

1 Manuscript

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| 33 NOTE I'm still working on integrating with Zotero and importing all the necessary references (thanks | |
| 34 Jim for the help and instructions!). Don't worry about the ?? references in the rendered version for now. | |
| 35 Savina I'm particularly keen to get your feedback on this. I think you mentioned including some other | |
| 36 references and discussion in Jeff's Bayesian model paper. If you think they fit in here please let me know or | |
| 37 feel free to include them. Likewise, please include any other comments you have, or if you have any insight | |
| 38 into how the interpretation of results from different methods would vary. | |

39 **Abstract**

- 40 1. Size spectra represent a fundamental attribute of community organization and are increasingly being
41 used in assessments of fresh waters.
- 42 2. Many methods have been proposed for constructing size spectra relationships, but recent work has
43 shown that varied methods return biased estimates of relationship parameters, and different methods
44 in fact are not estimating the same parameter. Despite this variability in estimates, it is unclear if
45 the relative change across environmental gradients is consistent across methodologies. Here, we simu-
46 late data sets across an hypothetical environmental gradient and estimate the size spectra parameter
47 (slope or exponent, depending on method) at each site, and, importantly, estimate the relationship of
48 parameters across the hypothetical gradient. We also use two previously published body size datasets
49 across an anthropogenic stress gradient and an environmental temperature gradient and assess how
50 the conclusions of these studies would vary based on methods used.
- 51 3. We find that maximum likelihood methods always perform as well or better than common binning
52 methods. Additionally, the variance in estimates using MLE methods is markedly reduced when
53 compared to binning methods.
- 54 4. The uncertainty and variation in estimates when using binning methods is often greater than or equal
55 to the variation previously published in experimental and observational studies, bringing into question
56 the effect size of previously published results. However, re-analysis of two previously published datasets
57 does not markedly alter the conclusions reached. Further study is needed to identify how and when
58 these estimates may be biased, and when the general pattern of results can be considered to be “true”.

59 **Introduction**

60 Body size distributions are a fundamental characteristic of communities. The remarkable consistency of
61 these relationships across spatiotemporal scales and habitat types has led them to be recommended as a “uni-
62 versal” indicator of ecological status (Petchey and Belgrano 2010)(Petchey and Belgrano 2010). Size metrics
63 have commonly been used in marine systems, and are increasingly being applied to assess the condition of
64 freshwater ecosystems (Martinez et al. 2016, Pomeranz 2018)(Pomeranz et al. 2019, **martinez2016?**).

65 Individual size distributions (ISD, also referred to as abundance size spectra) are commonly used. Gen-
66 erally, there is a negative relationship between body size (M) and abundance (N). Theoretical and empirical
67 data support this relationship being described as a simple power law with exponent λ in the form of $N \sim M^\lambda$.
68 Commonly, N is the count of body sizes grouped into bins, and λ is estimated from OLS regressions of log
69 transformed data of N and the mid point of the body size bin M_{bin} : $\log(N_{count}) = \lambda \log(M_{bin})$. Myriad
70 binning methods have been proposed, including linear and logarithmic bin widths. Likewise, methods can
71 rely on the absolute count in the bins as well as normalization techniques where the count is divided by the
72 bin width (especially common with logarithmic binning). Alternatively, λ can be estimated directly using
73 maximum likelihood techniques.

74 Previous work has shown that the estimates of λ differ between MLE and OLS techniques. OLS methods
75 are particularly sensitive to decisions made in the binning process. Simulation studies have shown that MLE
76 offers consistently more accurate estimates of λ (White et al. 2007, Edwards et al. 2017)(White et al. 2007,
77 Edwards et al. 2017), and reanalysis of empirical datasets also indicates that the conclusions are dependent
78 on the methodology used (White et al. 2007, Edwards et al. 2020)(White et al. 2007, Edwards et al. 2020).
79 However, recent empirical analysis of stream macroinvertebrate communities across the NAtional Ecological
80 Observatory Network (NEON, USA) showed that while the estimates of λ varied, the relative change across
81 the environmental gradient was consistent regardless of method used (Pomeranz et al. 2022)(Pomeranz et
82 al. 2022). While there is a growing consensus that MLE methods offer more reliable estimates of λ , and
83 binning methods result in biased estimates, it remains unclear if these biases are consistent and systematic or
84 stochastic, and whether or not the relative change in ISD parameters is consistent across space and time. In
85 other words, if the data within a study are all treated the same, does a relative change of OLS slope parameters
86 of 0.1 coincide with a relative change of MLE estimates of 0.1? In order to answer this question, we simulate
87 body size observations from bounded power law distributions with varied λ exponents across a hypothetical
88 environmental gradient and compare the results obtained using three different methodologies common in
89 the literature. In addition, we re-analyze two previously published datasets of stream community body sizes

90 across a stress and environmental gradient. We find that the MLE method more accurately estimates the
91 site-specific λ exponents as well as the relative change in exponents across the hypothetical gradient, as
92 well as having smaller variation. The logarithmic binning methods generally perform well, but have larger
93 confidence intervals around the estimates. However, the overall conclusions of previously published empirical
94 data sets are not dependent on the method used.

95 Methods

96 Data Simulation

97 In order to investigate the performance of commonly used methods, we simulate body size observations
98 from a bounded power law distribution using the inverse method, as described in Edwards et al. (2017)(2017).
99 Let M be a random variable of body sizes described by the probability density function:

$$f(M) = CM^\lambda, M_{min} \leq M \leq M_{max}$$

100 $M_{min} = 0.0026$ and $M_{max} = 1.2 * 10^3$. These values are based on empirical body sizes of stream benthic
101 communities reported in Pomeranz et al. (2020)(Pomeranz et al. 2022). Our results are not dependent on
102 the range of body sizes, and we show the results of other body size ranges in the supplemental information.

103 For the main analysis, presented here, we sampled $n = 1000$ body sizes from distributions described by
104 five different λ 's: (-1.5, -1.75, -2.00, -2.25, -2.5), such that our model for λ is:

$$\lambda = Norm(\alpha - 0.5X, \sigma)$$

105 Each value of λ was assumed to come from a community across a hypothetical environmental gradient
106 X . Values of X for each community are uniformly distributed from -1 to 1. We repeated the data simulation
107 1000 times (reps). The main results presented here were not dependent on the range of x -values or the
108 number of sites (supplemental information).

109 Sample size, n

110 The number of observations in our simulations may bias the results. Therefore, we repeated the simulations
111 described above, but varied the sample size n . We tested values of $n = 200, 500, 1000, 5000, 10000$.

112 “No” relationship

113 It is possible that our sampling or simulation framework could bias the results obtained. In order to
114 assess whether or not our simulations are robust to this, we performed independent random samples from
115 a bounded power law for five communities across a hypothetical gradient as described above, but we set
116 $\lambda = -2$ for all five communities. Hence, we are simulating a scenario where the size spectra relationship
117 is invariant to our hypothetical environmental gradient, and we should expect the estimated relationship
118 coefficient $\beta_1 = 0$.

119 Finer resolution of λ values

120 For the main analysis, we were interested in determining whether or not the methods were able to
121 detect relatively coarse changes to size spectra parameters, specifically, a change of 0.5 units across each
122 value of X , or an absolute change in λ values of 1.0 units. However, many seminal works on variation
123 in size spectra relationships in freshwater are on a much finer scale (on the order of 0.1 to 0.25 units).
124 Therefore, we performed simulations as described above, but varied λ from -1.9 to -2.1 across the hypothetical
125 environmental gradient. This equates to a know relationship coefficient of $\beta_1 = 0.1$ across the hypothetical
126 gradient, and an absolute change of 0.2 units.

127 **Estimation of Size spectra parameter λ**

128 For each sample of M and each of the 1000 replicates, we estimated the exponent λ using MLE methods
129 modified from the the `sizeSpectra` package (Edwards et al. 2020). In addition, we use two common binning
130 methods to estimate the OLS slope parameter in log-log space. For the first binning method, we created 6
131 equal logarithmic bins covering the range of body sizes. The count in each bin was normalized by dividing
132 by the bin width. This method has been used by (refs here, I think a lot of Woodward's group uses this...)
133 Throughout the manuscript, the normalized equal logarithmic binning method will be referred to as ELBn.
134 The second method was similar to ELBn, but bins of Log_2 widths are used. The count in each bin is
135 normalized in the same way. This method has been used by (Pomeranz et al. 2019, McGarvey et al. 2019)
136 and is referred to as the Normalized Abundance Spectrum (NAS). Previous work has investigated the biases
137 in λ estimates when using different methods (Edwards et al. (2017)(2017), Edwards et al. 2020(2020), White
138 et al. 2008(2007)). The focus of the present work is to investigate biases when estimating the relationship or
139 relative change in parameters across an environmental gradient. Hence, we do not discuss the λ estimates in
140 the main text, but the results are presented in the Supplemental Information (**NOTE** I'm thinking about
141 dropping this from SI. Might be too much info, and it's already been published on by Edwards. Does this
142 add anything to the story??).

143 **Estimation of relationship across gradient, β_1**

144 For each simulation replicate, we estimated how the size spectra parameters varied across the hypothetical
145 gradient. Simple OLS regression were conducted in the form $\lambda_{estimate} \beta_0 + \beta_1 * X$. The distribution of the
146 relationship coefficient, β_1 , were plotted compared to the known relationship.

147 **Empirical Data**

148 We re-analyze two data sets of benthic macroinvertebrate communities from stream habitats across two
149 different gradients. In the first, quantitative macroinvertebrate samples were collected from streams across
150 an acid mine drainage (AMD) stress gradient. Details of the sample collection and processing can be found
151 in (Pomeranz et al. 2019). Briefly, all individuals from each sample were identified to the lowest practical
152 taxonomic unit and body lengths were measured using image processing software from photos taken with a
153 camera mounted to a dissecting microscope. Body mass was estimated using taxon-specific published length
154 weight regressions.

155 The second data set was from the wadeable stream sites of the National Ecological Observatory Network
156 (National Ecological Observatory Network (NEON) 2022). NEON stream sites are located across a wide
157 temperature gradient in the United States, from Puerto Rico to Alaska. Quantitative macroinvertebrate
158 samples were collected using the most appropriate method based on the local habitat. All individuals were
159 identified and had their body lengths measured, and body mass was estimated using published length weight
160 regressions. This data has been analyzed previously using size spectra methods as described in (Pomer-
161 anz et al. 2022). Detailed methods of the sampling collection and data processing methods can be found
162 on the NEON website ([<https://www.neonscience.org/data-collection/macroinvertebrates>], or macroinverte-
163 brate DPI pubs). Coefficient estimates (± 1 standard deviation) are compared across methods. This allows
164 us to determine whether or not the main results would differ depending on the method used.

165 **Results**

166 **Relationship across the hypothetical environmental gradient**

167 Alternate plot version

168 All methods performed reasonably well in detecting the known relationship across the hypothetical en-
169 vironmental gradient (mean β_1 estimates ranged from -0.419 to -0.498). However, the SD for the NAS and
170 ELBn methods were 2-3x larger than that of the MLE method (Figure xx). This result was consistent across
171 simulation parameters, including sample size, number of sites, and λ values.

PLB

$m_{\text{range}} = (0.0026, 1200)$

$b = (-2.5, -1.5)$

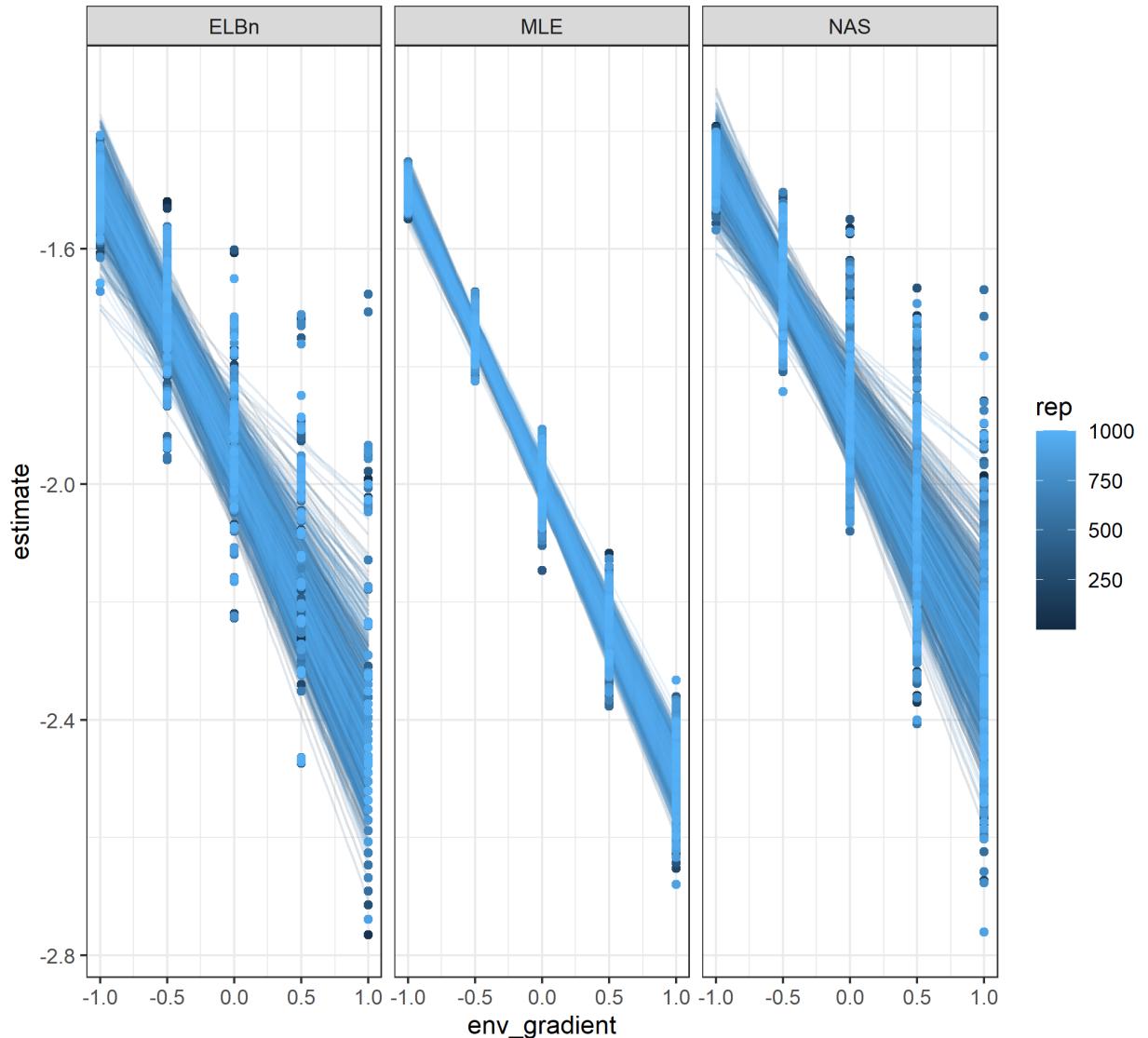


Figure 1: Relationship estimates across the hypothetical gradient for each replicate. Each panel is a different method for estimating the size spectra parameter.

