Vernal pool mercury analysis

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Introduction

This document details a preliminary analysis of data collected on methylmercury levels in vernal pools in the Upper Valley.

Data file summary

```
data.df <- read.csv(url("https://raw.githubusercontent.com/5355693/VP_Mercury/master/
hg_data.csv"))
summary(data.df)
```

```
##          Pool         Sample_Date      Spp        Amphib_MeHg
##   Downer      :32    5/12/15:28    SPSA: 96    Min.   :  0.8317
##   Mauran      :34    5/11/15:16    WOFR:100    1st Qu.:  5.2345
##   Podunk Conif:32    7/8/15 :14                Median : 43.1660
##   Podunk Decid:34    4/30/15:13                Mean   : 86.1619
##   Pomfret     :32    7/7/15 :13                3rd Qu.:118.4400
##   Shen        :32    (Other):79                Max.   :382.8120
##                      NA's   :33                NA's   :85
##     Amphib_THg            Life_Stage        Habitat         Water_Al
##   Min.   : 16.03    Adult       :52    Coniferous: 96    Min.   : 15.56
##   1st Qu.:105.25    Early Larvae:48    Deciduous :100    1st Qu.: 24.26
##   Median :198.30    Eggs        :48                      Median : 41.00
##   Mean   :216.04    Late Larvae :48                      Mean   : 77.24
##   3rd Qu.:311.75                                         3rd Qu.:132.63
##   Max.   :561.80                                         Max.   :236.37
##   NA's   :121                                            NA's   :67
##     Water_S            Water_DOC         Water_pH        Water_MeHg
##   Min.   : 244.3    Min.   : 2695    Min.   :4.900    Min.   :0.00006
##   1st Qu.: 448.8    1st Qu.: 8838    1st Qu.:5.800    1st Qu.:0.00038
##   Median : 613.5    Median :13117    Median :6.000    Median :0.00070
##   Mean   : 671.8    Mean   :13845    Mean   :5.939    Mean   :0.00102
##   3rd Qu.: 796.0    3rd Qu.:15982    3rd Qu.:6.100    3rd Qu.:0.00171
##   Max.   :1305.5    Max.   :28371    Max.   :7.300    Max.   :0.00277
##   NA's   :67        NA's   :67       NA's   :67       NA's   :33
##    Season_MeHg        Tissue_MeHg        Blood_MeHg          S.V
##   Min.   :0.00038    Min.   : 13.30    Min.   : 11.26    Min.   : 44.00
##   1st Qu.:0.00043    1st Qu.: 29.82    1st Qu.: 26.50    1st Qu.: 47.75
##   Median :0.00055    Median : 43.07    Median : 50.90    Median : 53.50
##   Mean   :0.00082    Mean   : 63.49    Mean   : 49.82    Mean   : 65.62
##   3rd Qu.:0.00102    3rd Qu.: 85.07    3rd Qu.: 61.91    3rd Qu.: 85.00
##   Max.   :0.00175    Max.   :212.03    Max.   :147.79    Max.   :101.00
##   NA's   :121        NA's   :147       NA's   :161       NA's   :144
##    Tot_Length          Mass           Water_THg
##   Min.   :140.0    Min.   : 8.60    Min.   :0.00226
##   1st Qu.:162.2    1st Qu.:11.50    1st Qu.:0.00418
##   Median :170.0    Median :13.50    Median :0.00538
##   Mean   :171.3    Mean   :14.07    Mean   :0.00516
##   3rd Qu.:185.0    3rd Qu.:16.15    3rd Qu.:0.00614
##   Max.   :200.0    Max.   :23.70    Max.   :0.00828
##   NA's   :172      NA's   :145      NA's   :121
```

There are 6 pools, 3 of which are surrounded by deciduous forest (Downer, Mauran, and Podunk Decid) and 3 of which are surrounded by coniferous forest (Podunk Conif, Pomfret, and Shen). The number of samples differs by date and by pool because sample number is determined by the number of amphibians collected.

```
##               Sample_Date
## Pool           4/19/16 4/20/15 4/20/16 4/21/15 4/24/15 4/28/15 4/29/15
##    Downer            0       0       0       0       0       5       1
##    Mauran            0       4       0       3       0       3       0
##    Podunk Conif      0       0       0       0       0       0       0
##    Podunk Decid      2       0       0       0       1       0       0
##    Pomfret           0       0       0       0       0       0       4
##    Shen              0       0       2       0       0       0       0
##               Sample_Date
## Pool           4/30/15 5/1/15 5/11/15 5/12/15 5/13/15 5/18/15 5/2/16
##    Downer            0      0       0      10       0       0       0
##    Mauran            0      0       0       4       0       0       0
##    Podunk Conif      8      0       8       0       0       4       0
##    Podunk Decid      5      0       0      10       0       0       0
##    Pomfret           0      0       0       4       4       0       2
##    Shen              0      6       8       0       0       0       0
##               Sample_Date
## Pool           5/26/15 5/3/16 5/9/15 6/1/15 6/7/16 7/6/15 7/7/15 7/8/15
##    Downer            0      0      0      4      0      0      8      0
##    Mauran            0      0      8      4      0      8      0      0
##    Podunk Conif      0      0      0      0      0      0      0      6
##    Podunk Decid      0      0      0      0      0      0      0      8
##    Pomfret           0      2      0      0      4      0      5      0
##    Shen              4      0      0      0      0      4      0      0
```

Exploratory analysis

Methlymercury levels in water by pool, over time

Levels of methylmercury in water differ among pools and habitats and by time of sample. In general, coniferous pools have higher levels of methylmercury, but variation among pools is substantial. In addition, a seasonal trend is evident in pools surrounded by deciduous forest, but not among pools surrounded by coniferous forest.
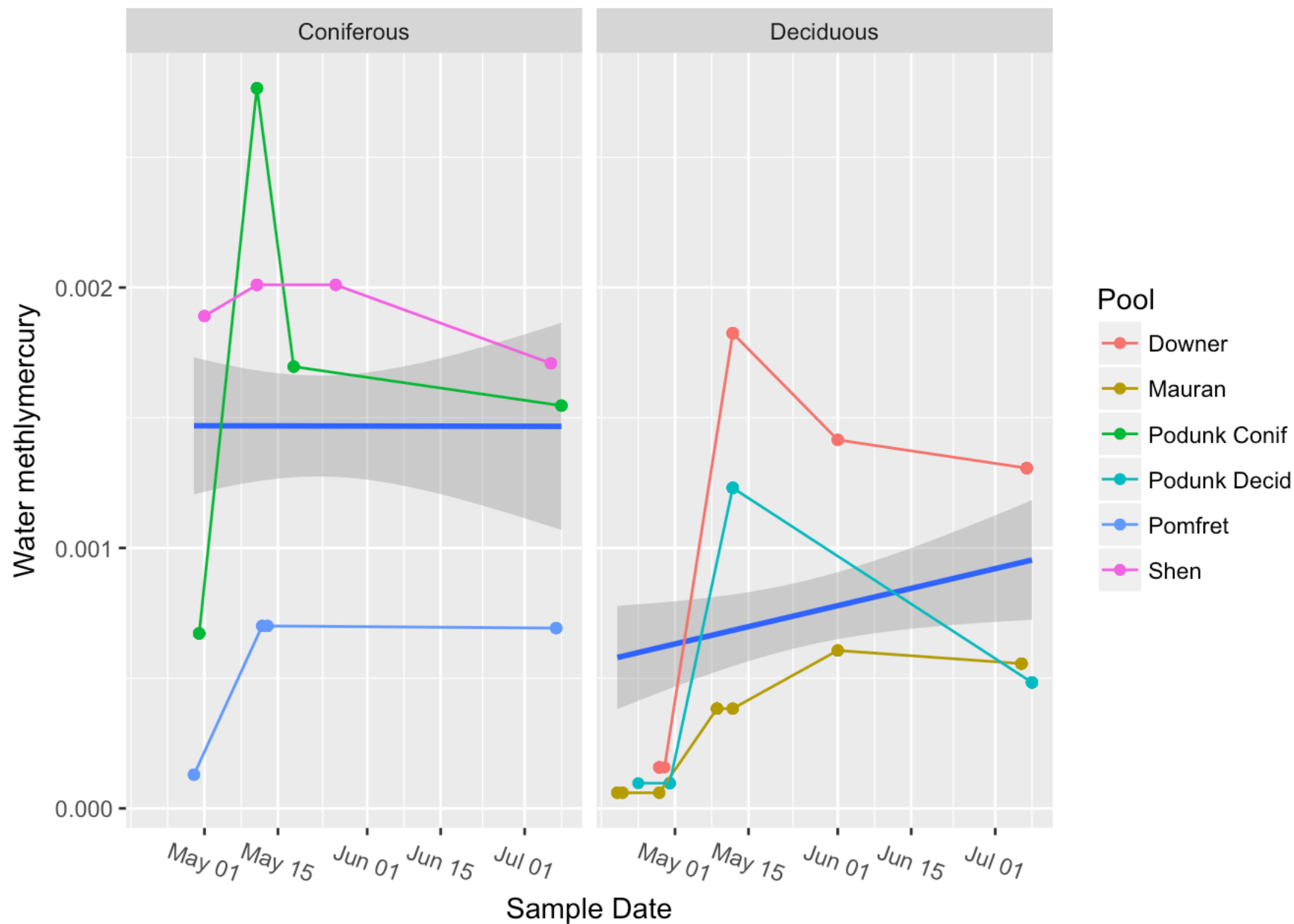
Total mercury levels in water tend to be higher in coniferous pools and tend to decrease over the course of a season. However, sample size is substantially lower for measures of total mercury because not every sampling

period has an associated total mercury level.
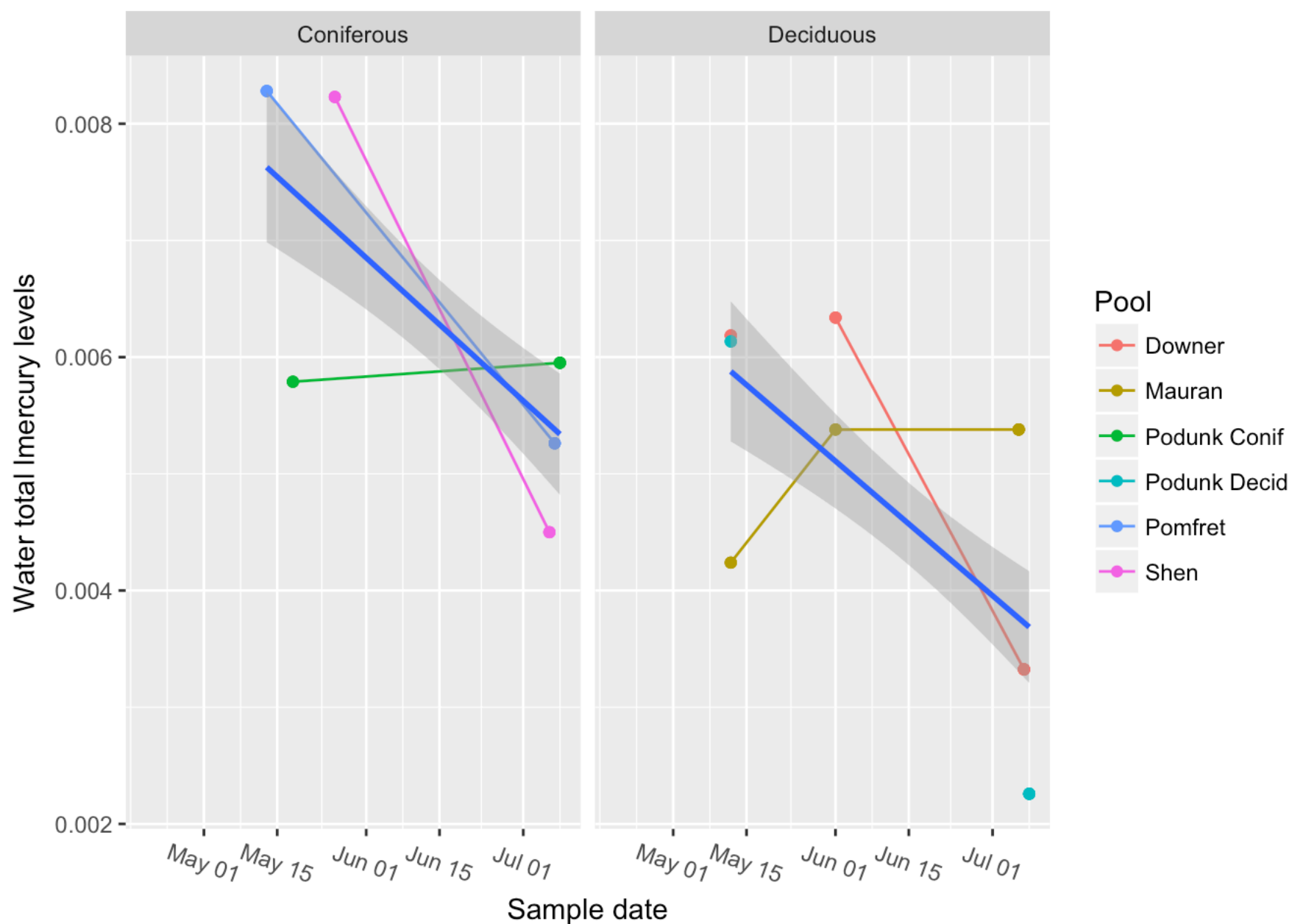
Performing a t-test assuming unequal variances, we find that the 95% credible interval for the difference in methylmercury (the parameter named "delta") between the two habitats includes zero (-0.00013, 0.00131).

```
## Loading required package: rjags
```

```
## Loading required package: coda
```

```
## Linked to JAGS 4.2.0
```

```
## Loaded modules: basemod,bugs
```

```
##
## Attaching package: 'R2jags'
```

```
## The following object is masked from 'package:coda':
##
##      traceplot
```

```
## module glm loaded
```

```
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 22
##    Unobserved stochastic nodes: 4
##    Total graph size: 43
##
## Initializing model
```
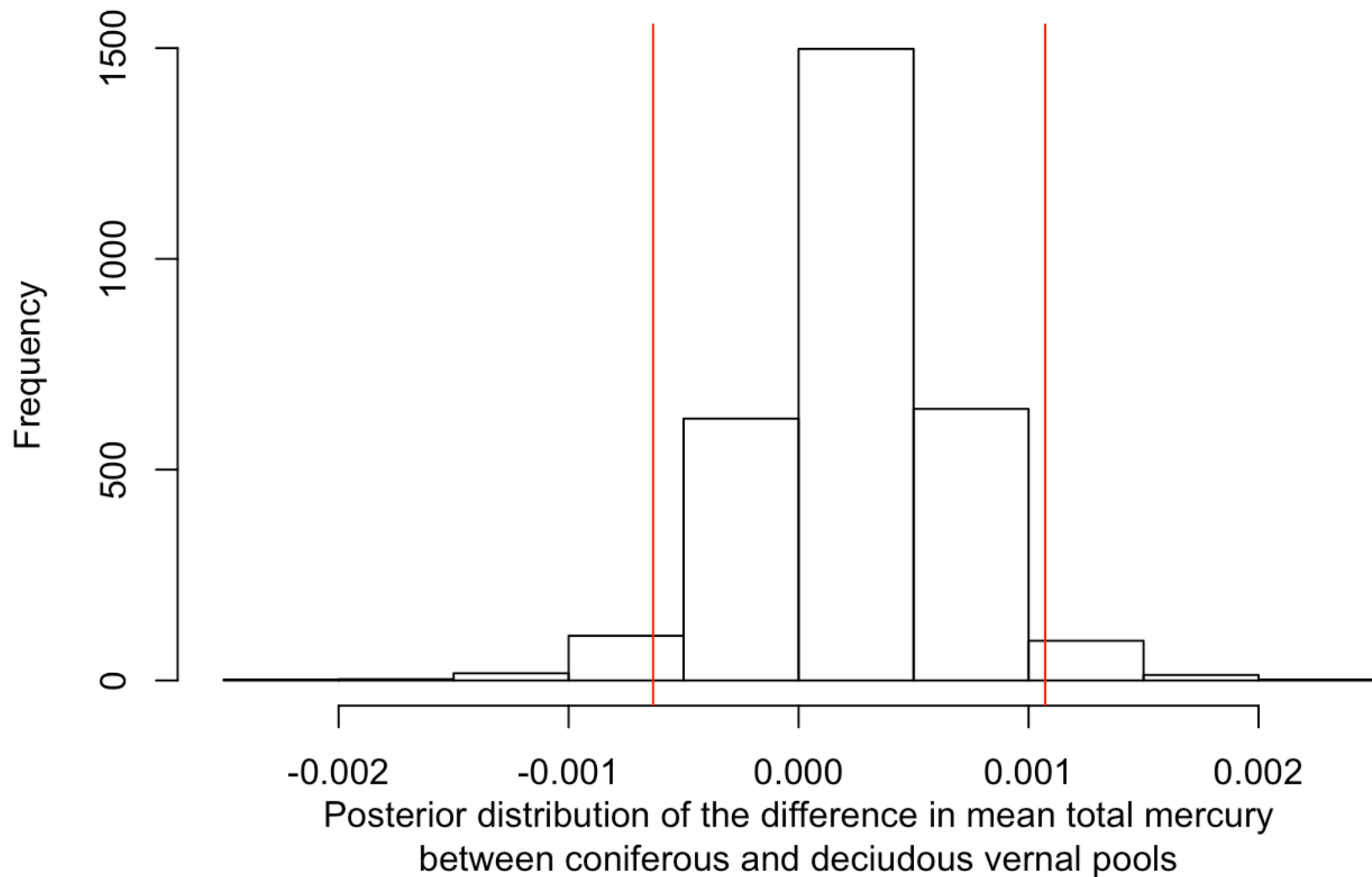
```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb16c024c7.txt", fit using jags,
##  3 chains, each with 5000 iterations (first 2500 discarded), n.thin = 2
##  n.sims = 3750 iterations saved
##              mu.vect sd.vect       2.5%       25%       50%       75%
## delta        0.00058 0.00036   -0.00014   0.00035   0.00058   0.00080
## mu1          0.00074 0.00021    0.00032   0.00060   0.00074   0.00087
## mu2          0.00132 0.00028    0.00075   0.00115   0.00132   0.00150
## sigma1       0.00069 0.00019    0.00044   0.00056   0.00066   0.00078
## sigma2       0.00091 0.00025    0.00058   0.00075   0.00087   0.00103
## deviance  -254.97263 3.53923 -259.25965 -257.50890 -255.80432 -253.35307
##              97.5%    Rhat n.eff
## delta       0.00129 1.00143  2400
## mu1         0.00116 1.00071  3800
## mu2         0.00187 1.00134  2700
## sigma1      0.00118 1.00110  3800
## sigma2      0.00153 1.00286  1200
## deviance -245.71961 1.00187  1600
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 6.3 and DIC = -248.7
## DIC is an estimate of expected predictive error (lower deviance is better).
```

