Supplemental Materials for: Heat increment of feeding in the common bottlenose dolphin (*Tursiops truncatus*) is an insignificant quantity in estimating field metabolic rate

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R Setup

```
library(mgcv)
library(ggformula)
library(tidyverse)
library(readxl)
library(ggeffects)
library(pracma)
library(DHARMa)
library(gt)
library(gtsummary)
library(mixedup)

knitr::opts_chunk$set(echo = TRUE,
                      fig.width = 6,
                      fig.height = 4.5,
                      fig.path = "figures/HIF-",
                      dev = "jpeg",
                      dpi = 300)

theme_set(theme_minimal(base_size = 12))
```

Data Preparation

```
HIF_data <- read_xlsx('data/updated_data.xlsx',
                      .name_repair = 'unique_quiet') |>
  mutate(animal = factor(animal),
         day = factor(interaction(animal, date, drop = TRUE)),
         is_male = if_else(sex == "M", 1,0))
```

HIF Model

Model fitting

```
HIF_model <- gam(oxygen_cons ~
                   # smooth terms
                   s(exact, k = 4, bs = "ts") +
                   s(percentdailytotal, k = 4, bs = "ts") +
                   ti(exact, percentdailytotal, k = 4, bs = "ts") +
                   s(body_mass, k = 4, bs = "ts") +
                   s(age, k = 4, bs = "ts") +
                   # other predictor(s)
                # treat sex as logical 0/1 for convenience later when predicting
                   is_male +
                   s(pool_temp, k = 4, bs = "ts") +
                   # random effects
                   s(animal, bs = "re") +
                   s(day, bs = "re"),
                 data = HIF_data,
                 method = "ML",
                 select = TRUE)
```

Standard model summary output from `gam()`:

```
summary(HIF_model)
```

```

Family: gaussian
Link function: identity

Formula:
oxygen_cons ~ s(exact, k = 4, bs = "ts") + s(percentdailytotal,
    k = 4, bs = "ts") + ti(exact, percentdailytotal, k = 4, bs = "ts") +
    s(body_mass, k = 4, bs = "ts") + s(age, k = 4, bs = "ts") +
    is_male + s(pool_temp, k = 4, bs = "ts") + s(animal, bs = "re") +
    s(day, bs = "re")

Parametric coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   0.6920     0.1025   6.751 7.21e-10 ***
is_male       0.1819     0.1189   1.530    0.129
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                                  edf Ref.df      F  p-value
s(exact)                    2.000e+00      3  9.077 5.42e-05 ***
s(percentdailytotal)        2.463e-01      3  0.797   0.2530
ti(exact,percentdailytotal) 6.440e-01      9  0.186   0.1127
s(body_mass)                2.199e-06      3  0.000   0.5413
s(age)                      5.244e-07      3  0.000   0.6708
s(pool_temp)                1.572e-06      3  0.000   0.9907
s(animal)                   4.654e+00      6 14.341 4.10e-07 ***
s(day)                      1.347e+01     46  0.525   0.0223 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.531   Deviance explained = 60.9%
-ML = -4.0912  Scale est. = 0.040124  n = 133
```

A prettier model summary table:

```
tbl_regression(HIF_model)
```

Characteristic	Beta	95% CI	p-value
is_male	0.18	–0.05, 0.41	0.13
s(exact)			<0.001
s(percentdailytotal)			0.3
ti(exact,percentdailytotal)			0.11
s(body_mass)			0.5
s(age)			0.7
s(pool_temp)			>0.9
s(animal)			<0.001
s(day)			0.022

Abbreviation: CI = Confidence Interval

Model Checking

Below are `mgcv` package checks for GAMs, presented mainly to view model convergence and basis dimension checking results.

```
gam.check(HIF_model)
```

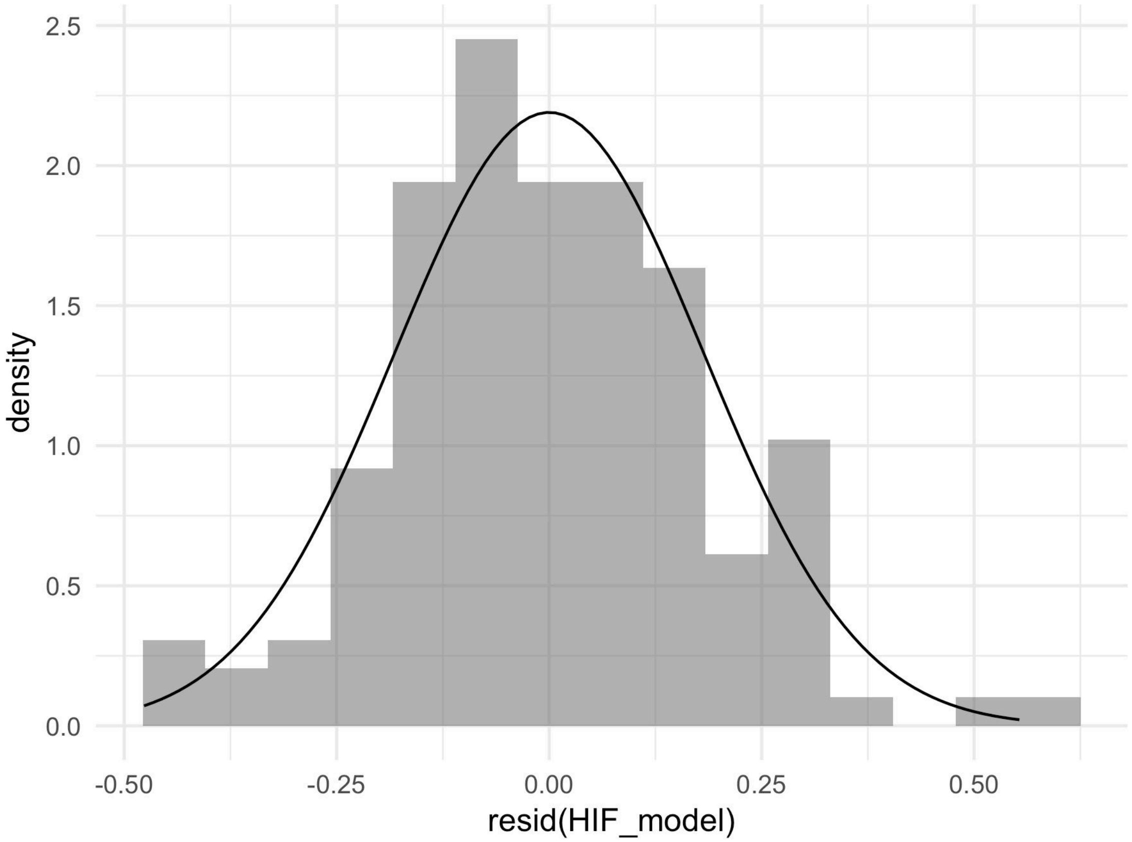
```

Method: ML   Optimizer: outer newton
full convergence after 19 iterations.
Gradient range [-7.932546e-07,4.7294e-07]
(score -4.091216 & scale 0.04012398).
Hessian positive definite, eigenvalue range [4.039797e-07,67.38306].
Model rank =  82 / 82

Basis dimension (k) checking results. Low p-value (k-index<1) may
indicate that k is too low, especially if edf is close to k'.

                                  k'      edf k-index p-value
s(exact)                    3.00e+00 2.00e+00    1.02    0.58
s(percentdailytotal)        3.00e+00 2.46e-01    0.97    0.29
ti(exact,percentdailytotal) 9.00e+00 6.44e-01    1.02    0.55
s(body_mass)                3.00e+00 2.20e-06    1.00    0.45
s(age)                      3.00e+00 5.24e-07    1.00    0.51
s(pool_temp)                3.00e+00 1.57e-06    0.98    0.32
```

```
s(animal)                        8.00e+00 4.65e+00       NA       NA
s(day)                           4.80e+01 1.35e+01       NA       NA
```

Residual Histogram

This graph allows us to verify the residual normality condition.

