

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)))
```

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)
  sex +
  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") +
  sex + 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 ***
sexM           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
sex			
F	—	—	
M	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'.

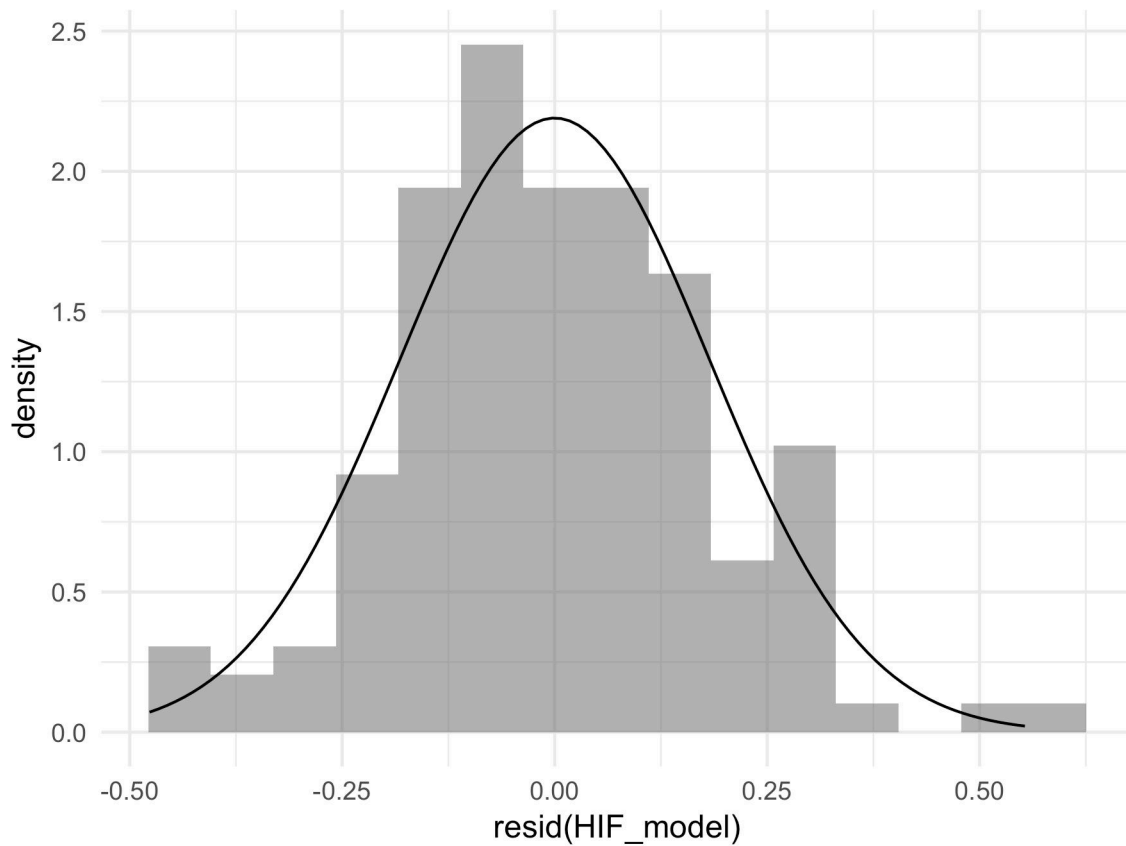
	k'	edf	k-index	p-value
s(exact)	3.00e+00	2.00e+00	1.02	0.59
s(percentdailytotal)	3.00e+00	2.46e-01	0.97	0.33
ti(exact,percentdailytotal)	9.00e+00	6.44e-01	1.02	0.56

s(body_mass)	3.00e+00	2.20e-06	1.00	0.45
s(age)	3.00e+00	5.24e-07	1.00	0.48
s(pool_temp)	3.00e+00	1.57e-06	0.98	0.38
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.

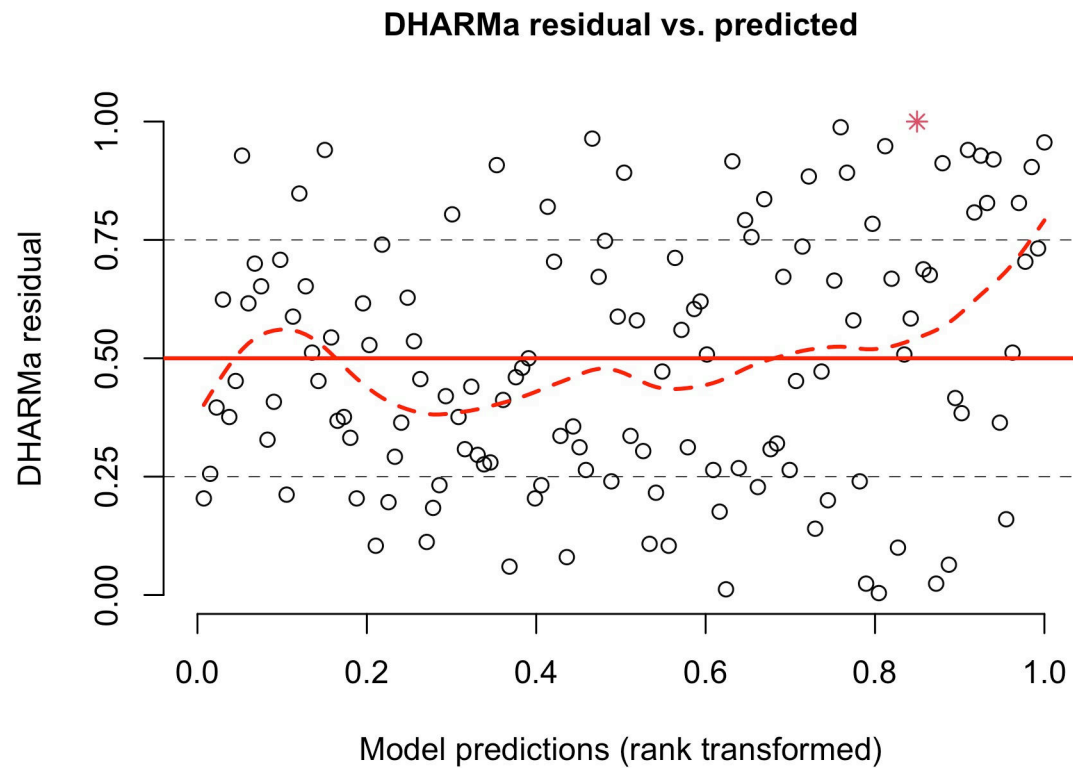
```
gf_dhistogram(~resid(HIF_model), bins = 15) |>
  gf_fitdistr(dist = "dnorm")
```



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)
```

