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
```

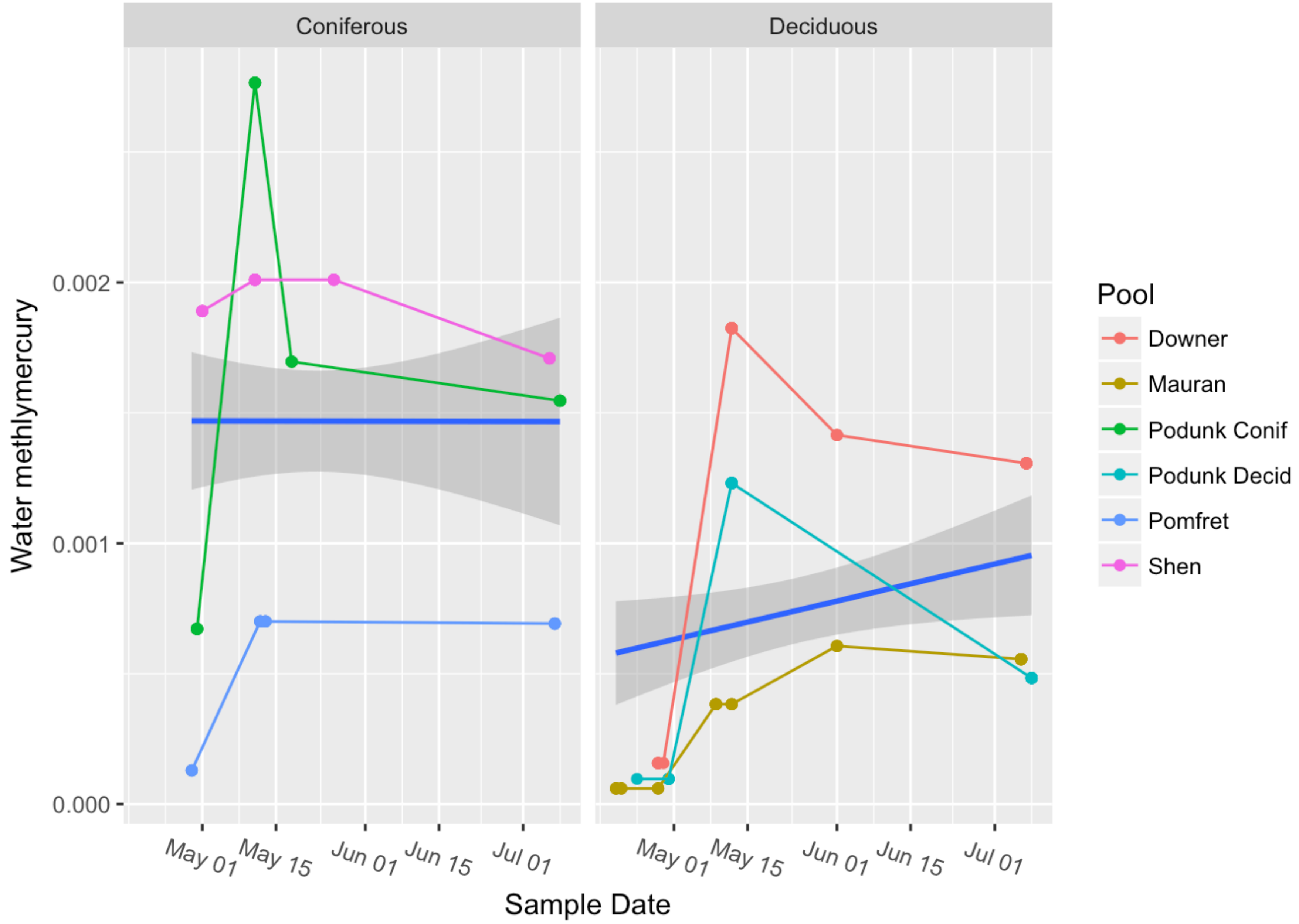
There are 6 pools, 3 of which are surrounded by deciduous forest (Downer, Mauran, and Podunk Decid) and 3 of which are surrounded by coniferous forest (Podunk Conif, Pomfret, and Shen). The number of samples differs by date and by pool because sample number is determined by the number of amphibians collected.

```
##                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
```

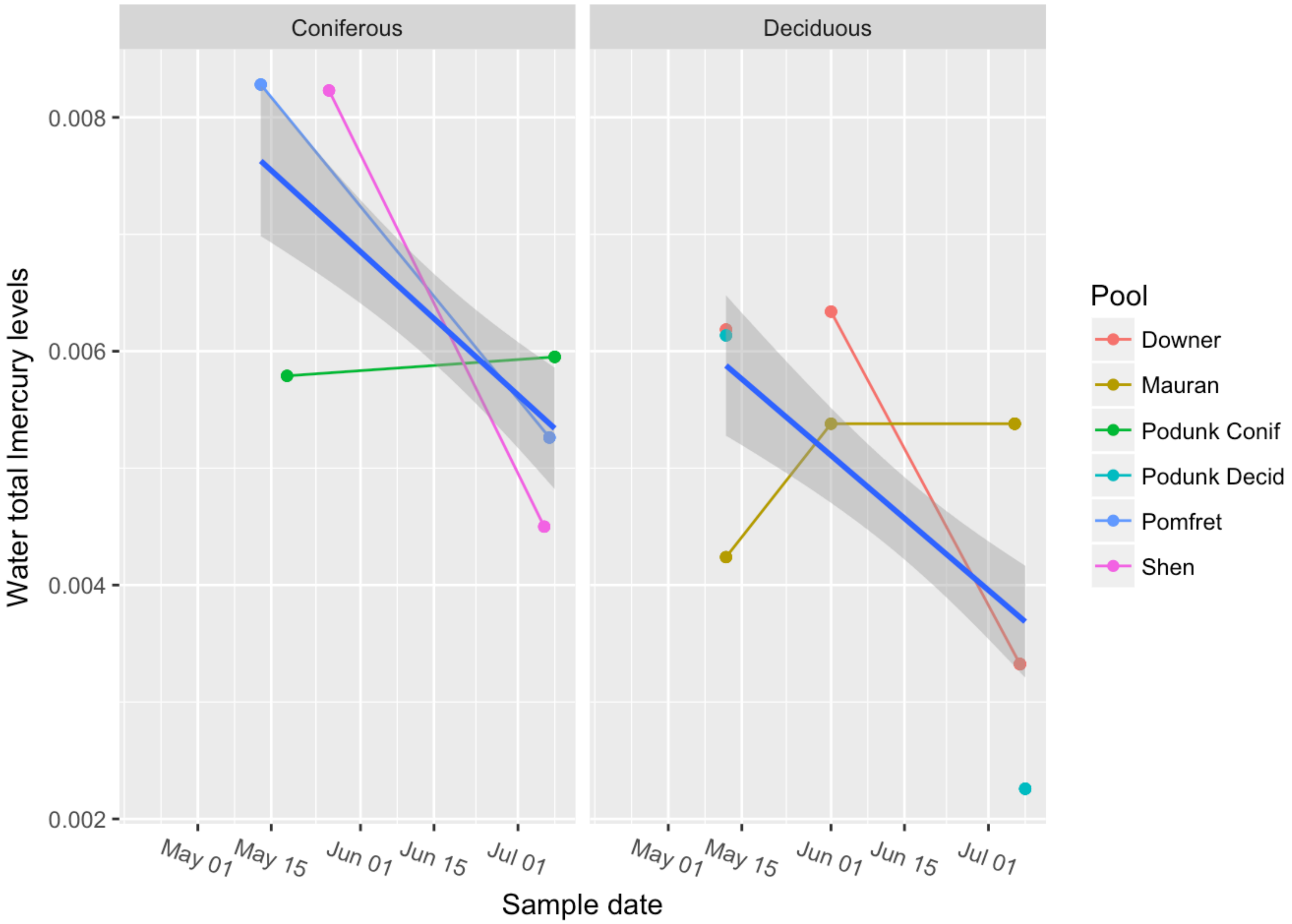
Exploratory analysis

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 (-0.00015, 0.00132).

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

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

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

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

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

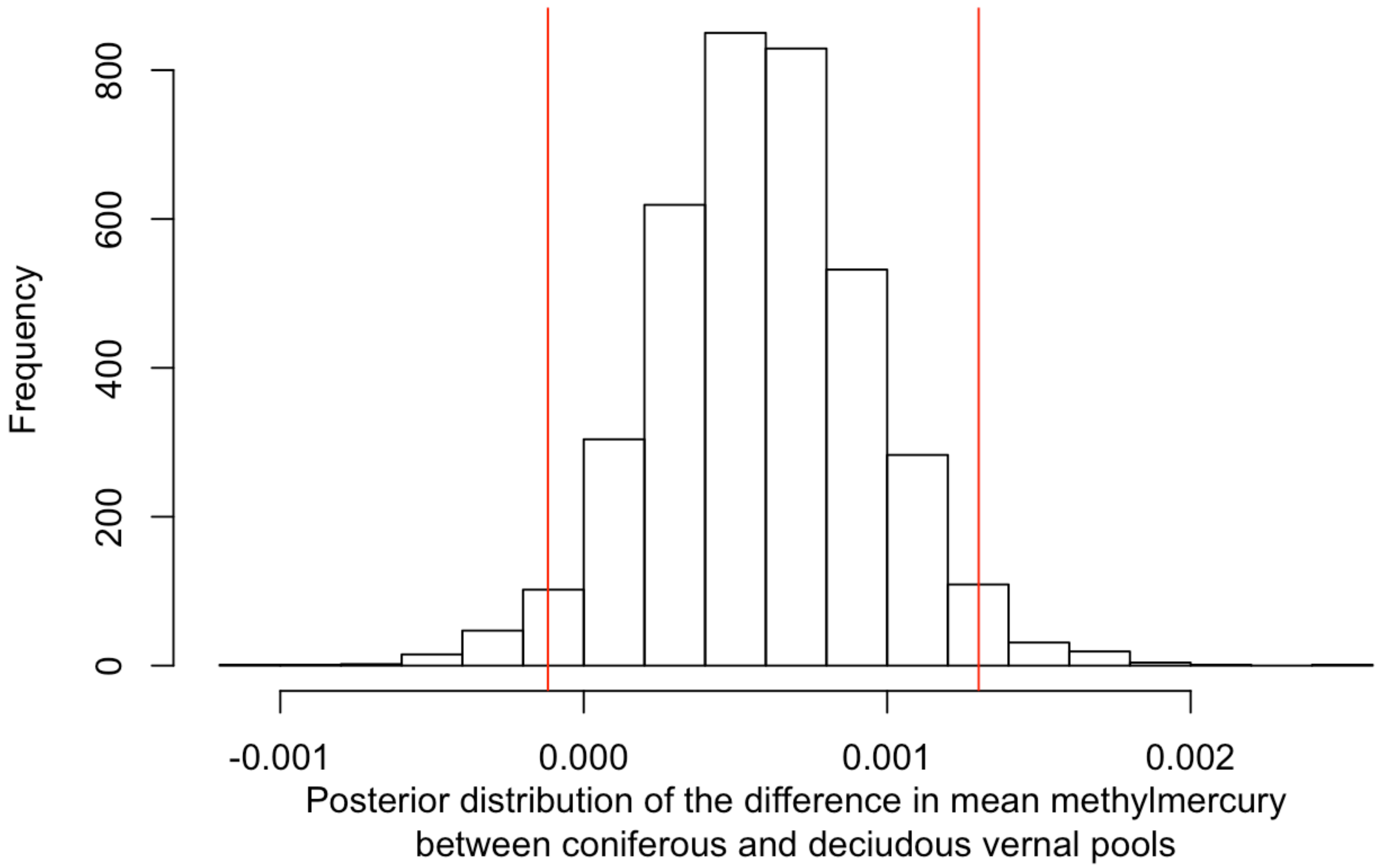
```
## The following object is masked from 'package:coda':
##
##     traceplot
```

```
## 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
pKfFp9k/model153416f7f16ed.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       0.00059 0.00036   -0.00012    0.00036    0.00059    0.00081
## mu1         0.00073 0.00022    0.00031    0.00060    0.00074    0.00087
## mu2         0.00132 0.00029    0.00073    0.00115    0.00132    0.00150
## sigma1      0.00070 0.00018    0.00044    0.00057    0.00066    0.00078
## sigma2      0.00091 0.00025    0.00057    0.00074    0.00086    0.00103
## deviance -254.92619 3.53702 -259.31288 -257.50657 -255.73502 -253.24879
##               97.5%    Rhat n.eff
## delta       0.00130 1.00111  3800
## mu1         0.00116 1.00093  3800
## mu2         0.00190 1.00317  3800
## sigma1      0.00113 1.00393  1200
## sigma2      0.00155 1.00117  3500
## deviance -245.70931 1.00177  1700
##
## 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.

