

1 A Retrospective Analysis of Mussel Monitoring in the
2 Puget Sound

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10 **Introduction**

11 The purpose of this report is to provide a retrospective analysis of data generated by pre-
12 vious mussel monitoring surveys coordinated under Washington Department of Fish and
13 Wildlife's (WDFW) Toxics Biological Obsevation System (TBiOS). We determine how ex-
14 isting historical bay mussel (*Mytilus trossulus*) contaminant data can be used for in a Toxics
15 in the Nearshore Vital Sign indicator. In addition, we assess the predictive ability of existing
16 sampling rate to predict expected contaminant trends.

17 Toxics data was obtained by transplanting relatively uncontaminated mussels from a local
18 aquaculture source to locations along the Puget Sound shoreline, covering a broad range
19 of upland land-use types from rural to highly urban. Mussels were then recovered, and
20 concentrations of several major contaminant classes were measured. Four mussels surveys
21 were performed, with mussels being retrieved in 2013, 2016, 2018, and 2020. Our analysis
22 focuses on polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PB-
23 DEs), and polychlorinated biphenyls(PCBs) due to their significance in both ecosystem and
24 human health.

25 All materials used to prepare this report can be found in the following GitHub repository:
26 https://github.com/cwangen/mussel_toxics/.

²⁷ **Methods**

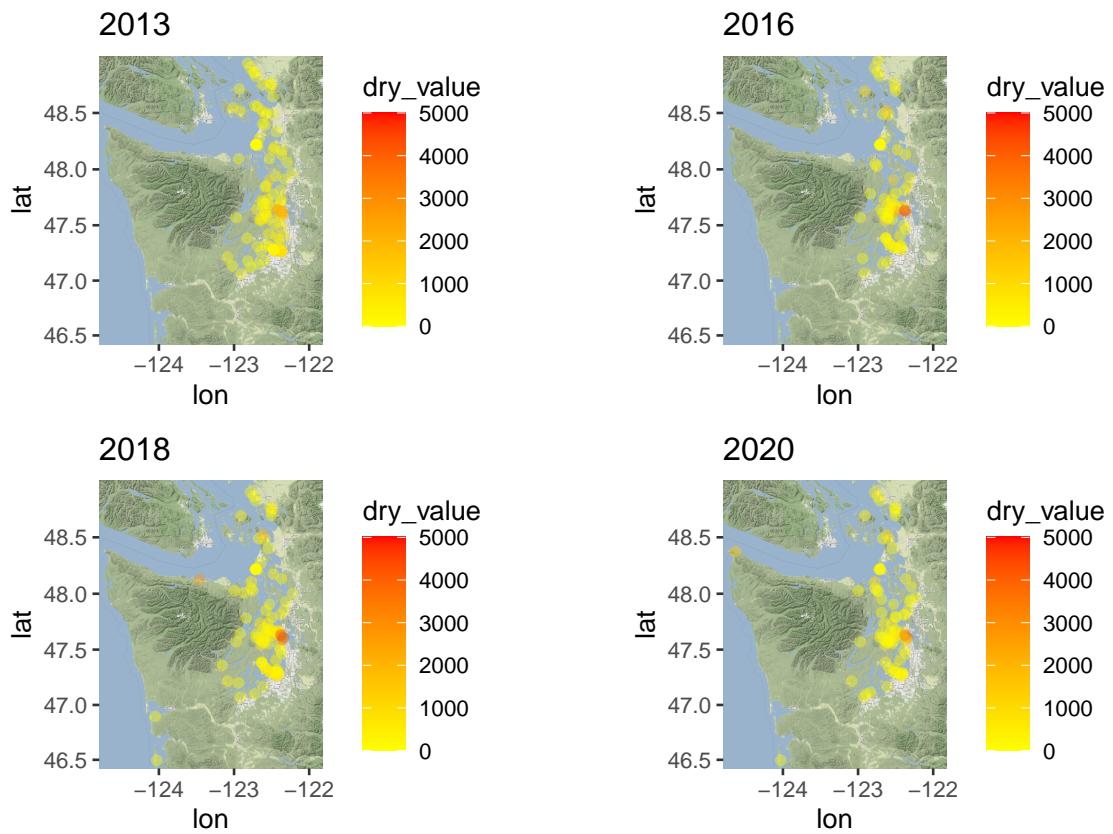
²⁸ **Data Analysis**

²⁹ The data used in this analysis originated in the form of an Microsoft Excel file, titled “2013-20MusselCagesPOPsPAHs_Cnty_WRIA_LIO_Coverages.xlsx.” The data
³⁰ included more fields than used in our analysis, and can be found in its entirety in “~mussel_toxics/data/raw/.” The data was cleaned in order to correct minor inconsistencies and
³¹ errors, resulting in “~mussel_toxics/data/clean/totals_all.csv.”

³⁴ Dry weights of toxics found in each sample were used for analysis. Though samples did record
³⁵ the concentration of lipids, as well as wet weight, dry weight is the standard for reporting
³⁶ toxicology data. Summary tables were created for dry weights and categorical variables. Dry
³⁷ weight concentrations were also plotted on maps. Raincloud plots were for subcategories of
³⁸ WRIA and year. Raincloud plots consist of a box plot, an approximate probability density,
³⁹ and individual data points.

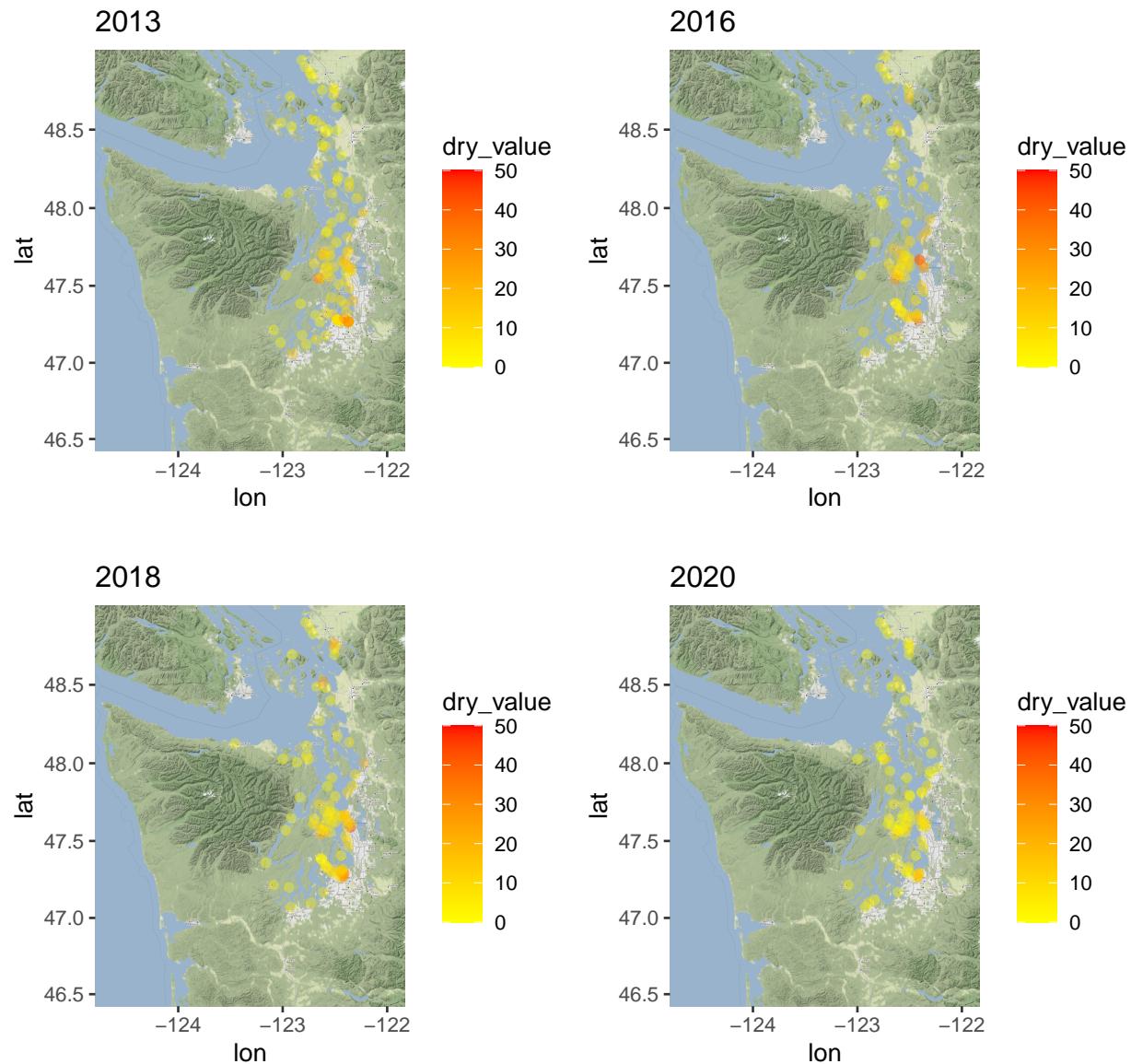
⁴⁰ Samples from the original aquaculture source used as reference were removed except for
⁴¹ the creation of maps. Samples outside of Puget Sound (latitude less than -123.5) were also
⁴² removed from sampling, but remain in map figures. Figures ??, ??, and ?? display the
⁴³ concentrations of PAHs, PBDEs, and PCBs over time in mussel samples. They also shed
⁴⁴ light on how sampling is inconsistent both spatially and temporally.

PAHs over time

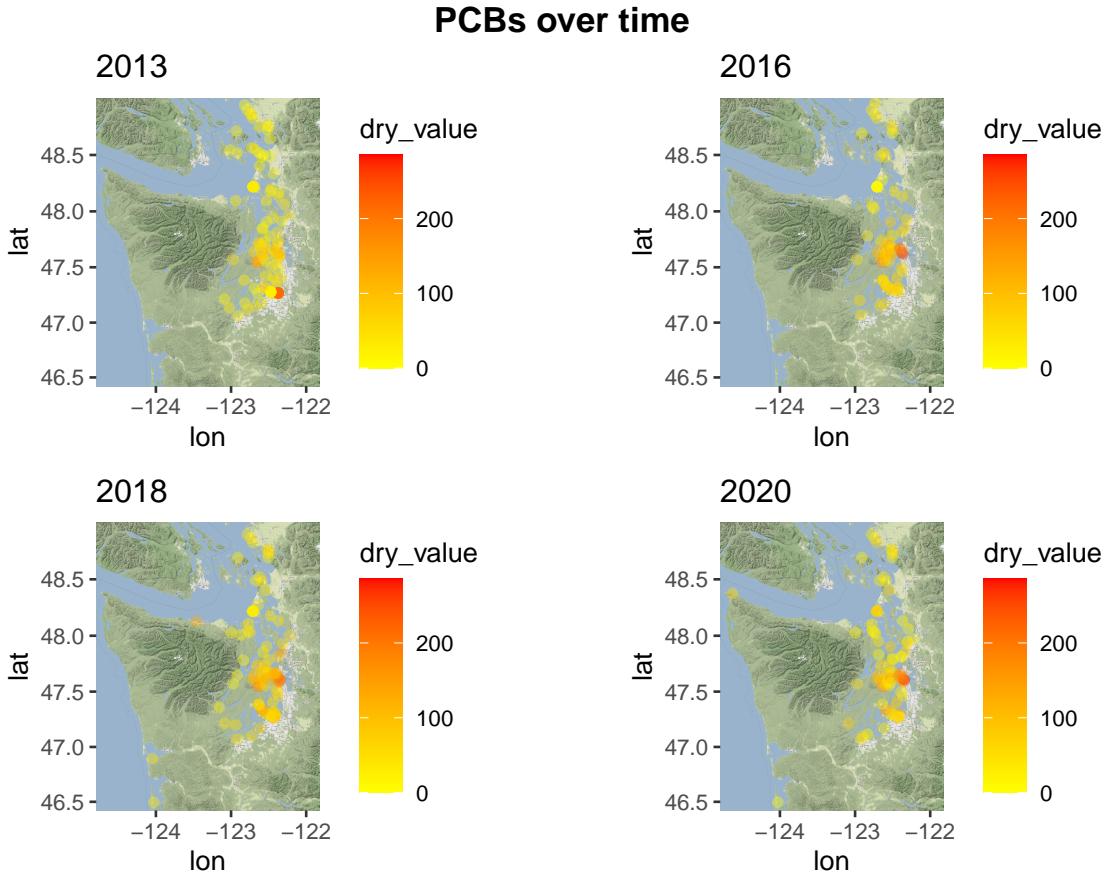


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PBDEs over time



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48 Modeling

49 We modeled the dry weight of the analytes found in mussels using a linear mixed model
 50 (LMM). These models consist of fixed effects, which remain constant, and random effects
 51 that follow a normal distribution and can correspondingly vary by individual. Our goals
 52 were to evaluate 1) The effect of year on dry weight of the relevant toxic analyte 2) If any
 53 other factors significantly affected dry weight, and 3) the effect of WRIA by year.

54 Variables considered in analysis included time (of mussels spent in water), latitude, lon-
 55 gitude, year, county, LIO, WRIA, and mean percent AU. Other extraneous variables (IE,
 56 funding source) were not used as there is no possible relationship between these and analyte
 57 content, though this information could be helpful when we begin to focus on future sampling
 58 plans in future reports. Exploratory analysis of the data allowed us to remove extraneous
 59 factors, while including those we wished to investigate. Models were created for each of the
 60 independent analytes as their nature and distribution should not be assumed to be the same.

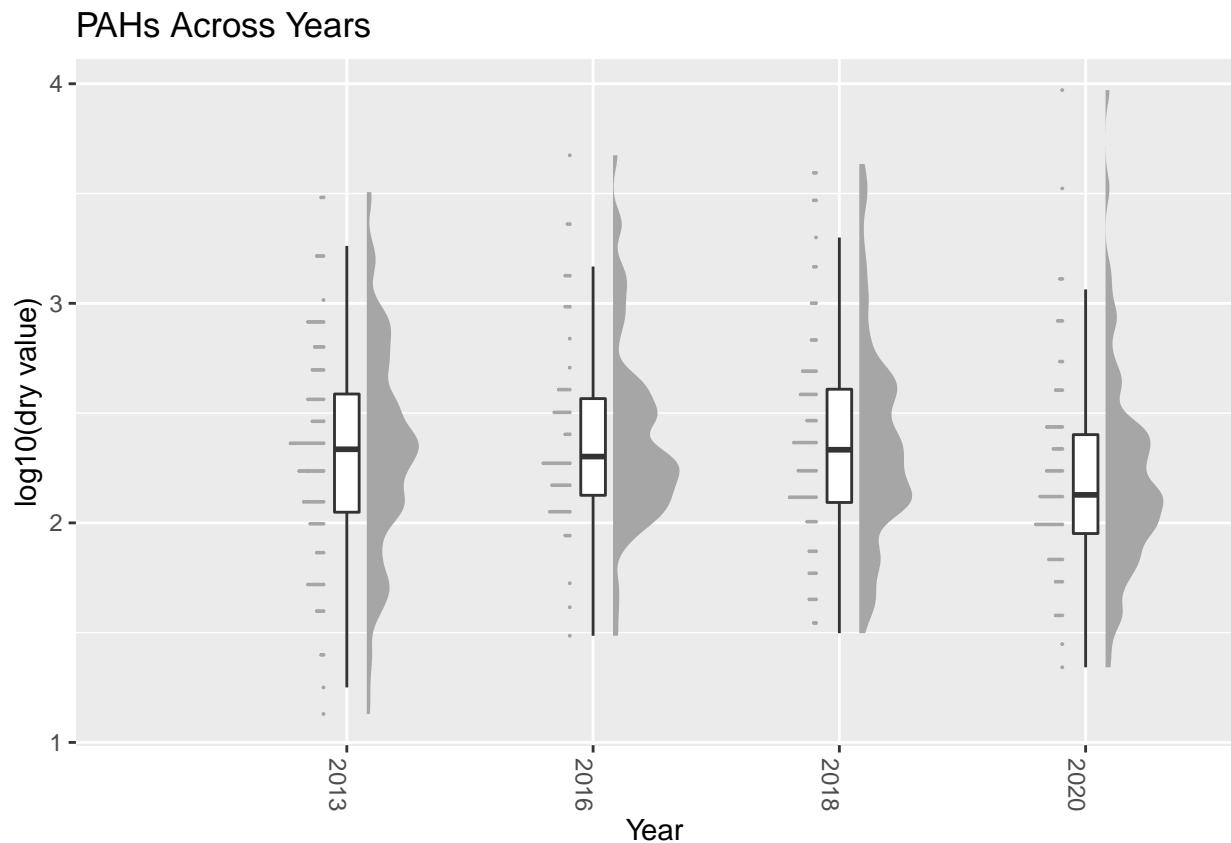
61 Initial model selection occurred by comparing Akaike information criterion (AIC) of all
 62 feasible possible models. Models that were unsuitable due to singular fits (overfitting) or
 63 violations or model assumptions such as non-normality were removed. The final model was

64 chosen by taking into account AIC, noninfringement of model assumptions, and variables
65 critical to project goals.

