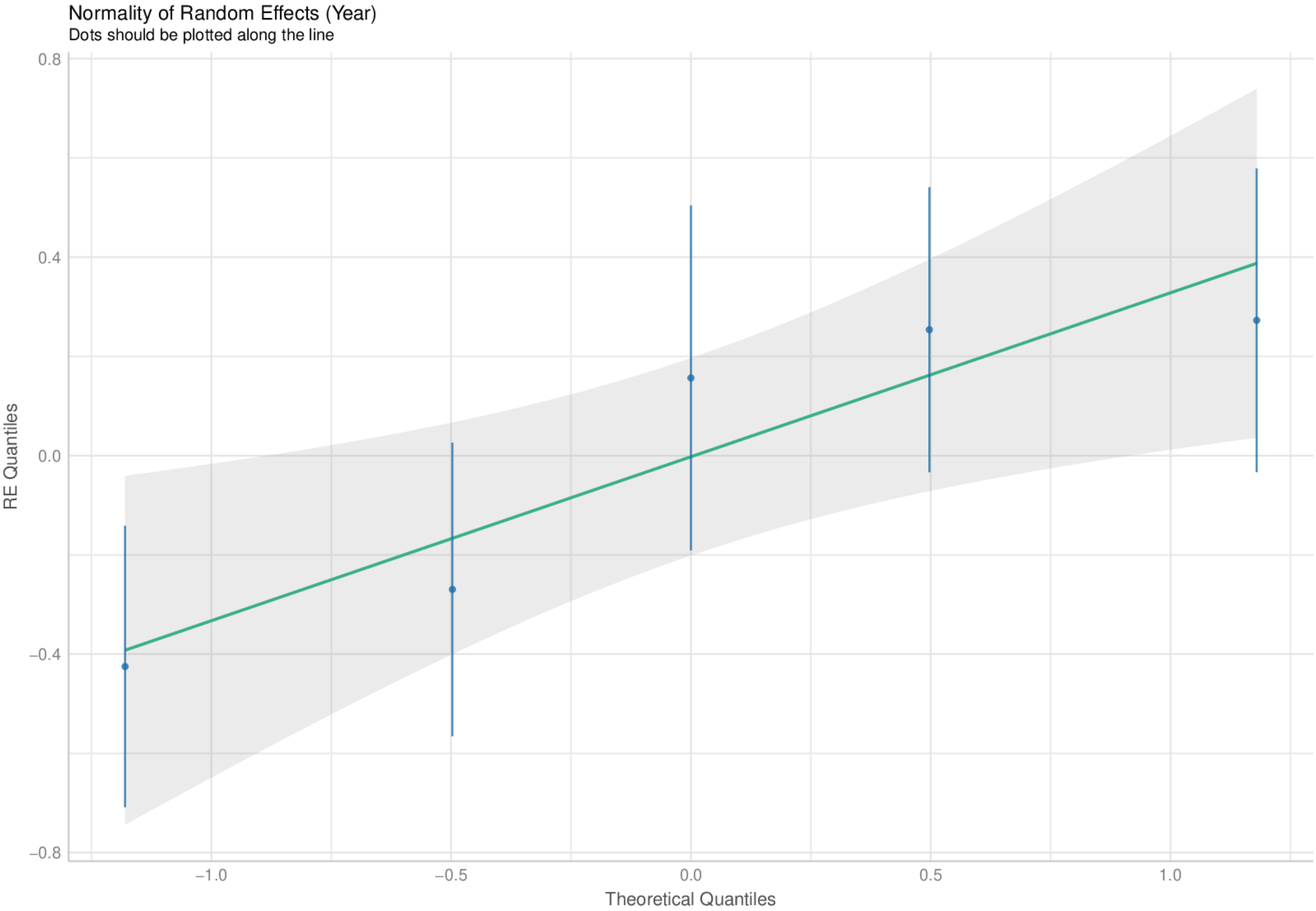
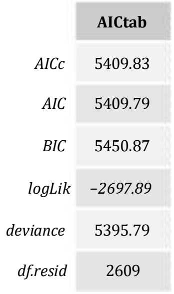