PLB

m_range =(0.0026,1200)
 $b = (-2.5, -1.5)$

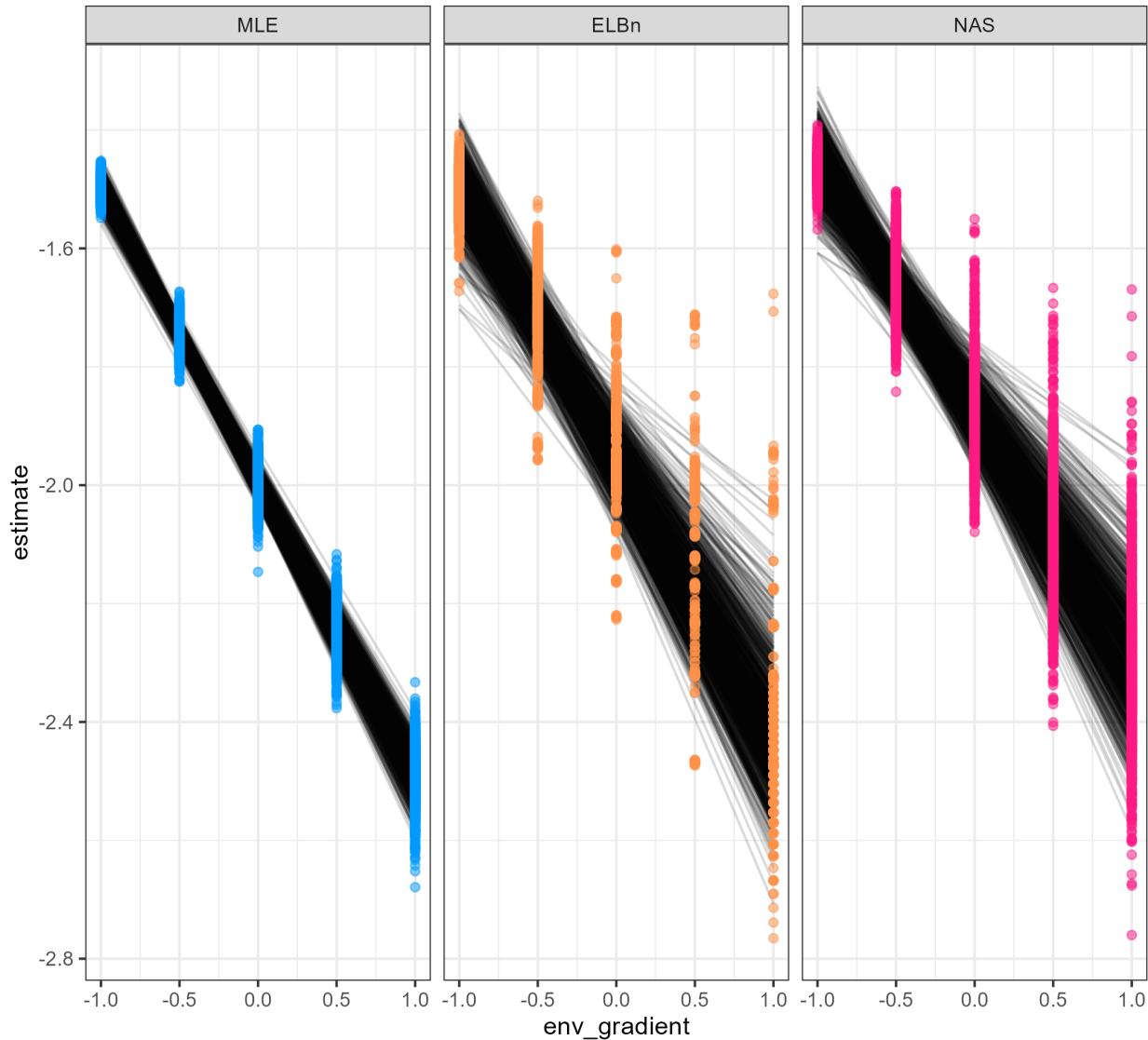


Figure 2: alternate plot version, thoughts on which you prefer??

```
172 ## Model p50 p25 p975  
173 ## 1 MLE -0.49 -0.50 -0.48  
174 ## 2 ELBn -0.49 -0.52 -0.29  
175 ## 3 NAS -0.44 -0.46 -0.30
```

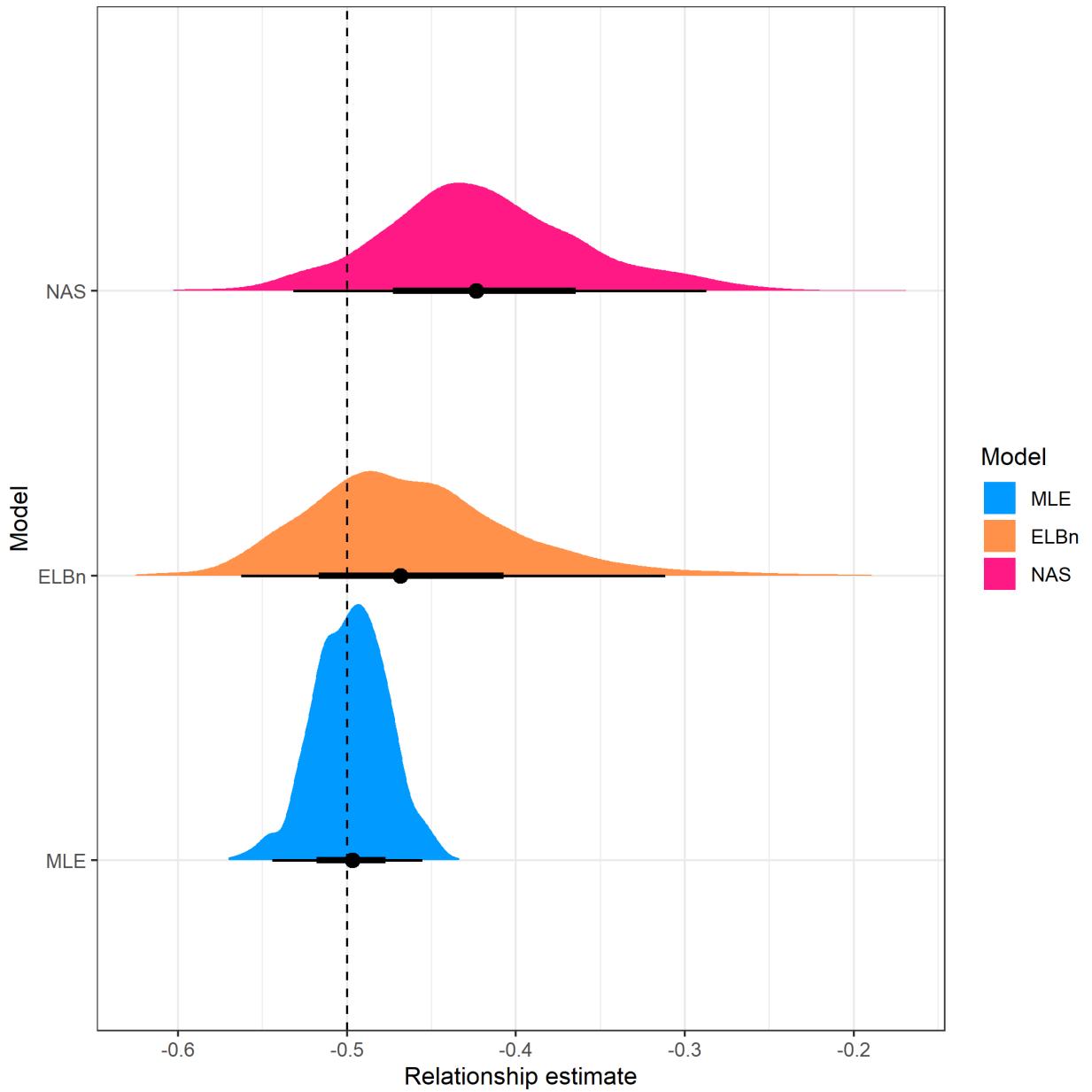


Figure 3: Halfeye plots for the estimated relationship coefficient estimates across 1000 replicates and three different methods. The point below each distribution plot is the median estimate, the thick and thin bars represent the 66th and 95th percentile, respectively. Vertical line is the known relationship. The median estimate for the MLE method was closest to the known relationship, and had the narrowest distribution across replicates.

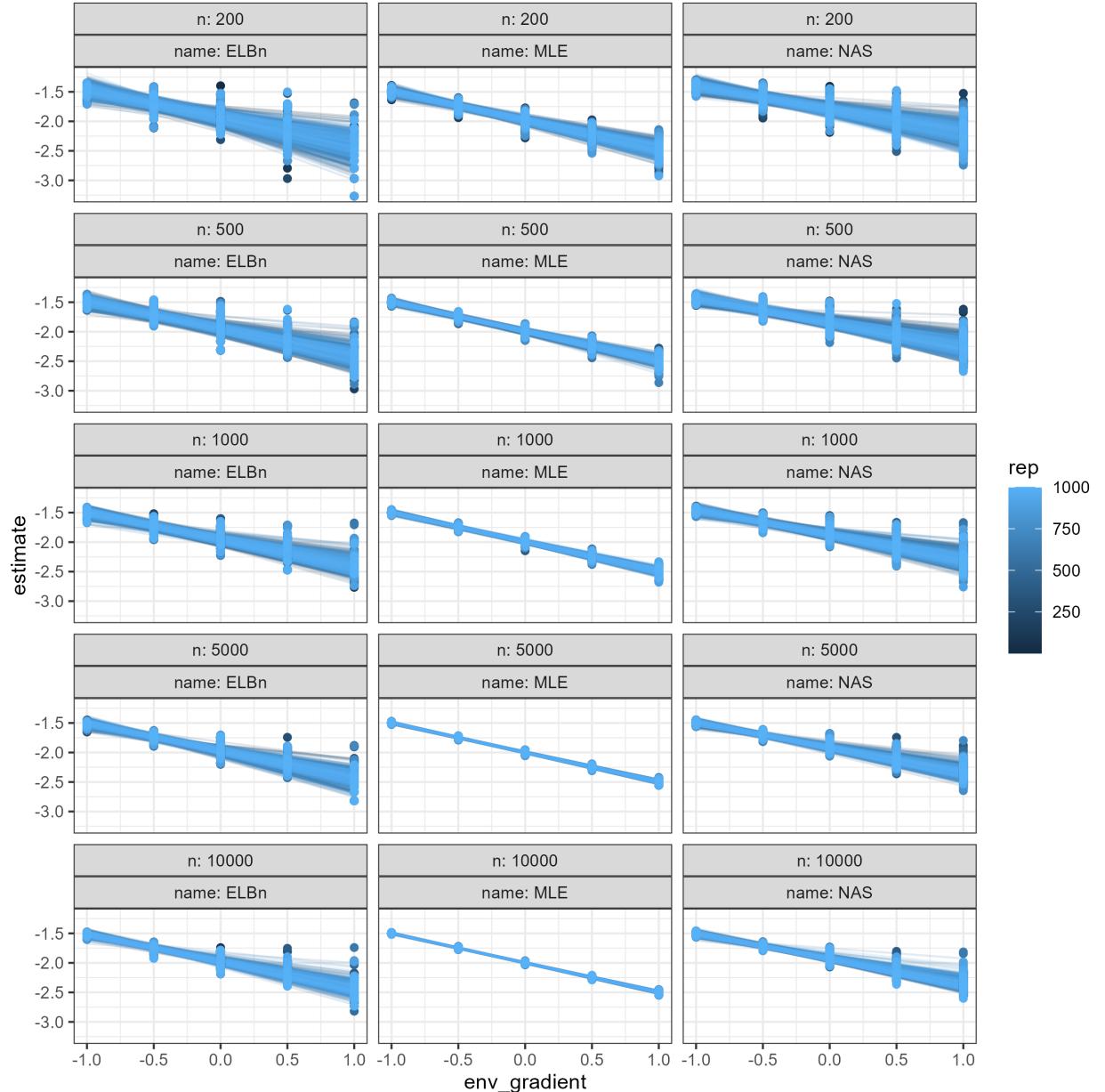


Figure 4: Individual regression estimates across the hypothetical gradient based on sample size (rows) and methodology used (columns).

176 **No relationship**

177 All of the methods performed similarly when there was no relationship across the hypothetical gradient.
178 The mean β_1 estimates for all three methods ranged from -0.0005 to 0.0002. However, the distribution
179 of coefficient estimates was much wider for the two binning methods; the 95th percentile of the MLE
180 distributions is narrower than the 66th percentiles for the NAS and ELBn methods.

```
181 ## Model p50 p25 p975
182 ## 1 MLE 0 -0.01 0.04
183 ## 2 NAS 0 -0.04 0.12
184 ## 3 ELBn 0 -0.05 0.13
```

185 **Small variation in lambda**

186 The performance of all methods to detect small variation in the λ parameter (between -1.9 to -2.1) across
187 the environmental gradient was also investigated. The median β_1 coefficient estimates for the MLE method
188 was -0.1, which exactly matched the value for the hypothetical relationship, while the other two methods
189 both had median estimates of 0.9. The upper 97.5 percentile of the distribution was below 0 for the MLE
190 method, meaning that most of the estimates had the correct sign. In contrast, the upper 97.5 percentile for
191 both the NAS and ELBn methods were greater than 0 at 0.027 and 0.052, respectively. (Figure 4).

```
192 ## Model p50 p25 p975
193 ## 1 MLE -0.10 -0.11 -0.06
194 ## 2 NAS -0.09 -0.13 0.03
195 ## 3 ELBn -0.09 -0.13 0.05
```

196 **Empirical data**

197 For both empirical data sets, the direction and magnitude of change (i.e. β_1 coefficients) are generally in
198 agreement. Size spectra parameters consistently increase (become flatter) in the AMD data (Fig. XX A).
199 Likewise, the size spectra parameters consistently increase (become steeper) with increasing temperature
200 across the NEON sites (Fig XX D)

201 **NOTE** I need to re-plot these in ggplot and stitch together. Currently stitching pre-made png files,
202 I think that's why it looks so funky.

