Supplement 4

FO2Hb and SaO2 with other co-oximetry covariates

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Contents

1   Research question

1. Determine whether along with functional oxyhaemoglobin saturation (SaO2, %Hb) and fractional oxyhaemoglobin saturation (FO2Hb, %Hb), total haemoglobin concentration (Hb, g/dl), carboxyhaemoglobin (COHb, %Hb), and methaemoglobin (MetHb, %Hb) are significant covariates in the prediction of each CIE L*a*b* component.

2   Load data

```
data <- read_rds('data-cleaned/clean-data.rds')
```

3   Quick look at the data

```
dim(data)
```

```
## [1] 163  22
```

```
names(data)
```

```
##  [1] "Animal_ID"        "Trial"            "Dpat"
##  [4] "Time_min"         "l_star"           "a_star"
##  [7] "b_star"           "PaO2"             "cal_SaO2"
## [10] "PaCO2"            "Cl"               "HCO3"
## [13] "Temp"             "pH"               "Cartridge_expired"
## [16] "Coox_SaO2"        "Hct"              "total_Hb"
## [19] "O2Hb"             "COHb"             "MetHb"
## [22] "O2_suppl"
```

```
glimpse(data)
```

```
## Rows: 163
## Columns: 22
## $ Animal_ID         <chr> "I1", "I1", "I1", "I1", "I1", "I1", "I1", "I1", "...
## $ Trial             <dbl> 1, 1, 1, 1, 1, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5...
## $ Dpat              <dbl> 0, 0, 0, 0, 0, 5, 5, 5, 5, 5, 0, 0, 0, 0, 0, 0, 0...
## $ Time_min          <dbl> 5, 10, 15, 20, 30, 5, 10, 15, 20, 30, 5, 10, 15, ...
## $ l_star            <dbl> 11.3, 12.9, 13.5, 14.6, 15.6, 12.6, 13.6, 13.8, 1...
## $ a_star            <dbl> 24.5, 31.5, 31.4, 34.1, 34.8, 30.7, 33.0, 31.4, 3...
## $ b_star            <dbl> 11.5, 17.5, 14.7, 19.9, 20.1, 16.5, 18.1, 18.2, 2...
## $ PaO2              <dbl> 27.9, 40.4, 35.3, 41.7, 40.3, 33.9, 34.9, 36.5, 4...
## $ cal_SaO2          <dbl> 45.6, 71.0, 62.3, 73.4, 71.9, 56.7, 60.1, 61.5, 7...
## $ PaCO2             <dbl> 54.9, 55.6, 58.5, 56.3, 57.6, 63.4, 62.4, 63.8, 6...
## $ Cl                <dbl> 113, 113, 111, 111, 109, 107, 106, 106, 105, 105,...
## $ HCO3              <dbl> 27.9, 29.9, 31.3, 31.3, 33.0, 31.0, 32.0, 31.0, 3...
## $ Temp              <dbl> 39.2, 39.7, 39.7, 39.7, 39.6, 39.2, 39.6, 39.6, 3...
## $ pH                <dbl> 7.31, 7.34, 7.34, 7.35, 7.37, 7.30, 7.32, 7.29, 7...
## $ Cartridge_expired <chr> "No", "No", "No", "No", "No", "No", "No", "No", "...
## $ Coox_SaO2         <dbl> 53.0, 67.8, 63.3, 74.1, 73.1, 53.8, 64.2, 56.2, 7...
## $ Hct               <dbl> 23, 24, 21, 20, 18, 27, 23, 26, 20, 19, 21, 20, 1...
## $ total_Hb          <dbl> 8.7, 7.8, 7.6, 7.2, 6.7, 9.6, 8.9, 9.8, 7.7, 7.3,...
```

```
## $ O2Hb                <dbl> 51.0, 65.4, 60.0, 71.0, 69.4, 51.9, 61.7, 54.2, 6...
## $ COHb                <dbl> 0.0, 1.2, 2.4, 1.0, 1.7, 0.0, 0.8, 0.0, 1.5, 1.4,...
## $ MetHb               <dbl> 3.7, 2.3, 2.8, 3.2, 3.3, 3.4, 3.1, 3.4, 2.4, 2.9,...
## $ O2_suppl            <chr> "No", "No", "No", "No", "No", "No", "No", "No", "...
```

---

4 Prepare data

```r
# Convert Animal ID to a factor
data %<>%
    mutate(Animal_ID = factor(Animal_ID,
                              levels = c('I1', 'I2', 'I3', 'I4', 'I5',
                                         'I6', 'I8', 'I9', 'I10', 'I11')))
```

---

5 FO2Hb

5.1 L*

5.1.1 Exploratory plots

```r
lstar <- data %>%
    select(Animal_ID, l_star, O2Hb, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(O2Hb, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
                             levels = c('O2Hb', 'total_Hb', 'COHb', 'MetHb'),
                             labels = c('FO2Hb (%)', 'Hb (g/dl)',
                                        'COHb (%)', 'MetHb (%)'))) %>%
    group_by(Variable) %>%
    nest() %>%
    mutate(plot = map2(.x = data,
                       .y = Variable,
                       ~ .x %>%
                           ggplot(data = .) +
                           aes(x = Values,
                               y = l_star) +
                           geom_point(aes(fill = Animal_ID),
                                      shape = 21,
                                      size = 3,
                                      alpha = 0.8) +
                           geom_smooth(se = FALSE,
                                       colour = '#000000') +
                           labs(title = str_glue('L* vs {.y}'),
                                subtitle = 'Black line: LOESS curve',
                                x = .y,
                                y = 'L*') +
                           scale_fill_viridis_d(name = 'Animal')))

walk(lstar$plot, ~print(.x))
```
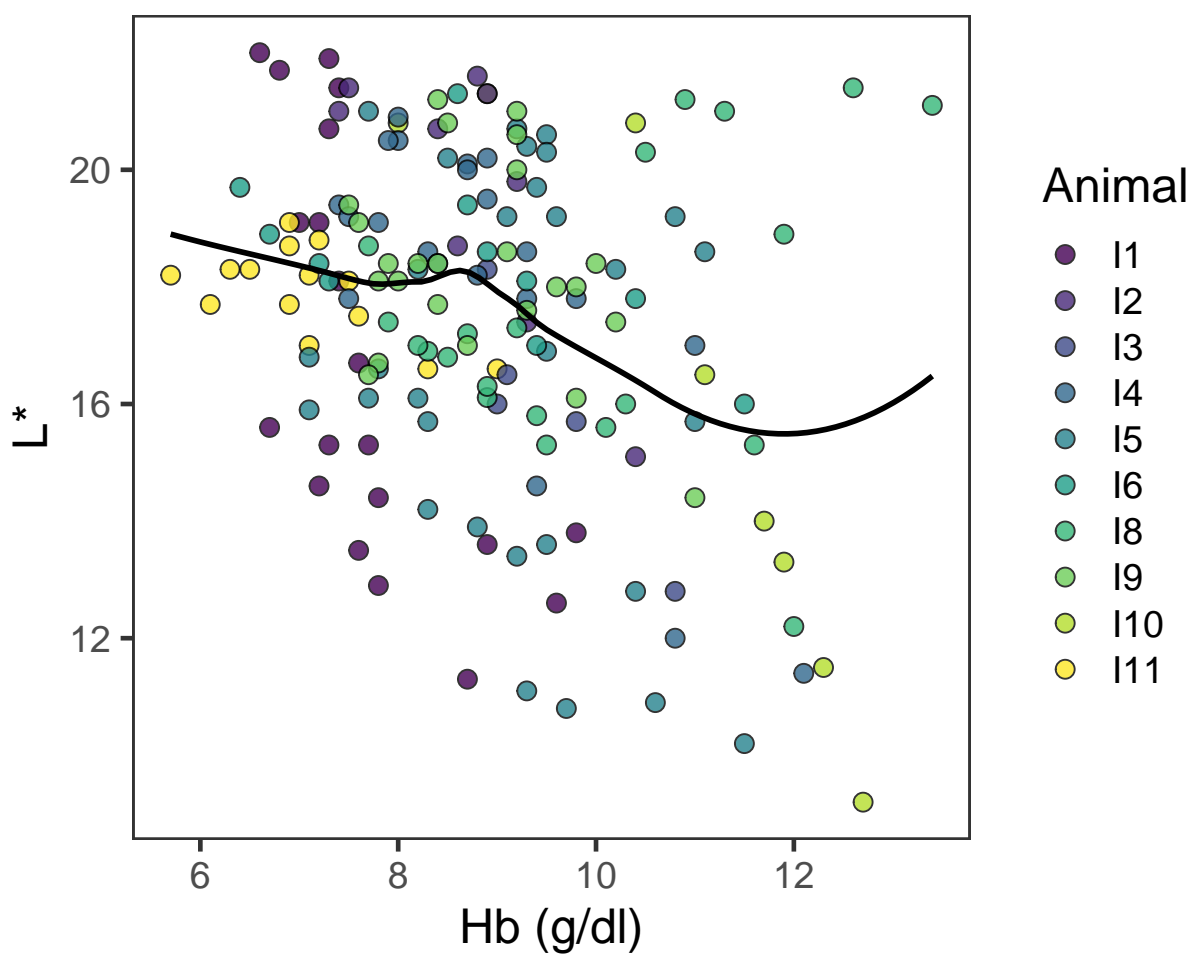
L* vs FO2Hb (%)

Black line: LOESS curve
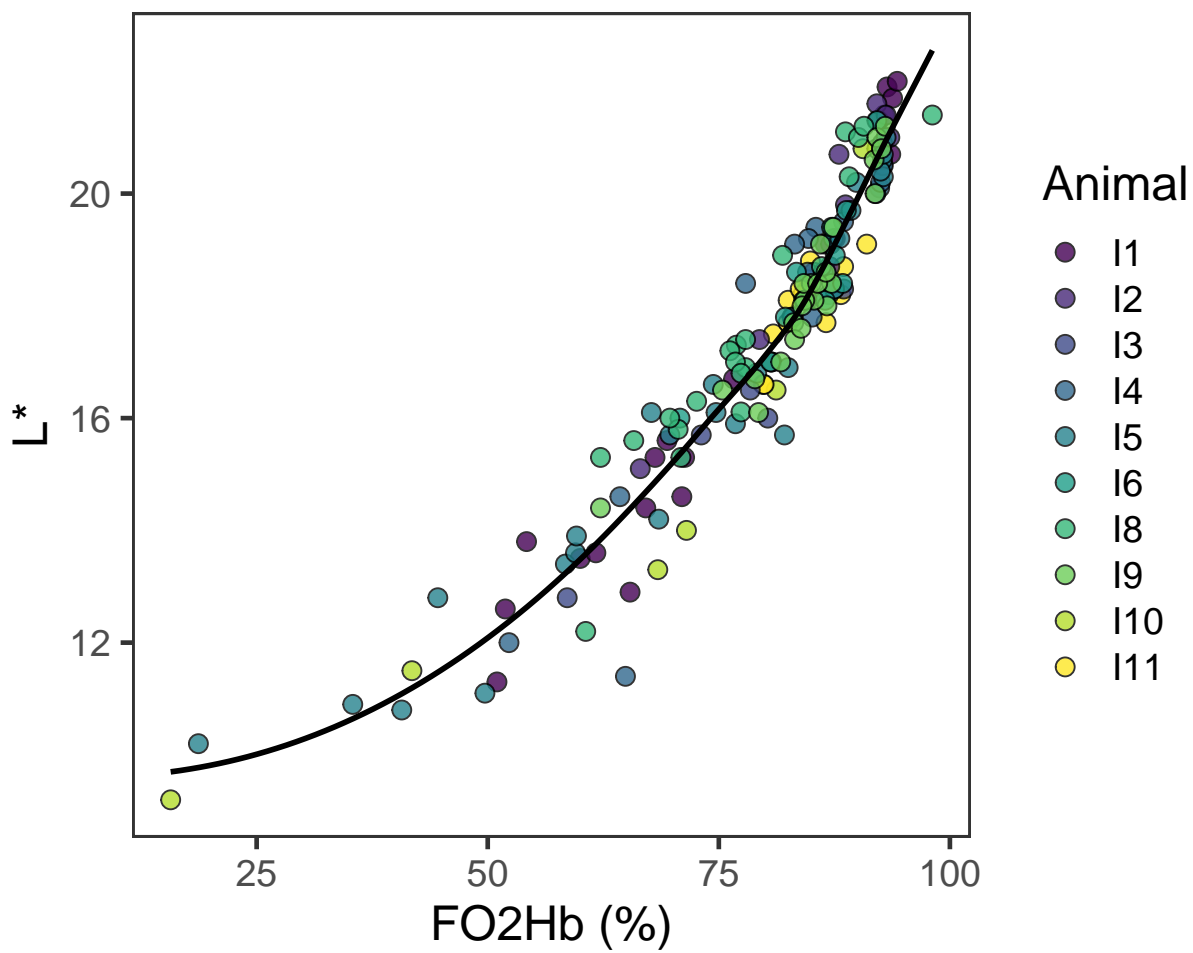
L* vs Hb (g/dl)

Black line: LOESS curve

L* vs COHb (%)