We can visualize this by showing a histogram of the posterior distribution of delta, with the 95% credible interval shown as vertical red lines.

Posterior distribution of the difference in mean methylmercury between coniferous and deciudous vernal pools
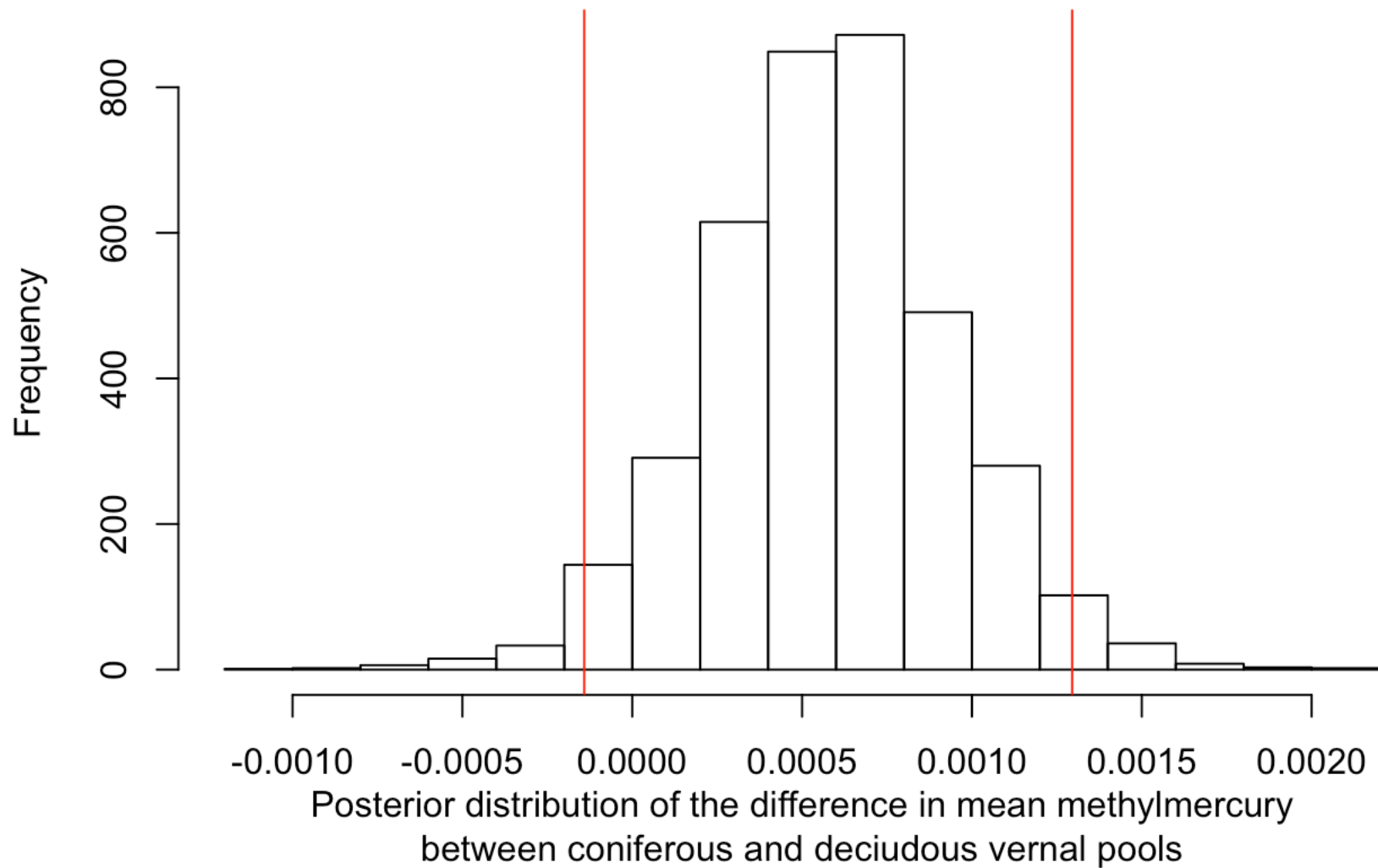
We can conclude that coniferous pools tend to have higher levels of methylmercury, but that the difference is not significant.

Repeating the analysis using total mercury levels in water produces a similar result: the difference in total mercury between coniferous and deciduous pools is approximately zero (mean difference = 0.00026, 95% credible interval = -0.00057 - 0.00108).

```
## Compiling model graph
##     Resolving undeclared variables
##     Allocating nodes
## Graph information:
##     Observed stochastic nodes: 14
##     Unobserved stochastic nodes: 4
##     Total graph size: 35
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb5d06a1b1.txt", fit using jags,
##  3 chains, each with 50000 iterations (first 25000 discarded), n.thin = 25
##  n.sims = 3000 iterations saved
##             mu.vect sd.vect      2.5%       25%       50%       75%
## delta        0.00025 0.00043   -0.00063   0.00000   0.00026   0.00050
## mu1          0.00103 0.00030    0.00047   0.00086   0.00104   0.00120
## mu2          0.00128 0.00031    0.00065   0.00111   0.00129   0.00146
## sigma1       0.00072 0.00033    0.00037   0.00052   0.00064   0.00082
## sigma2       0.00074 0.00031    0.00039   0.00055   0.00067   0.00086
## deviance  -166.26988 4.10074 -171.28638 -169.28916 -167.21232 -164.22276
##              97.5%    Rhat n.eff
## delta        0.00107 1.00062  3000
## mu1          0.00164 1.00057  3000
## mu2          0.00190 1.00083  3000
## sigma1       0.00146 1.00133  2400
## sigma2       0.00151 1.00106  3000
## deviance  -156.25731 1.00255  3000
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 8.4 and DIC = -157.9
## DIC is an estimate of expected predictive error (lower deviance is better).
```

Posterior distribution of the difference in mean total mercury between coniferous and deciudous vernal pools

Mercury levels in amphibians.

Adults

For adults, we need to choose which measure of mercury to use. More samples are missing blood mercury (n = 17) than are missing tissue mercury (n = 3). Therefore, for consistency, we should use tissue mercury as our measure.

```
summary(data.df$Blood_MeHg[data.df$Life_Stage=="Adult"])
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
##   11.26   26.50   50.90   49.82   61.91  147.79      17
```

```
summary(data.df$Tissue_MeHg[data.df$Life_Stage=="Adult"])
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
##   13.30   29.82   43.07   63.49   85.07  212.03       3
```