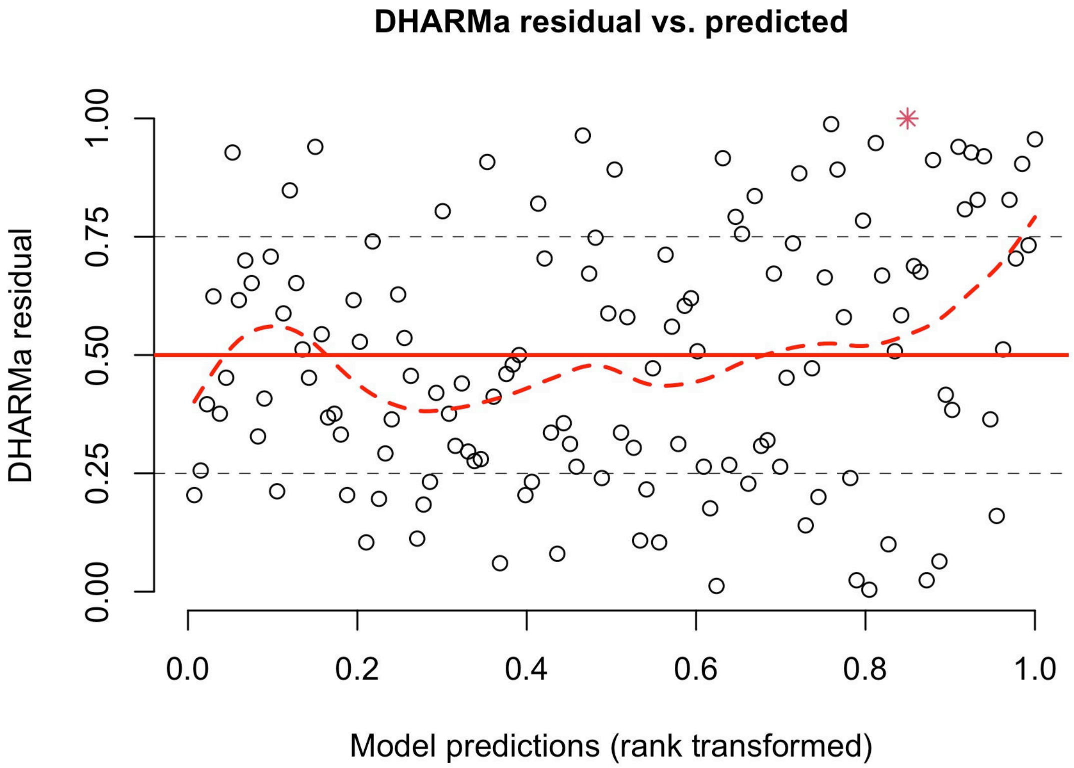
```
gf_dhistogram(~resid(HIF_model), bins = 15) |>
  gf_fitdistr(dist = "dnorm")
```



Scaled Residual Plot

The scaled residual plot (Hartig, 2024) allows verification that the mean-variance relationship is as expected, particularly for mixed-effect models and generalized linear models. If all is well, scaled residuals will be trendless and will have uniform spread between 0-1 for all fitted values.

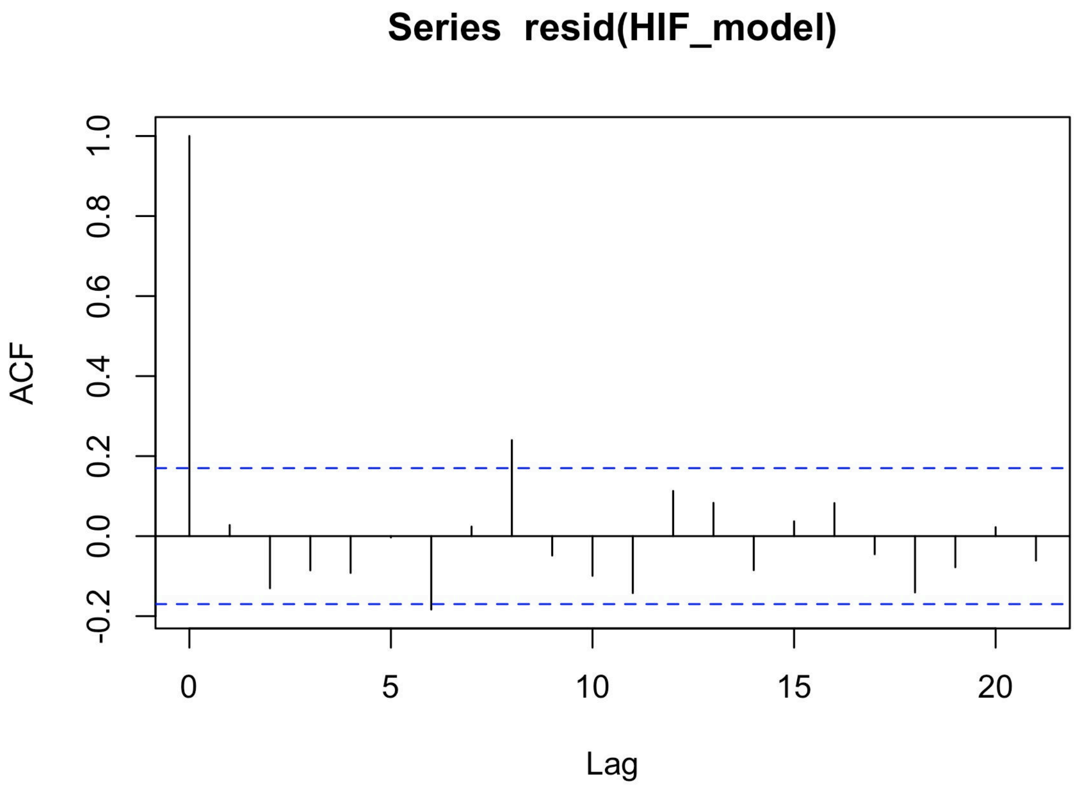
```
plotResiduals(simulateResiduals(HIF_model), quantreg = FALSE)
```



Residual ACF Plot

The ACF plot allows us to verify the residual independence condition.

```
acf(resid(HIF_model))
```



Model Interpretation

Hypothesis Testing

Type II ANOVA allows us to test the null hypothesis of no association with the response, for each predictor (and interaction) included in the model.

```
anova(HIF_model)
```

```


Family: gaussian
Link function: identity

Formula:
oxygen_cons ~ s(exact, k = 4, bs = "ts") + s(percentdailytotal,
    k = 4, bs = "ts") + ti(exact, percentdailytotal, k = 4, bs = "ts") +
    s(body_mass, k = 4, bs = "ts") + s(age, k = 4, bs = "ts") +
    is_male + s(pool_temp, k = 4, bs = "ts") + s(animal, bs = "re") +
    s(day, bs = "re")

Parametric Terms:
        df     F p-value
is_male  1 2.342   0.129

Approximate significance of smooth terms:
                                  edf     Ref.df      F  p-value
s(exact)                    2.000e+00 3.000e+00  9.077 5.42e-05
s(percentdailytotal)        2.463e-01 3.000e+00  0.797   0.2530
ti(exact,percentdailytotal) 6.440e-01 9.000e+00  0.186   0.1127
s(body_mass)                2.199e-06 3.000e+00  0.000   0.5413
s(age)                      5.244e-07 3.000e+00  0.000   0.6708
s(pool_temp)                1.572e-06 3.000e+00  0.000   0.9907
s(animal)                   4.654e+00 6.000e+00 14.341 4.10e-07
s(day)                      1.347e+01 4.600e+01  0.525   0.0223
```