Black line: LOESS curve
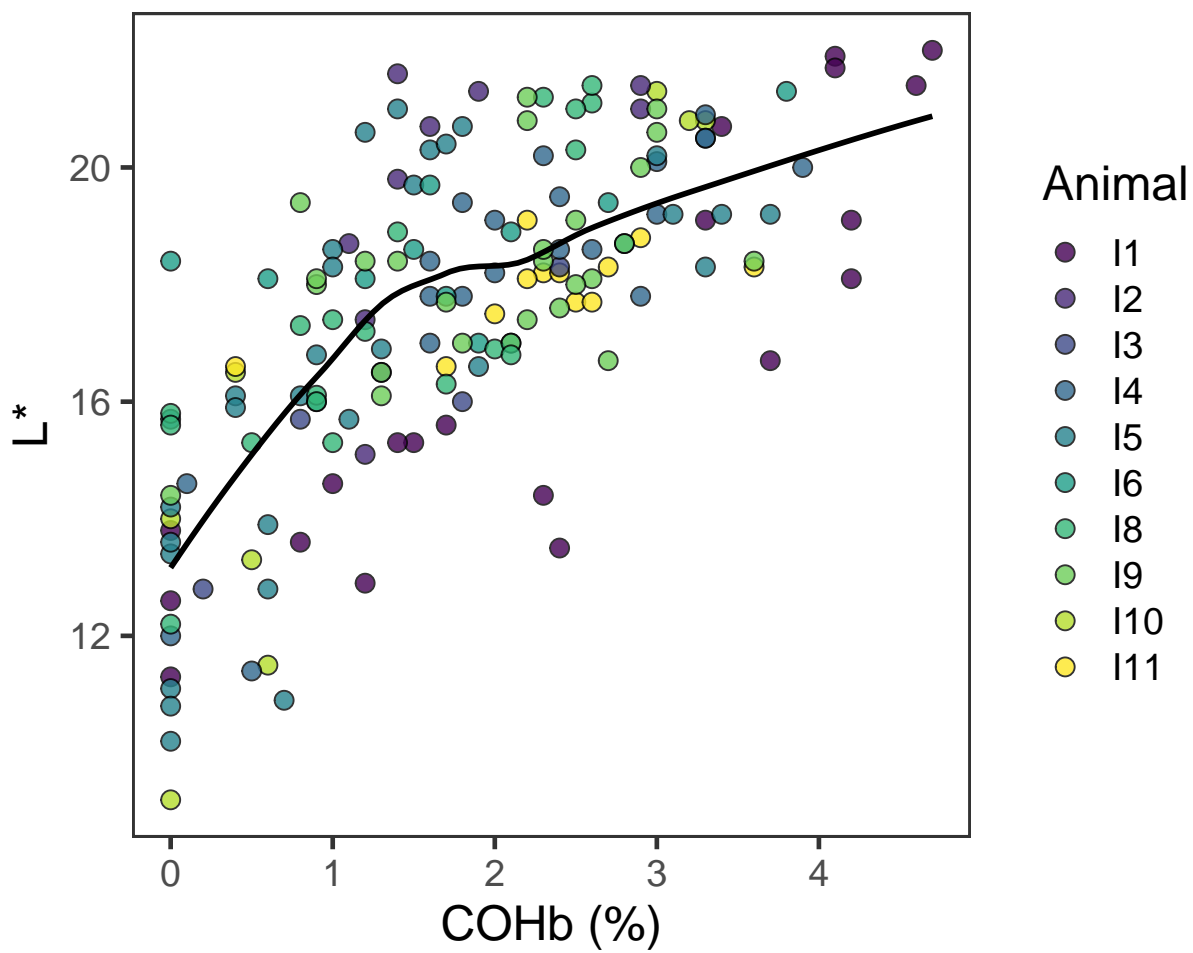
L* vs MetHb (%)
Black line: LOESS curve
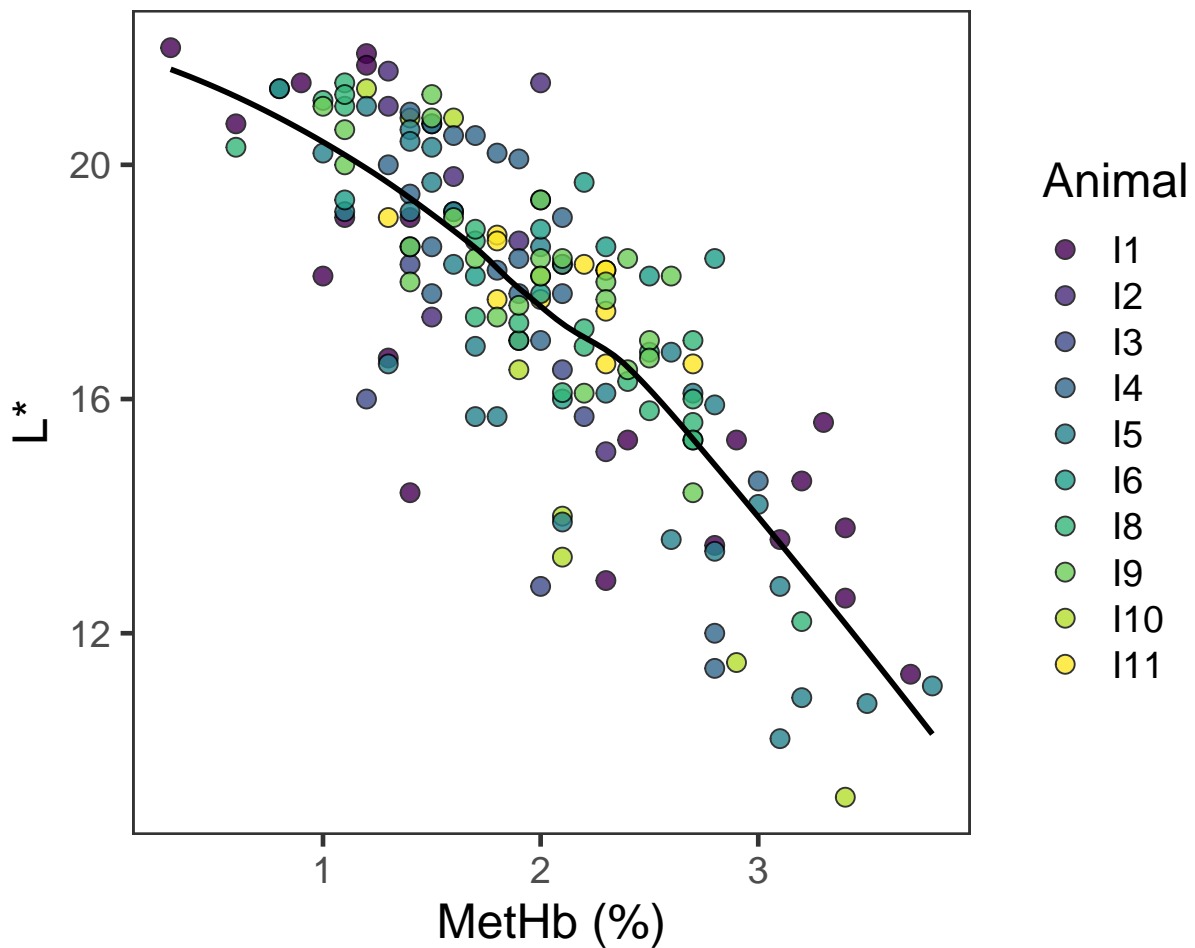
Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

5.1.2 Model

```r
# Specify model
lstar_full <- lmer(l_star ~ poly(O2Hb, 2) +
                        poly(COHb, 2) +
                        MetHb +
                        total_Hb +
                        (1 | Animal_ID),
                   data = data)

# Summarise model
summary(lstar_full)
```

5.1.2.1 Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: l_star ~ poly(O2Hb, 2) + poly(COHb, 2) + MetHb + total_Hb + (1 |
##     Animal_ID)
##    Data: data
##
```

```
## REML criterion at convergence: 340.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.5807 -0.6036  0.0237  0.5584  2.3899
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.1224   0.3499
##  Residual              0.4376   0.6615
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                 Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)     19.11188    0.63345 153.20676  30.171   <2e-16 ***
## poly(O2Hb, 2)1  28.96566    1.32561 154.79076  21.851   <2e-16 ***
## poly(O2Hb, 2)2   7.64044    0.77662 154.32295   9.838   <2e-16 ***
## poly(COHb, 2)1   1.23166    1.25835 155.93756   0.979   0.3292
## poly(COHb, 2)2   0.26814    0.76770 154.16860   0.349   0.7274
## MetHb           -0.40964    0.15718 154.15009  -2.606   0.0101 *
## total_Hb        -0.09064    0.04948 153.97419  -1.832   0.0689 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(O2H,2)1 p(O2H,2)2 p(COH,2)1 p(COH,2)2 MetHb
## ply(O2H,2)1 -0.535
## ply(O2H,2)2 -0.003  0.260
## ply(COH,2)1 -0.455 -0.165    -0.285
## ply(COH,2)2 -0.007  0.212    -0.080    -0.104
## MetHb       -0.736  0.604     0.143     0.498     0.031
## total_Hb    -0.870  0.346    -0.099     0.311    -0.008     0.362
```

```r
# Fixed effect with 95%CI
cbind(fixef(lstar_full), confint(lstar_full)[3:9, ]) %>%
    kable(caption = 'L* full model: FO2Hb, COHb, MetHb, and [Hb]',
        digits = 3,
        col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 1: L* full model: FO2Hb, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	19.112	17.872	20.325
poly(O2Hb, 2)1	28.966	26.427	31.587
poly(O2Hb, 2)2	7.640	6.144	9.145
poly(COHb, 2)1	1.232	-1.234	3.643
poly(COHb, 2)2	0.268	-1.223	1.745
MetHb	-0.410	-0.713	-0.106
total_Hb	-0.091	-0.185	0.008

```r
# FO2Hb only
lstar_FO2Hb <- lmer(l_star ~ poly(O2Hb, 2) +
                    (1 | Animal_ID),
                data = data)


# FO2Hb + MetHB
```

```r
lstar_MetHb <- lmer(l_star ~ poly(O2Hb, 2) +
                        MetHb +
                        (1 | Animal_ID),
                    data = data)

# Compare model fits
anova(lstar_MetHb, lstar_FO2Hb)
```

5.1.2.2 Compare model with and without methaemoglobin

```
## Data: data
## Models:
## lstar_FO2Hb: l_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
## lstar_MetHb: l_star ~ poly(O2Hb, 2) + MetHb + (1 | Animal_ID)
##             Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## lstar_FO2Hb  5 362.57 378.04 -176.29   352.57
## lstar_MetHb  6 354.96 373.52 -171.48   342.96 9.6129      1   0.001932 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Compare model R-squared values
r.squaredLR(lstar_FO2Hb)
```

```
## [1] 0.9338965
## attr(,"adj.r.squared")
## [1] 0.9410464
```

```r
r.squaredLR(lstar_MetHb)
```

```
## [1] 0.9376807
## attr(,"adj.r.squared")
## [1] 0.9448596
```

```r
# Compare model RMSE
RMSE.merMod(lstar_FO2Hb)
```

```
## [1] 0.6711249
```

```r
RMSE.merMod(lstar_MetHb)
```

```
## [1] 0.6506168
```

Likelihood ratio test found model with MetHb had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

5.2 a*

5.2.1 Exploratory plots

```r
astar <- data %>%
    select(Animal_ID, a_star, O2Hb, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(O2Hb, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
                             levels = c('O2Hb', 'total_Hb', 'COHb', 'MetHb'),
                             labels = c('FO2Hb (%)', 'Hb (g/dl)',
                                        'COHb (%)', 'MetHb (%)'))) %>%
    group_by(Variable) %>%
    nest() %>%
    mutate(plot = map2(.x = data,
```

```
                    .y = Variable,
                ~ .x %>%
                    ggplot(data = .) +
                    aes(x = Values,
                        y = a_star) +
                    geom_point(aes(fill = Animal_ID),
                                shape = 21,
                                size = 3,
                                alpha = 0.8) +
                    geom_smooth(se = FALSE,
                                colour = '#000000') +
                    labs(title = str_glue('a* vs {.y}'),
                        subtitle = 'Black line: LOESS curve',
                        x = .y,
                        y = 'a*') +
                    scale_fill_viridis_d(name = 'Animal')))

walk(astar$plot, ~print(.x))
```
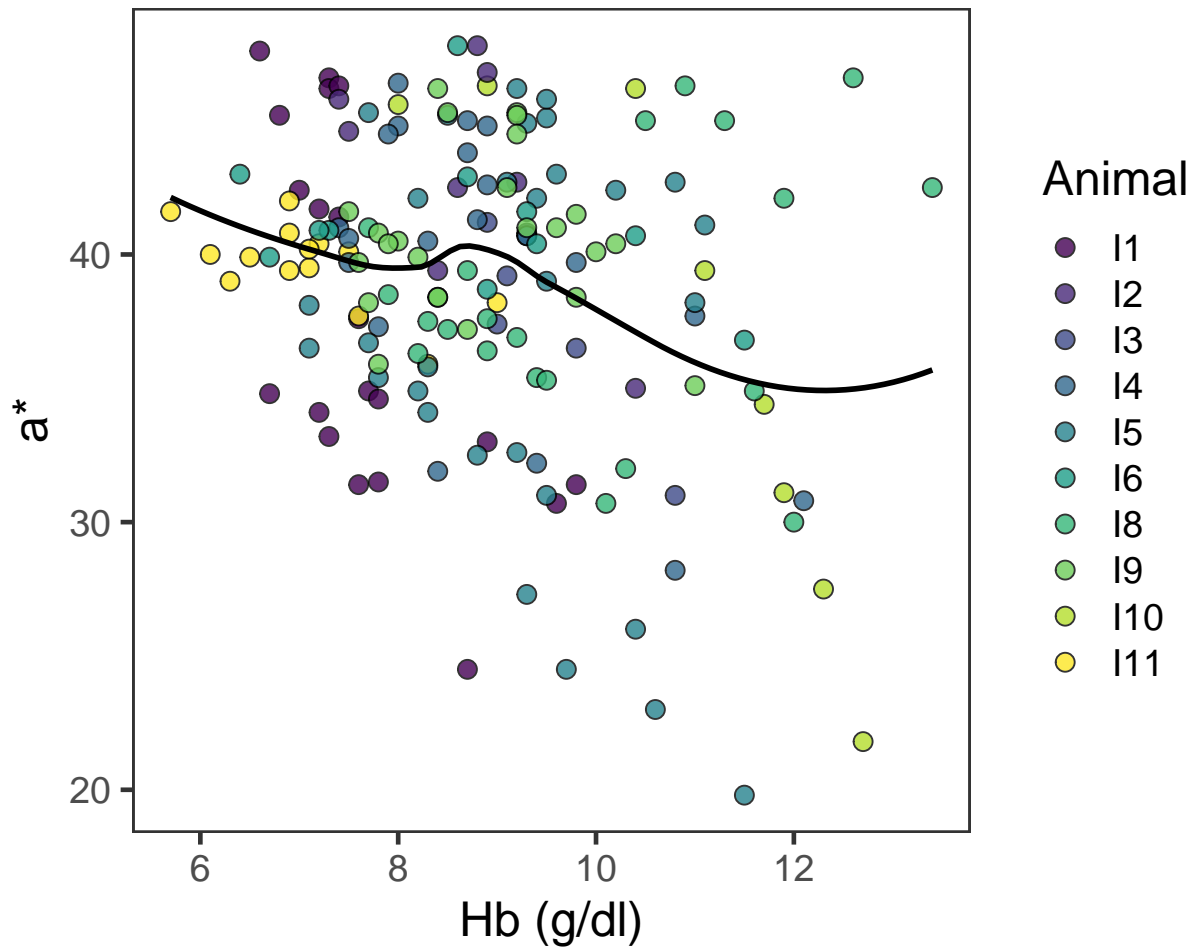
a* vs FO2Hb (%)

Black line: LOESS curve

a* vs Hb (g/dl)

Black line: LOESS curve

a* vs COHb (%)

Black line: LOESS curve

a* vs MetHb (%)