In addition, mercury levels measured in blood and tissue taken from the same individual show little correspondence and are not interchangeable. In this figure, mercury levels estimated from the same individual are connected by a line. Notice that tissue tends to be produce higher estimates of mercury load, but not always. Thus, mercury levels measured using the two different sample types aren't comparable.
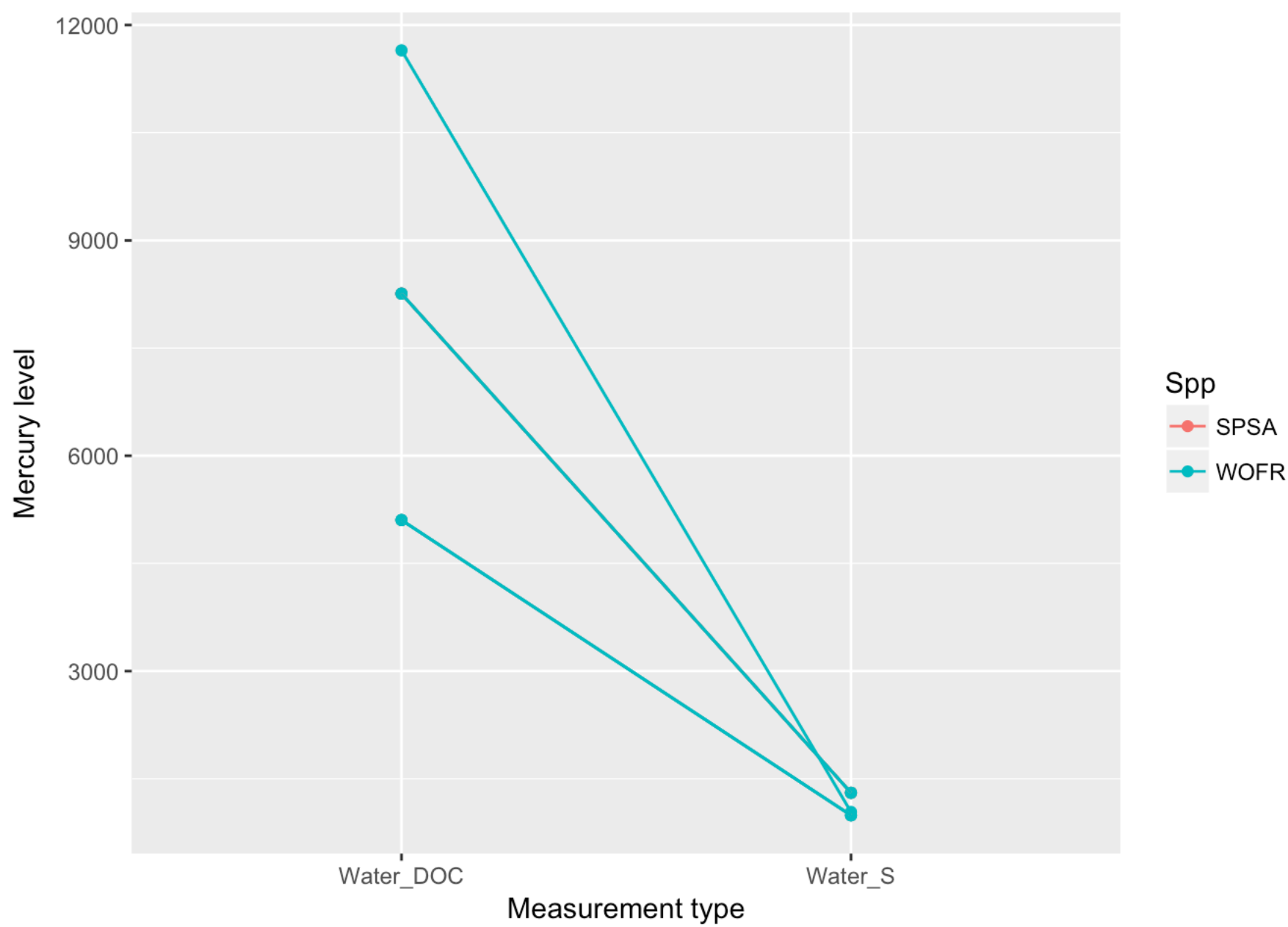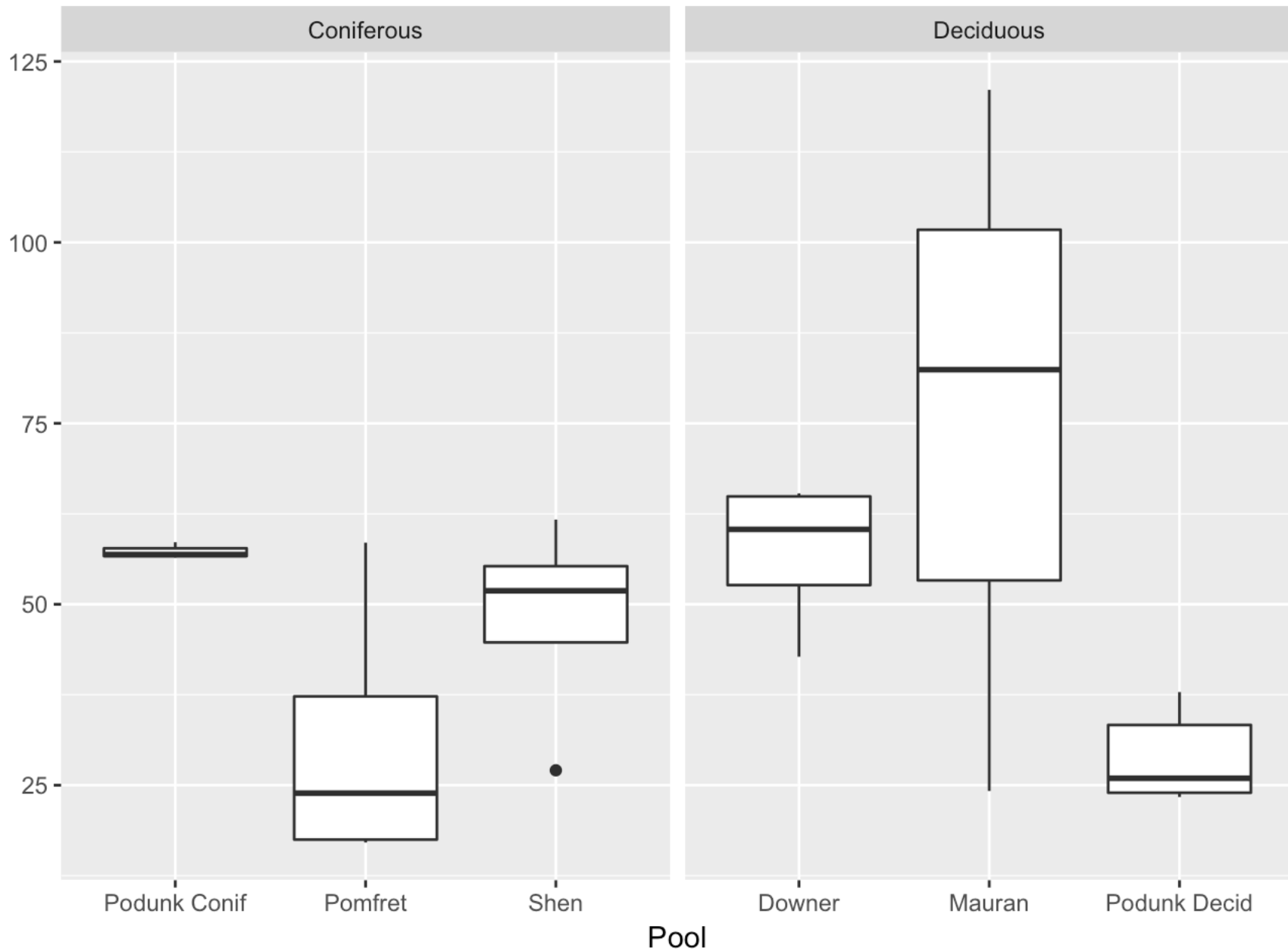
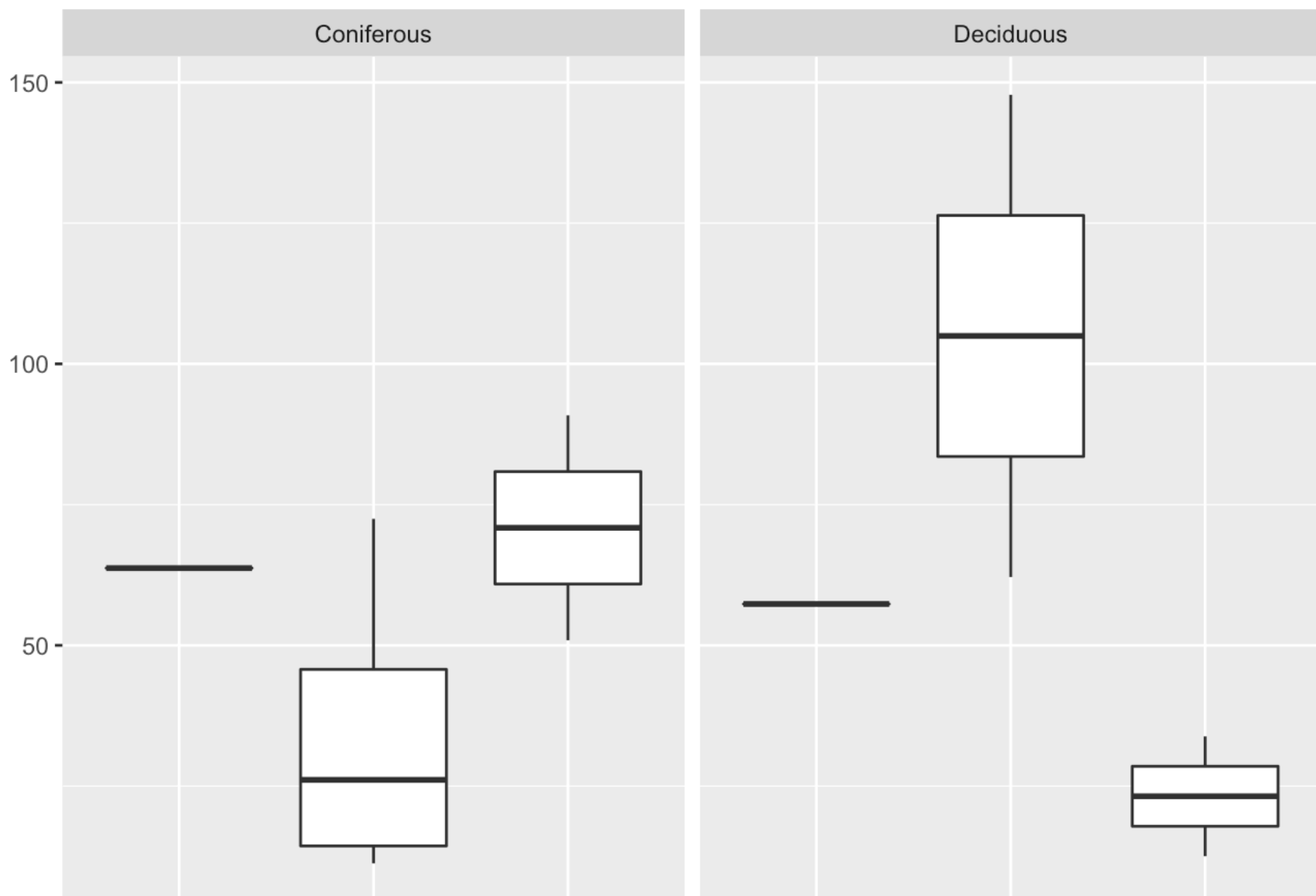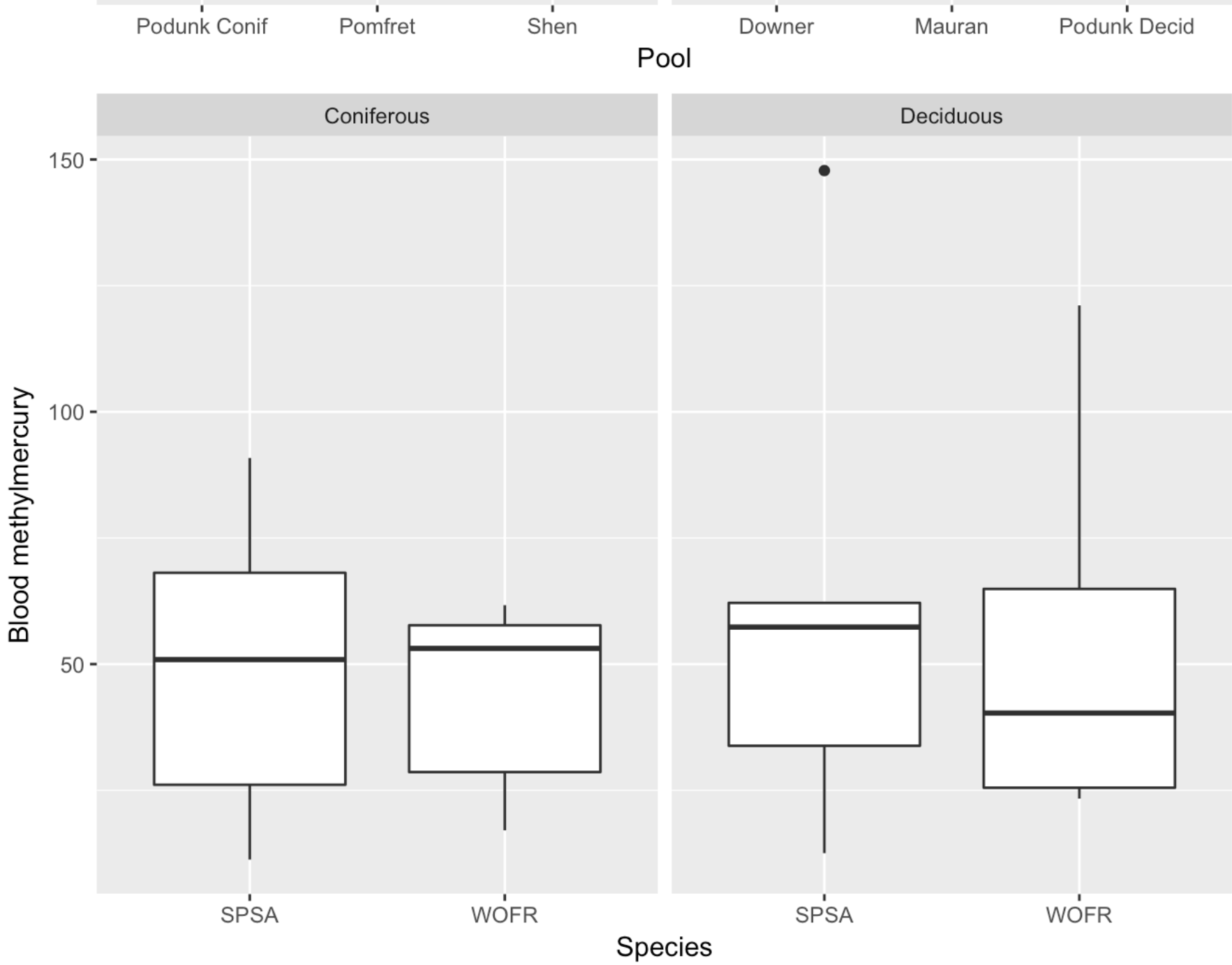Patterns of tissue methylmercury vary among pools and species.
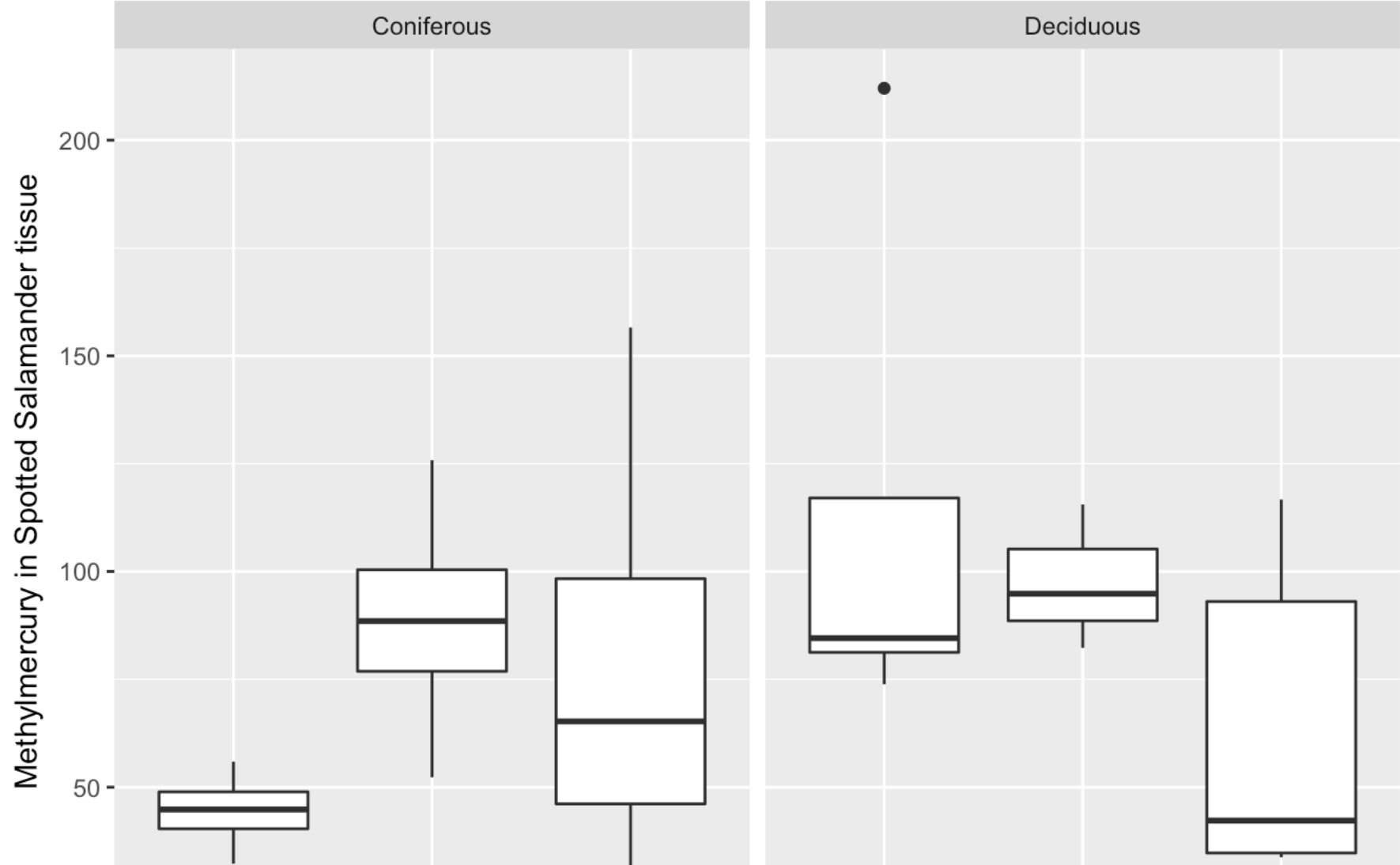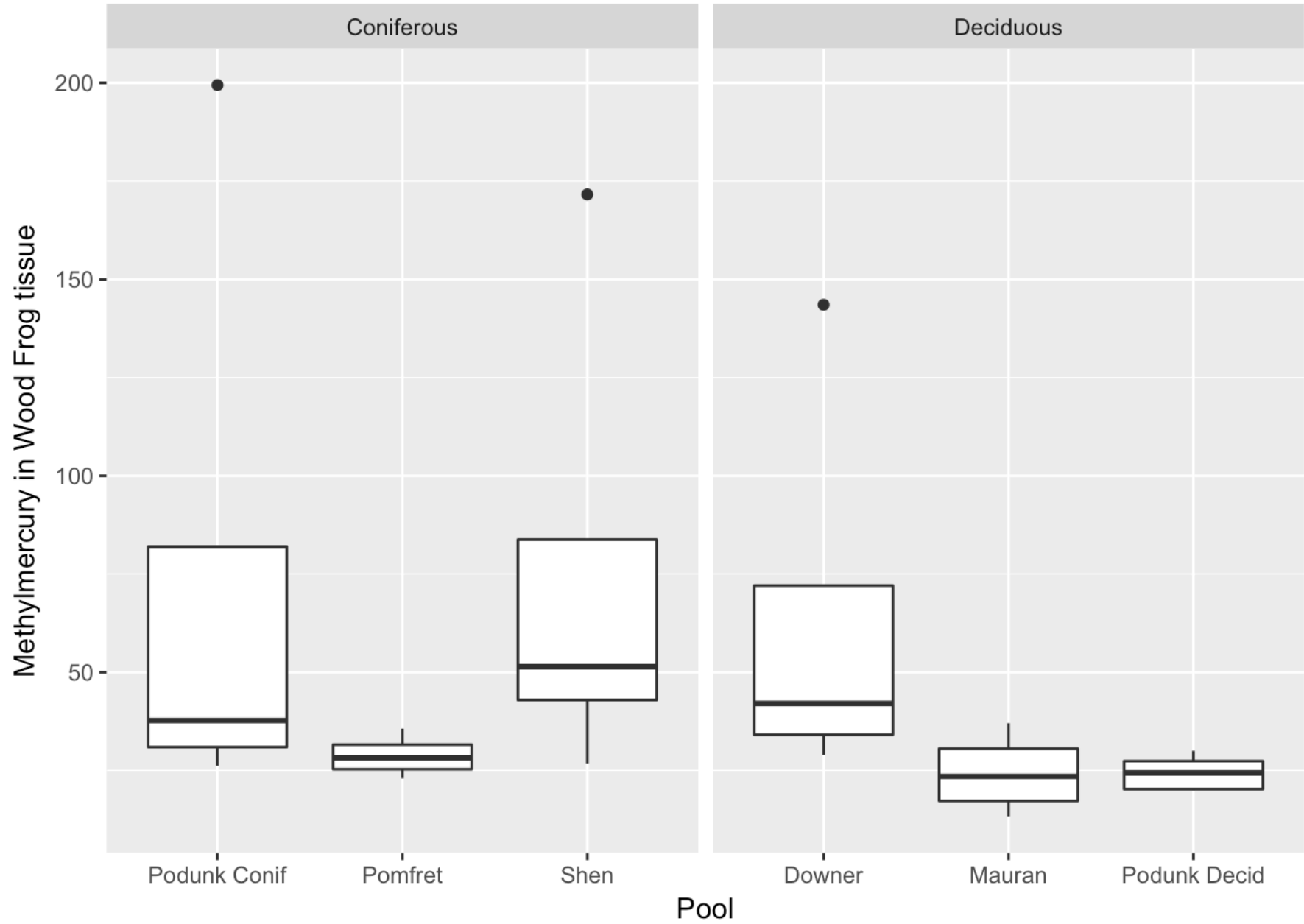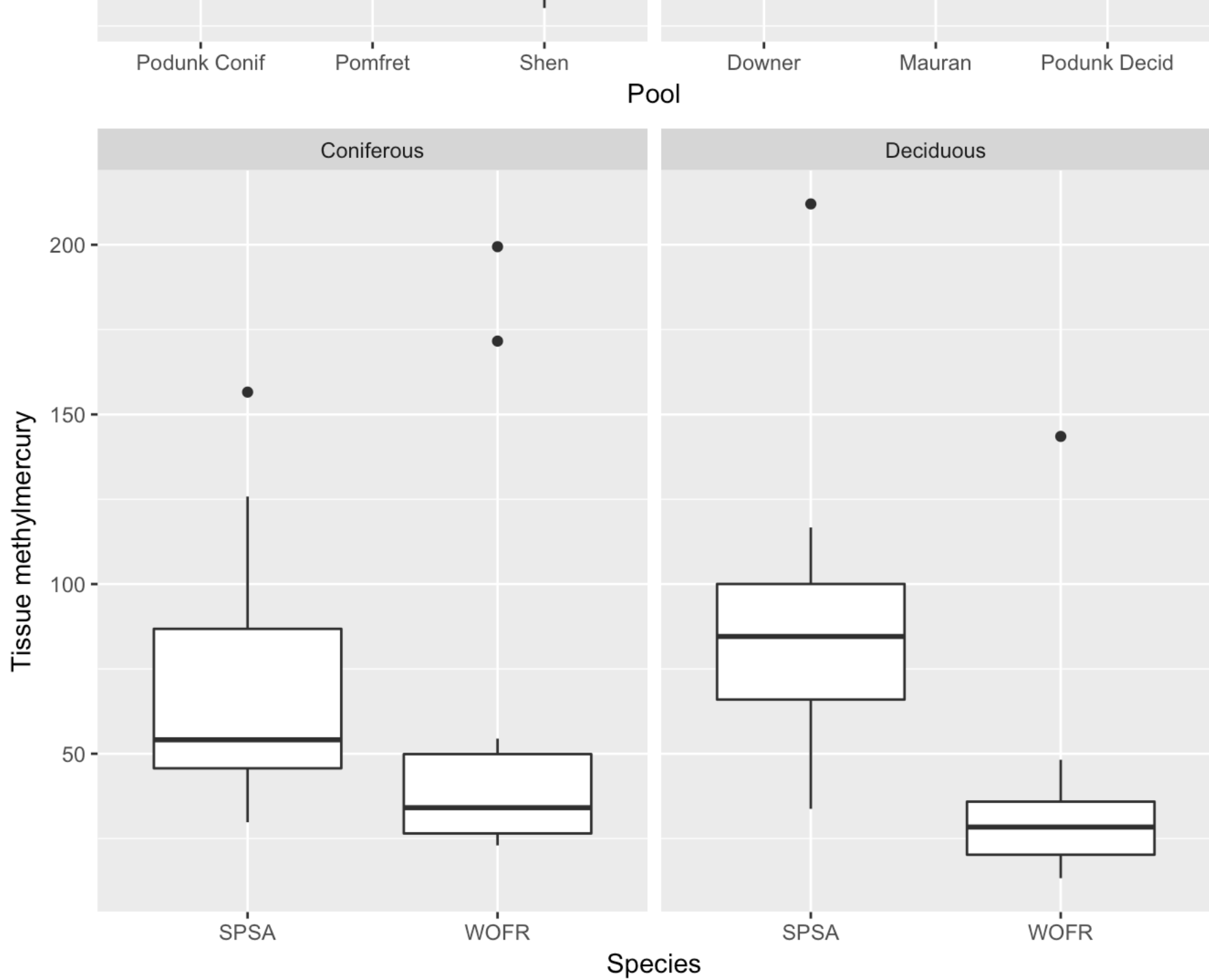
If we look at blood levels of methylmercury in adults:

Neither species shows a strong relationship between levels of methylmercury in tissue and levels of methylmercury in the water at the time of the sample.
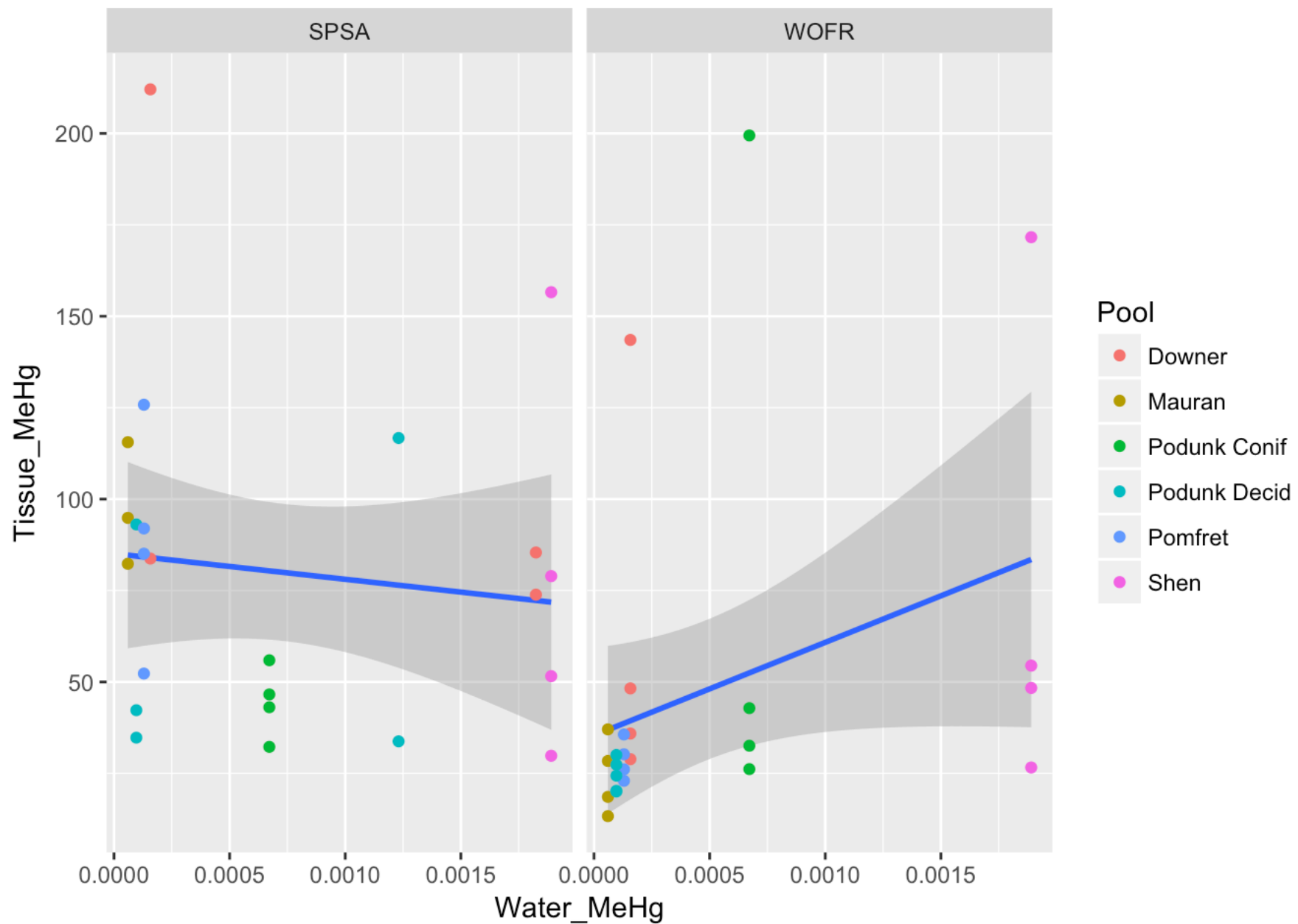
We see the same relationship if we look at blood methylmercury:

```
## Warning: Removed 17 rows containing non-finite values (stat_smooth).
```

```
## Warning: Removed 17 rows containing missing values (geom_point).
```
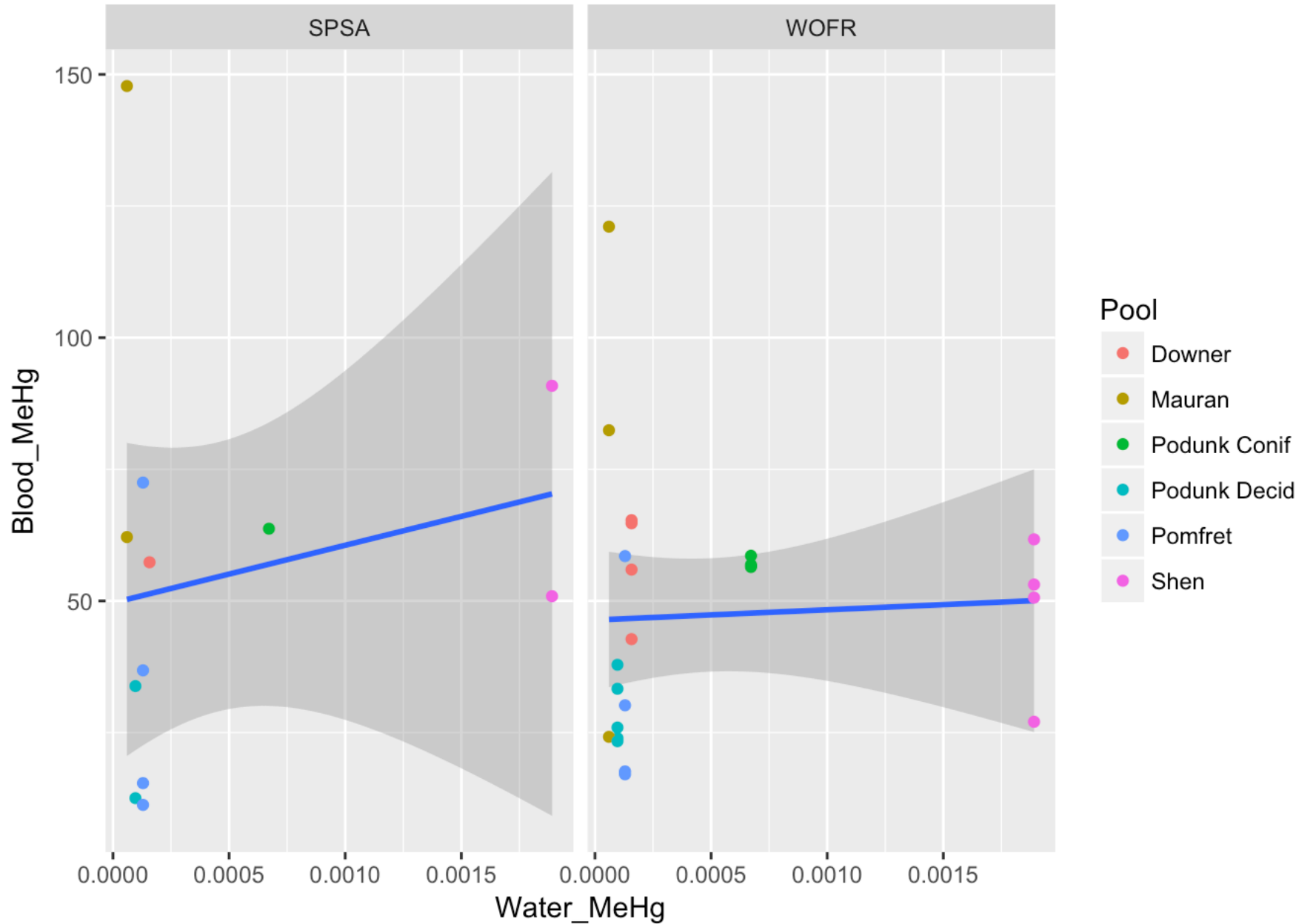
Larval stages