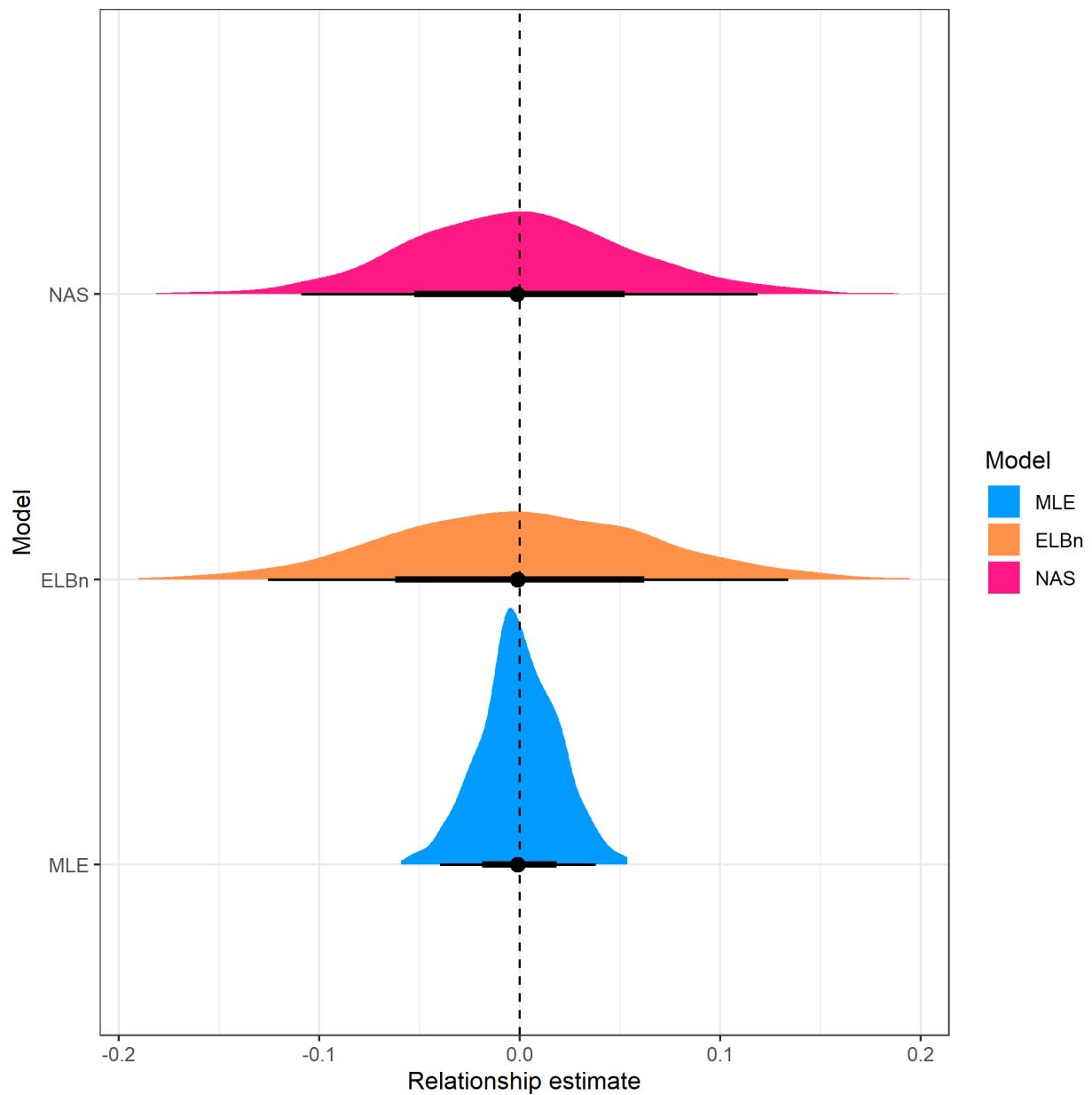


Figure 5: Individual regression estimates when no relationship exists across the hypothetical environmental gradient. The methods are separated by rows. The median for all methods is approximately zero, but the distribution for the MLE method is narrower than the two binning methods.

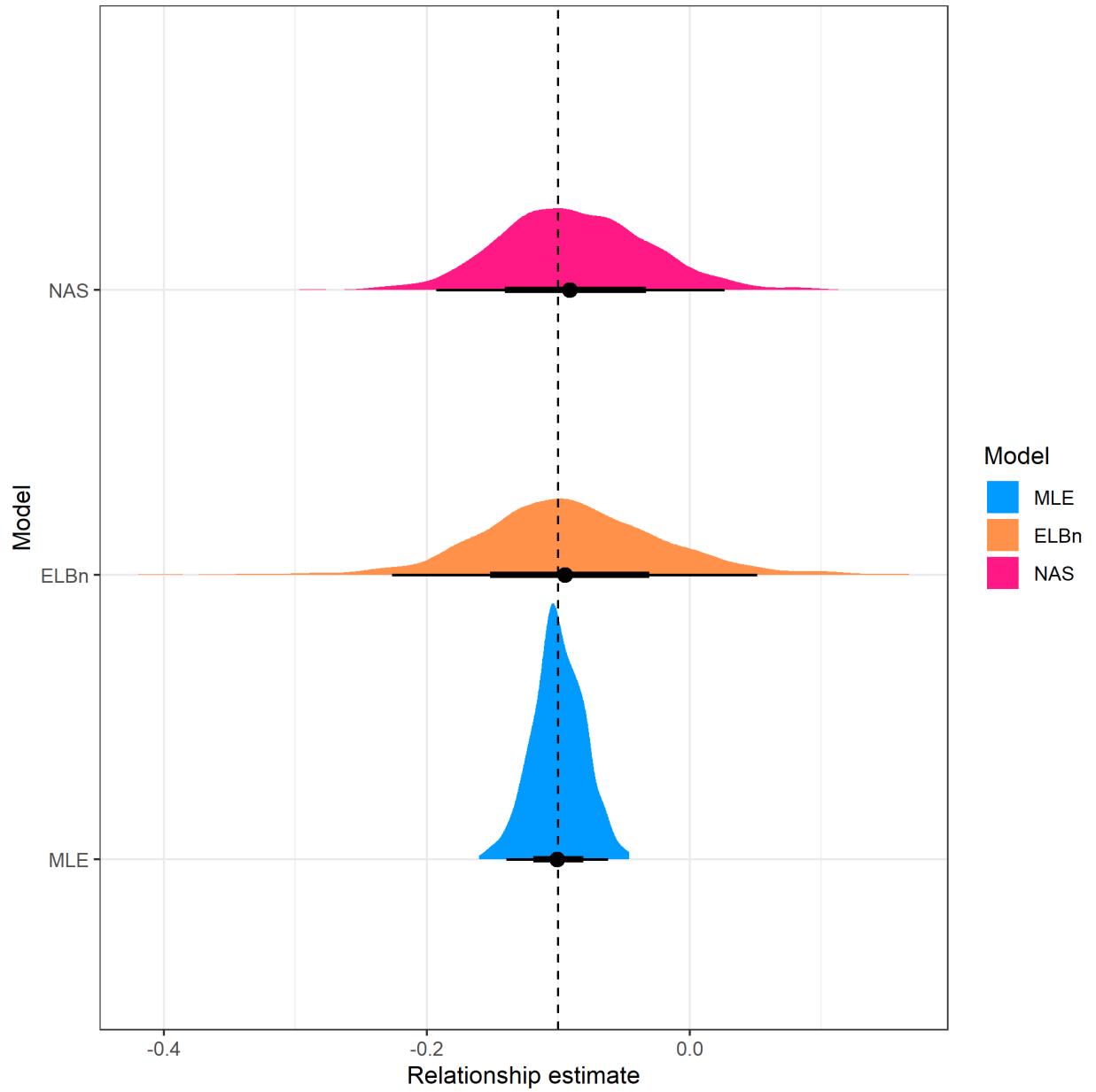


Figure 6: Distribution of the estimated relationship for each method. Here, λ varied from -1.9 to -2.1, with a known relationship of -0.1 across the gradient indicated by the dashed vertical line. The distribution of relationship estimates was approximately centered at the known relationship for all methods, with a narrower distribution observed for the MLE estimates.

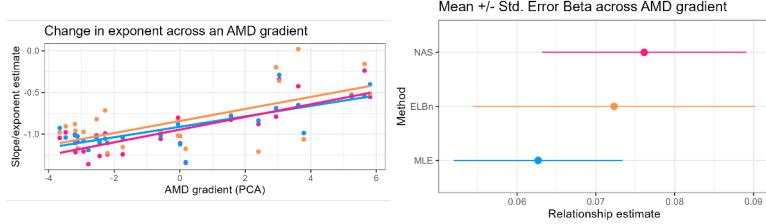
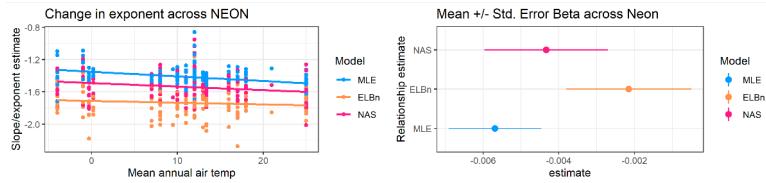
A**B****C****D**

Figure 7: Figure of empirical data estimates. All of the methods estimate the same sign of the relationship, but the estimates from the binning methods are generally greater than the MLE estimates.

203 The β_1 coefficient estimates for the AMD data are all positive and of relatively similar magnitudes across
204 methods. The MLE estimate has the smallest magnitude and SD (0.063 ± 0.011 SD), The ELBn method is
205 slightly larger and has a wider SD : (0.072 ± 0.018). Finally, the NAS method has the largest estimate and
206 an intermediate standard deviation (0.076 ± 0.013). The range of the gradient in the AMD data is relatively
207 small (9.5), meaning that the absolute change in the size spectra parameter (λ) across the AMD gradient
208 are similar regardless of method used (range of absolute: 0.59 to 0.72).

209 Similar to the AMD results, the β_1 coefficient estimates for the NEON data are all negative and have
210 similar magnitudes across methods. The MLE estimate is once again the smallest (-0.006 ± 0.001 SD).
211 The NAS and ELBn methods are larger at -0.004 ± 0.002 , and -0.002 ± 0.002 , respectively. Although the
212 estimates are similar and have a small magnitude, the range of the gradient in this data is larger (~30°C),
213 meaning that the absolute change in λ estimates ranges from 0.06 to 0.17, depending on the method used.

214 Simulating shallower lambdas

215 Estimates of the relationship between lambda and a hypothetical gradient varied depending on the method
216 used. However, interpretations of empirical data were broadly consistent across methodologies. Upon closer
217 inspection, the values of size spectra parameters in the empirical data were considerably shallower than -2.
218 Given that we found the performance of all methods increased with shallower λ 's we wanted to investigate
219 how the methods performed with simulated values closer to the empirical estimates of parameters describing
220 size spectra relationships. Therefore, we repeated the simulation process as in the main analysis, but used
221 λ values ranging from -1.1 to -1.5, with a known relationship of $\beta_1 = -0.2$

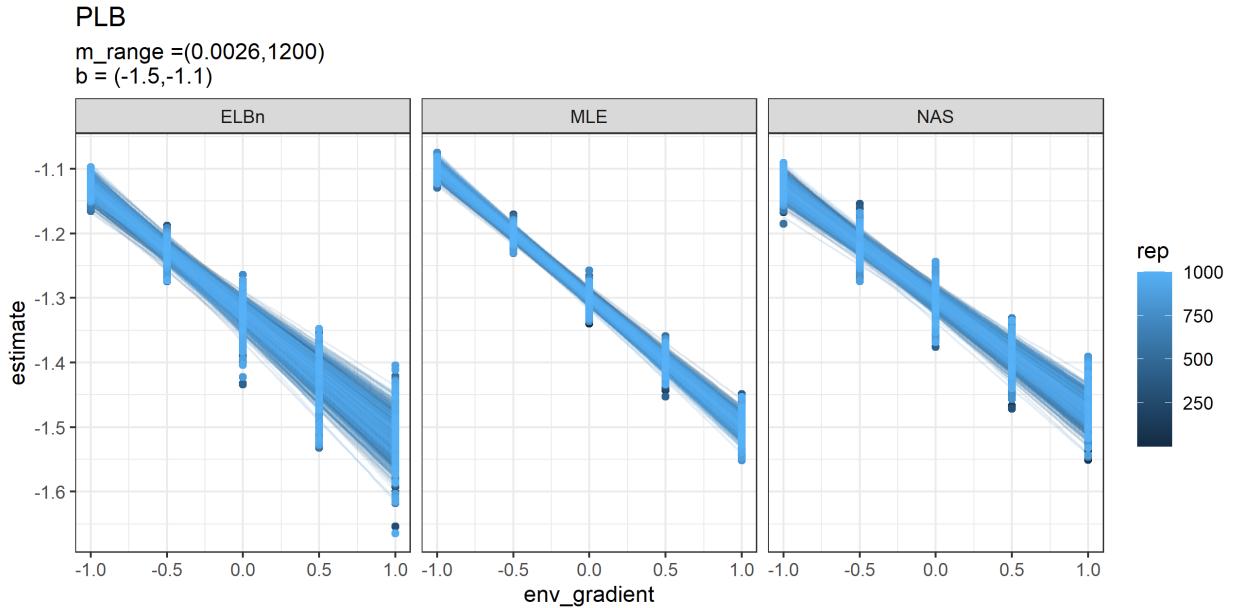


Figure 8: Relationship estimates across the hypothetical gradient for each replicate. Each panel is a different method for estimating the size spectra parameter.

222 We found generally less variability in the relationship estimates (β_1) across a gradient of distributions
 223 described with shallower λ parameters. However, the median MLE estimate was once again closest to the
 224 hypothetical relationship and had the narrowest distribution. The median ELBn estimate was close to the
 225 hypothetical relationship but had a slightly wider distribution. Finally, 95% of the NAS estimates were
 226 greater than the hypothetical relationship. In general, the distributions from the different methodologies
 227 followed the same overall pattern, with the MLE estimates being the smallest, and the binning methods
 228 being larger (i.e., more positive).

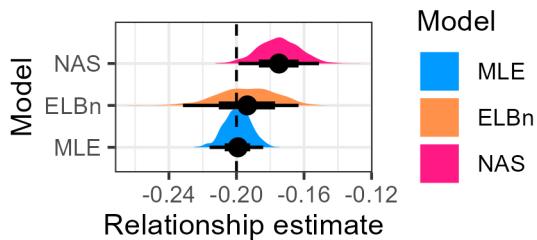


Figure 9: Distribution of relationship coefficient estimates. Vertical line is the known relationship. All methods under estimate the value, but the mean magnitude and distribution of values is greater for the ELBn and NAS methods.

```
229 ##   Model   p50   p25   p975
230 ## 1   MLE -0.20 -0.21 -0.18
231 ## 2   ELBn -0.19 -0.21 -0.16
232 ## 3   NAS -0.17 -0.18 -0.15
```

233 **Discussion**

234 Measuring parameters describing the decline in abundance with increasing body size in communities
235 is being done with increasing frequency across ecology. Previous work has investigated the accuracy and
236 inherent biases associated with different estimation methods. However, how these inaccuracies and biases
237 compound across environmental gradients remains uncertain, making it difficult to detect variation in size
238 spectra parameters across environmental gradients with confidence. Here, we sampled body sizes from known
239 distributions with varying parameters (λ) and estimated the coefficient of the relationship (β_1) across a hy-
240 pothetical gradient in order to assess how the results would vary depndng on the method used. Likewise, we
241 compared how the interpretation of previously published results could change depending on the methodology
242 used to estimate the relationship.