Variance Term Estimation

For random intercept terms, by analogy with the way that mixed-effect model results are usually reported and light of the fact that we wish to control for any individual or spatio-temporal effects rather than performing hypothesis testing for them, it is possible to report the standard variance components associated with each term. These are shown here in units of standard deviation. Note that since we used maximum-likelihood estimation, these estimates may be biased for finite sample size, but since variance term estimation is not the focus of inference (and smooth term hypothesis testing is) this seems like the right choice in our case.

```
vars <- invisible(gam.vcomp(HIF_model))

vars <- vars |>
  data.frame() |>
  mutate(
    Term = rownames(vars),
    Term = ifelse(Term == "scale", "scale (residuals)", Term)
  ) |>
rename(`Standard Deviation` = std.dev,
       `CI (lower)` = lower,
       `CI (upper)` = upper
       ) |>
  select(Term,
         `Standard Deviation`,
         `CI (lower)`,
         `CI (upper)`) |>
  mutate(across(where(is.numeric), function(x) signif(x, 3))) |>
  gt()
```

```
vars
```

Term	Standard Deviation	CI (lower)	CI (upper)
s(exact)	4.79e-04	1.48e-04	0.00154
s(percentdailytotal)	2.42e-01	8.11e-04	72.20000
ti(exact,percentdailytotal)1	8.24e-05	7.27e-07	0.00934
ti(exact,percentdailytotal)2	1.38e-03	1.98e-06	0.96400
s(body_mass)	1.11e-07	0.00e+00	Inf
s(age)	2.69e-07	0.00e+00	Inf
s(pool_temp)	4.97e-06	0.00e+00	Inf
s(animal)	1.31e-01	7.24e-02	0.23600
s(day)	8.68e-02	4.50e-02	0.16700
scale (residuals)	2.00e-01	1.72e-01	0.23200

Model predictions

Another way of visualizing the results of a GAM (or other regression model) is a prediction plot, where you select specific values at which to fix all the predictors other than `exact` time (age, proportion total calories, etc.) and then show predicted oxygen consumption given those values, and varying values of exact. Such a plot might be preferable to a partial plot because the y-axis is in terms of expected response variable values (in total, not a contribution to the whole from one predictor).

We used marginal means of "other" predictors; in this approach quantitative predictors are set to the mean value observed in the data, and for categorical predictors a weighted average across the observed values is used. The idea is to try to show the expected response value for "an average observation" (in some sense) in the actual data. For this reason, it may make the most sense as a set of predictions that can be overlaid on a plot of the data.

Marginal Means of Model Variables

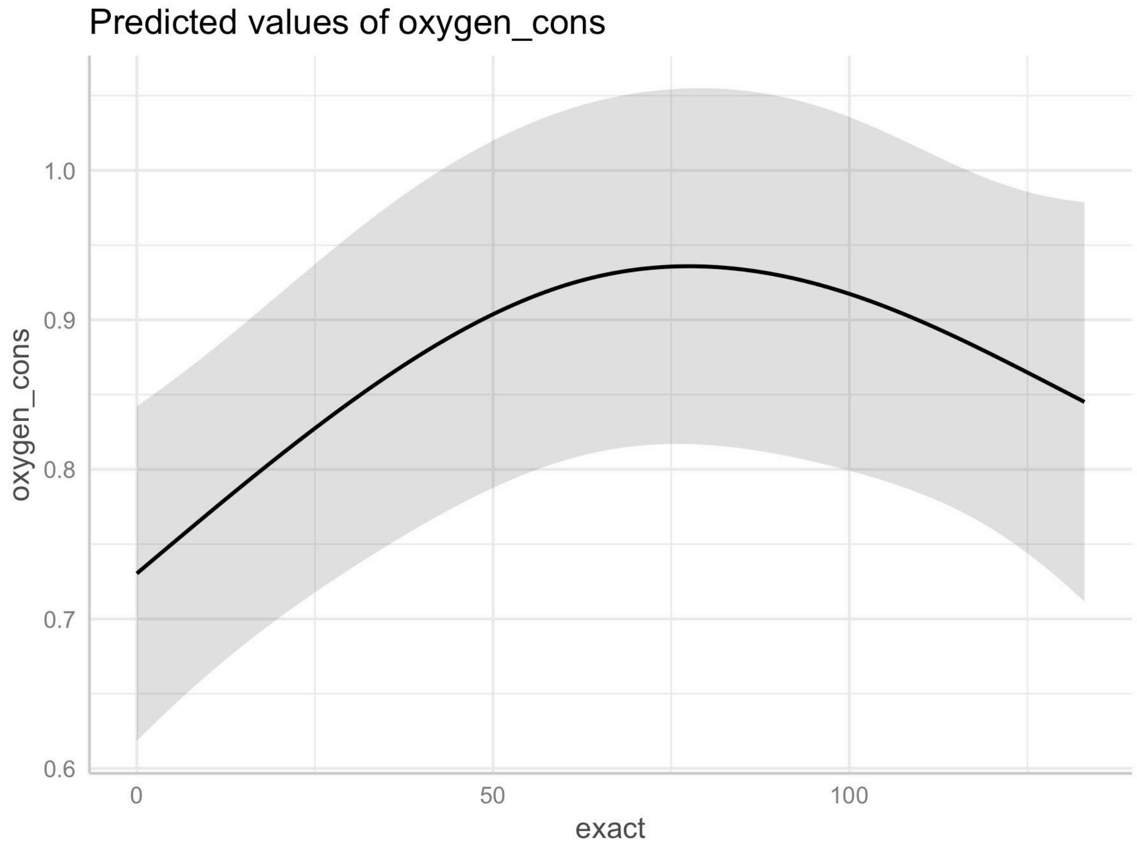
```r
model_vars <- c('exact',
                'percentdailytotal',
                'body_mass',
                'pool_temp',
                'age',
                'sex')

tbl_summary(
    data = HIF_data,
    statistic = list(all_continuous() ~ "{mean} ({sd})",
                     all_categorical() ~ "{n} ({p}%)"),
    type = list(all_continuous() ~ "continuous",
                all_categorical() ~ "categorical",
                age ~ "continuous"),
    # type = assign_summary_type(variables = model_vars,
    #                            cat_threshold = 3,
    #                            value = NULL),
    include = all_of(model_vars)
  )
```

Characteristic	**N = 133**[1]
exact	52 (49)
percentdailytotal	0.23 (0.06)
body_mass	179 (22)
pool_temp	22.80 (1.91)
age	21 (14)
sex	
F	32 (24%)
M	101 (76%)

[1] Mean (SD); n (%)

Model Predictions

```
mmeans <- predict_response(HIF_model, terms = 'exact [0:133]',
                 margin = 'marginalmeans')

mmeans |>
  plot()
```