Unclear pattern of accumulation of mercury for juvenile stages. Eggs tend to have low values for both species. For Wood Frog, mercury levels accumulate as might be expected if tissue mercury depends on cumulative exposure to water mercury. In Spotted Salamanders, however, early larvae in deciduous pools tend to have higher levels than late larvae.

```
## Warning: Removed 33 rows containing non-finite values (stat_boxplot).
```
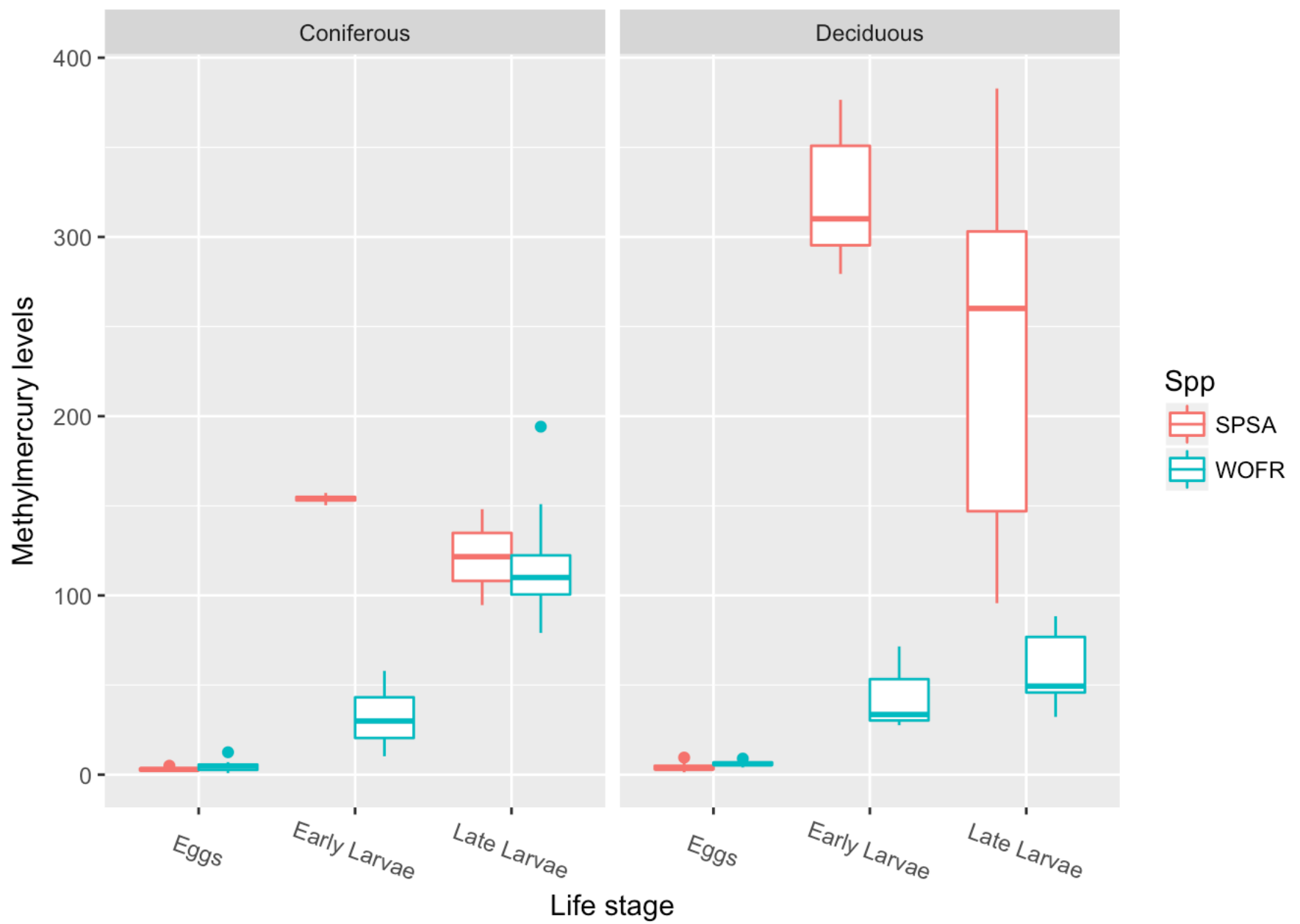
```
p7 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p7 + geom_boxplot(aes(x = Life_Stage, y = Amphib_MeHg, color = Spp)) +
  scale_x_discrete(limits = c("Eggs","Early Larvae","Late Larvae")) +
  theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
  xlab("Life stage") + ylab("Methylmercury levels")
```
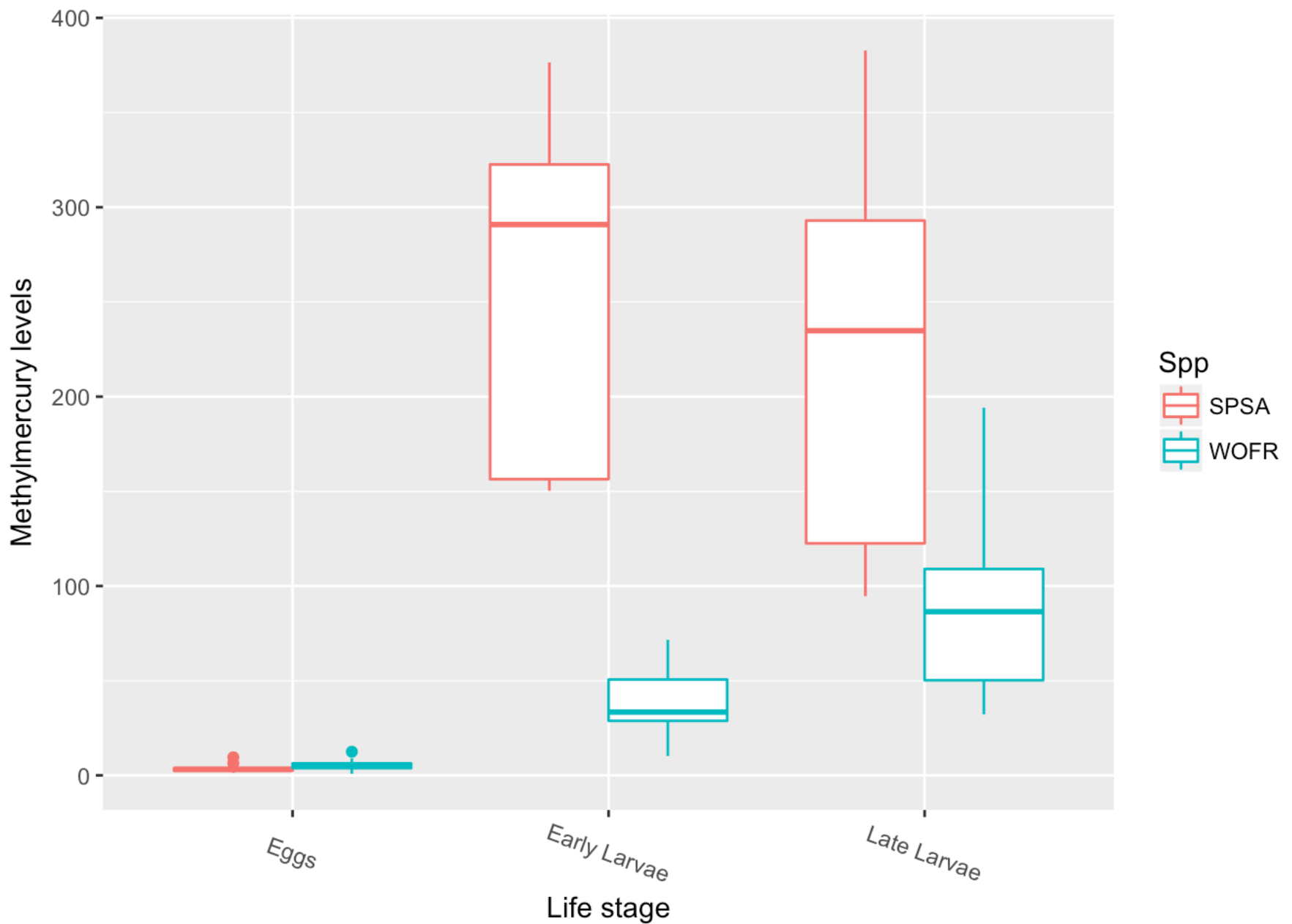
The odd pattern of higher mercury in early-stage larvae for SPSA doesn't appear to be strictly a pool effect. For example, the only two pools with early larvae sampled - Downer and Mauran - also show a decreased level of mercury in late-stage larvae.

```
p8 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p8 + geom_point(aes(x = Life_Stage, y = Amphib_MeHg, color = Pool)) +
   scale_x_discrete(limits = c("Eggs","Early Larvae","Late Larvae")) +
   theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
   xlab("Life stage") + ylab("Methylmercury levels") + facet_wrap(~Spp)
```
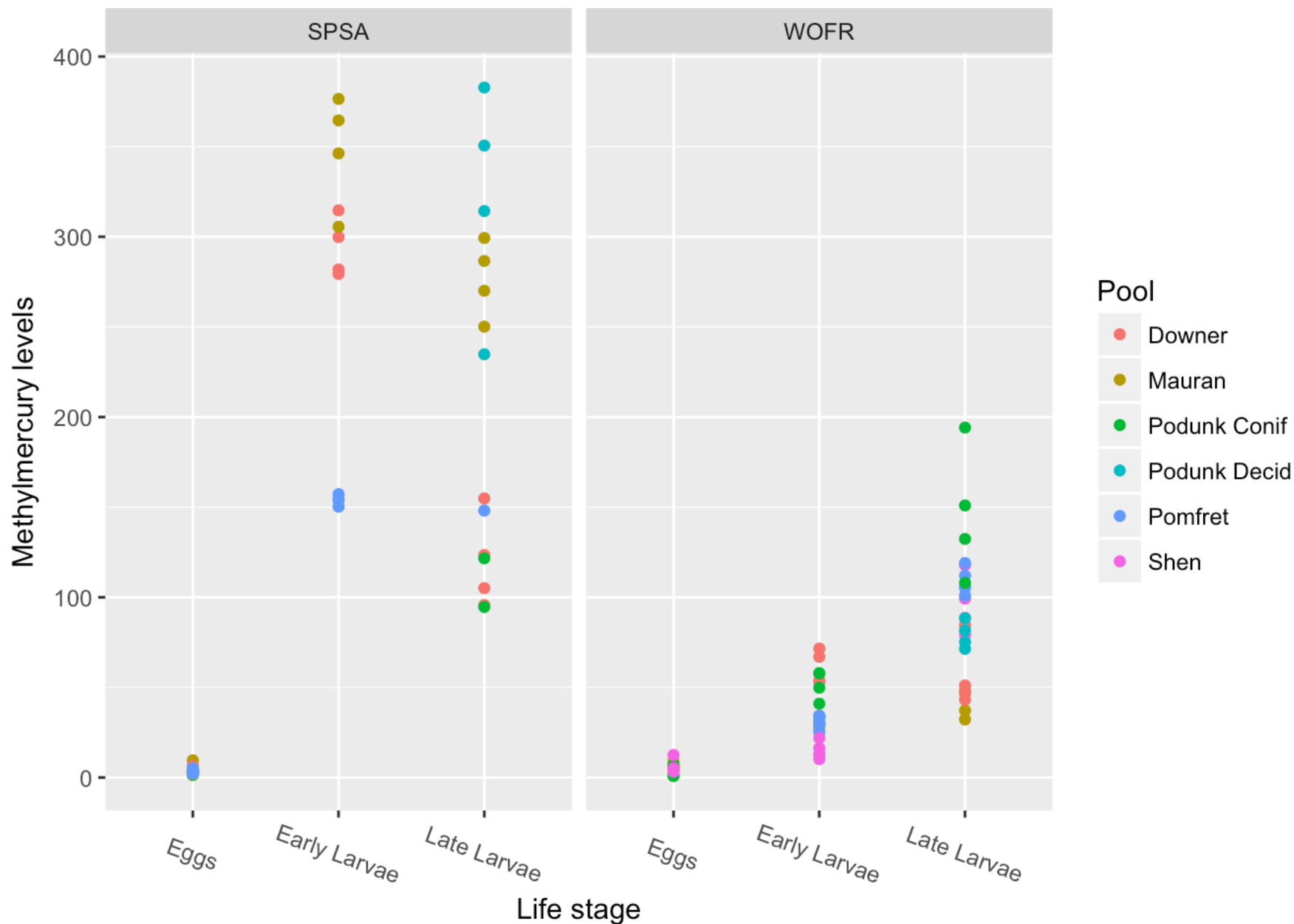
There is no consistent relationship between water mercury levels and levels of methylmercury in the tissue or blood of larval amphibians. There is a weak tendendcy for Spotted Salamander larvae to show a negative relationship with water mercury, but Wood Frogs show a contrasting pattern: lower levels of mercury in the body when water mercury is higher.
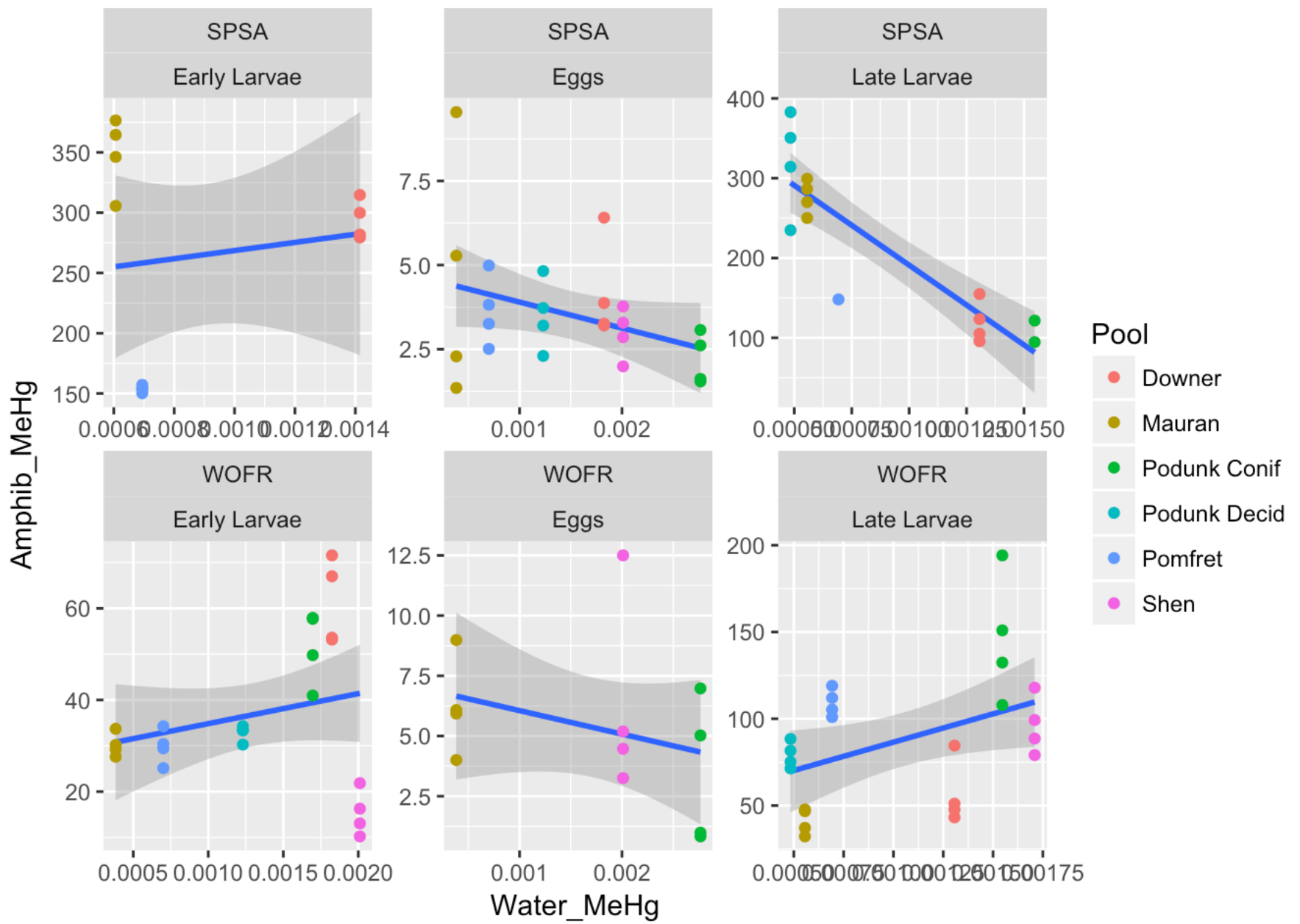
```
p10 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p10 + geom_smooth(aes(x = Water_MeHg, y = Amphib_MeHg), method = "lm") +
  geom_point(aes(x = Water_MeHg, y = Amphib_MeHg, color = Life_Stage)) + facet_wrap(~
Spp)
```
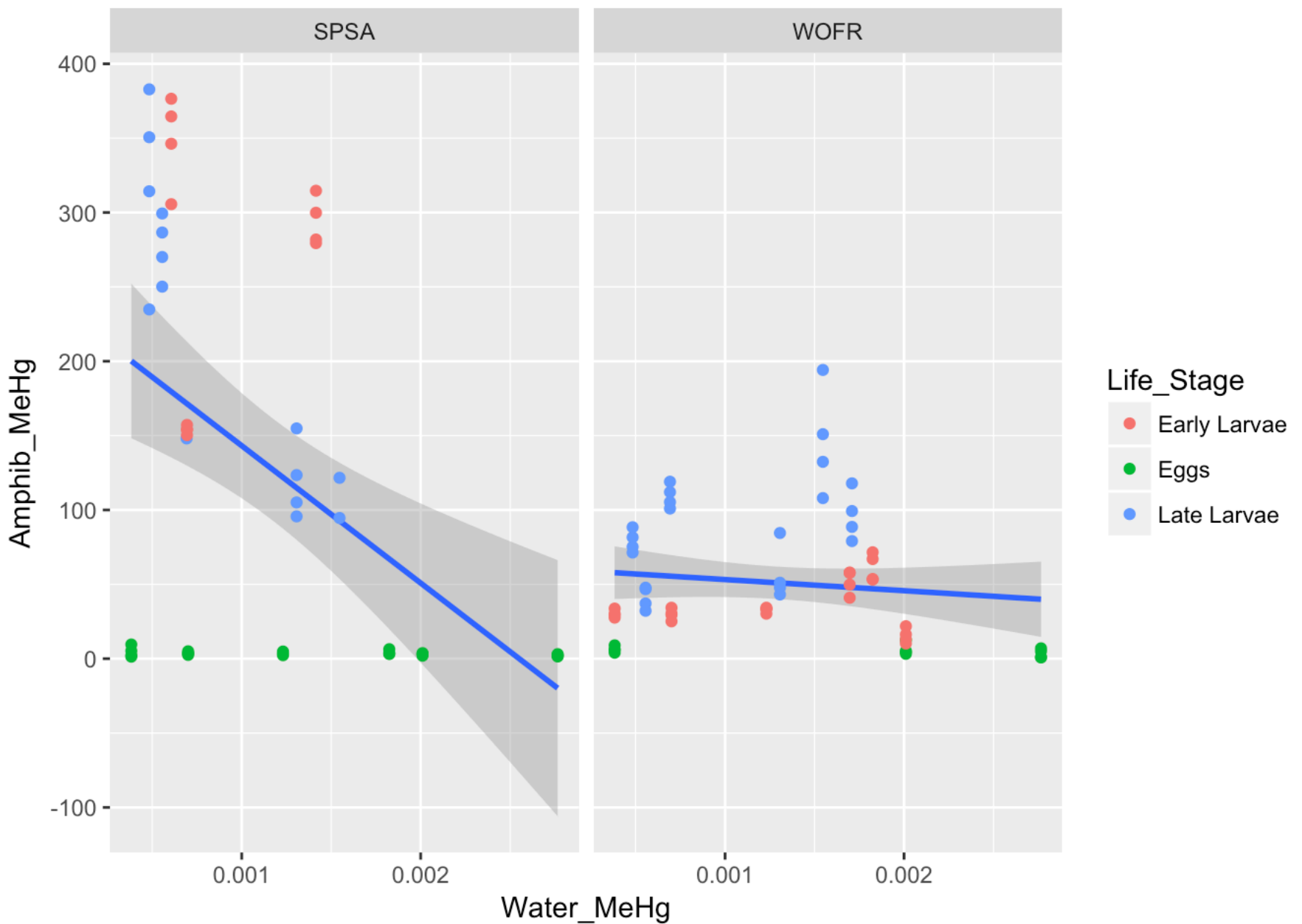
Exploratory analysis summary

1. A non-significant trend exists towards higher levels of mercury in water of coniferous pools.
2. Adult amphibians show no relationship between mercury in the body and mercury in the water. Adults of both species tend to have similar levels of mercury in the body.
3. Wood Frogs show a trend towards increasing mercury in the body as they mature, but Spotted Salamanders do not. In fact, for Spotted Salamanders, levels of mercury are highest in the early larval stage.
4. Spotted Salamanders have lower levels of mercury in their body when water mercury is higher, and this pattern holds across life stages (i.e., it is not confounded by stage of life).
5. Wood Frogs have higher levels of mercury in their body when water mercury is higher, and this pattern holds across life stages.
6. Thus, mercury levels in the body vary as a function of water mercury*species + life stage.