66 Results and Discussion

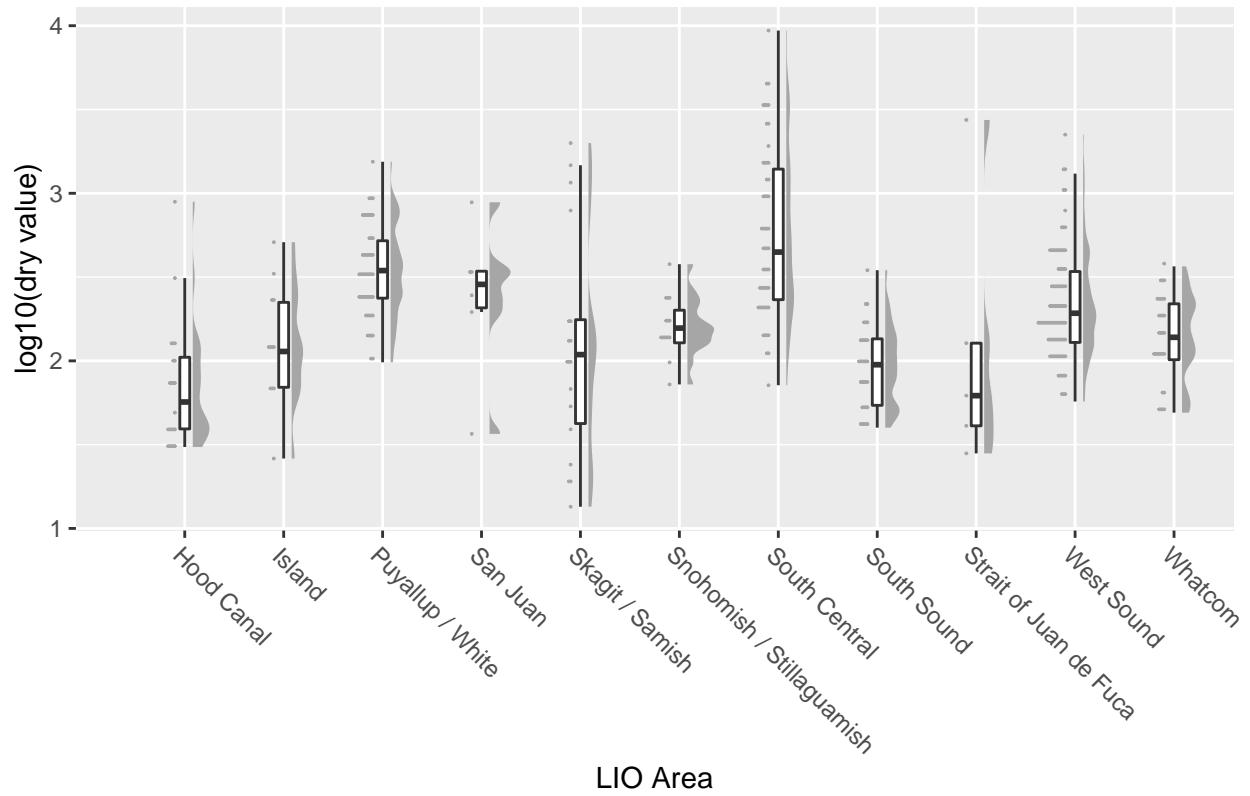
67 Data Analysis

68 Here's the code that makes these



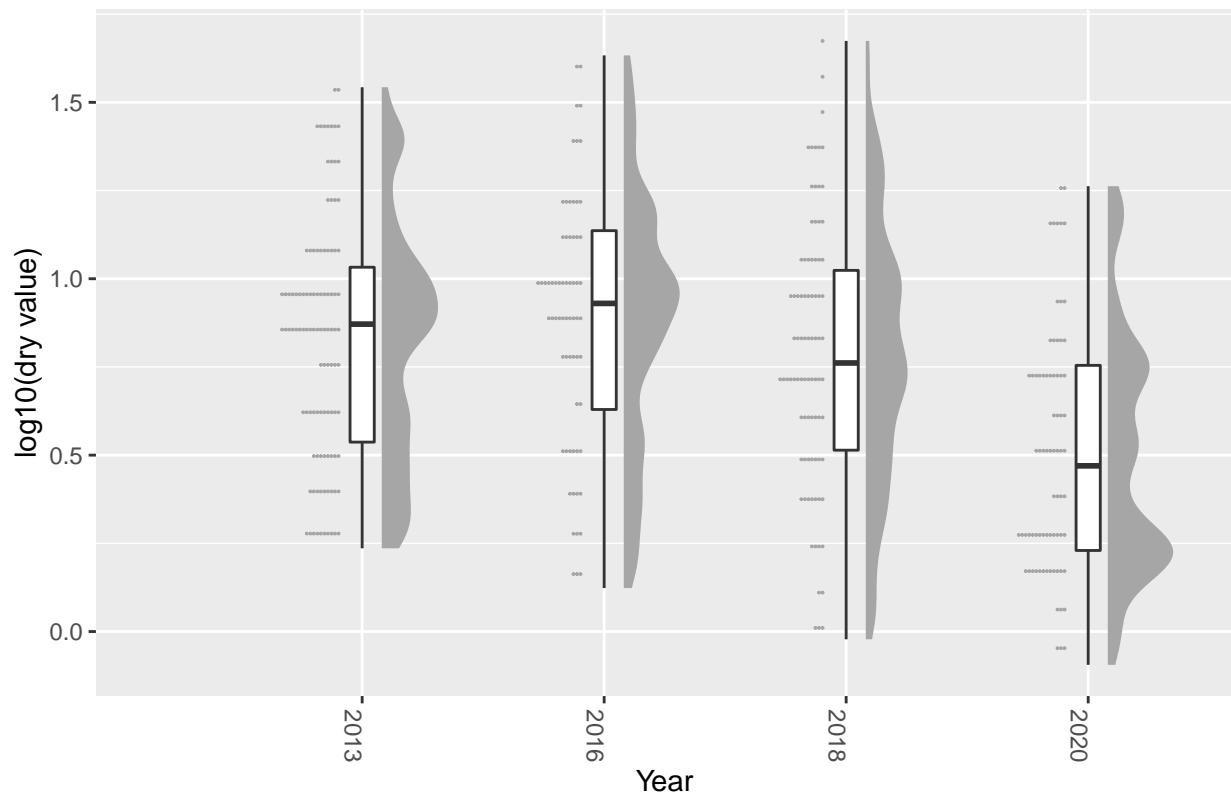
69

PAHs Across LIOs



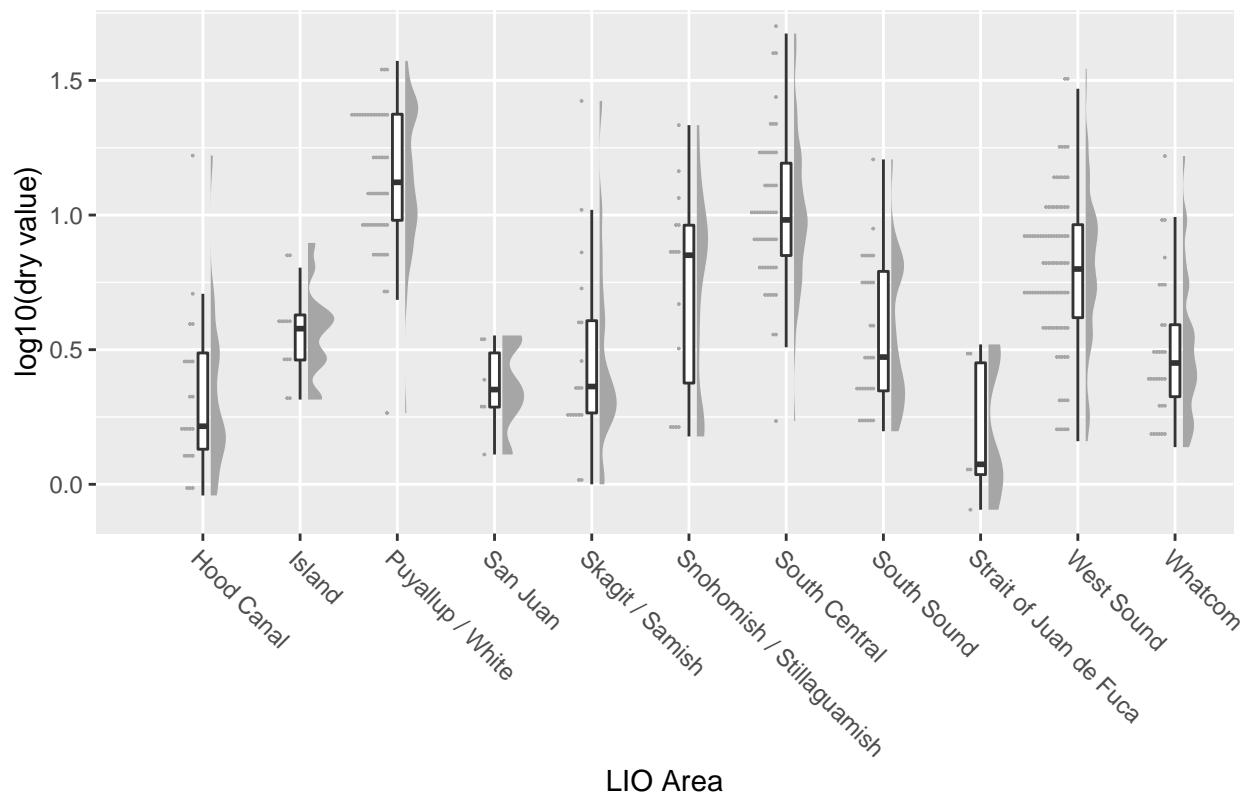
70

PBDEs Across Years



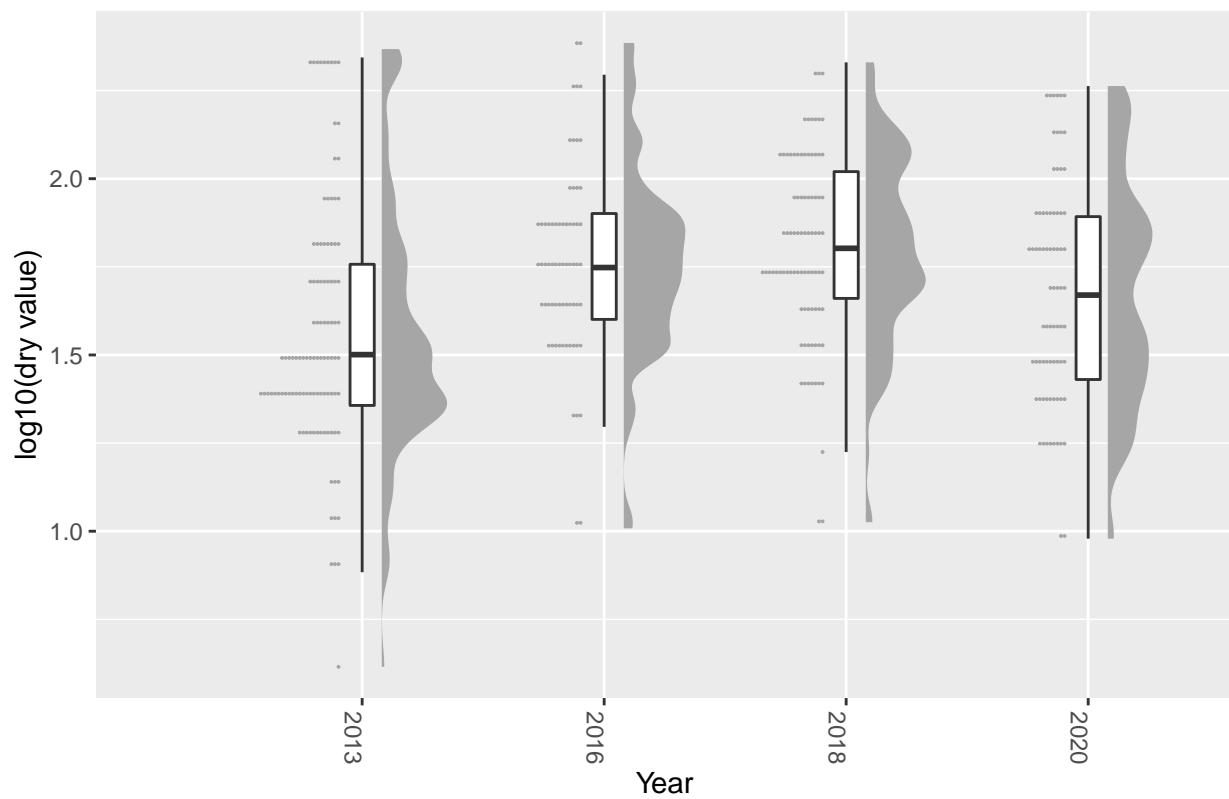
71

PBDEs Across LIOs



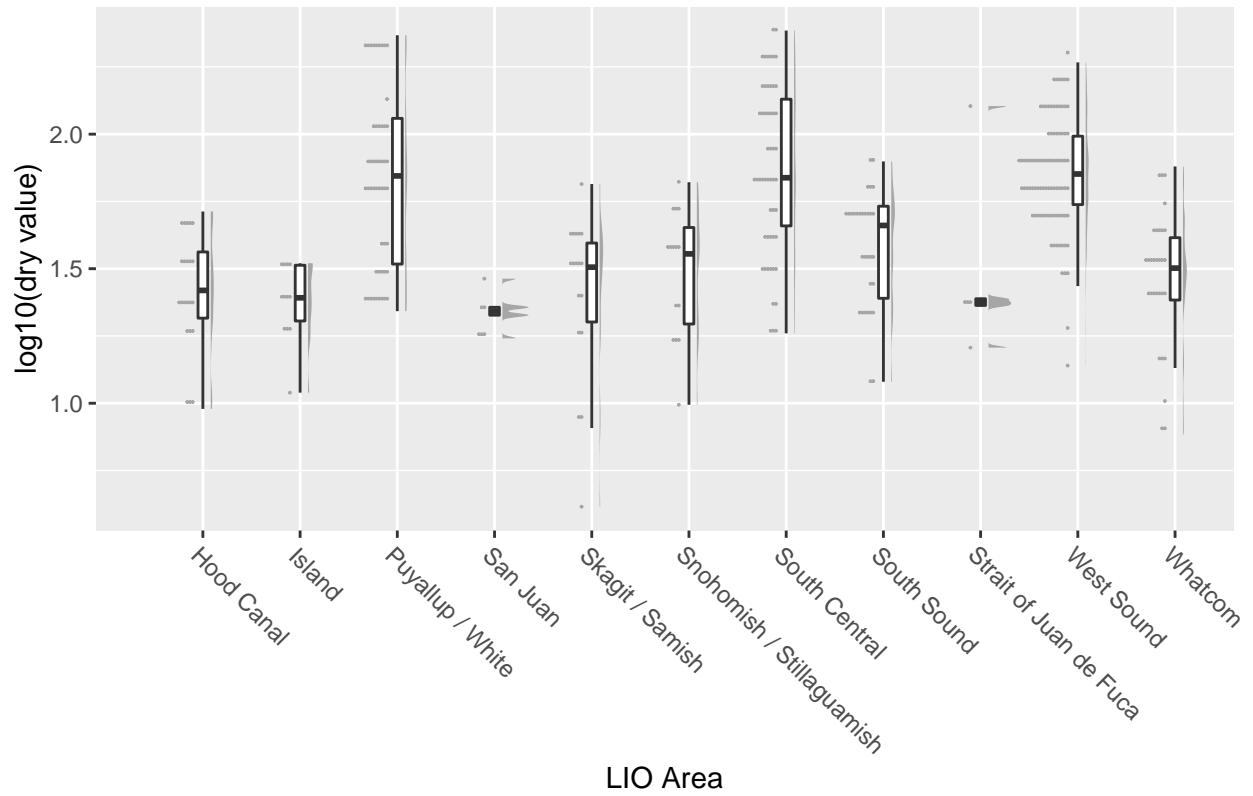
72

PCBs Across Years



73

PCBs Across LIOs



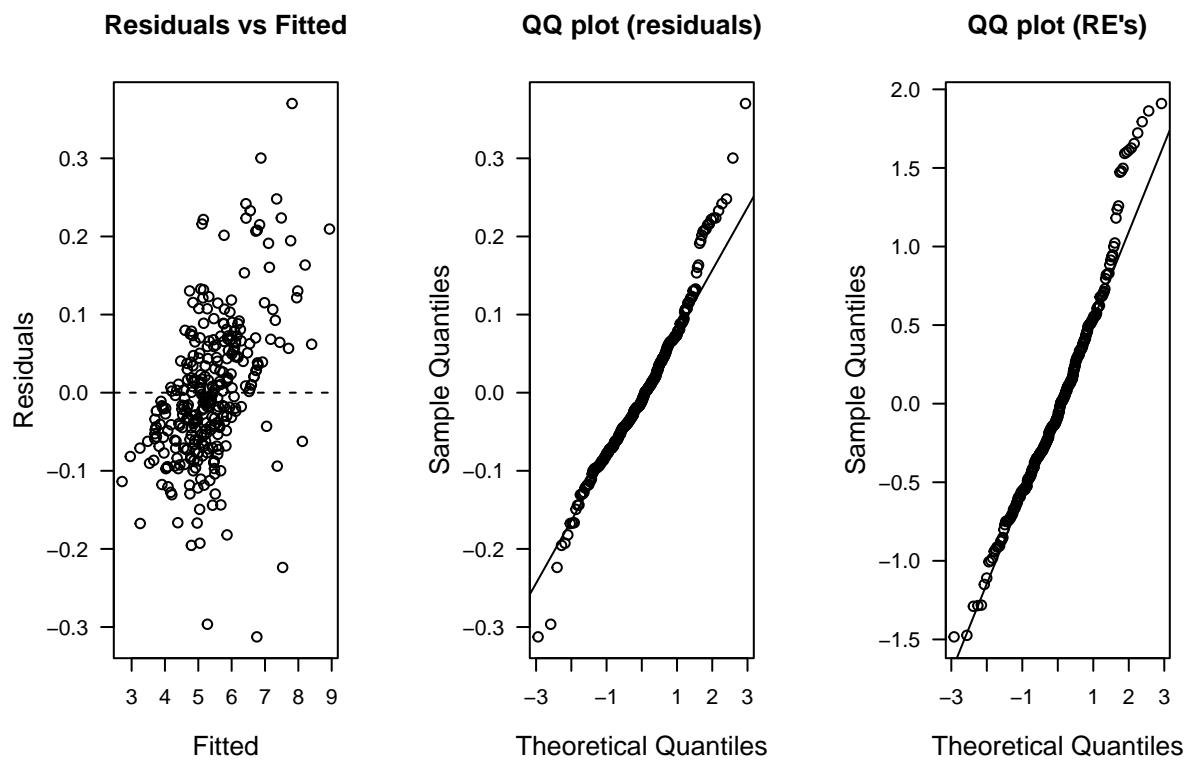
74

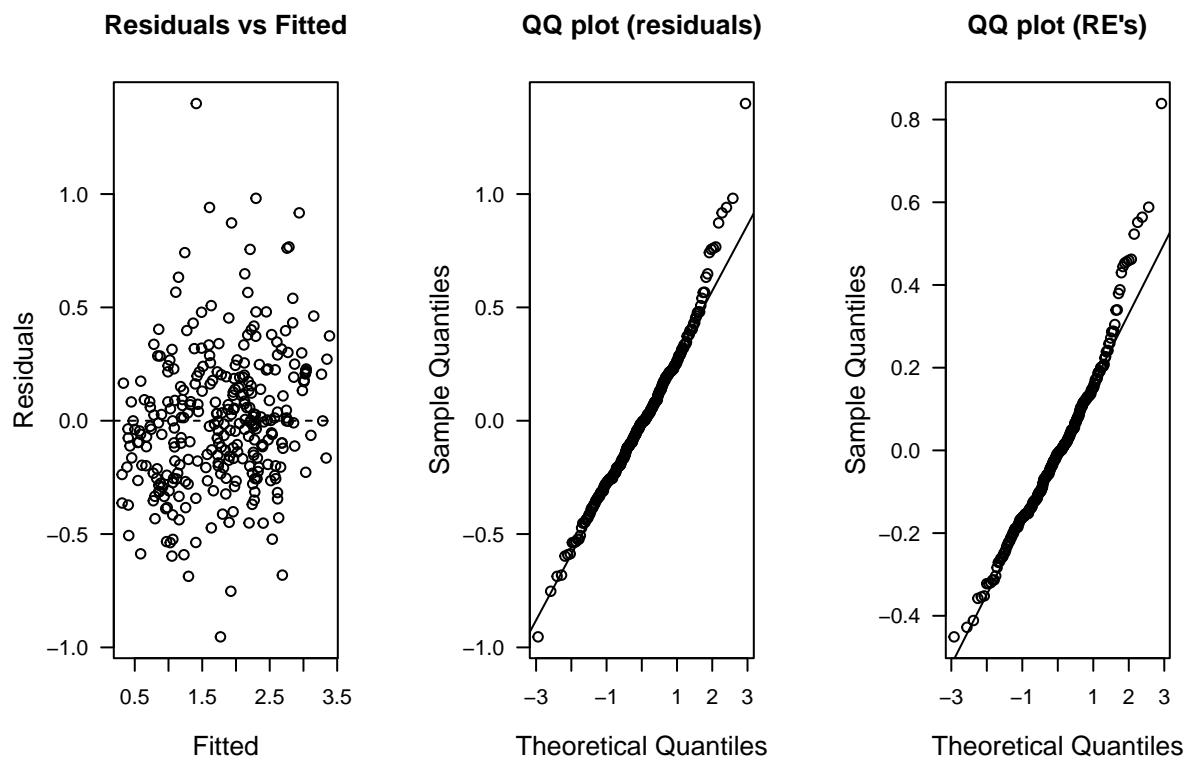
75 Modelling

76 The final model takes the form,

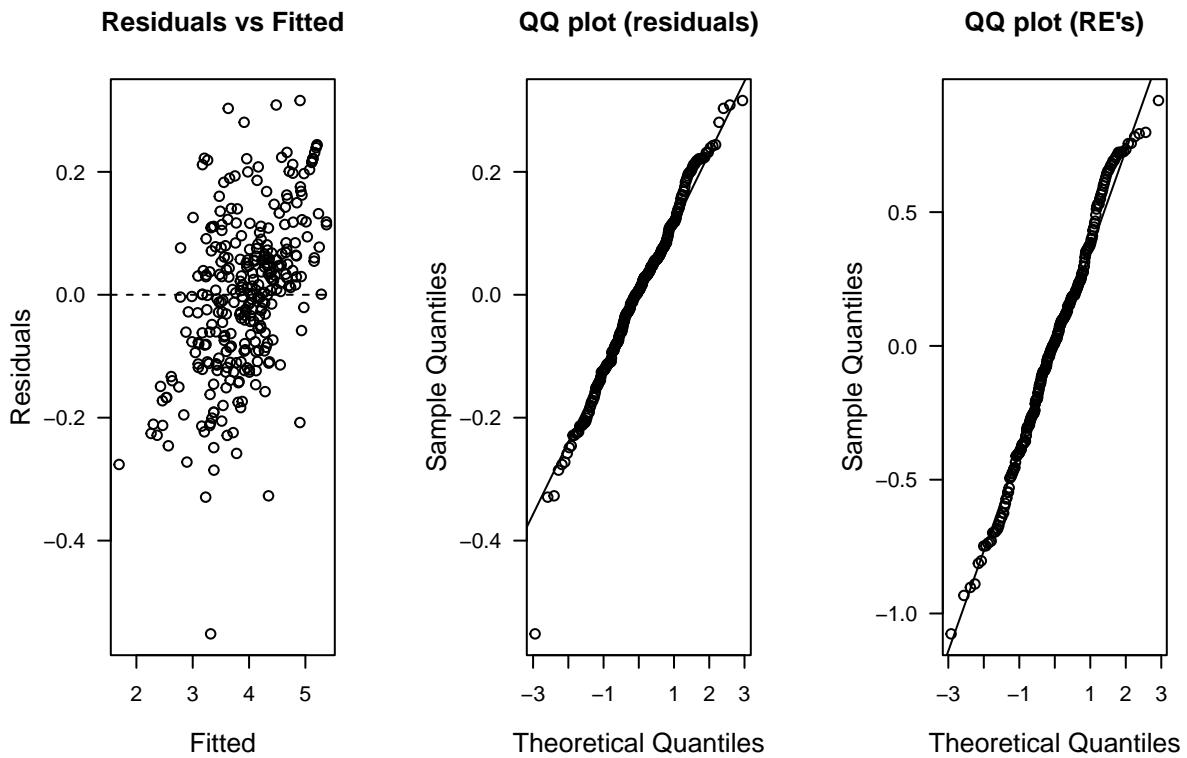
$$D_i = \beta_{1,year} + \beta_{2,year \times LIO} + \beta_3 \text{surface}_i + \beta_4 \text{time}_i + \mu_{i,j} + \epsilon_i \quad (1)$$

77 Where D_i is the natural logarithm of the dry weight of the analyze from a sample site, β_1 is
 78 the categorical effect of year, β_2 is the interaction effect of year and LIO, β_3 is the coefficient
 79 for mean percent AU of the nearest watershed region, β_4 is the coefficient for the time the
 80 mussels remained in the water (which varied by sampling year), μ is the random effect of
 81 longitude nested in LIO $\mu_i \sim N(0, \sigma_\mu^2)$, and ϵ is the error distributed $\epsilon_i \sim N(0, \sigma_\epsilon^2)$.





83



84

```

85 ## Warning in aictab.AIClmerMod(cand.set = models, modnames = model.names, :
86 ## Model selection for fixed effects is only appropriate with ML estimation:
87 ## REML (default) should only be used to select random effects for a constant set of fix

```

	Random Effect of Model	AIC	Delta AIC
5	(1 longitude)	731.4414	0.000000
2	(1 LIO/longitude)	733.4414	2.000000
4	(1 latitude)	739.3850	7.943634
1	(1 LIO/latitude)	741.3850	9.943634
3	(1 LIO)	763.8950	32.453640

	npar	Sum Sq	Mean Sq	F value
time	1	0.0228454	0.0228454	0.3815897
year	3	2.1121122	0.7040374	11.7596479
mean_is_au	1	11.7407703	11.7407703	196.1079397
year:lio_areas	28	6.3306777	0.2260956	3.7765111

```

88 ## Warning in aictab.AIClmerMod(cand.set = models, modnames = model.names, :
89 ## Model selection for fixed effects is only appropriate with ML estimation:
90 ## REML (default) should only be used to select random effects for a constant set of fix

```

	Random Effect of Model	AIC	Delta AIC
5	1 longitude)	566.8031	0.0000000
4	1 latitude)	567.7357	0.9326201
2	1 LIO/longitude)	568.8031	2.0000000
1	1 LIO/latitude)	569.7357	2.9326201
3	1 LIO)	569.8586	3.0555193

	npar	Sum Sq	Mean Sq	F value
time	1	0.1609362	0.1609362	0.9750445
year	3	23.7643340	7.9214447	47.9926976
mean_is_au	1	39.6328810	39.6328810	240.1189370
year:lio_areas	28	34.5947127	1.2355255	7.4855285

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
PB_notime	35	497.4085	627.9620	-213.7042	427.4085	NA	NA	NA
PB_LMM	36	496.2293	630.5128	-212.1146	424.2293	3.179225	1	0.0745801

```

91 ## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
92 ## unable to evaluate scaled gradient

93 ## Warning in checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :
94 ## Model failed to converge: degenerate Hessian with 1 negative eigenvalues

95 ## Warning in aictab.AIClmerMod(cand.set = models, modnames = model.names, :
96 ## Model selection for fixed effects is only appropriate with ML estimation:
97 ## REML (default) should only be used to select random effects for a constant set of fix

```

	Random Effect of Model	AIC	Delta AIC
5	(1 longitude)	578.6010	0.000000
2	(1 LIO/longitude)	580.6010	2.000000
4	(1 latitude)	584.1236	5.522596

	Random Effect of Model	AIC	Delta AIC
1	(1 LIO/latitude)	586.1236	7.522596
3	(1 LIO)	592.6684	14.067482

	npar	Sum Sq	Mean Sq	F value
time	1	2.311266	2.3112656	34.232954
year	3	1.678516	0.5595055	8.287029
mean_is_au	1	6.632512	6.6325123	98.236431
year:lio_areas	28	9.999718	0.3571328	5.289617

98 While we decided to fit the same model for each analyte, the parameter estimates are different
 99 in each case (REF TABLE HERE). Most notably, in each case the variable with the highest
 100 t-value is mean percent AU, indicating that it is the strongest and most significant effect on
 101 dry weight.