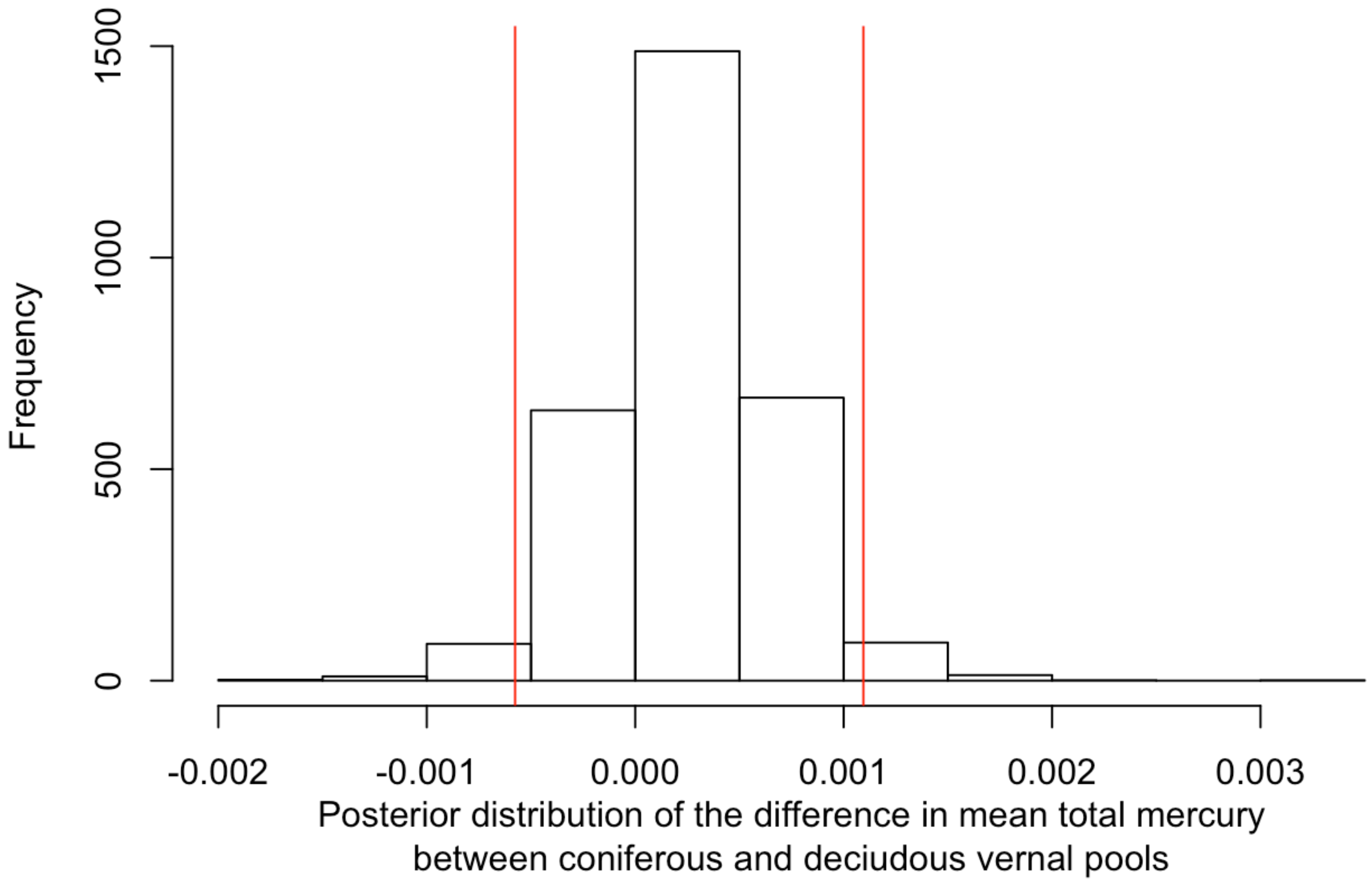


We can conclude that coniferous pools tend to have higher levels of methylmercury, but that the difference is not significant.