Formal analysis.

Juvenile life stages.

For a more formal analysis, I compared 3 linear models within a Bayesian framework. For the juvenile life stages, these models included: 1. Amphibian mercury ~ species*water methlymercury. In this model, mercury level measured in amphibian larvae reflects an interaction between species and water mercury levels.

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 5
##      Total graph size: 408
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb4a107bb5.txt", fit using jags,
##   3 chains, each with 50000 iterations (first 25000 discarded), n.thin = 25
##   n.sims = 3000 iterations saved
##           mu.vect sd.vect      2.5%        25%        50%        75%
## alpha[1]  -6.88930 2.71147 -12.30719  -8.72598   -6.87078   -5.05781
## alpha[2]   1.25256 2.24799  -3.18741  -0.29670    1.24523    2.79110
## beta[1]   -1.48440 0.39076  -2.25450  -1.75688   -1.47910   -1.21565
## beta[2]   -0.32058 0.32803  -0.96074  -0.54755   -0.32327   -0.10130
## sigma      1.63688 0.11464   1.42556   1.55864    1.62742    1.70885
## deviance 423.16469 3.21876 418.77555 420.85191 422.53198 424.83914
##              97.5%    Rhat n.eff
## alpha[1]  -1.65525 1.00221  1200
## alpha[2]   5.59646 1.00060  3000
## beta[1]   -0.73313 1.00230  1100
## beta[2]    0.31820 1.00060  3000
## sigma      1.87938 1.00101  3000
## deviance 430.91782 1.00084  3000
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 5.2 and DIC = 428.3
## DIC is an estimate of expected predictive error (lower deviance is better).
```

2. Amphibian mercury ~ species*water methlymercury + life stage. In this model, mercury level measured in amphibian larvae reflects an interaction between species and water mercury levels and an additive effect of life stage.

```r
spp <- data_subset2$Spp
hg <- log(data_subset2$Amphib_MeHg)
stage <- data_subset2$Life_Stage
stage <- droplevels(stage)
waterhg <- log(data_subset2$Water_MeHg) #could consider scaling this to get smaller B
etas
n <- nrow(data_subset2)
n.groups <- length(levels(data_subset2$Spp))
n.stages <- 3
jags.params <- c("alpha","beta.spp", "beta.stage", "sigma")
jags.inits <- function(){
  list(sigma=rlnorm(1))
}
#Model
logmercuryanova2 <- function () {
  for(i in 1:n){
    hg[i] ~ dnorm(mu[i], tau)
    mu[i] <- alpha[spp[i]] + beta.spp[spp[i]]*waterhg[i] + beta.stage[stage[i]]
  }
  for(i in 1:n.groups){
    alpha[i] ~ dnorm(0, 0.001)
    beta.spp[i] ~ dnorm(0, 0.001)
  }
  for(i in 1:n.stages){
    beta.stage[i] ~ dnorm(0,0.001)
  }
  sigma ~ dunif(0,100)
  tau <- 1/(sigma*sigma)
}

jagsfitlogm2 <- jags(data = c("spp","hg","waterhg","n","n.groups","stage", "n.stages"
), inits = jags.inits, jags.params,
              n.iter = 20000, model.file = logmercuryanova2)
```

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 8
##      Total graph size: 531
##
## Initializing model
```

```r
print(jagsfitlogm2,digits = 5)
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb38977c85.txt", fit using jags,
##  3 chains, each with 20000 iterations (first 10000 discarded), n.thin = 10
##  n.sims = 3000 iterations saved
##                   mu.vect   sd.vect        2.5%        25%        50%        75%
## alpha[1]         -0.23803  13.97289  -27.30881   -9.48233   -0.25633    9.00286
## alpha[2]          2.49401  13.93225  -24.76124   -6.84877    2.42390   11.62955
## beta.spp[1]      -0.48963   0.16169   -0.80603   -0.60136   -0.49032   -0.37593
## beta.spp[2]       0.02664   0.13555   -0.24113   -0.06103    0.02934    0.11658
## beta.stage[1]     1.55720  13.93749  -26.17162   -7.47096    1.56932   10.83863
## beta.stage[2]    -1.54504  13.93772  -29.27061  -10.57703   -1.60321    7.78338
## beta.stage[3]     2.04431  13.94194  -25.72103   -6.96154    2.05991   11.32138
## sigma             0.65968   0.04521    0.57798    0.62835    0.65821    0.68735
## deviance        221.17563   3.99817  215.63546  218.26215  220.41847  223.26000
##                    97.5%     Rhat  n.eff
## alpha[1]          27.64914  1.00150   2000
## alpha[2]          30.26403  1.00126   2600
## beta.spp[1]       -0.17403  1.00126   2600
## beta.spp[2]        0.29103  1.00138   2300
## beta.stage[1]     28.67072  1.00136   2300
## beta.stage[2]     25.58973  1.00138   2300
## beta.stage[3]     29.25111  1.00138   2300
## sigma              0.75879  1.00080   3000
## deviance         230.94303  1.00100   3000
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 8.0 and DIC = 229.2
## DIC is an estimate of expected predictive error (lower deviance is better).
```

3. Amphibian mercury ~ species*water methlymercury + life stage + habitat. In this model, mercury level measured in amphibian larvae reflects an interaction between species and water mercury levels and additive effects of life stage and habitat (deciduous vs coniferous).

```
## Compiling model graph
##     Resolving undeclared variables
##     Allocating nodes
## Graph information:
##     Observed stochastic nodes: 111
##     Unobserved stochastic nodes: 122
##     Total graph size: 2036
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
   pF1pfkK/model2abb7720219.txt", fit using jags,
##  3 chains, each with 20000 iterations (first 10000 discarded), n.thin = 10
##  n.sims = 3000 iterations saved
##                     mu.vect  sd.vect       2.5%       25%       50%       75%
## alpha[1]           -0.22001 17.44203 -36.22376 -11.50921  -0.41072  11.44582
## alpha[2]            2.68661 17.42713 -32.48717  -8.73437   2.69582  14.34475
## beta.habitat[1]     0.09212 17.49481 -34.61100 -11.15070   0.27148  11.46574
## beta.habitat[2]     0.21263 17.49682 -34.56005 -11.04738   0.42359  11.53919
## beta.spp[1]        -0.38885 18.26218 -35.61864 -12.74260  -0.27938  12.45760
## beta.spp[2]         0.15012 18.25732 -35.02432 -12.22734   0.25636  12.99051
## beta.stage[1]       1.56724 16.09429 -30.18838  -9.50657   1.32134  12.36052
## beta.stage[2]      -1.53029 16.09362 -33.37789 -12.57760  -1.80774   9.20755
## beta.stage[3]       2.04828 16.09352 -29.77377  -8.96261   1.77714  12.77618
## beta.waterhg       -0.07220 18.25983 -35.48133 -12.85661  -0.16426  12.23858
## bpvalue             0.53000  0.49918   0.00000   0.00000   1.00000   1.00000
## sigma               0.66227  0.04644   0.57901   0.62958   0.65945   0.69204
## deviance          221.53384  4.23681 215.30929 218.43227 220.79840 223.89280
##                      97.5%    Rhat n.eff
## alpha[1]           33.38394 1.00106  3000
## alpha[2]           36.27603 1.00114  3000
## beta.habitat[1]    34.79453 1.00155  1900
## beta.habitat[2]    35.04371 1.00155  1900
## beta.spp[1]        35.02438 1.00051  3000
## beta.spp[2]        35.46901 1.00050  3000
## beta.stage[1]      33.55844 1.00089  3000
## beta.stage[2]      30.21687 1.00088  3000
## beta.stage[3]      33.82712 1.00088  3000
## beta.waterhg       35.09772 1.00050  3000
## bpvalue             1.00000 1.00067  3000
## sigma               0.75964 1.00097  3000
## deviance          231.88571 1.00069  3000
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 9.0 and DIC = 230.5
## DIC is an estimate of expected predictive error (lower deviance is better).
```

The best model is model 2 (it has the smallest Deviance Information Criterion (DIC), similar to AIC in that it reflects a balance between model fit and parsimony). In other words, mercury levels in larval amphibians depend on mercury levels in the water (although not in the expected way!) and on the life stage.

Adding an effect of habitat increases the DIC, indicating that this parameter is not useful in explaining variation in mercury levels in amphibians:

```
cat(paste(c("DIC for Spp*WaterHg:"), jagsfitm1$BUGSoutput$DIC))
```

```
## DIC for Spp*WaterHg: 428.347006887934
```

```
cat(paste(c("DIC for Spp*WaterHg + Stage"), jagsfitlogm2$BUGSoutput$DIC))
```

```
## DIC for Spp*WaterHg + Stage 229.169596061473
```

```
cat(paste(c("DIC for Spp*WaterHg + Stage + Habitat:"),jagsfitlogm3$BUGSoutput$DIC))
```

```
## DIC for Spp*WaterHg + Stage + Habitat: 230.513349936155
```

Using that model for inference, we can generate predictions and figures that show the expected relationships.

```
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 111
##    Unobserved stochastic nodes: 119
##    Total graph size: 2417
##
## Initializing model
```

Predictions for each life stage, from the best model:
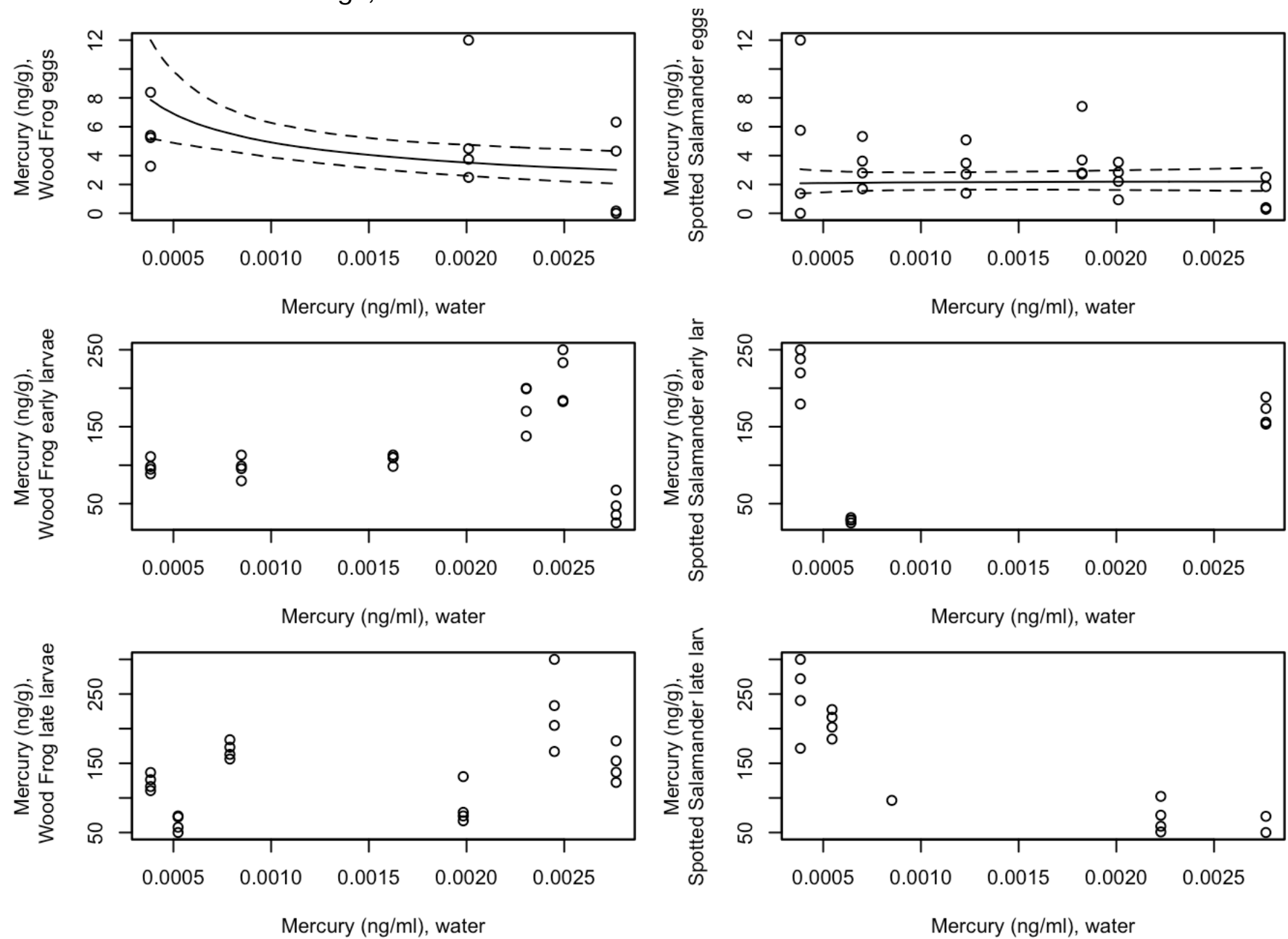
Adults.

For adults, there are only 2 sensible models: 1) Tissue_MeHg ~ Species 2) Tissue_MeHg ~ Species + Habitat

I did not include any models with an effect of water mercury on tissue mercury because it is not biologically clear why this relationship should exist. Adults of both species are not resident within the pool, so I would not expect to find any effect of water mercury on the tissue mercury. Exploratory analyses support this assumption.

As with the juveniles, I analyzed both models in a Bayesian framework. First, the model that simply partitions variation among species:

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 49
##      Unobserved stochastic nodes: 3
##      Total graph size: 116
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb3112440f.txt", fit using jags,
##  3 chains, each with 50000 iterations (first 25000 discarded), n.thin = 25
##  n.sims = 3000 iterations saved
##            mu.vect sd.vect      2.5%       25%       50%       75%      97.5%
## alpha[1]   4.24631 0.12714   3.99661   4.16016   4.24621   4.33470   4.49062
## alpha[2]   3.58136 0.12425   3.33794   3.49908   3.58064   3.66436   3.82891
## delta     34.21236 9.98466  16.00774  27.10019  33.61481  40.65894  54.44065
## sigma      0.61927 0.06545   0.51066   0.57183   0.61296   0.65960   0.76246
## spsa      70.41285 8.96975  54.41361  64.08159  69.84050  76.30194  89.17680
## wofr      36.20049 4.50897  28.16106  33.08514  35.89646  39.03123  46.01258
## deviance  90.43772 2.52849  87.50737  88.52805  89.79014  91.66503  96.70302
##              Rhat n.eff
## alpha[1] 1.00057  3000
## alpha[2] 1.00119  2900
## delta    1.00050  3000
## sigma    1.00233  1100
## spsa     1.00057  3000
## wofr     1.00116  3000
## deviance 1.00057  3000
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 3.2 and DIC = 93.6
## DIC is an estimate of expected predictive error (lower deviance is better).
```

Next, the model that includes an effect of habitat type:

```
## Compiling model graph
##     Resolving undeclared variables
##     Allocating nodes
## Graph information:
##     Observed stochastic nodes: 49
##     Unobserved stochastic nodes: 5
##     Total graph size: 169
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pF1pfkK/model2abb309be1c3.txt", fit using jags,
##  3 chains, each with 20000 iterations (first 10000 discarded), n.thin = 10
##  n.sims = 3000 iterations saved
##                    mu.vect sd.vect     2.5%      25%      50%      75%
## group.mean[1,1]    4.13171 0.17656  3.79590  4.01392  4.13071  4.24452
## group.mean[2,1]    3.79589 0.17615  3.44959  3.67572  3.79405  3.91444
## group.mean[1,2]    4.36537 0.18198  4.02219  4.24719  4.36008  4.48352
## group.mean[2,2]    3.39844 0.17071  3.07659  3.28174  3.39806  3.50657
## sigma              0.61042 0.06861  0.49758  0.56231  0.60408  0.65132
## deviance          88.95227 3.59919 84.28918 86.39947 88.24726 90.74144
##                      97.5%    Rhat n.eff
## group.mean[1,1]    4.47583 1.00052  3000
## group.mean[2,1]    4.13732 1.00143  2200
## group.mean[1,2]    4.72801 1.00150  2000
## group.mean[2,2]    3.74340 1.00050  3000
## sigma              0.75932 1.00058  3000
## deviance          97.68281 1.00130  2500
##
## For each parameter, n.eff is a crude measure of effective sample size,
## and Rhat is the potential scale reduction factor (at convergence, Rhat=1).
##
## DIC info (using the rule, pD = var(deviance)/2)
## pD = 6.5 and DIC = 95.4
## DIC is an estimate of expected predictive error (lower deviance is better).
```

Adding an effect of habitat does not improve the model fit, suggesting that habitat is not an important predictor of variation in mercury levels among adult amphibians.