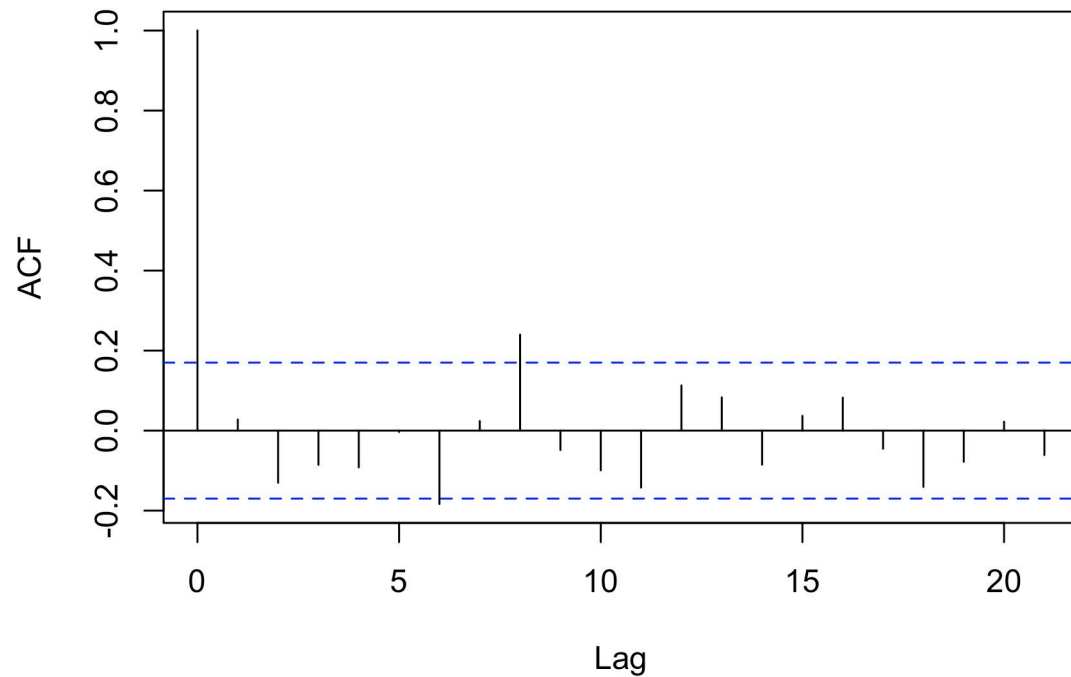


Residual ACF Plot

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

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

Series 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") +  
  sex + s(pool_temp, k = 4, bs = "ts") + s(animal, bs = "re") +  
  s(day, bs = "re")
```