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 = -0.00053 - 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
pKfFp9k/model153417dcbfa59.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       0.00026 0.00041   -0.00058    0.00000    0.00026    0.00051
## mu1         0.00103 0.00027    0.00049    0.00087    0.00104    0.00120
## mu2         0.00129 0.00031    0.00070    0.00112    0.00130    0.00147
## sigma1      0.00070 0.00028    0.00037    0.00052    0.00064    0.00081
## sigma2      0.00075 0.00031    0.00039    0.00054    0.00068    0.00086
## deviance -166.46625 3.88193 -171.39816 -169.31674 -167.34901 -164.46467
##               97.5%    Rhat n.eff
## delta       0.00109 1.00057  3000
## mu1         0.00156 1.00086  3000
## mu2         0.00191 1.00074  3000
## sigma1      0.00140 1.00150  2900
## sigma2      0.00152 1.00096  3000
## deviance -156.55740 1.00213  1300
##
## 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 = 7.5 and DIC = -158.9
## DIC is an estimate of expected predictive error (lower deviance is better).
```



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.

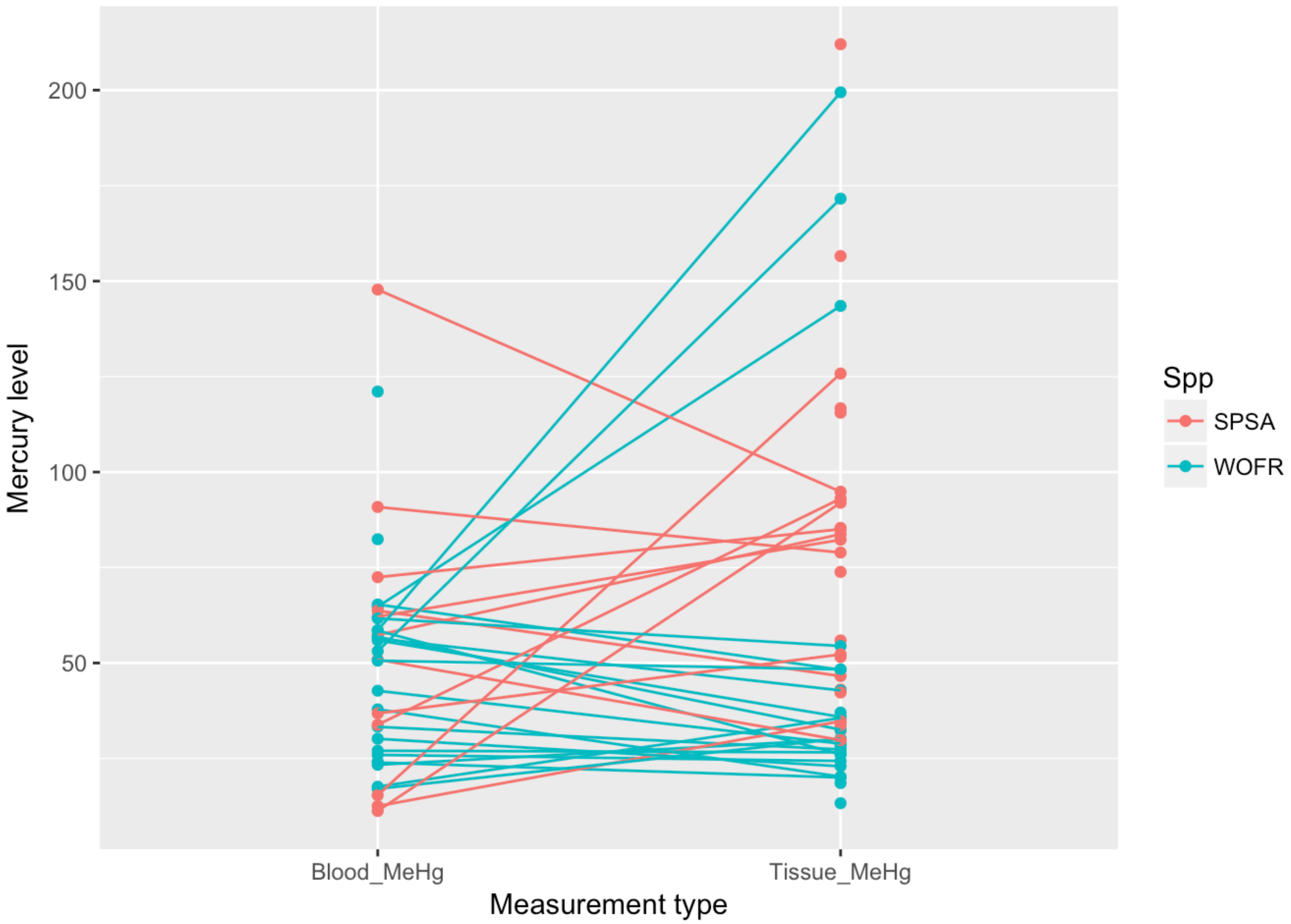
```
summary(data.df$Blood_MeHg[data.df$Life_Stage=="Adult"])
```

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

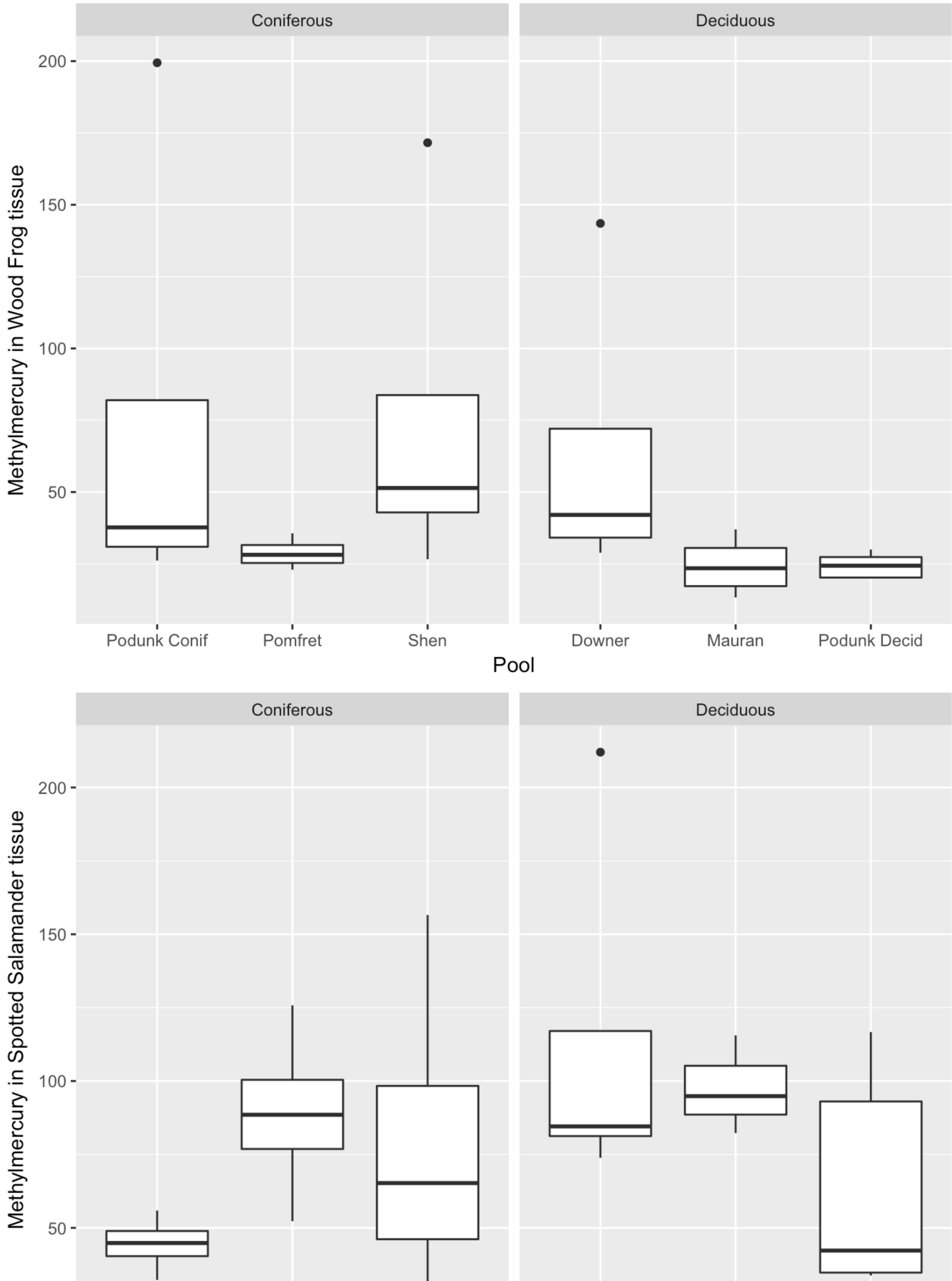
```
summary(data.df$Tissue_MeHg[data.df$Life_Stage=="Adult"])
```

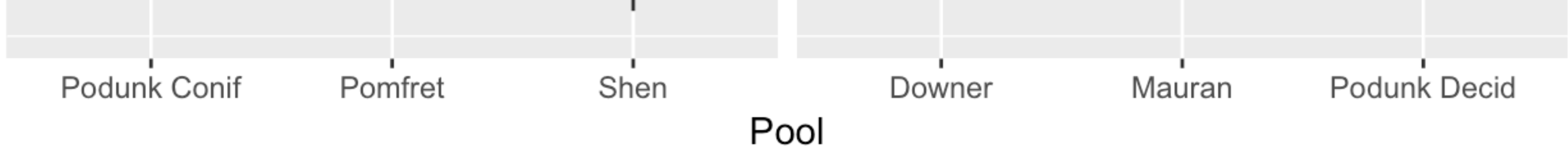
```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's 
##   13.30   29.82   43.07   63.49   85.07  212.03       3
```

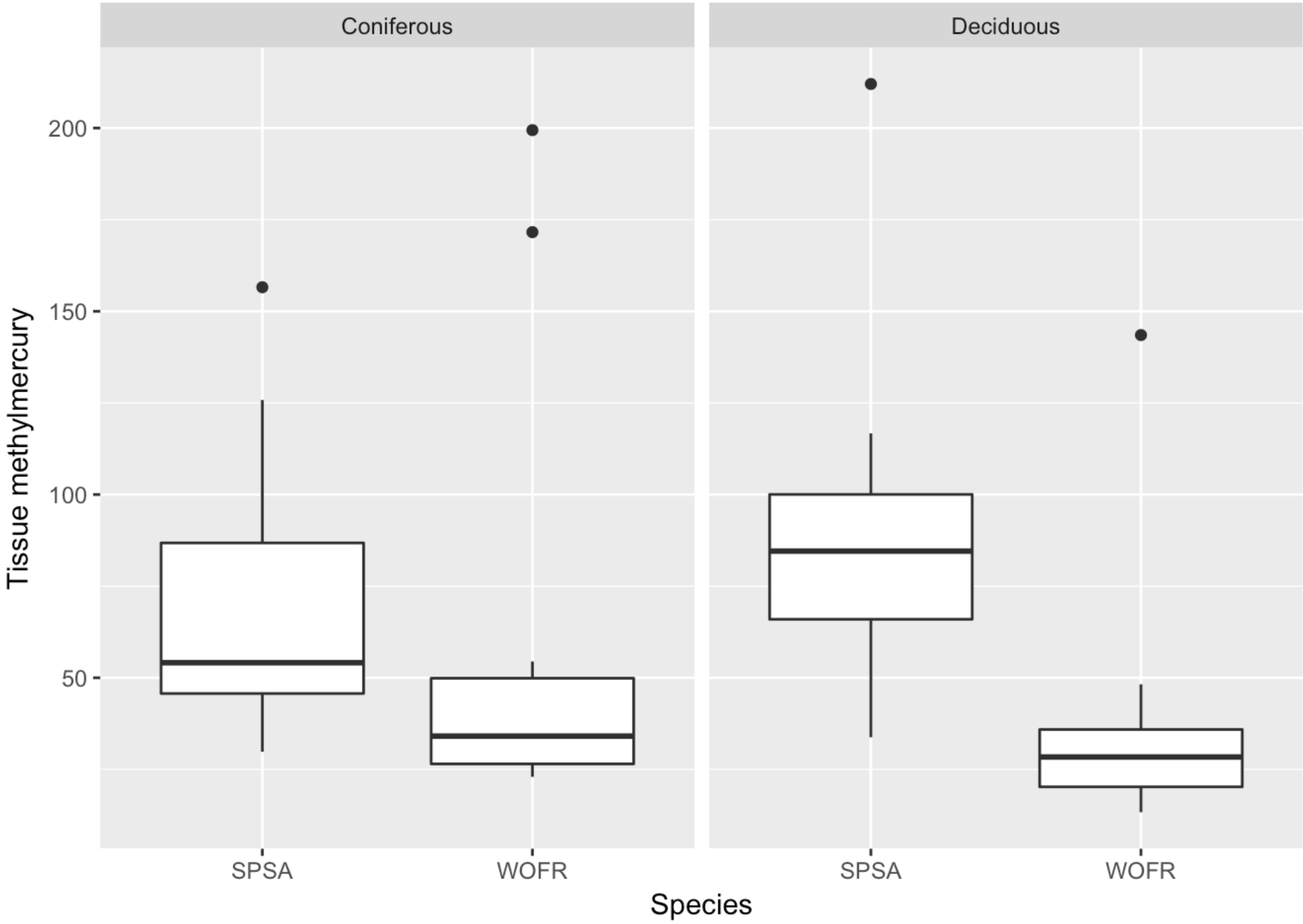
In addition, mercury levels measured in blood and tissue taken from the same individual show little correspondence and are not interchangeable. In this figure, mercury levels estimated from the same individual are connected by a line. Notice that tissue tends to be produce higher estimates of mercury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



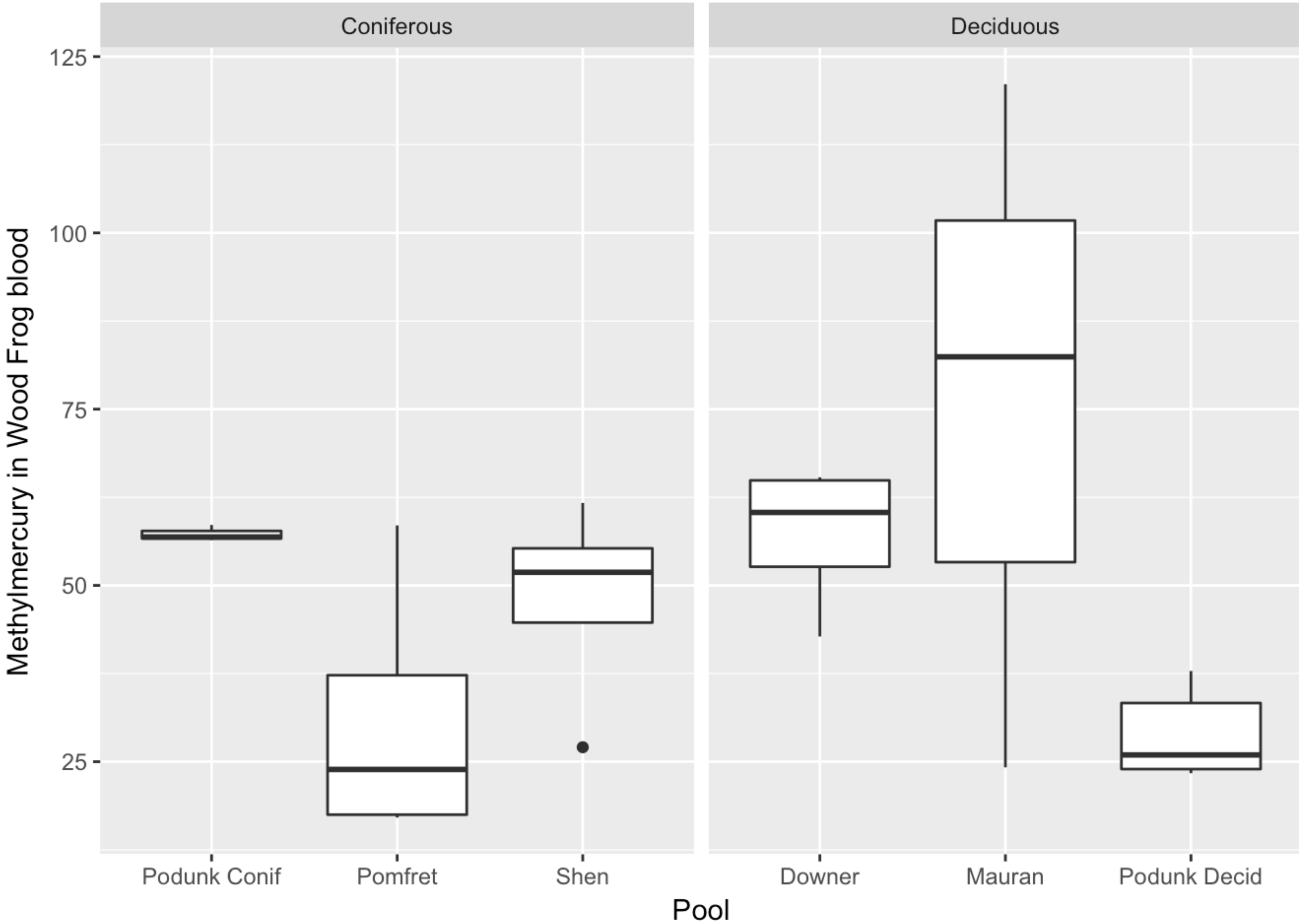
Patterns of tissue methylmercury vary among pools and species.

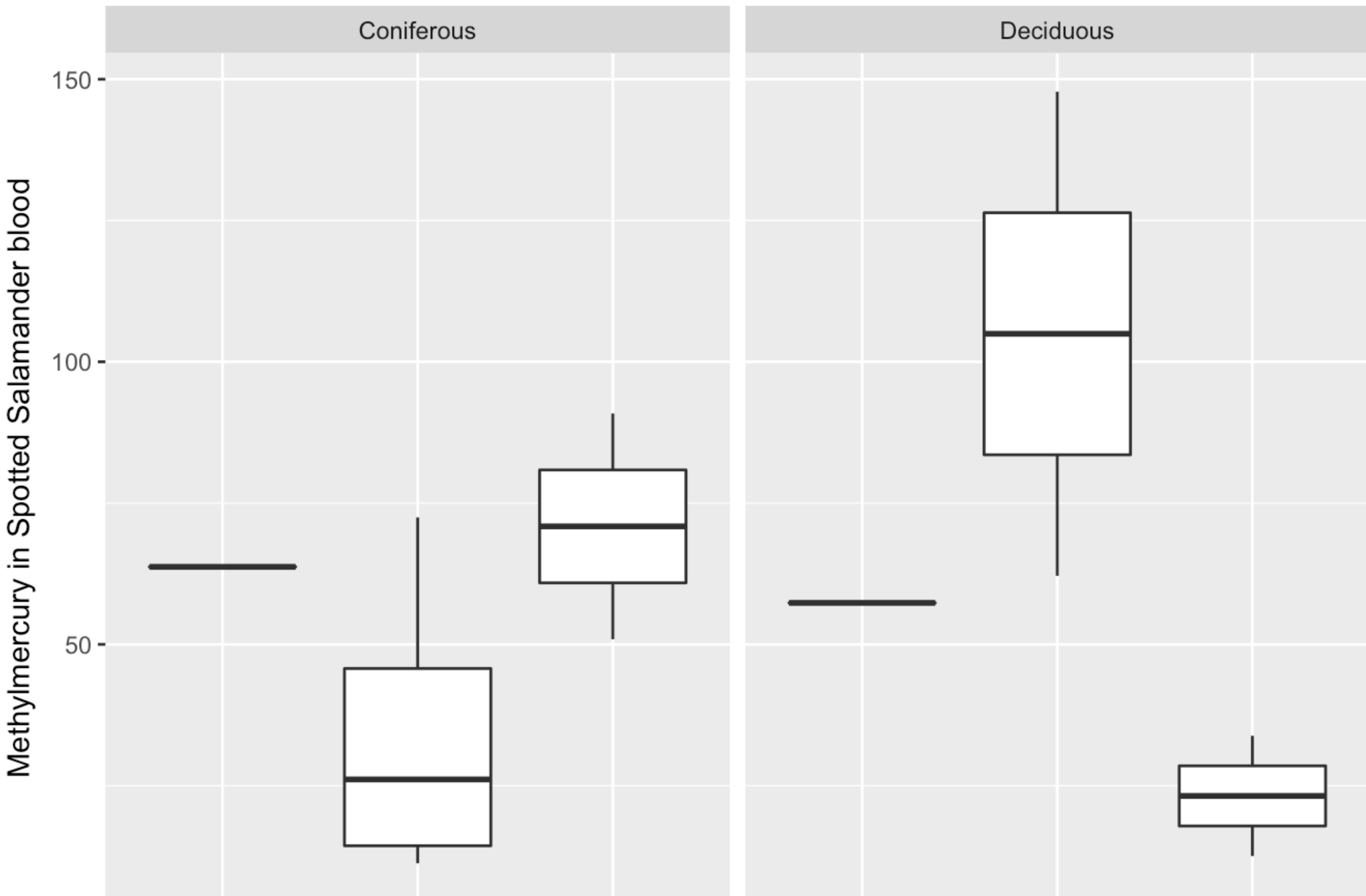


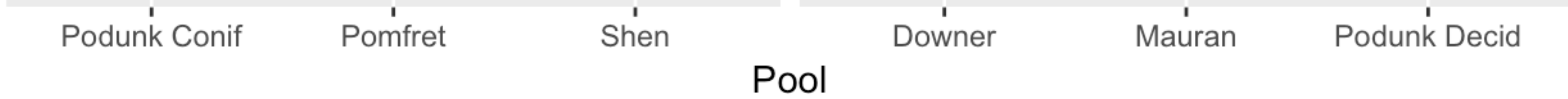


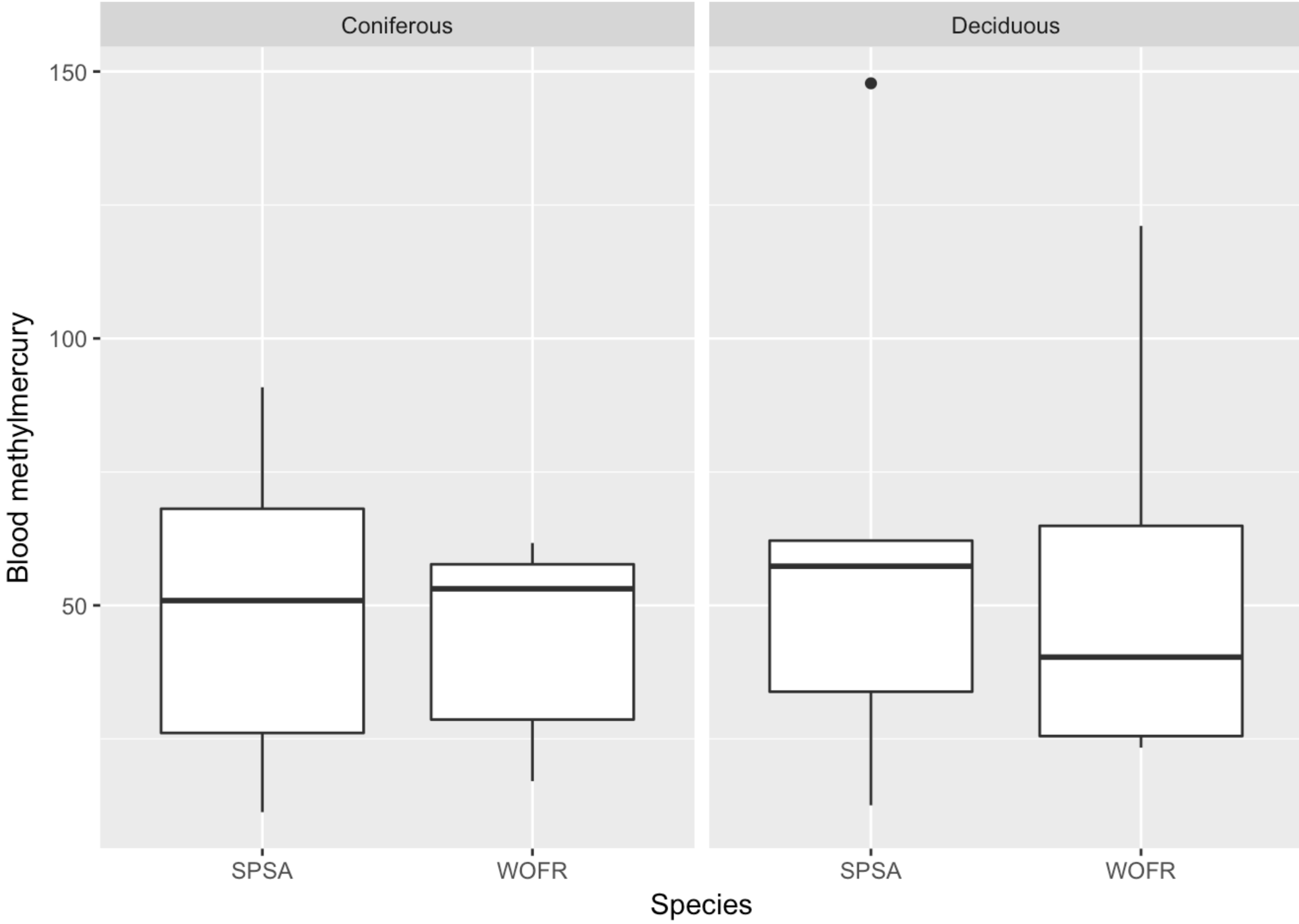


If we look at blood levels of methylmercury in adults:

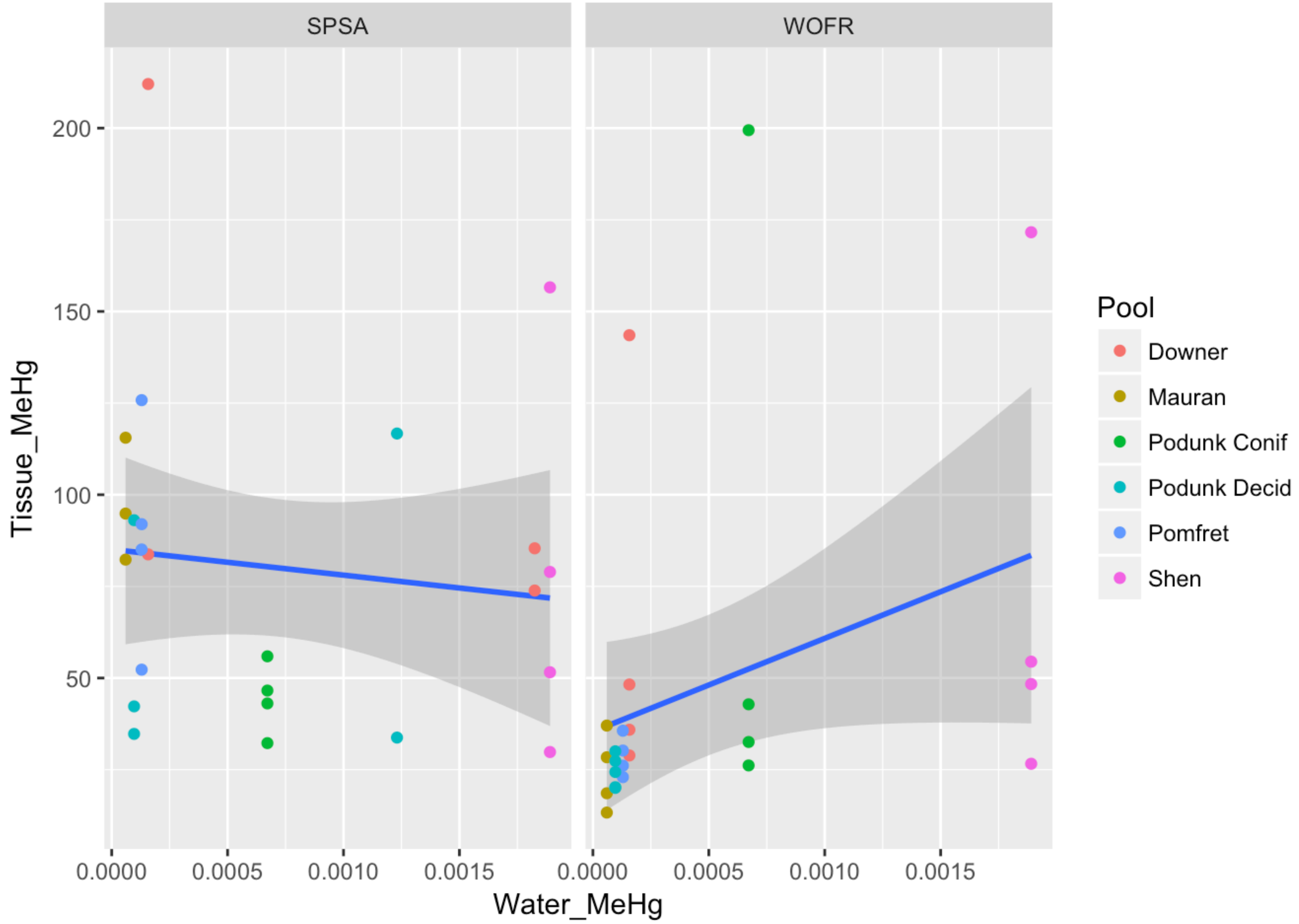

Coniferous
Deciduous
125
100
75
50
25
Methylmercury in Wood Frog blood
Podunk Conif
Pomfret
Shen
Downer
Mauran
Podunk Decid
Pool


Coniferous
Deciduous
150
100
50
Methylmercury in Spotted Salamander blood





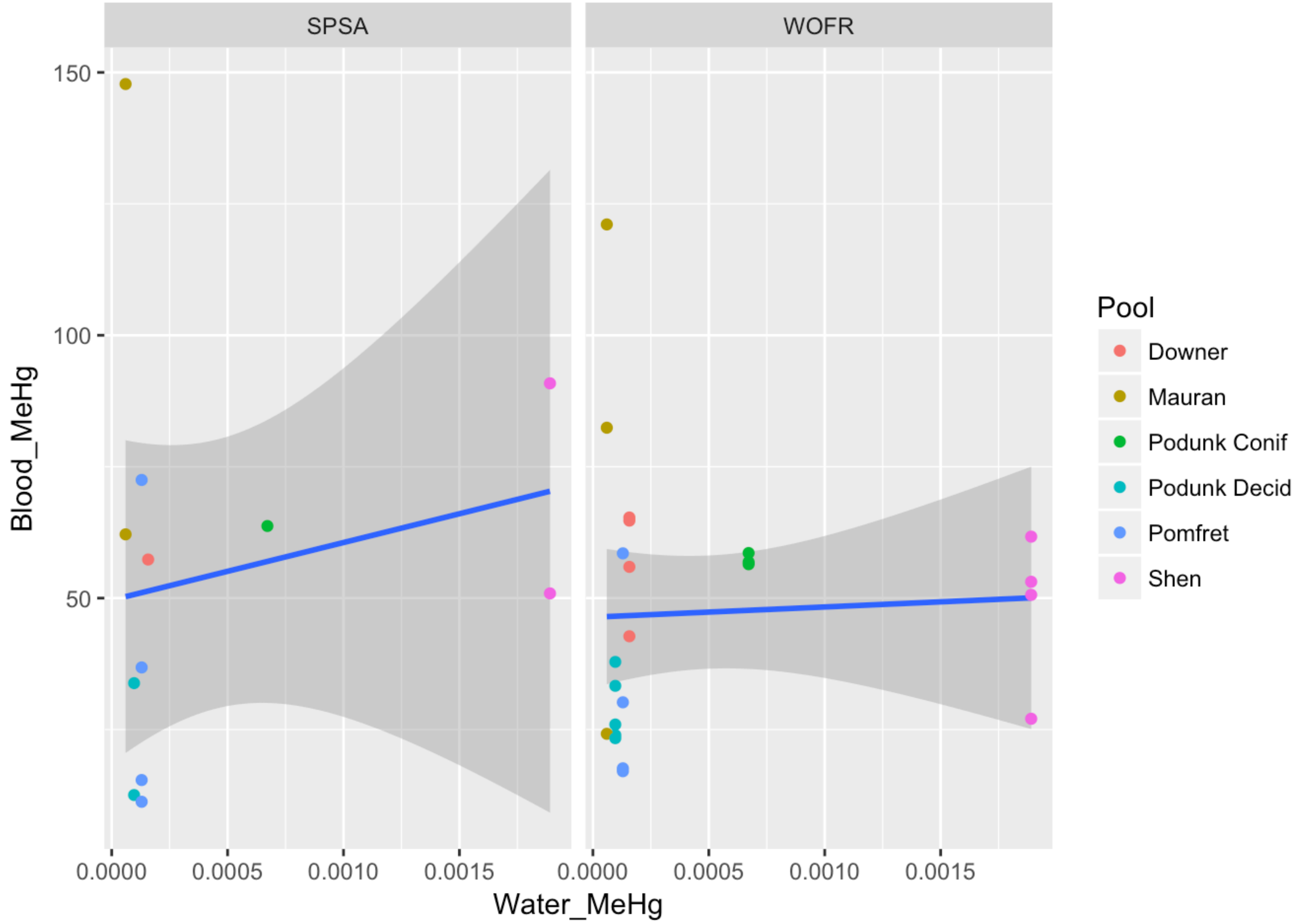
Neither species shows a strong relationship between levels of methylmercury in tissue and levels of methylmercury in the water at the time of the sample.



We see the same relationship if we look at blood methylmercury:

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

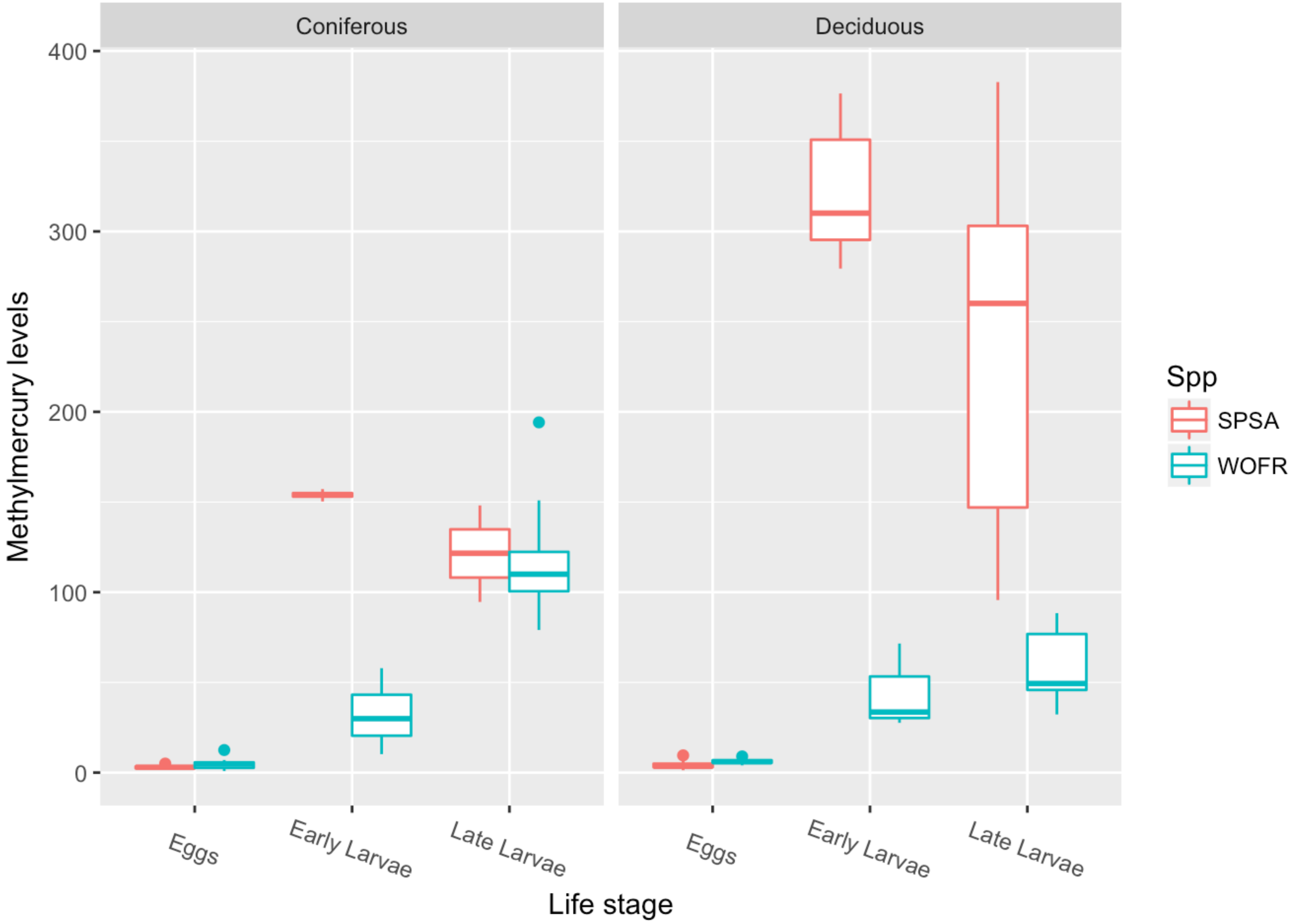
```
## Warning: Removed 17 rows containing missing values (geom_point).
```



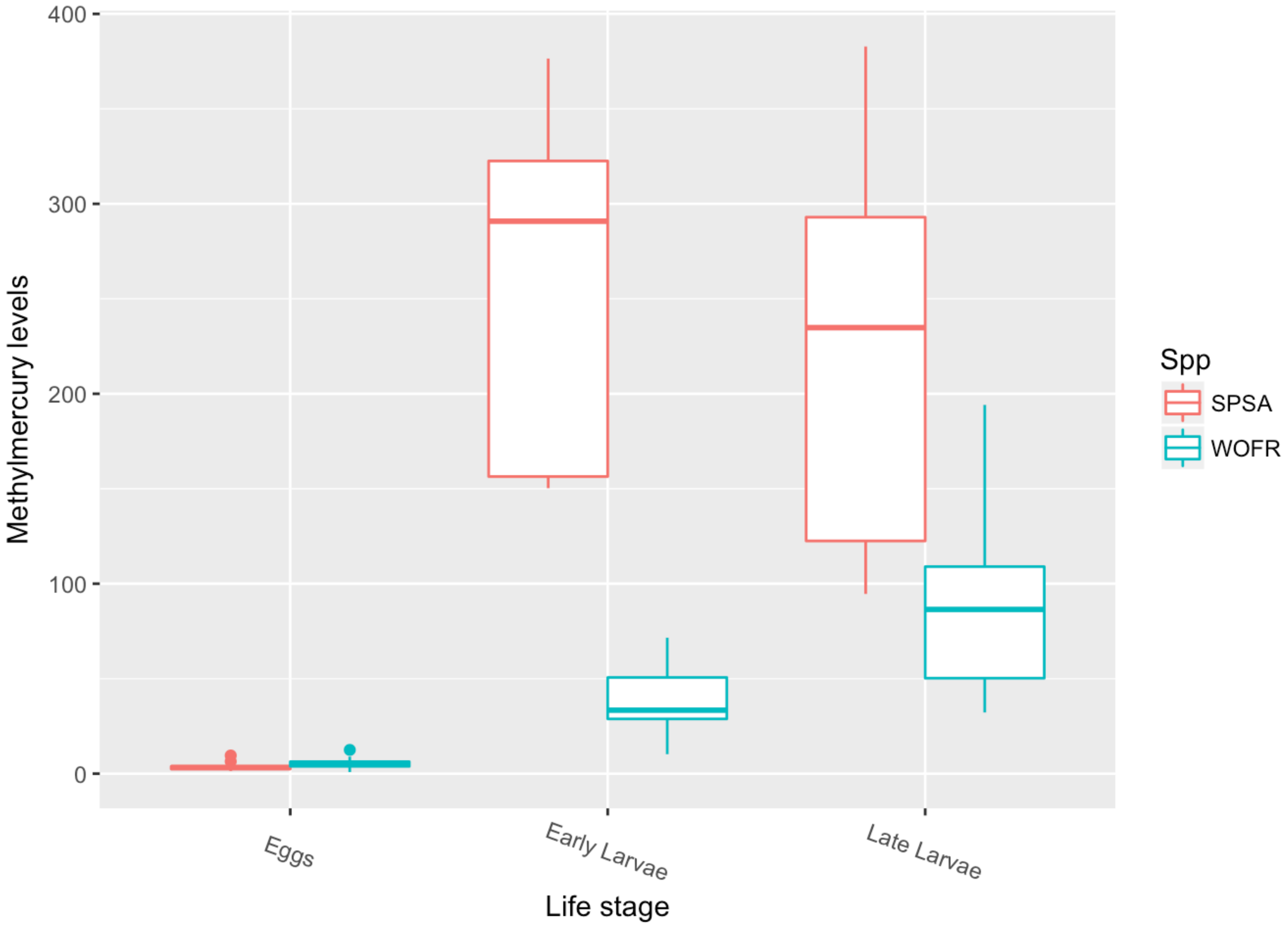
Larval stages

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).
```