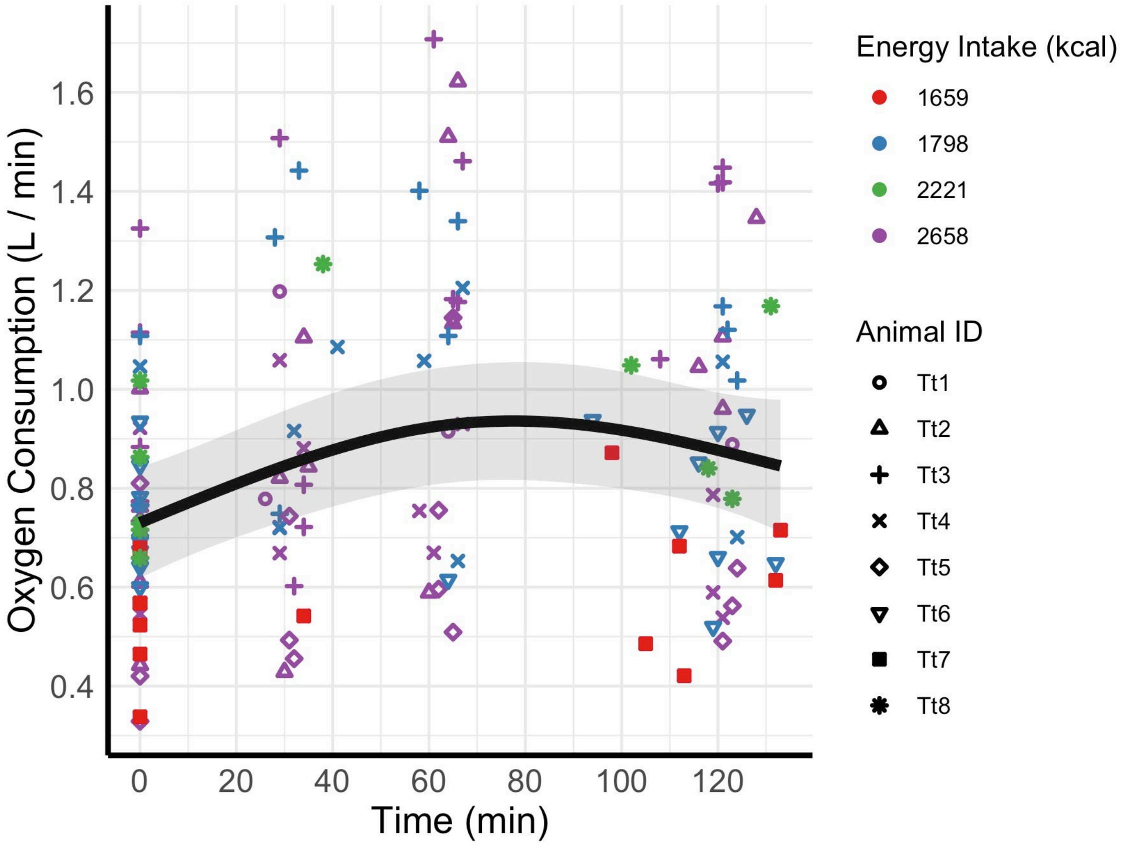
Visualization

Data Plot with Model Fit

Define colors and shapes to use for plotting

```
kcal_colors <- c("#E41A1C", "#377EB8", "#4DAF4A", "#984EA3")
shape_vector <- c("Tt1" = 1, "Tt2" = 2, "Tt3" = 3, "Tt4" = 4,
                  "Tt5" = 5, "Tt6" = 6, "Tt7" = 7, "Tt8" = 8)
```

```
oxygen_time_plot <- ggplot(HIF_data,
                           aes(x = exact, y = oxygen_cons)) +
  geom_point(aes(color = factor(kcal), shape = animal),
             size = 1,
             stroke = 1.2) +
  geom_line(data = mmeans,
            aes(x = x, y = predicted),
            color = 'black',
            linewidth = 2) +
  geom_ribbon(data = mmeans,
              aes(ymin = conf.low, ymax = conf.high, x = x),
              inherit.aes = FALSE,
              fill = 'grey44', alpha = 0.2) +
  labs(x = "Time (min)",
       y = "Oxygen Consumption (L / min)",
       color = "Energy Intake (kcal)",
       shape = "Animal ID") +
  scale_color_manual(values = kcal_colors) +
  scale_shape_manual(values = shape_vector) +
  theme_minimal() +
  theme(axis.text = element_text(size = 12),
        axis.title = element_text(size = 14),
        axis.line = element_line(linewidth = 1)) +
  scale_x_continuous(breaks = seq(0, 120, by = 20)) +
  scale_y_continuous(breaks = seq(0, 2, by = 0.2))

print(oxygen_time_plot)
```



Area Under Curve

The calculations below show the AUC under the black line in the graph above.

For additional reference if needed, the initial value of oxygen consumption at time 0 is: 0.7303654

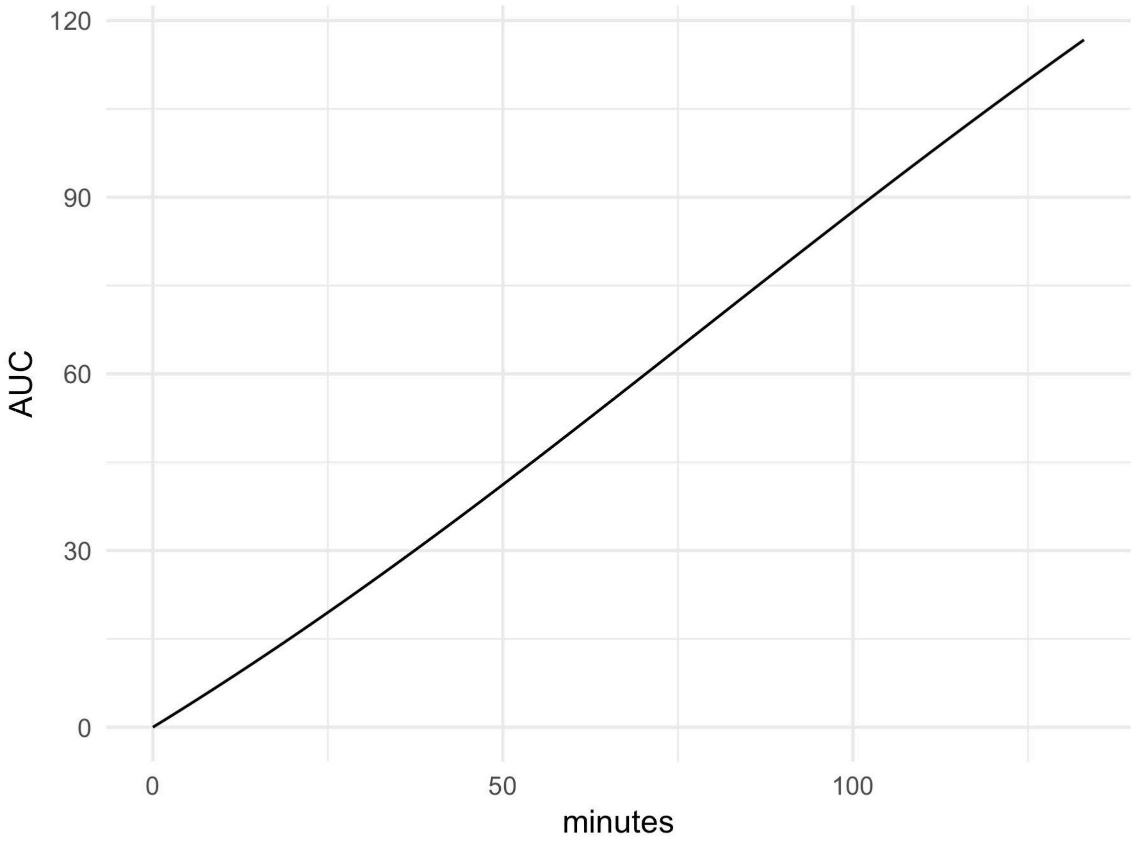
To find the cumulative area under the curve, using the marginal mean expected 0_2 consumption values, we could do something like:

```r
predicted_AUC <- data.frame(minutes = mmeans$x,
                            AUC = cumtrapz(x = mmeans$x,
                                           y = mmeans$predicted))
max(predicted_AUC$AUC)
```

```
[1] 116.7265
```

The total at 133 minutes is: 116.7265361. Below the cumulative sums are shown:

```r
ggplot(predicted_AUC,
       aes(x = minutes, y = AUC)) +
  geom_path()
```



Uncertainty Estimation

To compute this quantity with uncertainty, we could probably employ a parametric bootstrap.