```
Parametric Terms:
```

	df	F	p-value
sex	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

```
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 (%)

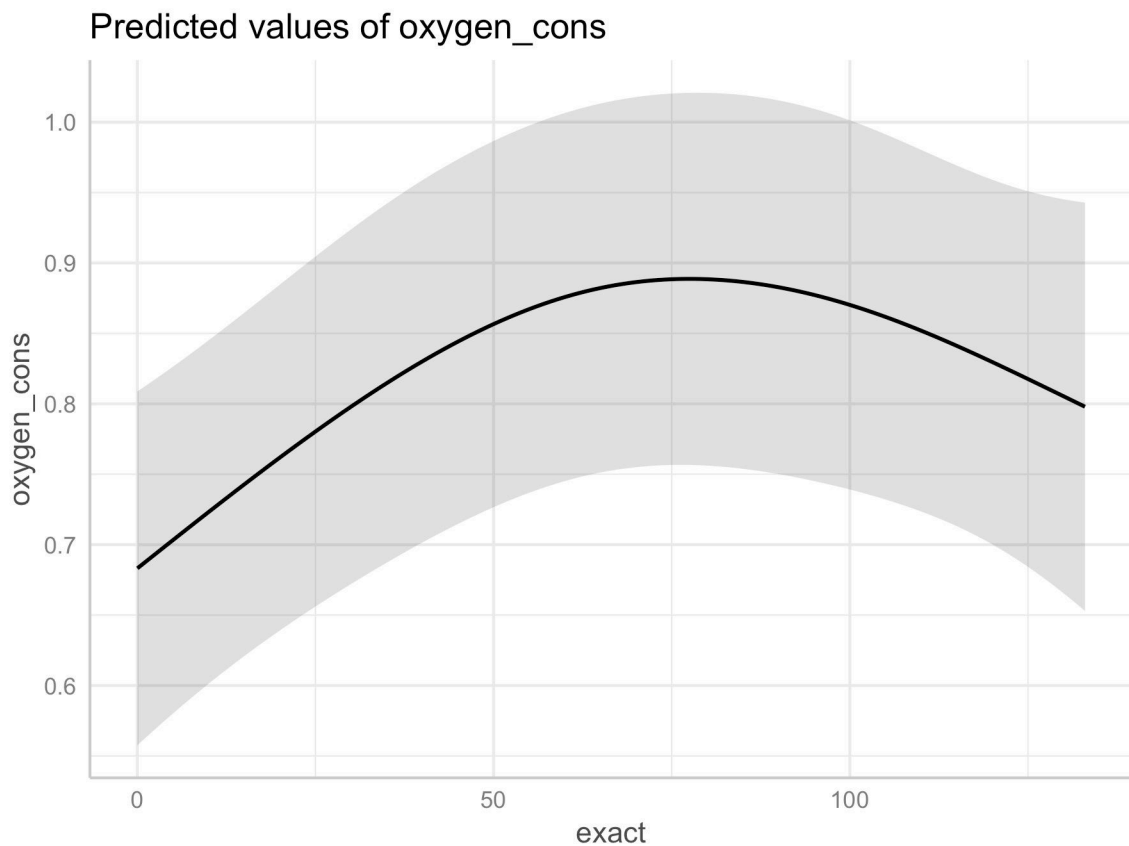
Model Predictions

```

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)
```