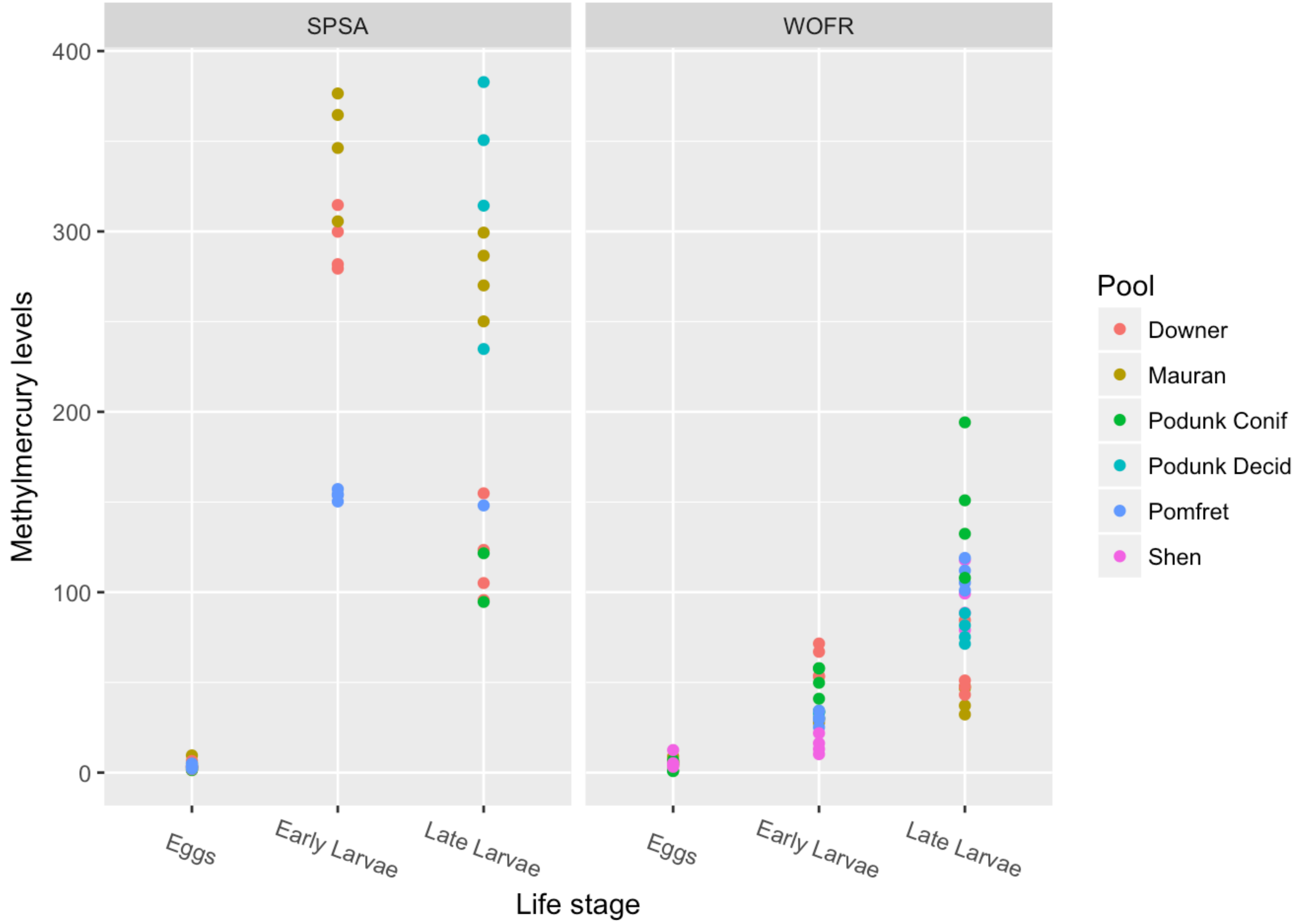



```
p7 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p7 + geom_boxplot(aes(x = Life_Stage, y = Amphib_MeHg, color = Spp)) +
  scale_x_discrete(limits = c("Eggs","Early Larvae","Late Larvae")) +
  theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
  xlab("Life stage") + ylab("Methylmercury levels")
```