243 Generally, the estimate from the MLE method was always closer to the known relationship in simulated
244 data, and always had the smallest variation around the estimate.

245 Binning methods are easy to use and interpret, which most likely accounts for their wide use in ecological
246 studies. However, aggregating individuals into logarithmic bins removes a large source of the variation within
247 the data by collapsing body size variation into a single values within bins. For example, all individuals placed
248 into a bin that ranges from 2-4 grams of mass are all treated as having a mass of 3 grams, the midpoint
249 of that bin. Likewise, a single abundance value is taken for this bin, despite that fact that there is almost
250 certainly variation in the abundance of individuals that weigh ~2 grams or ~4 grams. By homogenizing
251 the data in this way, it seems that binning methods will always produce noisier results than MLE from the
252 binning process alone, since binning is akin to deleting information that the model could otherwise use.

253 One of the benefits of using MLE is that all data points are retained for use within the model.

254 The performance of all methods improved as sample size increases, and as the exponent of the size
255 spectra relationship gets larger (more shallow). As either or both of these variables change, the difference
256 in estimated relationship coefficients declines across the methods. This is particularly interesting given the
257 fact that both empirical data sets of stream communities examined here are shallower than the expected
258 relationship of $\lambda = -2$, and the conclusions of the change in size spectra relationships across the gradients
259 are not dependent on method used. If communities under study are in fact described by shallower exponents,
260 the method used may not be critical in the conclusions reached. However, the MLE method performs as
261 well or better than the two binning methods examined here under all contexts and should be the preferred
262 method in future studies. At a minimum, future studies should report MLE estimates to ensure that the
263 results are not dependent on the methodology used.

264 Despite the drop in performance with reduced variation in λ values, when a significant relationship was
265 observed, it was generally in the correct direction and of a similar magnitude. This suggests that previously
266 reported significant changes in size spectra parameters across environmental gradients and in experimental
267 manipulations are plausible, and the magnitude of the relative change is a reasonable estimate. Given that
268 all of the data within a study is treated identically, the the over all change in size spectra parameters is
269 likely reasonable. However, the biases and inconsistencies in relationship estimates presented here suggest
270 that it would be difficult if not impossible to compare the relative changes across different published studies
271 which use different methods. Publication of individual body size data with future studies of size spectra
272 relationships would greatly aid in our ability to generalize changes to this fundamental aspect of community
273 organization across spatiotemporal scales and in response to environmental stressors and perturbations.

274 **Concluding Remarks**

275 Both of the binning methods can perform as well as MLE under certain circumstances (large sample size,
276 shallow λ). However, it would be extremely difficult to know if this was the case beforehand. Furthermore,
277 in order to confirm this result, MLE methods would likely need to be employed. With the publication of the
278 sizespectra package (2017), producing MLE estimates of size spectra parameters is a relatively easy task.
279 Therefore, we recommend using this in all future studies of size spectra relationships.

280 We reiterate the recommendations of White et al. (2007)(2007), Sprules and Barth (2016)(2016) and
281 Edwards et al. (2017)(2017) to estimate size spectra relationships using MLE methods due to their superior
282 performance in nearly every context. Furthermore, we strongly encourage authors to publish individual size
283 data whenever possible. This will allow for the consistent re-analysis of existing data sets as methodologies

²⁸⁴ develop and improve. This will aid in the ability for size spectra work to be synthesized between research
²⁸⁵ groups and across scales.

286 **References**

- 287 Edwards, A. M., J. P. W. Robinson, J. L. Blanchard, J. K. Baum, and M. J. Plank. 2020. Accounting for the
288 bin structure of data removes bias when fitting size spectra. *Marine Ecology Progress Series* 636:19–33.
- 289 Edwards, A. M., J. P. W. Robinson, M. J. Plank, J. K. Baum, and J. L. Blanchard. 2017. Testing and
290 recommending methods for fitting size spectra to data. *Methods in Ecology and Evolution* 8:57–67.
- 291 McGarvey, D. J., T. E. Woods, and A. J. Kirk. 2019. Modeling the Size Spectrum for Macroinvertebrates
292 and Fishes in Stream Ecosystems. *Journal of Visualized Experiments: JoVE*.
- 293 National Ecological Observatory Network (NEON). 2022. Macroinvertebrate collection (DP1.20120.001).
294 National Ecological Observatory Network (NEON).
- 295 Petchey, O. L., and A. Belgrano. 2010. Body-size distributions and size-spectra: Universal indicators of
296 ecological status? *Biology Letters* 6:434–437.
- 297 Pomeranz, J. P. F., J. R. Junker, and J. S. Wesner. 2022. Individual size distributions across North American
298 streams vary with local temperature. *Global Change Biology* 28:848–858.
- 299 Pomeranz, J. P. F., H. J. Warburton, and J. S. Harding. 2019. Anthropogenic mining alters macroinverte-
300 brate size spectra in streams. *Freshwater Biology* 64:81–92.
- 301 Sprules, W. G., and L. E. Barth. 2016. Surfing the biomass size spectrum: Some remarks on history, theory,
302 and application. *Canadian Journal of Fisheries and Aquatic Sciences* 73:477–495.
- 303 White, E. P., S. K. M. Ernest, A. J. Kerkhoff, and B. J. Enquist. 2007. Relationships between body size
304 and abundance in ecology. *Trends in Ecology & Evolution* 22:323–330.

305 **Supplementary material**

306 **Range of body sizes, M**

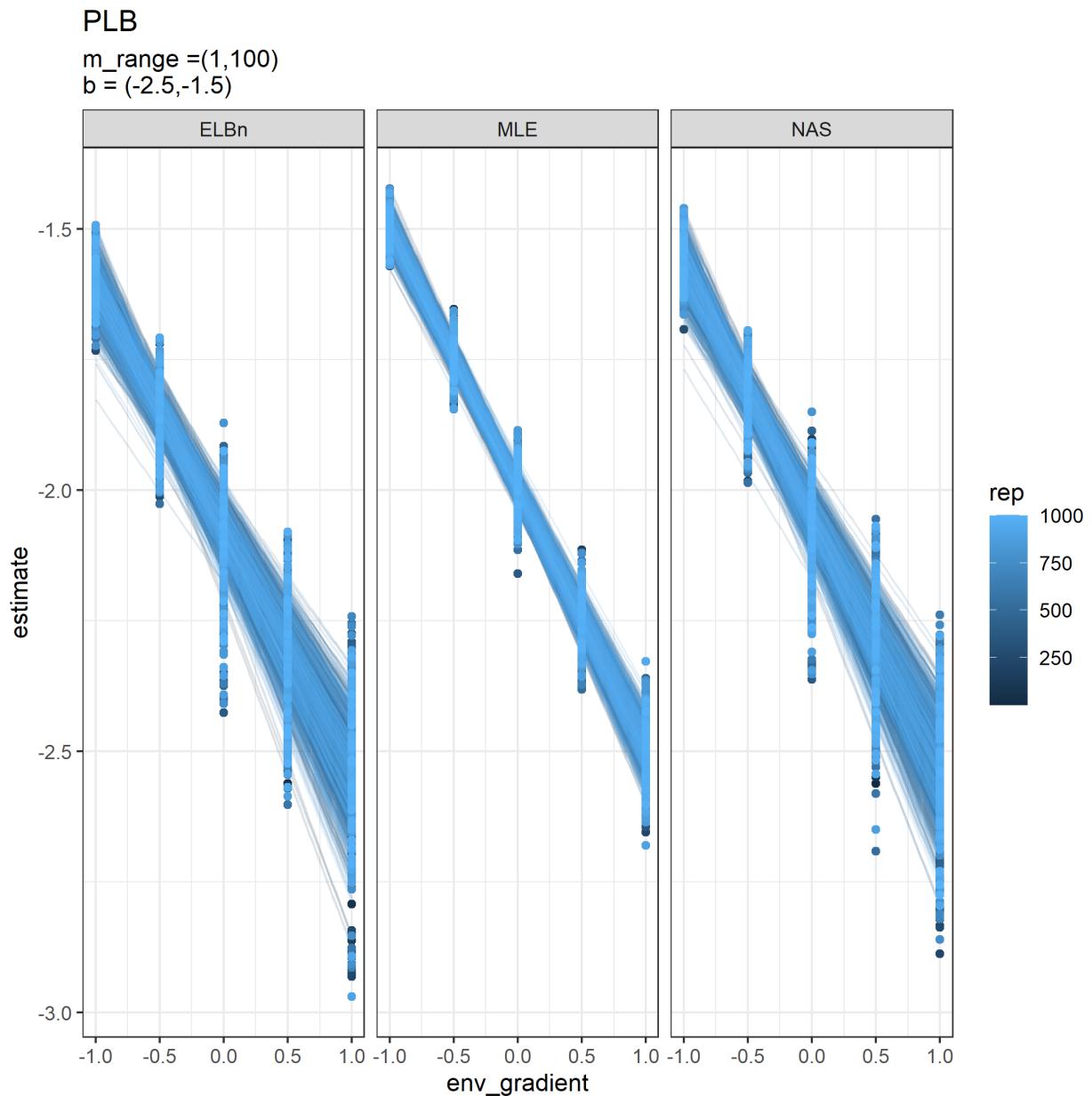


Figure S1: Individual regressions for five sites across a hypothetical gradient with a known relationship of 0.5. Range of body sizes is reduced and is from 1, to 100.

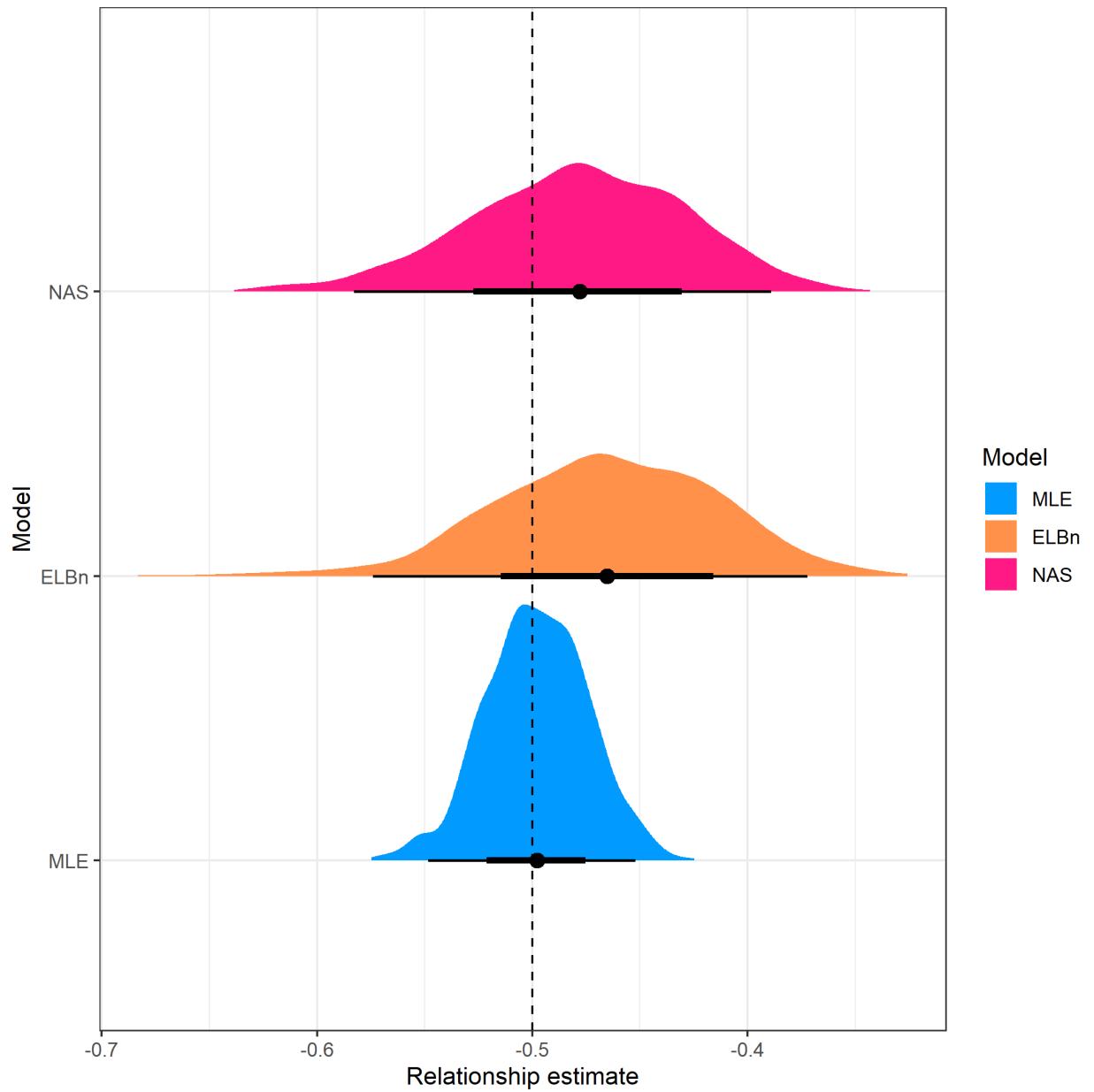


Figure S2: Distribution of estimated relationship (β_1) coefficient's for five sites across a hypothetical gradient with known value of 0.5. Range of body sizes is reduced and is from 1, to 100.