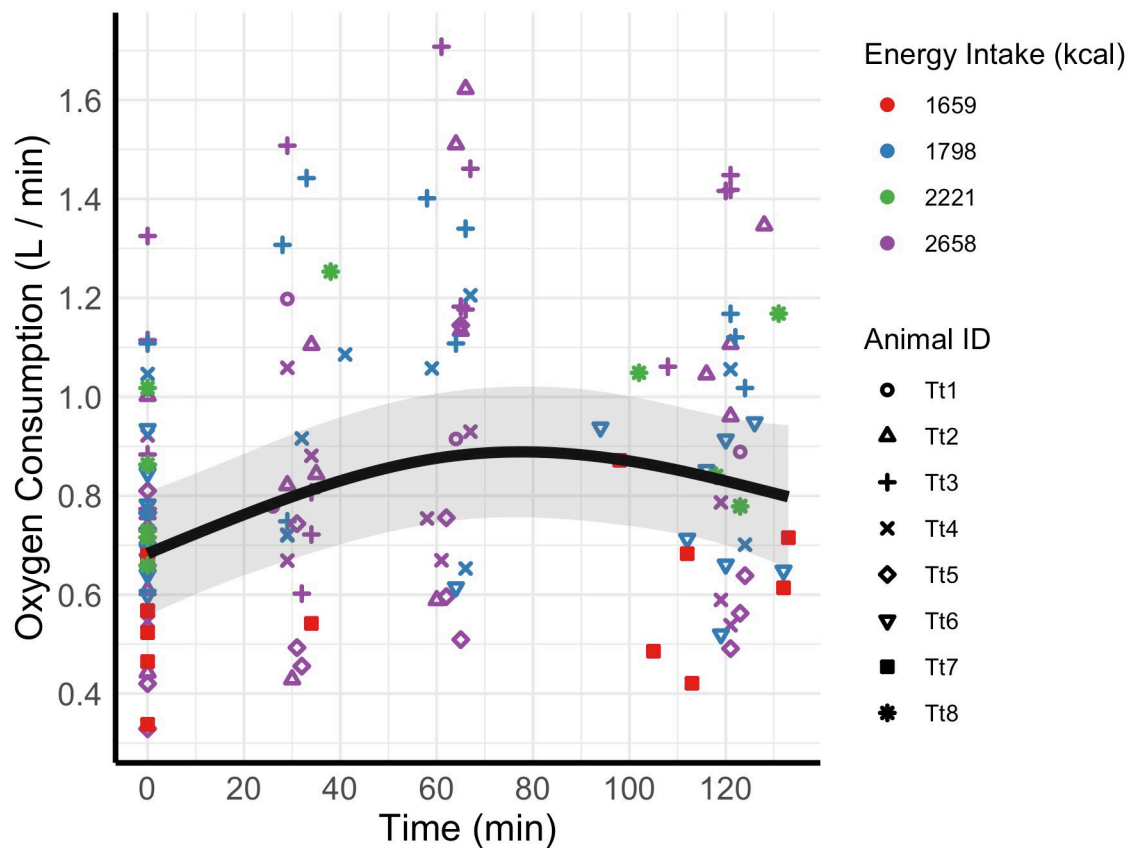
```
oxygen_time_plot <- ggplot(HIF_data,
                           aes(x = exact, y = oxygen_cons)) +
  geom_point(aes(color = factor(kcal), shape = animal),
            size = 1,
            stroke = 1.2) +
  geom_line(data = mmeans,
            aes(x = x, y = predicted),
            color = 'black',
            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: 0.6831761

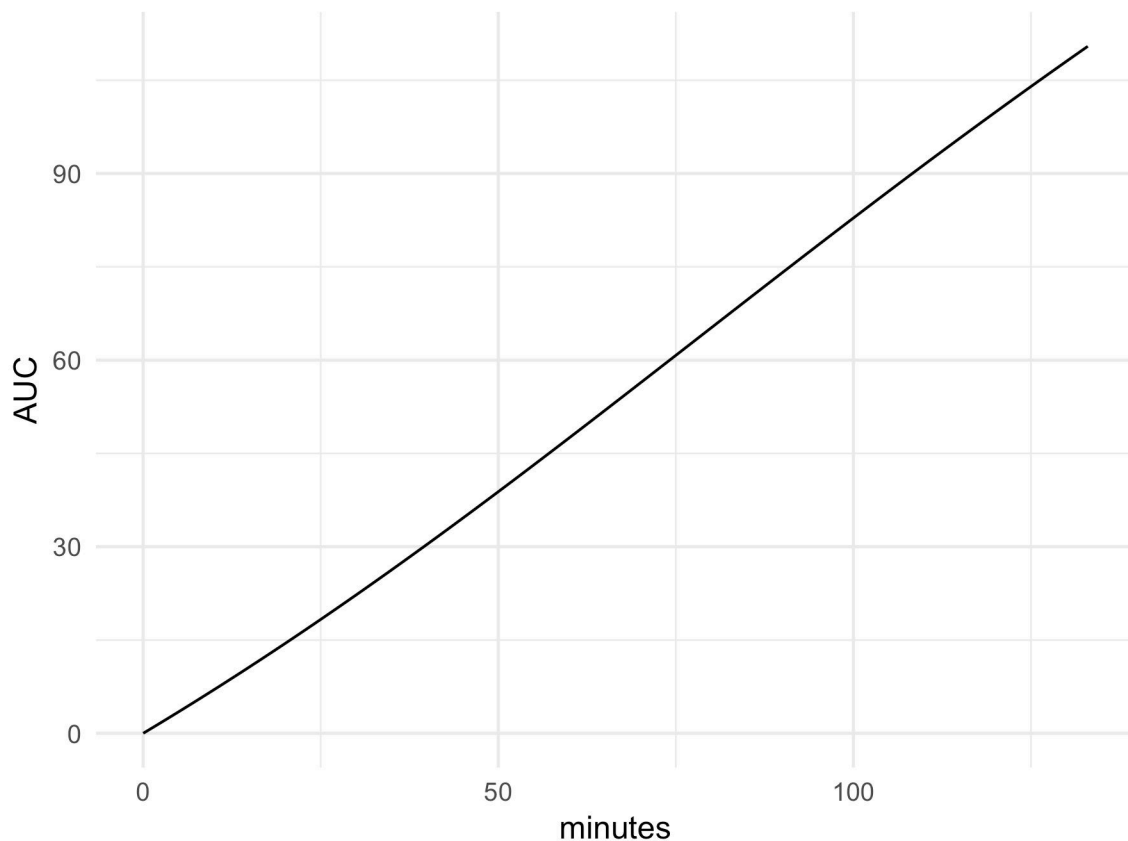
To find the cumulative area under the curve, using the marginal mean expected O_2 consumption values, we could do something like:

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