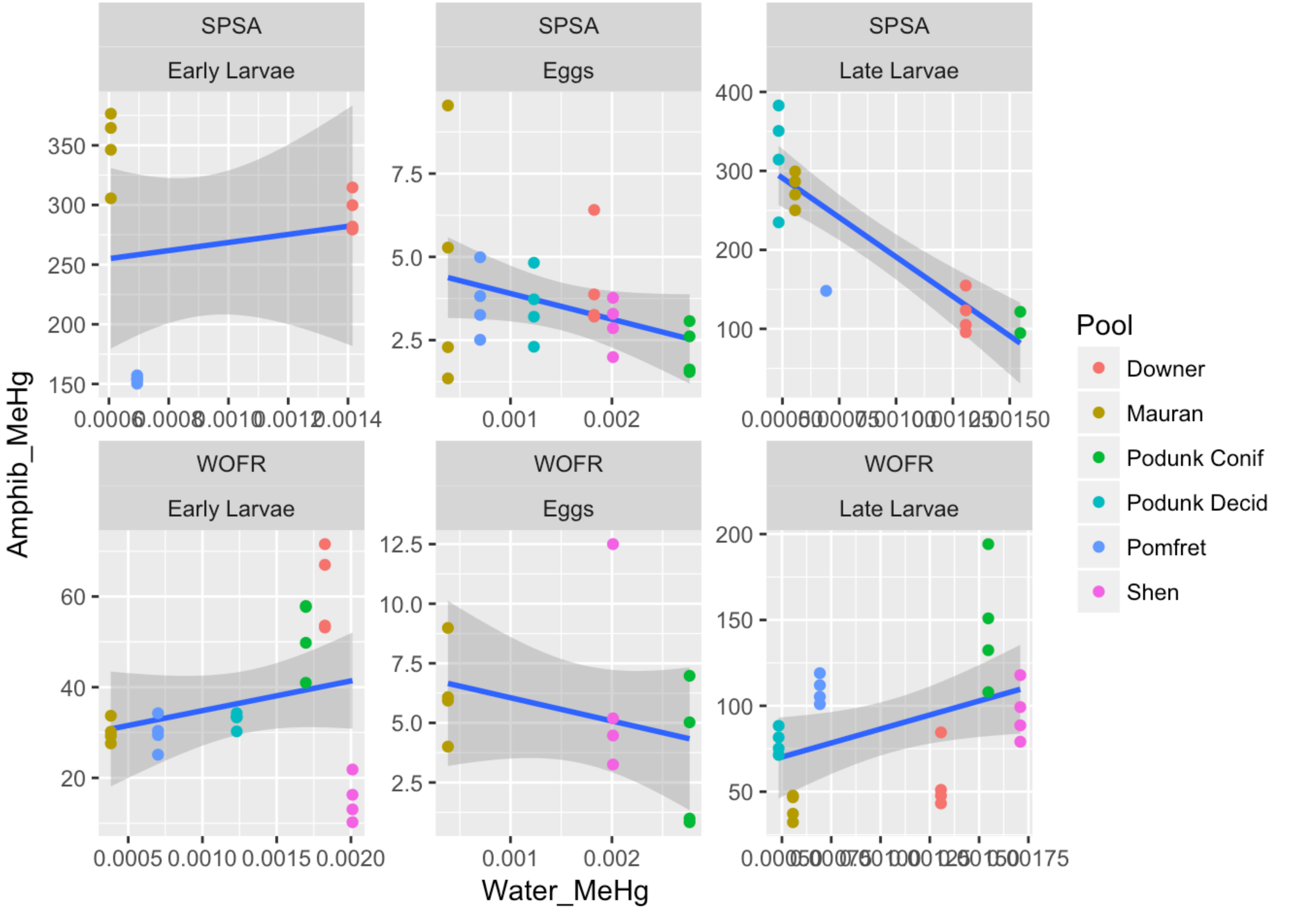


The odd pattern of higher mercury in early-stage larvae for SPSA doesn't appear to be strictly a pool effect. For example, the only two pools with early larvae sampled - Downer and Mauran - also show a decreased level of mercury in late-stage larvae.

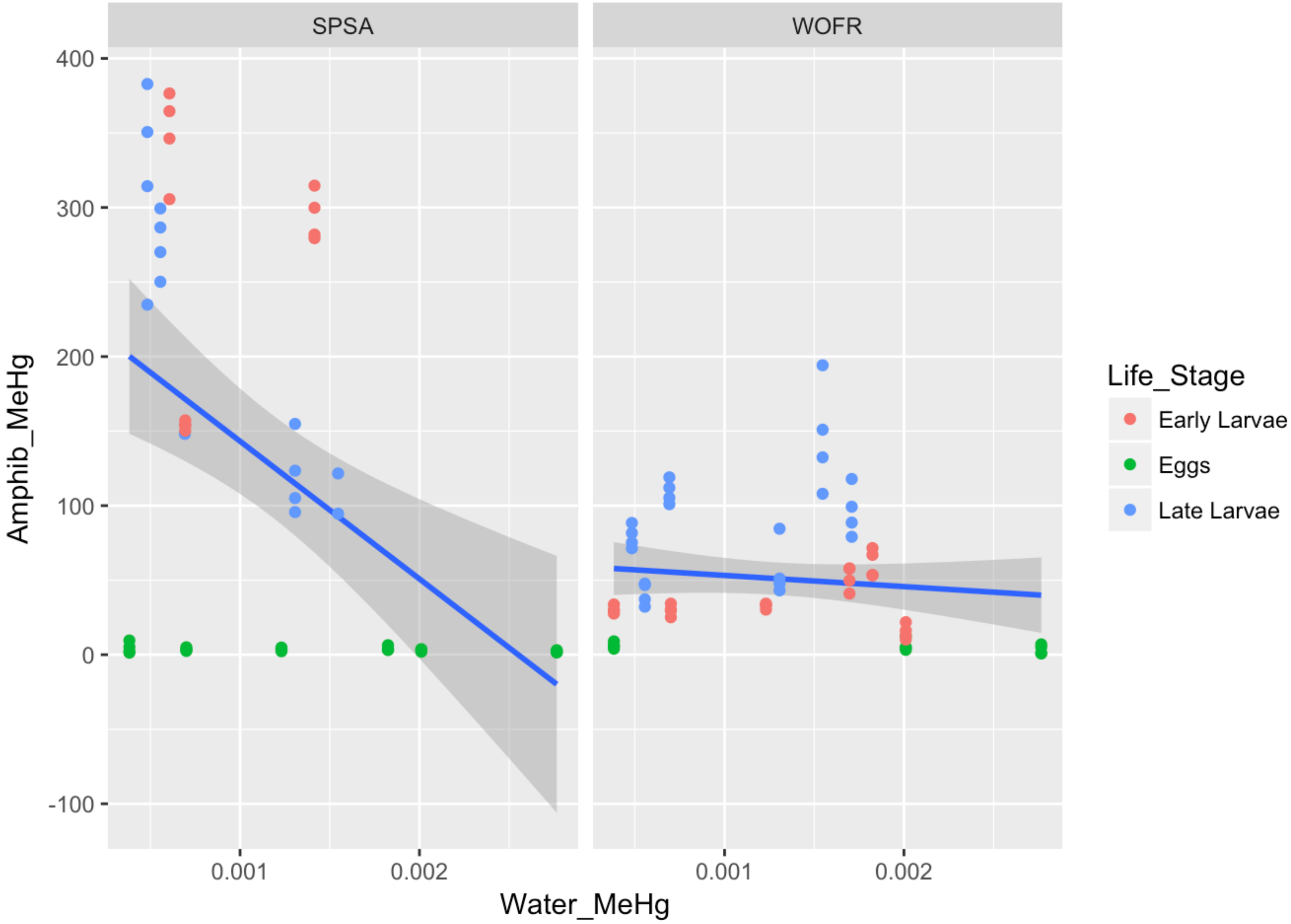
```
p8 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p8 + geom_point(aes(x = Life_Stage, y = Amphib_MeHg, color = Pool)) +
  scale_x_discrete(limits = c("Eggs","Early Larvae","Late Larvae")) +
  theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
  xlab("Life stage") + ylab("Methylmercury 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
pKfFp9k/model1534113228199.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]  -6.88336 2.69438 -12.16724  -8.74432  -6.83990  -5.02849
## alpha[2]   1.20740 2.30236  -3.36076  -0.37877   1.17836   2.72465
## beta[1]   -1.48443 0.38696  -2.24887  -1.75180  -1.47606  -1.21613
## beta[2]   -0.32630 0.33549  -0.98724  -0.55539  -0.33009  -0.09753
## sigma      1.63921 0.11371   1.43505   1.56080   1.63553   1.71047
## deviance 423.14091 3.18978 418.86270 420.77391 422.50135 424.84530
##              97.5%    Rhat n.eff
## alpha[1]  -1.73854 1.00082  3000
## alpha[2]   5.85676 1.00275   890
## beta[1]   -0.74099 1.00087  3000
## beta[2]    0.35158 1.00288   840
## sigma      1.87835 1.00057  3000
## deviance 430.85823 1.00141  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 = 5.1 and DIC = 428.2
## 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.