102 -model parameters (this might require a table)

103 -what we can and can't say

104 Toxics in the Nearshore Recommendation

105 Acknowledgments

¹⁰⁶ **References**

¹⁰⁷ **Appendix**

¹⁰⁸ **Parameter Summary Tables**

effect	group	term	estimate	std.error	statistic
fixed	NA	(Intercept)	6.2765624	0.9487517	6.6156006
fixed	NA	time	-	0.0150282	-
			0.0333872		2.2216399
fixed	NA	year2016	1.1438858	0.8861281	1.2908809
fixed	NA	year2018	0.6525321	0.6528914	0.9994497
fixed	NA	year2020	0.6519114	0.6671822	0.9771115
fixed	NA	mean_is_au	0.0176958	0.0022143	7.9916168
fixed	NA	year2013:lio_areasPuyallup / White	0.6350302	0.4605221	1.3789355
fixed	NA	year2016:lio_areasPuyallup / White	0.8226249	0.4608683	1.7849457
fixed	NA	year2018:lio_areasPuyallup / White	0.9387117	0.3287235	2.8556272
fixed	NA	year2020:lio_areasPuyallup / White	0.3918281	0.3613502	1.0843446
fixed	NA	year2013:lio_areasSkagit / Samish	-	0.4993974	-
			0.6166944		1.2348770
fixed	NA	year2016:lio_areasSkagit / Samish	1.1390929	0.5549795	2.0524955
fixed	NA	year2018:lio_areasSkagit / Samish	0.7056200	0.4445397	1.5873047
fixed	NA	year2020:lio_areasSkagit / Samish	0.3159018	0.4544027	0.6952025
fixed	NA	year2013:lio_areasSnohomish / Stillaguamish	0.2129240	0.5309136	0.4010522
fixed	NA	year2016:lio_areasSnohomish / Stillaguamish	0.6179667	0.5038938	1.2263828
fixed	NA	year2018:lio_areasSnohomish / Stillaguamish	0.3392289	0.4148951	0.8176255
fixed	NA	year2020:lio_areasSnohomish / Stillaguamish	0.2575482	0.3967836	0.6490898
fixed	NA	year2013:lio_areasSouth Central	1.0081942	0.4506951	2.2369761
fixed	NA	year2016:lio_areasSouth Central	1.7884073	0.4058285	4.4068052
fixed	NA	year2018:lio_areasSouth Central	1.7074824	0.3099487	5.5089189
fixed	NA	year2020:lio_areasSouth Central	1.1627493	0.3245431	3.5827266
fixed	NA	year2013:lio_areasSouth Sound	-	0.4765611	-
			0.4336633		0.9099847

effect	group	term	estimate	std.error	statistic
fixed	NA	year2016:lio_areasSouth Sound	0.5687781	0.4541705	1.2523448
fixed	NA	year2018:lio_areasSouth Sound	0.2316511	0.3613974	0.6409871
fixed	NA	year2020:lio_areasSouth Sound	0.1074163	0.4019557	0.2672341
fixed	NA	year2013:lio_areasWest Sound	0.6192862	0.4463476	1.3874527
fixed	NA	year2016:lio_areasWest Sound	1.1437725	0.3836862	2.9810104
fixed	NA	year2018:lio_areasWest Sound	0.9120430	0.2813508	3.2416574
fixed	NA	year2020:lio_areasWest Sound	0.5893960	0.3026935	1.9471711
fixed	NA	year2013:lio_areasWhatcom	0.0458001	0.4625833	0.0990094
fixed	NA	year2016:lio_areasWhatcom	0.7457055	0.4175714	1.7858153
fixed	NA	year2018:lio_areasWhatcom	0.5302650	0.3590290	1.4769418
fixed	NA	year2020:lio_areasWhatcom	0.2506166	0.3538604	0.7082357
ran_pars longitude		sd__(Intercept)	0.6789580	NA	NA
ran_pars Residual		sd__Observation	0.2446813	NA	NA

effect	group	term	estimate	std.error	statistic
fixed	NA	(Intercept)	0.3496092	0.6847147	0.5105911
fixed	NA	time	0.0183058	0.0108812	1.6823311
fixed	NA	year2016	-	0.6374253	-
			1.6640937		2.6106487
fixed	NA	year2018	-	0.4682145	-
			1.4257793		3.0451417
fixed	NA	year2020	-	0.4784976	-
			1.3802527		2.8845549
fixed	NA	mean_is_au	0.0110729	0.0015511	7.1388899
fixed	NA	year2013:lio_areasPuyallup / White	0.6090696	0.3274634	1.8599627
fixed	NA	year2016:lio_areasPuyallup / White	1.6521041	0.3279461	5.0377309
fixed	NA	year2018:lio_areasPuyallup / White	1.7843219	0.2402129	7.4280858
fixed	NA	year2020:lio_areasPuyallup / White	1.0128938	0.2709207	3.7387100
fixed	NA	year2013:lio_areasSkagit / Samish	-	0.3555111	-
			0.7160361		2.0141030
fixed	NA	year2016:lio_areasSkagit / Samish	0.6040931	0.3950230	1.5292605
fixed	NA	year2018:lio_areasSkagit / Samish	0.7085928	0.3164560	2.2391512
fixed	NA	year2020:lio_areasSkagit / Samish	-	0.3234838	-
			0.1749629		0.5408708
fixed	NA	year2013:lio_areasSnohomish / Stillaguamish	0.3697962	0.3779179	0.9785095
fixed	NA	year2016:lio_areasSnohomish / Stillaguamish	1.5495702	0.4332002	3.5770302

effect	group	term	estimate	std.error	statistic
fixed	NA	year2018:lio_areasSnohomish / Stillaguamish	1.4640905	0.3437459	4.2592230
fixed	NA	year2020:lio_areasSnohomish / Stillaguamish	-	0.3007902	-
fixed	NA	year2013:lio_areasSouth Central	0.3711061	0.3274746	1.1332362
fixed	NA	year2016:lio_areasSouth Central	1.8077996	0.3122823	5.7889909
fixed	NA	year2018:lio_areasSouth Central	1.4610804	0.2323144	6.2892369
fixed	NA	year2020:lio_areasSouth Central	0.7270140	0.2453562	2.9630961
fixed	NA	year2013:lio_areasSouth Sound	-	0.3392384	-
			0.2228129		0.6568035
fixed	NA	year2016:lio_areasSouth Sound	0.7579557	0.3233312	2.3442085
fixed	NA	year2018:lio_areasSouth Sound	0.5119190	0.2572732	1.9897874
fixed	NA	year2020:lio_areasSouth Sound	0.0974607	0.2861554	0.3405865
fixed	NA	year2013:lio_areasWest Sound	0.6006529	0.3219181	1.8658561
fixed	NA	year2016:lio_areasWest Sound	1.2598560	0.2788534	4.5179864
fixed	NA	year2018:lio_areasWest Sound	1.1107103	0.2095135	5.3013769
fixed	NA	year2020:lio_areasWest Sound	0.3615617	0.2220291	1.6284427
fixed	NA	year2013:lio_areasWhatcom	-	0.3382538	-
			0.5319712		1.5726986
fixed	NA	year2016:lio_areasWhatcom	0.7440840	0.3204689	2.3218605
fixed	NA	year2018:lio_areasWhatcom	0.6274997	0.2777402	2.2593043
fixed	NA	year2020:lio_areasWhatcom	-	0.2742560	-
			0.0891794		0.3251686
ran_pars	longitude	sd__(Intercept)	0.3145257	NA	NA
ran_pars	Residual	sd__Observation	0.4062699	NA	NA

effect	group	term	estimate	std.error	statistic
fixed	NA	(Intercept)	3.5134807	0.7116380	4.9371740
fixed	NA	time	-	0.0112948	-
			0.0032171		0.2848276
fixed	NA	year2016	0.2027042	0.6633304	0.3055856
fixed	NA	year2018	-	0.4878430	-
			0.1659919		0.3402567
fixed	NA	year2020	-	0.4985390	-
			0.1270023		0.2547491
fixed	NA	mean_is_a	0.0098035	0.0016433	5.9655876
fixed	NA	year2013:lio_areasPuyallup / White	0.2613301	0.3424850	0.7630409
fixed	NA	year2016:lio_areasPuyallup / White	0.2645550	0.3427918	0.7717658

effect	group	term	estimate	std.error	statistic
fixed	NA	year2018:lio_areasPuyallup / White	0.8427605	0.2479702	3.3986363
fixed	NA	year2020:lio_areasPuyallup / White	0.5381814	0.2763396	1.9475360
fixed	NA	year2013:lio_areasSkagit / Samish	-	0.3714599	-
			0.8328908		2.2422091
fixed	NA	year2016:lio_areasSkagit / Samish	-	0.4127899	-
			0.0482016		0.1167703
fixed	NA	year2018:lio_areasSkagit / Samish	0.2964648	0.3306639	0.8965742
fixed	NA	year2020:lio_areasSkagit / Samish	0.1489861	0.3379946	0.4407944
fixed	NA	year2013:lio_areasSnohomish / Stillaguamish	-	0.3948991	-
			0.1928837		0.4884379
fixed	NA	year2016:lio_areasSnohomish / Stillaguamish	0.4012922	0.4037738	0.9938540
fixed	NA	year2018:lio_areasSnohomish / Stillaguamish	0.5818442	0.3295202	1.7657313
fixed	NA	year2020:lio_areasSnohomish / Stillaguamish	-	0.3031069	-
			0.4992896		1.6472396
fixed	NA	year2013:lio_areasSouth Central	0.0841193	0.3385743	0.2484514
fixed	NA	year2016:lio_areasSouth Central	0.7404221	0.3113267	2.3782804
fixed	NA	year2018:lio_areasSouth Central	1.0454146	0.2366393	4.4177562
fixed	NA	year2020:lio_areasSouth Central	0.7845679	0.2484273	3.1581381
fixed	NA	year2013:lio_areasSouth Sound	-	0.3544673	-
			0.3823504		1.0786621
fixed	NA	year2016:lio_areasSouth Sound	0.3472277	0.3378299	1.0278181
fixed	NA	year2018:lio_areasSouth Sound	0.7140612	0.2688102	2.6563766
fixed	NA	year2020:lio_areasSouth Sound	0.3279004	0.2989813	1.0967255
fixed	NA	year2013:lio_areasWest Sound	0.3936854	0.3344735	1.1770302
fixed	NA	year2016:lio_areasWest Sound	0.6954890	0.2883086	2.4123074
fixed	NA	year2018:lio_areasWest Sound	1.1397819	0.2136310	5.3352819
fixed	NA	year2020:lio_areasWest Sound	0.9059338	0.2286909	3.9613893
fixed	NA	year2013:lio_areasWhatcom	-	0.3488255	-
			0.3977492		1.1402528
fixed	NA	year2016:lio_areasWhatcom	-	0.3212329	-
			0.0802677		0.2498737
fixed	NA	year2018:lio_areasWhatcom	0.3493509	0.2791823	1.2513361
fixed	NA	year2020:lio_areasWhatcom	-	0.2748077	-
			0.1266581		0.4608971
ran_pars longitude	sd	(Intercept)	0.4697339	NA	NA
ran_pars Residual	sd	Observation	0.2598380	NA	NA

109

Raincloud plots

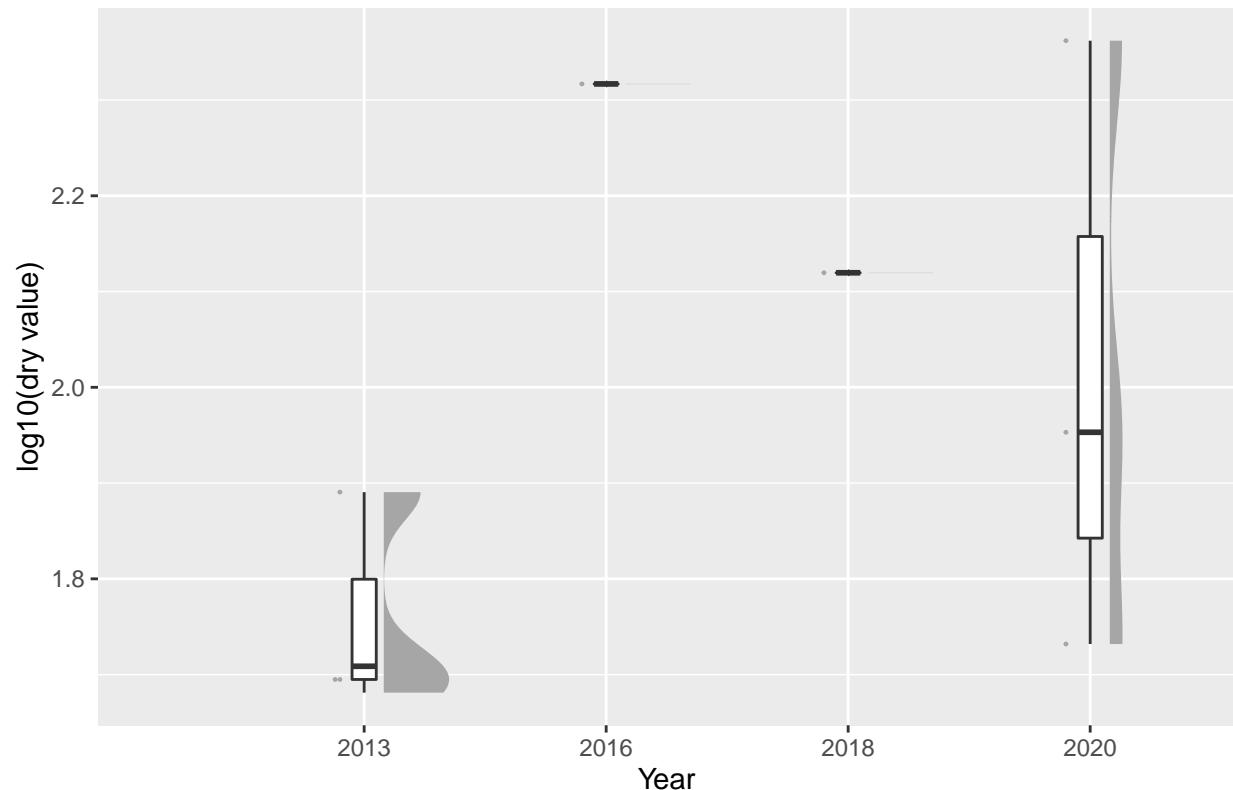
110

These also exist as PDFs or could probably exist as individual files/plots etc.