```
[1] 110.4504
```

The total at 130 minutes is: 110.4503529. Below the cumulative sums are shown:

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



To compute this quantity with uncertainty, we would probably employ a parametric bootstrap (not done yet).

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.

```
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()`:

```
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(percentdailyto- tal)			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

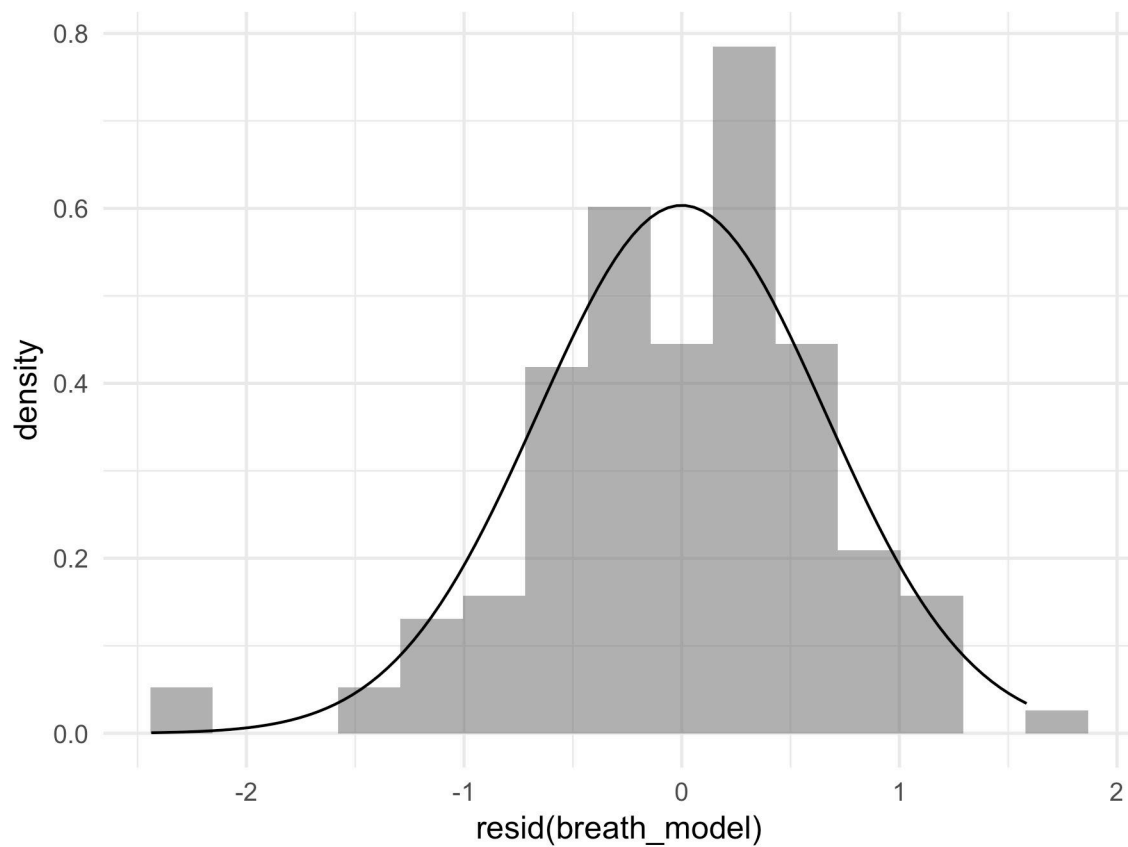
