

# 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. : 3.762	Adult :52	Coniferous: 96	Min. : 15.56
##	1st Qu.: 9.229	Early Larvae:48	Deciduous :100	1st Qu.: 24.26
##	Median :105.452	Eggs :48		Median : 41.00
##	Mean :148.406	Late Larvae :48		Mean : 77.24
##	3rd Qu.:275.302			3rd Qu.:132.63
##	Max. :561.799			Max. :236.37
##	NA's :85			NA's :67
##	Water_S	Water_DOC	Water_pH	Water_MeHg
##	Min. : 244.3	Min. : 2695	Min. :4.900	Min. :0.00004
##	1st Qu.: 448.8	1st Qu.: 8838	1st Qu.:5.800	1st Qu.:0.00038
##	Median : 613.5	Median :13117	Median :6.000	Median :0.00081
##	Mean : 671.8	Mean :13845	Mean :5.939	Mean :0.00105
##	3rd Qu.: 796.0	3rd Qu.:15982	3rd Qu.:6.100	3rd Qu.:0.00182
##	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. :0.00022	Min. : 13.30	Min. : 11.26	Min. : 44.00
##	1st Qu.:0.00041	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 :0.00080	Mean : 63.49	Mean : 49.82	Mean : 65.62
##	3rd Qu.:0.00100	3rd Qu.: 85.07	3rd Qu.: 61.91	3rd Qu.: 85.00
##	Max. :0.00195	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. :0.00103	
##	1st Qu.:162.2	1st Qu.:11.50	1st Qu.:0.00381	
##	Median :170.0	Median :13.50	Median :0.00538	
##	Mean :171.3	Mean :14.07	Mean :0.00517	
##	3rd Qu.:185.0	3rd Qu.:16.15	3rd Qu.:0.00634	
##	Max. :200.0	Max. :23.70	Max. :0.01025	
##	NA's :172	NA's :145	NA's :47	

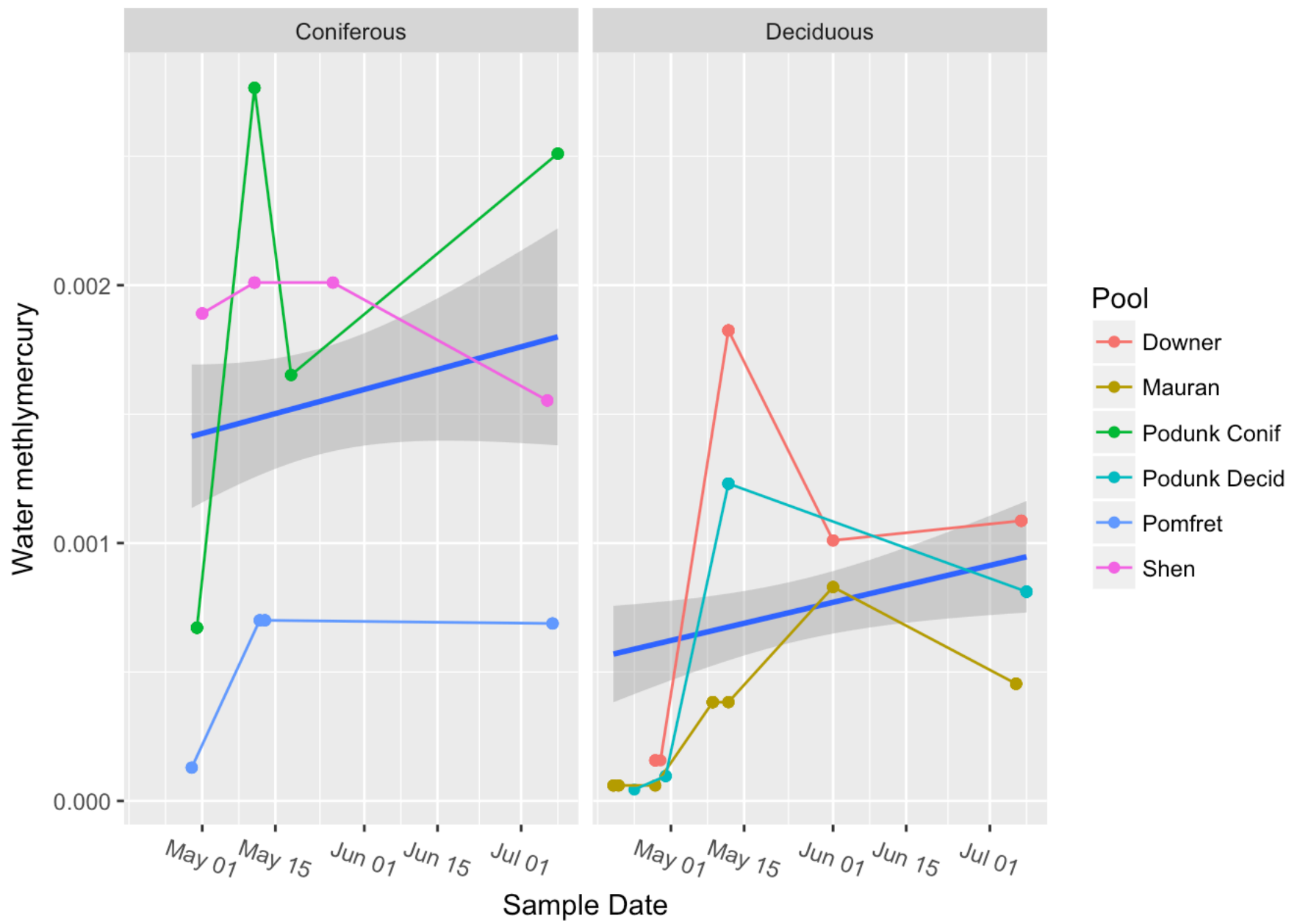
There are 6 pools, 3 of which are surrounded by deciduous forest (Downer, Mauran, and Podunk Decid) and 3 of which are surrounded by coniferous forest (Podunk Conif, Pomfret, and Shen). The number of samples differs by date and by pool because sample number is determined by the number of amphibians collected.

##		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

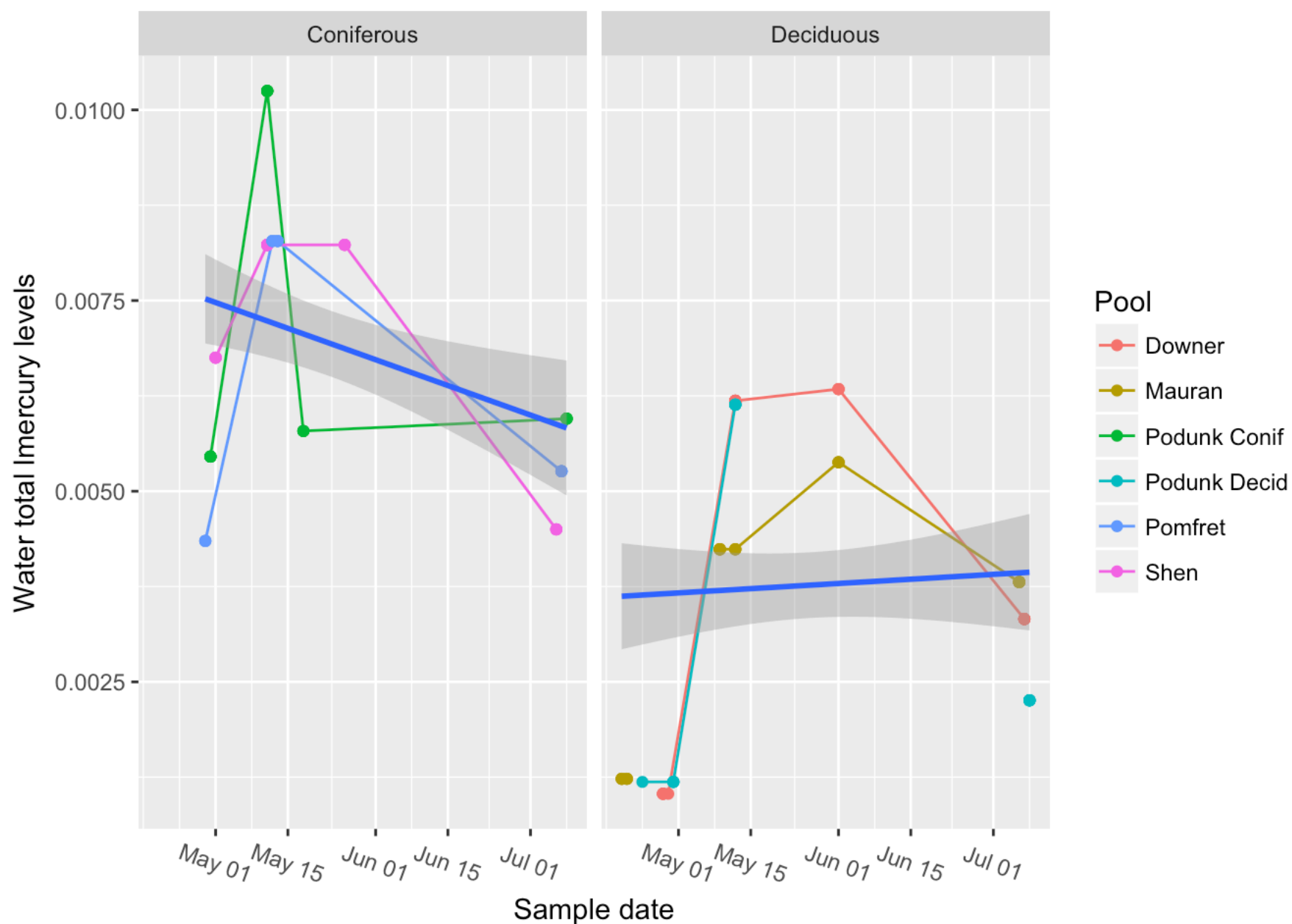
## Methlymercury levels in water by pool, over time

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 (0.000, 0.00146).

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

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

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

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

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

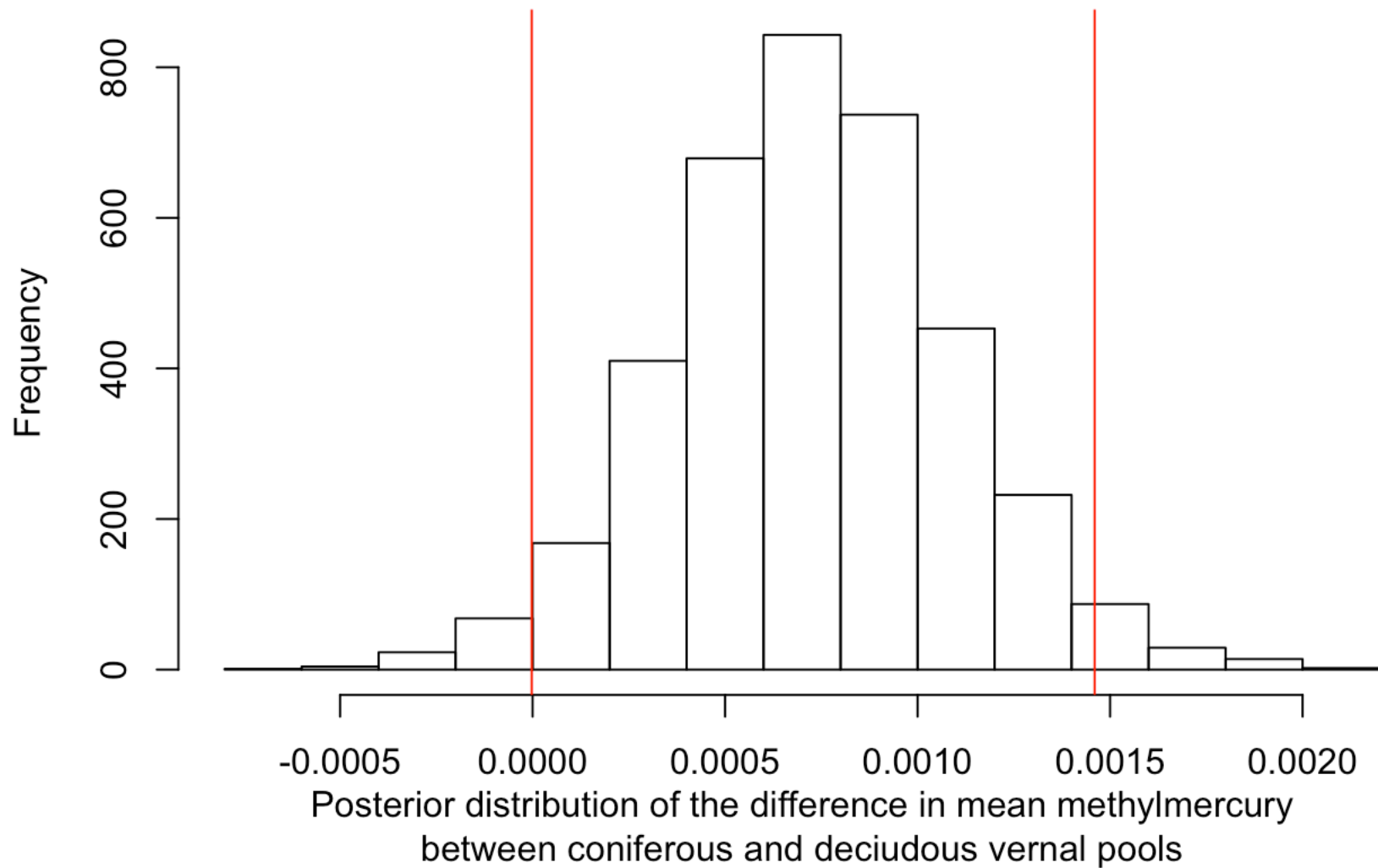
```
## The following object is masked from 'package:coda':  
##  
## traceplot
```

```
## module glm loaded
```

```
## Compiling model graph
##   Resolving undeclared variables
##   Allocating nodes
## Graph information:
##   Observed stochastic nodes: 23
##   Unobserved stochastic nodes: 4
##   Total graph size: 44
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
puFqews/model153ebld6412f4.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      0.00073 0.00037   0.00000   0.00048   0.00073   0.00096
## mu1        0.00067 0.00019   0.00028   0.00055   0.00067   0.00079
## mu2        0.00139 0.00031   0.00078   0.00119   0.00140   0.00159
## sigma1     0.00064 0.00016   0.00041   0.00053   0.00061   0.00072
## sigma2     0.00100 0.00027   0.00062   0.00082   0.00095   0.00113
## deviance -266.69224 3.51504 -271.09470 -269.30729 -267.44321 -264.96032
##
##           97.5%      Rhat n.eff
## delta      0.00146 1.00150  2200
## mu1        0.00104 1.00135  2700
## mu2        0.00202 1.00173  1800
## sigma1     0.00102 1.00135  3000
## sigma2     0.00167 1.00150  2200
## deviance -257.63514 1.00144  2400
##
## 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 = 6.2 and DIC = -260.5
## 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 small, with a weak tendency for higher mercury in coniferous pools (mean difference = 0.00072, 95% credible interval = -0.00002 - 0.00147).