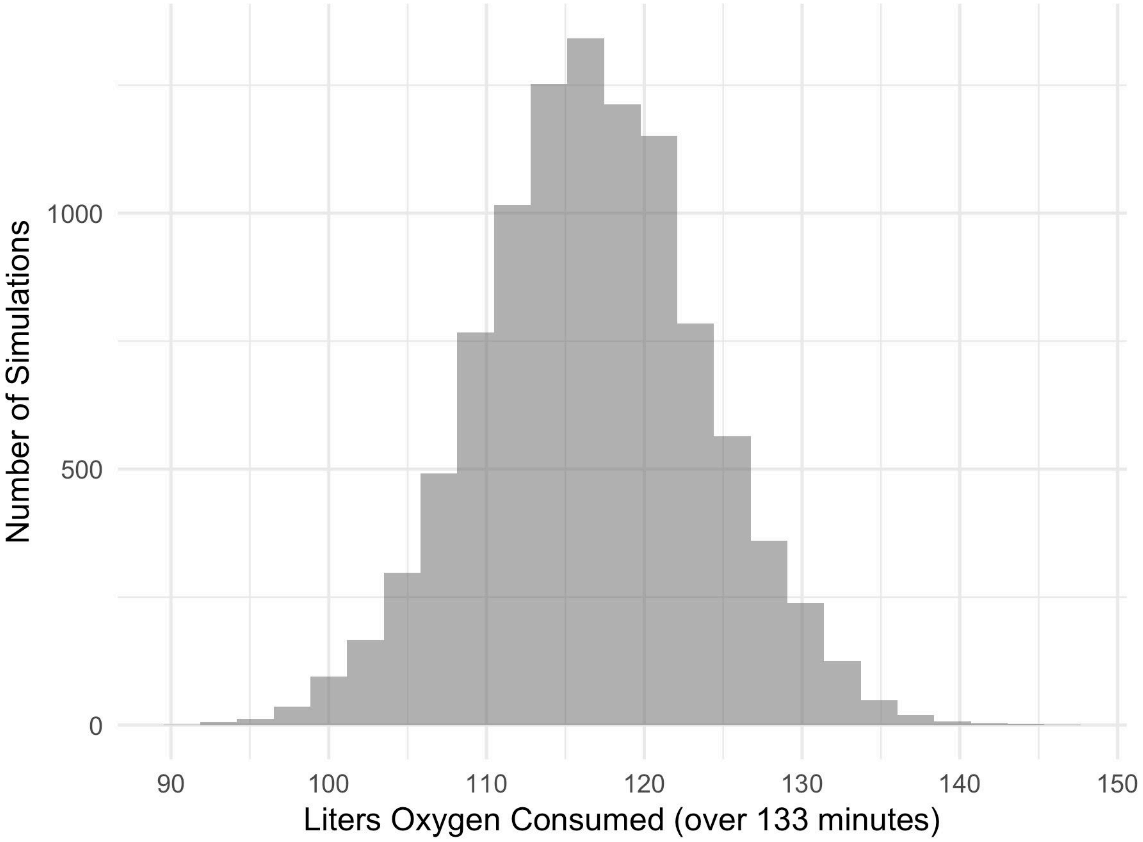
First we need a function that takes a fitted model and from it computes the total AUC for 133 minutes:

```r
compute_auc <- function(predicted_smooth, x = mmeans$x){
  AUC = cumtrapz(x = x,
                 y = predicted_smooth)
  total_AUC <- max(AUC)
  return(total_AUC)
}
```

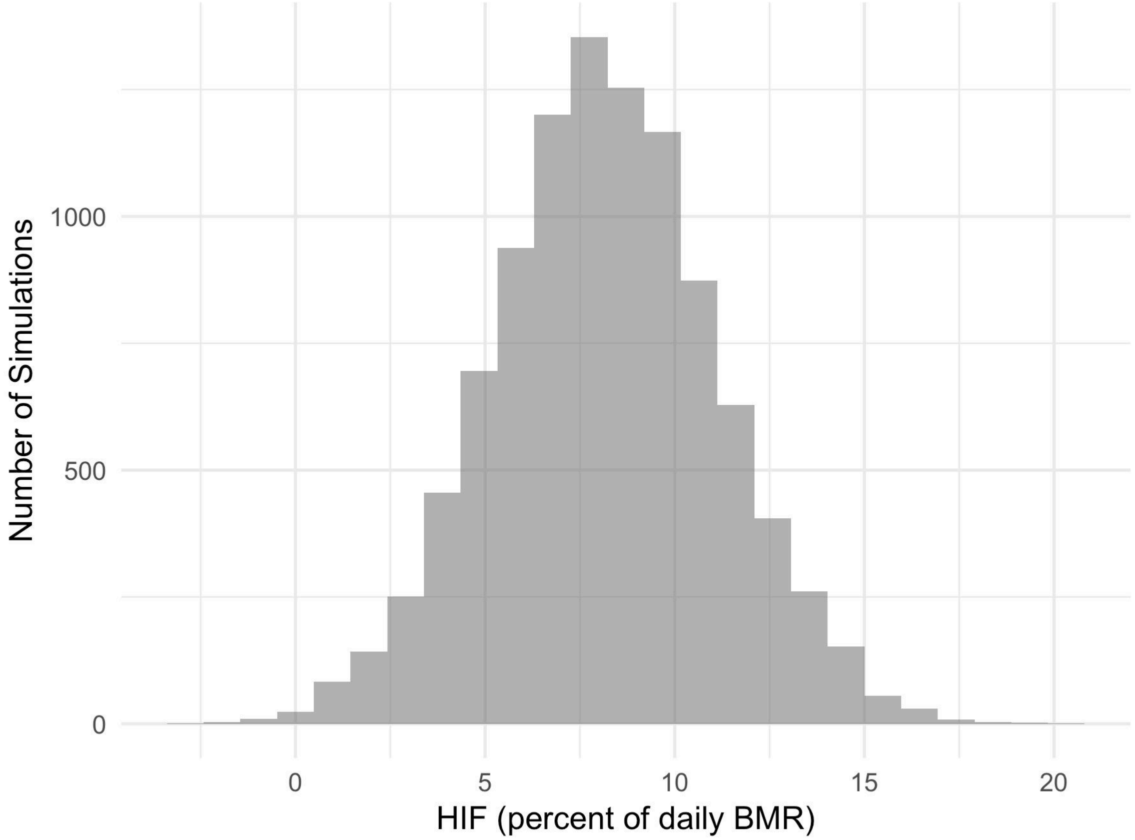
```r
nsim <- 10000
set.seed(14)

# draws of parameter estimates from the posterior
post_params <- gam.mh(HIF_model, ns = nsim*2, burn = 2000, thin = 2)$bs
# yields matrix with cols = model params and rows = sim #s

# get linear predictor matrix for "mm data"
mm_const <- attr(x = mmeans, which = "constant.values") |>
  data.frame()
mm_df <- expand_grid(mm_const,
                     exact = unique(mmeans$x))

mm_lpmat <- predict(HIF_model,
        newdata = mm_df,
        type="lpmatrix",
        exclude = c("s(animal)", "s(day)"),
        newdata.guaranteed = TRUE)

# get predicted vo2 values (each col is one sim)
vo2_curve <- mm_lpmat %*% t(post_params) ## posterior curve samples

vo2_totals <- apply(vo2_curve,
                    MARGIN = 2,
                    FUN = compute_auc)

# as percent, assuming BMR is constant
BMR <- 1051.2 # L O2 in one day
BMR_133 <- 97.1

HIF_133 <- vo2_totals - BMR_133
daily_HIF <- HIF_133 / mm_const$percentdailytotal
daily_HIF_percent <- (daily_HIF / BMR) * 100
```