Basis dimension (k) checking results. Low p-value (k-index<1) may indicate that k is too low, especially if edf is close to k'.

	k'	edf	k-index	p-value
s(Breath_freq)	3.00e+00	1.83e+00	0.94	0.24
s(exact)	3.00e+00	1.81e+00	1.07	0.77
s(age)	3.00e+00	1.02e-09	0.97	0.34
s(body_mass)	3.00e+00	1.00e+00	0.94	0.20
s(percentdailytotal)	3.00e+00	1.89e+00	0.89	0.10
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.

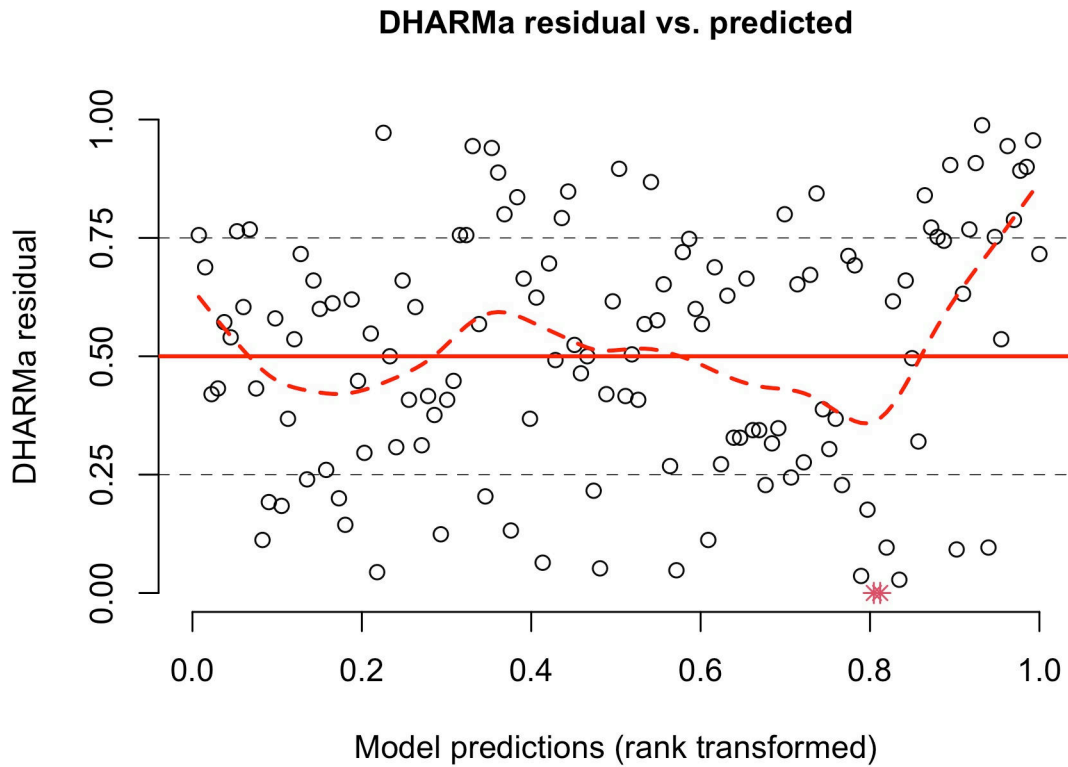
```
gf_dhistogram(~resid(breath_model), bins = 15) |>  
  gf_fitdistr(dist = "dnorm")
```



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)
```