```
## Compiling model graph
##   Resolving undeclared variables
##   Allocating nodes
## Graph information:
##   Observed stochastic nodes: 23
##   Unobserved stochastic nodes: 4
##   Total graph size: 44
##
## Initializing model
```

```
## Inference for Bugs model at "/var/folders/83/ltgpdkyd4js5ld5pnht4cjcw0000gn/T//Rtm
puFqews/model153eb49313bce.txt", fit using jags,
## 3 chains, each with 50000 iterations (first 25000 discarded), n.thin = 25
## n.sims = 3000 iterations saved
##
```

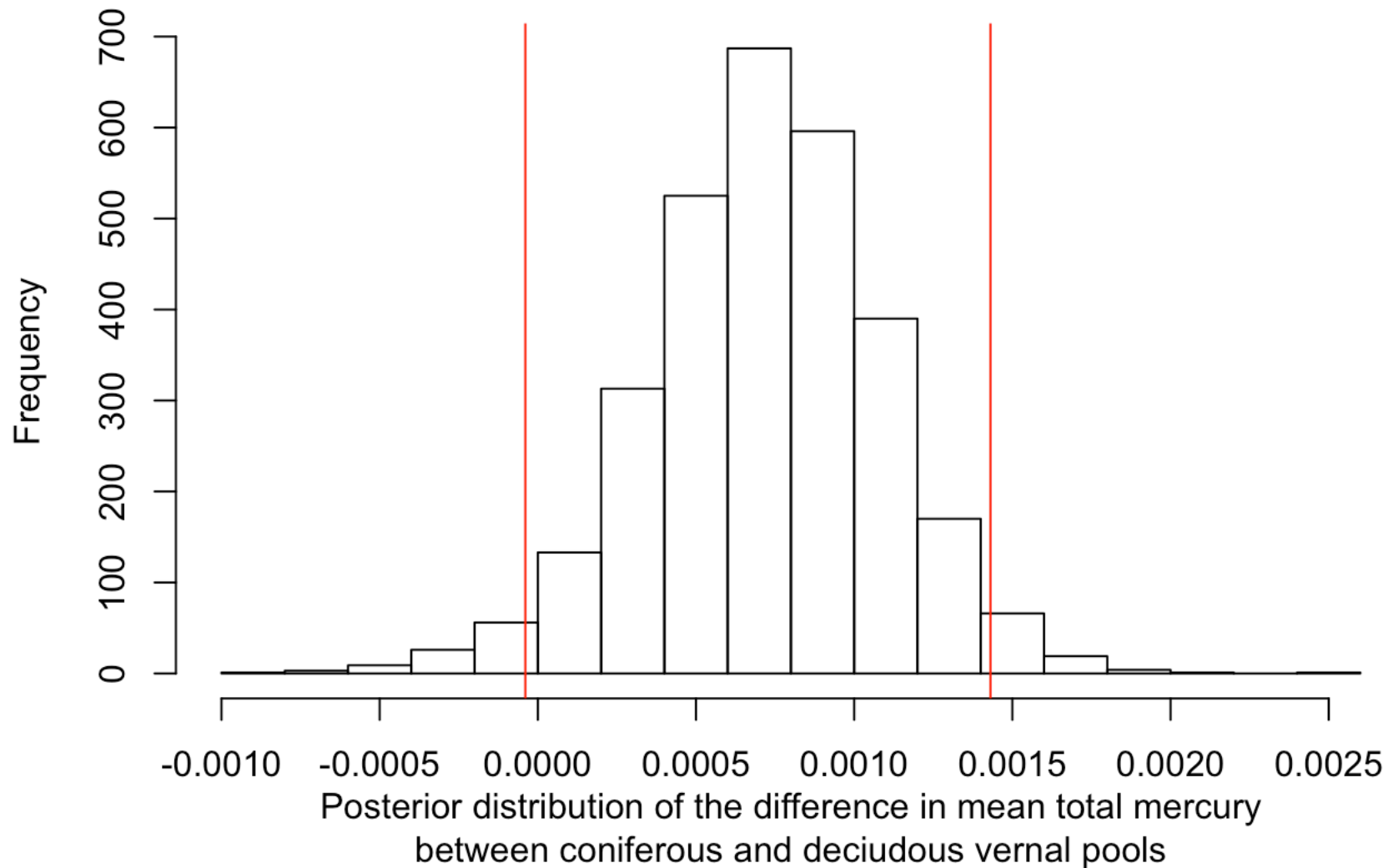
	mu.vect	sd.vect	2.5%	25%	50%	75%
## delta	0.00072	0.00037	-0.00004	0.00050	0.00072	0.00097
## mu1	0.00067	0.00019	0.00028	0.00054	0.00067	0.00079
## mu2	0.00139	0.00032	0.00072	0.00121	0.00139	0.00158
## sigma1	0.00064	0.00017	0.00041	0.00052	0.00061	0.00071
## sigma2	0.00099	0.00026	0.00062	0.00081	0.00094	0.00112
## deviance	-266.73956	3.53277	-270.99724	-269.36634	-267.51113	-265.00652