111

```
## $‘13‘
```

PAHs – WRIA # 13

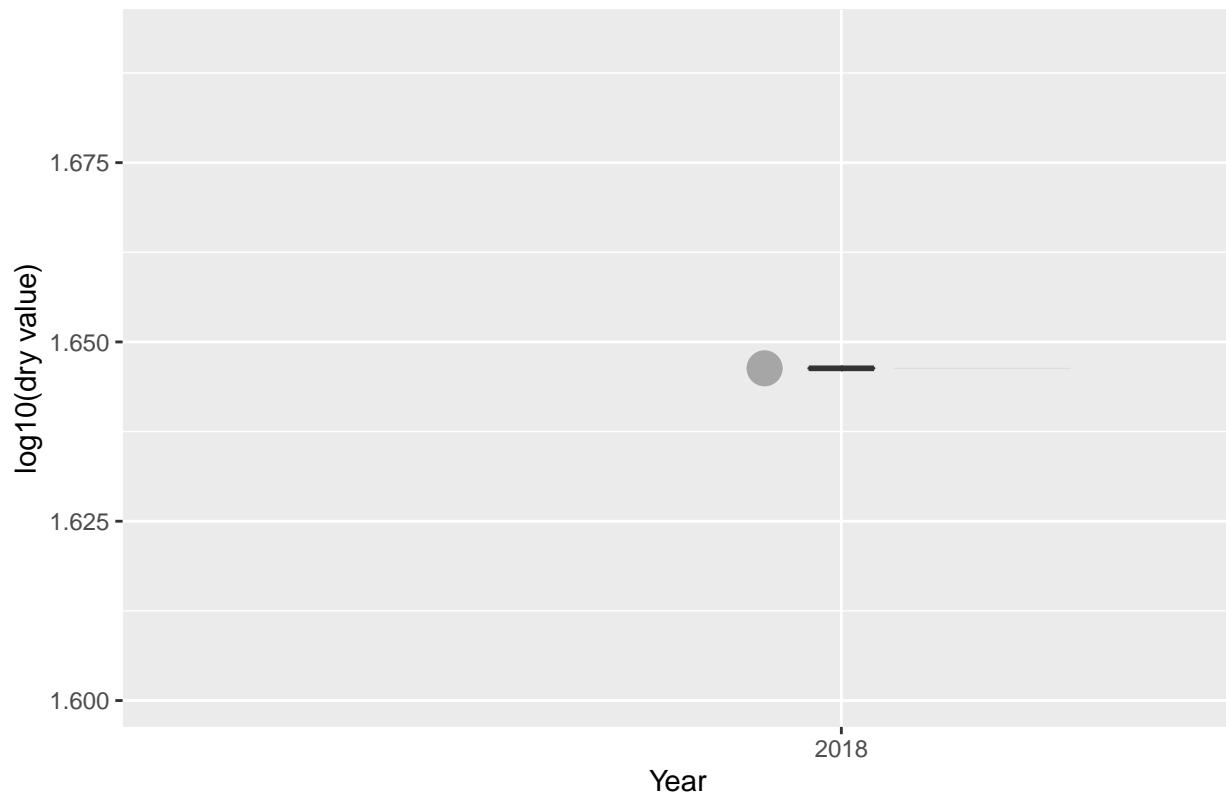


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113 ##

114 ## \$‘11‘

PAHs – WRIA # 11

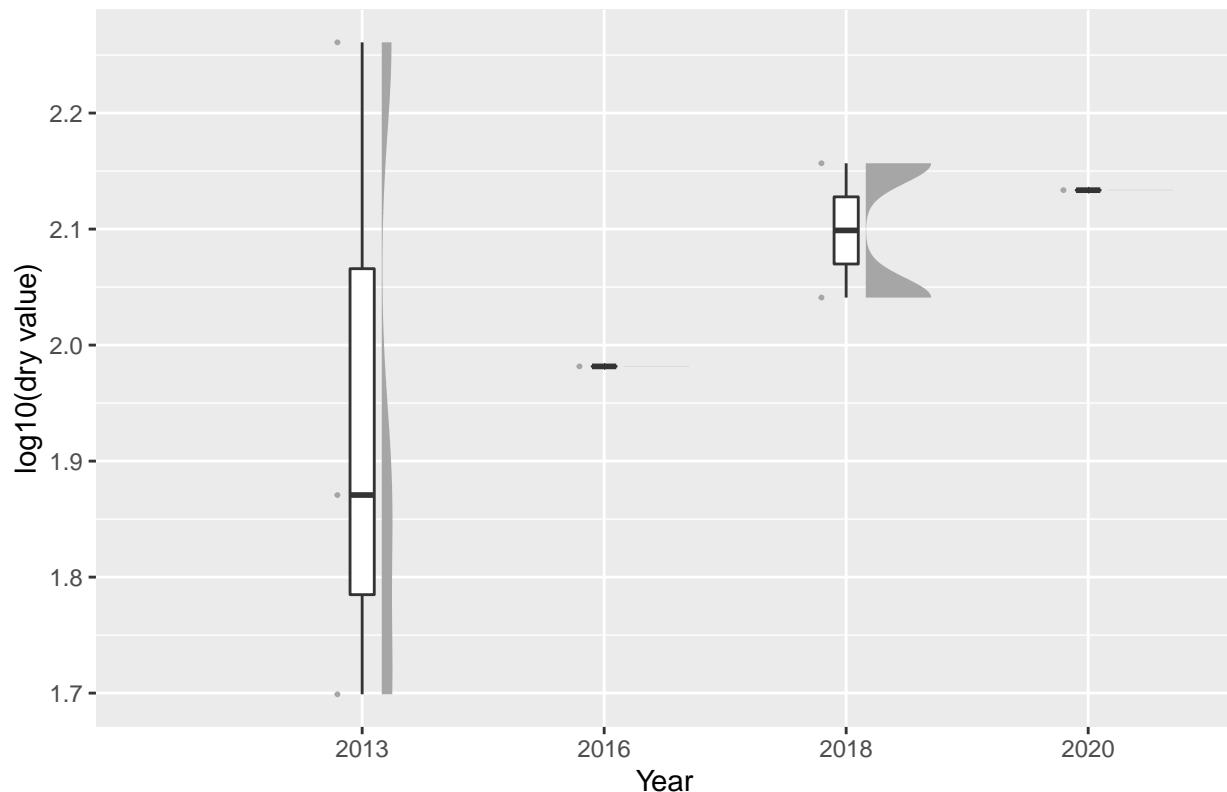


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116 ##

117 ## \$`14`

PAHs – WRIA # 14

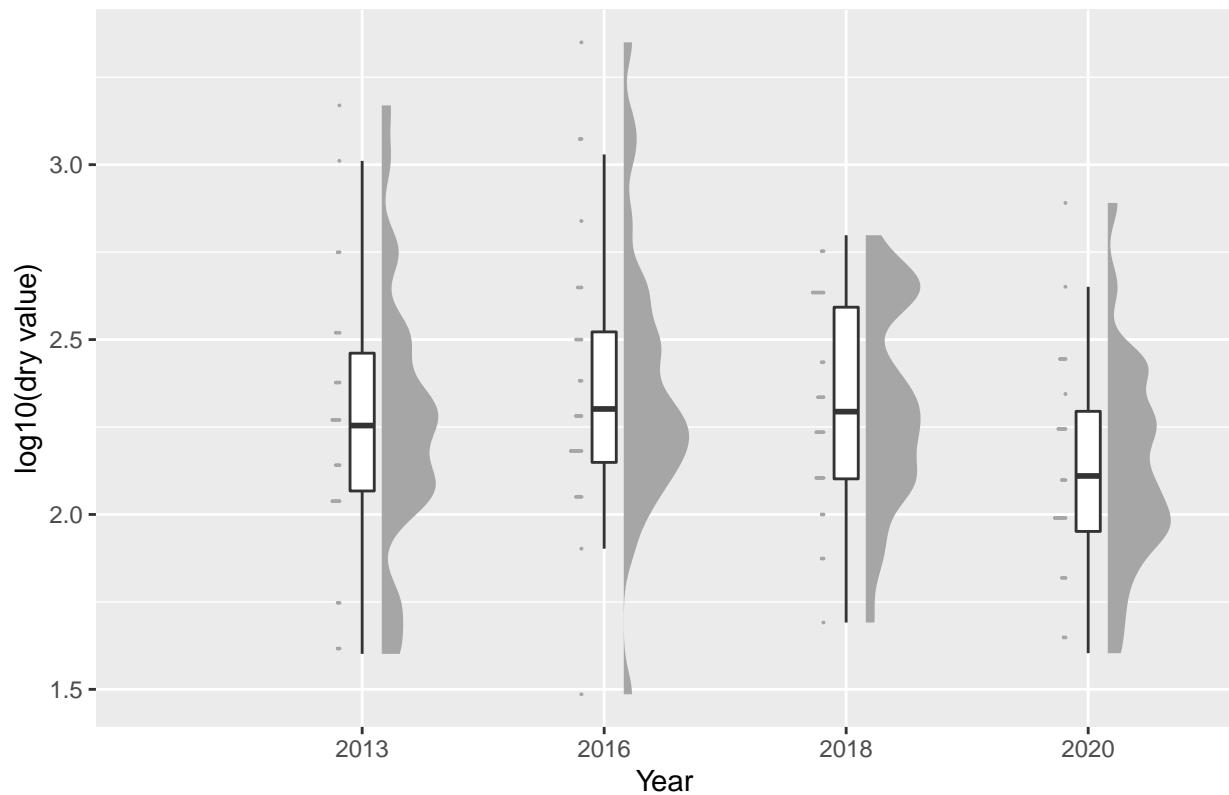


118

119 ##

120 ## \$‘15‘

PAHs – WRIA # 15

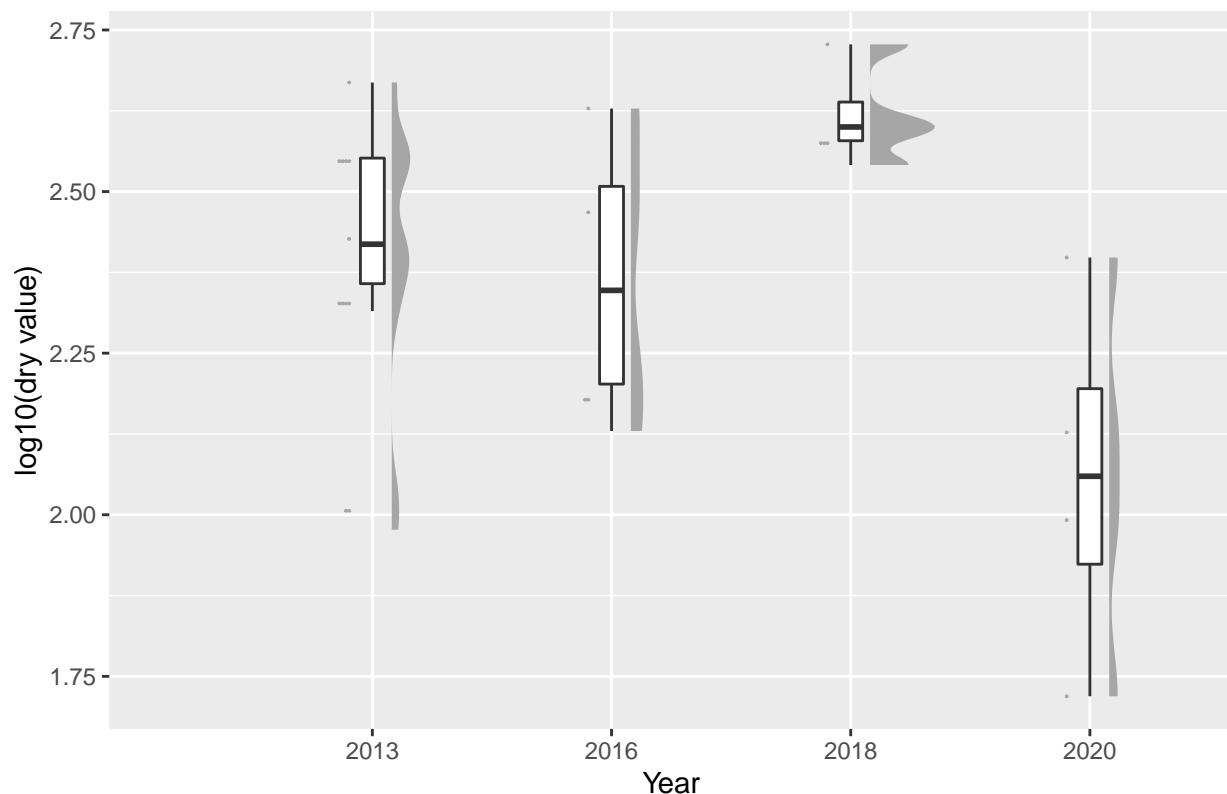


121

122 ##

123 ## \$‘12‘

PAHs – WRIA # 12

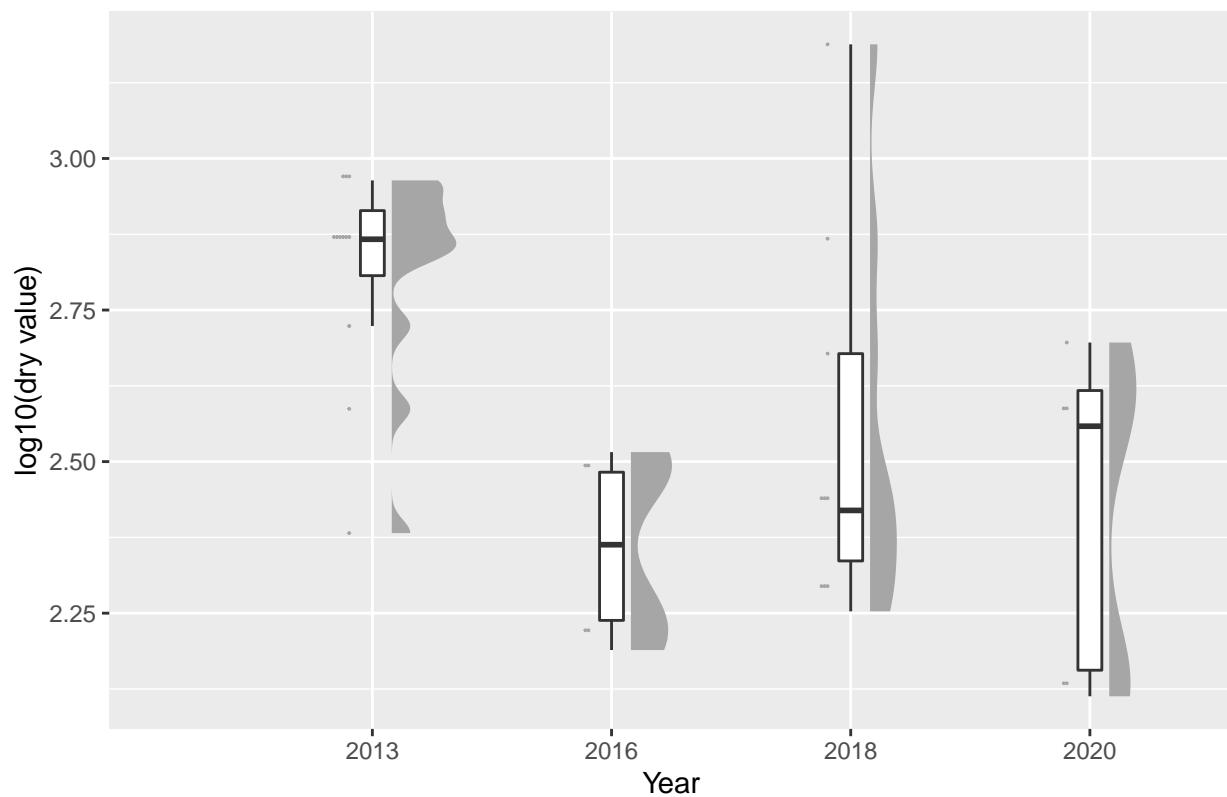


124

125 ##

126 ## \$‘10‘

PAHs – WRIA # 10

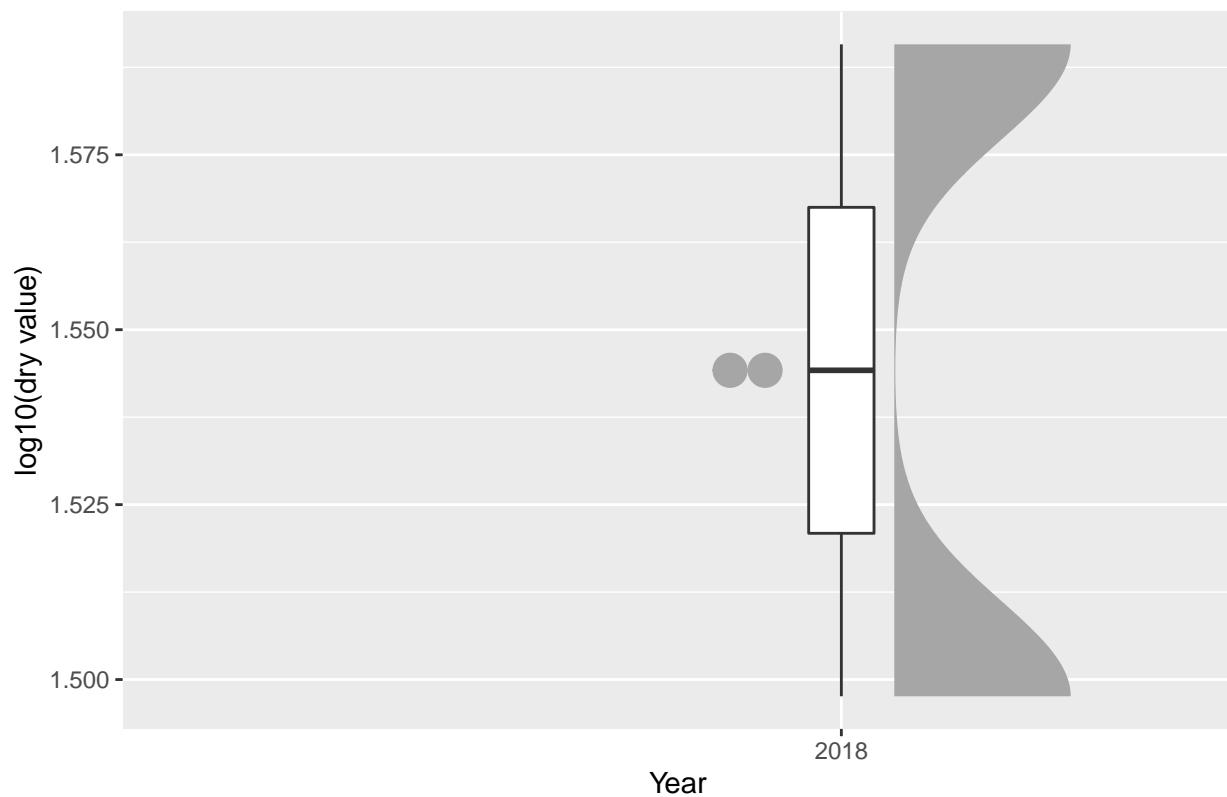


127

128 ##

129 ## \$‘16‘

PAHs – WRIA # 16

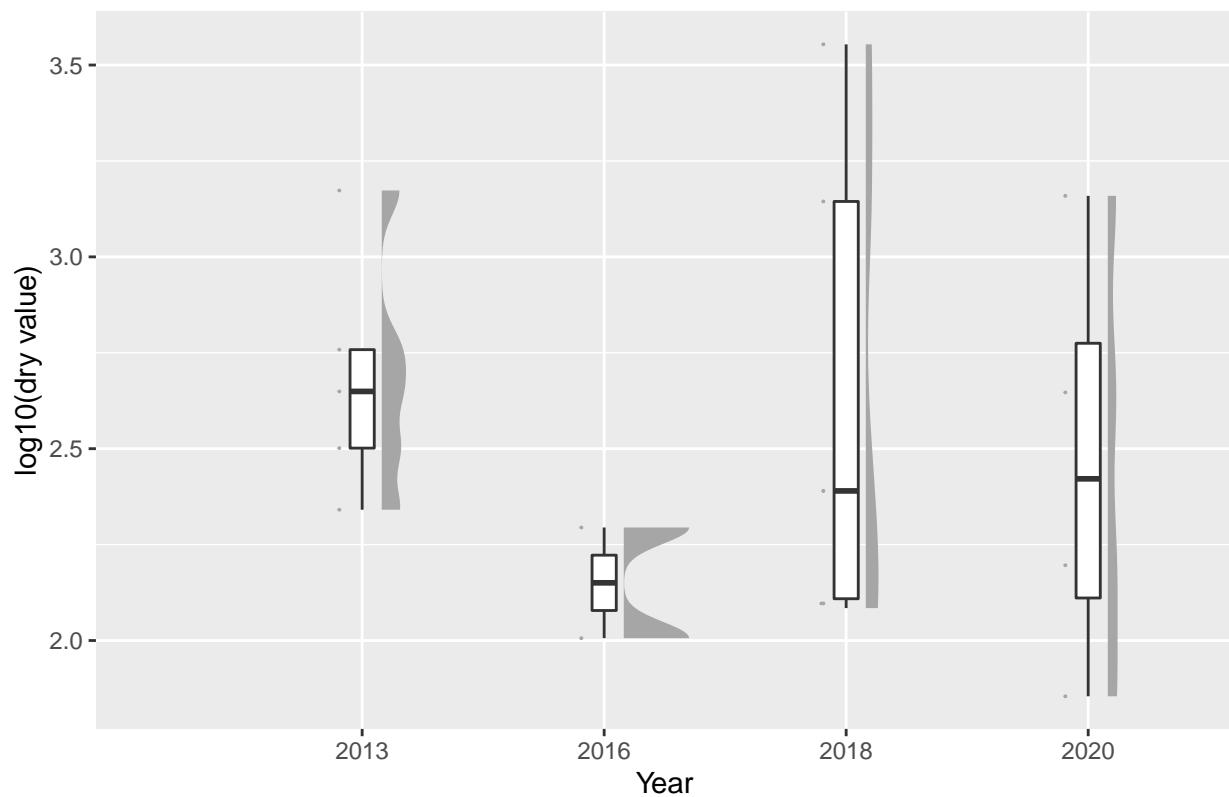


130

131 ##

132 ## \$‘9‘

PAHs – WRIA # 9

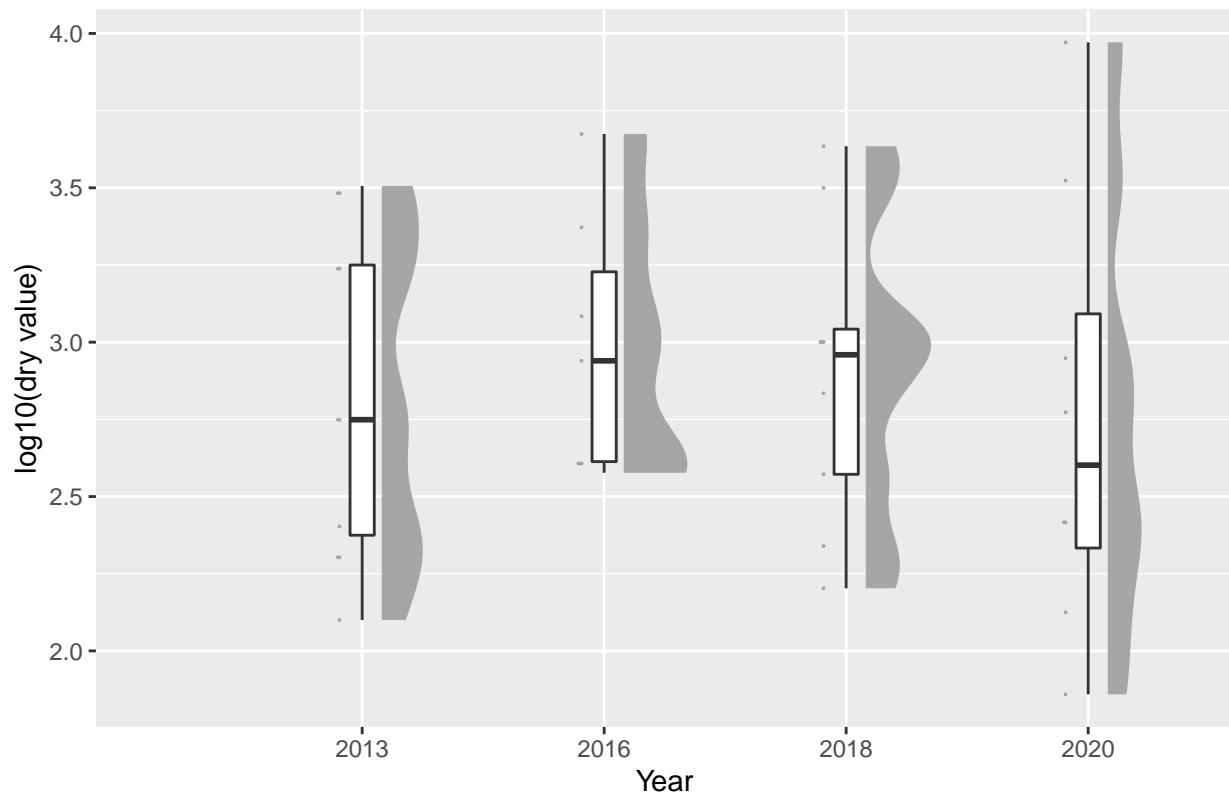