```
## DIC, Model 1: 93.6362613391778
```

```
## DIC, Model 2: 95.4287031076965
```

The conclusion? Adult Wood Frogs have lower mercury levels than Spotted Salamanders, and habitat does not explain significant amounts of variation.

```
## Mean difference, Spotted Salamander - Wood Frog: 34.2123566508267
```

```
## 95% CI: 16.0077416372491 - 54.4406468582676
```

Conclusion

Mercury levels - either methylmercury or total mercury - in the water of deciduous and coniferous vernal pools do not differ in this sample of pools. In part, the lack of variation probably reflects the rather small sample size.

Methylmercury levels in larval stages differ among species, among life stages, and as a function of water mercury. Eggs have the lowest levels of methylmercury, early larvae the highest, and late larvae are intermediate in methylmercury loads.

For Spotted Salamanders, methylmercury loads tended to decrease as water methylmercury increased. For Wood Frogs, I found no relationship between methylmercury load and water methylmercury levels.
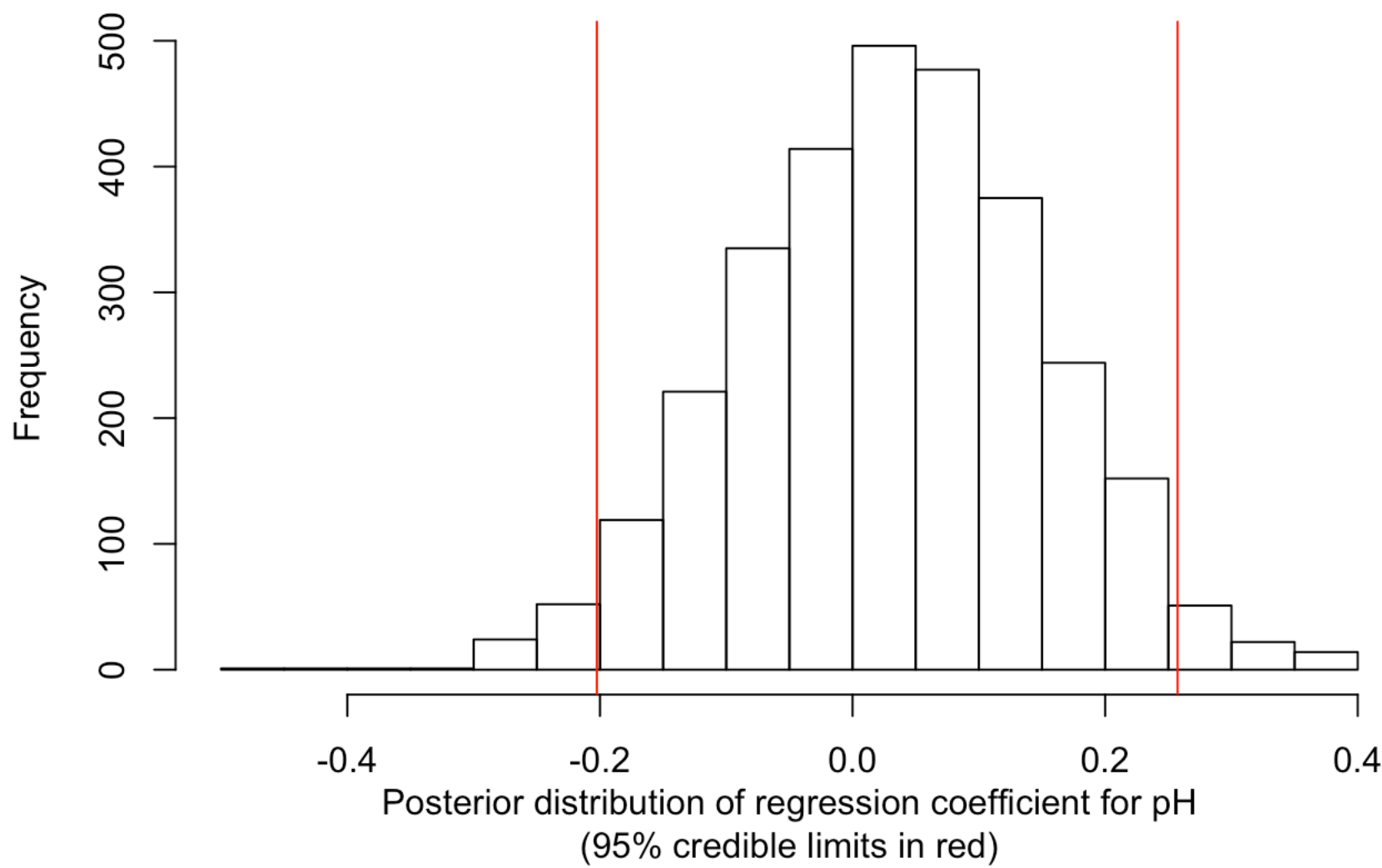
Among adults, tissue methylmercury varied among species. Spotted Salamanders had higher average loads than did Wood Frogs. Habitat did not explain variation in mercury loads among samples.

Addendum

Steve asked that I consider 4 additional covariates of methylmercury: DOC, S, Al, and pH. I first examined whether adding any of these variables to the best model of methlymercury levels in the egg and larval amphibians.

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 120
##      Total graph size: 2022
##
## Initializing model
```

The regression coefficient for water pH is not significantly different from zero, and including it doesn't improve the model fit (the Deviance Information Criterion is higher).

Posterior distribution of regression coefficient for pH
(95% credible limits in red)

```
## DIC for Spp*WaterHg + Stage 229.169596061473
```

```
## DIC for Spp*WaterHg + Stage + pH: 230.489002315367
```

The same is true for Aluminum.

```
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 111
##    Unobserved stochastic nodes: 120
##    Total graph size: 2028
##
## Initializing model
```
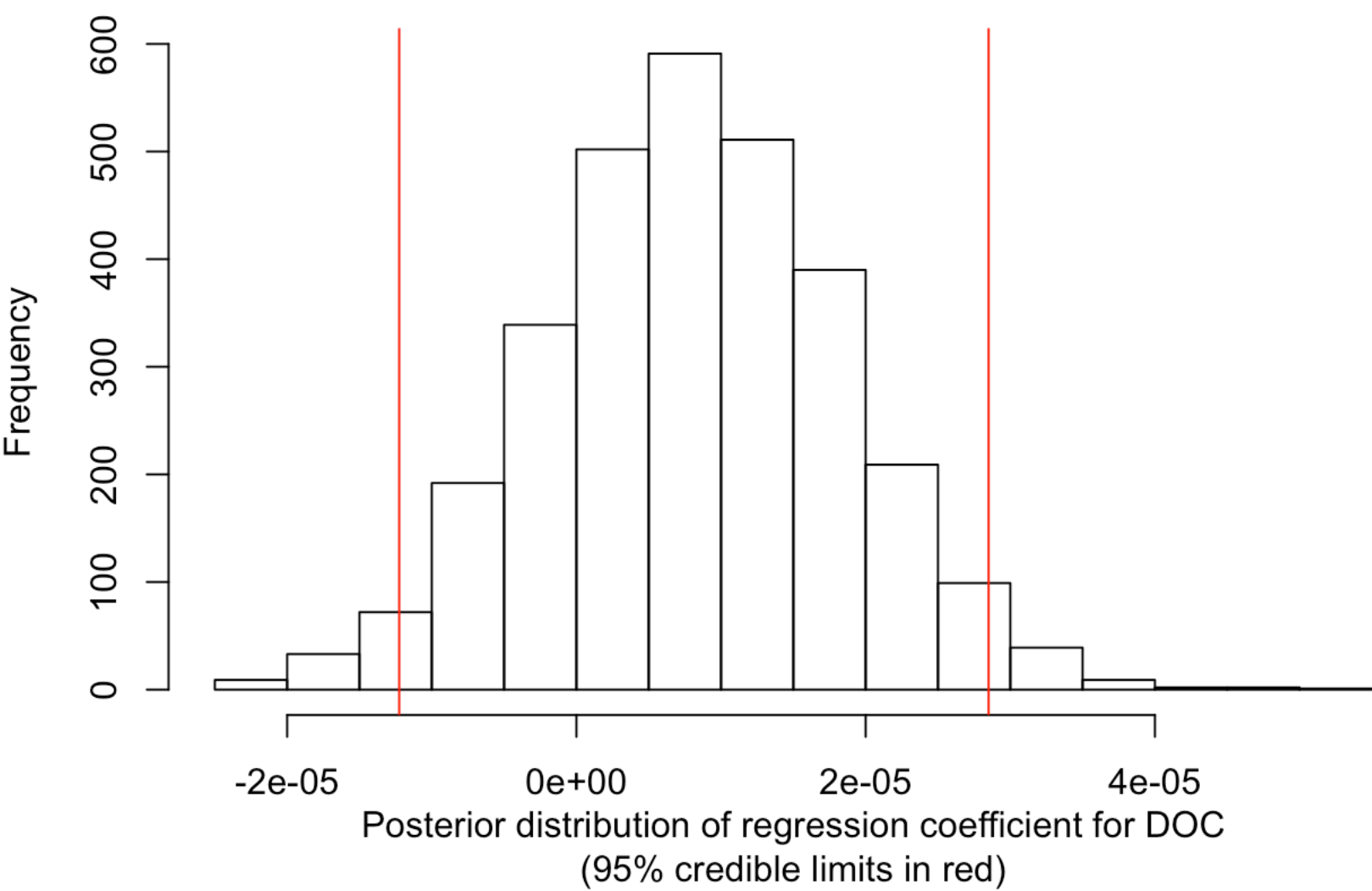


```
## DIC for Spp*WaterHg + Stage 229.169596061473
```
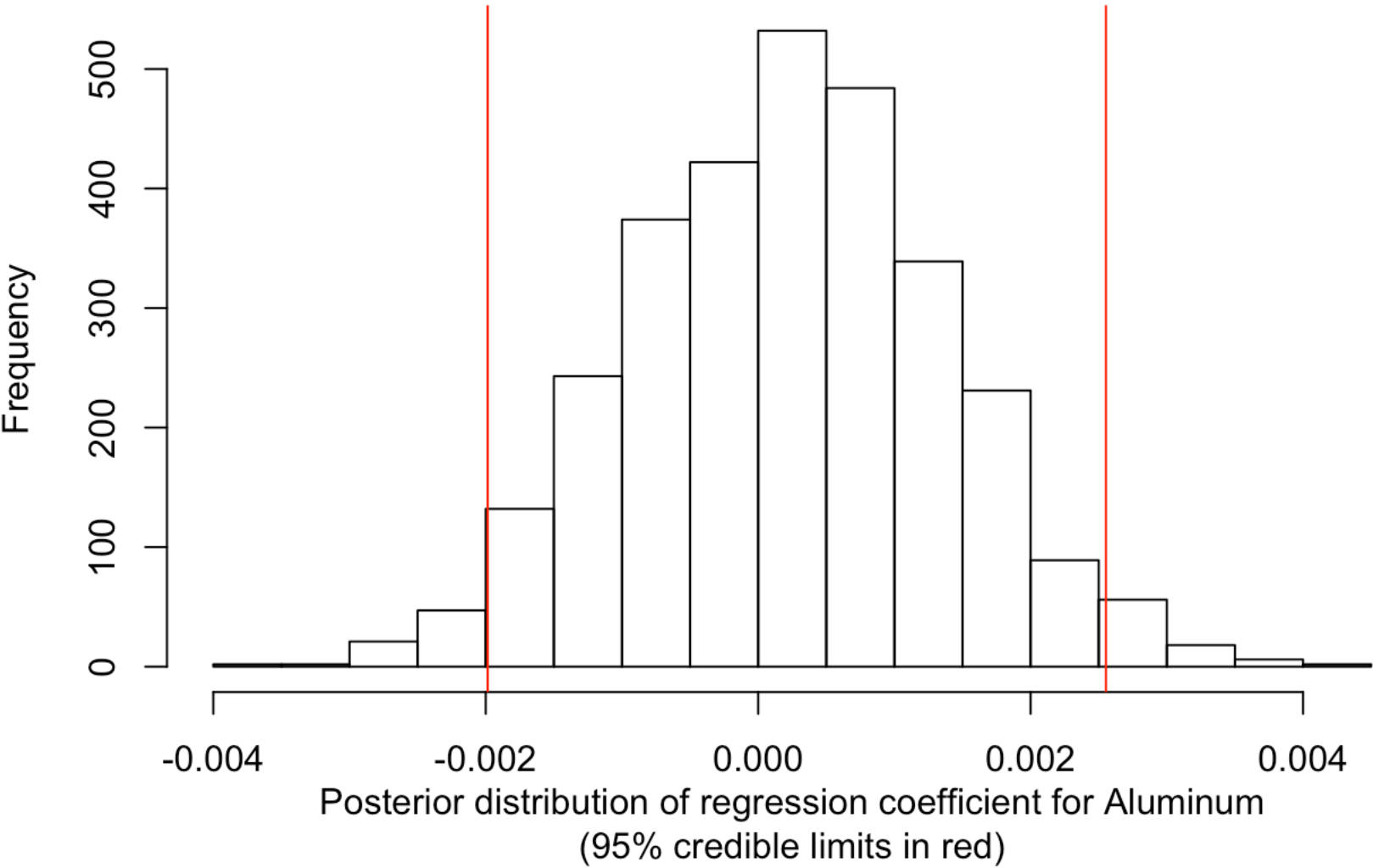
```
## DIC for Spp*WaterHg + Stage + Al: 230.203951249116
```

And DOC.

```
## Compiling model graph
##    Resolving undeclared variables
##    Allocating nodes
## Graph information:
##    Observed stochastic nodes: 111
##    Unobserved stochastic nodes: 120
##    Total graph size: 2028
##
## Initializing model
```