307 Large environmental gradient

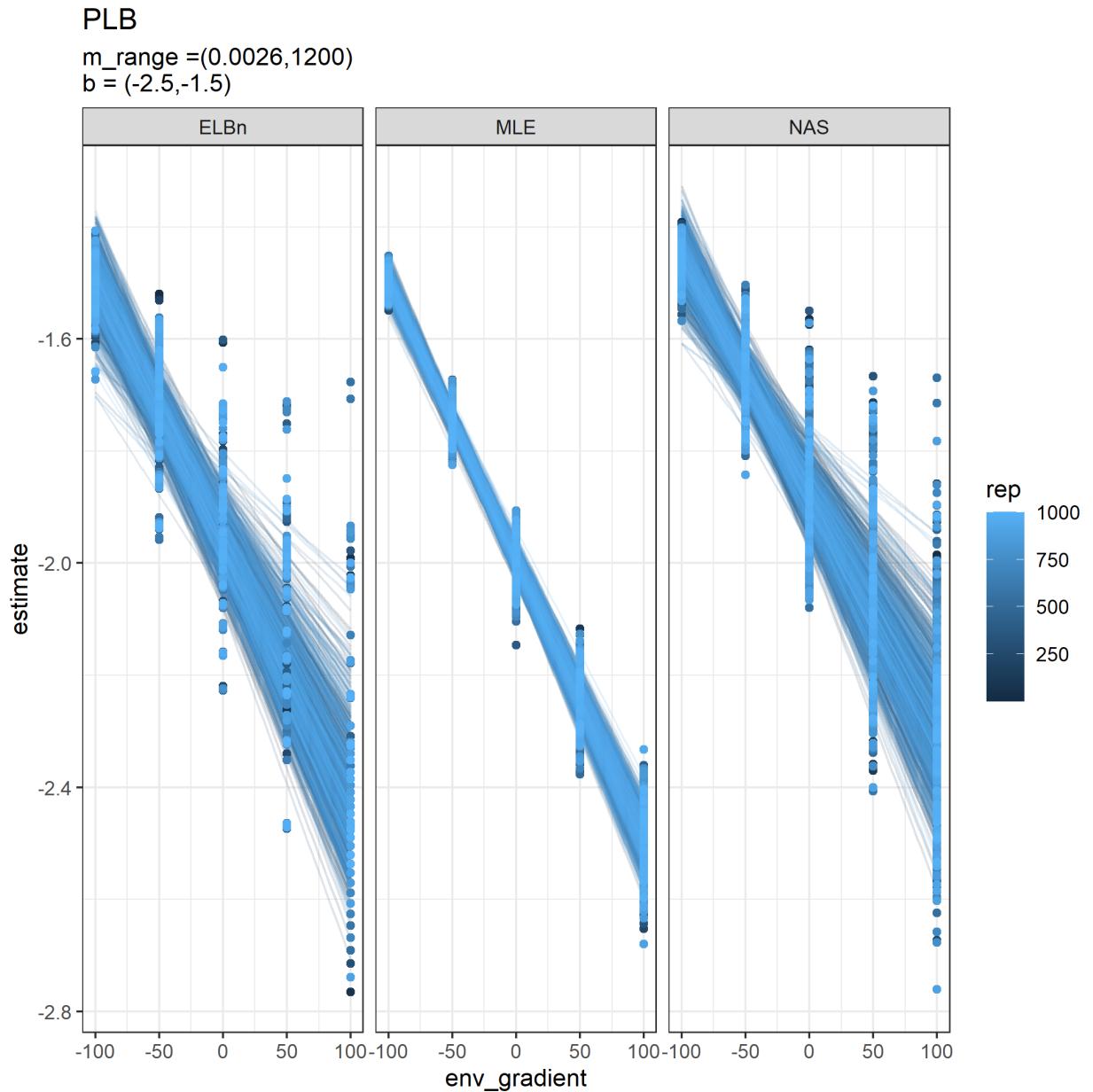


Figure S3: Individual regressions for five sites across a hypothetical gradient with a known relationship of 0.5. Range of environmental values (x -axis) increased to be -1000, to 1000.

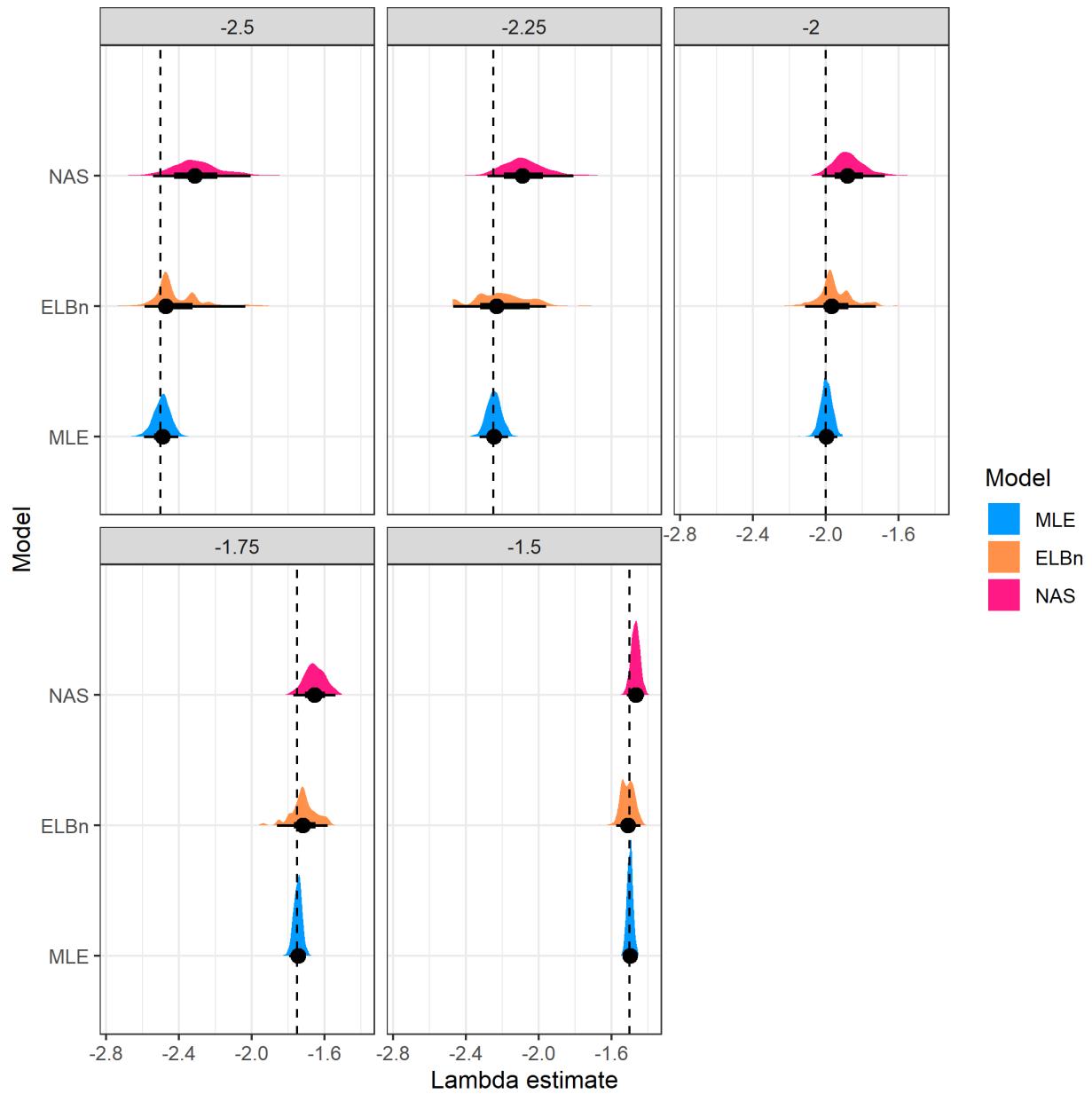


Figure S4: Distribution of estimated λ coefficient for five sites across a hypothetical gradient with known values. Range of environmental values (x -axis) increased to be -1000, to 1000.

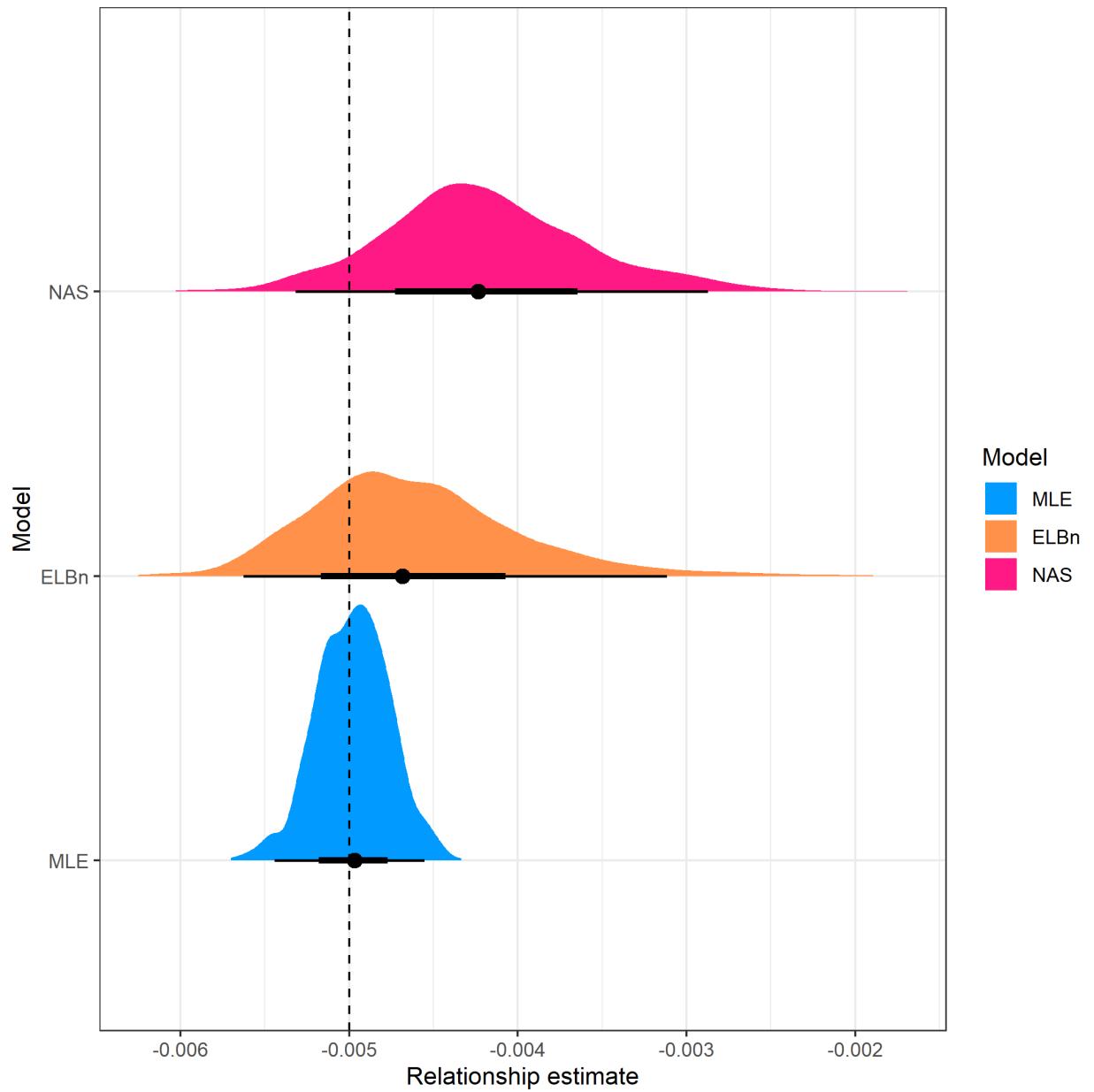


Figure S5: Distribution of estimated relationship (β_1) coefficient's for five sites across a hypothetical gradient with known value of 0.5. Range of environmental values (x -axis) increased to be -1000, to 1000.

308 Varying number of sites

309 10 sites

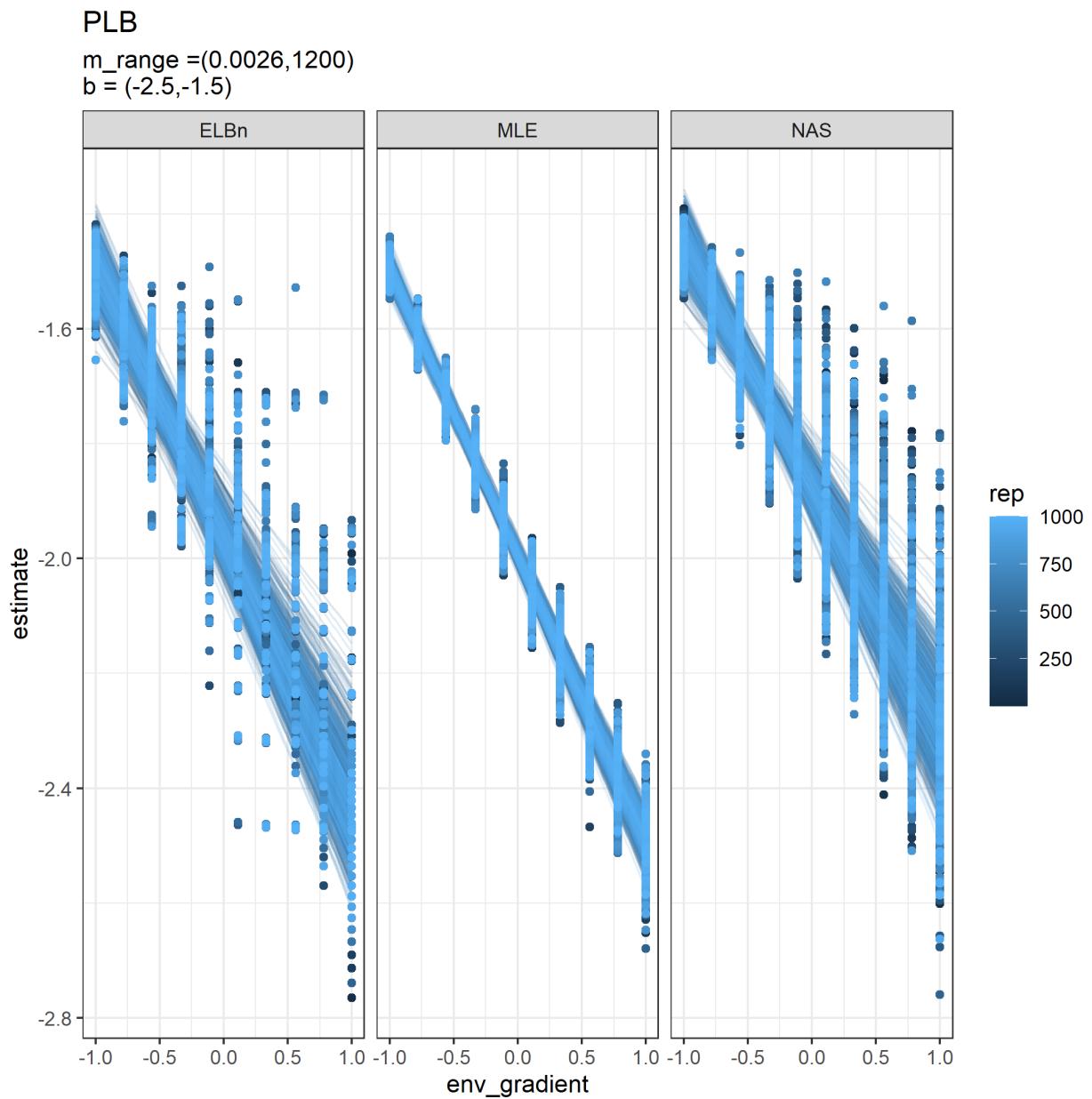


Figure S6: Individual regressions for ten sites across a hypothetical gradient with a known relationship of 0.5. All other parameters are the same as in the main analysis