Black line: LOESS curve

Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

5.2.2   Model

```
# Specify model
astar_full <- lmer(a_star ~ poly(O2Hb, 2) +
                       poly(COHb, 2) +
                       MetHb +
                       total_Hb +
                       (1 | Animal_ID),
                  data = data)

# Summarise model
summary(astar_full)
```

5.2.2.1   Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: a_star ~ poly(O2Hb, 2) + poly(COHb, 2) + MetHb + total_Hb + (1 |
##       Animal_ID)
##     Data: data
##
```

```
## REML criterion at convergence: 553.9
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -4.1134 -0.5428  0.0747  0.5395  2.1956
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.06454  0.254
##  Residual              1.83673  1.355
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                 Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)     39.78411    1.21852 132.56569  32.650  < 2e-16 ***
## poly(O2Hb, 2)1  63.34899    2.61365 148.81905  24.238  < 2e-16 ***
## poly(O2Hb, 2)2   9.75421    1.53782 151.62846   6.343 2.46e-09 ***
## poly(COHb, 2)1  -0.76606    2.44235 131.92920  -0.314  0.75428
## poly(COHb, 2)2   3.06330    1.51844 150.26694   2.017  0.04543 *
## MetHb           -1.00423    0.31226 153.96480  -3.216  0.00158 **
## total_Hb         0.13361    0.09342 103.28725   1.430  0.15570
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(O2H,2)1 p(O2H,2)2 p(COH,2)1 p(COH,2)2 MetHb
## ply(O2H,2)1 -0.537
## ply(O2H,2)2  0.007  0.239
## ply(COH,2)1 -0.505 -0.153    -0.255
## ply(COH,2)2 -0.035  0.283    -0.061    -0.143
## MetHb       -0.773  0.612     0.154     0.488     0.049
## total_Hb    -0.878  0.335    -0.126     0.381     0.017     0.394
```

```r
# Fixed effect with 95%CI
cbind(fixef(astar_full), confint(astar_full)[3:9, ]) %>%
    kable(caption = 'a* full model: FO2Hb, COHb, MetHb, and [Hb]',
          digits = 3,
          col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 2: a* full model: FO2Hb, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	39.784	37.482	42.188
poly(O2Hb, 2)1	63.349	58.187	68.301
poly(O2Hb, 2)2	9.754	6.773	12.691
poly(COHb, 2)1	-0.766	-5.639	3.847
poly(COHb, 2)2	3.063	0.196	6.154
MetHb	-1.004	-1.646	-0.415
total_Hb	0.134	-0.046	0.311

```r
# FO2Hb only
astar_FO2Hb <- lmer(a_star ~ poly(O2Hb, 2) +
                    (1 | Animal_ID),
                data = data)


# FO2Hb + MetHB + COHb
```

```
astar_MetCOHb <- lmer(a_star ~ poly(O2Hb, 2) +
                       poly(COHb, 2) +
                       MetHb +
                       (1 | Animal_ID),
                   data = data)

# Compare model fits
anova(astar_MetCOHb, astar_FO2Hb)
```

5.2.2.2 Compare model with and without methaemoglobin and carboxyhaemoglobin

```
## Data: data
## Models:
## astar_FO2Hb: a_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
## astar_MetCOHb: a_star ~ poly(O2Hb, 2) + poly(COHb, 2) + MetHb + (1 | Animal_ID)
##               Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## astar_FO2Hb    5 592.68 608.15 -291.34   582.68
## astar_MetCOHb  8 576.88 601.63 -280.44   560.88 21.808      3  7.151e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Compare model R-squared values
r.squaredLR(astar_FO2Hb)
```

```
## [1] 0.932471
## attr(,"adj.r.squared")
## [1] 0.9342383
```

```
r.squaredLR(astar_MetCOHb)
```

```
## [1] 0.9408995
## attr(,"adj.r.squared")
## [1] 0.9426827
```

```
# Compare model RMSE
RMSE.merMod(astar_FO2Hb)
```

```
## [1] 1.383574
```

```
RMSE.merMod(astar_MetCOHb)
```

```
## [1] 1.322884
```

Likelihood ratio test found model with MetHb and COHb had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

5.3 b*

5.3.1 Exploratory plots

```
bstar <- data %>%
    select(Animal_ID, b_star, O2Hb, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(O2Hb, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
                        levels = c('O2Hb', 'total_Hb', 'COHb', 'MetHb'),
                        labels = c('FO2Hb (%)', 'Hb (g/dl)',
                                   'COHb (%)', 'MetHb (%)'))) %>%
    group_by(Variable) %>%
    nest() %>%
```

```
    mutate(plot = map2(.x = data,
                       .y = Variable,
                       ~ .x %>%
                           ggplot(data = .) +
                           aes(x = Values,
                               y = b_star) +
                           geom_point(aes(fill = Animal_ID),
                                      shape = 21,
                                      size = 3,
                                      alpha = 0.8) +
                           geom_smooth(se = FALSE,
                                       colour = '#000000') +
                           labs(title = str_glue('b* vs {.y}'),
                                subtitle = 'Black line: LOESS curve',
                                x = .y,
                                y = 'b*') +
                           scale_fill_viridis_d(name = 'Animal')))

walk(bstar$plot, ~print(.x))
```
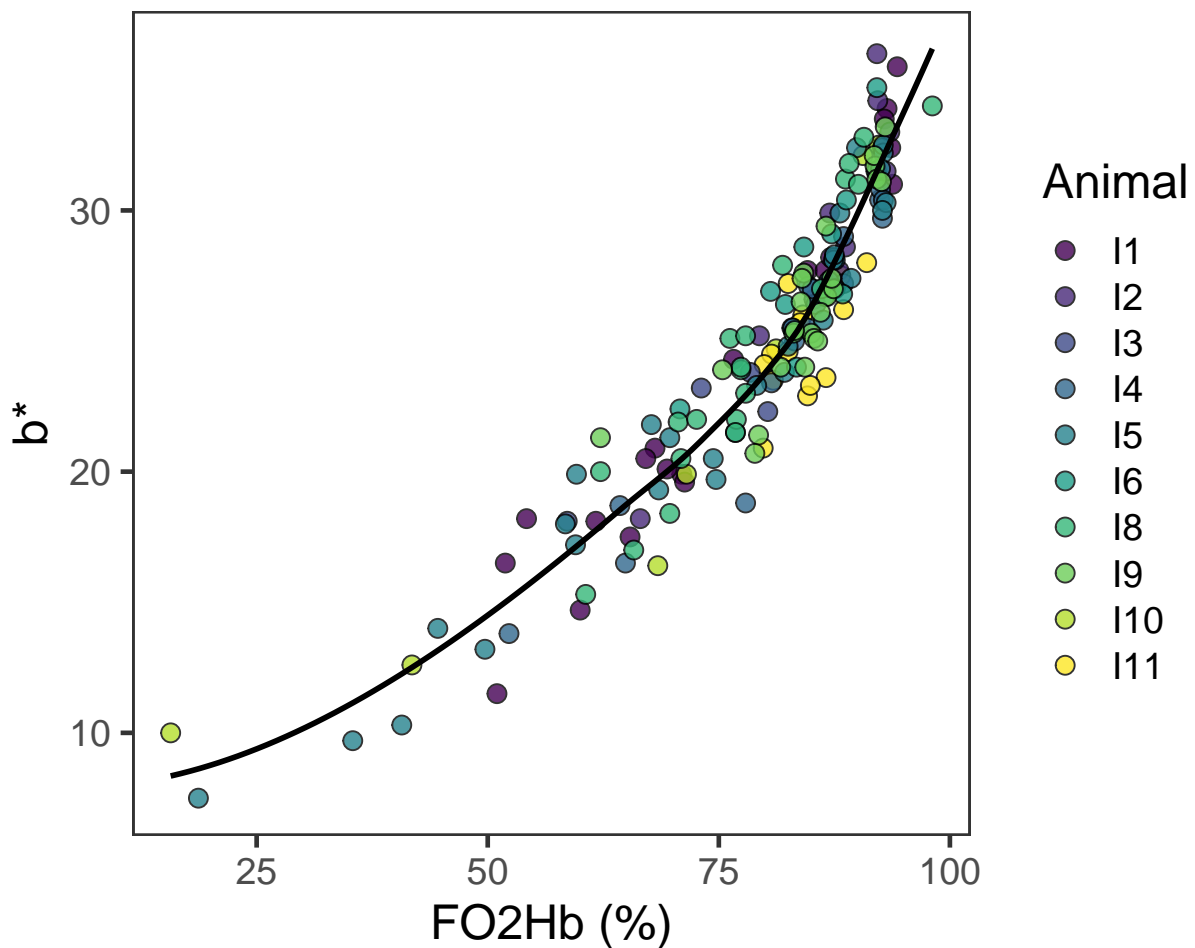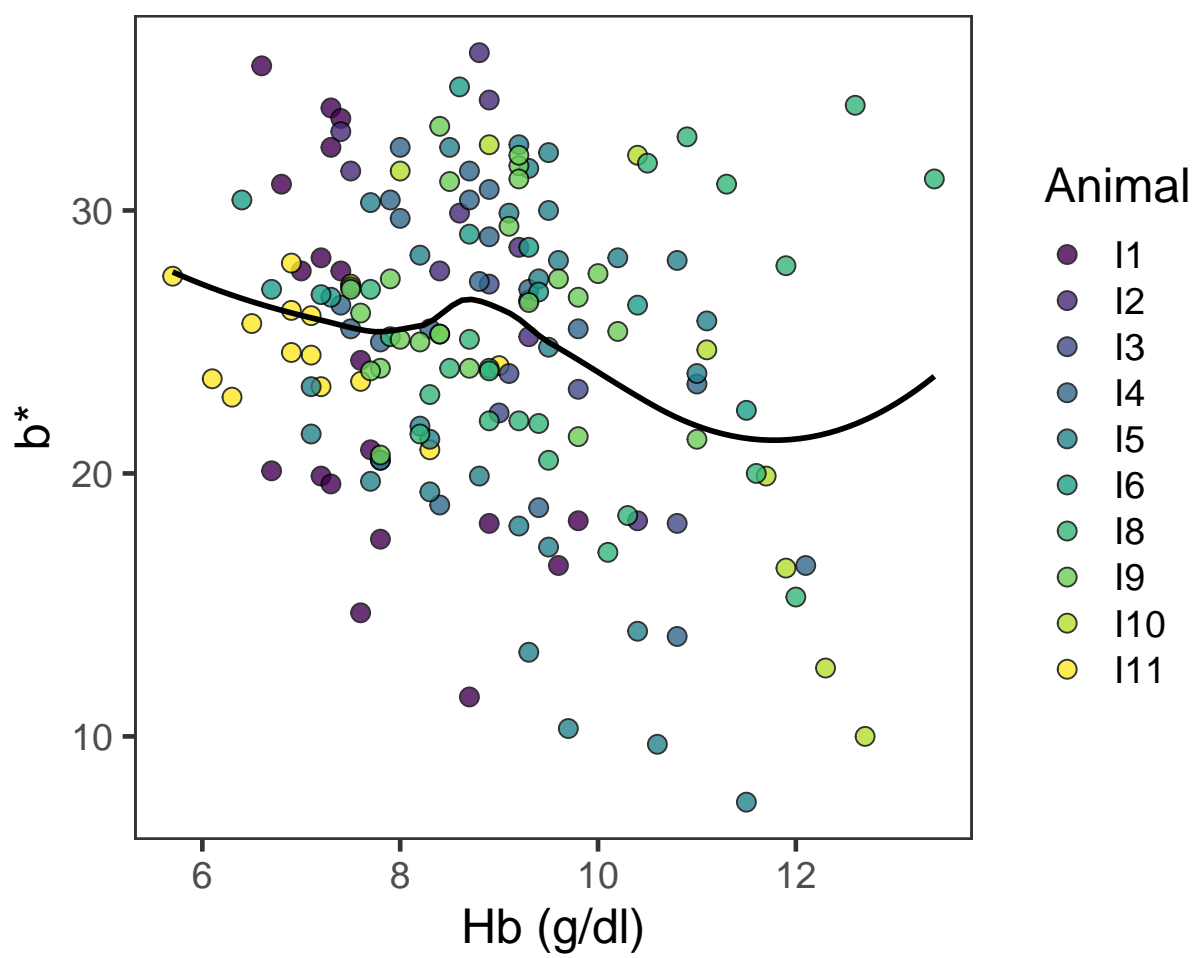
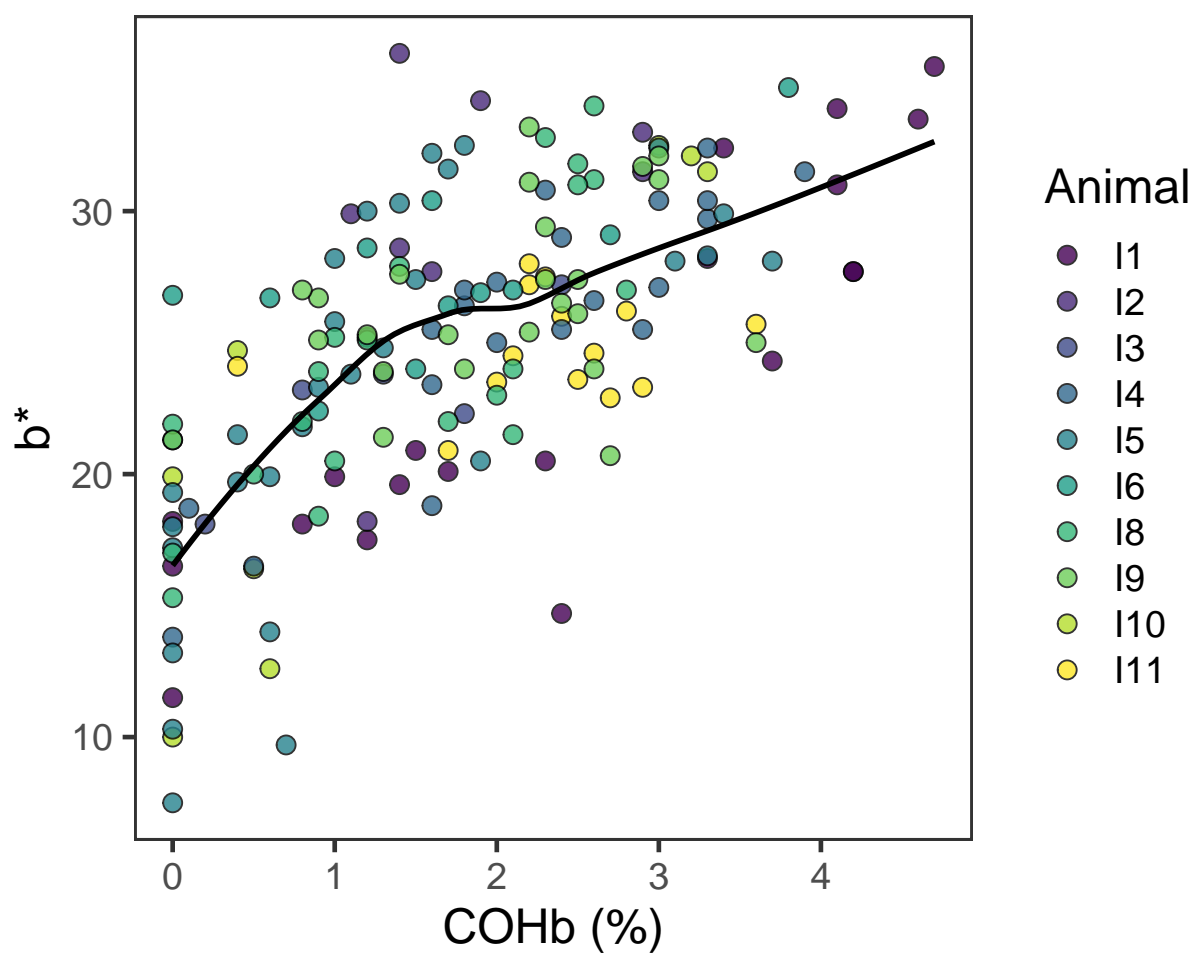## b* vs FO2Hb (%)