Histogram of simulation results:

```
gf_histogram(~vo2_totals) |>
  gf_labs(x = "Liters Oxygen Consumed (over 133 minutes)",
          y = "Number of Simulations")
```



```
gf_histogram(~daily_HIF_percent) |>
  gf_labs(x = "HIF (percent of daily BMR)",
          y = "Number of Simulations")
```



Based on the simulations, the mean HIF during the 133 minutes and also as a percent of the BMR over a full day are *higher* than the estimates based on the data (showing in Figure 1, computed based on the marginal mean model predictions). One reason for the difference may be that the way that individual and day are treated is slightly different in the two cases. By analogy to other resampling/simulation-based approaches the standard errors obtained here could still be appropriate estimates (and better than showing no uncertainty at all).

```
vo2_se <- round(sd(vo2_totals), digits = 2)
vo2_percent_se <- round(sd(daily_HIF_percent), digits = 2)

vo2_se
```

```
[1] 7.08
```

```
vo2_percent_se
```

```
[1] 2.95
```

The standard deviation of the simulated values is: `vo2_sd` liters.

The standard deviation of the simulated percentages is: `vo2_percent_se`.

Breathing Frequency Model

Based on the discussion in the manuscript, I think maybe this is what we need…

Methods would say,

> We also fitted a normal GAM to quantify the association (if any) between the dependent variable tidal volume and breathing frequency. The model included smooths of breathing frequency as well as time since feeding, energy intake, age, and body mass, plus the categorical predictor sex and random effects of animal ID and day of trial. Settings, including basis type and dimension, were all the same as for the oxygen consumption GAM.

```r
breath_model <- gam(tidal_vol ~ s(Breath_freq, bs = "ts", k = 4) +
                      s(exact, bs = "ts", k = 4) +
                      s(age, bs = "ts", k = 4) +
                      s(body_mass, bs = "ts", k = 4) +
                      s(percentdailytotal, k = 4, bs = "ts") +
                      # s(pool_temp, k = 4, bs = "ts") +
                      # sex +
                      # random effects
                      s(animal, bs = "re") +
                      s(day, bs = "re"), data = HIF_data)
```

Standard model summary output from `gam()`:

```r
summary(breath_model)
```

```

Family: gaussian 
Link function: identity 

Formula:
tidal_vol ~ s(Breath_freq, bs = "ts", k = 4) + s(exact, bs = "ts", 
    k = 4) + s(age, bs = "ts", k = 4) + s(body_mass, bs = "ts", 
    k = 4) + s(percentdailytotal, k = 4, bs = "ts") + s(animal, 
    bs = "re") + s(day, bs = "re")

Parametric coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   4.1464     0.3361   12.34   <2e-16 ***
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                           edf Ref.df      F  p-value    
s(Breath_freq)       1.830e+00      3 47.223 2.51e-05 ***
s(exact)             1.806e+00      3  8.157 1.25e-05 ***
s(age)               1.019e-09      3  0.000   0.6499    
s(body_mass)         1.001e+00      3 85.682   0.2675    
s(percentdailytotal) 1.887e+00      3 53.658   0.2852    
s(animal)            5.400e+00      7 51.915  < 2e-16 ***
s(day)               9.363e+00     47  0.301   0.0575 .  
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.843   Deviance explained = 86.9%
GCV = 0.63065  Scale est. = 0.52497   n = 133
```

A prettier model summary table:

```
tbl_regression(breath_model)
```

Characteristic	Beta	95% CI	p-value
s(Breath_freq)			<0.001
s(exact)			<0.001
s(age)			0.6
s(body_mass)			0.3
s(percentdailytotal)			0.3
s(animal)			<0.001
s(day)			0.057

Abbreviation: CI = Confidence Interval

Model Checking

Below are `mgcv` package checks for GAMs, presented mainly to view model convergence and basis dimension checking results.

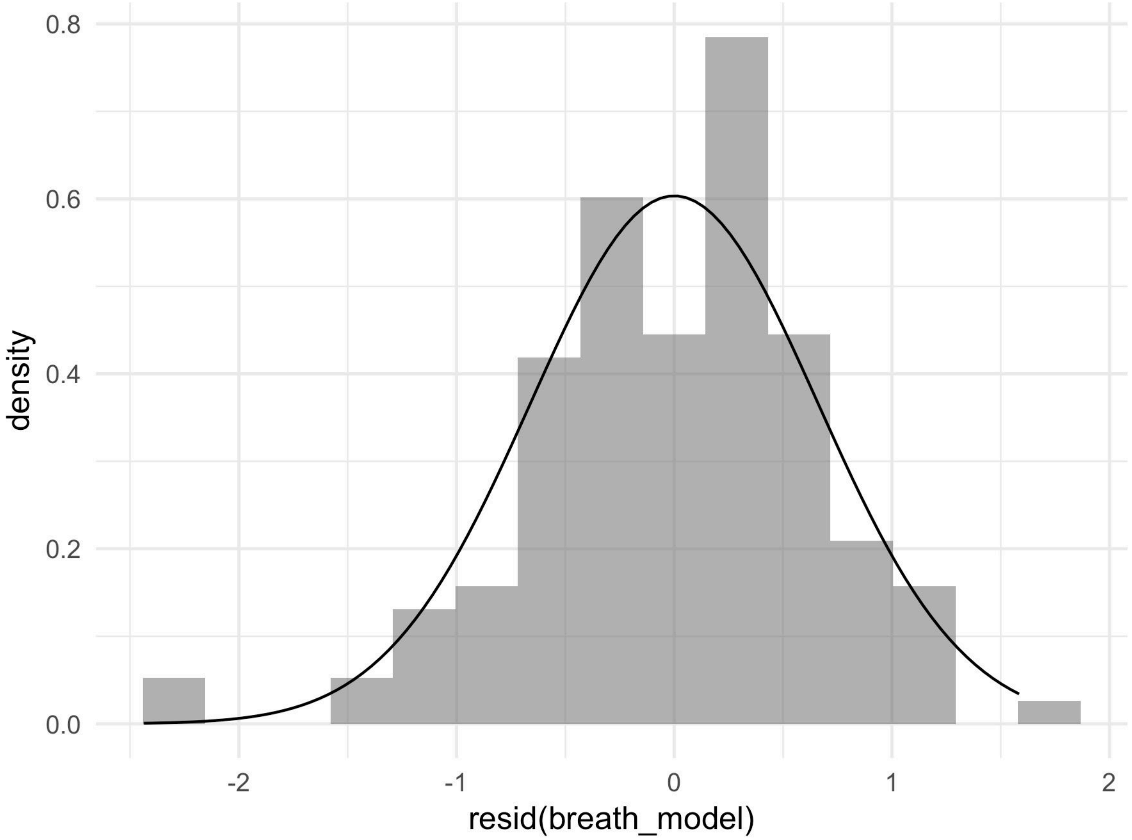
```
gam.check(breath_model)
```

```

Method: GCV   Optimizer: magic
Smoothing parameter selection converged after 76 iterations.
The RMS GCV score gradient at convergence was 1.093626e-06 .
The Hessian was positive definite.
Model rank =  72 / 72

Basis dimension (k) checking results. Low p-value (k-index<1) may
indicate that k is too low, especially if edf is close to k'.

                           k'      edf k-index p-value
s(Breath_freq)       3.00e+00 1.83e+00    0.94    0.20
s(exact)             3.00e+00 1.81e+00    1.07    0.80
s(age)               3.00e+00 1.02e-09    0.97    0.33
s(body_mass)         3.00e+00 1.00e+00    0.94    0.27
s(percentdailytotal) 3.00e+00 1.89e+00    0.89    0.12
s(animal)            8.00e+00 5.40e+00      NA      NA
s(day)               4.80e+01 9.36e+00      NA      NA
```

Residual Histogram

This graph allows us to verify the residual normality condition.