```
##
```

	97.5%	Rhat	n.eff
## delta	0.00143	1.00243	1000
## mu1	0.00106	1.00054	3000
## mu2	0.00200	1.00276	890
## sigma1	0.00104	1.00152	2000
## sigma2	0.00165	1.00056	3000
## deviance	-258.15889	1.00077	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 = 6.2 and DIC = -260.5
## 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.

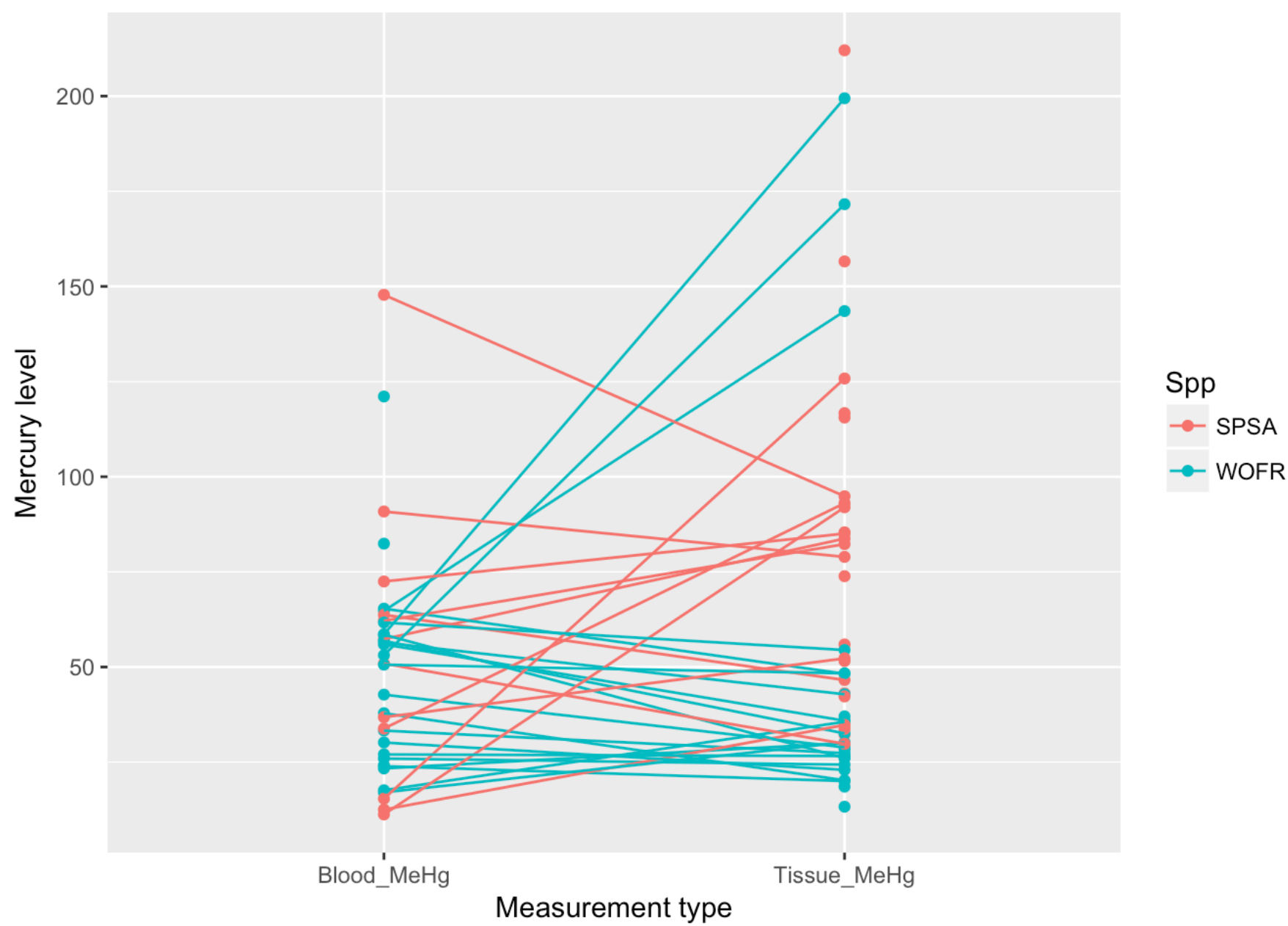
```
summary(data.df$Blood_MeHg[data.df$Life_Stage=="Adult"])
```

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

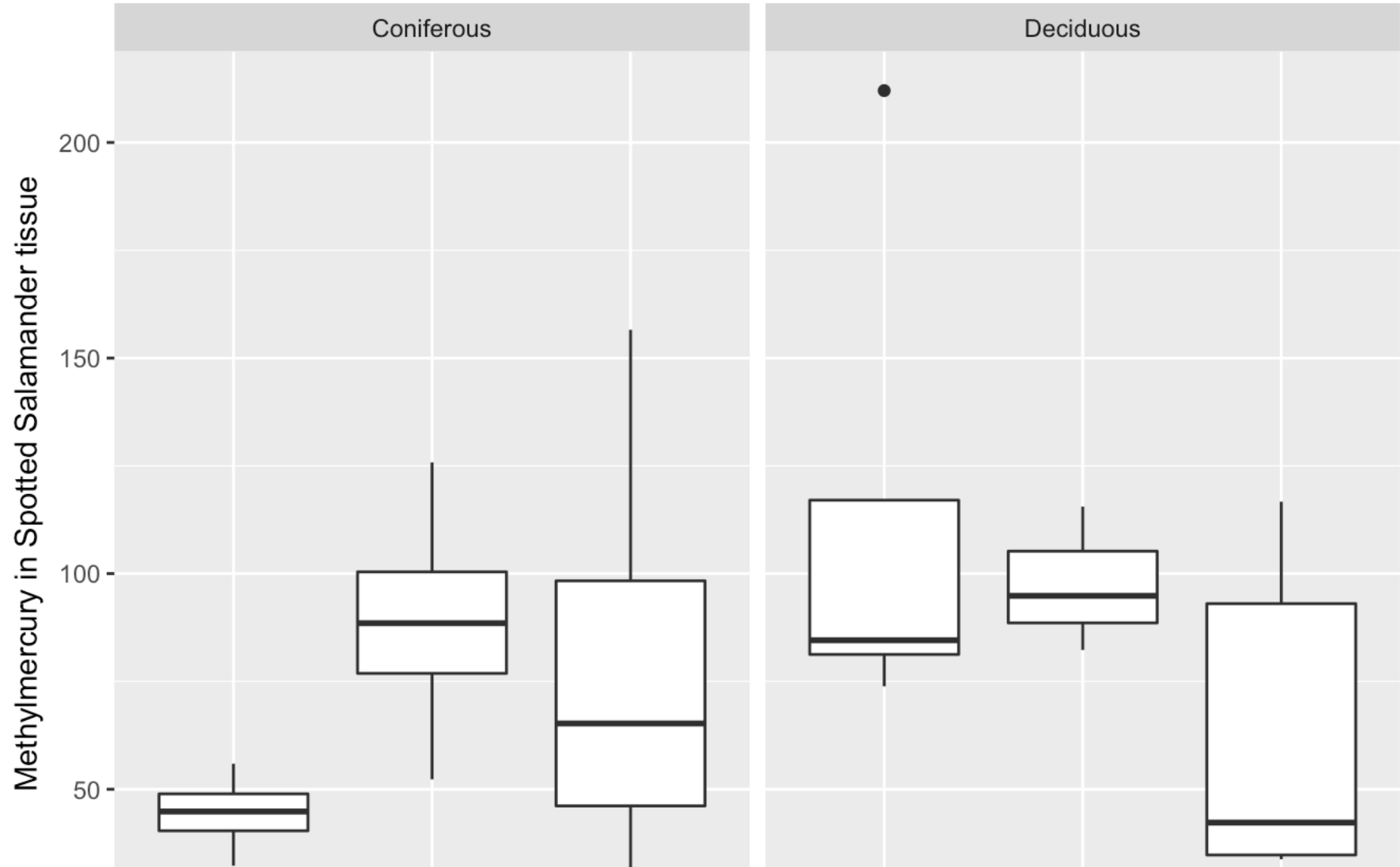
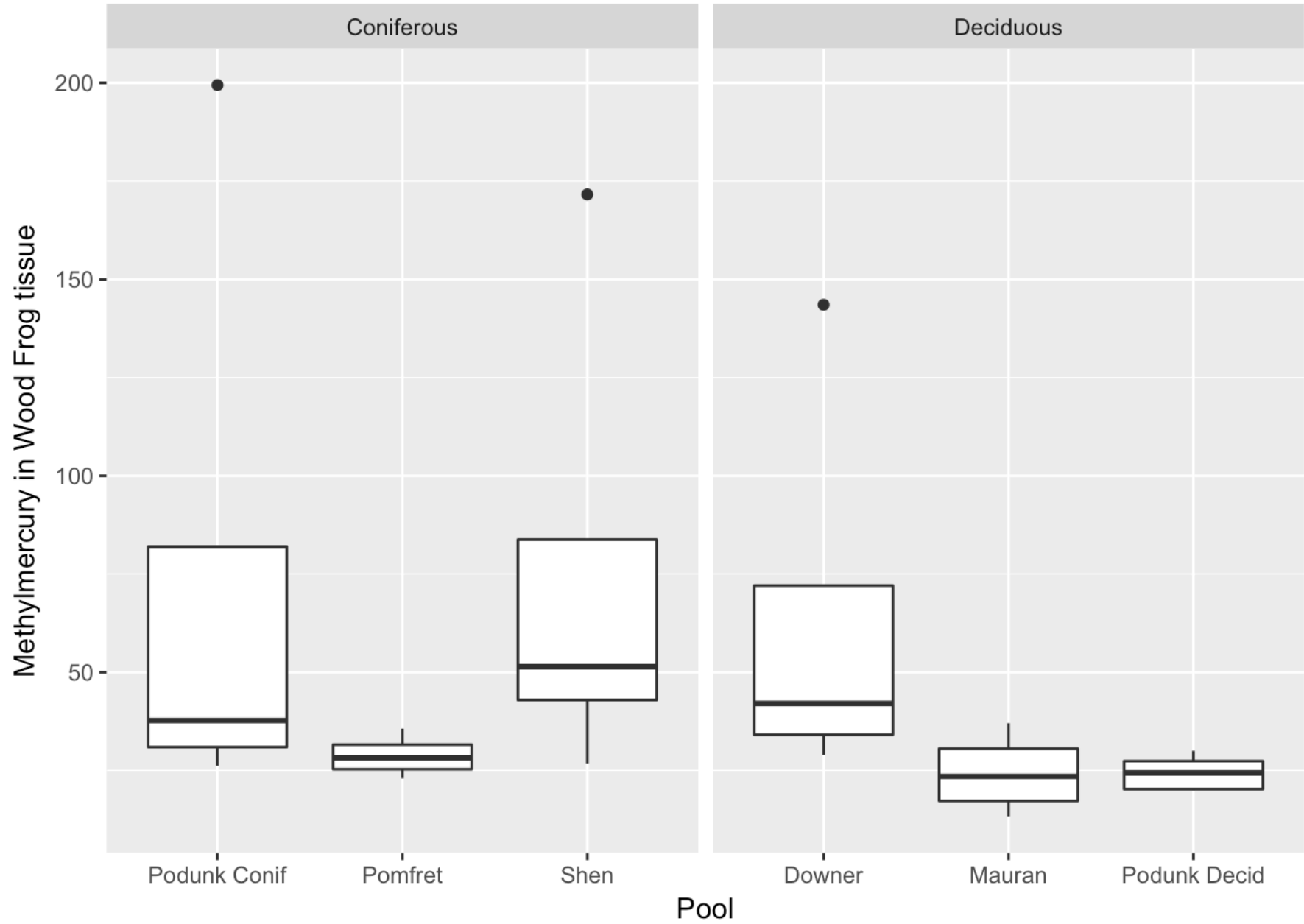
```
summary(data.df$Tissue_MeHg[data.df$Life_Stage=="Adult"])
```

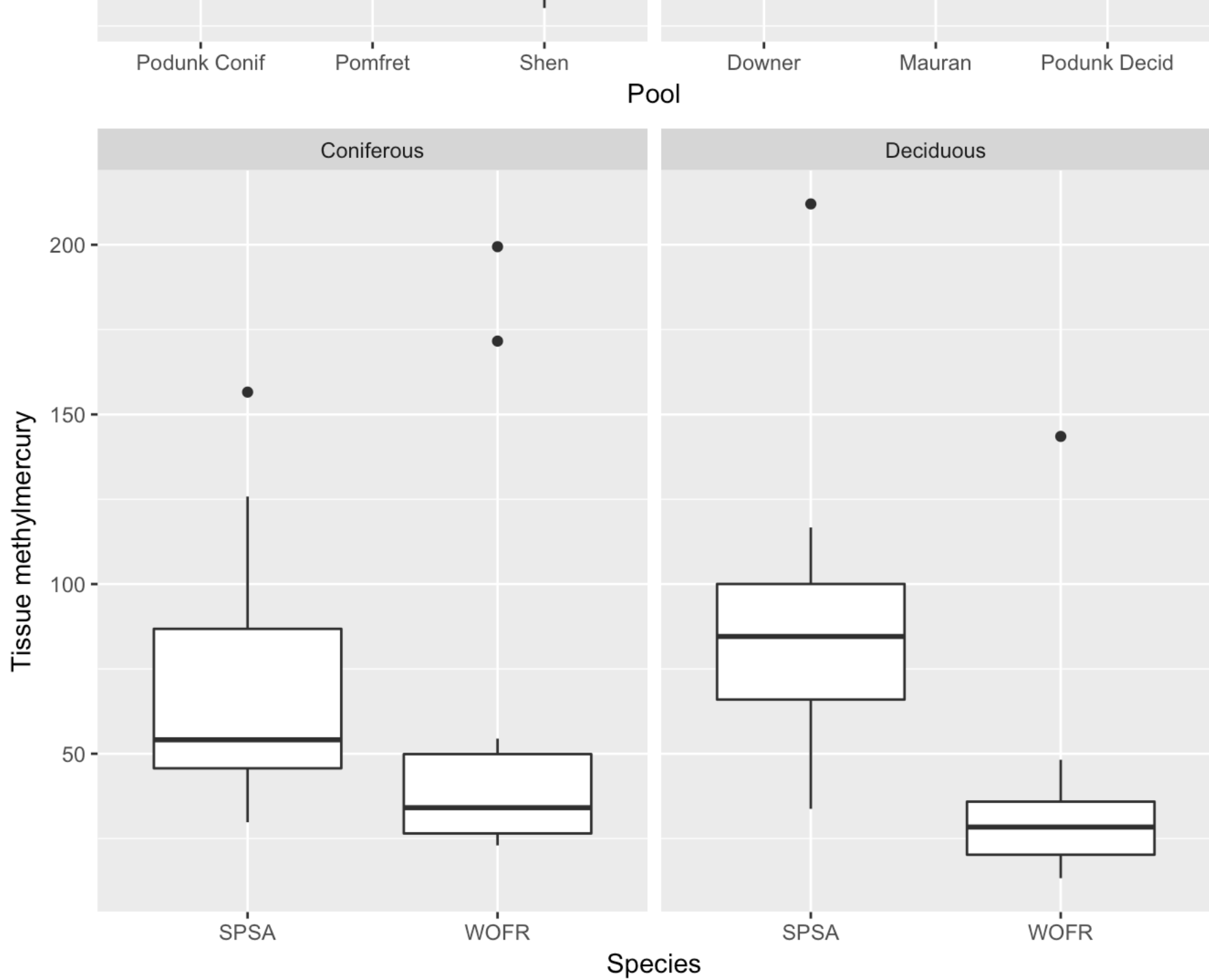