```
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
```

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

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
pKfFp9k/model153413d4301bc.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]        0.33624 14.16758 -27.10085  -9.21360   0.07701   9.89848
## alpha[2]        3.05161 14.18830 -24.71716  -6.49242   3.07908  12.91070
## beta.spp[1]    -0.48710  0.16269  -0.80238  -0.59631  -0.49058  -0.37924
## beta.spp[2]     0.02674  0.13369  -0.24141  -0.06110   0.02683   0.11768
## beta.stage[1]   1.00369 14.16607 -26.65714  -8.71731   1.02995  10.65111
## beta.stage[2]  -2.10543 14.16548 -29.81768 -11.83987  -2.04490   7.44028
## beta.stage[3]   1.48643 14.16484 -26.24690  -8.25095   1.58869  11.03731
## sigma           0.65946  0.04615   0.57630   0.62769   0.65631   0.68904
## deviance      221.07685  3.69897 215.64970 218.30416 220.50445 223.20564
##                   97.5%    Rhat n.eff
## alpha[1]       28.17253 1.00068  3000
## alpha[2]       30.55147 1.00064  3000
## beta.spp[1]    -0.16894 1.00103  3000
## beta.spp[2]     0.27998 1.00060  3000
## beta.stage[1]  28.75190 1.00063  3000
## beta.stage[2]  25.72268 1.00064  3000
## beta.stage[3]  29.21912 1.00063  3000
## sigma           0.75962 1.00054  3000
## deviance      229.76098 1.00080  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 = 6.8 and DIC = 227.9
## 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
pKfFp9k/model1534115b02270.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]         -0.93497 17.54269 -36.12788 -12.75717  -0.97684  11.04406
## alpha[2]          1.97512 17.53613 -32.95357  -9.86044   1.92164  13.94136
## beta.habitat[1]   1.09396 17.66512 -34.46333 -11.44366   1.18150  12.94940
## beta.habitat[2]   1.21374 17.66668 -34.33148 -11.25317   1.30601  13.07954
## beta.spp[1]      -0.15738 18.22157 -36.03552 -12.46413  -0.35230  12.18142
## beta.spp[2]       0.38183 18.22373 -35.64317 -11.89133   0.19723  12.73121
## beta.stage[1]     1.26583 15.85037 -29.59350  -9.54448   1.40605  11.77505
## beta.stage[2]    -1.82568 15.85144 -32.85846 -12.60602  -1.66647   8.78878
## beta.stage[3]     1.74952 15.84947 -29.29202  -8.95913   1.93316  12.30332
## beta.waterhg     -0.30541 18.22329 -35.88636 -12.65144  -0.11333  11.96629
## bpvalue           0.51567  0.49984   0.00000   0.00000   1.00000   1.00000
## sigma             0.66126  0.04619   0.57660   0.62880   0.65927   0.69033
## deviance        221.37739  4.14419 215.30891 218.31366 220.75278 223.75695
##                     97.5%    Rhat n.eff
## alpha[1]         33.36096 1.00268   920
## alpha[2]         36.40542 1.00281   870
## beta.habitat[1]  35.28727 1.00097  3000
## beta.habitat[2]  35.07798 1.00097  3000
## beta.spp[1]      35.40477 1.00179  1600
## beta.spp[2]      35.96744 1.00180  1500
## beta.stage[1]    31.65542 1.00221  1200
## beta.stage[2]    28.67601 1.00218  1200
## beta.stage[3]    32.23642 1.00219  1200
## beta.waterhg     35.59038 1.00181  1500
## bpvalue           1.00000 1.00122  2800
## sigma             0.75866 1.00173  1600
## deviance        231.13873 1.00326   730
##
## 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 = 8.6 and DIC = 229.9
## 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: 428.228432930762
```

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

```
## DIC for Spp*WaterHg + Stage 227.920549924618
```

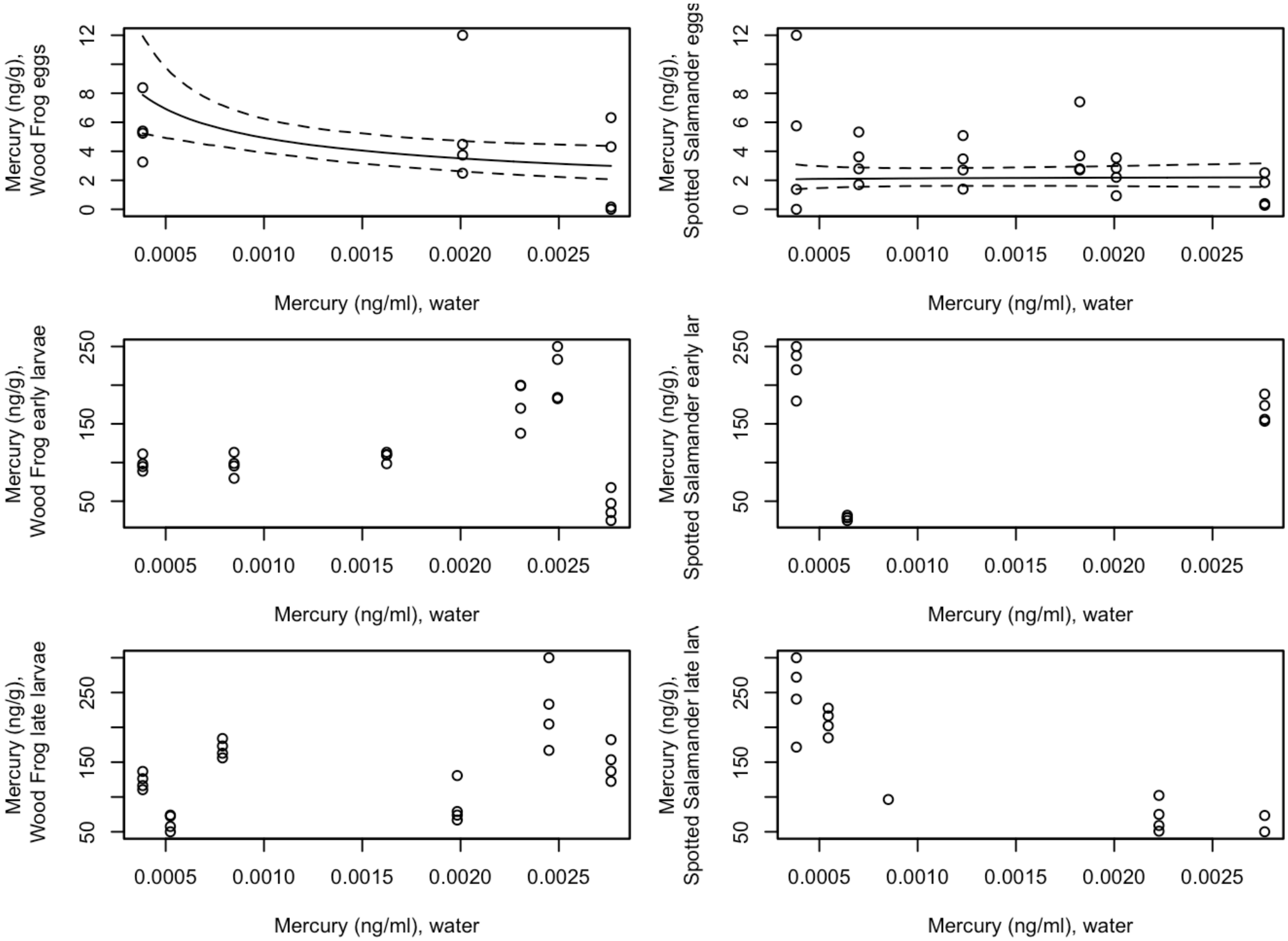
```
cat(paste(c("DIC for Spp*WaterHg + Stage + Habitat:"),jagsfitlogm3$BUGSoutput$DIC))
```

```
## DIC for Spp*WaterHg + Stage + Habitat: 229.946726080789
```

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
pKfFp9k/model1534124df85c.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]  4.25053  0.13082  3.99320  4.16278  4.24907  4.34051  4.50326
## alpha[2]  3.58766  0.12322  3.34247  3.50723  3.58955  3.67043  3.82171
## delta    34.32059 10.40852 14.73606 27.21051 33.91801 41.32835 55.04377
## sigma     0.62138  0.06747  0.50389  0.57395  0.61671  0.66129  0.76916
## spsa     70.74439  9.27486 54.22837 64.24970 70.04055 76.74692 90.31104
## wofr     36.42380  4.48723 28.28895 33.35588 36.21778 39.26882 45.68220
## deviance 90.54196  2.61706 87.53853 88.60318 89.92082 91.76221 97.08436
##             Rhat n.eff
## alpha[1] 1.00120  2800
## alpha[2] 1.00053  3000
## delta    1.00110  3000
## sigma    1.00056  3000
## spsa     1.00121  2800
## wofr     1.00053  3000
## deviance 1.00116  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 = 3.4 and DIC = 94.0
## 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
pKfFp9k/model1534132e14112.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]  4.13357 0.17615  3.78115  4.01570  4.13315  4.25130
## group.mean[2,1]  3.79037 0.17205  3.45435  3.67295  3.78936  3.90438
## group.mean[1,2]  4.36370 0.17351  4.02149  4.24902  4.36409  4.47783
## group.mean[2,2]  3.39385 0.17179  3.04775  3.27827  3.39003  3.50871
## sigma            0.60761 0.06597  0.49415  0.56098  0.60237  0.64772
## deviance        88.79490 3.34360 84.41224 86.30716 88.11071 90.46143
##                    97.5%    Rhat n.eff
## group.mean[1,1]  4.47883 1.00054  3000
## group.mean[2,1]  4.12804 1.00134  2400
## group.mean[1,2]  4.70667 1.00142  3000
## group.mean[2,2]  3.73251 1.00057  3000
## sigma            0.75324 1.00094  3000
## deviance        96.99658 1.00113  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 = 5.6 and DIC = 94.4
## 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: 93.9665461323738
```

```
## DIC, Model 2: 94.3850578516245
```

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: 34.3205900416876
```

```
## 95% CI: 14.7360599355035 - 55.0437723878811
```

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.

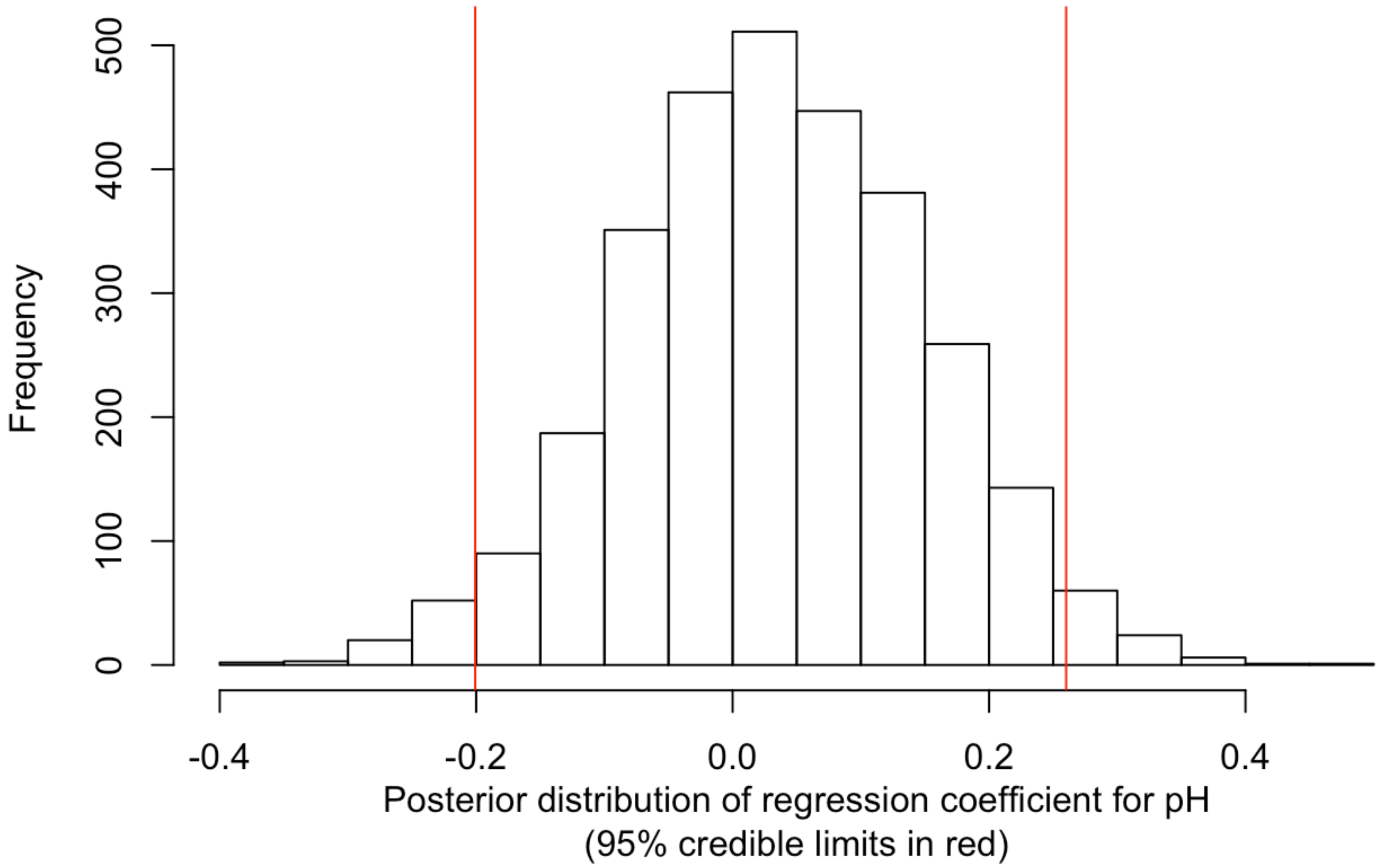
Among adults, tissue methylmercury varied among species. Spotted Salamanders had higher average loads than did Wood Frogs. Habitat did not explain variation in mercury loads among samples.