Black line: LOESS curve

b* vs Hb (g/dl)

Black line: LOESS curve

b* vs COHb (%)

Black line: LOESS curve

b* vs MetHb (%)

Black line: LOESS curve
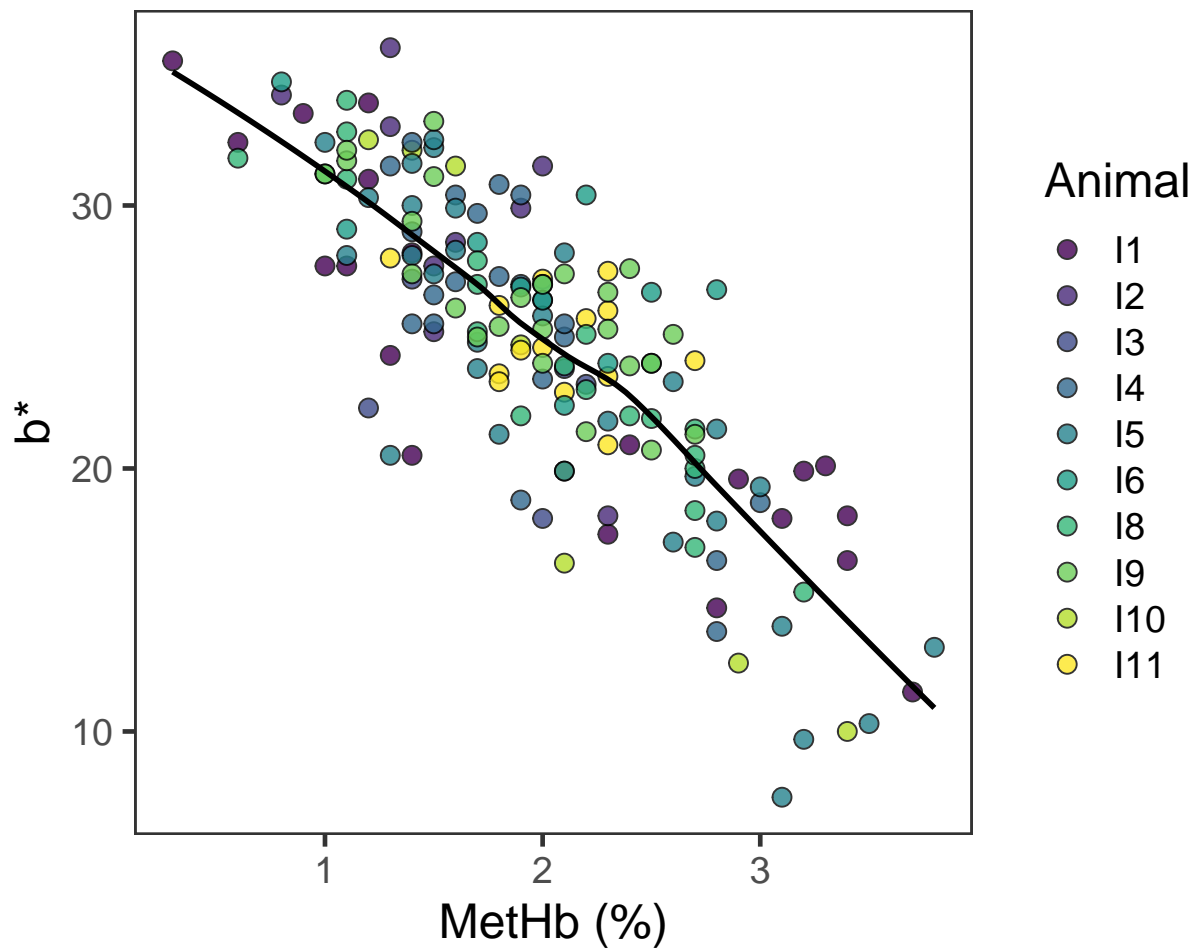
Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

5.3.2 Model

```r
# Specify model
bstar_full <- lmer(b_star ~ poly(O2Hb, 2) +
                      poly(COHb, 2) +
                      MetHb +
                      total_Hb +
                      (1 | Animal_ID),
                  data = data)

# Summarise model
summary(bstar_full)
```

5.3.2.1 Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: b_star ~ poly(O2Hb, 2) + poly(COHb, 2) + MetHb + total_Hb + (1 |
##     Animal_ID)
##    Data: data
##
```

```
## REML criterion at convergence: 602.2
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.7344 -0.7195  0.1167  0.6187  2.7541
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.2823   0.5314
##  Residual              2.4289   1.5585
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)     26.3339     1.4508 149.0396  18.152  < 2e-16 ***
## poly(O2Hb, 2)1  62.6552     3.0761 155.7481  20.368  < 2e-16 ***
## poly(O2Hb, 2)2  14.6236     1.8051 155.9913   8.101 1.47e-13 ***
## poly(COHb, 2)1  -2.4510     2.9025 152.3933  -0.844 0.399743
## poly(COHb, 2)2   3.8819     1.7843 155.9636   2.176 0.031089 *
## MetHb           -1.3288     0.3656 155.9615  -3.634 0.000378 ***
## total_Hb         0.1427     0.1128 137.6202   1.265 0.207897
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(O2H,2)1 p(O2H,2)2 p(COH,2)1 p(COH,2)2 MetHb
## ply(O2H,2)1 -0.539
## ply(O2H,2)2  0.003  0.251
## ply(COH,2)1 -0.478 -0.159    -0.274
## ply(COH,2)2 -0.016  0.238    -0.073    -0.120
## MetHb       -0.755  0.608     0.147     0.494     0.036
## total_Hb    -0.877  0.343    -0.112     0.340     0.000     0.377
```

```r
# Fixed effect with 95%CI
cbind(fixef(bstar_full), confint(bstar_full)[3:9, ]) %>%
    kable(caption = 'a* full model: FO2Hb, COHb, MetHb, and [Hb]',
          digits = 3,
          col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 3: a* full model: FO2Hb, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	26.334	23.556	29.139
poly(O2Hb, 2)1	62.655	56.719	68.578
poly(O2Hb, 2)2	14.624	11.099	18.080
poly(COHb, 2)1	-2.451	-8.135	3.088
poly(COHb, 2)2	3.882	0.475	7.401
MetHb	-1.329	-2.052	-0.631
total_Hb	0.143	-0.073	0.360

```r
# FO2Hb only
bstar_FO2Hb <- lmer(b_star ~ poly(O2Hb, 2) +
                      (1 | Animal_ID),
                    data = data)


# FO2Hb + MetHB + COHb
```

```r
bstar_MetCOHb <- lmer(b_star ~ poly(O2Hb, 2) +
                          poly(COHb, 2) +
                          MetHb +
                          (1 | Animal_ID),
                       data = data)

# Compare model fits
anova(bstar_MetCOHb, bstar_FO2Hb)
```

5.3.2.2 Compare model with and without methaemoglobin and carboxyhaemoglobin

```
## Data: data
## Models:
## bstar_FO2Hb: b_star ~ poly(O2Hb, 2) + (1 | Animal_ID)
## bstar_MetCOHb: b_star ~ poly(O2Hb, 2) + poly(COHb, 2) + MetHb + (1 | Animal_ID)
##               Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## bstar_FO2Hb    5 646.58 662.05 -318.29   636.58
## bstar_MetCOHb  8 627.82 652.57 -305.91   611.82 24.767      3  1.728e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
# Compare model R-squared values
r.squaredLR(bstar_FO2Hb)
```

```
## [1] 0.9115231
## attr(,"adj.r.squared")
## [1] 0.9131492
```

```r
r.squaredLR(bstar_MetCOHb)
```

```
## [1] 0.9239849
## attr(,"adj.r.squared")
## [1] 0.9256333
```

```r
# Compare model RMSE
RMSE.merMod(bstar_FO2Hb)
```

```
## [1] 1.603557
```

```r
RMSE.merMod(bstar_MetCOHb)
```

```
## [1] 1.505103
```

Likelihood ratio test found model with MetHb and COHb had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