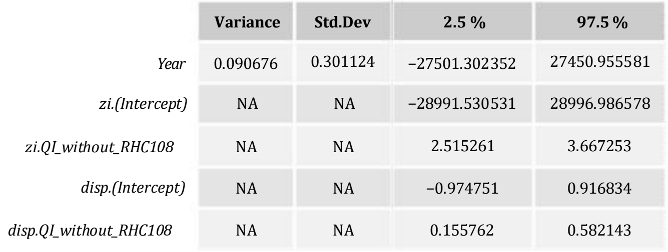
Figure Supp_20 4 Model fit and estimates. A) Different information criteria and model fit statistics of the fitted model. From top to bottom: Akaike information criterion for small sample size (AICc) and normal sample size (AIC), Bayesian information criterion (BIC), log-likelihood (logLik), "absolute unconditional" deviance (deviance=-2*logLik) and residual degrees-of-freedom (df.resid). B) Variance (Variance) and standard deviation (Std.Dev) as well as the lower (2.5%) and upper (97.5%) limit of the 95% confidence interval for the random effects and corrective model (where possible). C) Fixed effect estimates from left to right: 95% odds Ratio confidence interval (OR) lower (OR 2.5%) and upper (OR 97.5%) limit as well as the odds ratio (OR Estimate). 95% estimate confidence intervals (2.5 %, 97.5%) together with the model estimates (Estimate) and their standard errors (Std. Error), z values and p values (Pr(>|z|)) as well as holm corrected p values (holm). The last four columns report p values, estimates and lower and upper 95% confidence interval after corrections for simultaneous multiple hypothesis testing using the “multcomp” package and the “glht” function.

A



	AICtab
AICc	5409.83
AIC	5409.79
BIC	5450.87
logLik	*−2697.89*
deviance	5395.79
df.resid	2609

B



	Variance	Std.Dev	2.5 %	97.5 %
Year	0.090676	0.301124	−27501.302352	27450.955581
zi.(Intercept)	NA	NA	−28991.530531	28996.986578
zi.QI_without_RHC108	NA	NA	2.515261	3.667253
disp.(Intercept)	NA	NA	−0.974751	0.916834
disp.QI_without_RHC108	NA	NA	0.155762	0.582143

C

	OR 2.5 %	OR 97.5 %	OR Estimate	2.5 %	97.5 %	Estimate	Std. Error	z value	Pr(>|z|)	holm	p_glht	ghlt Estimate	ghlt lwr	ghlt upr
cond.(Intercept)	0.052635	0.101878	0.073228	−2.944368	−2.283977	−2.614173	0.16847	−15.517117	0	0	0	−2.614173	−2.985806	−2.242539
cond.QI_without_RHC108	944.145793	1731.772021	1278.688886	6.850281	7.4569	7.153591	0.154753	46.225918	0	0	0	7.153591	6.812217	7.494964

Figure Supp_20 5 Graphical representation of model estimates (coloured dots) with corresponding value above using the “sjPlot” package. Horizontal lines mark the 95 % confidence intervals. Significance after Holm-Bonferroni corrections are indicated by asterisks. *<0.05, **<0.01, *** <0.001. Note the strong positive effect of QI on PCR success rate in the conditional model. Dispersion and zero-inflation model terms did not have a significant effect but helped to model dispersion and zeroes.