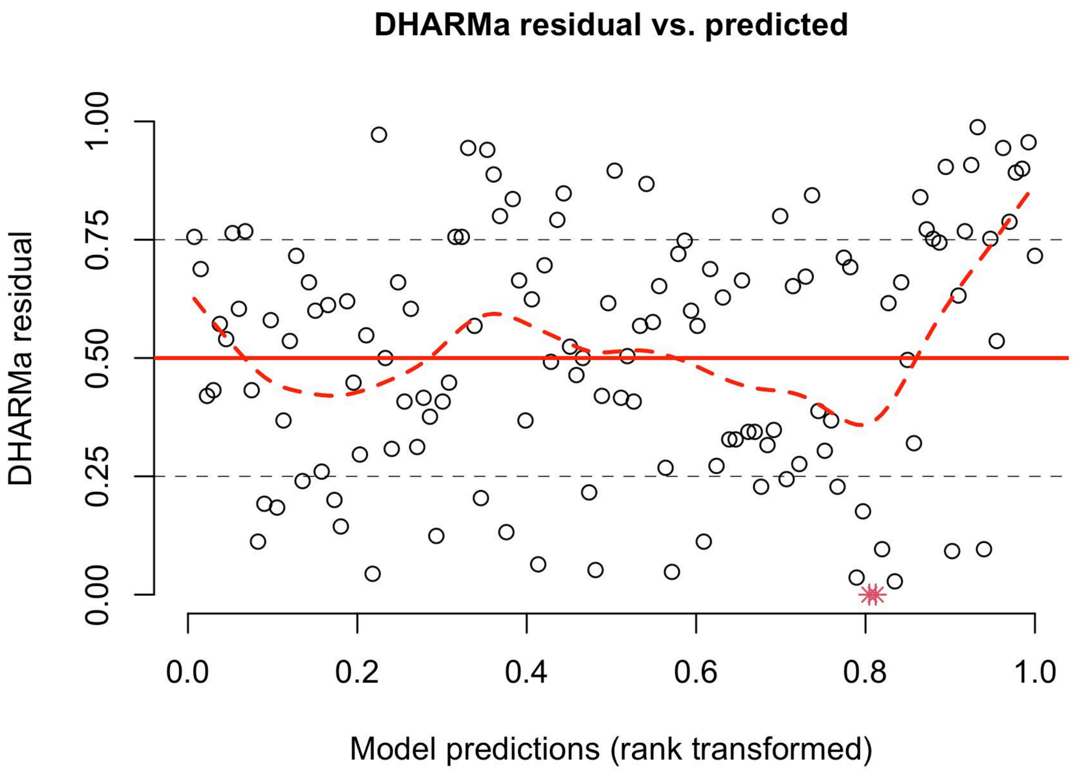
```r
gf_dhistogram(~resid(breath_model), bins = 15) |>
  gf_fitdistr(dist = "dnorm")
```



Scaled Residual Plot

The scaled residual plot (Hartig, 2024) allows verification that the mean-variance relationship is as expected, particularly for mixed-effect models and generalized linear models. If all is well, scaled residuals will be trendless and will have uniform spread between 0-1 for all fitted values.

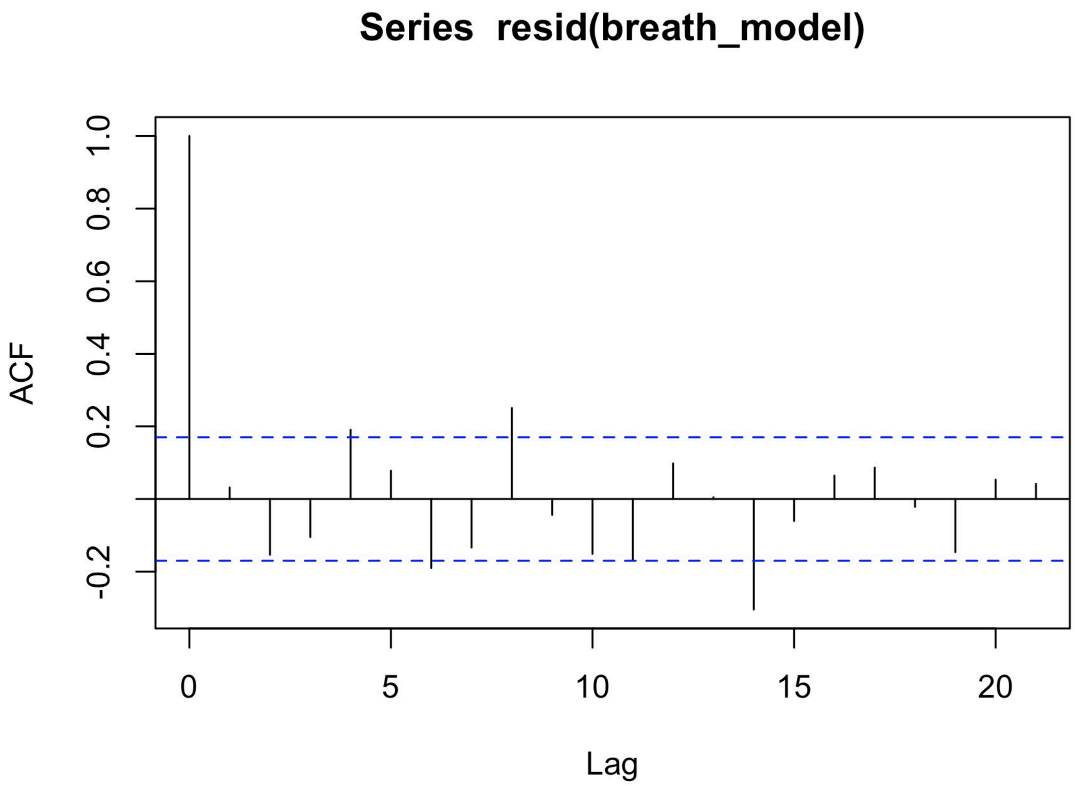
```
plotResiduals(simulateResiduals(breath_model), quantreg = FALSE)
```



Residual ACF Plot

The ACF plot allows us to verify the residual independence condition.

```r
acf(resid(breath_model))
```



Model Interpretation

Hypothesis Testing

Type II ANOVA allows us to test the null hypothesis of no association with the response, for each predictor (and interaction) included in the model.

```
anova(breath_model)
```