---

6 SaO2

6.1 L*

6.1.1 Exploratory plots

```r
lstar <- data %>%
    select(Animal_ID, l_star, Coox_SaO2, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(Coox_SaO2, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
```

```r
                       levels = c('Coox_SaO2', 'total_Hb', 'COHb', 'MetHb'),
                       labels = c('SaO2 (%)', 'Hb (g/dl)',
                                  'COHb (%)', 'MetHb (%)'))) %>%
    group_by(Variable) %>%
    nest() %>%
    mutate(plot = map2(.x = data,
                       .y = Variable,
                       ~ .x %>%
                           ggplot(data = .) +
                           aes(x = Values,
                               y = l_star) +
                           geom_point(aes(fill = Animal_ID),
                                      shape = 21,
                                      size = 3,
                                      alpha = 0.8) +
                           geom_smooth(se = FALSE,
                                       colour = '#000000') +
                           labs(title = str_glue('L* vs {.y}'),
                                subtitle = 'Black line: LOESS curve',
                                x = .y,
                                y = 'L*') +
                           scale_fill_viridis_d(name = 'Animal')))

walk(lstar$plot, ~print(.x))
```
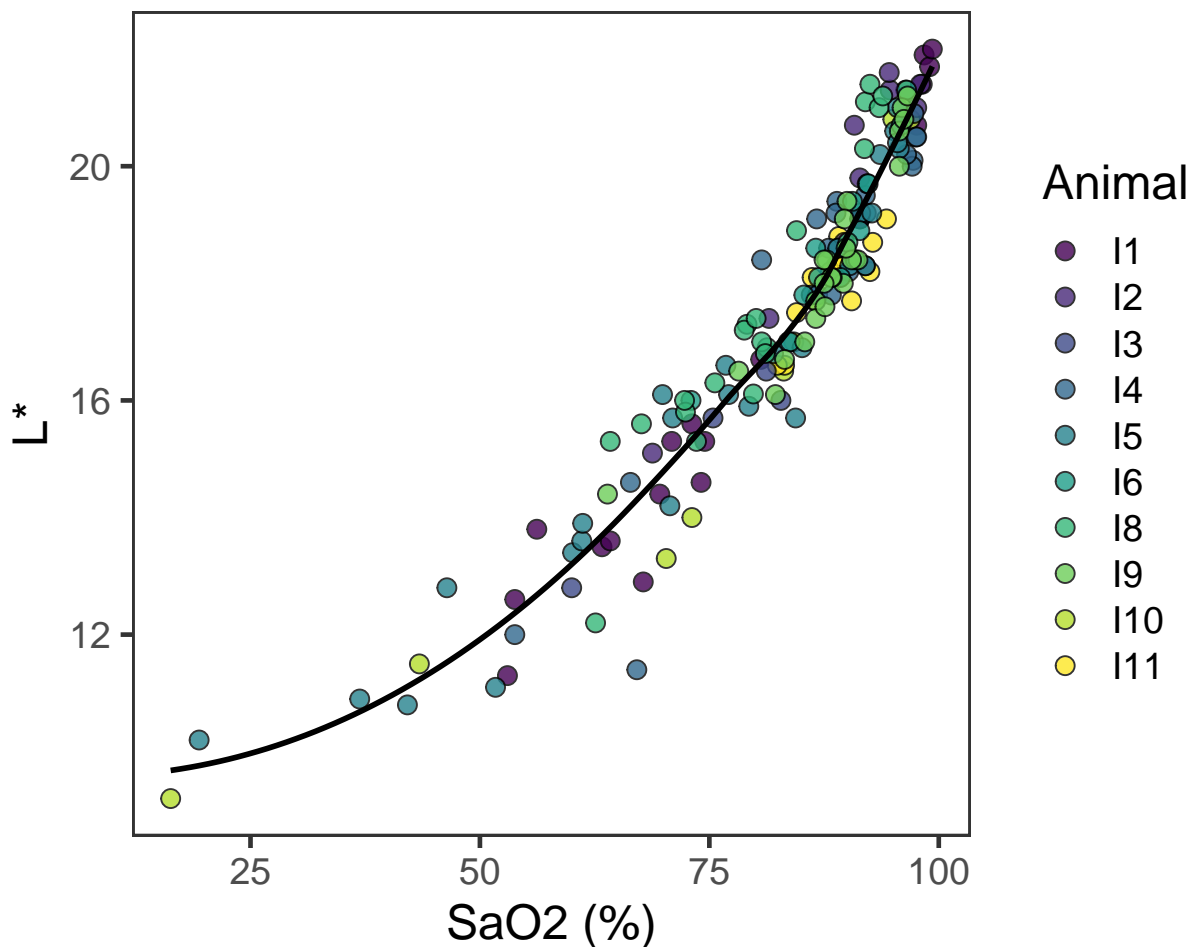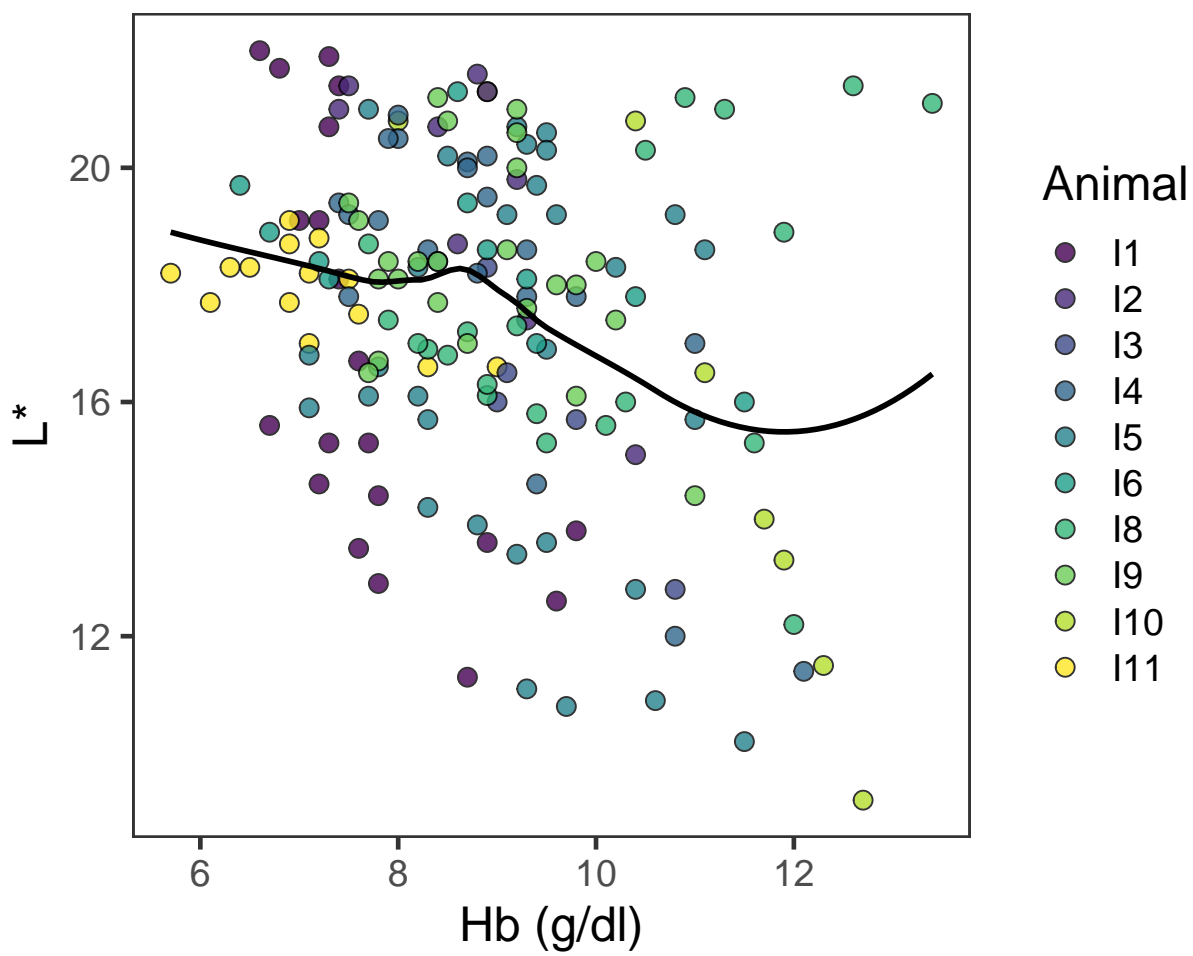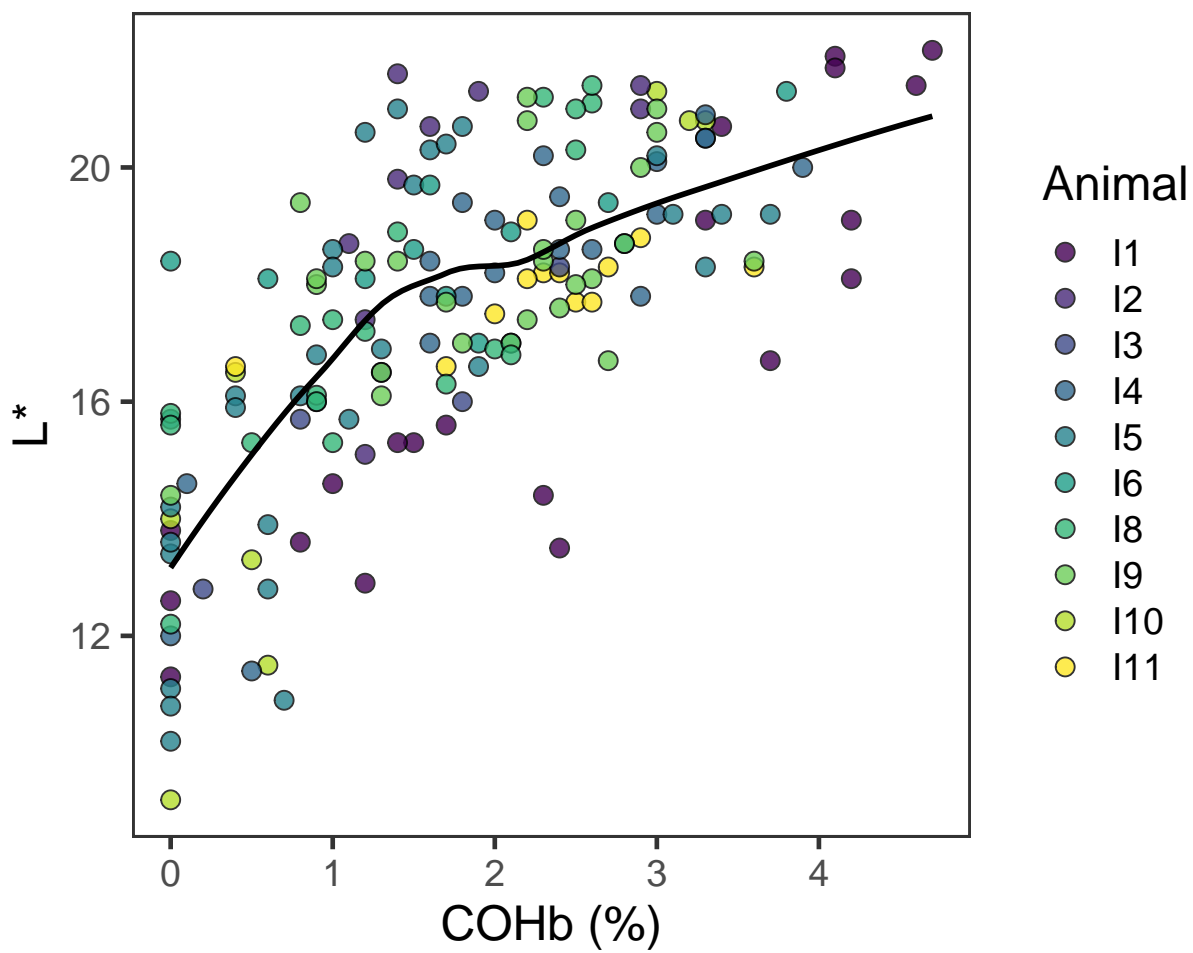

L* vs SaO2 (%)

Black line: LOESS curve

L* vs Hb (g/dl)

Black line: LOESS curve

L* vs COHb (%)

Black line: LOESS curve

L* vs MetHb (%)
Black line: LOESS curve
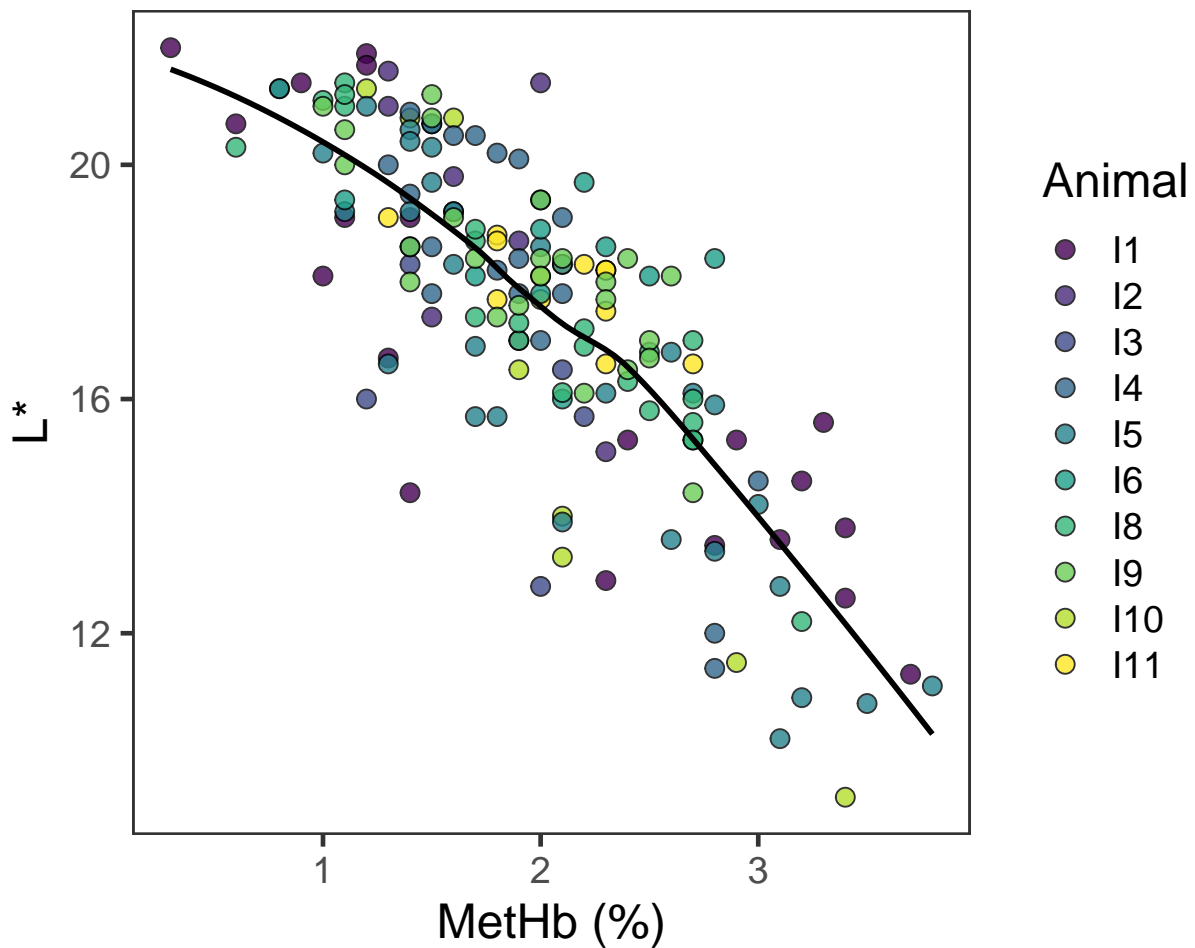
Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

6.1.2 Model

```r
# Specify model
lstar_full <- lmer(l_star ~ poly(Coox_SaO2, 2) +
                      poly(COHb, 2) +
                      MetHb +
                      total_Hb +
                      (1 | Animal_ID),
                  data = data)

# Summarise model
summary(lstar_full)
```

6.1.2.1 Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: l_star ~ poly(Coox_SaO2, 2) + poly(COHb, 2) + MetHb + total_Hb +
##      (1 | Animal_ID)
##    Data: data
##
```

```
## REML criterion at convergence: 341.3
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.6352 -0.6001  0.0188  0.6175  2.4220
##
## Random effects:
##  Groups    Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.1428   0.3778
##  Residual              0.4378   0.6617
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                     Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)         19.03880    0.63716 153.18773  29.881  < 2e-16 ***
## poly(Coox_SaO2, 2)1 30.03179    1.37369 154.77795  21.862  < 2e-16 ***
## poly(Coox_SaO2, 2)2  7.99529    0.80179 154.72208   9.972  < 2e-16 ***
## poly(COHb, 2)1      -1.39394    1.30402 155.87153  -1.069 0.286740
## poly(COHb, 2)2      -0.24375    0.77080 153.88065  -0.316 0.752253
## MetHb               -0.55385    0.15406 153.76959  -3.595 0.000436 ***
## total_Hb            -0.05119    0.05003 154.98381  -1.023 0.307768
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(C_SO2,2)1 p(C_SO2,2)2 p(COH,2)1 p(COH,2)2 MetHb
## p(C_SO2,2)1 -0.534
## p(C_SO2,2)2 -0.023  0.298
## ply(COH,2)1 -0.395 -0.253      -0.352
## ply(COH,2)2  0.002  0.179      -0.123      -0.090
## MetHb       -0.732  0.577       0.123       0.458     0.014
## total_Hb    -0.875  0.372      -0.054       0.260    -0.009     0.373
```

```r
# Fixed effect with 95%CI
cbind(fixef(lstar_full), confint(lstar_full)[3:9, ]) %>%
    kable(caption = 'L* full model: SaO2, COHb, MetHb, and [Hb]',
          digits = 3,
          col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 4: L* full model: SaO2, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	19.039	17.801	20.261
poly(Coox_SaO2, 2)1	30.032	27.397	32.720
poly(Coox_SaO2, 2)2	7.995	6.444	9.541
poly(COHb, 2)1	-1.394	-3.932	1.109
poly(COHb, 2)2	-0.244	-1.737	1.242
MetHb	-0.554	-0.852	-0.256
total_Hb	-0.051	-0.147	0.047

```r
# SaO2 only
lstar_SaO2 <- lmer(l_star ~ poly(Coox_SaO2, 2) +
                       (1 | Animal_ID),
                   data = data)


# SaO2 + MetHB
```

```
lstar_MetHb <- lmer(l_star ~ poly(Coox_SaO2, 2) +
                        MetHb +
                        (1 | Animal_ID),
                    data = data)

# Compare model fits
anova(lstar_MetHb, lstar_SaO2)
```

6.1.2.2 Compare model with and without methaemoglobin

```
## Data: data
## Models:
## lstar_SaO2: l_star ~ poly(Coox_SaO2, 2) + (1 | Animal_ID)
## lstar_MetHb: l_star ~ poly(Coox_SaO2, 2) + MetHb + (1 | Animal_ID)
##             Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## lstar_SaO2   5 361.75 377.22 -175.88   351.75
## lstar_MetHb  6 352.26 370.82 -170.13   340.26 11.495      1  0.0006977 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Compare model R-squared values
r.squaredLR(lstar_SaO2)
```

```
## [1] 0.9342318
## attr(,"adj.r.squared")
## [1] 0.9413842
```

```
r.squaredLR(lstar_MetHb)
```

```
## [1] 0.93871
## attr(,"adj.r.squared")
## [1] 0.9458968
```

```
# Compare model RMSE
RMSE.merMod(lstar_SaO2)
```

```
## [1] 0.6600265
```

```
RMSE.merMod(lstar_MetHb)
```

```
## [1] 0.6367252
```