133

134 ##

135 ## \$‘8‘

PAHs – WRIA # 8

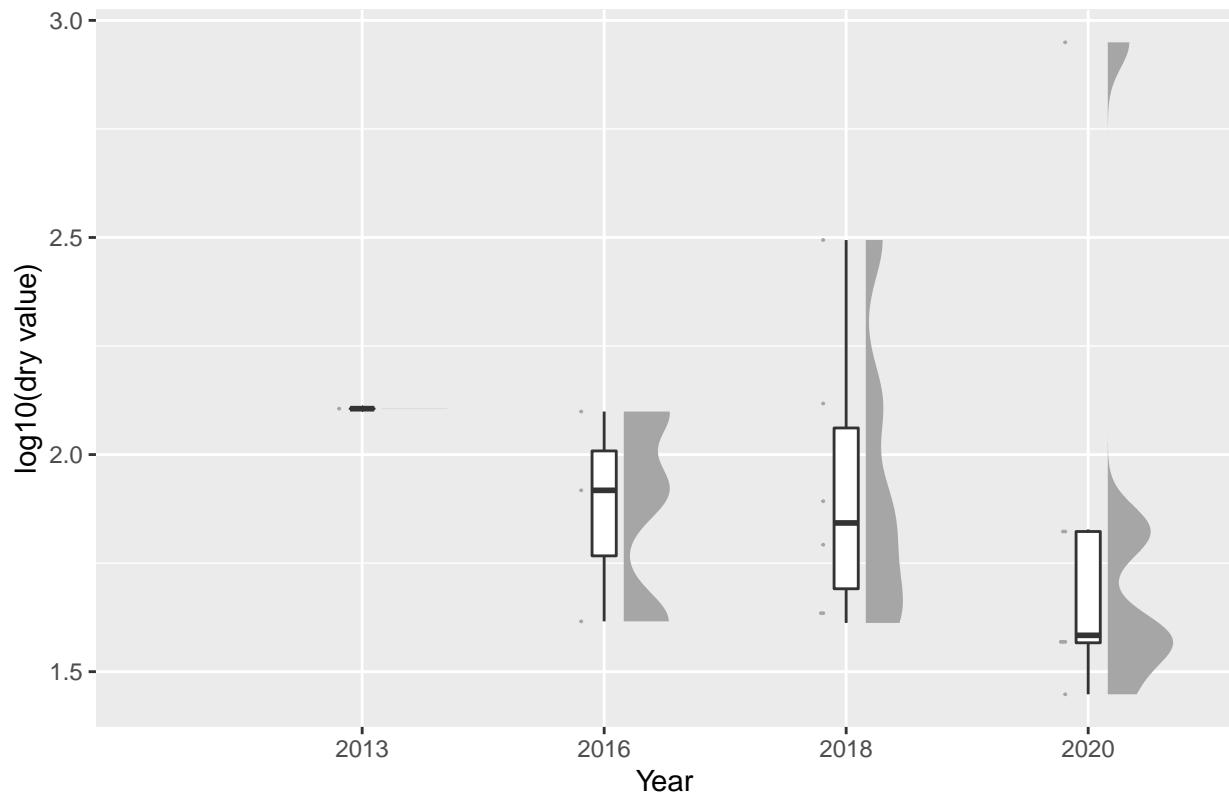


136

137 ##

138 ## \$‘17‘

PAHs – WRIA # 17

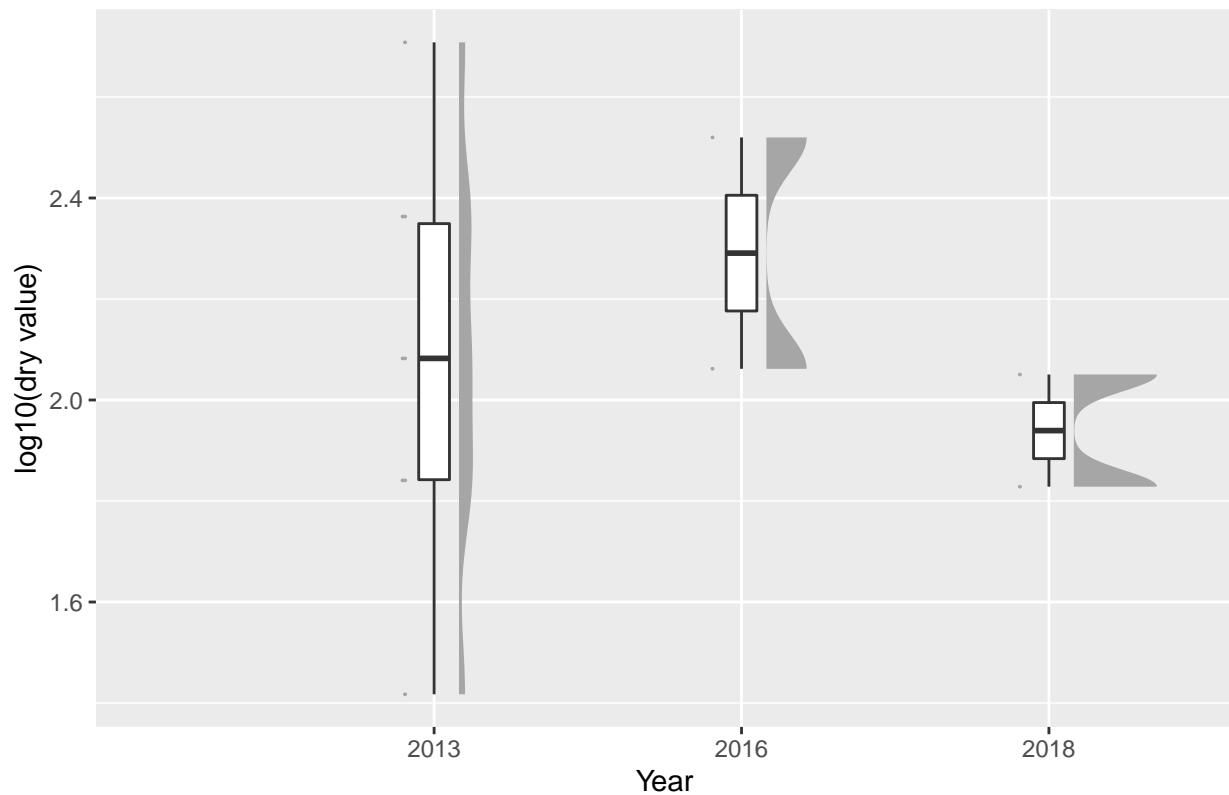


139

140 ##

141 ## \$‘6‘

PAHs – WRIA # 6

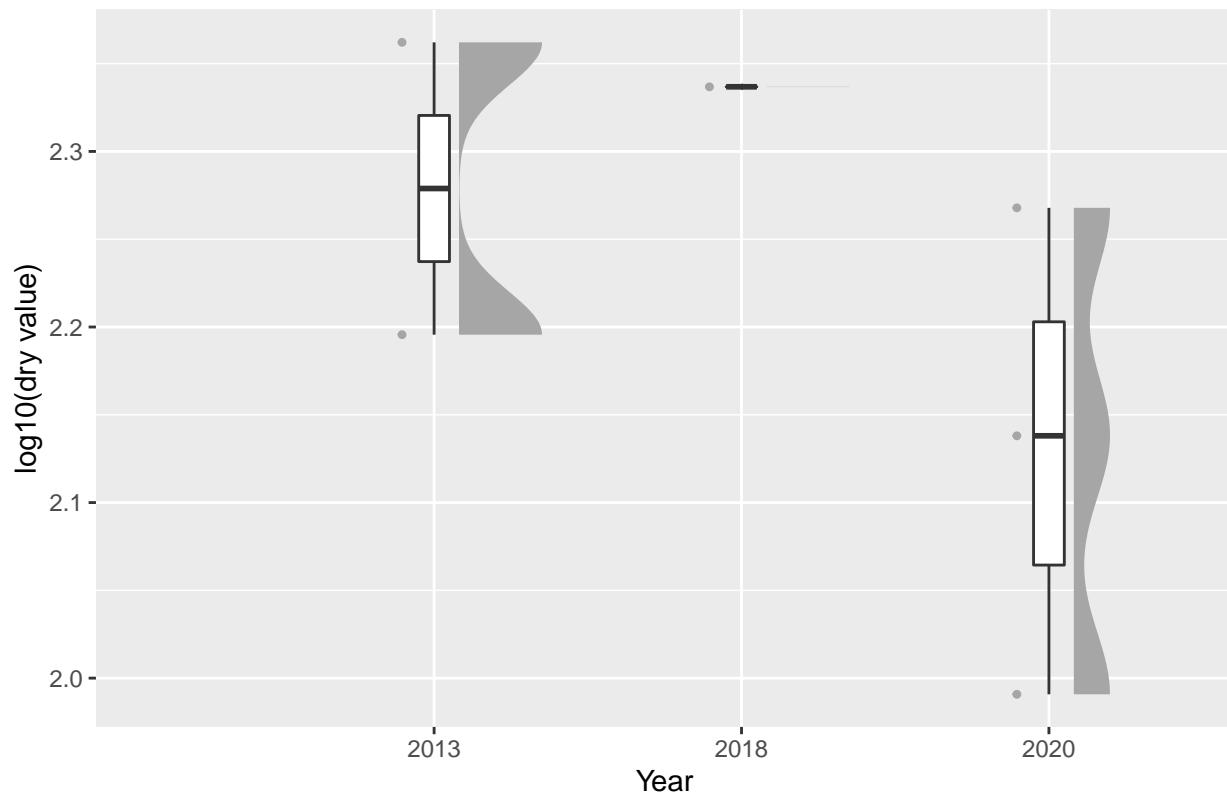


142

143 ##

144 ## \$‘7‘

PAHs – WRIA # 7



145

146 ##

147 ## \$‘5‘

PAHs – WRIA # 5

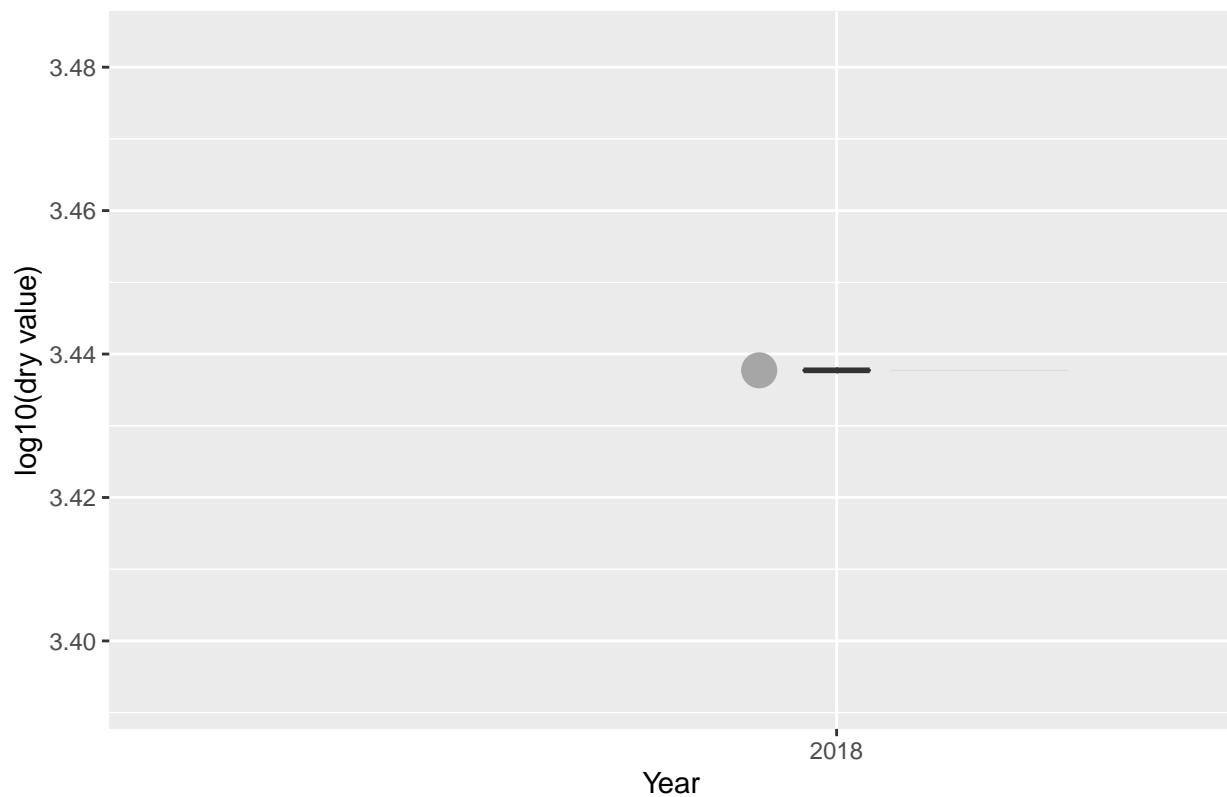


148

149 ##

150 ## \$‘18‘

PAHs – WRIA # 18

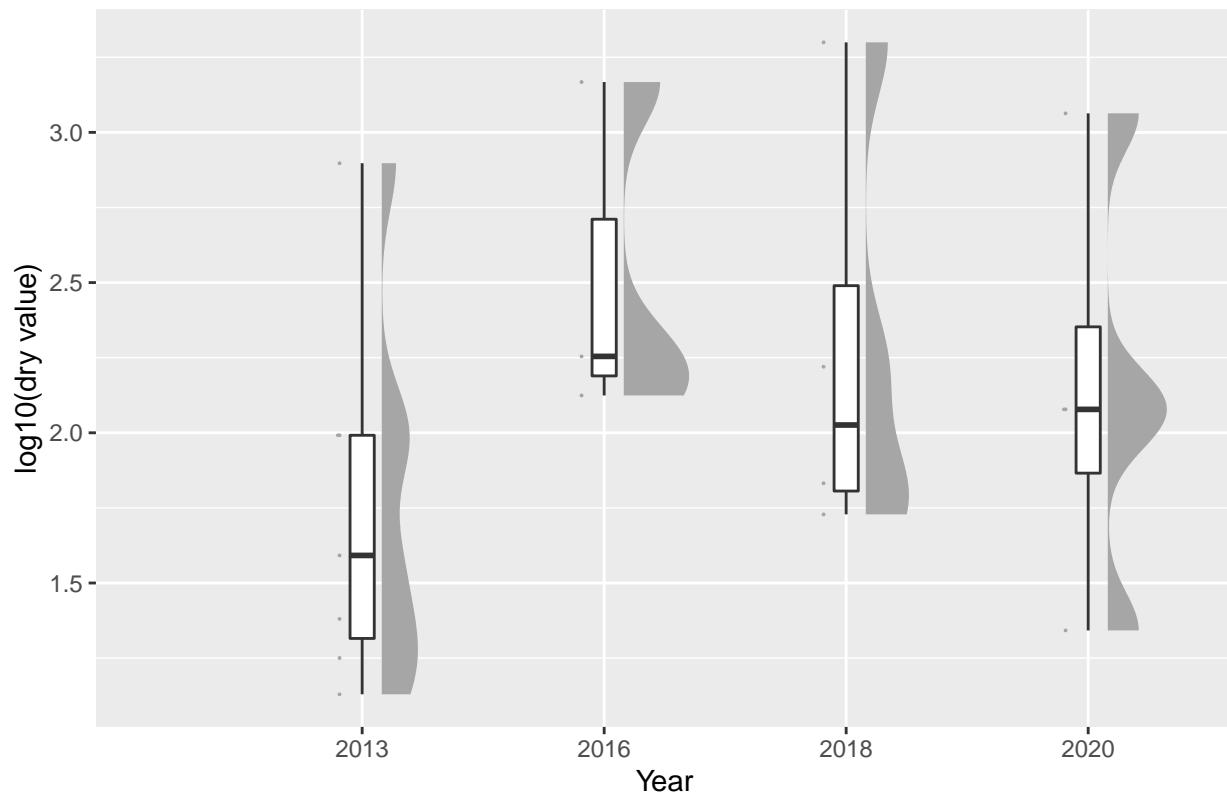


151

152 ##

153 ## \$‘3‘

PAHs – WRIA # 3

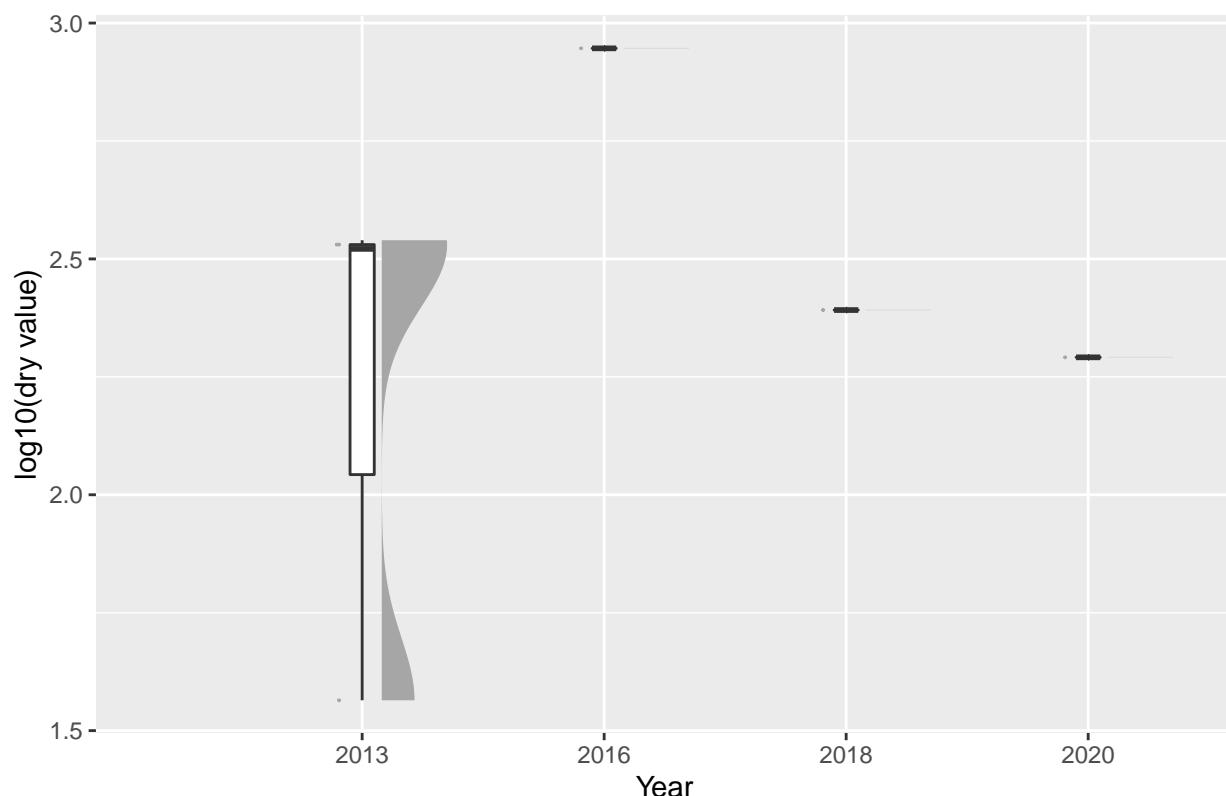


154

155 ##

156 ## \$‘2‘

PAHs – WRIA # 2

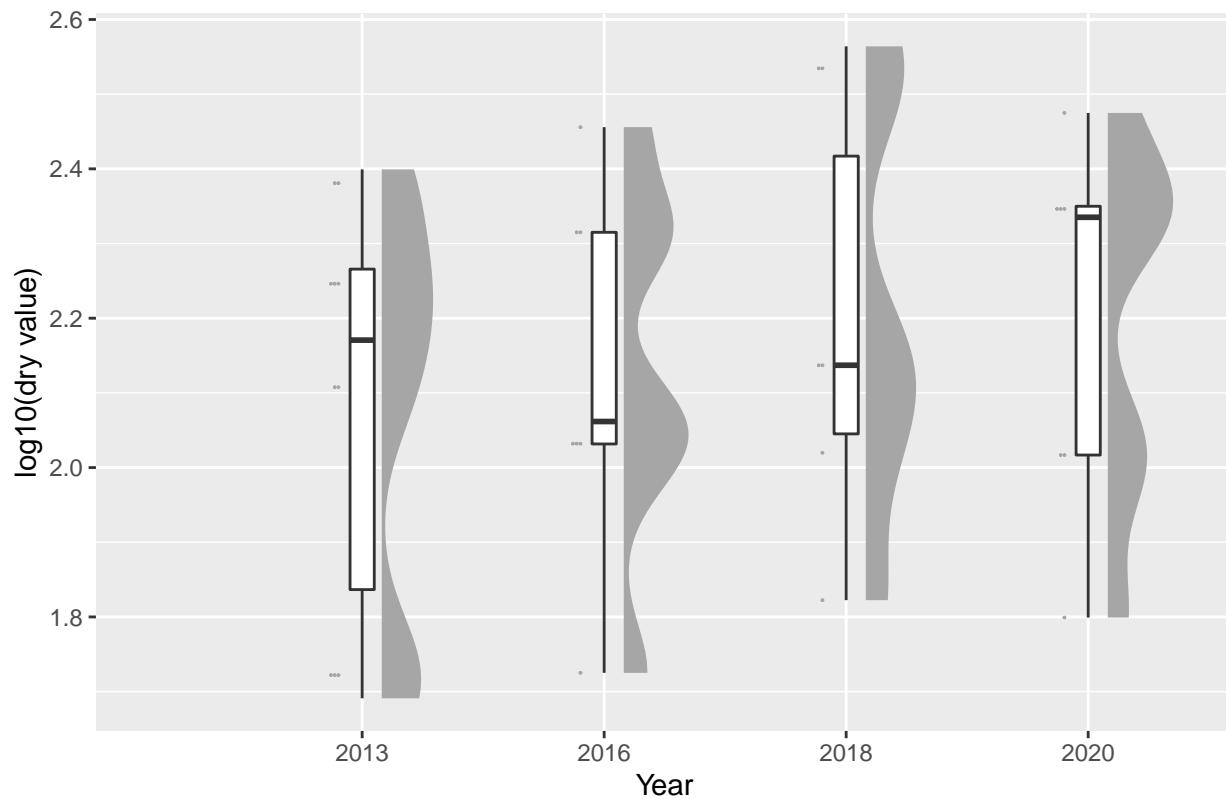


157

158 ##

159 ## \$‘1‘

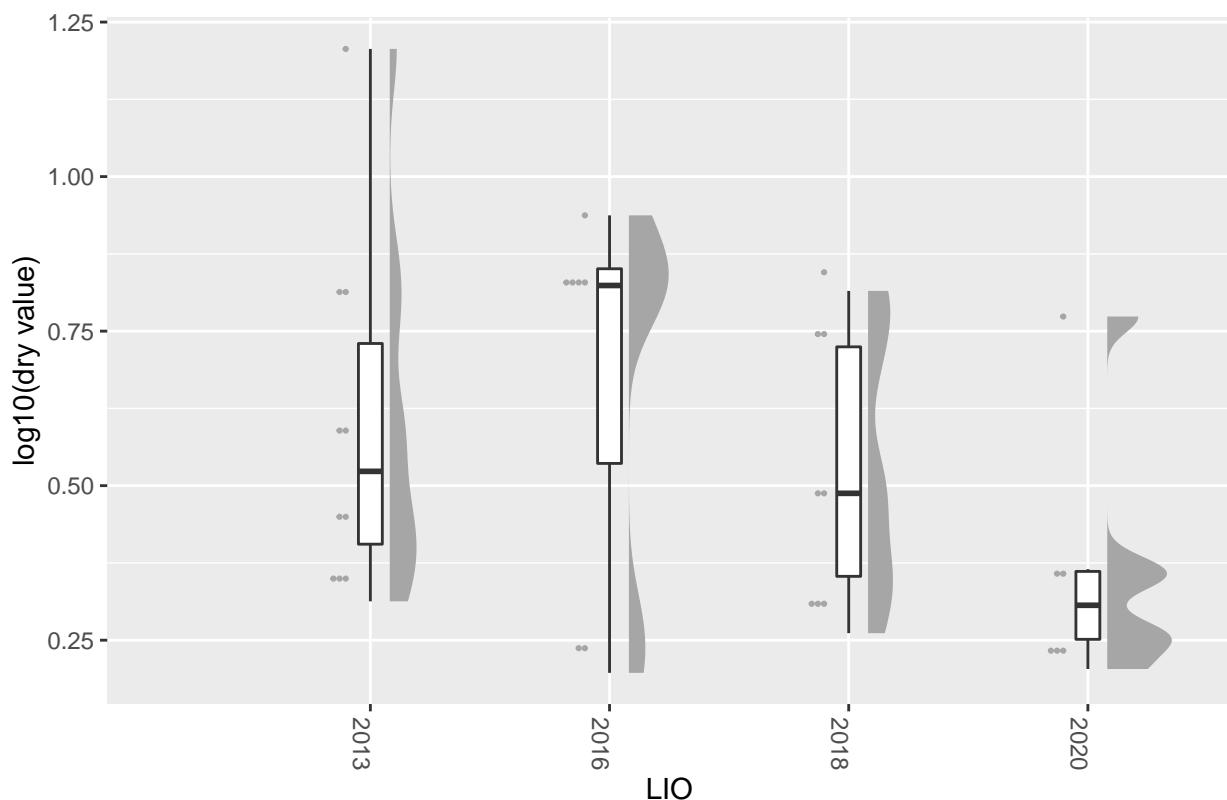
PAHs – WRIA # 1



160

161 ## \$‘South Sound‘