##	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	NA's
##	13.30	29.82	43.07	63.49	85.07	212.03	3

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.

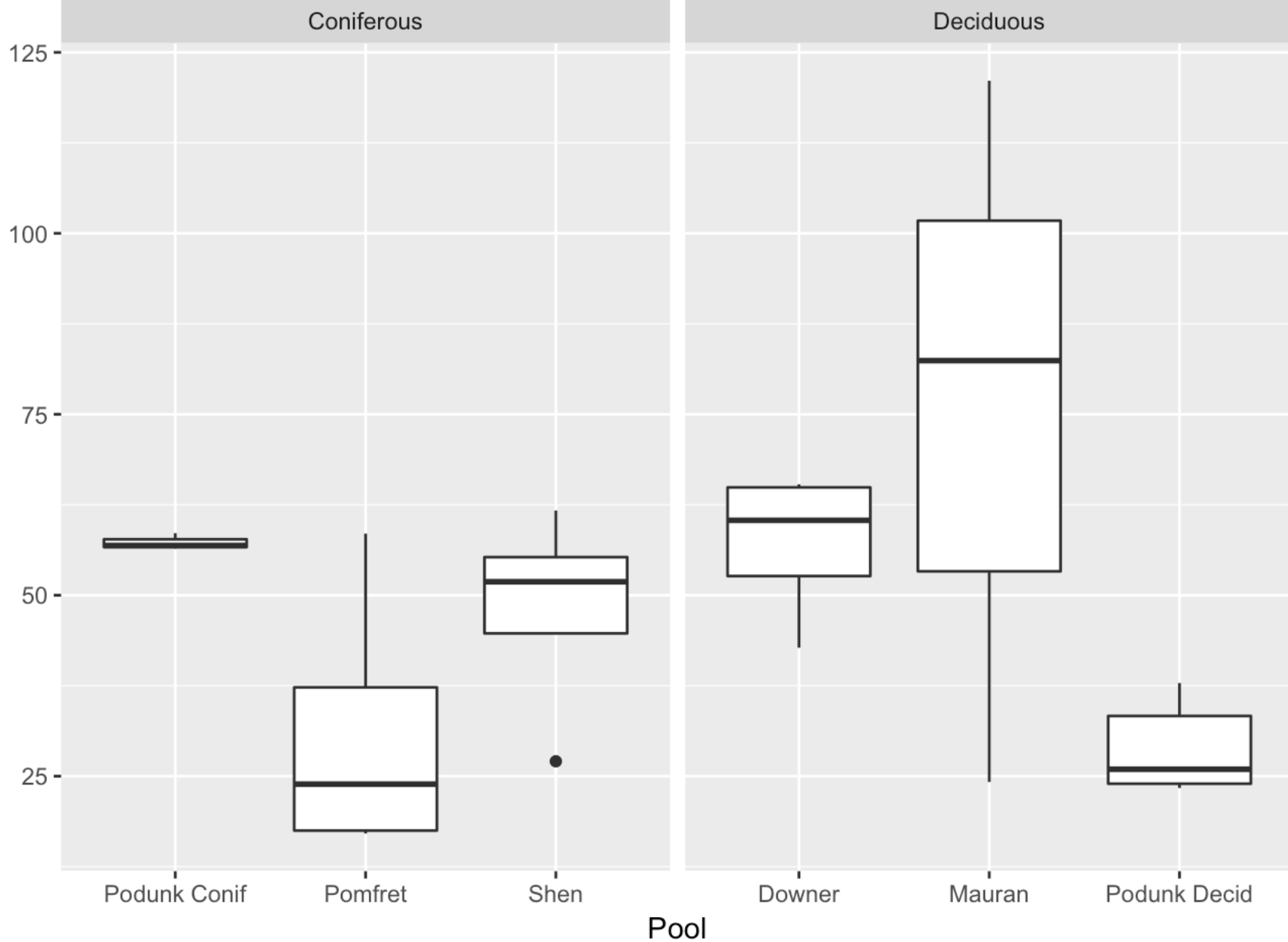




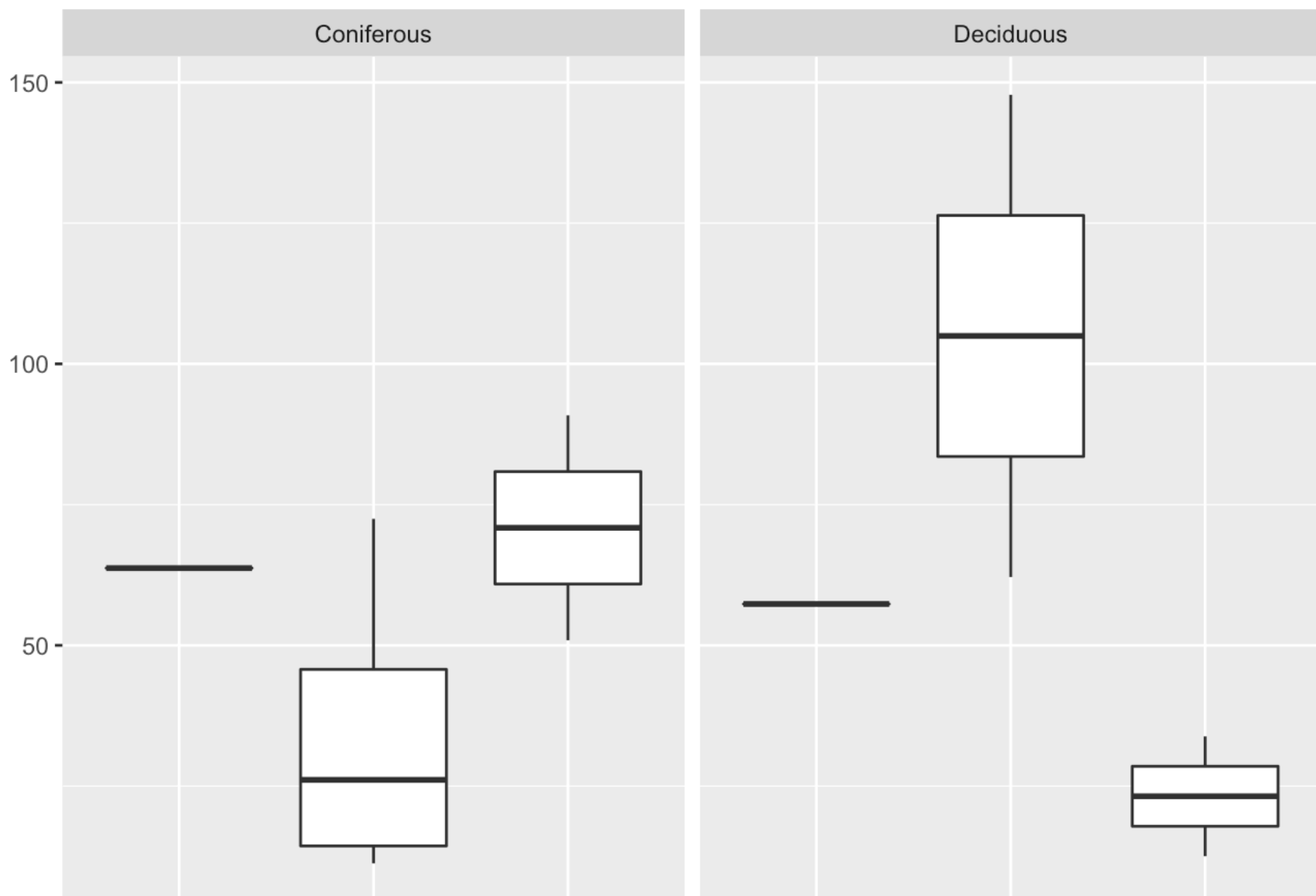
If we look at blood levels of methylmercury in adults:

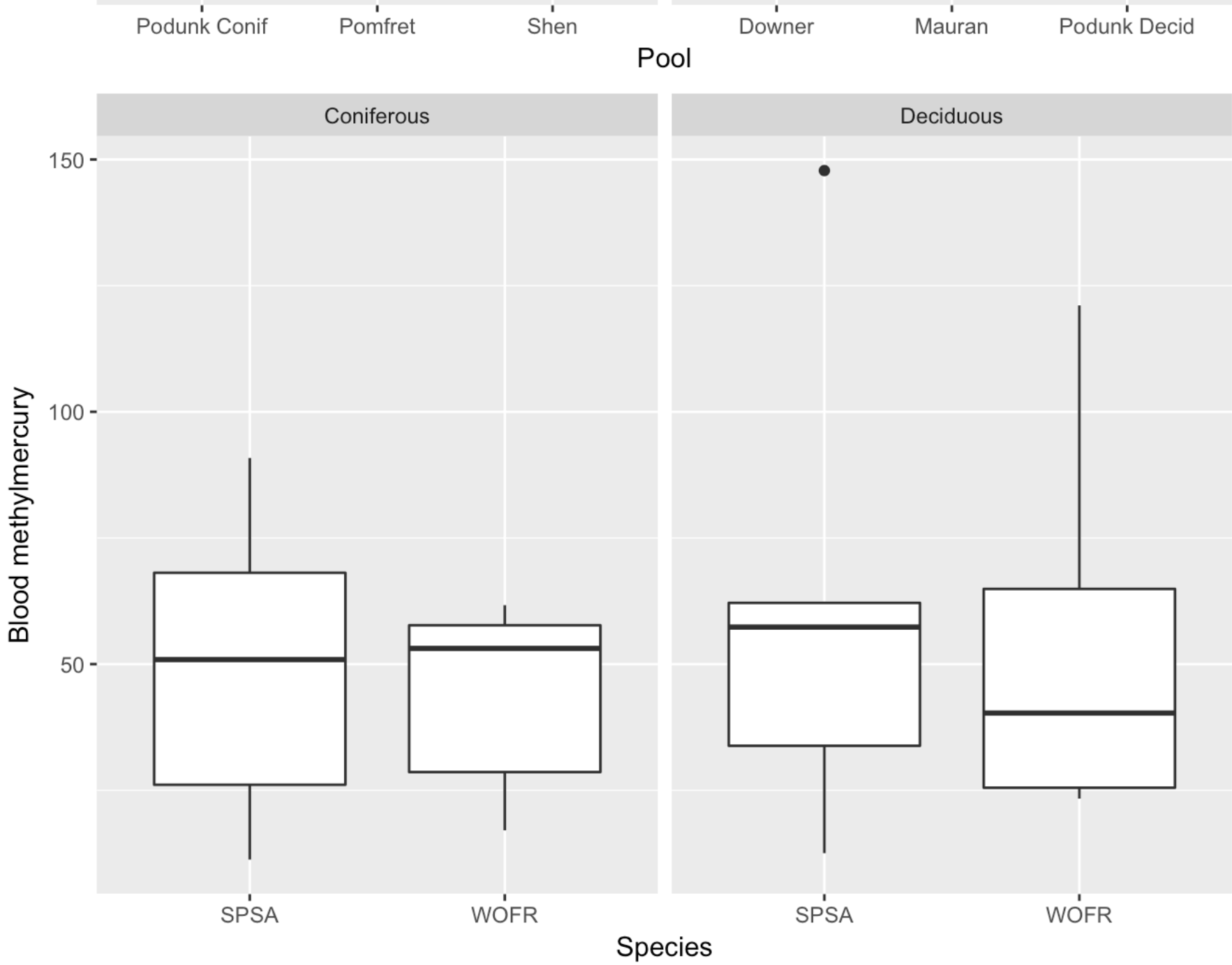


Methylmercury in Wood Frog blood



Methylmercury in Spotted Salamander blood

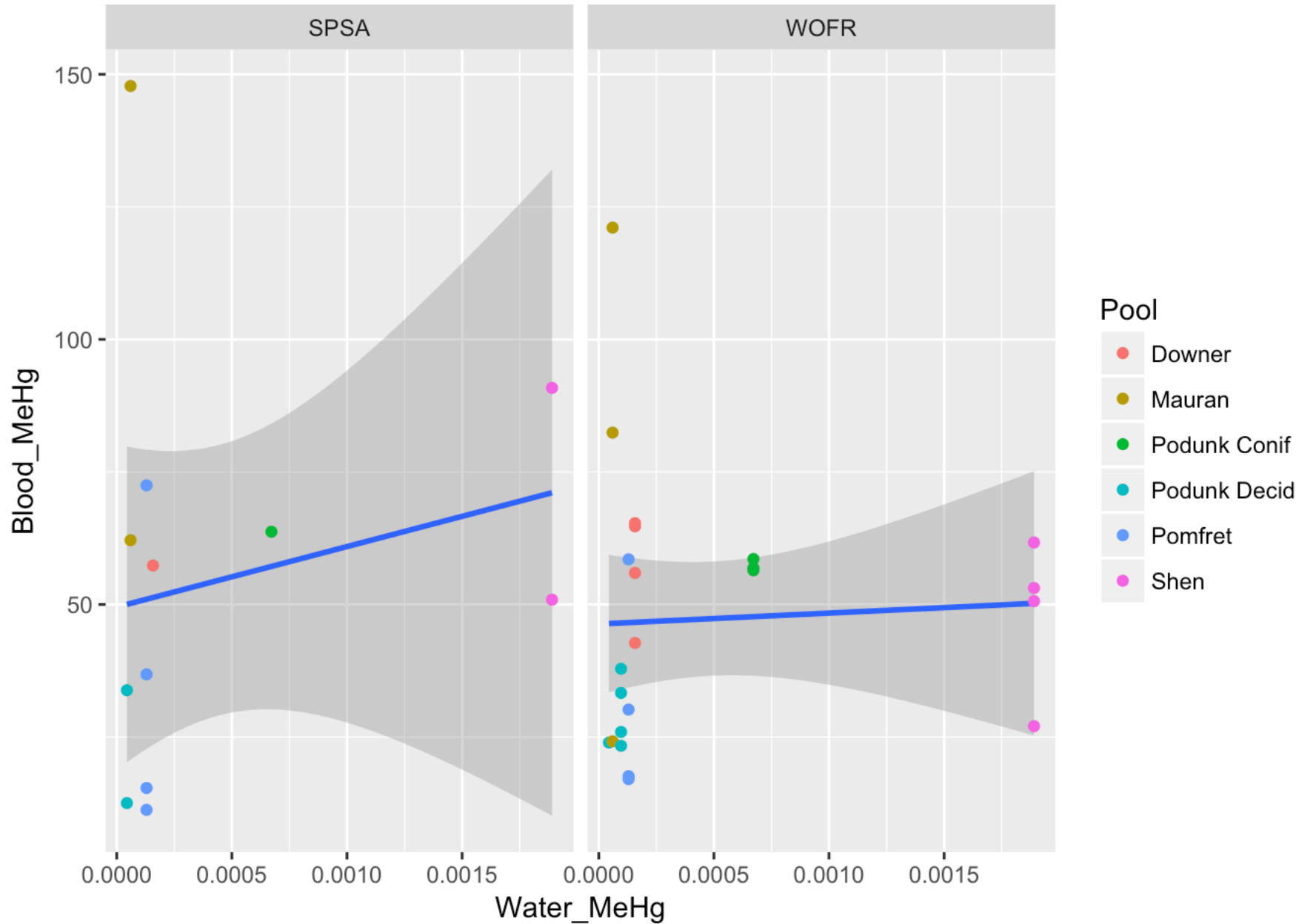




Neither species shows a strong relationship between levels of methylmercury in tissue and levels of methylmercury in the water at the time of the sample.



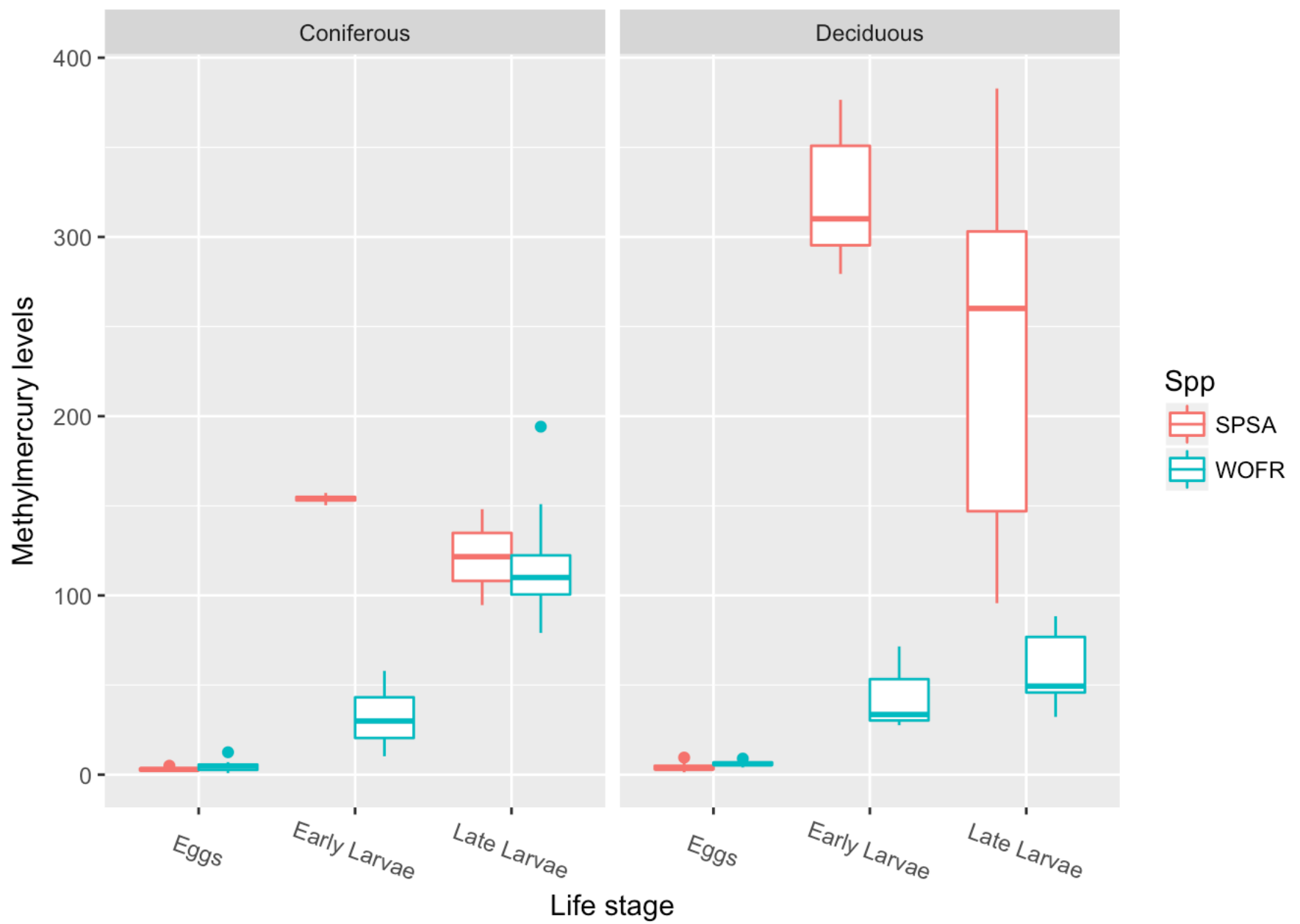




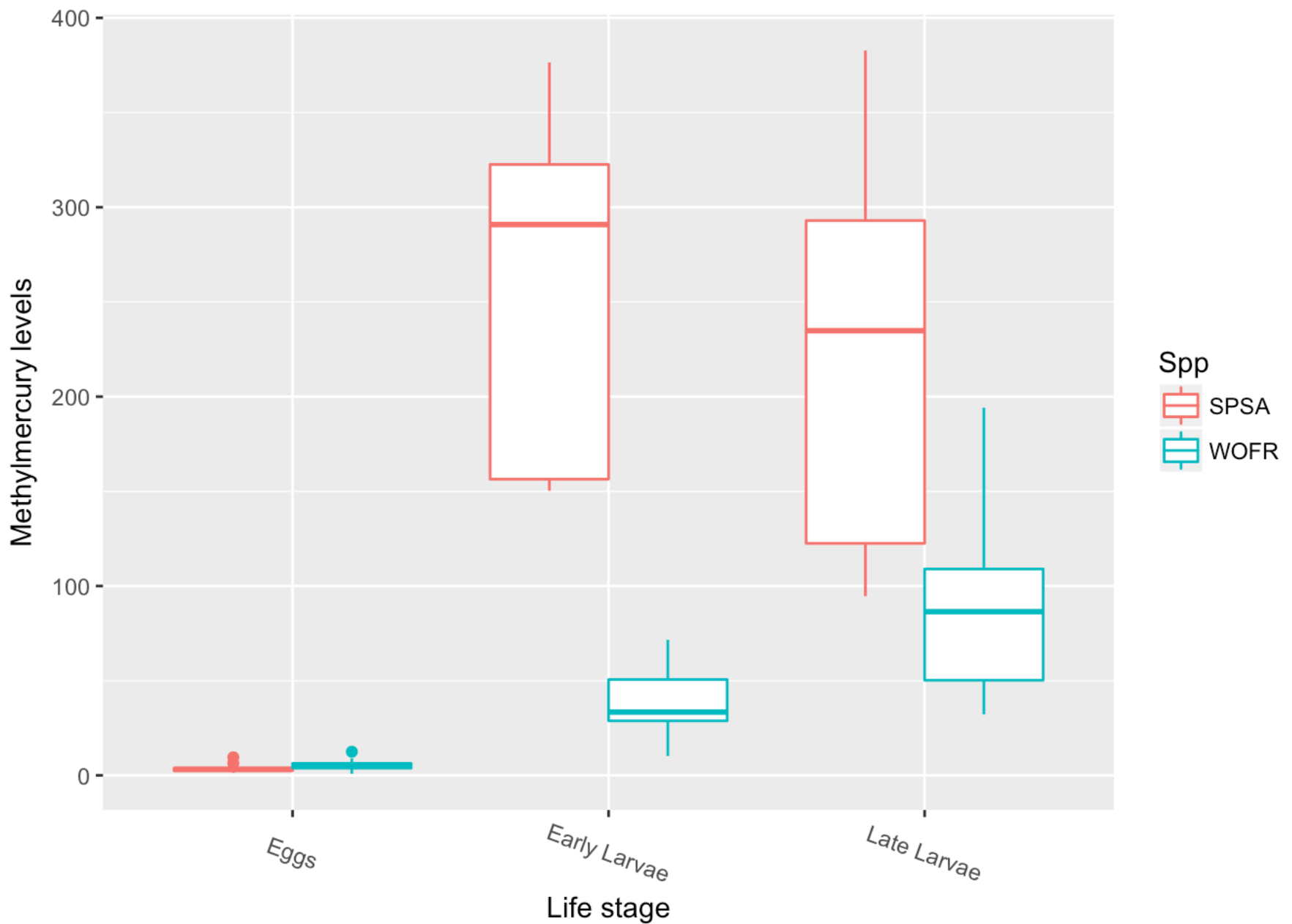
## Larval stages

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).
```