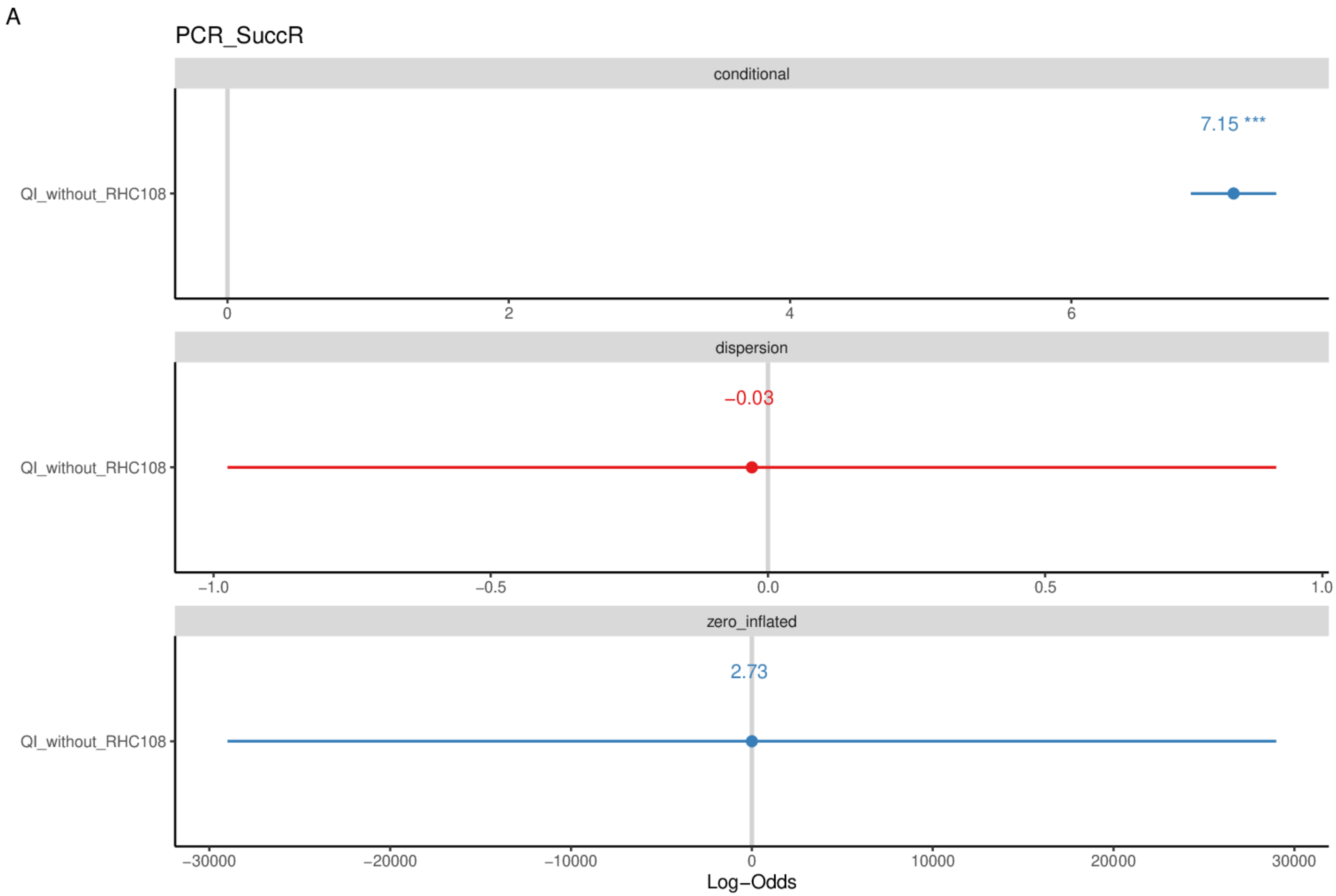
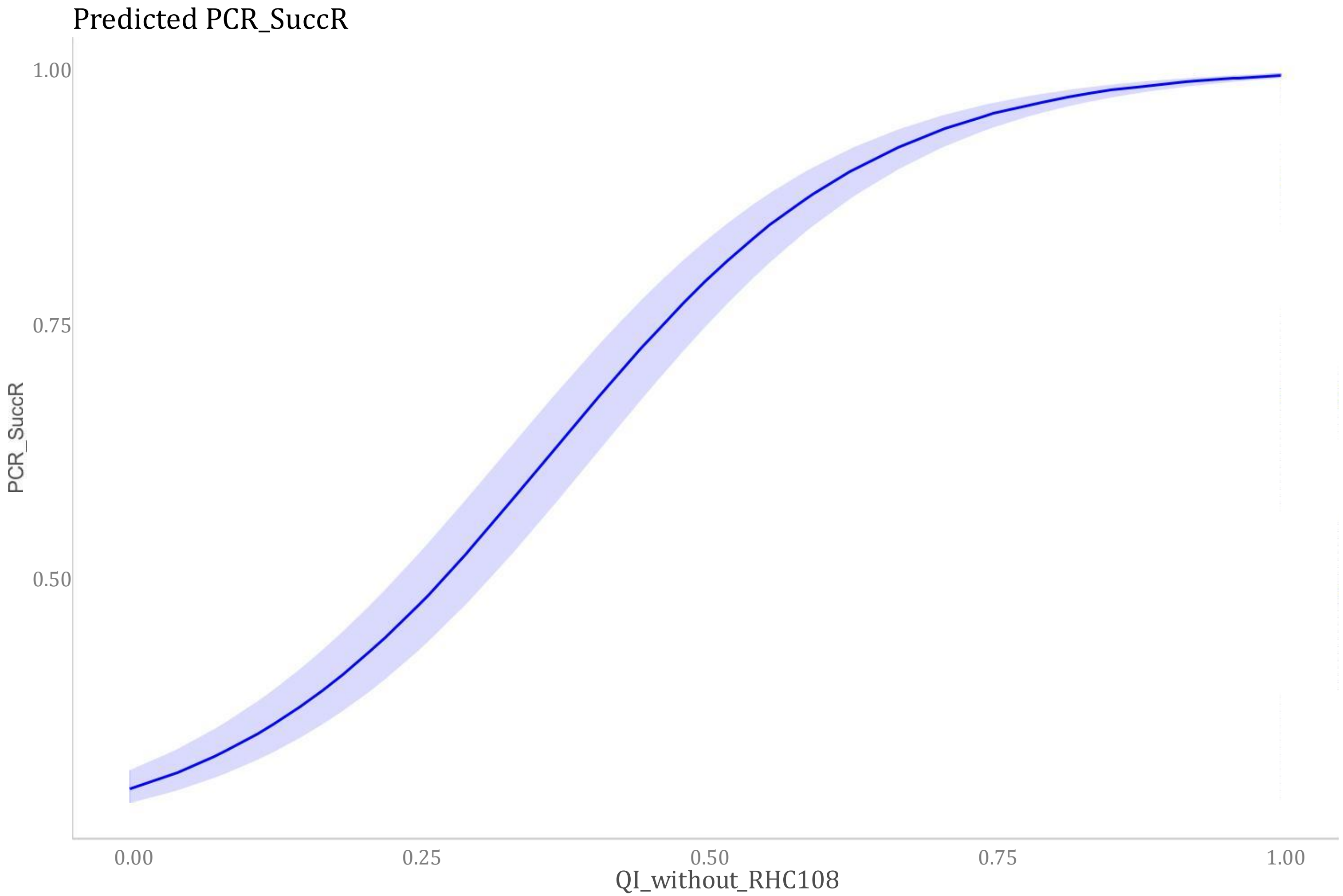


Figure Supp_20 6 Predicted marginal fixed effects (solid lines) on PCR success rate ("PCR_SuccR") using ggpredict() from the "ggeffects". 95 % confidence intervals are indicated by the lighter shape. Blue indicates a positive effect. "QI_without_RHC108" on the X-axis represents the observed unstandardized QI value per sample as used in the whole publication. "_without_RHC108" refers to the removal of locus RHC108 in 2018 from QI calculations due to marker issues of that year (see Supp_03). Note that with higher QI values the curve gets flatter indicating that the share of PCR success rate on QI values towards one decreases. This means on the other hand that the share of disagreement between loci contributing to QI is increasing with higher QI values. At the lower end of QI the curve is also flatter indicating that disagreement also plays a more important role here defining QI. Note the differences between the two extreme QI values one and zero. A QI of one is only possible with all loci amplified and agreeing while a QI of zero can range from complete amplification failure to complete disagreement between replicate loci. See also Figure Supp_11 1. QI is therefore superior to PCR success rate in measuring eoDNA quality and degradation since it additionally incorporates genotyping errors as disagreement between replicates and therefore increases resolution of eoDNA degradation.



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