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).

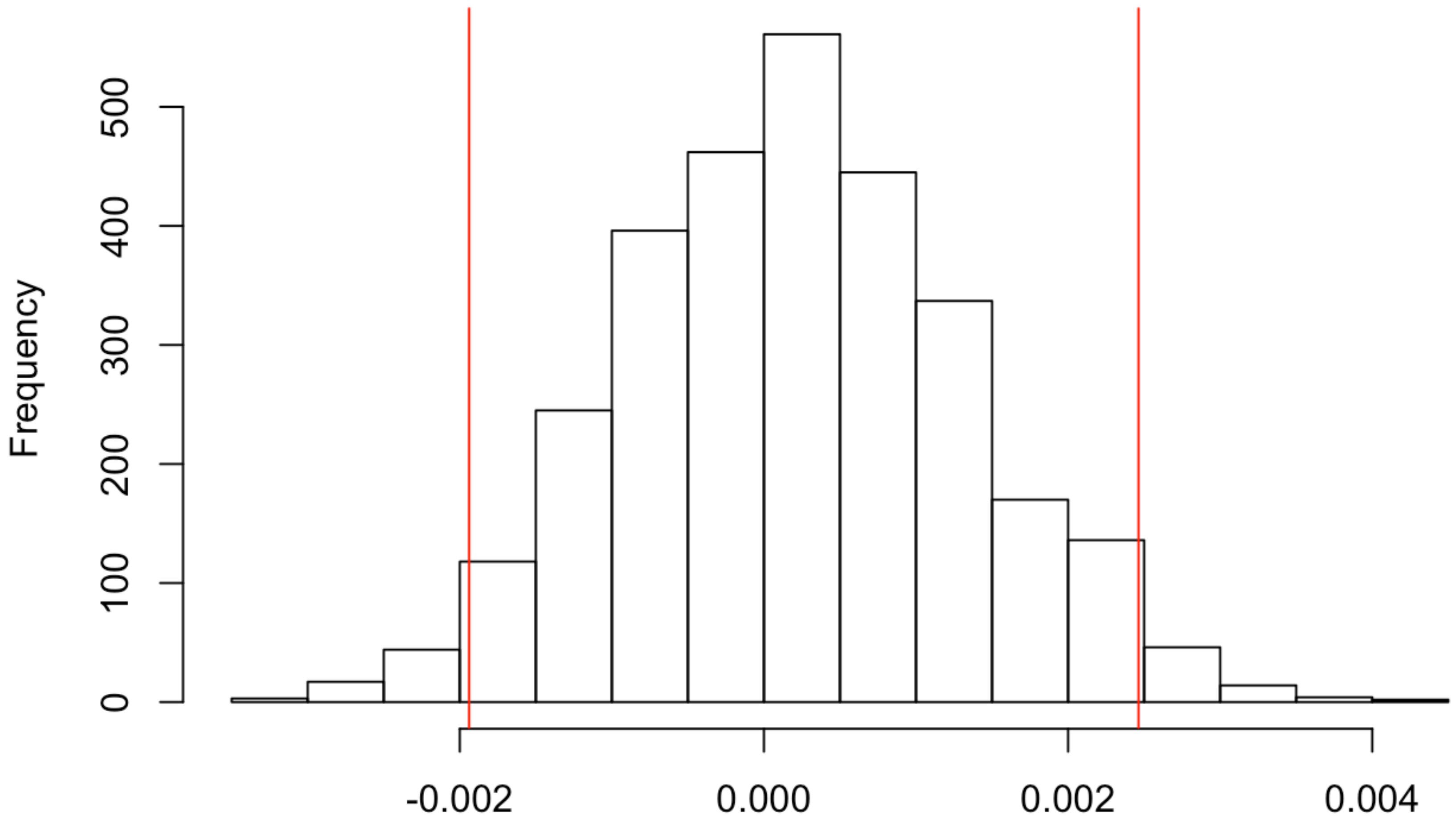



```
## DIC for Spp*WaterHg + Stage 227.920549924618
```

```
## DIC for Spp*WaterHg + Stage + pH: 231.093847852567
```

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
```



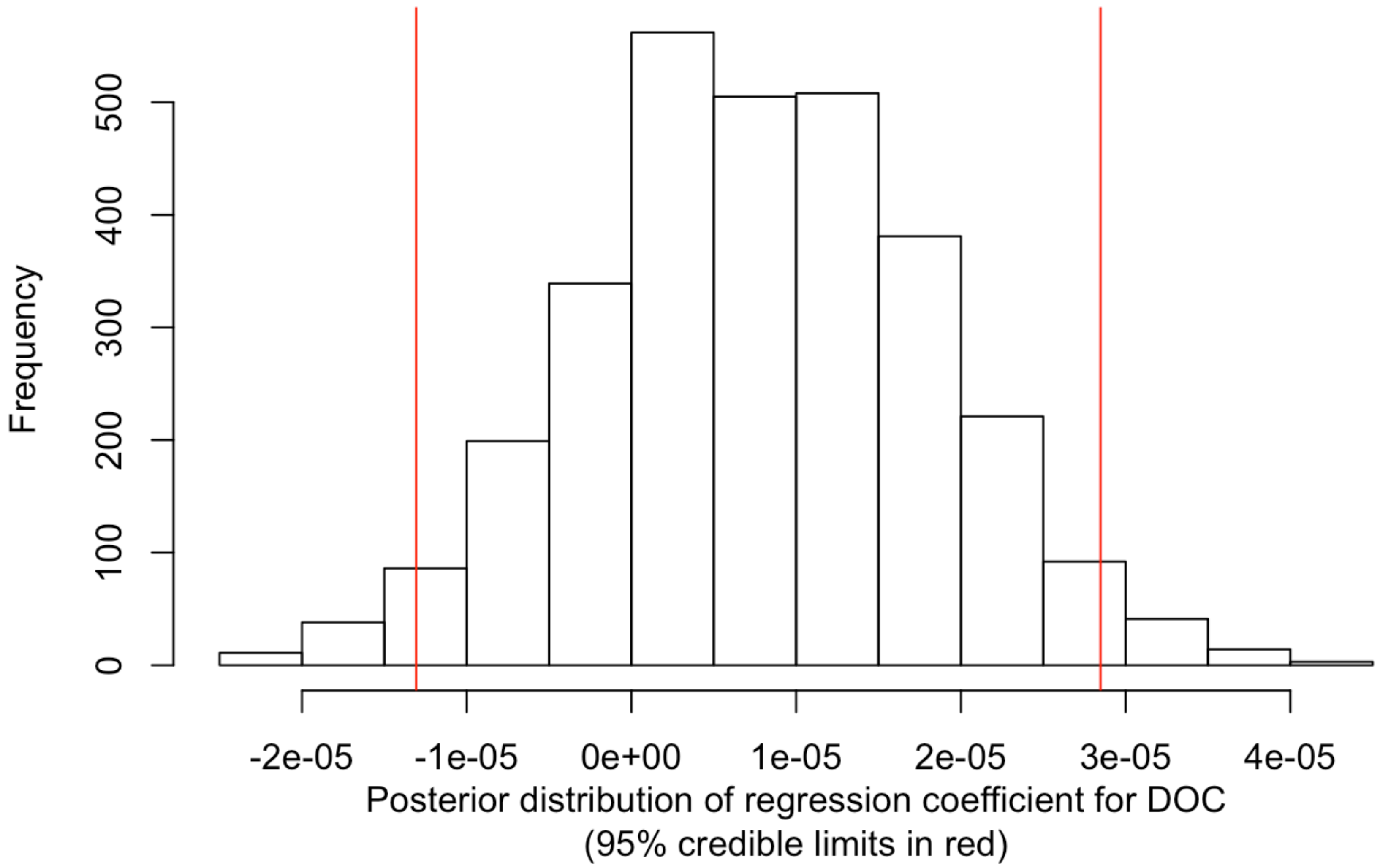
Posterior distribution of regression coefficient for Aluminum
(95% credible limits in red)

```
## DIC for Spp*WaterHg + Stage 227.920549924618
```

```
## DIC for Spp*WaterHg + Stage + Al: 231.211046320506
```

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
```

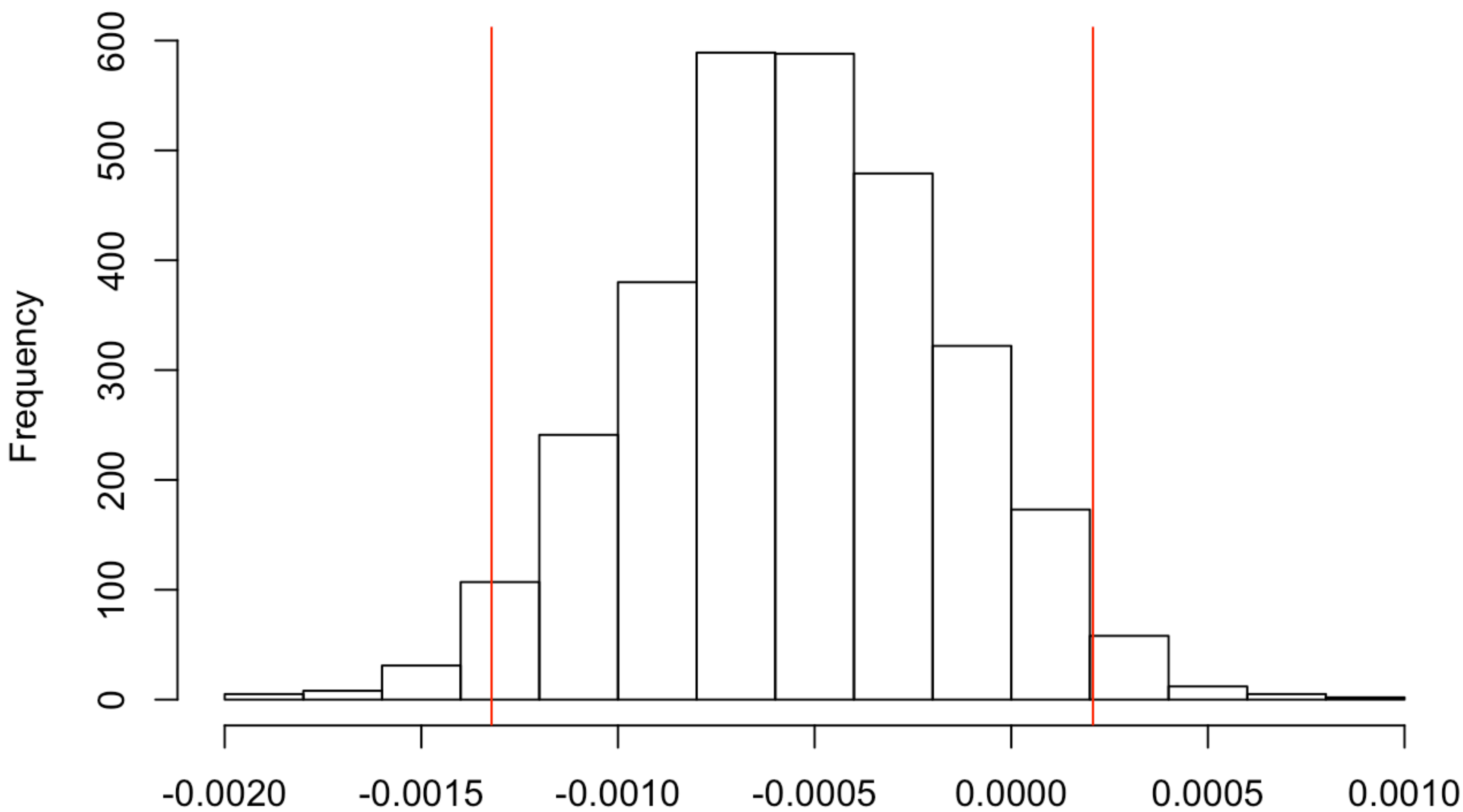



```
## DIC for Spp*WaterHg + Stage 227.920549924618
```

```
## DIC for Spp*WaterHg + Stage + DOC: 229.92966365511
```