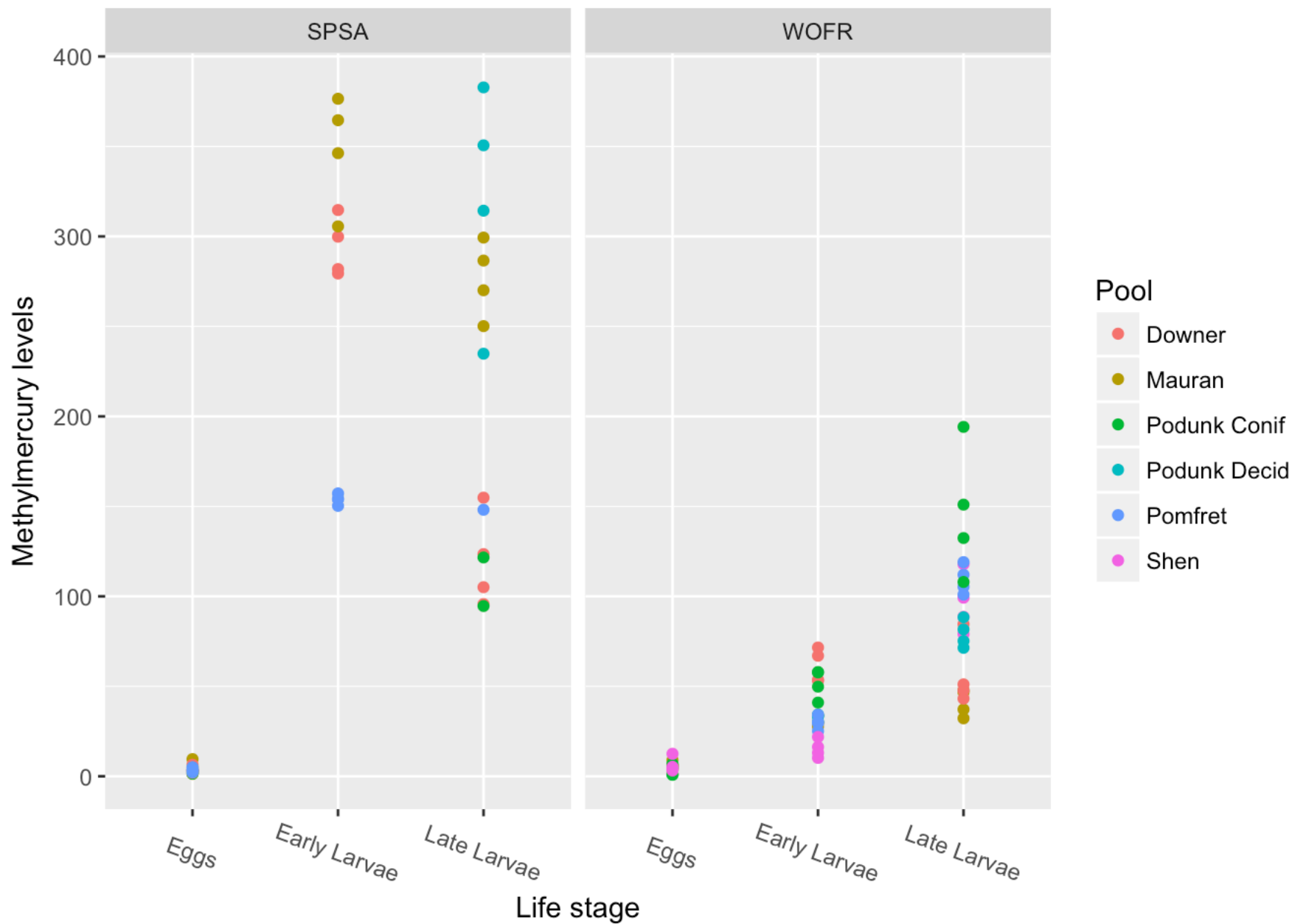


```
p7 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p7 + geom_boxplot(aes(x = Life_Stage, y = Amphib_MeHg, color = Spp)) +
  scale_x_discrete(limits = c("Eggs", "Early Larvae", "Late Larvae")) +
  theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
  xlab("Life stage") + ylab("Methylmercury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

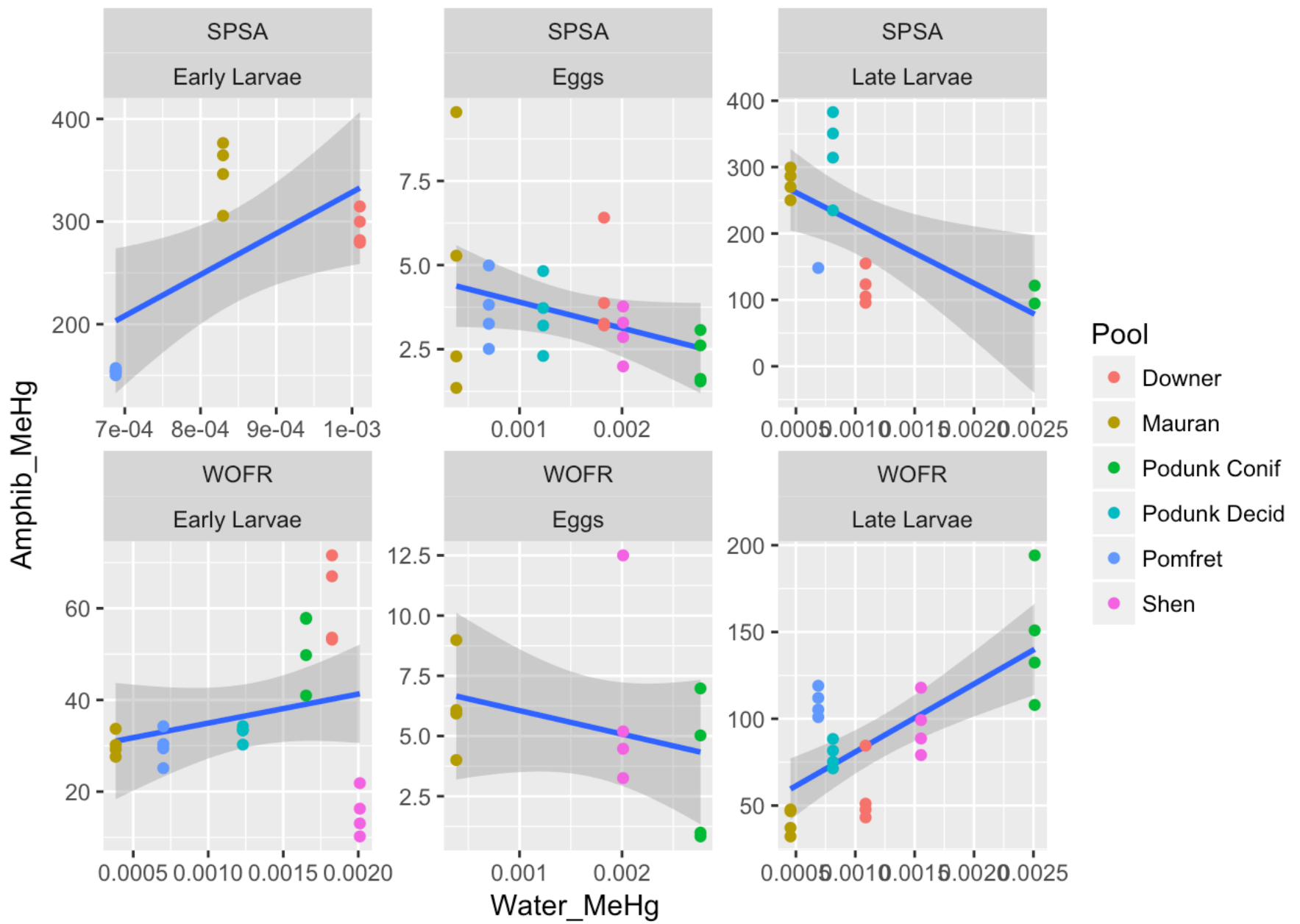


The odd pattern of higher mercury in early-stage larvae for SPSA doesn't appear to be strictly a pool effect. For example, the only two pools with early larvae sampled - Downer and Mauran - also show a decreased level of mercury in late-stage larvae.

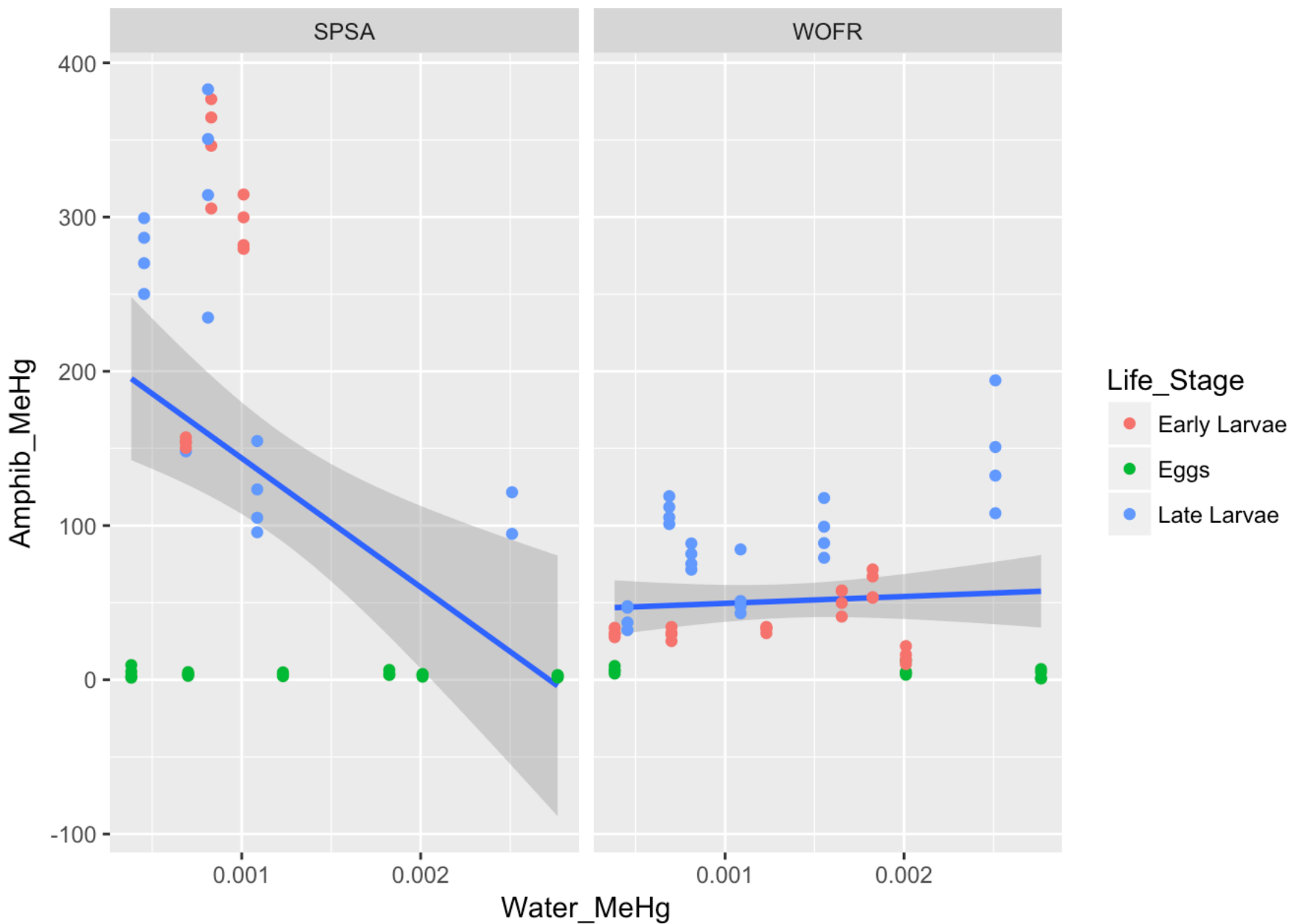
```
p8 <- ggplot(data = subset(data.df, Life_Stage != "Adult"))
p8 + geom_point(aes(x = Life_Stage, y = Amphib_MeHg, color = Pool)) +
  scale_x_discrete(limits = c("Eggs", "Early Larvae", "Late Larvae")) +
  theme(axis.text.x = element_text(angle = 340, vjust = 0.5)) +
  xlab("Life stage") + ylab("Methylmercury 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.



```
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
puFqews/model153eb310fcf00.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]  -6.12658 2.80635 -11.93649  -7.90748  -6.13713  -4.23789
## alpha[2]   2.39854 2.24151  -2.07557   0.92065   2.40264   3.87881
## beta[1]   -1.37860 0.40658  -2.21326  -1.63657  -1.37771  -1.10565
## beta[2]   -0.15374 0.32913  -0.80771  -0.36864  -0.15397   0.06465
## sigma     1.66330 0.11385   1.45914   1.58558   1.65798   1.73469
## deviance 426.53120 3.20556 422.38057 424.13509 425.81898 428.20450
##           97.5%      Rhat n.eff
## alpha[1]  -0.84947 1.00054  3000
## alpha[2]   6.77532 1.00101  3000
## beta[1]   -0.61664 1.00055  3000
## beta[2]    0.49598 1.00105  3000
## sigma     1.89383 1.00227  1100
## deviance 434.31172 1.00142  2200
##
## 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 = 431.7
## 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
puFqews/model153eb553bf5e5.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]    -0.56305  14.21700 -26.89245 -10.40181  -1.09174   8.96276
## alpha[2]     2.60303  14.16376 -23.60369  -7.23834   2.09932  12.13684
## beta.spp[1]  -0.47541   0.16251  -0.79090  -0.58509  -0.47594  -0.36416
## beta.spp[2]   0.10763   0.13079  -0.13847   0.01891   0.10409   0.19739
## beta.stage[1] 1.98970  14.16999 -25.88954  -7.73932   2.65899  11.77417
## beta.stage[2] -1.12333  14.17086 -28.82912 -10.82740  -0.49270   8.66749
## beta.stage[3] 2.49851  14.17146 -25.52409  -7.27704   3.15869  12.26252
## sigma        0.66272   0.04661   0.57913   0.63073   0.66006   0.69199
## deviance     221.66019   3.88310  216.17102  218.79067  221.02083  223.77544
##
##          97.5%      Rhat  n.eff
## alpha[1]    27.55091  1.00133   2400
## alpha[2]    30.27912  1.00126   2600
## beta.spp[1]  -0.14851  1.00136   3000
## beta.spp[2]   0.36796  1.00259    960
## beta.stage[1] 28.11009  1.00145   2100
## beta.stage[2] 24.84895  1.00144   2100
## beta.stage[3] 28.47932  1.00144   2100
## sigma        0.76176  1.00059   3000
## deviance     231.06567  1.00312    770
##
## 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 = 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
puFqews/model153eb3bcc84d0.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]    -0.42142 17.81978 -35.52128 -12.62957  -0.22595  11.66861
## alpha[2]     2.90262 17.81155 -32.74519  -9.26945   3.08278  15.08491
## beta.habitat[1] 0.88993 17.72000 -34.02339 -10.83365   0.86773  12.47705
## beta.habitat[2] 1.04731 17.72273 -33.79250 -10.67686   1.02591  12.62670
## beta.spp[1]    -0.27654 18.02063 -34.52058 -12.37324  -0.18376  11.89924
## beta.spp[2]     0.32609 18.01809 -34.04821 -11.71364   0.40000  12.58073
## beta.stage[1]   1.16022 15.88069 -29.73635  -9.76855   1.11267  12.05213
## beta.stage[2]  -1.93497 15.87998 -33.04594 -12.89266  -1.93737   8.93739
## beta.stage[3]   1.66483 15.88078 -29.20827  -9.20547   1.61299  12.59277
## beta.waterhg   -0.15461 18.02008 -35.81625 -12.41003  -0.28548  11.96364
## bpvalue        0.51500  0.49986   0.00000   0.00000   1.00000   1.00000
## sigma          0.66085  0.04596   0.57741   0.62981   0.65925   0.68990
## deviance       221.50538  4.10373 215.38427 218.48367 220.83804 223.85088
##
##          97.5%      Rhat  n.eff
## alpha[1]    35.33457 1.00133  2400
## alpha[2]    38.30630 1.00137  2300
## beta.habitat[1] 35.07896 1.00155  1900
## beta.habitat[2] 35.22109 1.00156  1900
## beta.spp[1]   35.05908 1.00095  3000
## beta.spp[2]   36.02380 1.00095  3000
## beta.stage[1] 31.90006 1.00071  3000
## beta.stage[2] 28.78026 1.00071  3000
## beta.stage[3] 32.49144 1.00071  3000
## beta.waterhg  34.04239 1.00095  3000
## bpvalue       1.00000 1.00095  3000
## sigma         0.75700 1.00138  2300
## deviance      231.43661 1.00075  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 = 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: 431.667697574029
```