Posterior distribution of regression coefficient for DOC
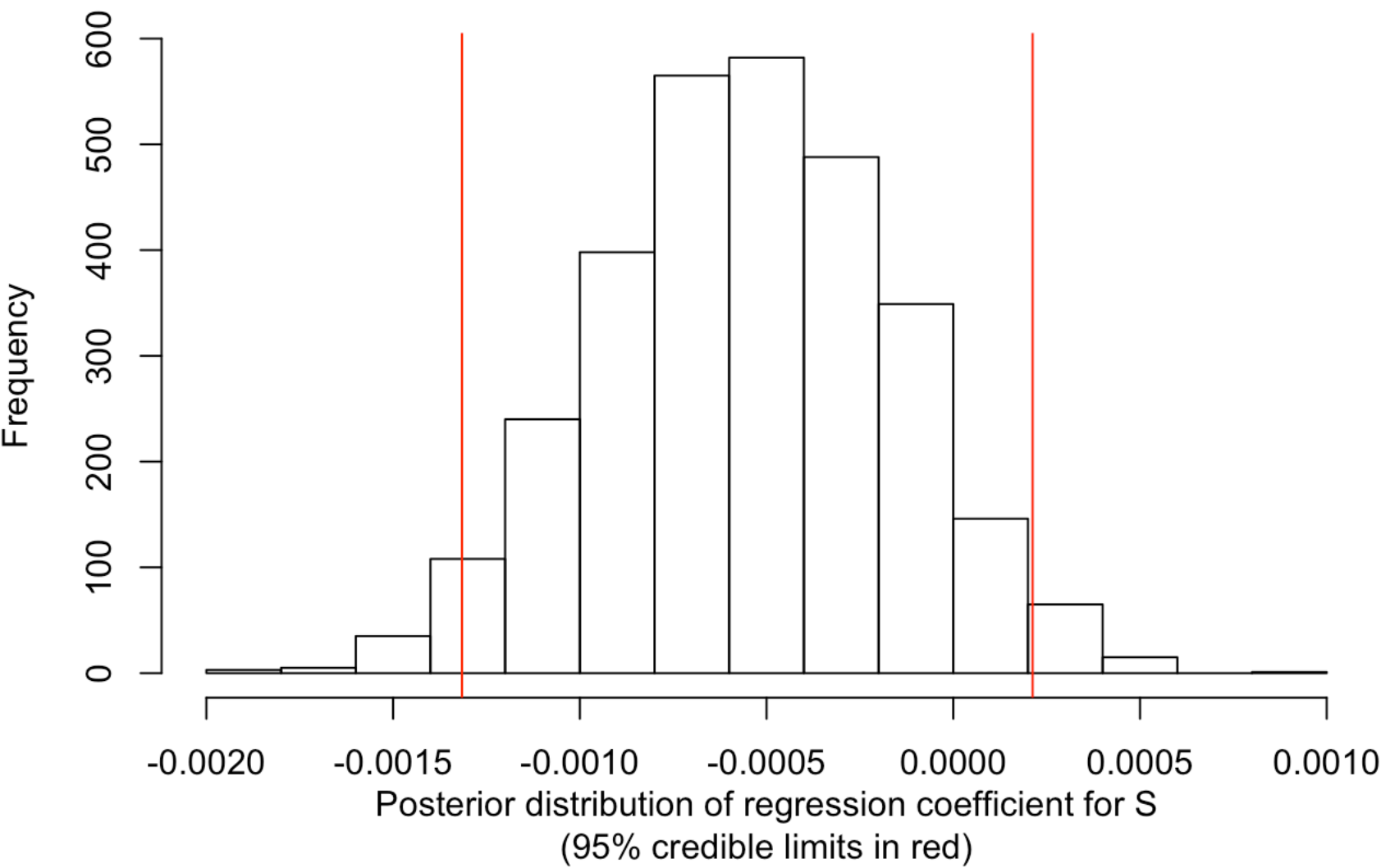(95% credible limits in red)

```
## DIC for Spp*WaterHg + Stage 229.169596061473
```

```
## DIC for Spp*WaterHg + Stage + DOC: 229.846446527041
```

And S.

```
## Compiling model graph
##     Resolving undeclared variables
##     Allocating nodes
## Graph information:
##     Observed stochastic nodes: 111
##     Unobserved stochastic nodes: 120
##     Total graph size: 2028
##
## Initializing model
```


Posterior distribution of regression coefficient for S
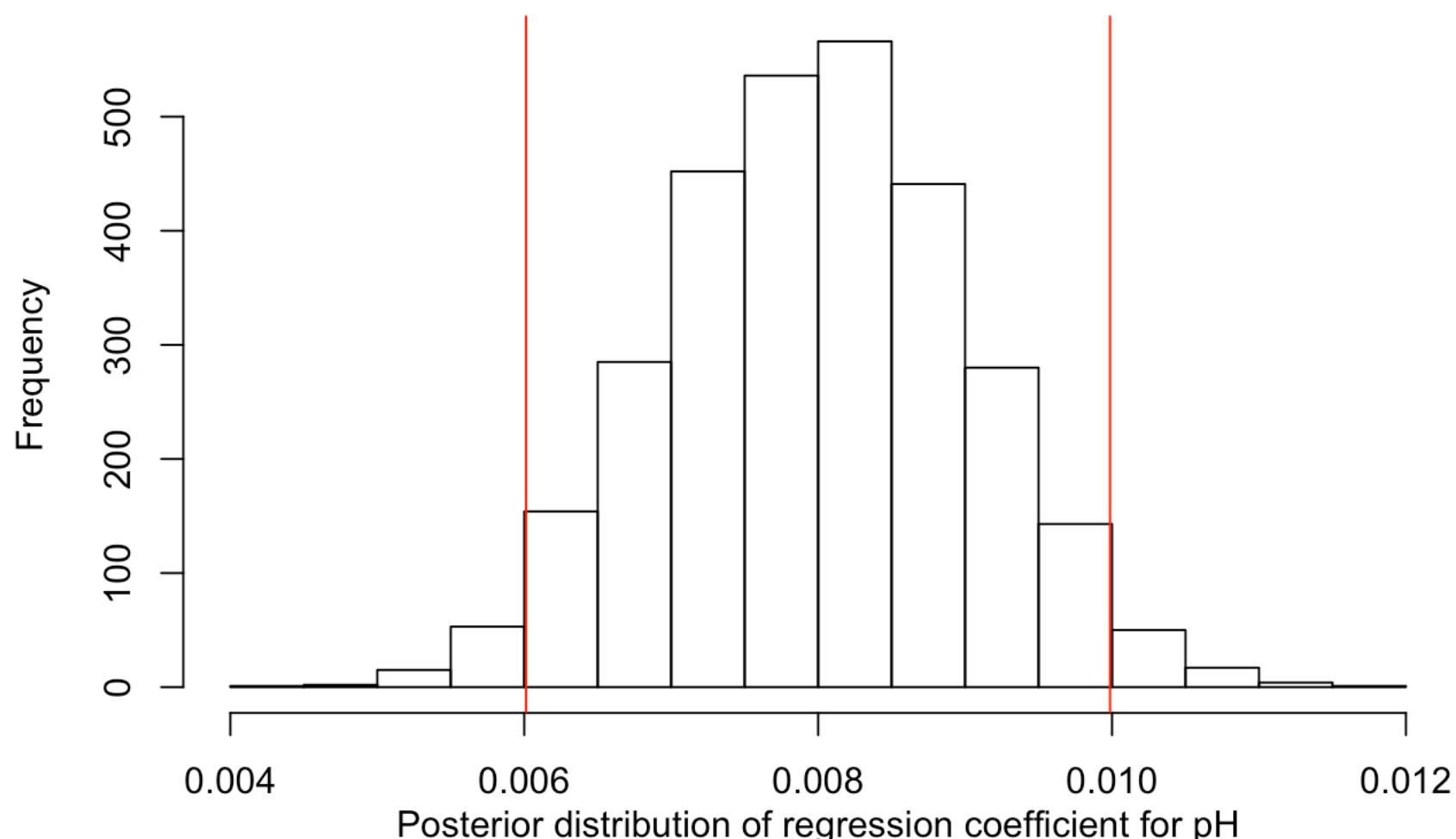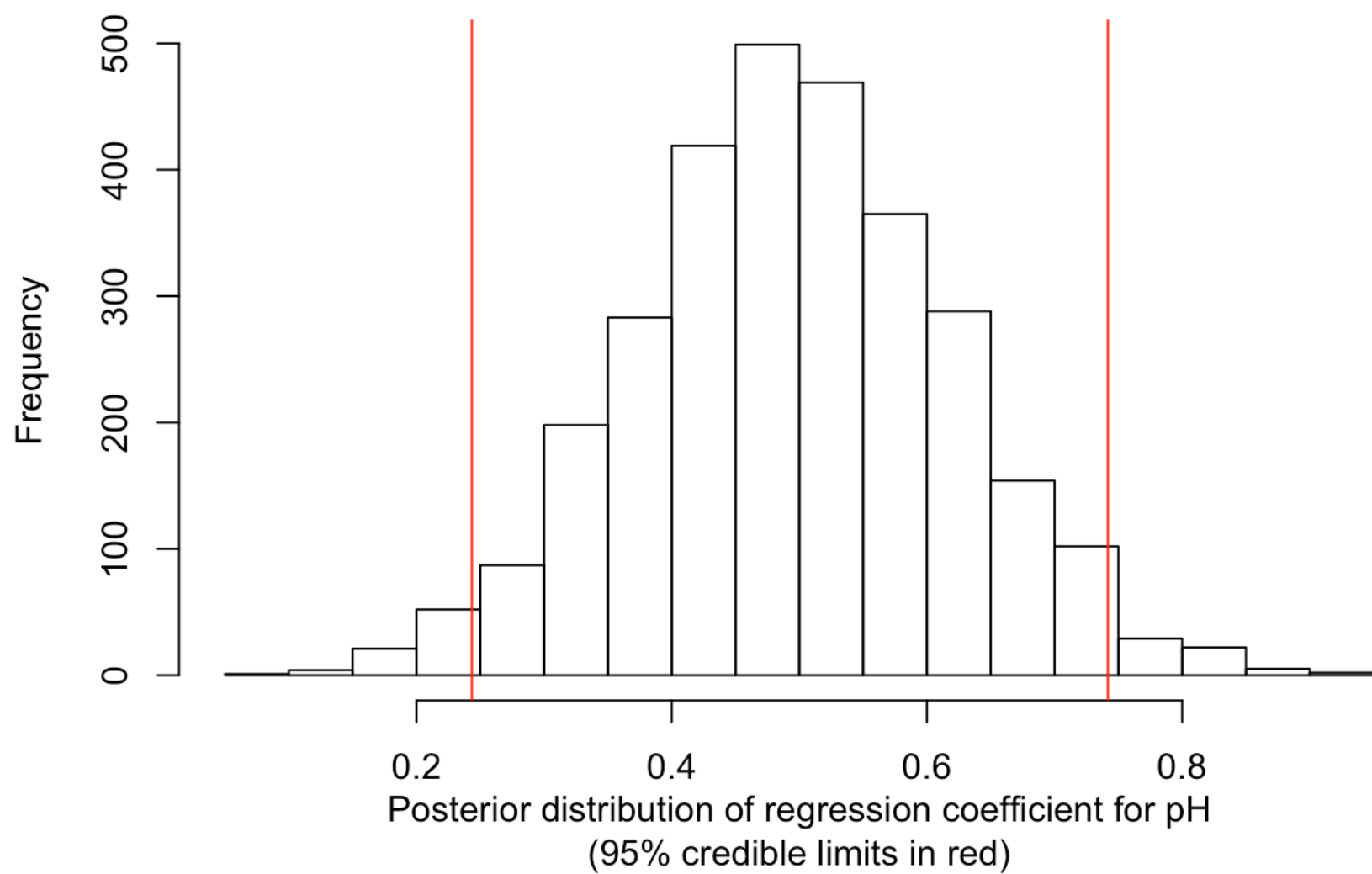(95% credible limits in red)

```
## DIC for Spp*WaterHg + Stage 229.169596061473
```

```
## DIC for Spp*WaterHg + Stage + S:  229.25133370525
```

Some of these variables do, however, show a relationship with water methylmercury. In particular, both water pH and water Aluminum are related to water methylmercury.

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 6
##      Total graph size: 661
##
## Initializing model
```

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 6
##      Total graph size: 661
```

Posterior distribution of regression coefficient for pH
(95% credible limits in red)

Posterior distribution of regression coefficient for pH

The predicted values suggest a positive relationship between methylmercury and pH, which is somewhat surprising. However, it appears that the relationship is driven by a single pool at Downer (observed points are shown as circles in these figures).

```
## Compiling model graph
##      Resolving undeclared variables
##      Allocating nodes
## Graph information:
##      Observed stochastic nodes: 111
##      Unobserved stochastic nodes: 4
##      Total graph size: 1275
##
## Initializing model
```

The relationship between water pH and water methylmercury (points are observed values, solid line is predicted relationship, and dashed lines are 95% credible limits):
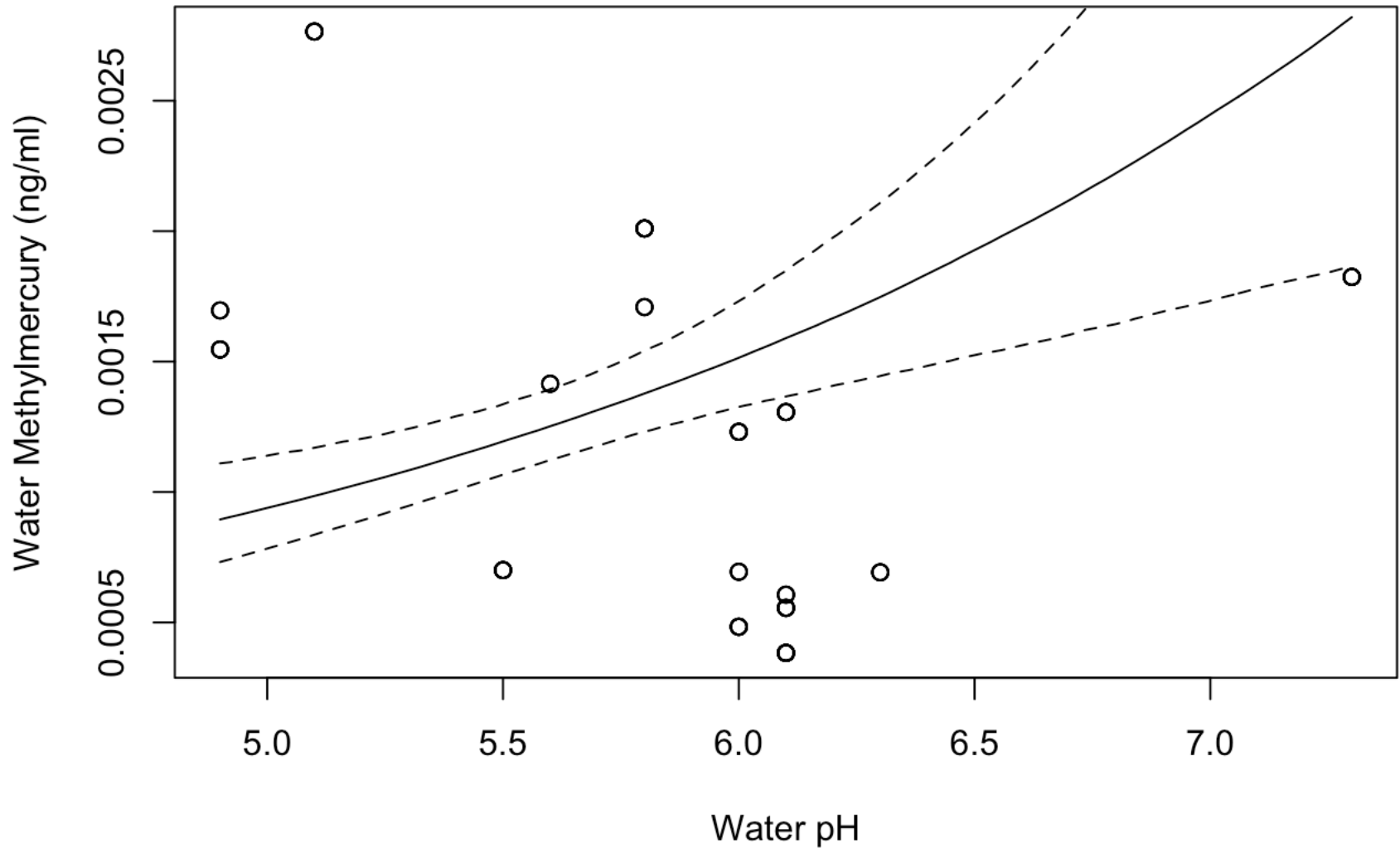
The relationship between water aluminum and water methylmercury (points are observed values, solid line is predicted relationship, and dashed lines are 95% credible limits):