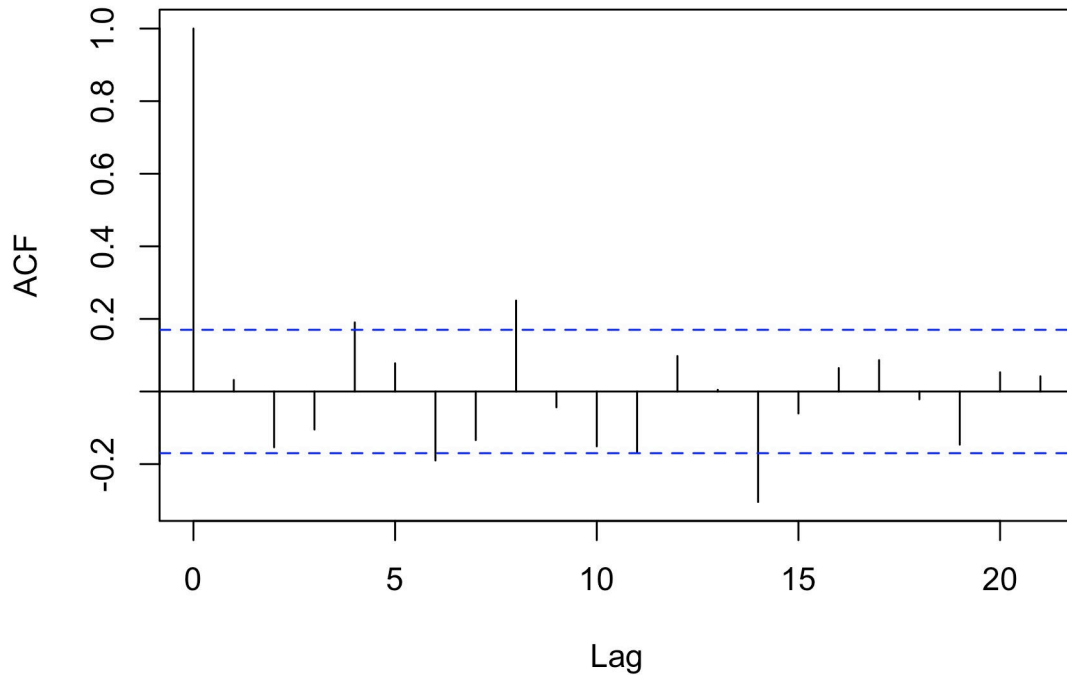


Residual ACF Plot

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

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

Series 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
```

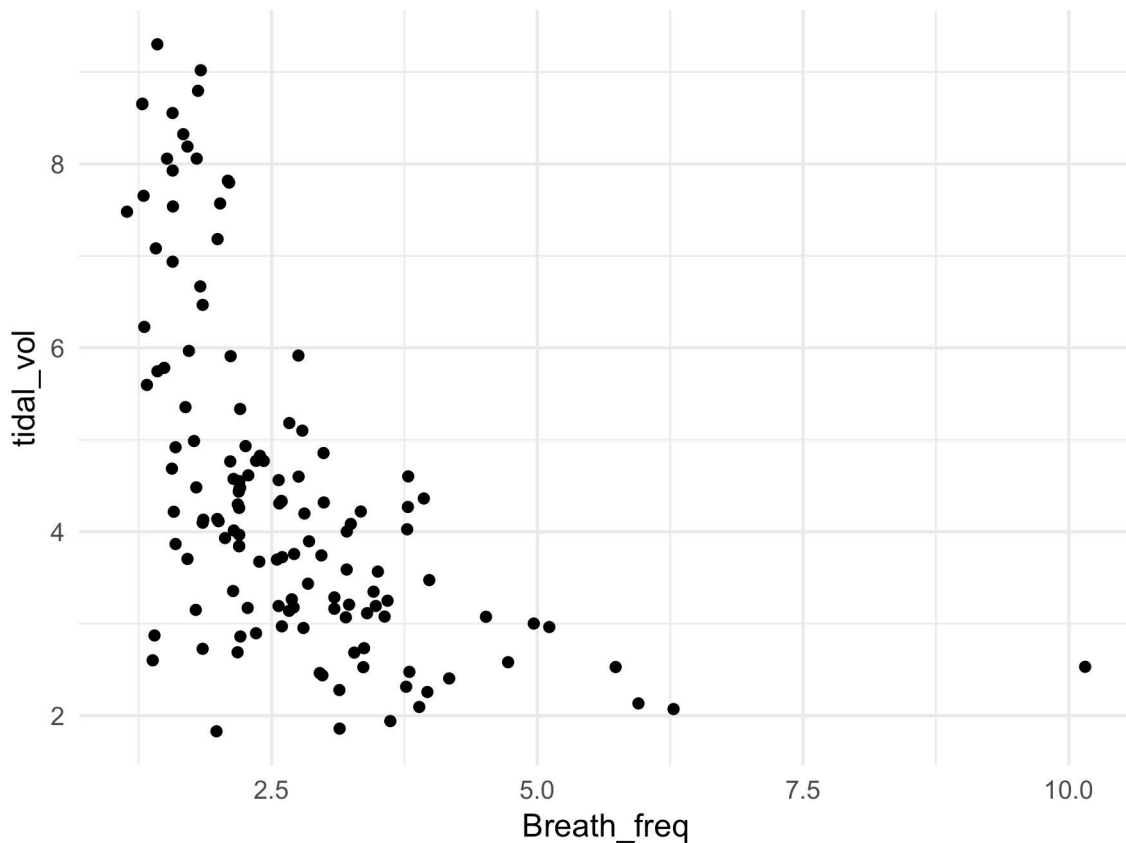
s(Breath_freq)	1.830e+00	3.000e+00	47.223	2.51e-05
s(exact)	1.806e+00	3.000e+00	8.157	1.25e-05
s(age)	1.019e-09	3.000e+00	0.000	0.6499
s(body_mass)	1.001e+00	3.000e+00	85.682	0.2675
s(percentdailytotal)	1.887e+00	3.000e+00	53.658	0.2852
s(animal)	5.400e+00	7.000e+00	51.915	< 2e-16