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

```
## DIC for Spp*WaterHg + Stage 229.184883257031
```

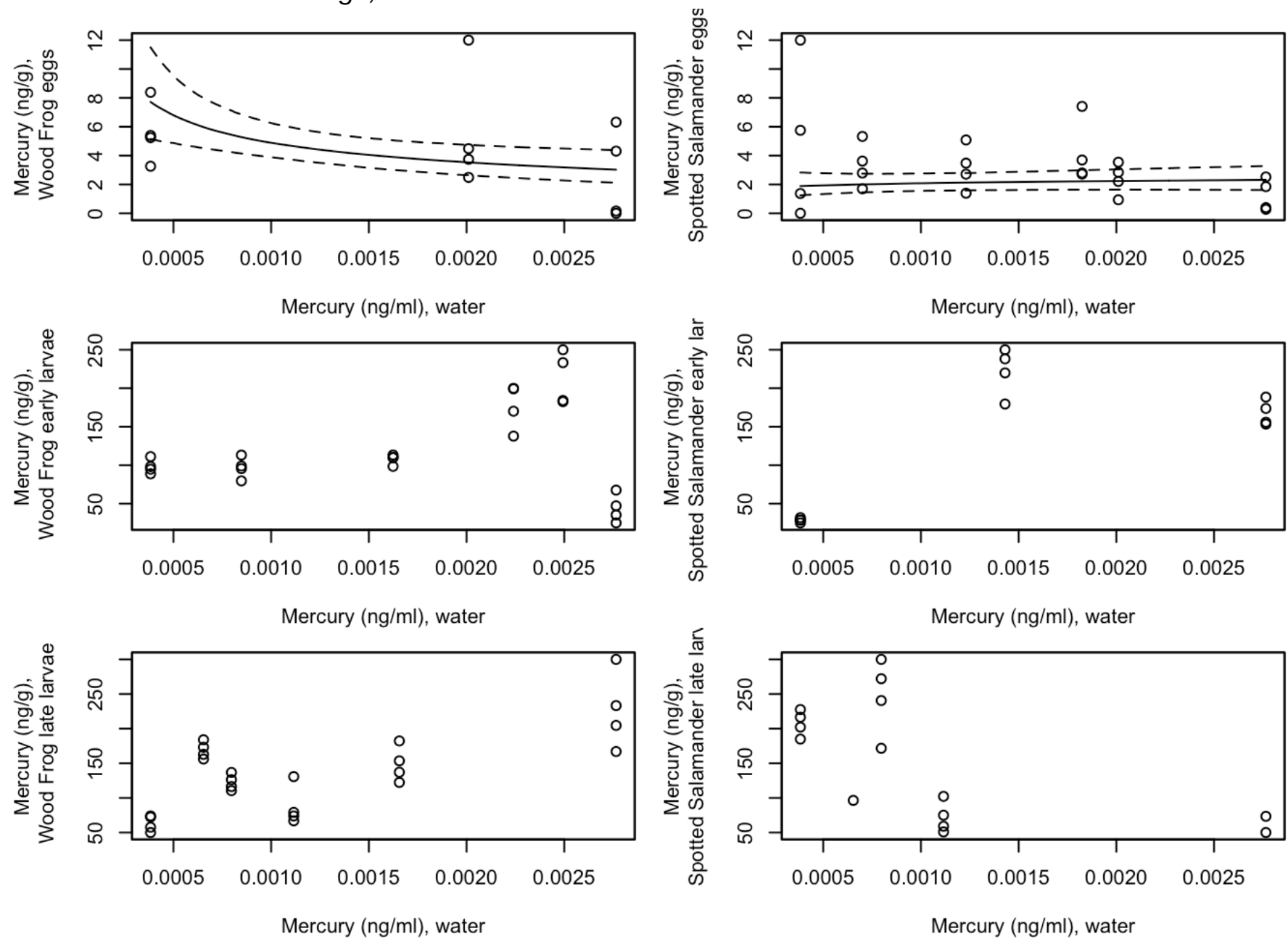
```
cat(paste(c("DIC for Spp*WaterHg + Stage + Habitat:"),jagsfitlogm3$BUGSoutput$DIC))
```

```
## DIC for Spp*WaterHg + Stage + Habitat: 229.929067090714
```

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)  $\text{Tissue\_MeHg} \sim \text{Species}$  2)  $\text{Tissue\_MeHg} \sim \text{Species} + \text{Habitat}$

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
puFqews/model153eb4ad8d359.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]  4.24504  0.12797   4.00338   4.15950   4.24658   4.32337   4.50364
## alpha[2]  3.59085  0.12435   3.34293   3.50829   3.59008   3.67614   3.83177
## delta     33.78761 10.21253  15.31855  26.89030  33.53968  40.15542  55.03394
## sigma      0.61955  0.06611   0.50399   0.57330   0.61504   0.65708   0.76339
## spsa      70.33442  9.09817  54.78299  64.03957  69.86636  75.44255  90.34561
## wofr      36.54680  4.57547  28.30196  33.39129  36.23680  39.49379  46.14407
## deviance  90.46918  2.63467  87.54111  88.54297  89.78061  91.65659  97.08799
##           Rhat n.eff
## alpha[1] 1.00066  3000
## alpha[2] 1.00094  3000
## delta     1.00052  3000
## sigma     1.00074  3000
## spsa      1.00067  3000
## wofr      1.00091  3000
## deviance  1.00075  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 = 3.5 and DIC = 93.9
## 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
puFqews/model153eb5c3f802c.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]  4.13220 0.17664  3.79133  4.01421  4.13194  4.24905
## group.mean[2,1]  3.79510 0.17698  3.43272  3.67753  3.79764  3.91604
## group.mean[1,2]  4.36424 0.17613  4.02386  4.24604  4.36425  4.47950
## group.mean[2,2]  3.39535 0.17098  3.05665  3.27783  3.39343  3.50971
## sigma           0.61026 0.06639  0.50172  0.56169  0.60388  0.65124
## deviance         88.85388 3.40506 84.45690 86.41542 88.15288 90.55366
##
##          97.5%      Rhat n.eff
## group.mean[1,1]  4.47209 1.00075  3000
## group.mean[2,1]  4.14328 1.00131  2500
## group.mean[1,2]  4.71241 1.00088  3000
## group.mean[2,2]  3.72641 1.00096  3000
## sigma           0.76129 1.00112  3000
## deviance         97.11096 1.00141  2200
##
## 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 = 5.8 and DIC = 94.6
## 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: 93.9413803248553
```

```
## DIC, Model 2: 94.6499606488611
```

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: 33.7876136557854
```

```
## 95% CI: 15.3185483740378 - 55.0339382413978
```