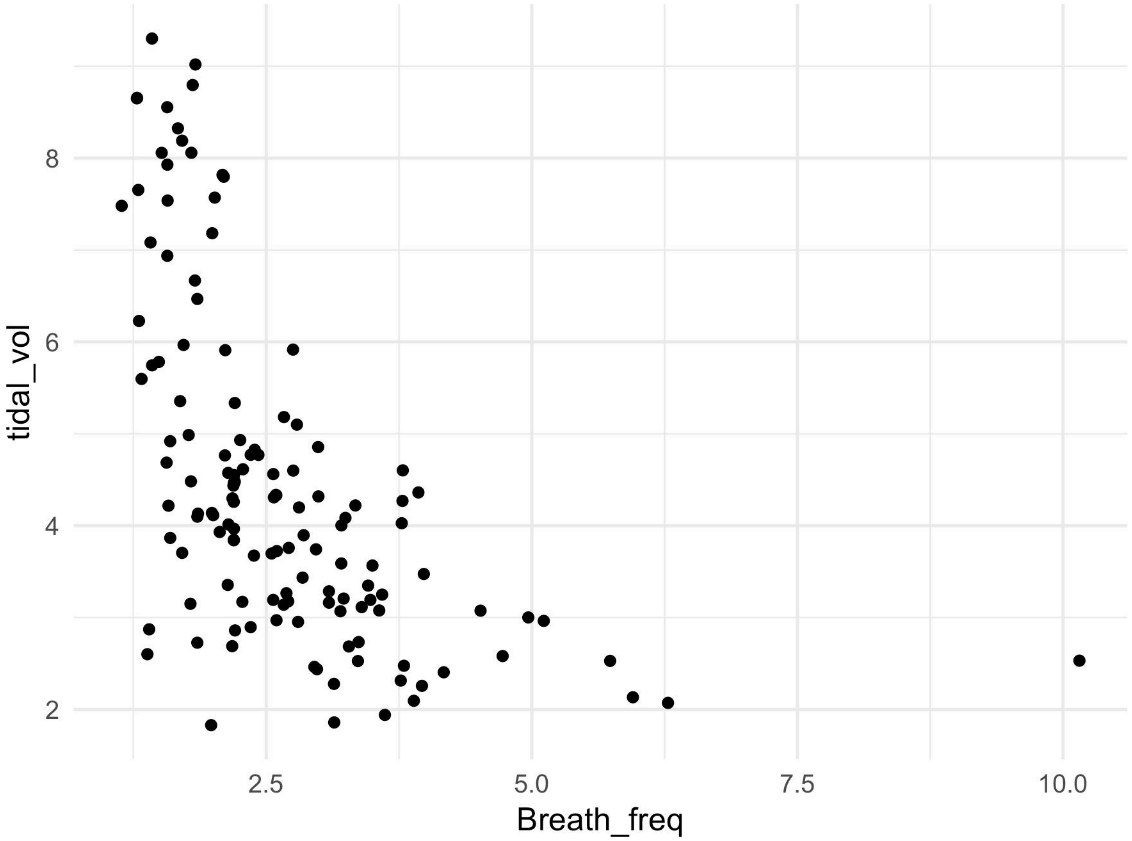
```

Family: gaussian
Link function: identity

Formula:
tidal_vol ~ s(Breath_freq, bs = "ts", k = 4) + s(exact, bs = "ts",
    k = 4) + s(age, bs = "ts", k = 4) + s(body_mass, bs = "ts",
    k = 4) + s(percentdailytotal, k = 4, bs = "ts") + s(animal,
    bs = "re") + s(day, bs = "re")

Approximate significance of smooth terms:
                           edf    Ref.df      F  p-value
```

```
s(Breath_freq)       1.830e+00 3.000e+00 47.223 2.51e-05
s(exact)             1.806e+00 3.000e+00  8.157 1.25e-05
s(age)               1.019e-09 3.000e+00  0.000   0.6499
s(body_mass)         1.001e+00 3.000e+00 85.682   0.2675
s(percentdailytotal) 1.887e+00 3.000e+00 53.658   0.2852
s(animal)            5.400e+00 7.000e+00 51.915  < 2e-16
s(day)               9.363e+00 4.700e+01  0.301   0.0575
```

The main result here is the p-value for `Breath_freq`, which is very small (6.7e-5). We can interpret this as strong evidence that tidal volume and breathing frequency are associated, (even after controlling for possible effets of time since feeding, age, mass, food amount, temperature, individual and trial day).

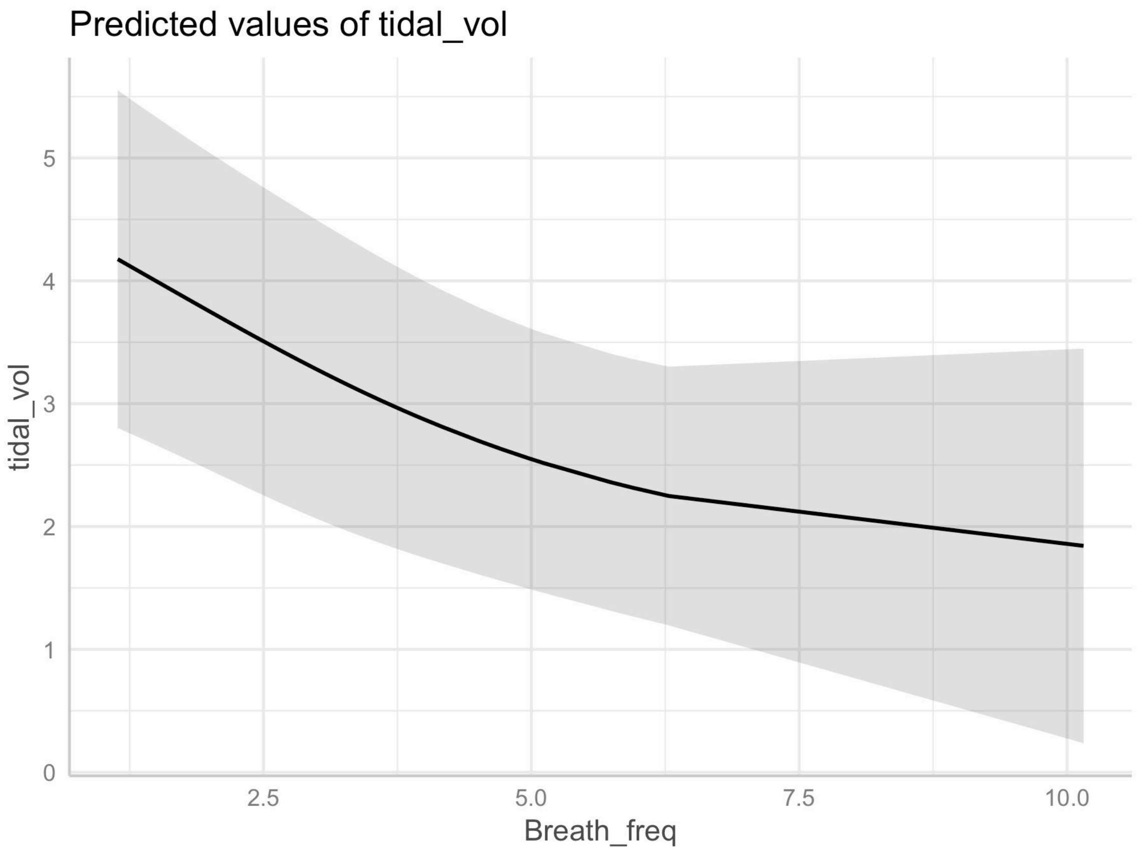
Visualization
Candidate for Figure 3?

```
gf_point(tidal_vol ~ Breath_freq, data = HIF_data)
```



What the model says (for average of all predictors in the dataset)

```
predict_response(breath_model, terms = "Breath_freq [all]",
                 condition = list(exact = 0)) |>
  plot()
```



References

Hartig F (2024). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. R package version 0.4.7, https://CRAN.R-project.org/package=DHARMa.