Likelihood ratio test found model with MetHb had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

6.2 a*

6.2.1 Exploratory plots

```
astar <- data %>%
    select(Animal_ID, a_star, Coox_SaO2, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(Coox_SaO2, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
                             levels = c('Coox_SaO2', 'total_Hb', 'COHb', 'MetHb'),
                             labels = c('SaO2 (%)', 'Hb (g/dl)',
                                        'COHb (%)', 'MetHb (%)'))) %>%
    group_by(Variable) %>%
    nest() %>%
    mutate(plot = map2(.x = data,
```

```
                    .y = Variable,
            ~ .x %>%
                ggplot(data = .) +
                aes(x = Values,
                    y = a_star) +
                geom_point(aes(fill = Animal_ID),
                           shape = 21,
                           size = 3,
                           alpha = 0.8) +
                geom_smooth(se = FALSE,
                            colour = '#000000') +
                labs(title = str_glue('a* vs {.y}'),
                     subtitle = 'Black line: LOESS curve',
                     x = .y,
                     y = 'a*') +
                scale_fill_viridis_d(name = 'Animal')))

walk(astar$plot, ~print(.x))
```
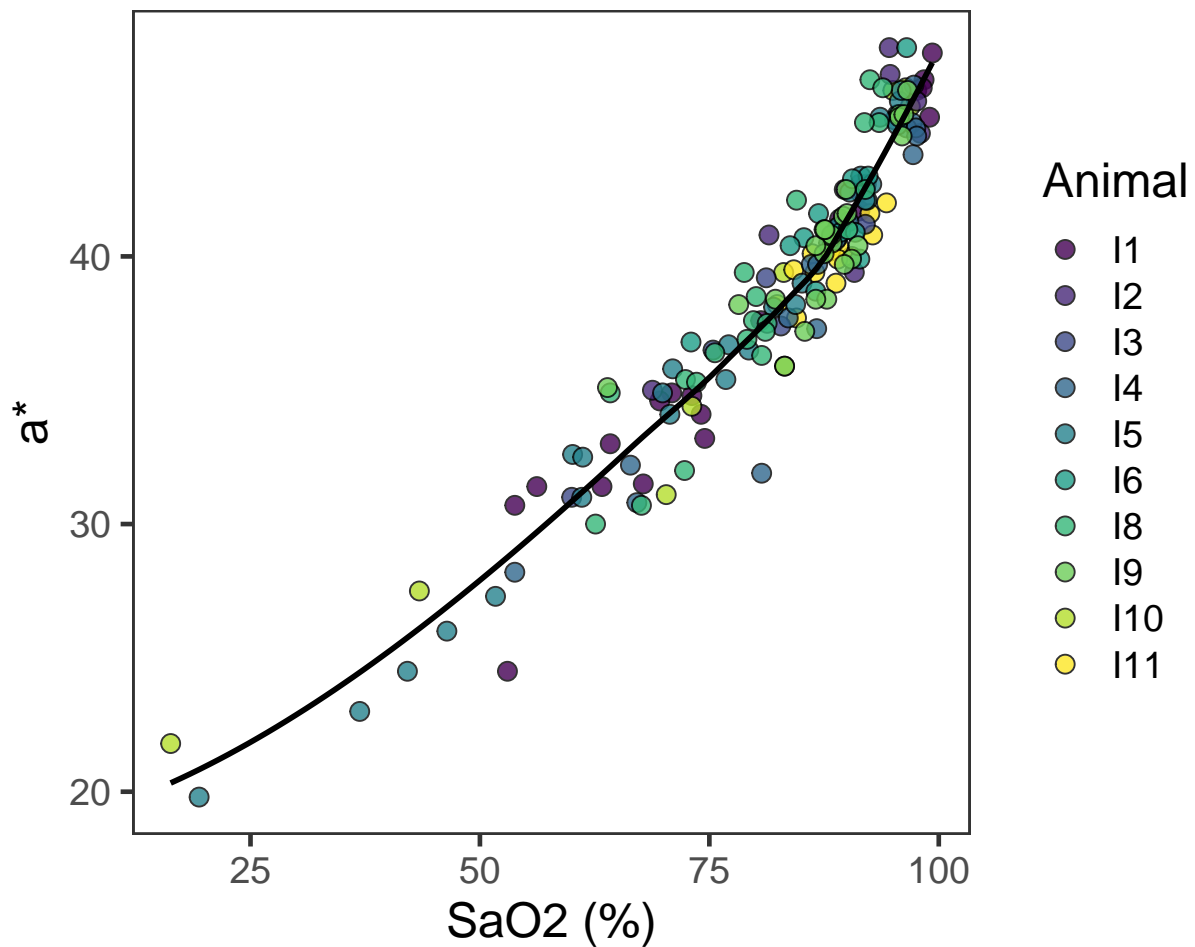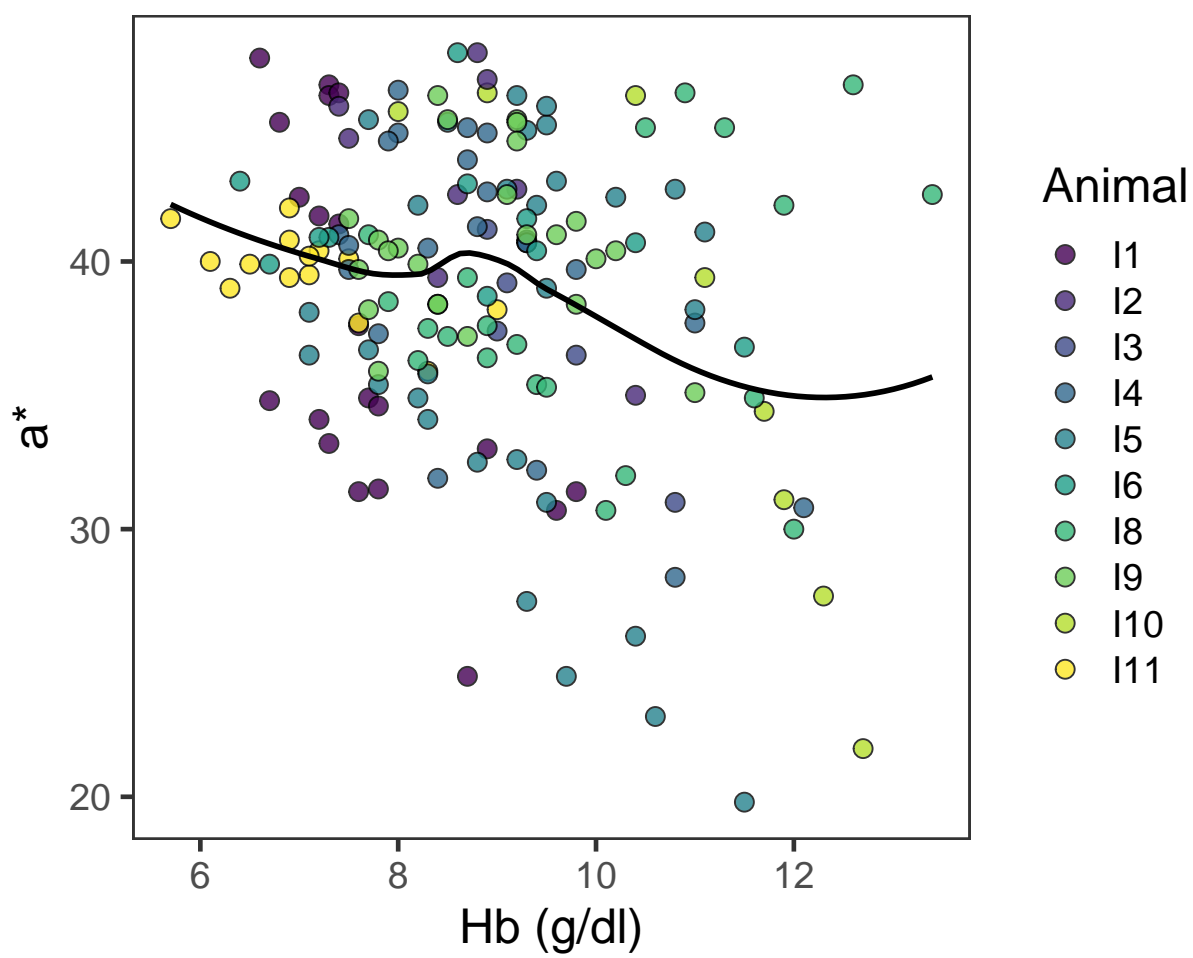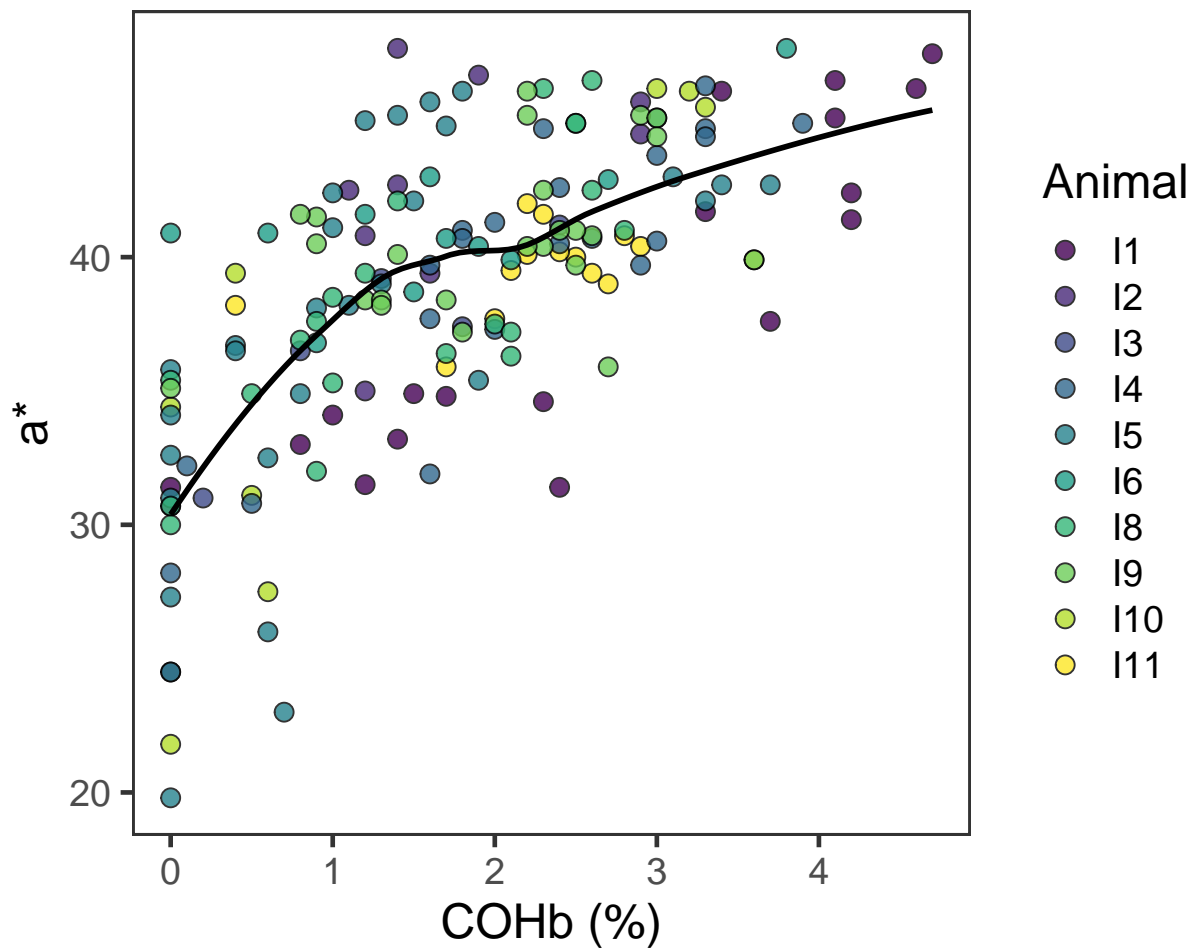

a* vs SaO2 (%)

Black line: LOESS curve

a* vs Hb (g/dl)
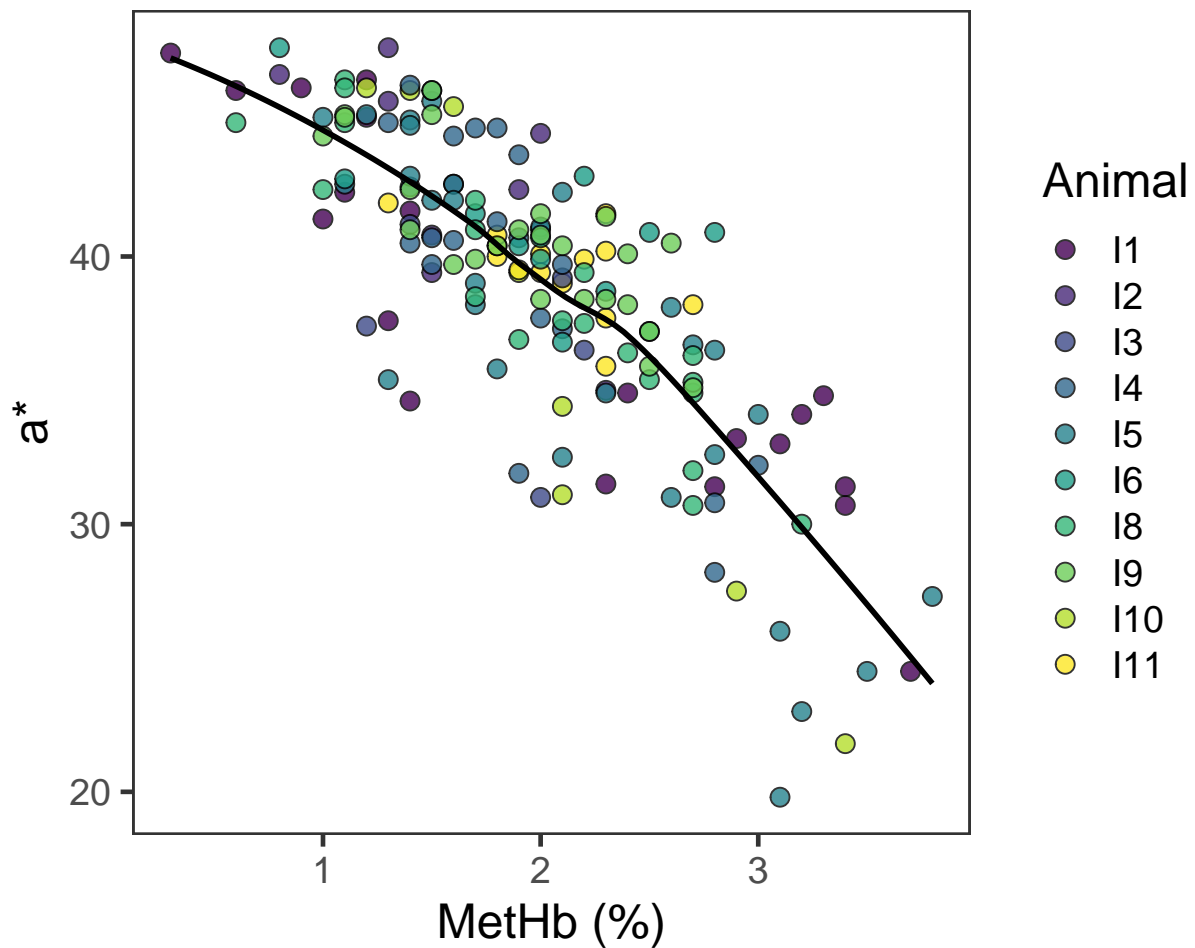
Black line: LOESS curve

a* vs COHb (%)