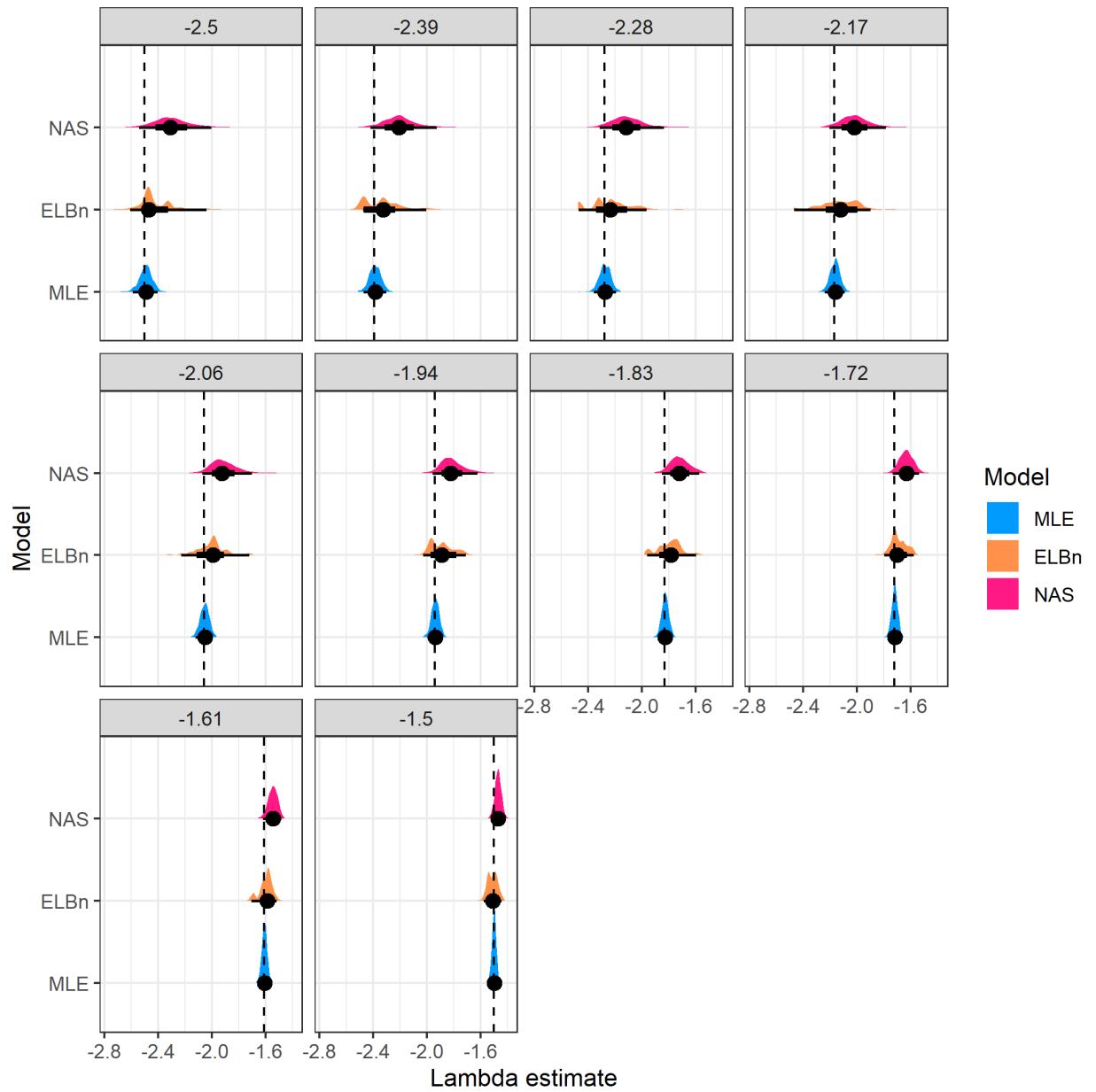


Figure S7: Distribution of estimated λ coefficient for ten sites across a hypothetical gradient with known values. All other parameters are the same as in the main analysis

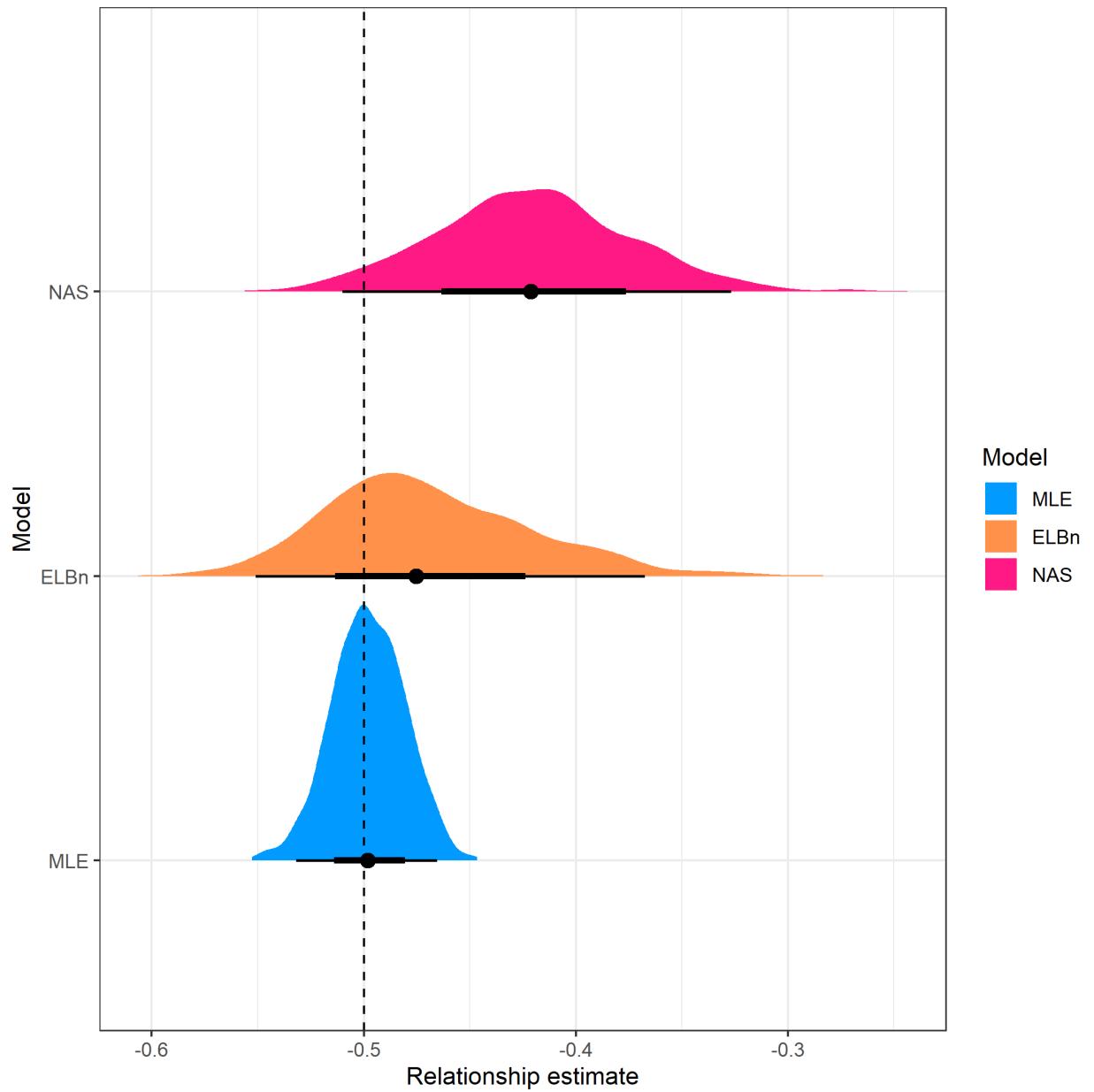


Figure S8: Distribution of estimated relationship (β_1) coefficient's for ten sites across a hypothetical gradient with known value of 0.5. All other parameters are the same as in the main analysis

310 Three sites

PLB

m_range =(0.0026,1200)

b = (-2.5,-1.5)

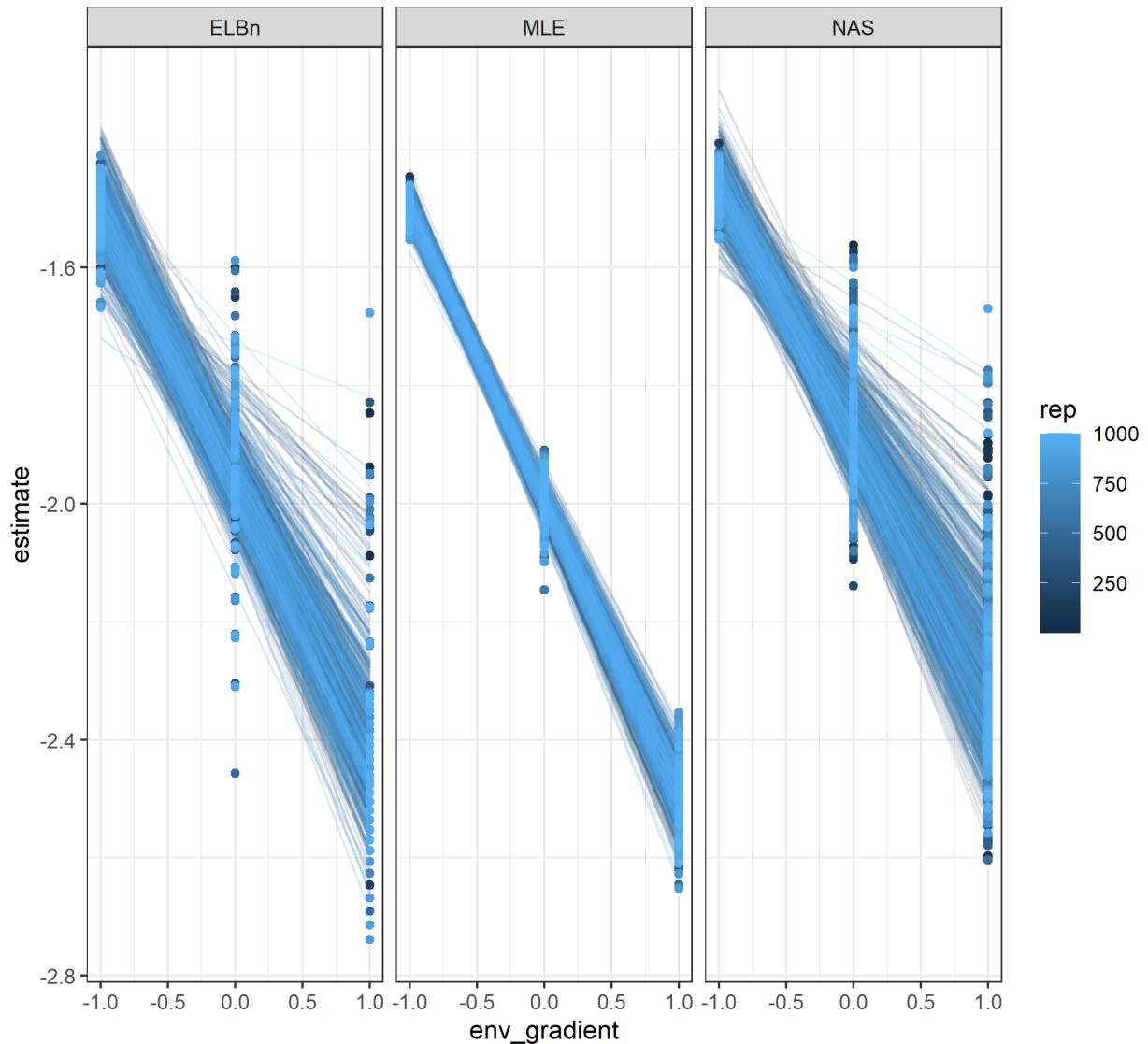


Figure S9: Individual regressions for three sites across a hypothetical gradient with a known relationship of 0.5. All other parameters are the same as in the main analysis.

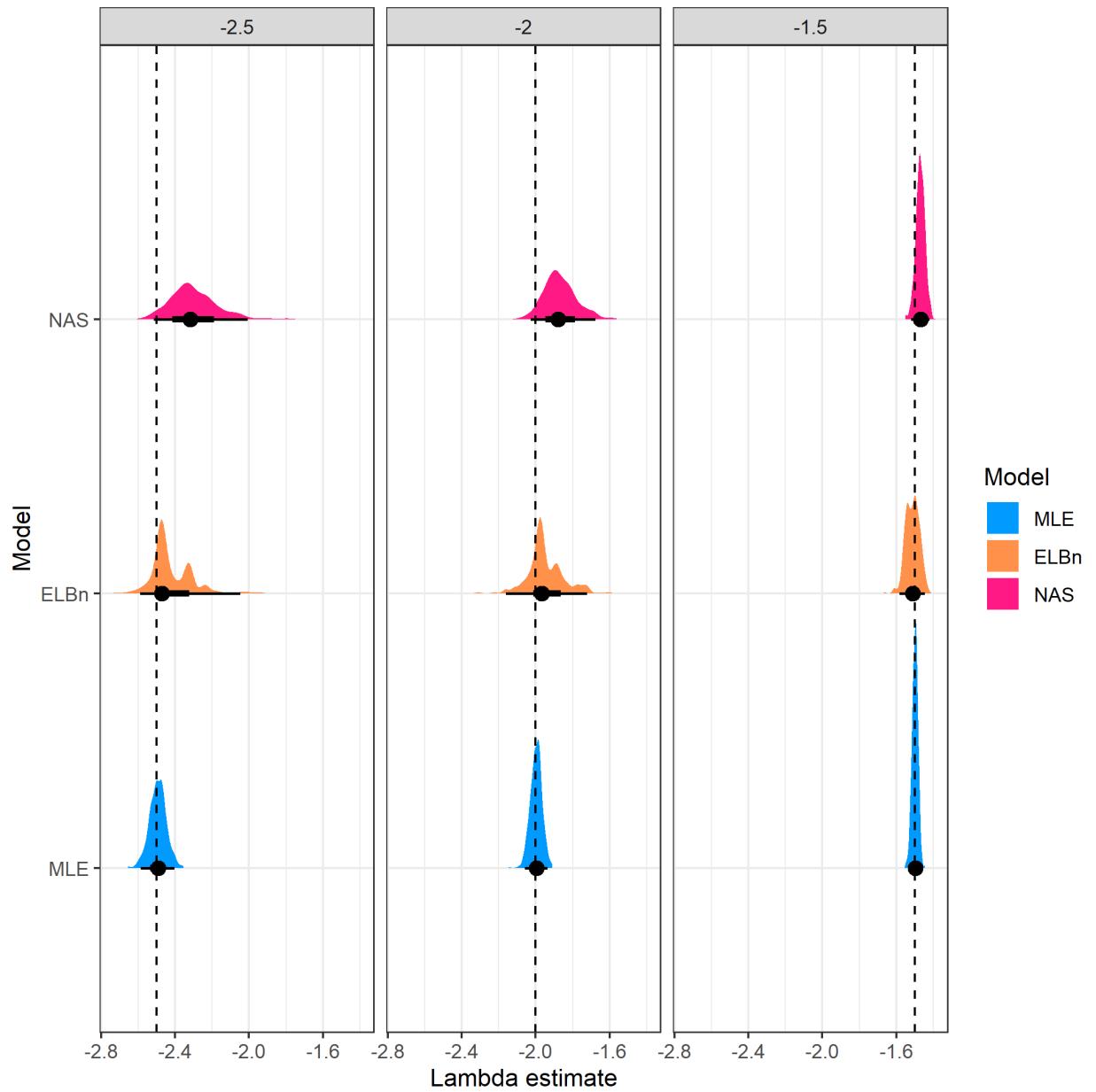


Figure S10: Distribution of estimated λ coefficient for three sites across a hypothetical gradient with known values. All other parameters are the same as in the main analysis.