## 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.

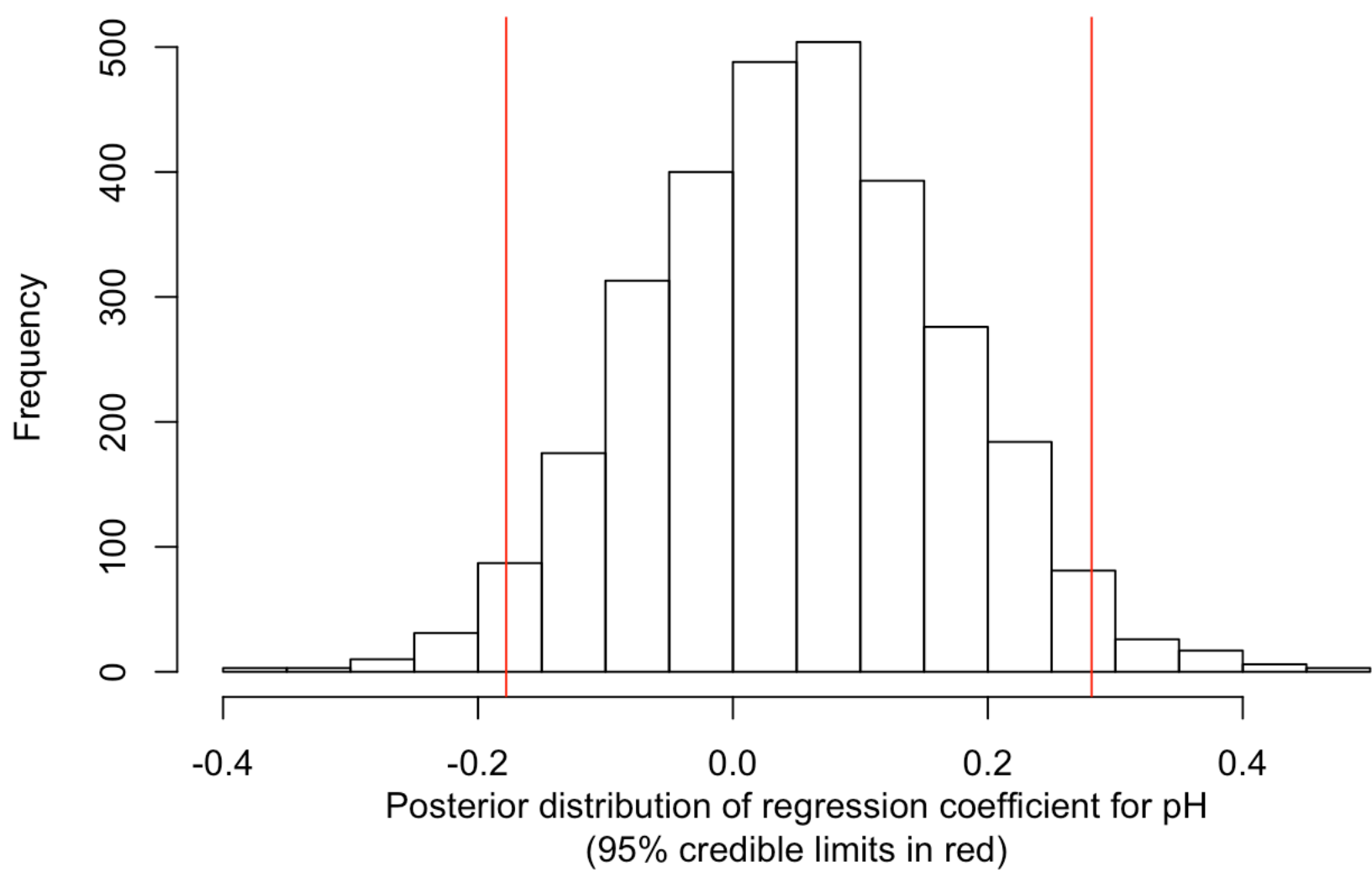
Among adults, tissue methylmercury varied among species. Spotted Salamanders had higher average loads than did Wood Frogs. Habitat did not explain variation in mercury loads among samples.

## 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 methylmercury 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).



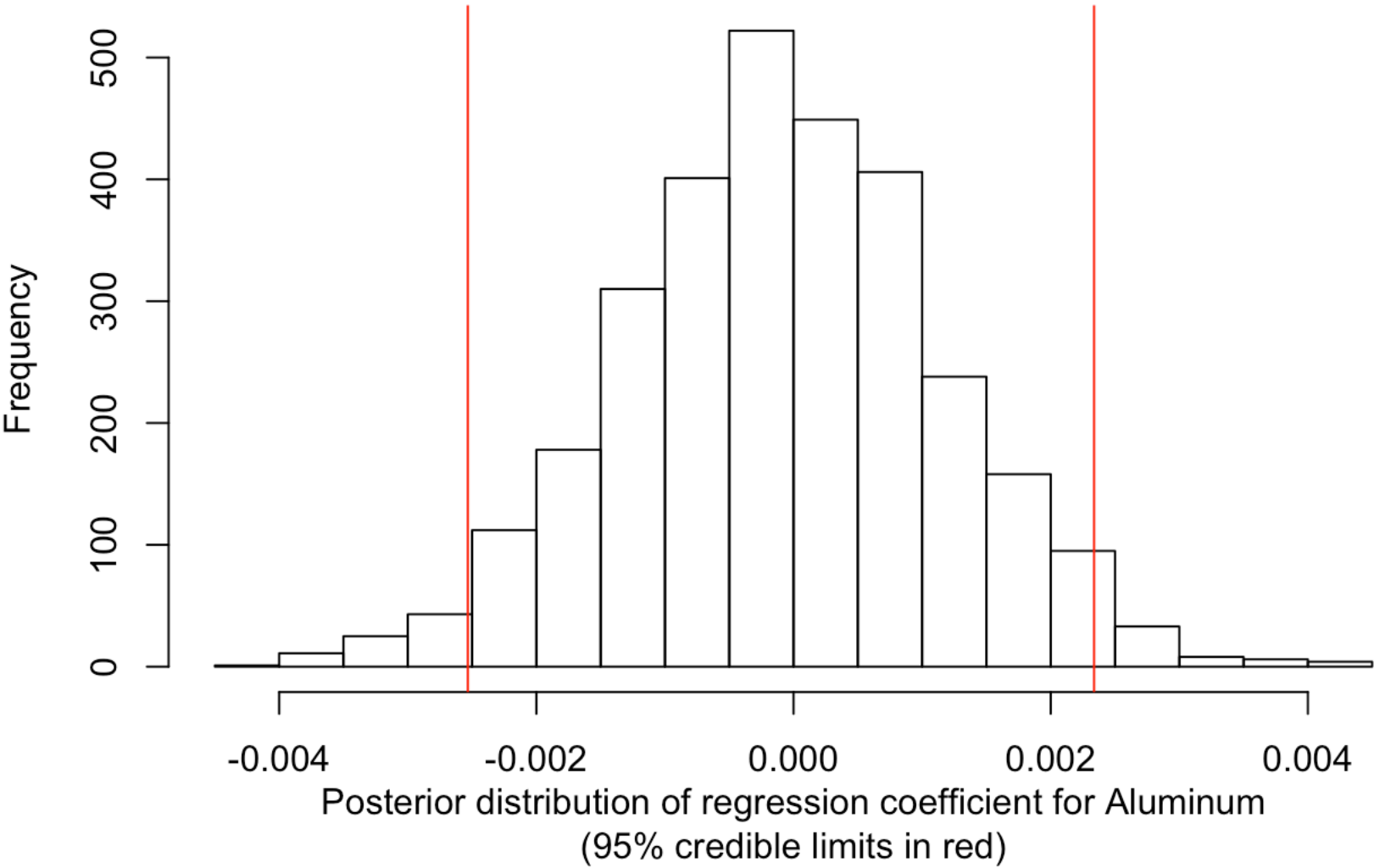
```
## DIC for Spp*WaterHg + Stage 229.184883257031
```

```
## DIC for Spp*WaterHg + Stage + pH: 231.465156112804
```

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
```

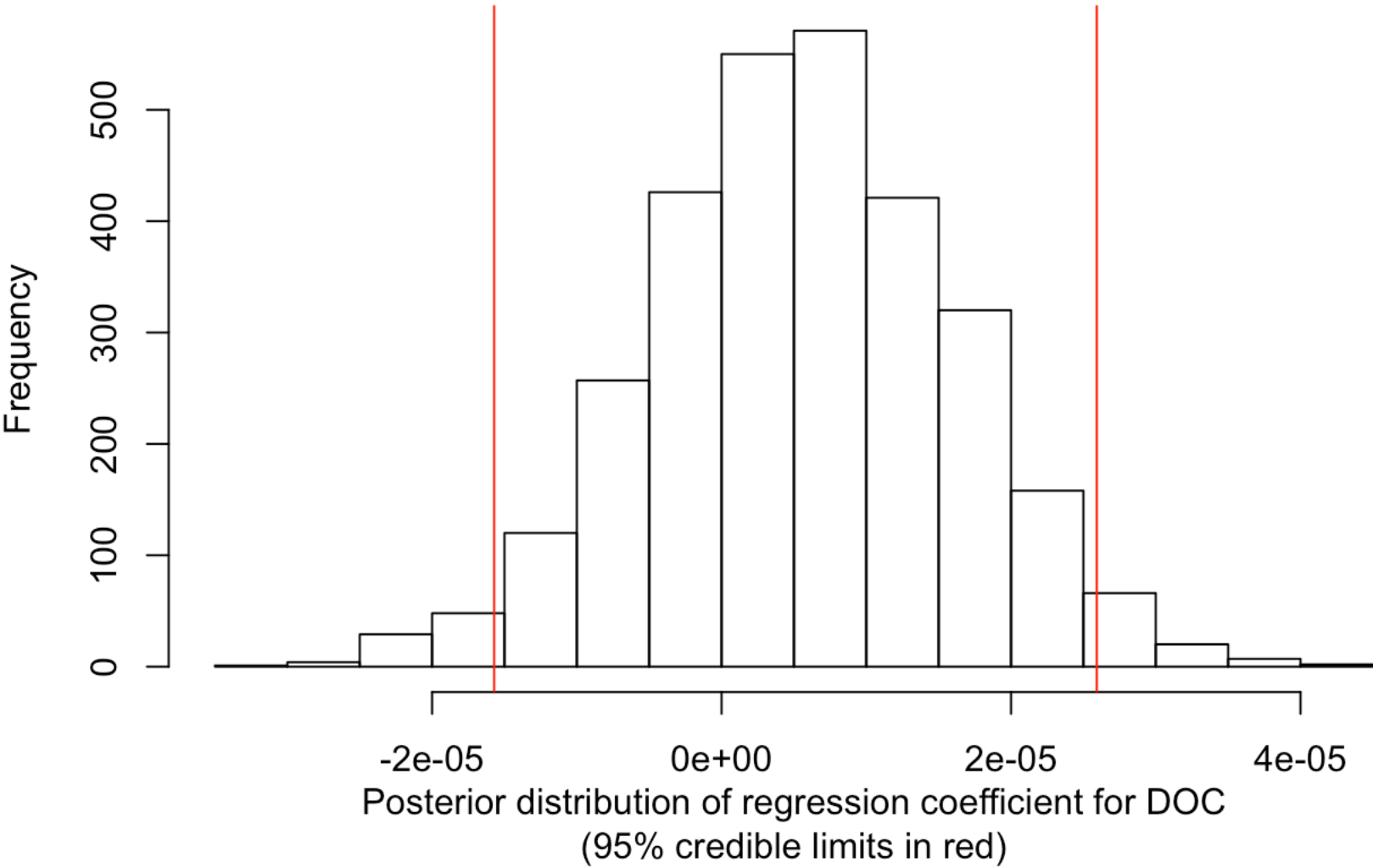


```
## DIC for Spp*WaterHg + Stage 229.184883257031
```

```
## DIC for Spp*WaterHg + Stage + Al: 231.0520577986
```

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
```