Black line: LOESS curve

a* vs MetHb (%)

Black line: LOESS curve

Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

6.2.2 Model

```
# Specify model
astar_full <- lmer(a_star ~ poly(Coox_SaO2, 2) +
                    poly(COHb, 2) +
                    MetHb +
                    total_Hb +
                    (1 | Animal_ID),
               data = data)

# Summarise model
summary(astar_full)
```

6.2.2.1 Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: a_star ~ poly(Coox_SaO2, 2) + poly(COHb, 2) + MetHb + total_Hb +
##     (1 | Animal_ID)
##     Data: data
##
```

```
## REML criterion at convergence: 555.6
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -3.9907 -0.5757  0.0795  0.5042  2.2019
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.1111   0.3332
##  Residual              1.8366   1.3552
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                      Estimate Std. Error        df t value Pr(>|t|)
## (Intercept)          39.68712    1.23593 141.48851  32.111  < 2e-16 ***
## poly(Coox_SaO2, 2)1  65.27890    2.72042 151.26553  23.996  < 2e-16 ***
## poly(Coox_SaO2, 2)2  10.18578    1.58957 152.07816   6.408 1.75e-09 ***
## poly(COHb, 2)1       -5.57469    2.55173 141.13637  -2.185   0.0306 *
## poly(COHb, 2)2        2.18026    1.53377 153.46056   1.422   0.1572
## MetHb                -1.27344    0.30767 155.42197  -4.139 5.70e-05 ***
## total_Hb              0.20445    0.09589 119.46569   2.132   0.0351 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) p(C_SO2,2)1 p(C_SO2,2)2 p(COH,2)1 p(COH,2)2 MetHb
## p(C_SO2,2)1 -0.537
## p(C_SO2,2)2 -0.008  0.275
## ply(COH,2)1 -0.440 -0.239      -0.327
## ply(COH,2)2 -0.019  0.238      -0.106      -0.129
## MetHb       -0.766  0.585       0.130       0.451     0.025
## total_Hb    -0.885  0.362      -0.083       0.320     0.012     0.402
```

```r
# Fixed effect with 95%CI
cbind(fixef(astar_full), confint(astar_full)[3:9, ]) %>%
    kable(caption = 'a* full model: SaO2, COHb, MetHb, and [Hb]',
          digits = 3,
          col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 5: a* full model: SaO2, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	39.687	37.339	42.123
poly(Coox_SaO2, 2)1	65.279	59.881	70.450
poly(Coox_SaO2, 2)2	10.186	7.057	13.211
poly(COHb, 2)1	-5.575	-10.551	-0.721
poly(COHb, 2)2	2.180	-0.734	5.271
MetHb	-1.273	-1.896	-0.689
total_Hb	0.204	0.019	0.387

```r
# SaO2 only
astar_SaO2 <- lmer(a_star ~ poly(Coox_SaO2, 2) +
                     (1 | Animal_ID),
                   data = data)


# SaO2 + MetHB + COHb + [Hb]
```

```
astar_MetCOHb <- lmer(a_star ~ poly(Coox_SaO2, 2) +
                              poly(COHb, 2) +
                              MetHb +
                              total_Hb +
                              (1 | Animal_ID),
                       data = data)

# Compare model fits
anova(astar_MetCOHb, astar_SaO2)
```

6.2.2.2   Compare model with and without methaemoglobin and carboxyhaemoglobin

```
## Data: data
## Models:
## astar_SaO2: a_star ~ poly(Coox_SaO2, 2) + (1 | Animal_ID)
## astar_MetCOHb: a_star ~ poly(Coox_SaO2, 2) + poly(COHb, 2) + MetHb + total_Hb +
## astar_MetCOHb:       (1 | Animal_ID)
##                Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## astar_SaO2      5 604.82 620.29 -297.41   594.82
## astar_MetCOHb   9 578.97 606.82 -280.49   560.97 33.851      4  7.995e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Compare model R-squared values
r.squaredLR(astar_SaO2)
```

```
## [1] 0.9272521
## attr(,"adj.r.squared")
## [1] 0.9290094
```

```
r.squaredLR(astar_MetCOHb)
```

```
## [1] 0.9408715
## attr(,"adj.r.squared")
## [1] 0.9426547
```

```
# Compare model RMSE
RMSE.merMod(astar_SaO2)
```

```
## [1] 1.416503
```

```
RMSE.merMod(astar_MetCOHb)
```

```
## [1] 1.30927
```

Likelihood ratio test found model with MetHb, COHb and [Hb] had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

6.3   b*

6.3.1   Exploratory plots

```
bstar <- data %>%
    select(Animal_ID, b_star, Coox_SaO2, total_Hb, COHb, MetHb) %>%
    pivot_longer(cols = c(Coox_SaO2, total_Hb, COHb, MetHb),
                 names_to = 'Variable',
                 values_to = 'Values') %>%
    mutate(Variable = factor(Variable,
                             levels = c('Coox_SaO2', 'total_Hb', 'COHb', 'MetHb'),
                             labels = c('SaO2 (%)', 'Hb (g/dl)',
                                        'COHb (%)', 'MetHb (%)'))) %>%
```

```
    group_by(Variable) %>%
    nest() %>%
    mutate(plot = map2(.x = data,
                       .y = Variable,
                       ~ .x %>%
                           ggplot(data = .) +
                           aes(x = Values,
                               y = b_star) +
                           geom_point(aes(fill = Animal_ID),
                                      shape = 21,
                                      size = 3,
                                      alpha = 0.8) +
                           geom_smooth(se = FALSE,
                                       colour = '#000000') +
                           labs(title = str_glue('b* vs {.y}'),
                                subtitle = 'Black line: LOESS curve',
                                x = .y,
                                y = 'b*') +
                           scale_fill_viridis_d(name = 'Animal')))

walk(bstar$plot, ~print(.x))
```
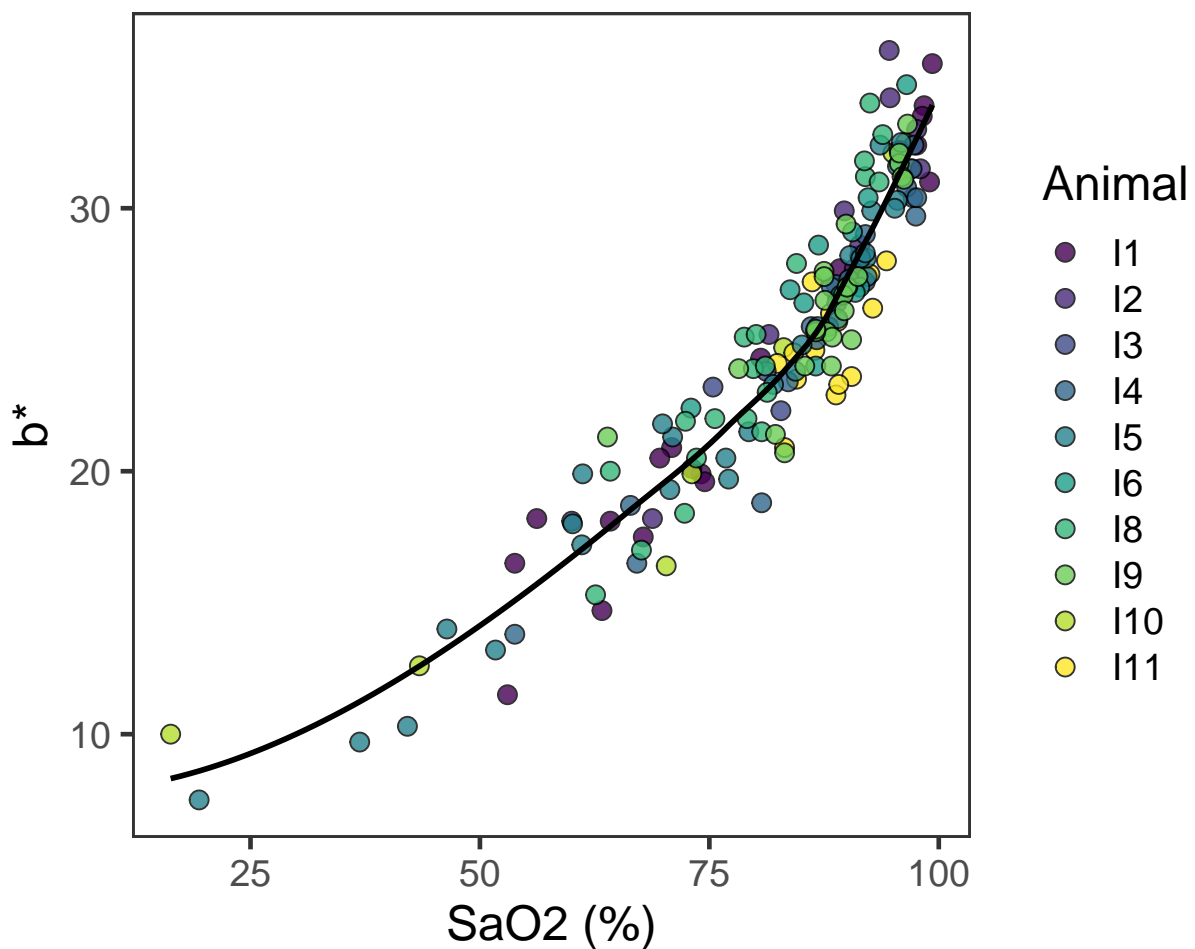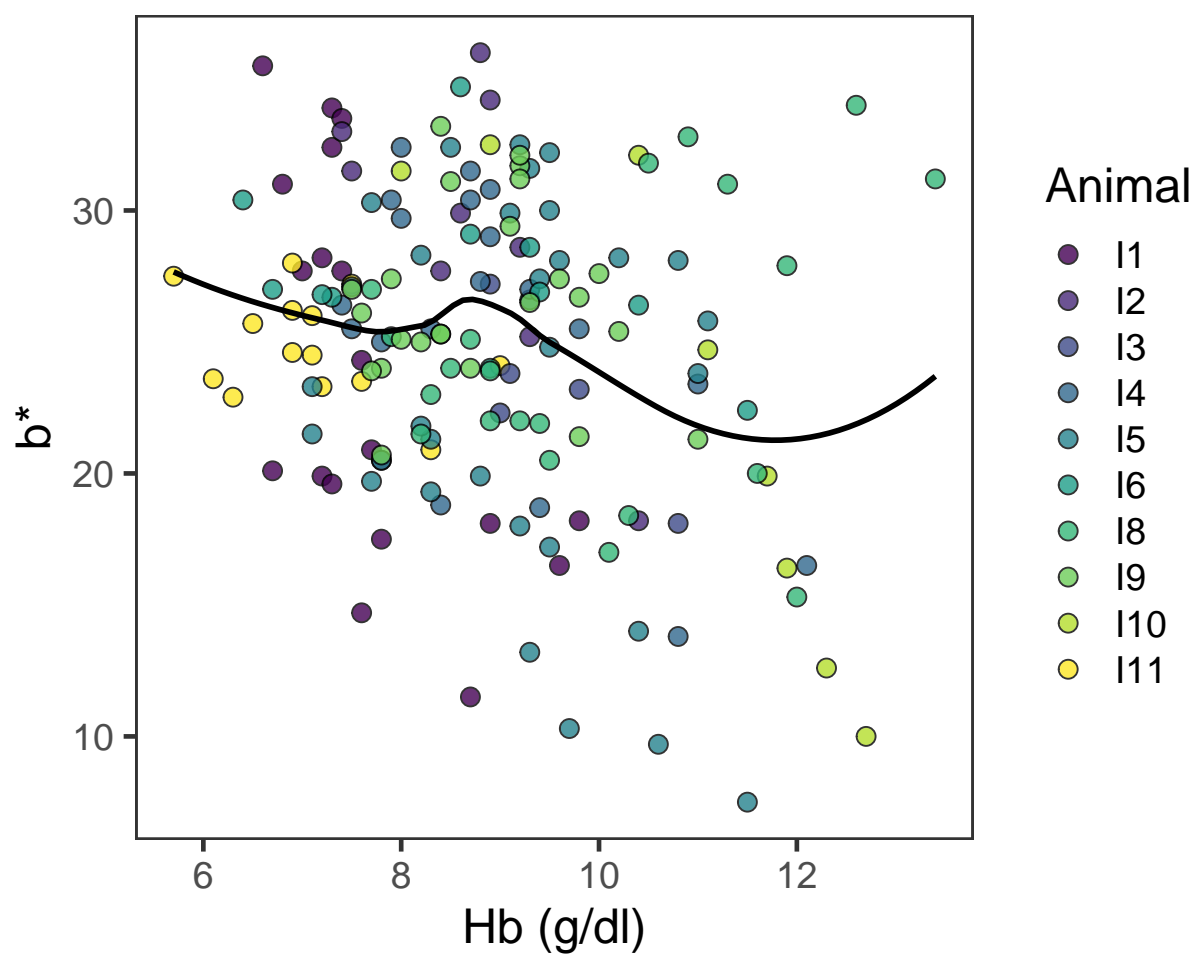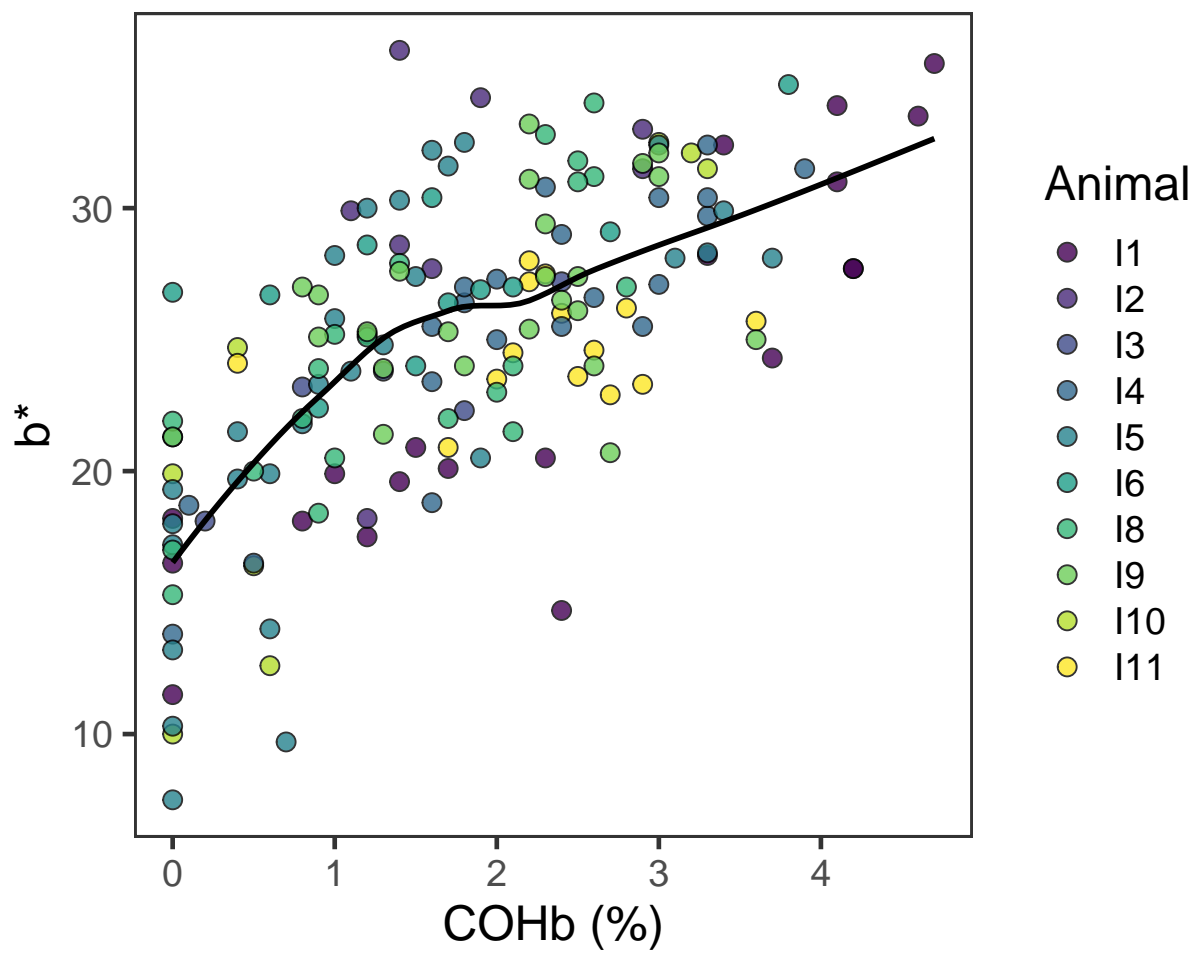