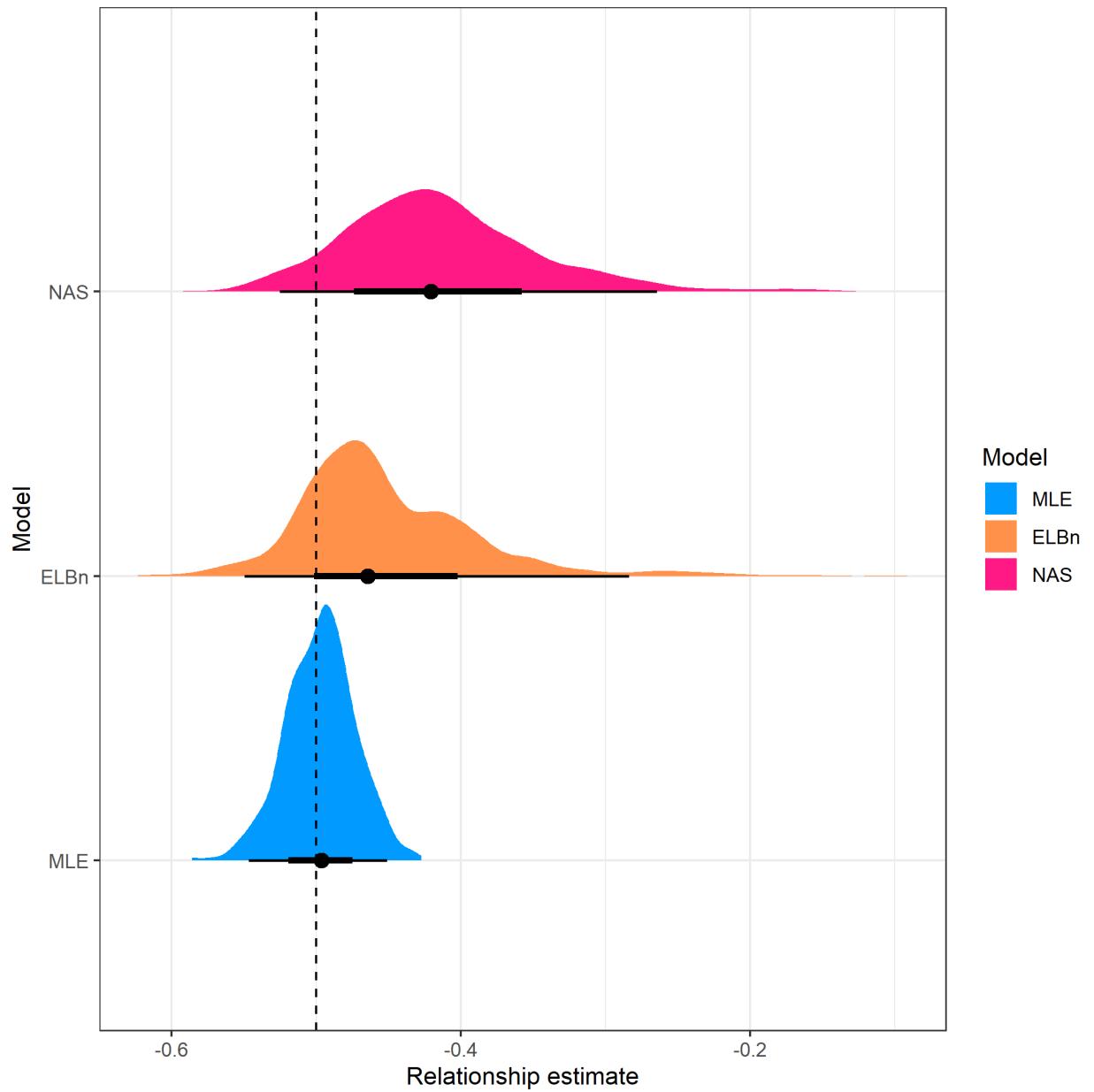


Figure S11: Distribution of estimated relationship (β_1) coefficient's for three sites across a hypothetical gradient with known value of 0.5. All other parameters are the same as in the main analysis

₃₁₁ **Sample size**

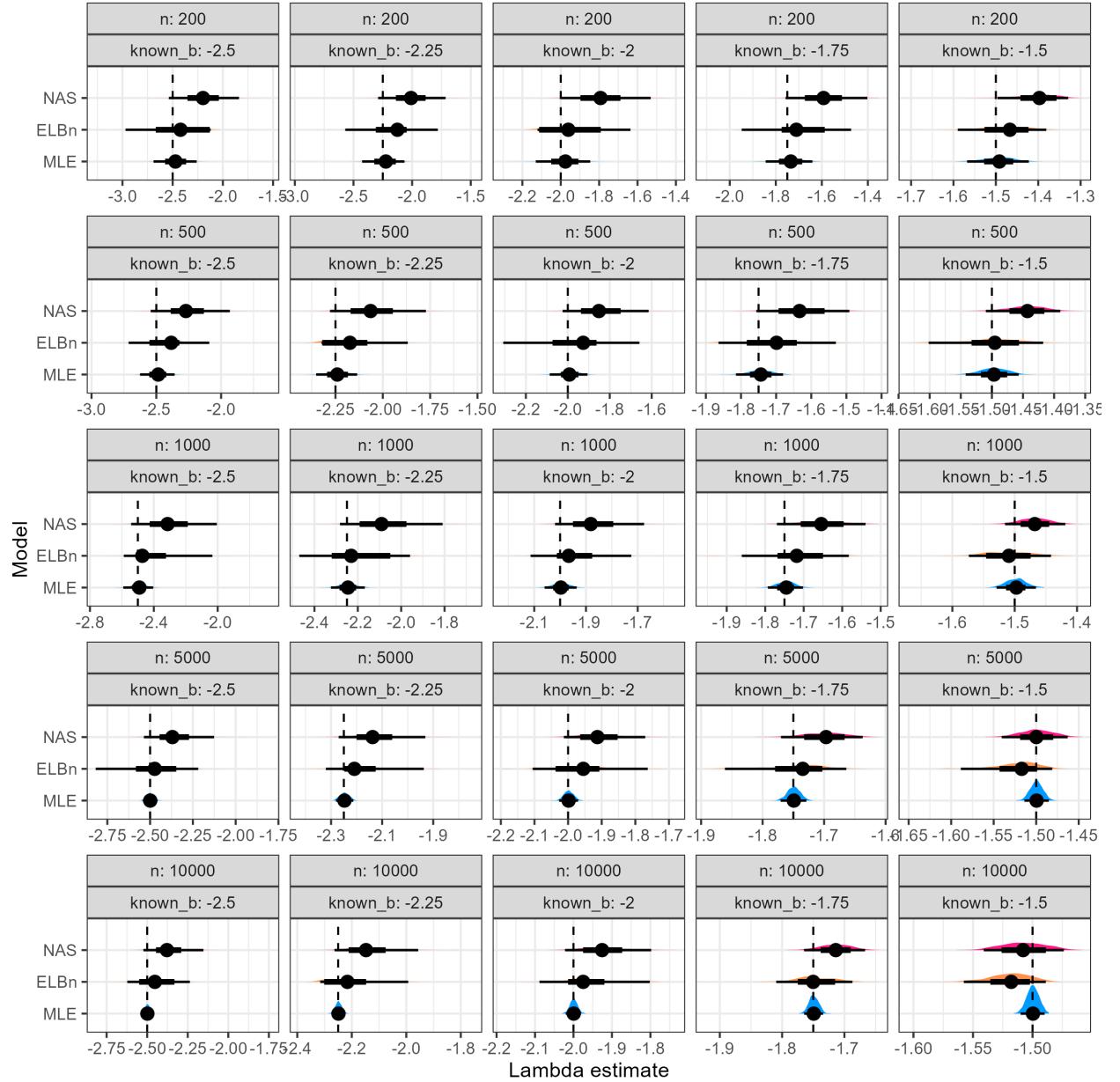


Figure S12: Distribution of size spectra parameter estimates. Vertical line is the known parameter (dashed line) which describes the bounded power law distribution from which the body size estimates were sampled. As n increases (top to bottom) and λ increases (left to right), the accuracy of the estimate improves across all methods.

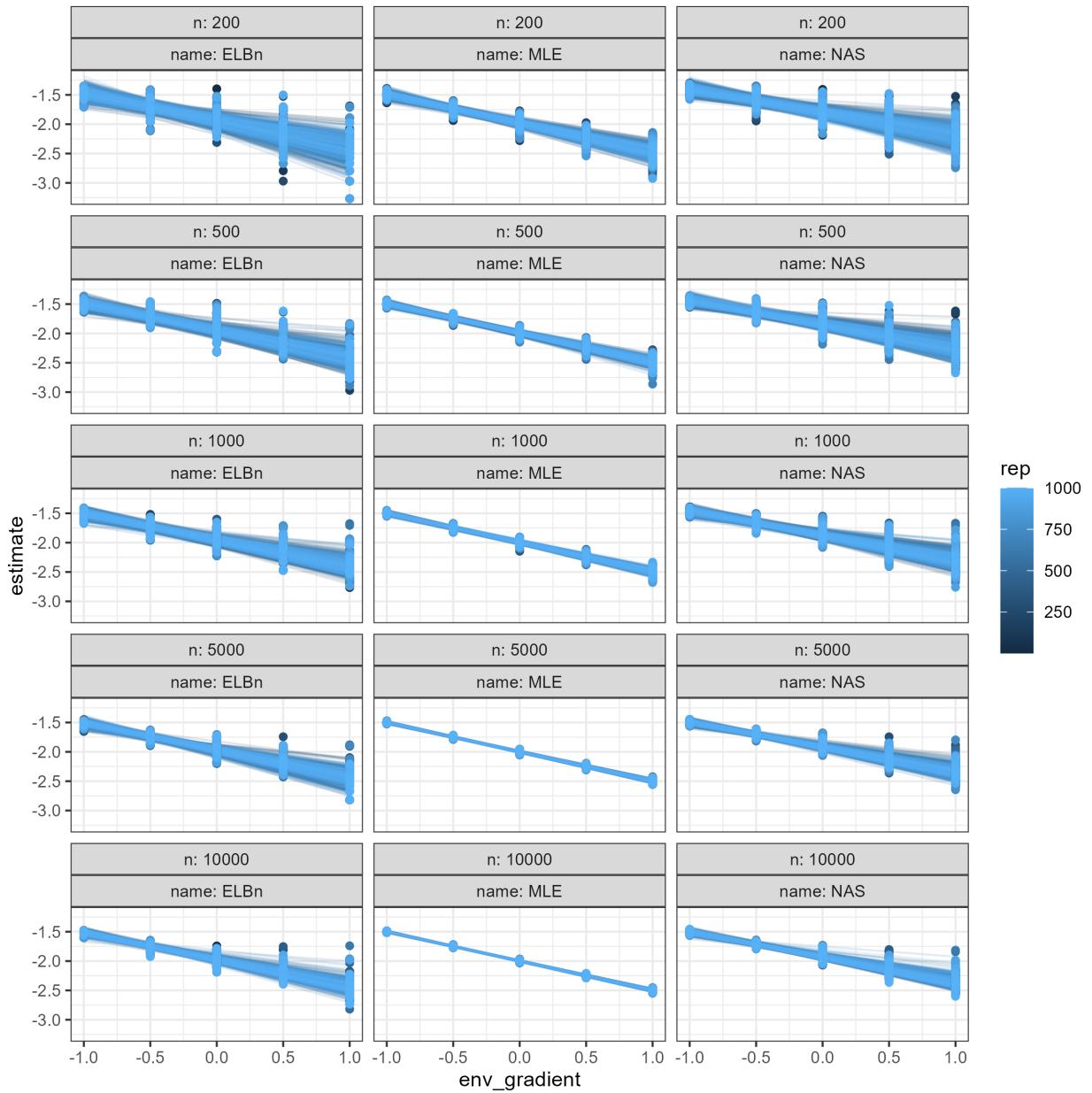


Figure S13: Individual regressions when varying sample size

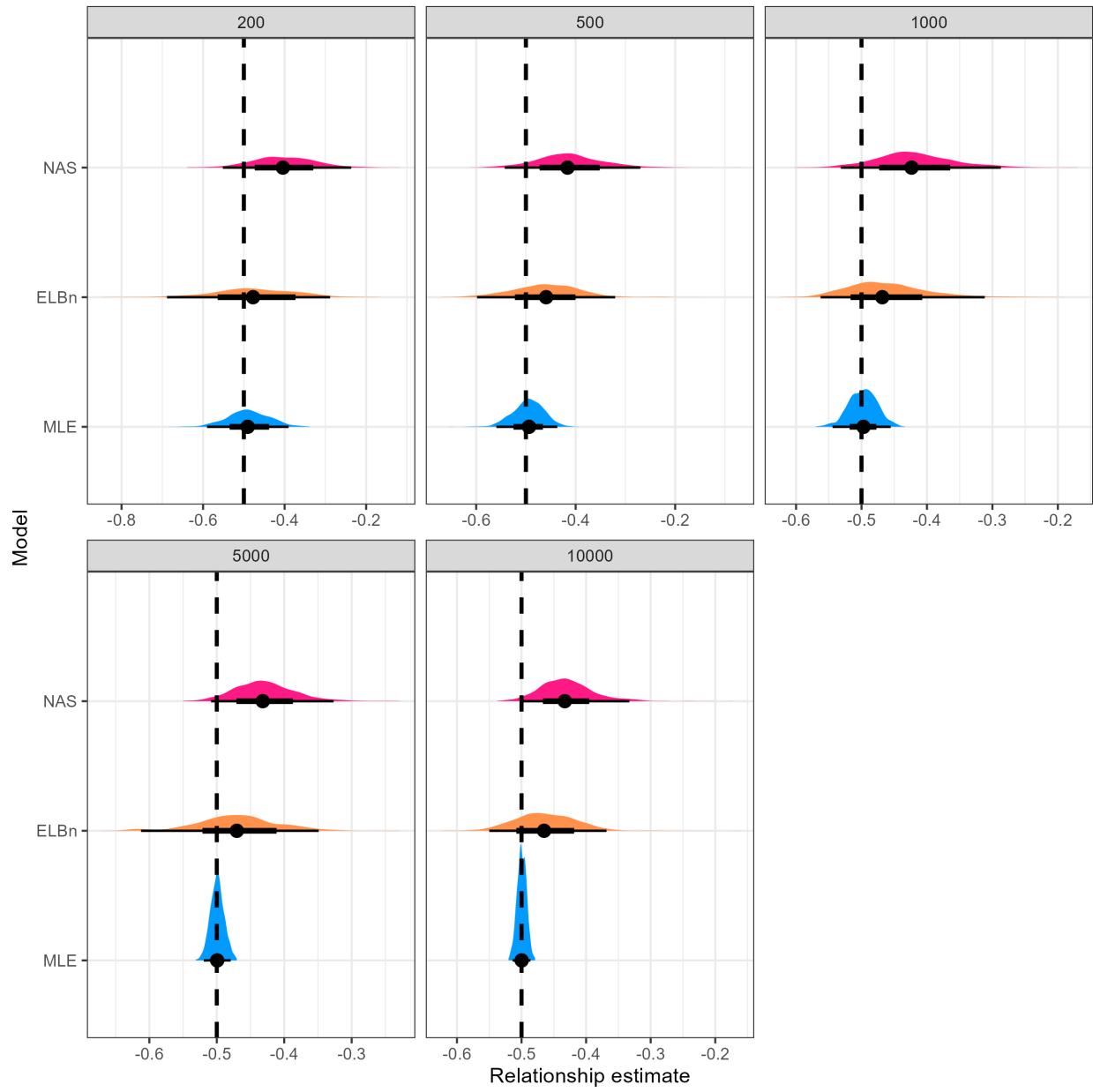


Figure S14: Distribution of the relationship coefficients with varying sample size