PBDEs – LIO – South Sound

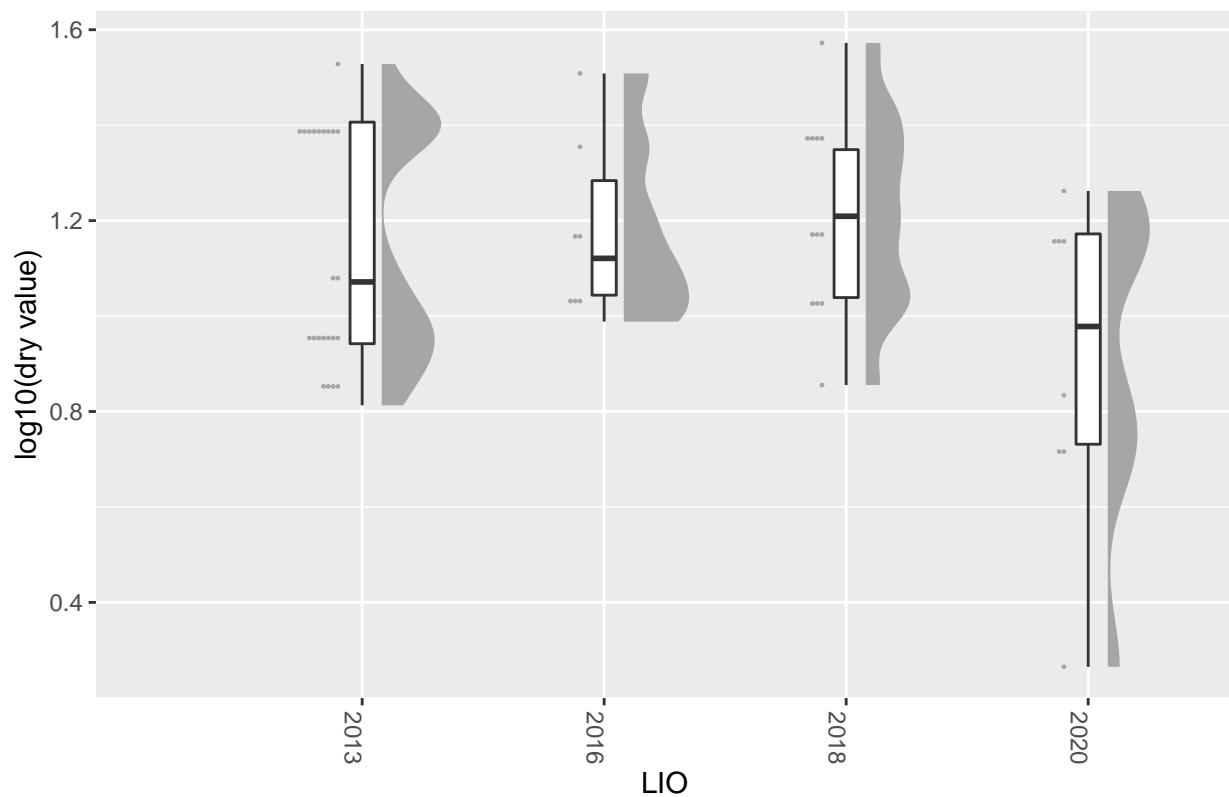


162

163 ##

164 ## \$‘Puyallup / White‘

PBDEs – LIO – Puyallup / White

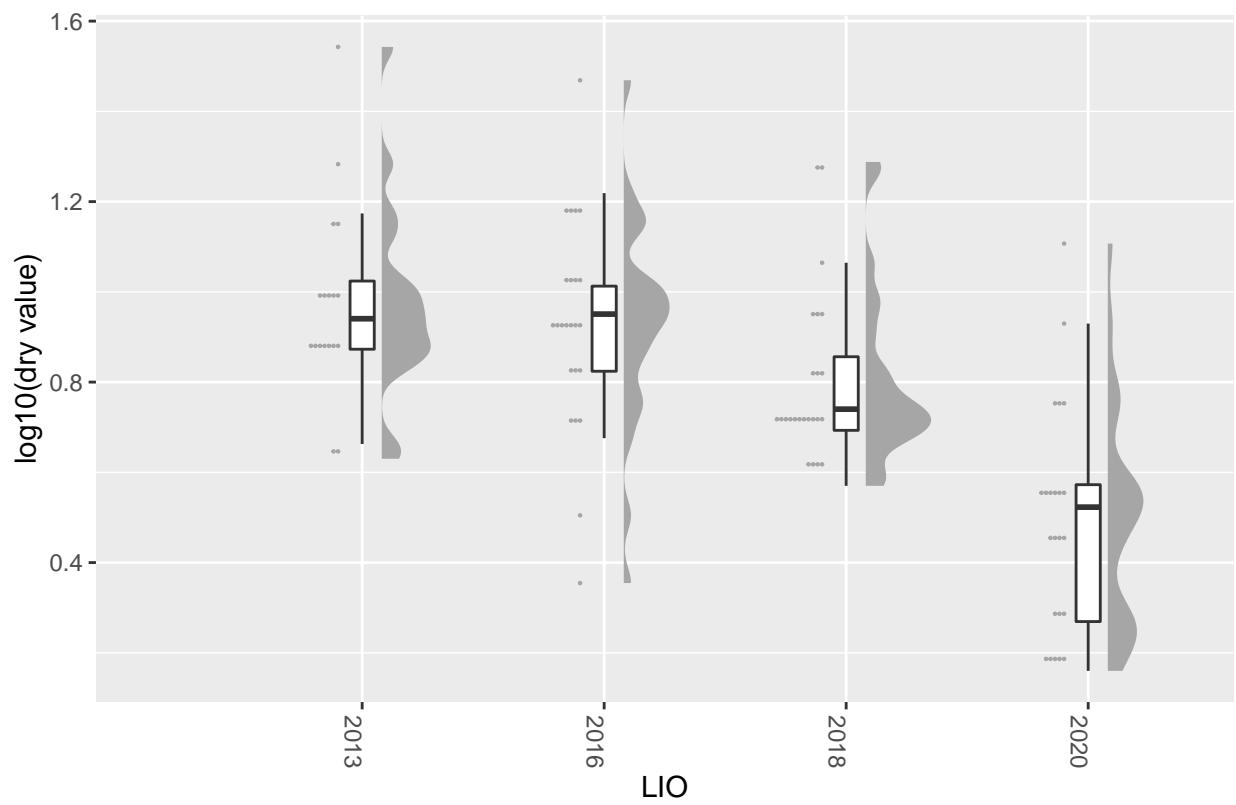


165

166 ##

167 ## \$'West Sound'

PBDEs – LIO – West Sound

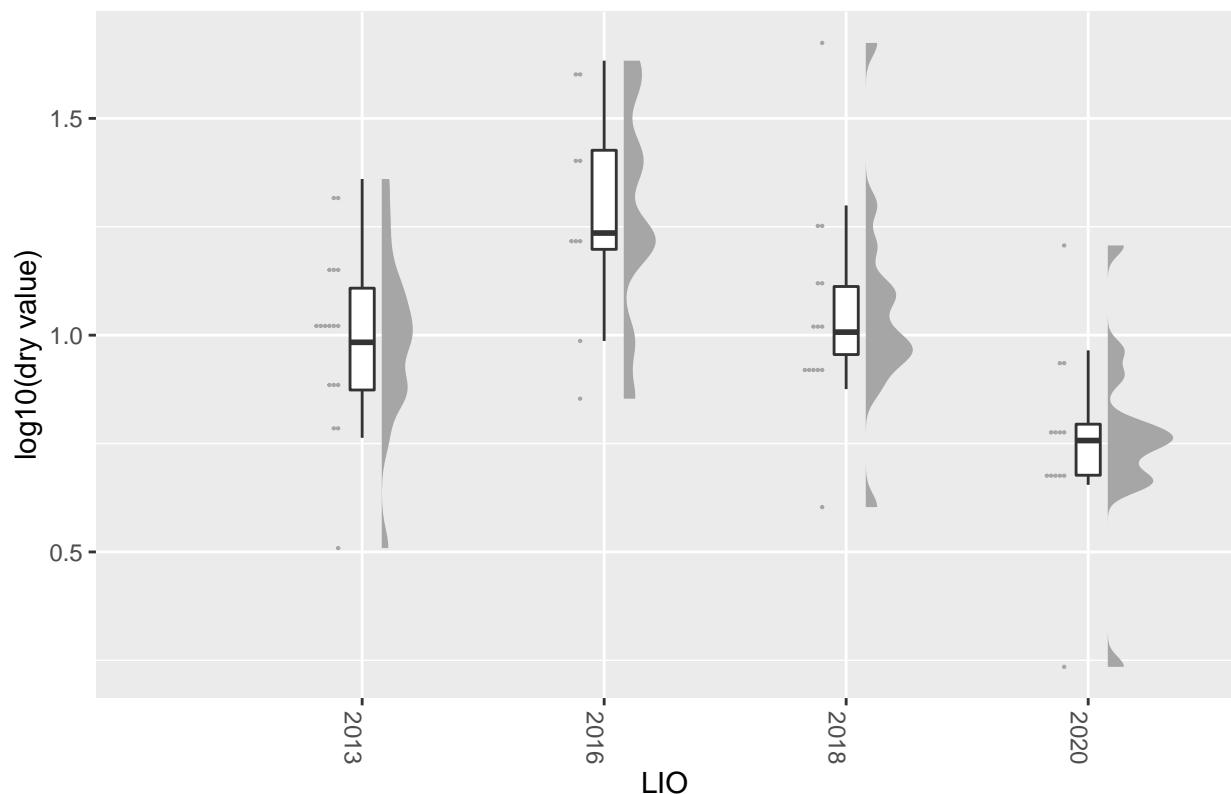


168

169 ##

170 ## \$‘South Central’

PBDEs – LIO – South Central

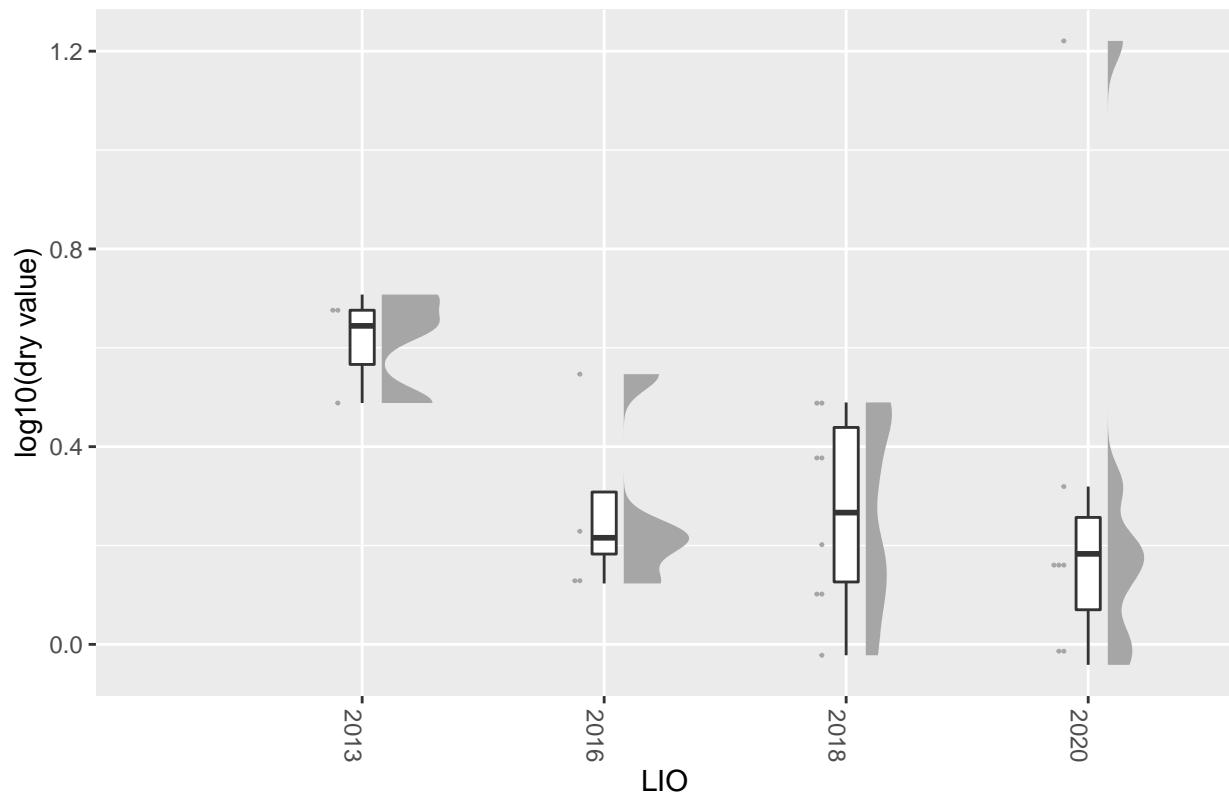


171

172 ##

173 ## \$‘Hood Canal’

PBDEs – LIO – Hood Canal

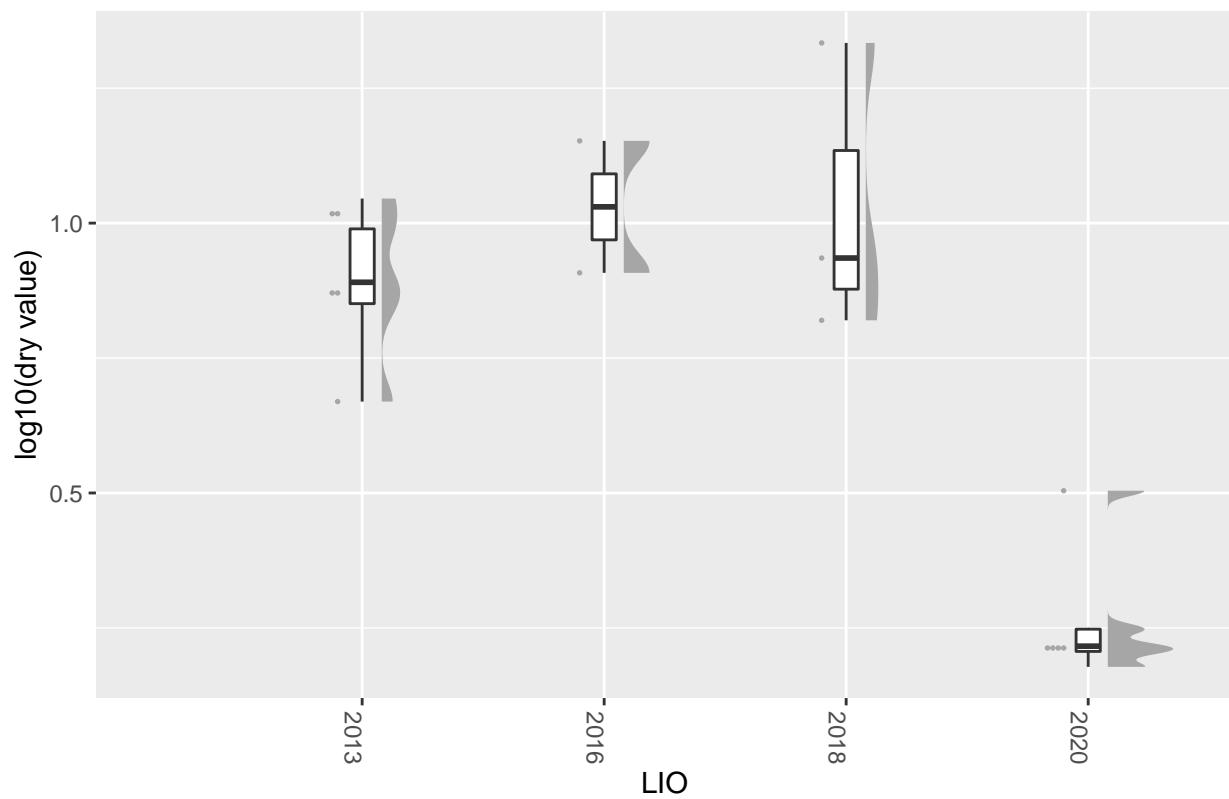


174

175 ##

176 ## \$‘Snohomish / Stillaguamish’

PBDEs – LIO – Snohomish / Stillaguamish

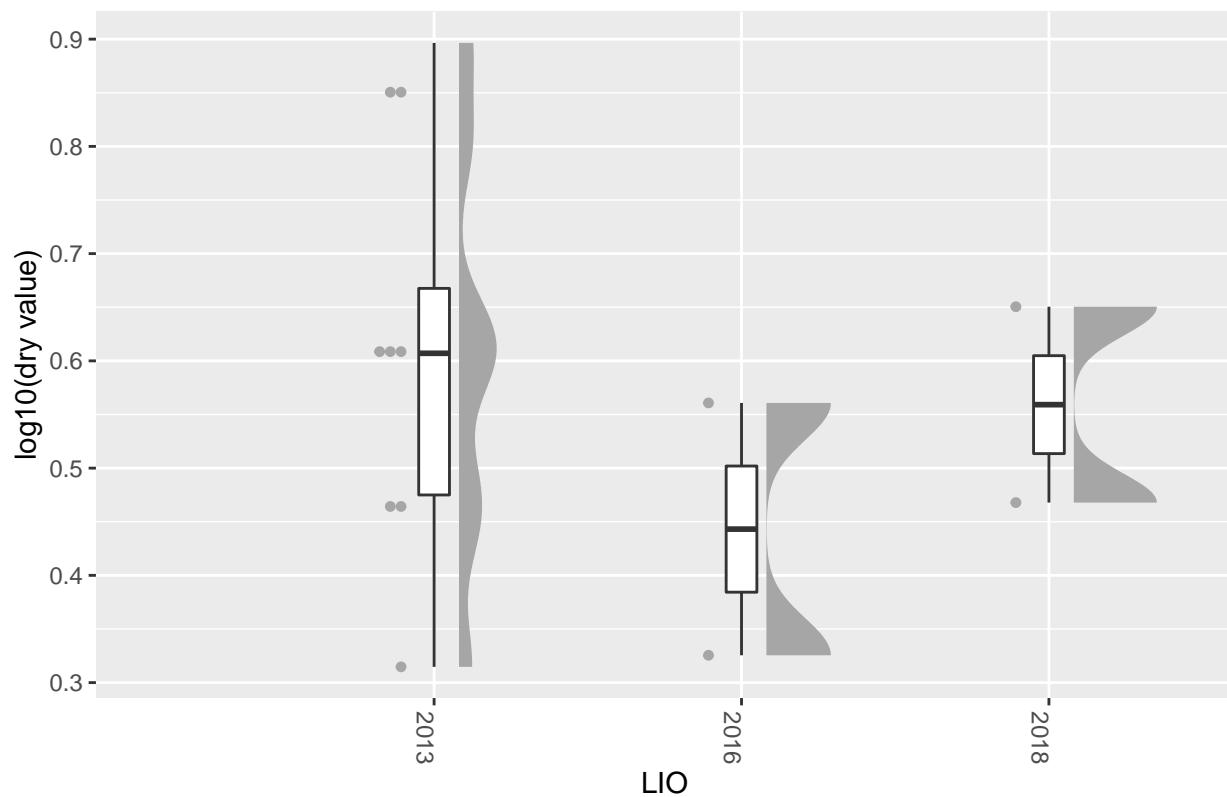


177

178 ##

179 ## \$Island

PBDEs – LIO – Island

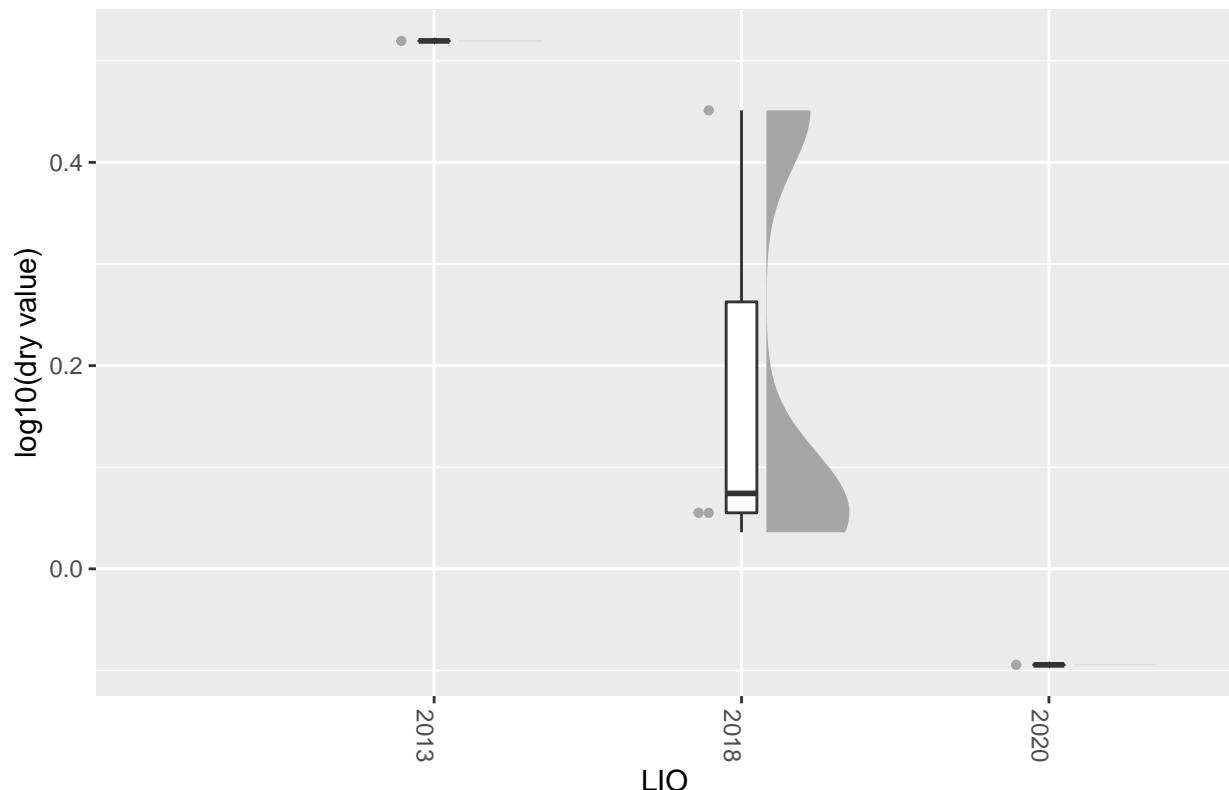


180

181 ##

182 ## \$‘Strait of Juan de Fuca’