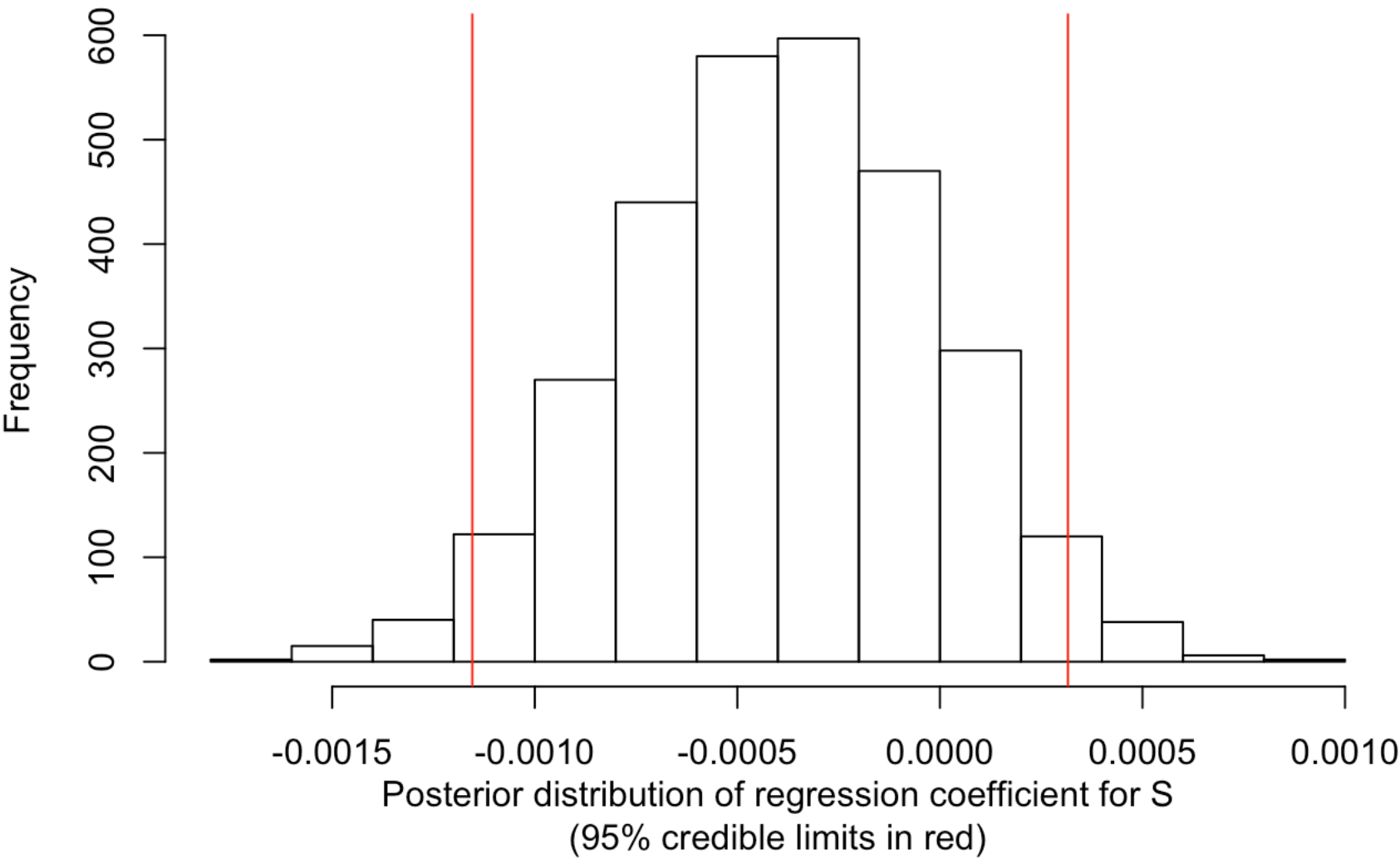


```
## DIC for Spp*WaterHg + Stage 229.184883257031
```

```
## DIC for Spp*WaterHg + Stage + DOC: 230.90269784412
```

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
```

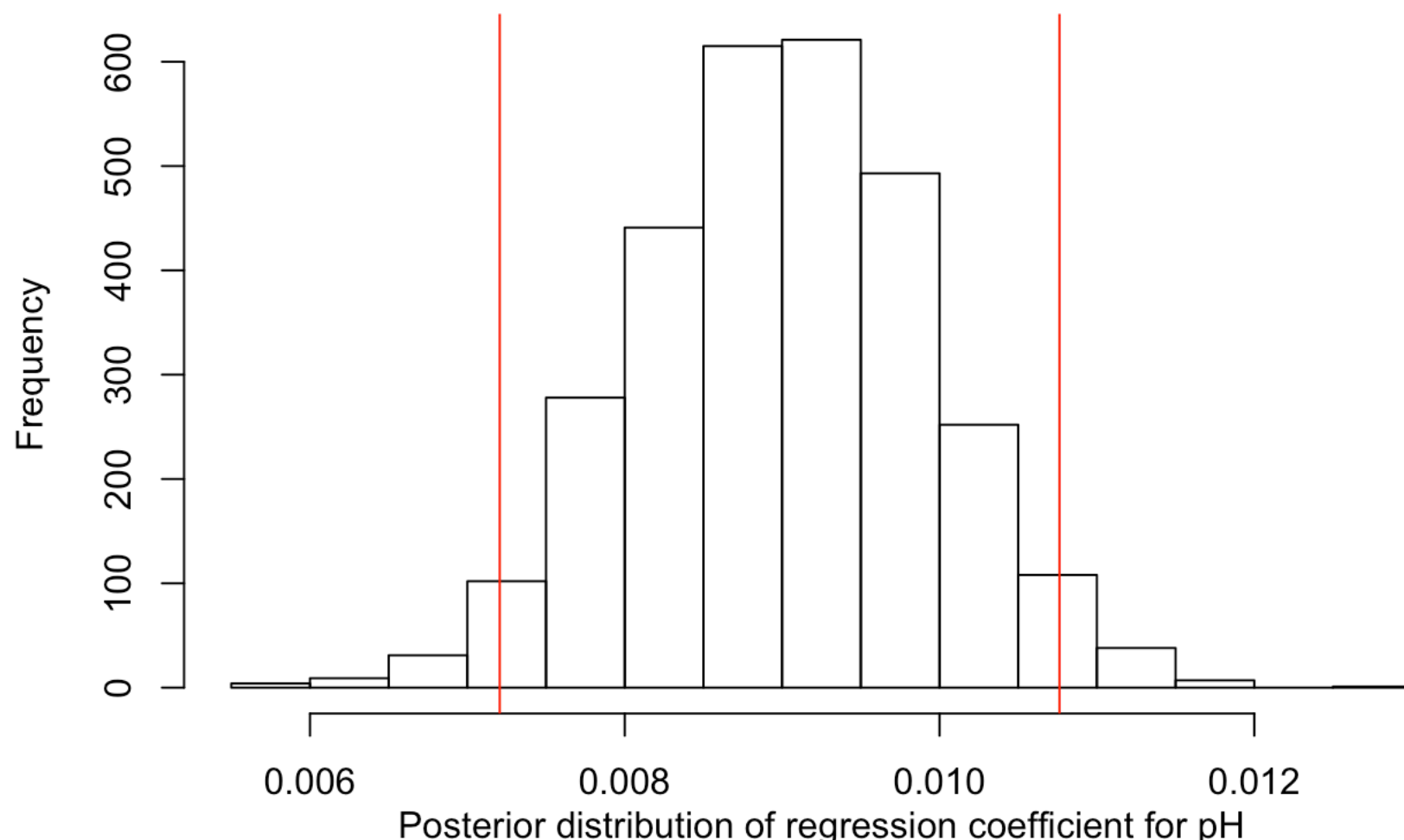
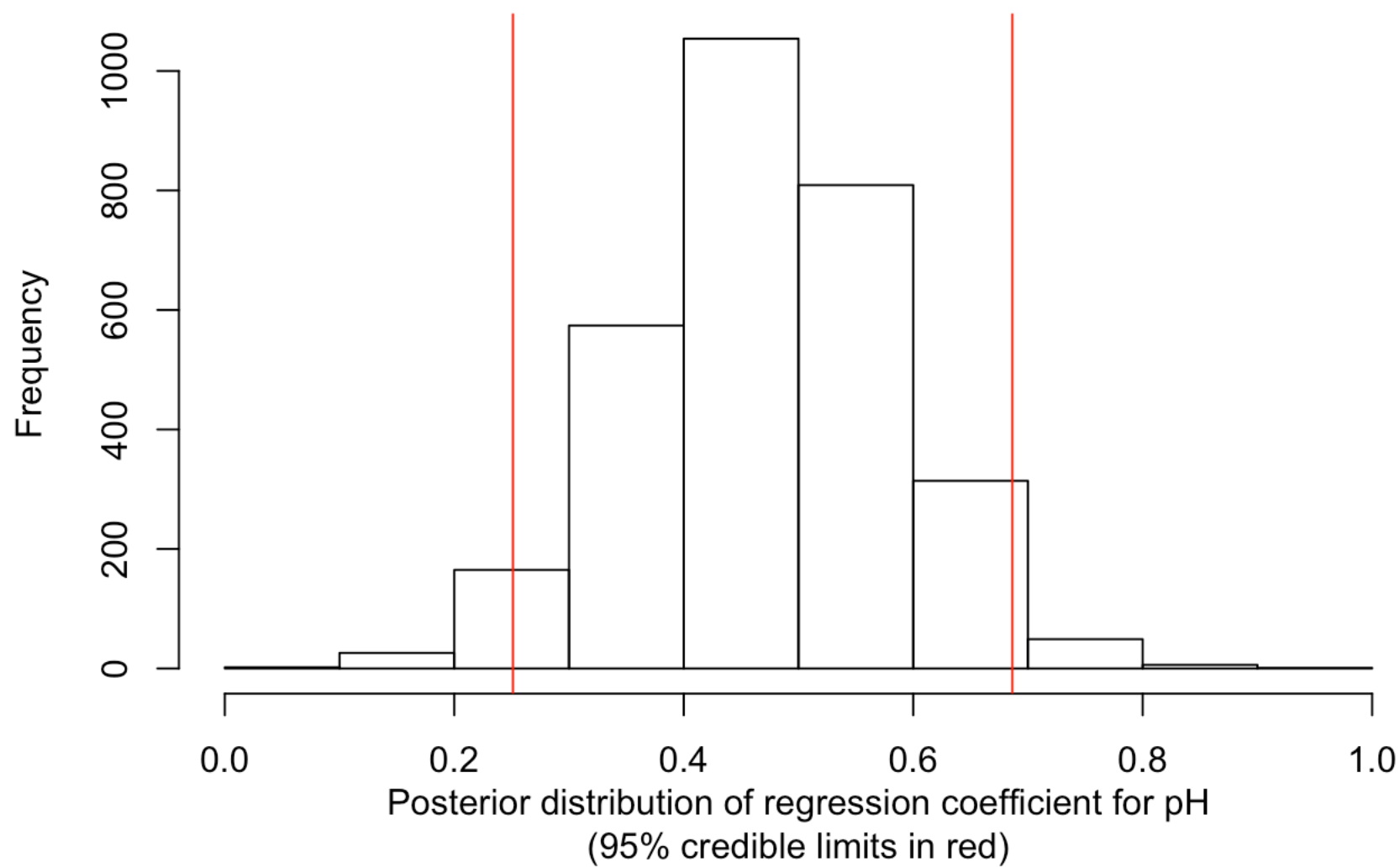


```
## DIC for Spp*WaterHg + Stage 229.184883257031
```

```
## DIC for Spp*WaterHg + Stage + S: 230.239975045208
```

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
```

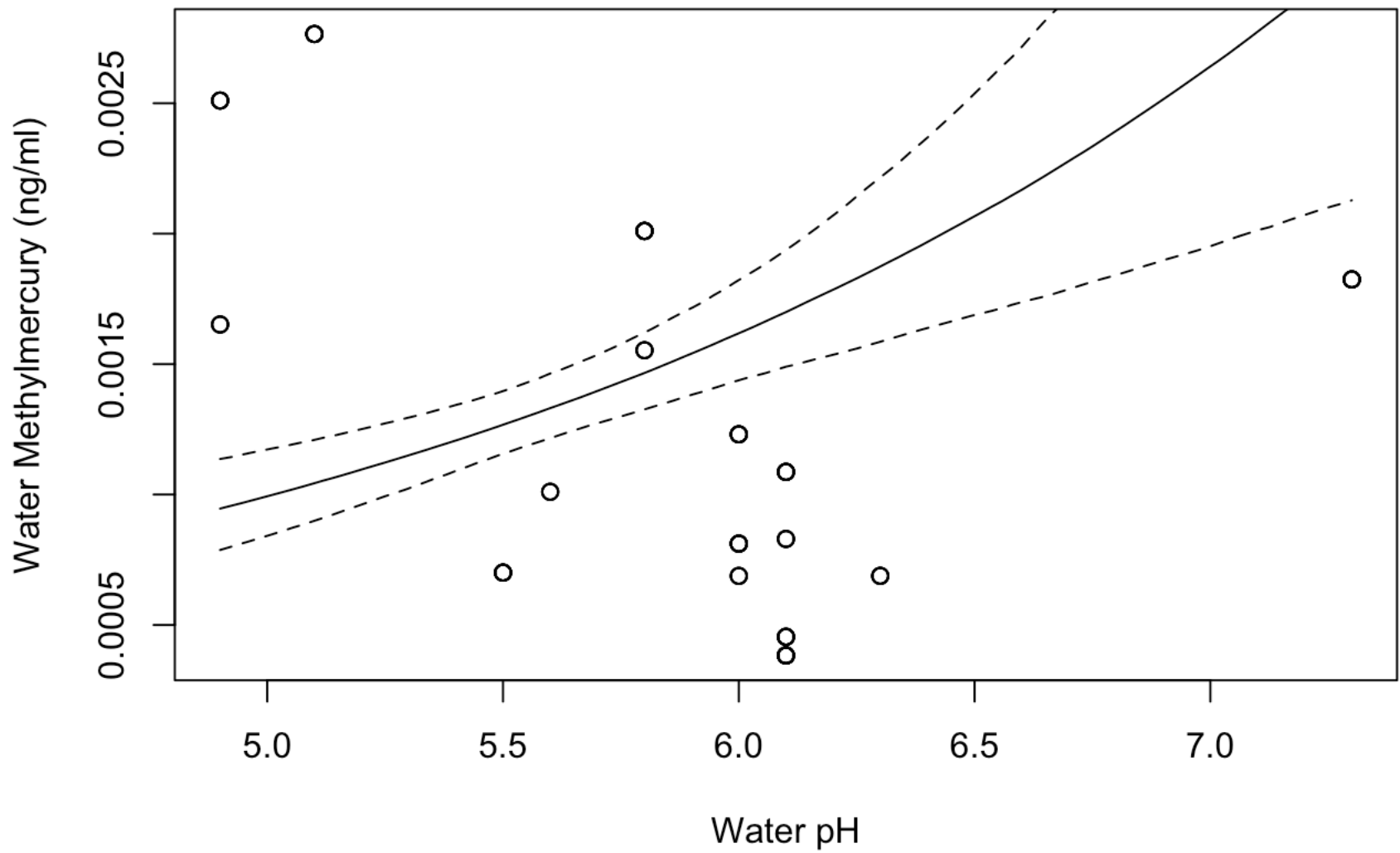


(95% credible limits in red)

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):