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
```



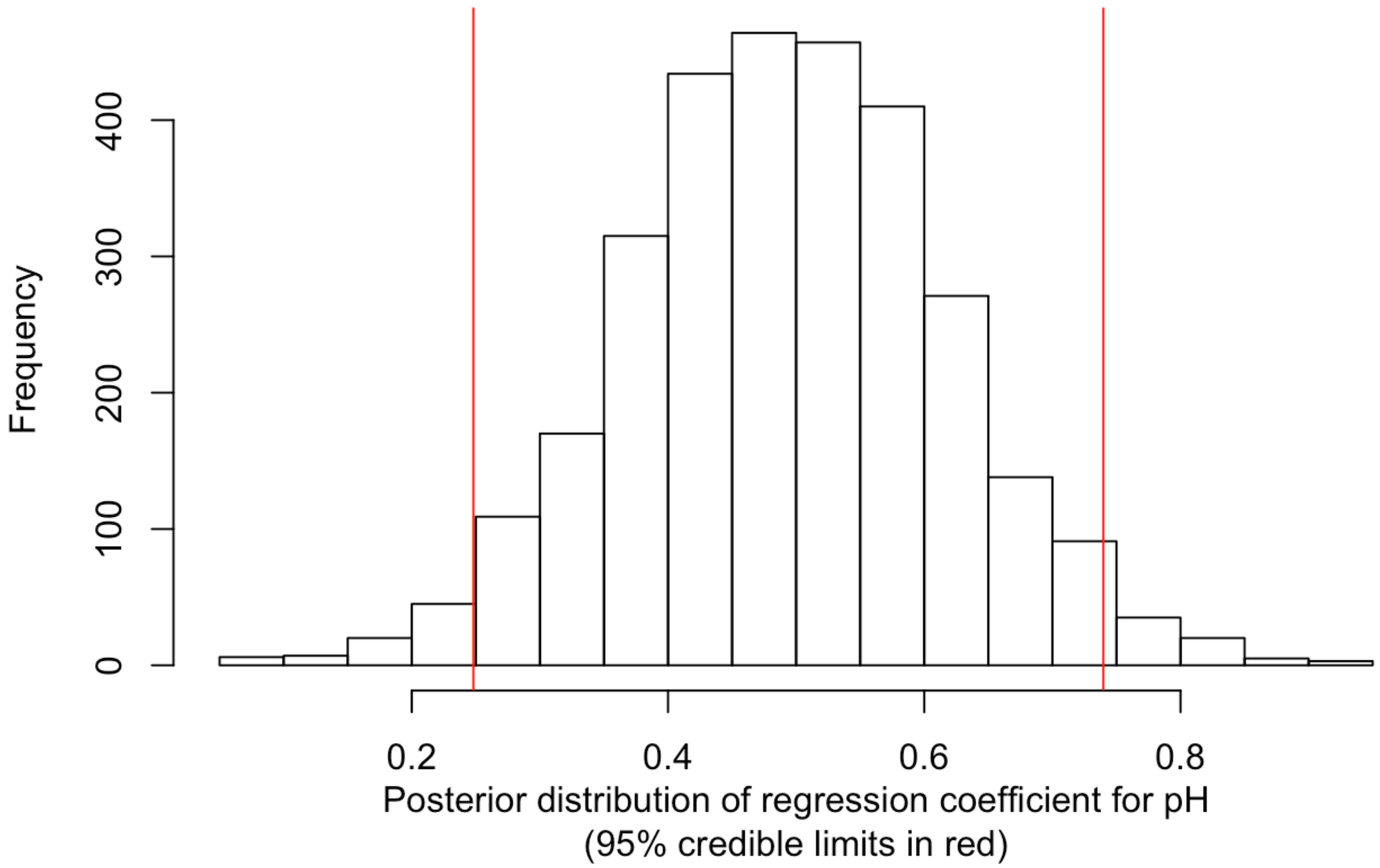
Posterior distribution of regression coefficient for S
(95% credible limits in red)

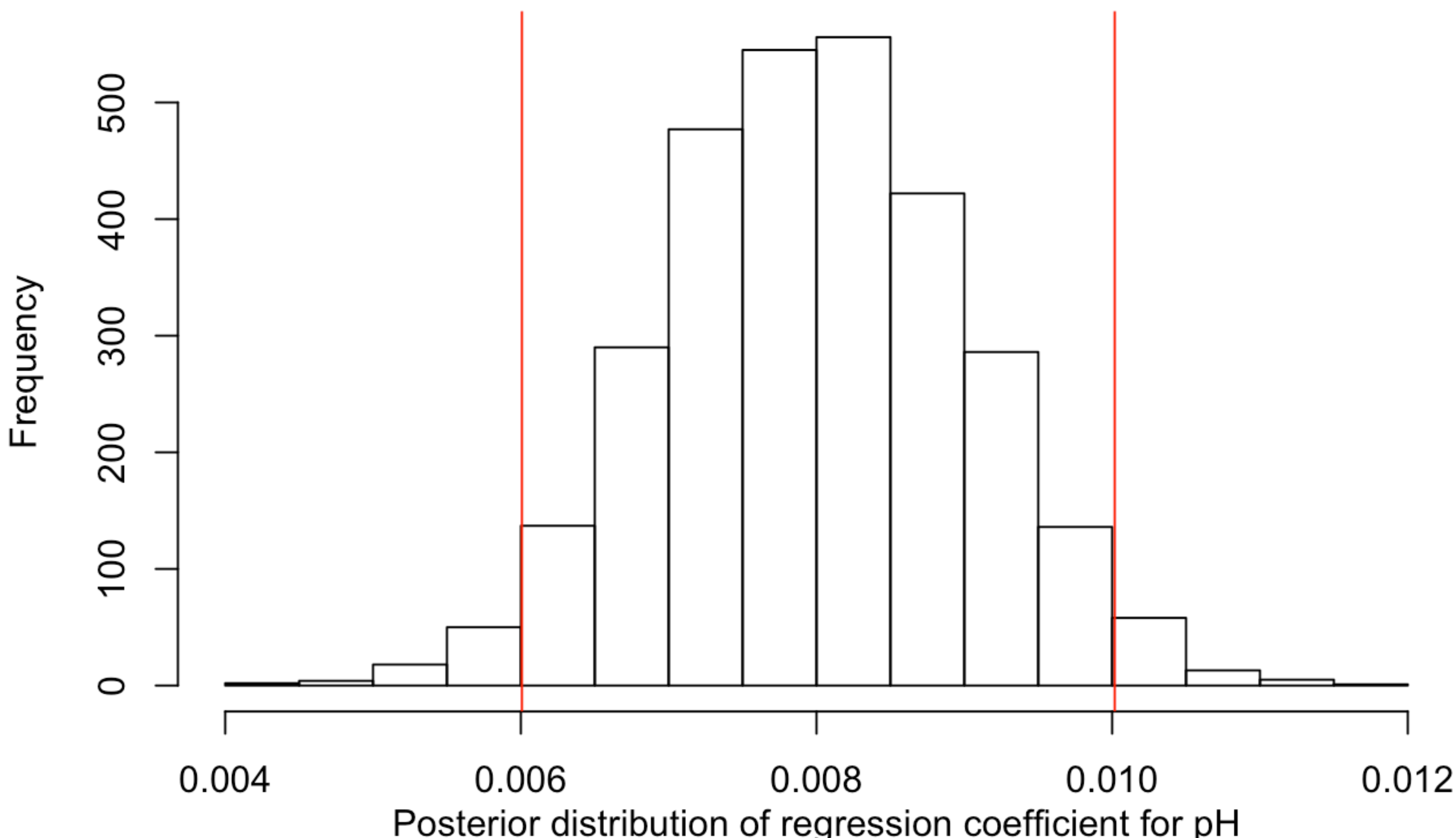
```
## DIC for Spp*WaterHg + Stage 227.920549924618
```

```
## DIC for Spp*WaterHg + Stage + S: 229.286519464278
```

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
```


Frequency
0
100
200
300
400
0.2
0.4
0.6
0.8
Posterior distribution of regression coefficient for pH
(95% credible limits in red)

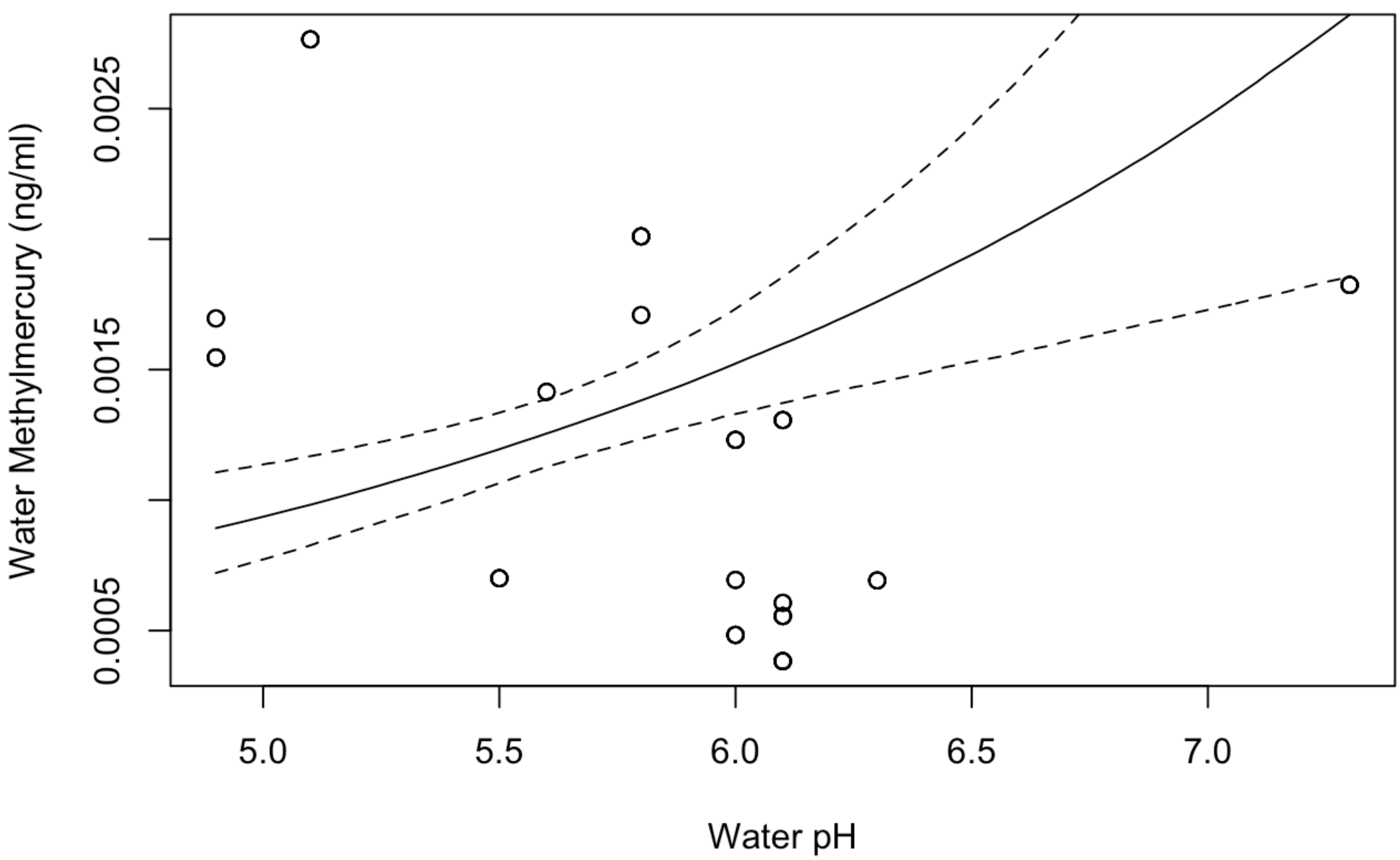

Frequency
0
100
200
300
400
500
0.004
0.006
0.008
0.010
0.012
Posterior distribution of regression coefficient for pH

(95% credible limits in red)

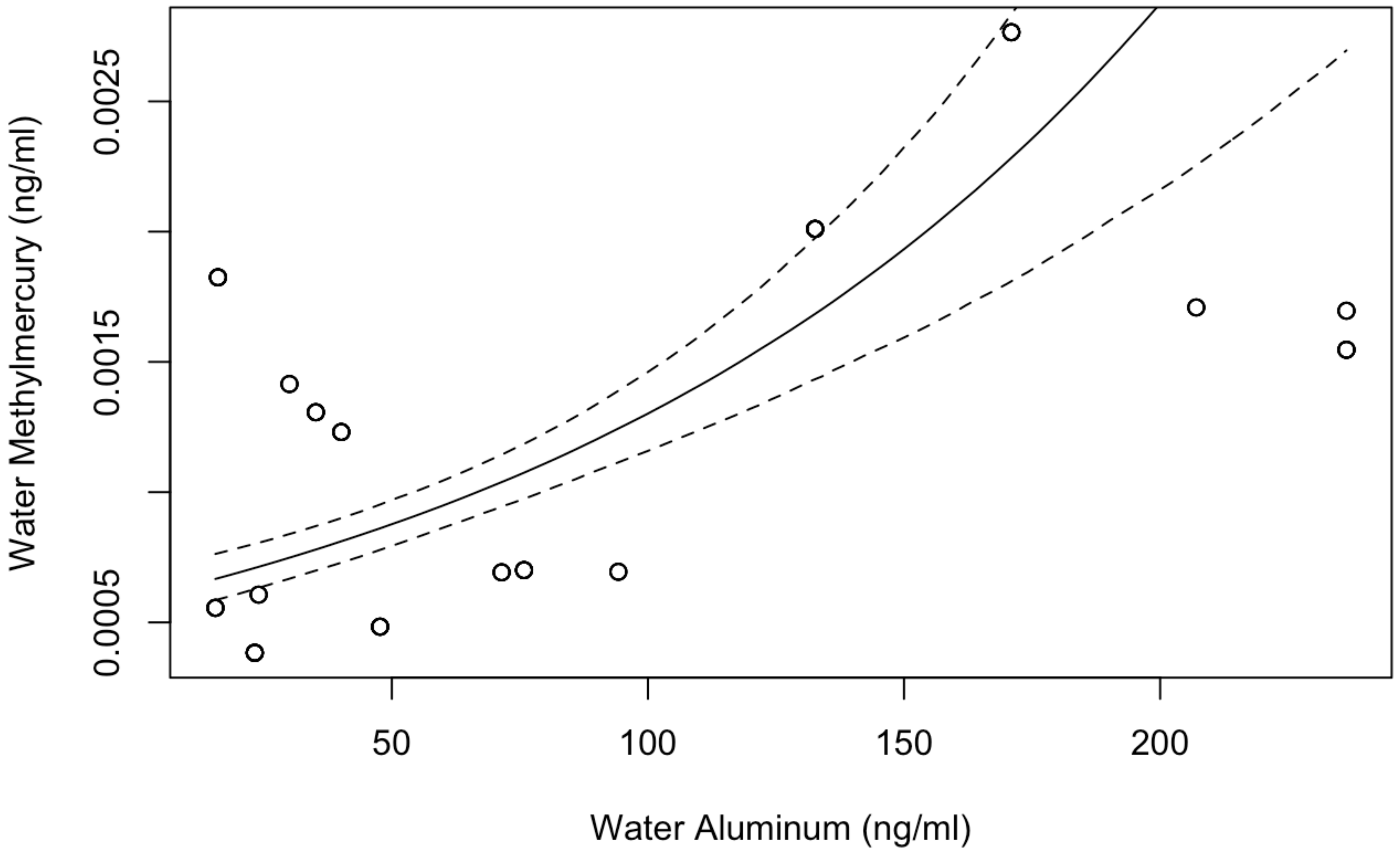
The predicted values suggest a positive relationship between methylmercury and pH, which is somewhat surprising. However, it appears that the relationship is driven by a single pool at Downer (observed points are shown as circles in these figures).

```
## 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):



Water Methylmercury (ng/ml)
0.0005
0.0015
0.0025
50
100
150
200
Water Aluminum (ng/ml)