s(day)	9.363e+00	4.700e+01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

The main result here is the p-value for Breath_freq, which is very small (6.7×10^{-5}). We can interpret this as strong evidence that tidal volume and breathing frequency are associated, (even after controlling for possible effects of time since feeding, age, mass, food amount, temperature, individual and trial day).

Visualization

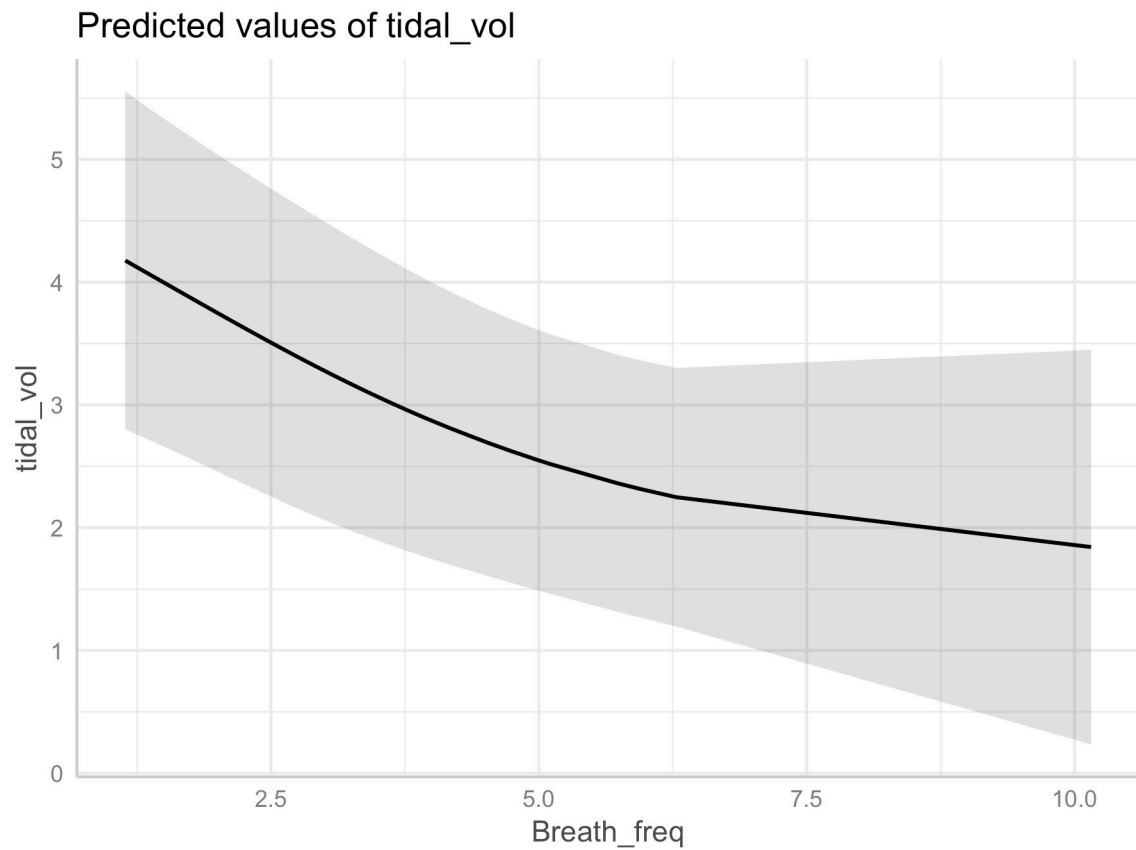
Candidate for Figure 3?

```
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>.