PBDEs – LIO – Strait of Juan de Fuca

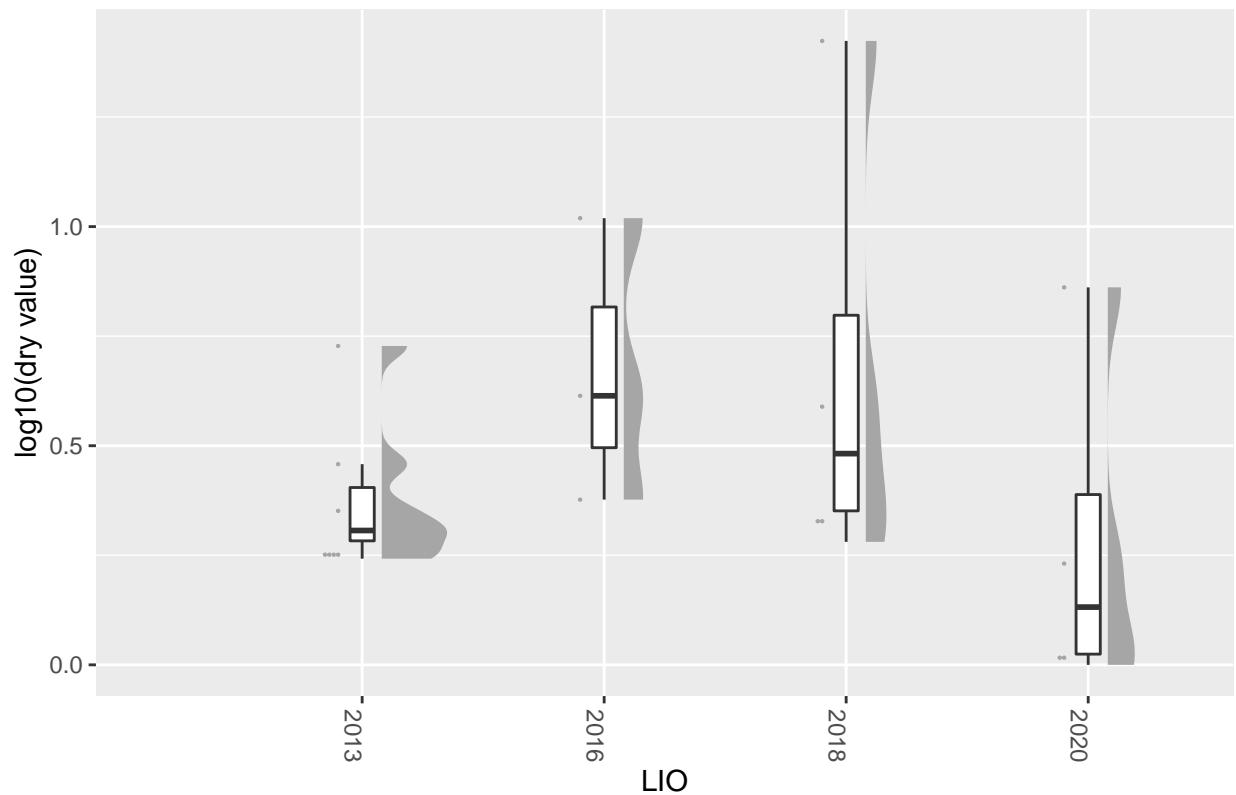


183

184 ##

185 ## \$‘Skagit / Samish’

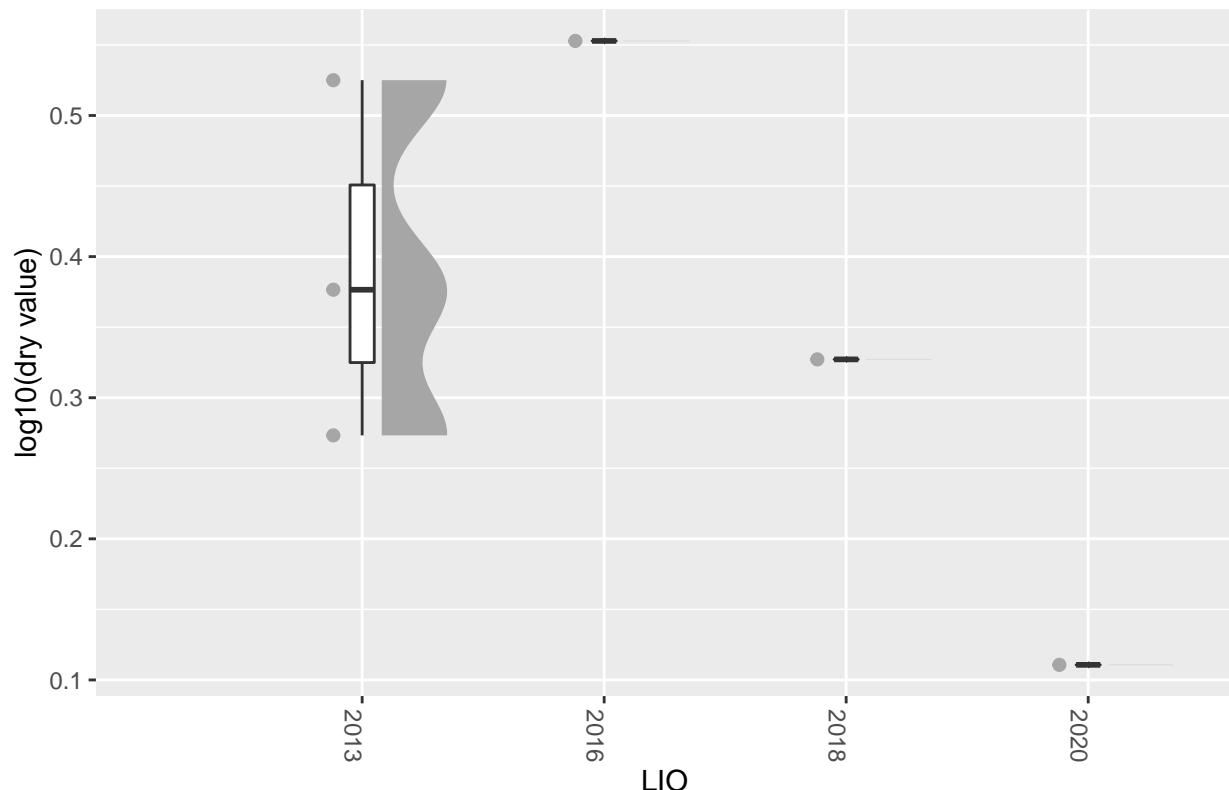
PBDEs – LIO – Skagit / Samish



186

```
187 ##  
188 ## $‘San Juan’
```

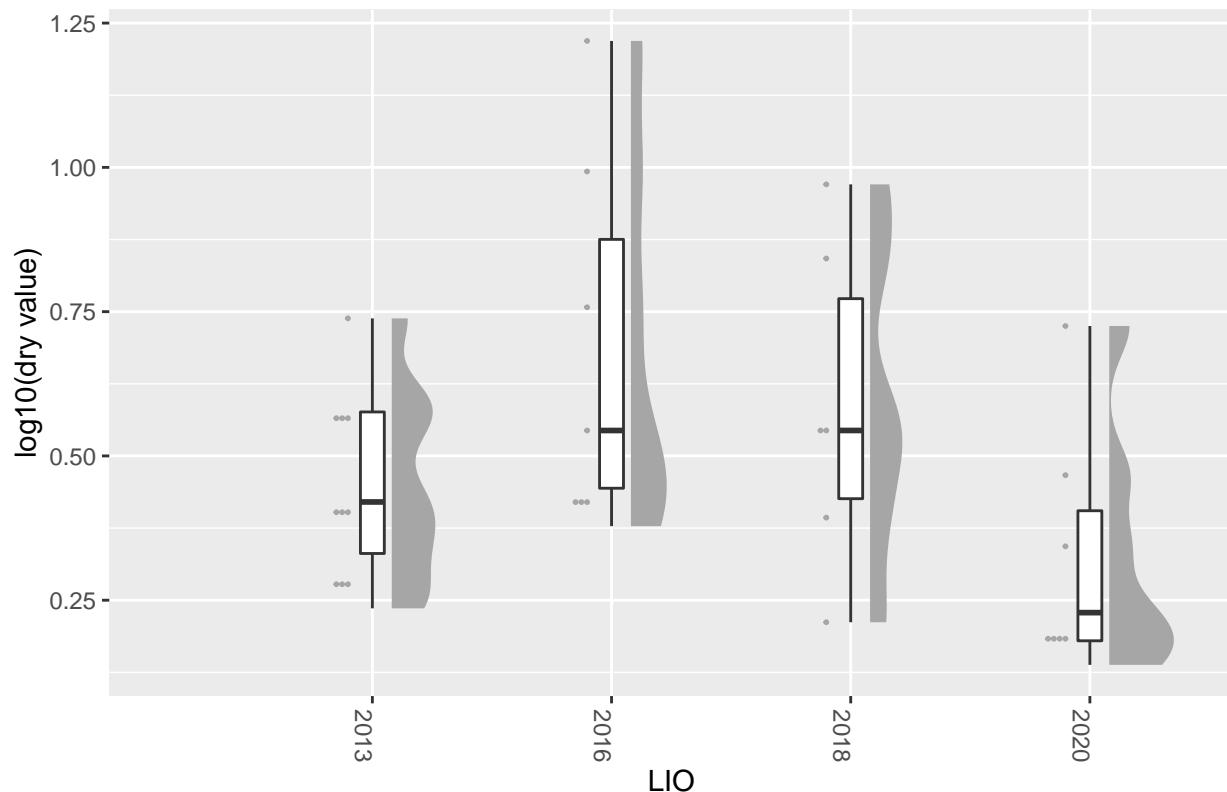
PBDEs – LIO – San Juan



189

```
190 ##  
191 ## $Whatcom
```

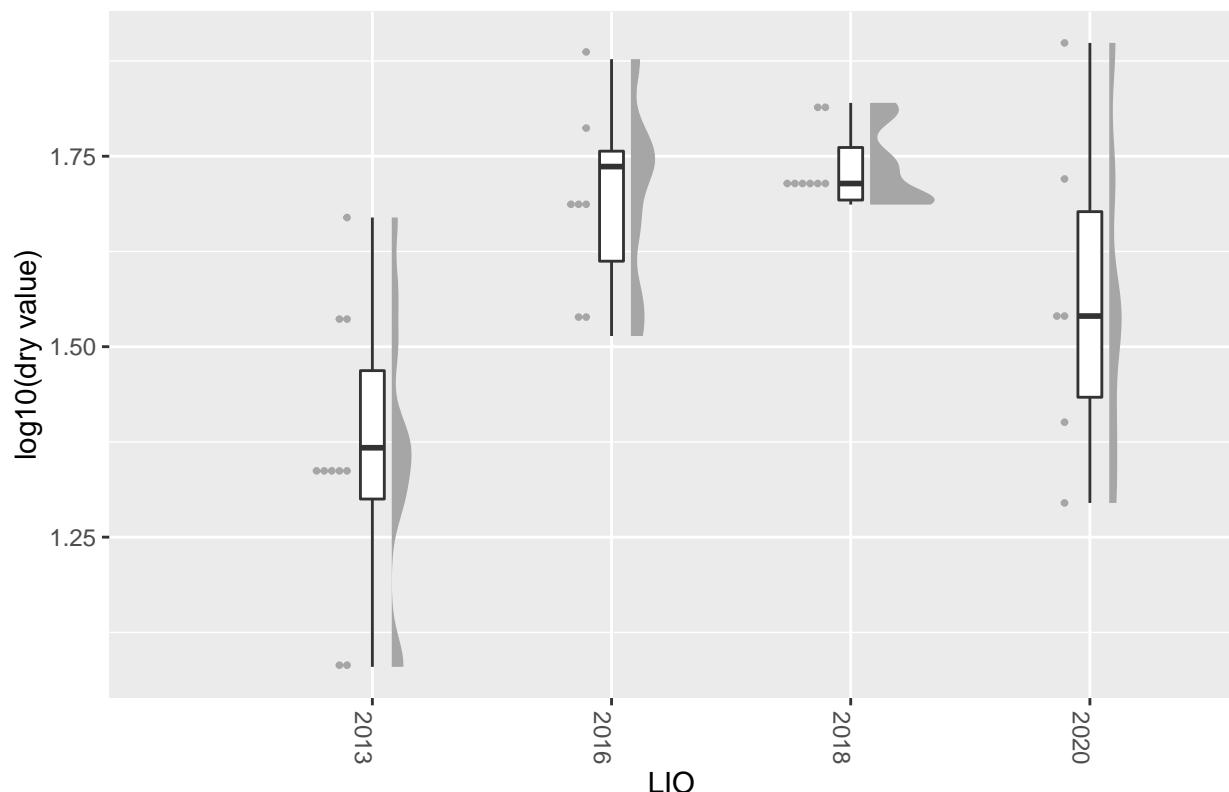
PBDEs – LIO – Whatcom



192

193 ## \$‘South Sound‘

PCBs – LIO – South Sound

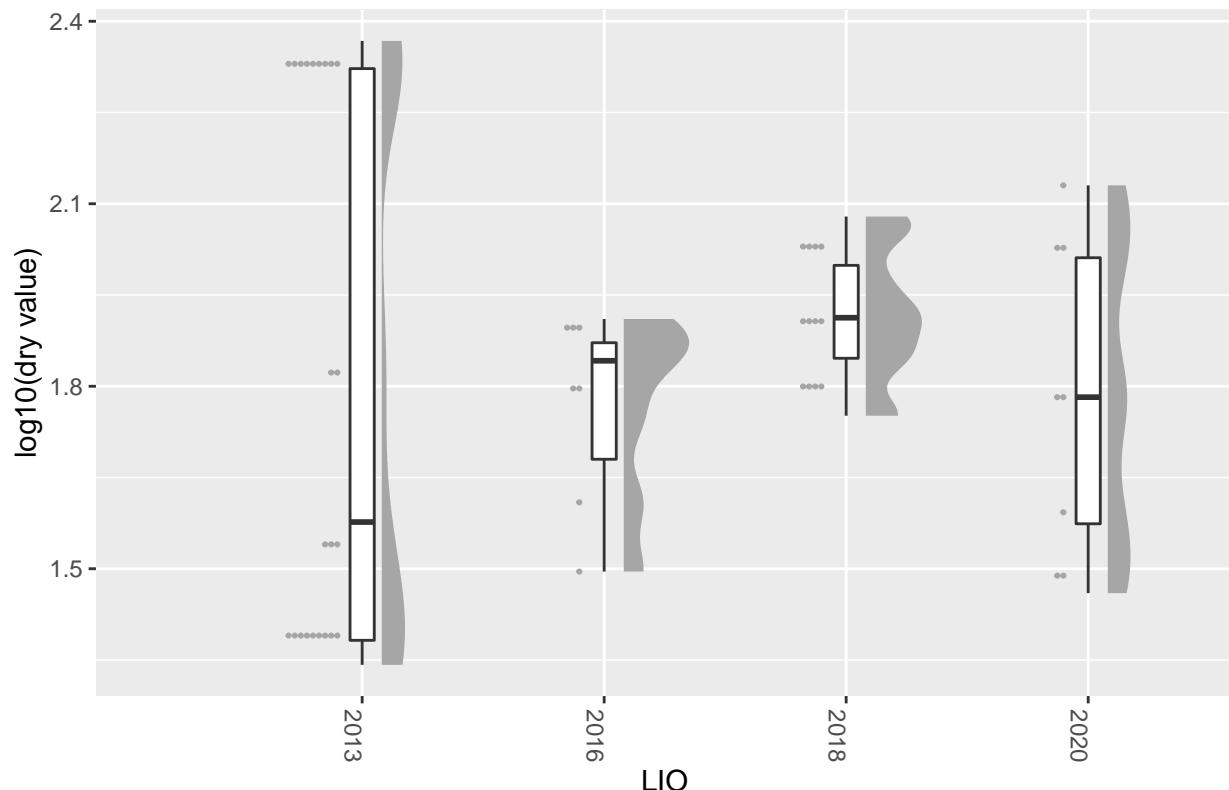


194

195 ##

196 ## \$‘Puyallup / White‘

PCBs – LIO – Puyallup / White

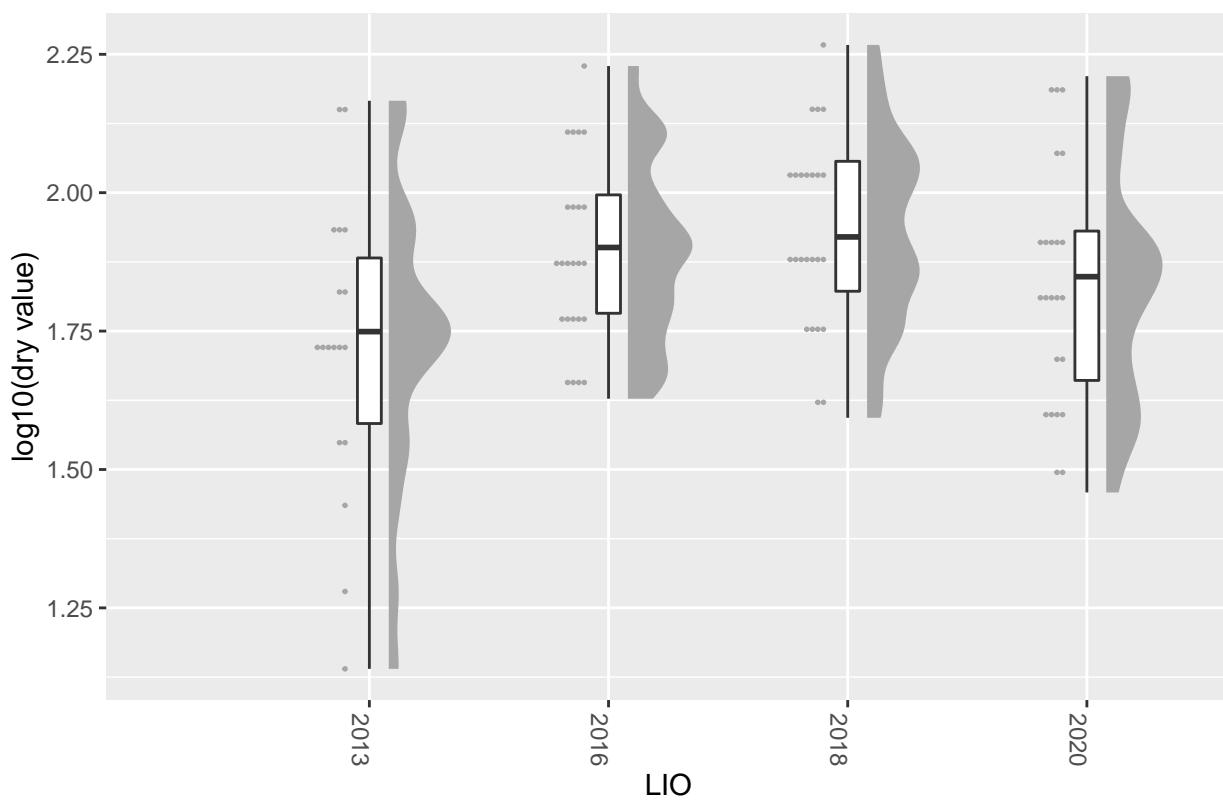


197

198 ##

199 ## \$'West Sound'

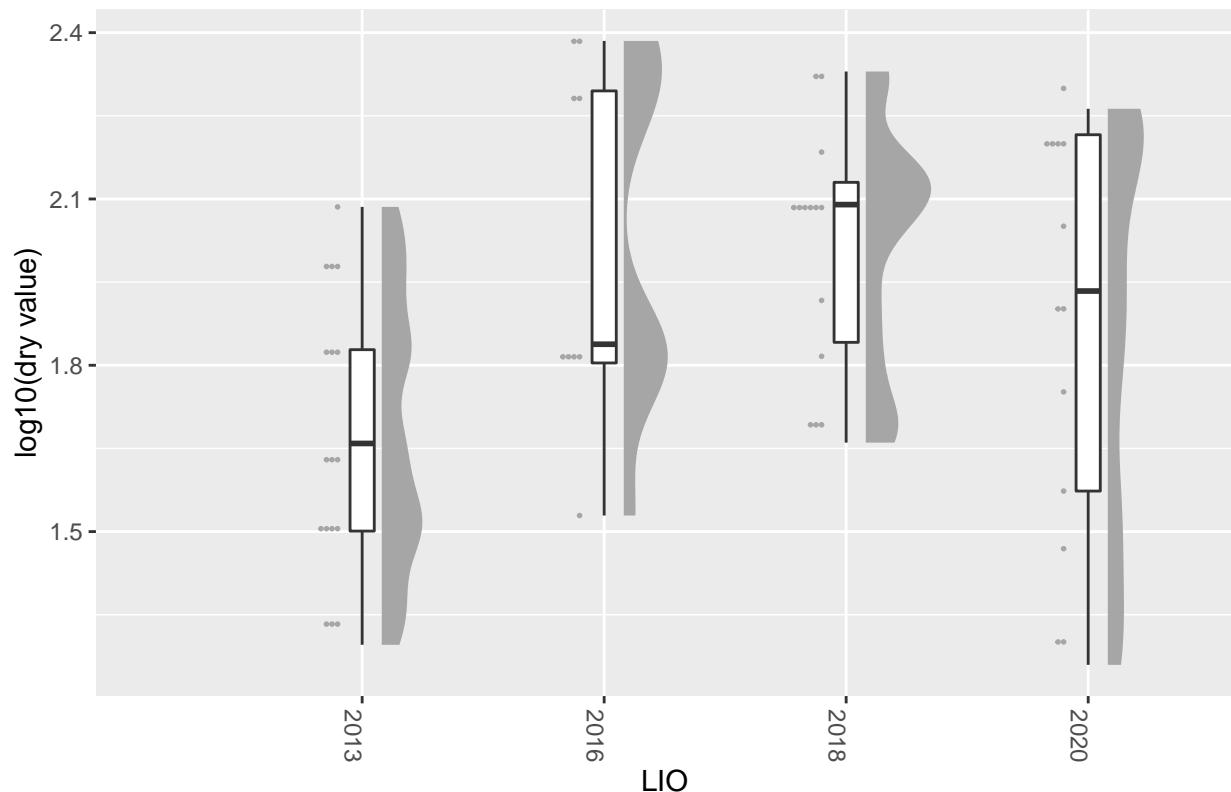
PCBs – LIO – West Sound



200

```
201 ##  
202 ## $‘South Central’
```

PCBs – LIO – South Central

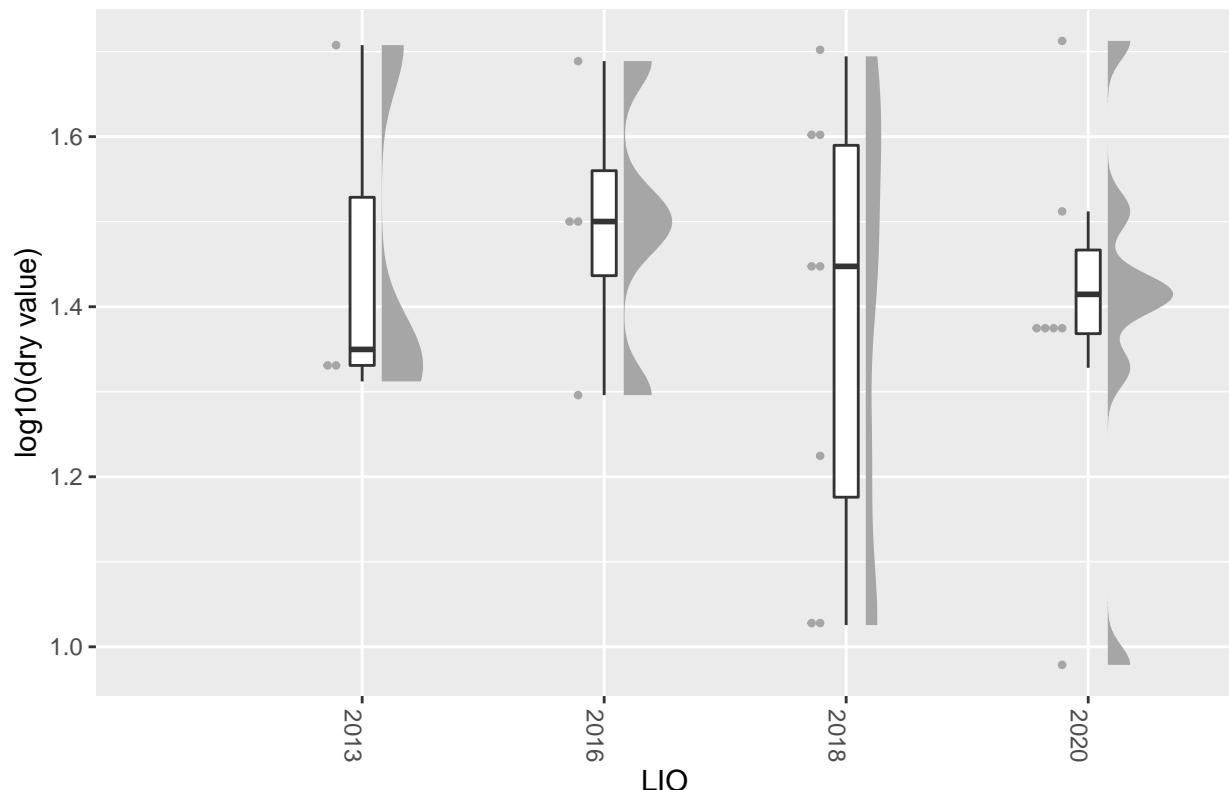


203

204 ##

205 ## \$‘Hood Canal’