b* vs SaO2 (%)

Black line: LOESS curve

b* vs Hb (g/dl)
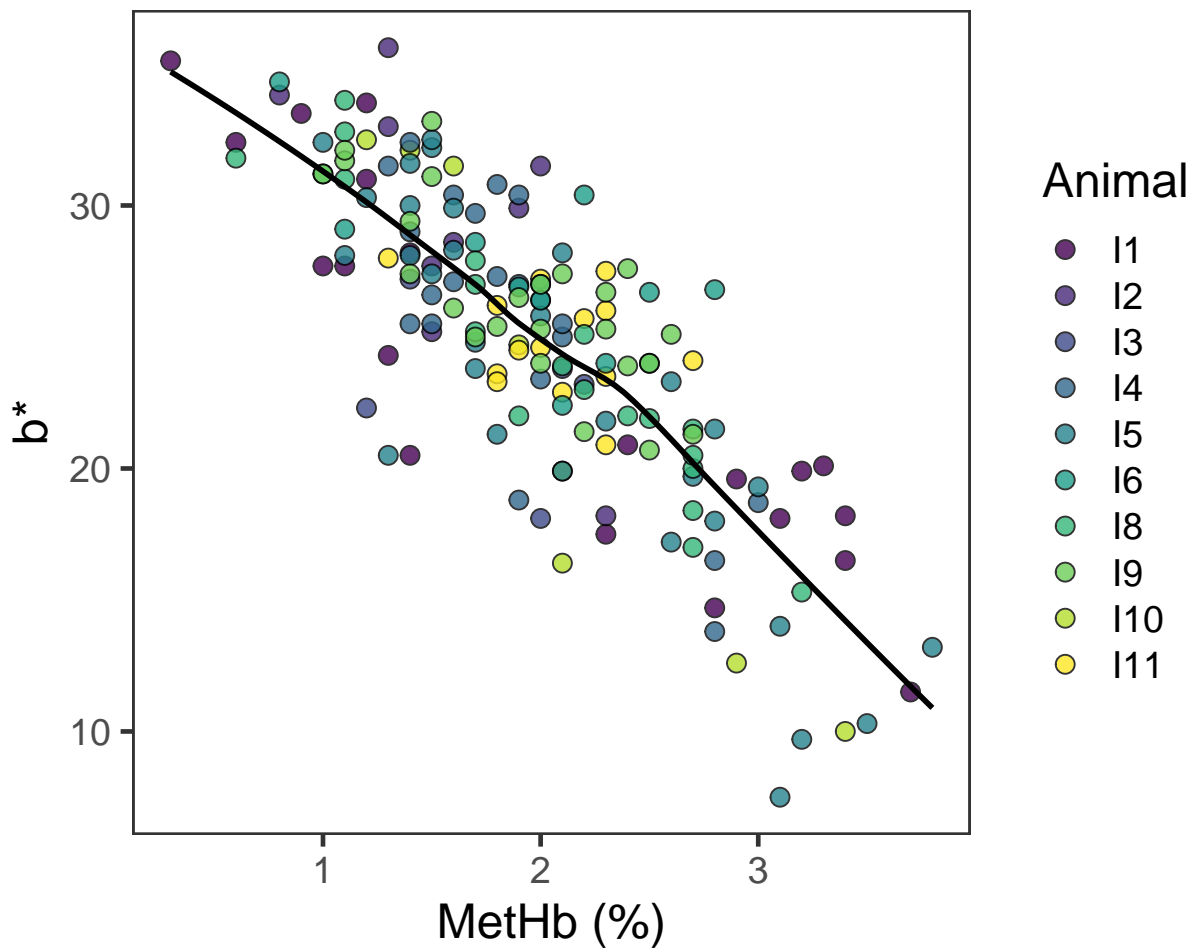
Black line: LOESS curve

b* vs COHb (%)

Black line: LOESS curve

b* vs MetHb (%)

Black line: LOESS curve

Appears to be a quadratic fit for COHb, linear fit for MetHb, and no real relationship between L* and [Hb].

6.3.2   Model

```r
# Specify model
bstar_full <- lmer(b_star ~ poly(Coox_SaO2, 2) +
                    poly(COHb, 2) +
                    MetHb +
                    total_Hb +
                    (1 | Animal_ID),
                data = data)

# Summarise model
summary(bstar_full)
```

6.3.2.1   Full model

```
## Linear mixed model fit by REML. t-tests use Satterthwaite's method [
## lmerModLmerTest]
## Formula: b_star ~ poly(Coox_SaO2, 2) + poly(COHb, 2) + MetHb + total_Hb +
##      (1 | Animal_ID)
##    Data: data
##
```

```
## REML criterion at convergence: 604.7
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.6364 -0.6824  0.1002  0.6006  2.7441
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Animal_ID (Intercept) 0.3643   0.6036
##  Residual              2.4473   1.5644
## Number of obs: 163, groups:  Animal_ID, 10
##
## Fixed effects:
##                     Estimate Std. Error       df t value Pr(>|t|)
## (Intercept)          26.2097     1.4676 150.7791  17.859  < 2e-16 ***
## poly(Coox_SaO2, 2)1  64.7462     3.2027 155.8744  20.216  < 2e-16 ***
## poly(Coox_SaO2, 2)2  15.2491     1.8699 155.9405   8.155 1.08e-13 ***
## poly(COHb, 2)1       -7.7977     3.0251 153.6398  -2.578   0.0109 *
## poly(COHb, 2)2        2.8154     1.8010 155.9819   1.563   0.1200
## MetHb                -1.6289     0.3603 155.7679  -4.521 1.21e-05 ***
## total_Hb              0.2226     0.1150 143.7248   1.936   0.0549 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##            (Intr) p(C_SO2,2)1 p(C_SO2,2)2 p(COH,2)1 p(COH,2)2 MetHb
## p(C_SO2,2)1 -0.538
## p(C_SO2,2)2 -0.016  0.288
## ply(COH,2)1 -0.416 -0.246      -0.342
## ply(COH,2)2 -0.005  0.202      -0.116      -0.107
## MetHb       -0.750  0.581       0.126       0.455     0.017
## total_Hb    -0.882  0.369      -0.068       0.286    -0.002     0.386
```

```r
# Fixed effect with 95%CI
cbind(fixef(bstar_full), confint(bstar_full)[3:9, ]) %>%
    kable(caption = 'a* full model: SaO2, COHb, MetHb, and [Hb]',
          digits = 3,
          col.names = c('Estimate', '2.5%', '97.5%'))
```

Table 6: a* full model: SaO2, COHb, MetHb, and [Hb]

	Estimate	2.5%	97.5%
(Intercept)	26.210	23.401	29.059
poly(Coox_SaO2, 2)1	64.746	58.529	70.892
poly(Coox_SaO2, 2)2	15.249	11.565	18.824
poly(COHb, 2)1	-7.798	-13.664	-2.002
poly(COHb, 2)2	2.815	-0.628	6.365
MetHb	-1.629	-2.340	-0.940
total_Hb	0.223	0.001	0.443

```r
# SaO2 only
bstar_SaO2 <- lmer(b_star ~ poly(Coox_SaO2, 2) +
                     (1 | Animal_ID),
                 data = data)


# SaO2 + MetHB + COHb
```

```
bstar_MetCOHb <- lmer(b_star ~ poly(Coox_SaO2, 2) +
                              poly(COHb, 2) +
                              MetHb +
                              (1 | Animal_ID),
                      data = data)

# Compare model fits
anova(bstar_MetCOHb, bstar_SaO2)
```

6.3.2.2   Compare model with and without methaemoglobin and carboxyhaemoglobin

```
## Data: data
## Models:
## bstar_SaO2: b_star ~ poly(Coox_SaO2, 2) + (1 | Animal_ID)
## bstar_MetCOHb: b_star ~ poly(Coox_SaO2, 2) + poly(COHb, 2) + MetHb + (1 | Animal_ID)
##                Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## bstar_SaO2      5 659.79 675.26 -324.90   649.79
## bstar_MetCOHb   8 632.84 657.59 -308.42   616.84 32.952      3  3.296e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Compare model R-squared values
r.squaredLR(bstar_SaO2)
```

```
## [1] 0.9040574
## attr(,"adj.r.squared")
## [1] 0.9056701
```

```
r.squaredLR(bstar_MetCOHb)
```

```
## [1] 0.9216114
## attr(,"adj.r.squared")
## [1] 0.9232555
```

```
# Compare model RMSE
RMSE.merMod(bstar_SaO2)
```

```
## [1] 1.655449
```

```
RMSE.merMod(bstar_MetCOHb)
```

```
## [1] 1.519256
```

Likelihood ratio test found model with MetHb and COHb had a better fit, but R-squared and RMSE indicate the difference is of marginal importance.

---

7   Session Information

```
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Catalina 10.15.4
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
```

```
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] knitr_1.28          forcats_0.5.0      stringr_1.4.0     dplyr_0.8.5
##  [5] purrr_0.3.3         readr_1.3.1        tidyr_1.0.2       tibble_3.0.0
##  [9] ggplot2_3.3.0.9000  tidyverse_1.3.0    MuMIn_1.43.15     merTools_0.5.0
## [13] arm_1.10-1          MASS_7.3-51.5      lmerTest_3.1-1    lme4_1.1-21
## [17] Matrix_1.2-18       magrittr_1.5
##
## loaded via a namespace (and not attached):
##  [1] nlme_3.1-145        fs_1.3.1           lubridate_1.7.4
##  [4] httr_1.4.1          numDeriv_2016.8-1.1 tools_3.6.3
##  [7] backports_1.1.5     utf8_1.1.4         R6_2.4.1
## [10] DBI_1.1.0           mgcv_1.8-31        colorspace_1.4-1
## [13] withr_2.1.2         tidyselect_1.0.0   compiler_3.6.3
## [16] cli_2.0.2           rvest_0.3.5        xml2_1.3.0
## [19] labeling_0.3        scales_1.1.0       mvtnorm_1.1-0
## [22] blme_1.0-4          digest_0.6.25      minqa_1.2.4
## [25] rmarkdown_2.1       pkgconfig_2.0.3    htmltools_0.4.0
## [28] dbplyr_1.4.2        fastmap_1.0.1      highr_0.8
## [31] rlang_0.4.5         readxl_1.3.1       rstudioapi_0.11
## [34] shiny_1.4.0.2       generics_0.0.2     farver_2.0.3
## [37] jsonlite_1.6.1      Rcpp_1.0.4         munsell_0.5.0
## [40] fansi_0.4.1         abind_1.4-5        lifecycle_0.2.0
## [43] stringi_1.4.6       yaml_2.2.1         grid_3.6.3
## [46] promises_1.1.0      crayon_1.3.4       lattice_0.20-38
## [49] haven_2.2.0         splines_3.6.3      hms_0.5.3
## [52] pillar_1.4.3        boot_1.3-24        codetools_0.2-16
## [55] stats4_3.6.3        reprex_0.3.0       glue_1.3.2
## [58] evaluate_0.14       modelr_0.1.6       vctrs_0.2.4
## [61] nloptr_1.2.2.1      httpuv_1.5.2       foreach_1.5.0
## [64] cellranger_1.1.0    gtable_0.3.0       assertthat_0.2.1
## [67] xfun_0.12           mime_0.9           xtable_1.8-4
## [70] broom_0.5.5         coda_0.19-3        later_1.0.0
## [73] viridisLite_0.3.0   iterators_1.0.12   ellipsis_0.3.0
```