PCBs – LIO – Hood Canal

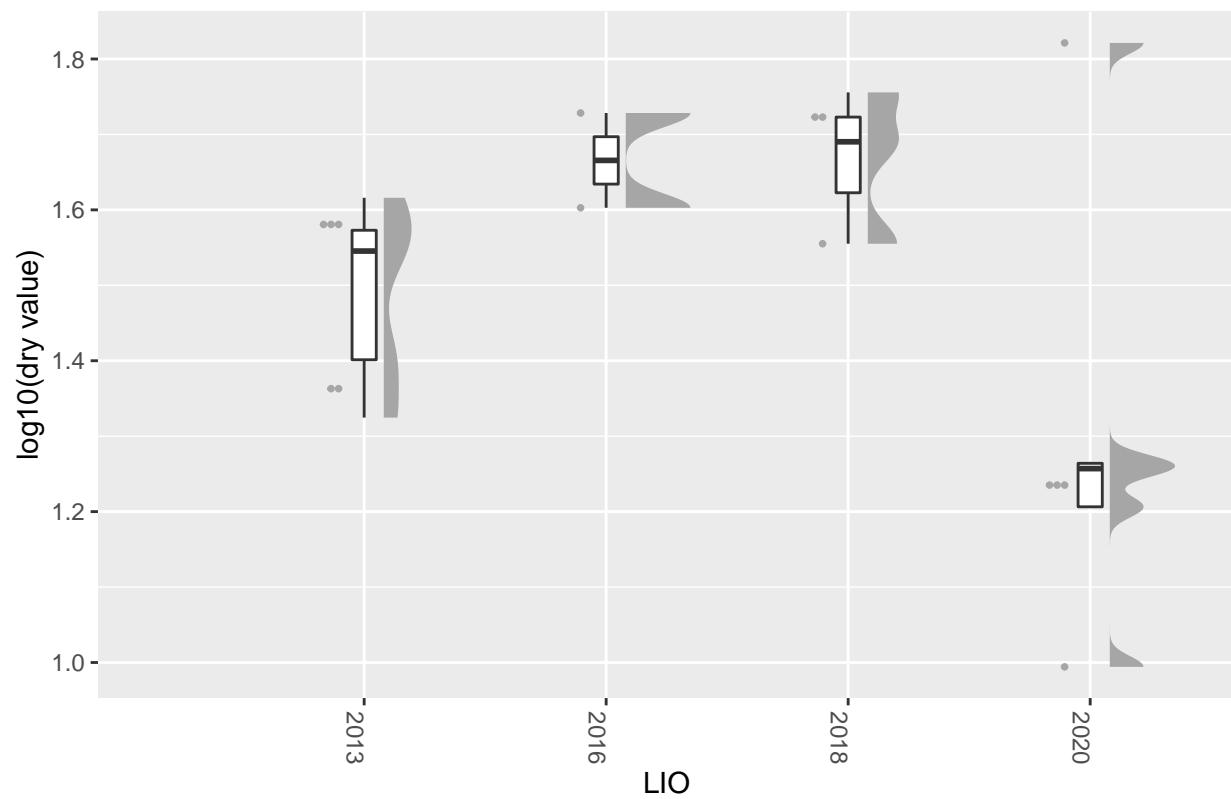


206

207 ##

208 ## \$‘Snohomish / Stillaguamish’

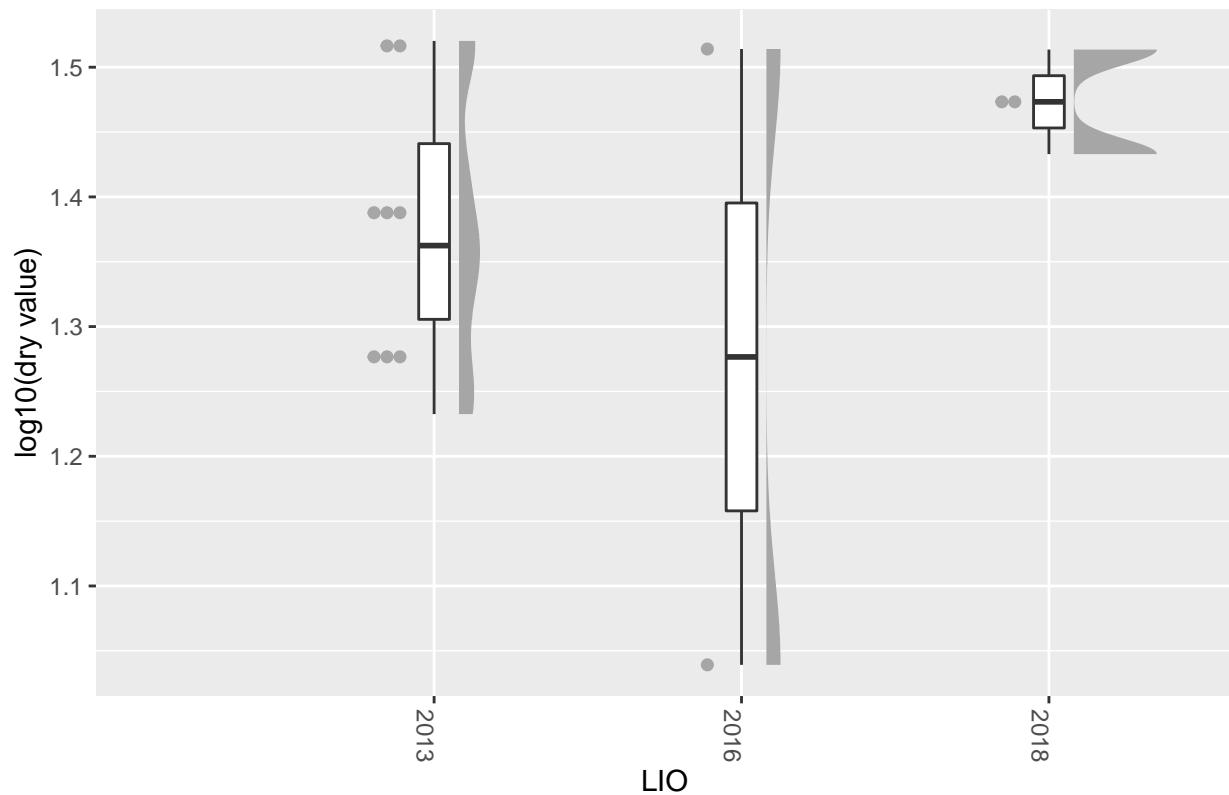
PCBs – LIO – Snohomish / Stillaguamish



209

```
210 ##  
211 ## $Island
```

PCBs – LIO – Island

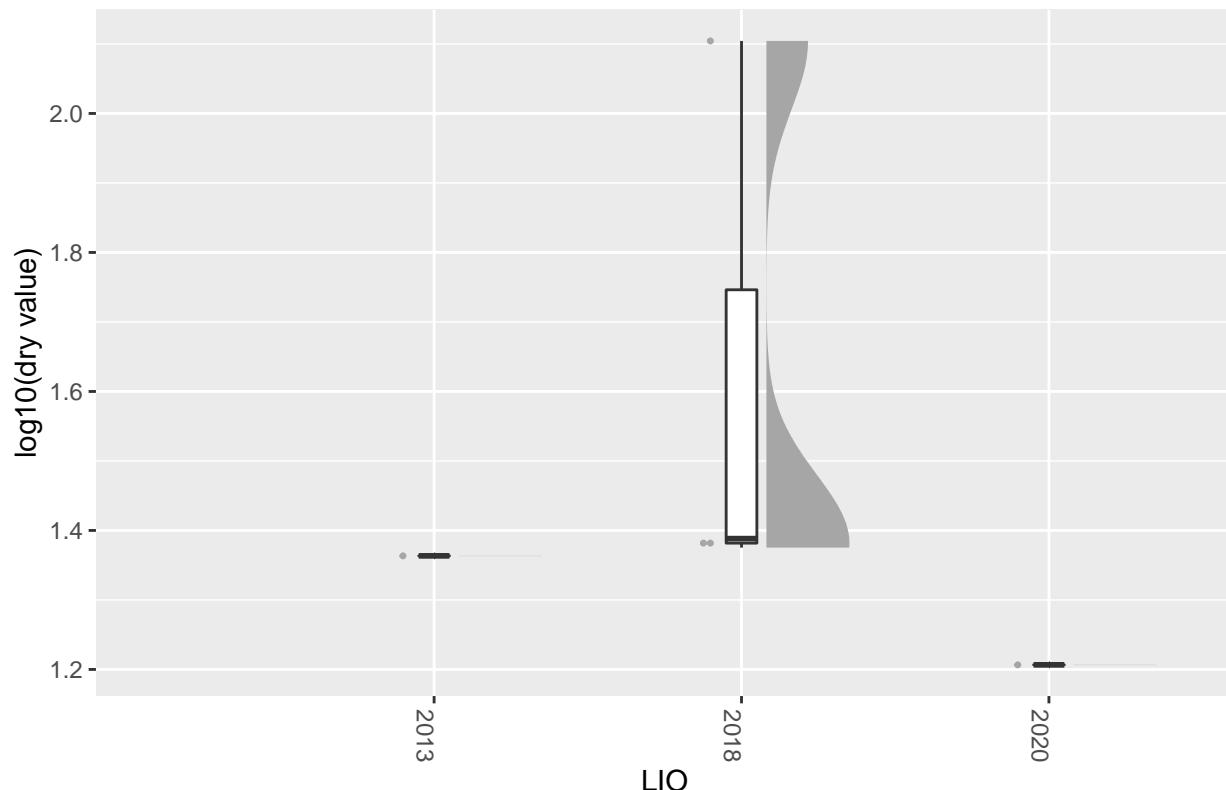


212

213 ##

214 ## \$‘Strait of Juan de Fuca’

PCBs – LIO – Strait of Juan de Fuca

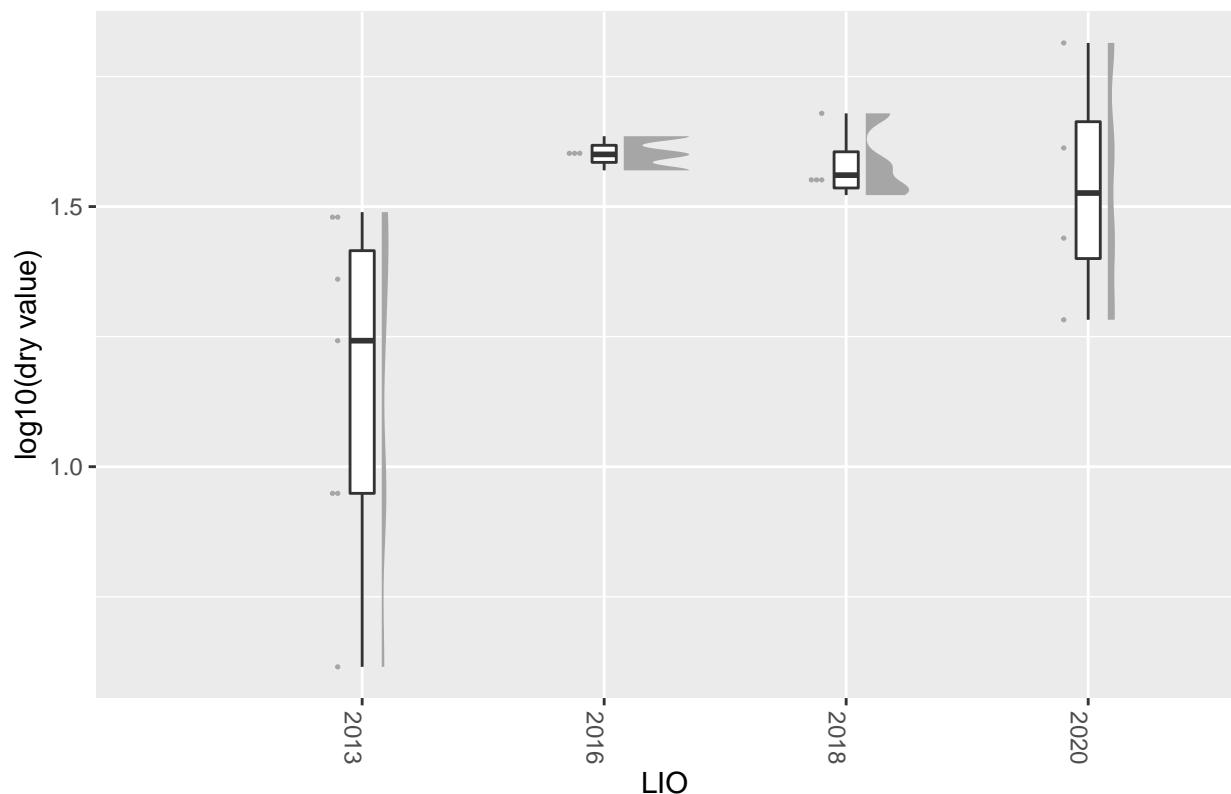


215

216 ##

217 ## \$‘Skagit / Samish‘

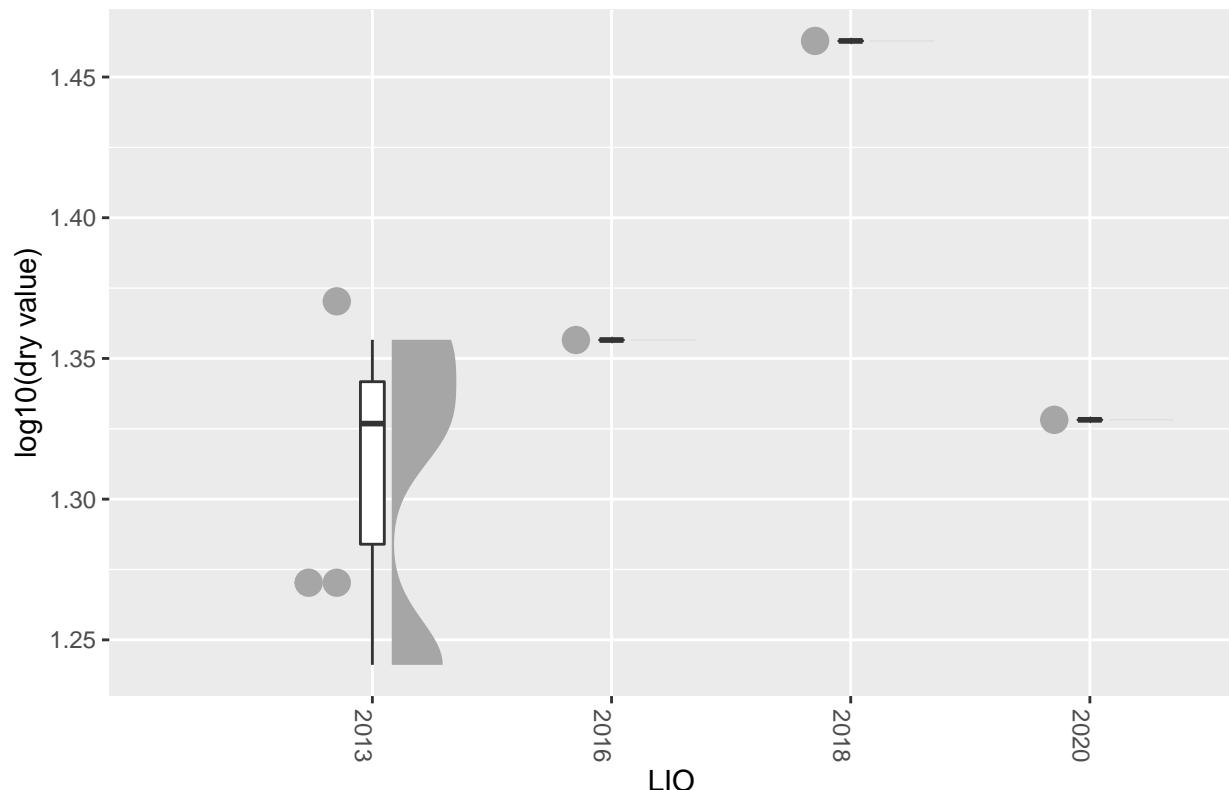
PCBs – LIO – Skagit / Samish



218

```
219 ##  
220 ## $‘San Juan’
```

PCBs – LIO – San Juan

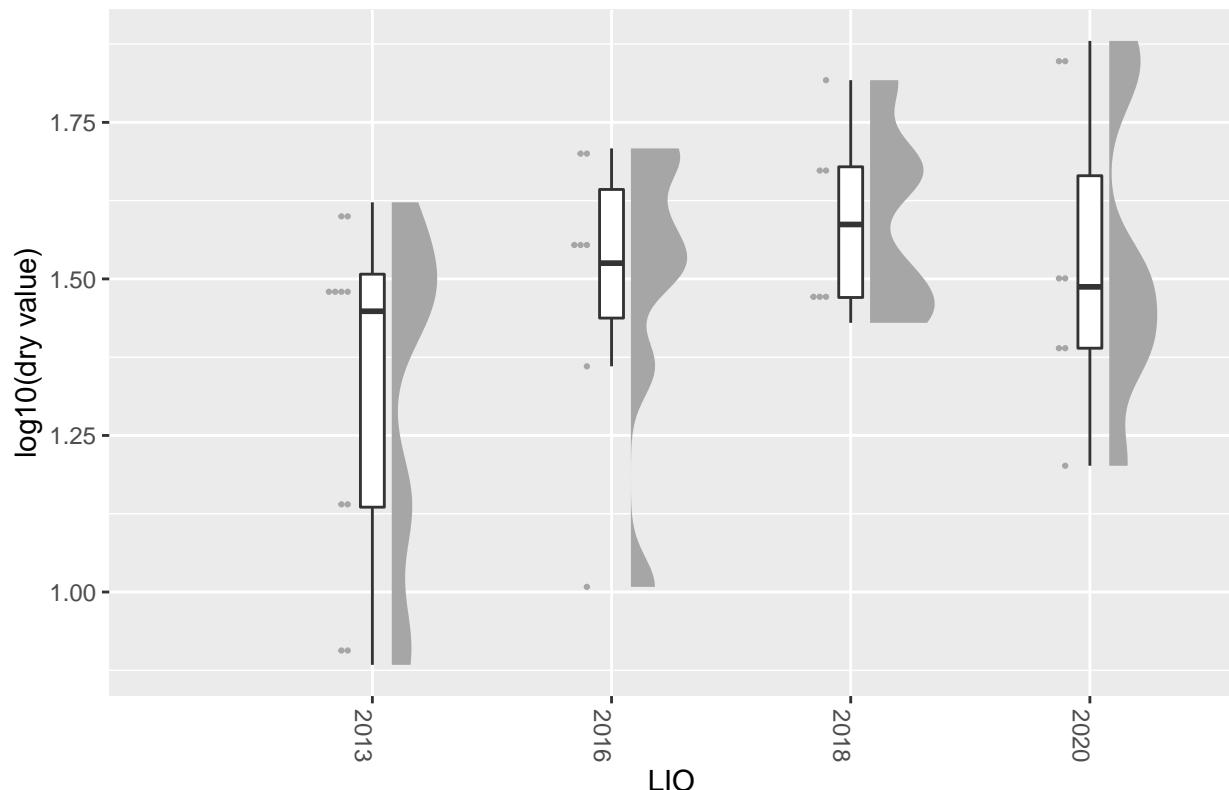


221

222 ##

223 ## \$Whatcom

PCBs – LIO – Whatcom



224