312 **λ and relationship estimates**

313 **NOTE** I'm considering dropping the λ estimate plots from the manuscript. I think it's too much info,
314 but if it adds to the story I'm happy to leave it in. **END**

315 In the main analysis, we simulate body size data from bounded power law distributions while varying
316 the λ exponent which describes the distribution. For the results presented in the main text, we held the
317 number of sites at 5, scaled the environmental gradient from -1 to 1, and set the minimum and maximum
318 body sizes to 0.0026 and $1.2 * 10^3$ respectively. Here, we plot the results of varying the number of sites (3,
319 10), increasing the scale of the environmental gradient (-100 to 100) and decreasing the range of body sizes
320 (min = 1, max = 100). Generally, the results reported in the main manuscript are robust to changing these
321 parameters: the MLE estimate is nearly always closer to the known parameters, and the variation in these
322 estimates is usually smaller than the binning methods.

323 Number of sites = 3

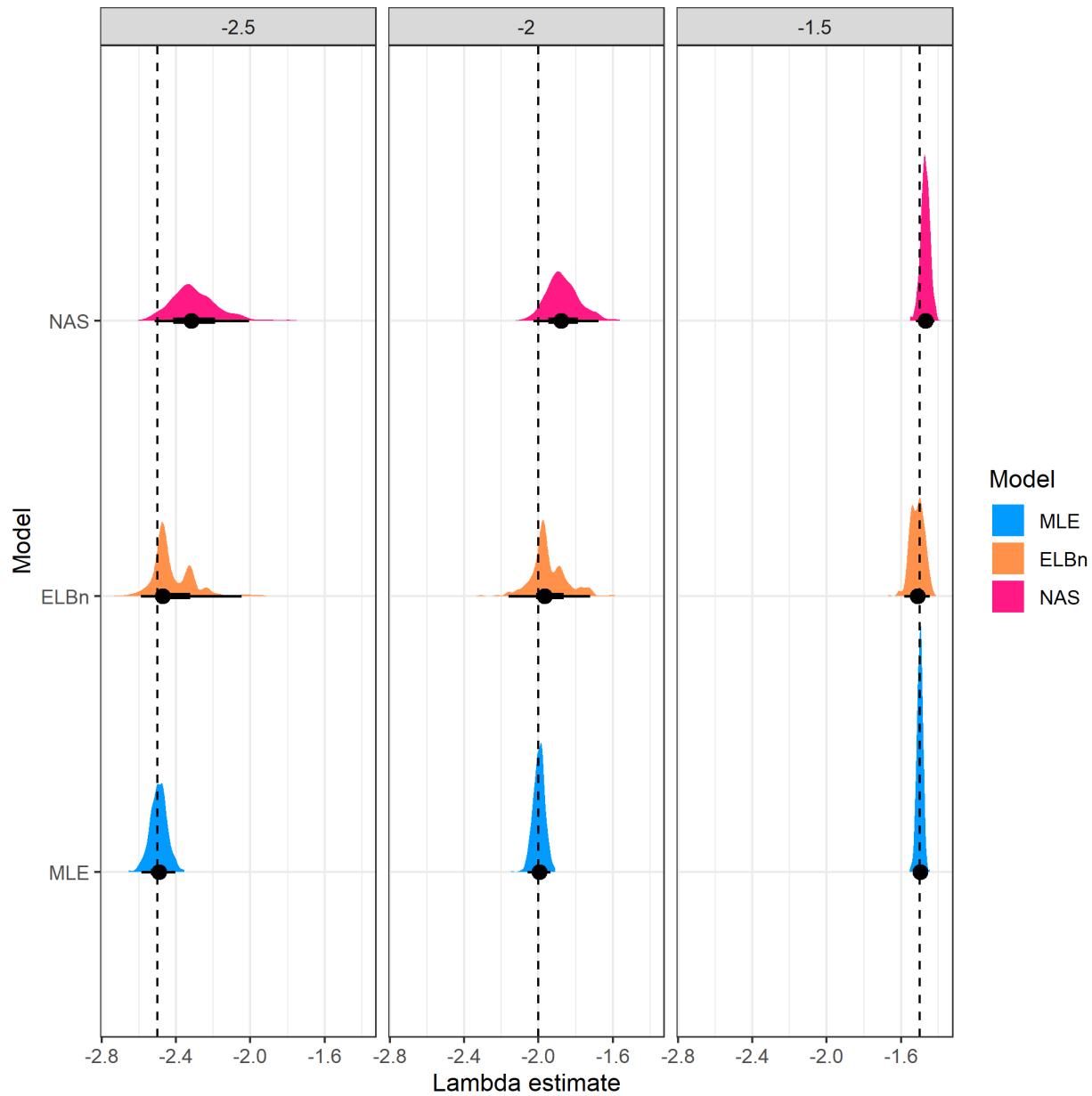


Figure S15: Distribution of estimated λ coefficient across a hypothetical gradient with known values (dashed line). The number of sites is reduced to 3, compared with 5 sites presented in the main analysis.

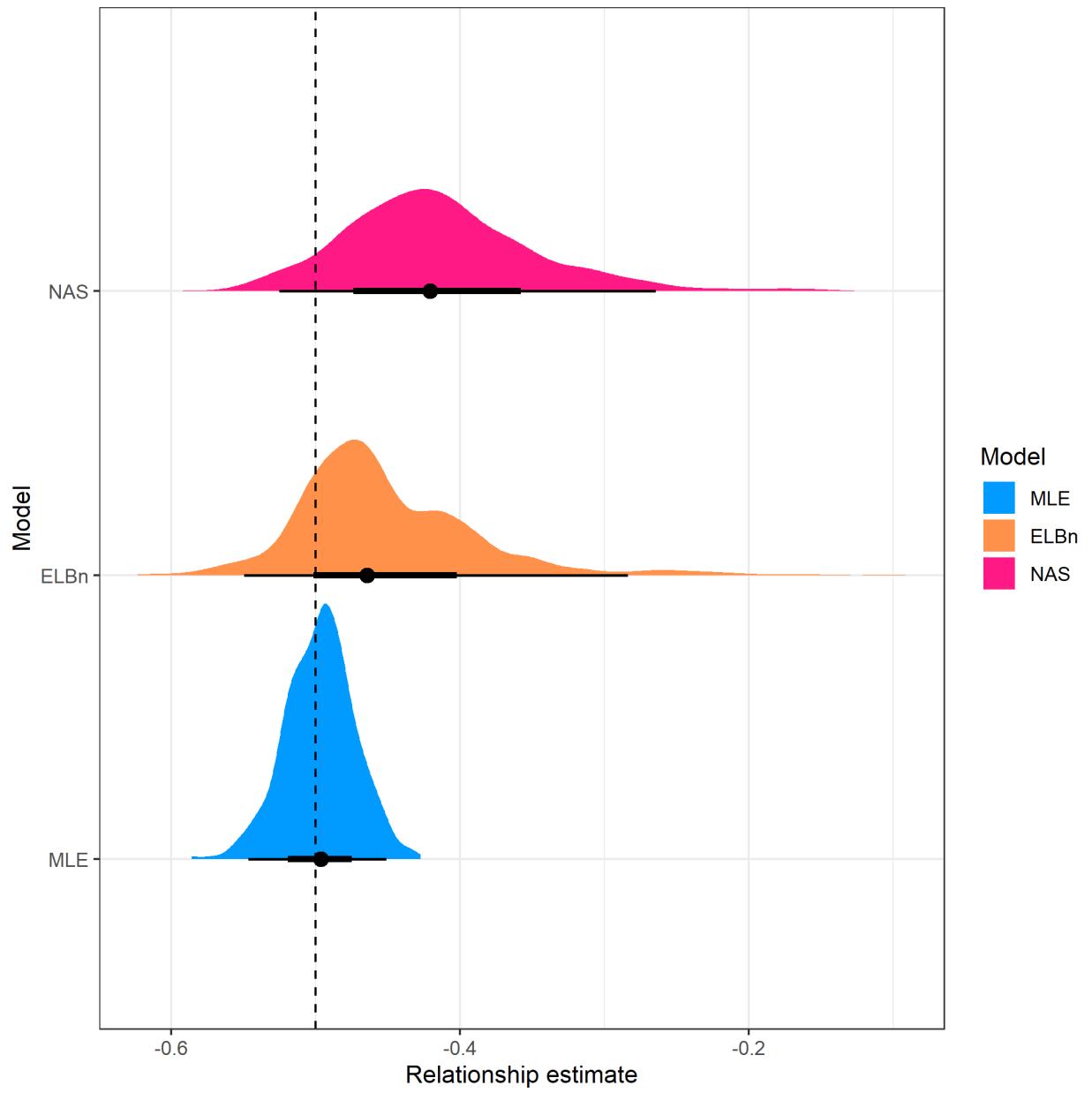


Figure S16: Relationship estimate

³²⁴ Number of sites = 10

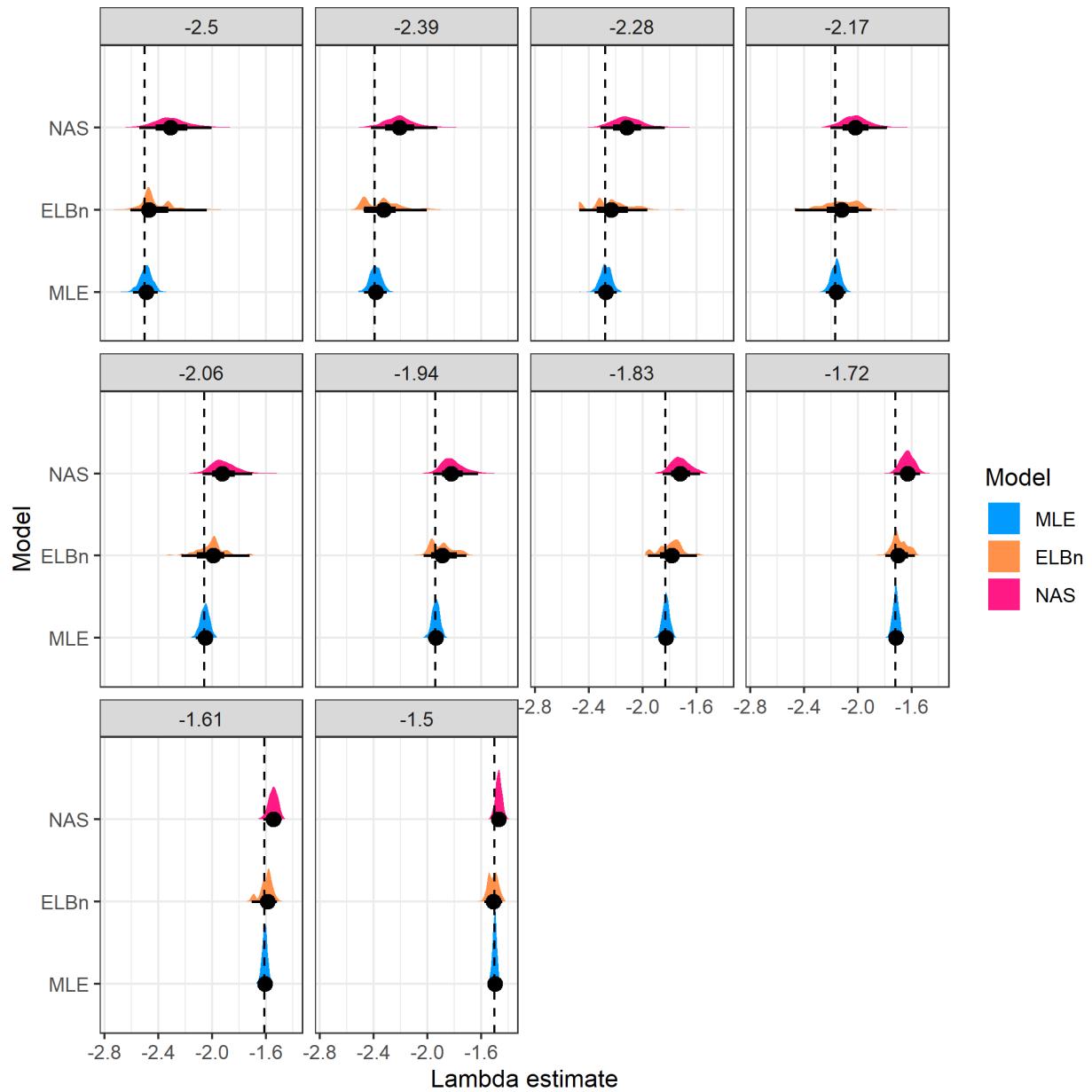


Figure S17: Distribution of estimated λ coefficient across a hypothetical gradient with known values(dashed line). The number of sites is increased to 10, compared with 5 sites presented in the main analysis.

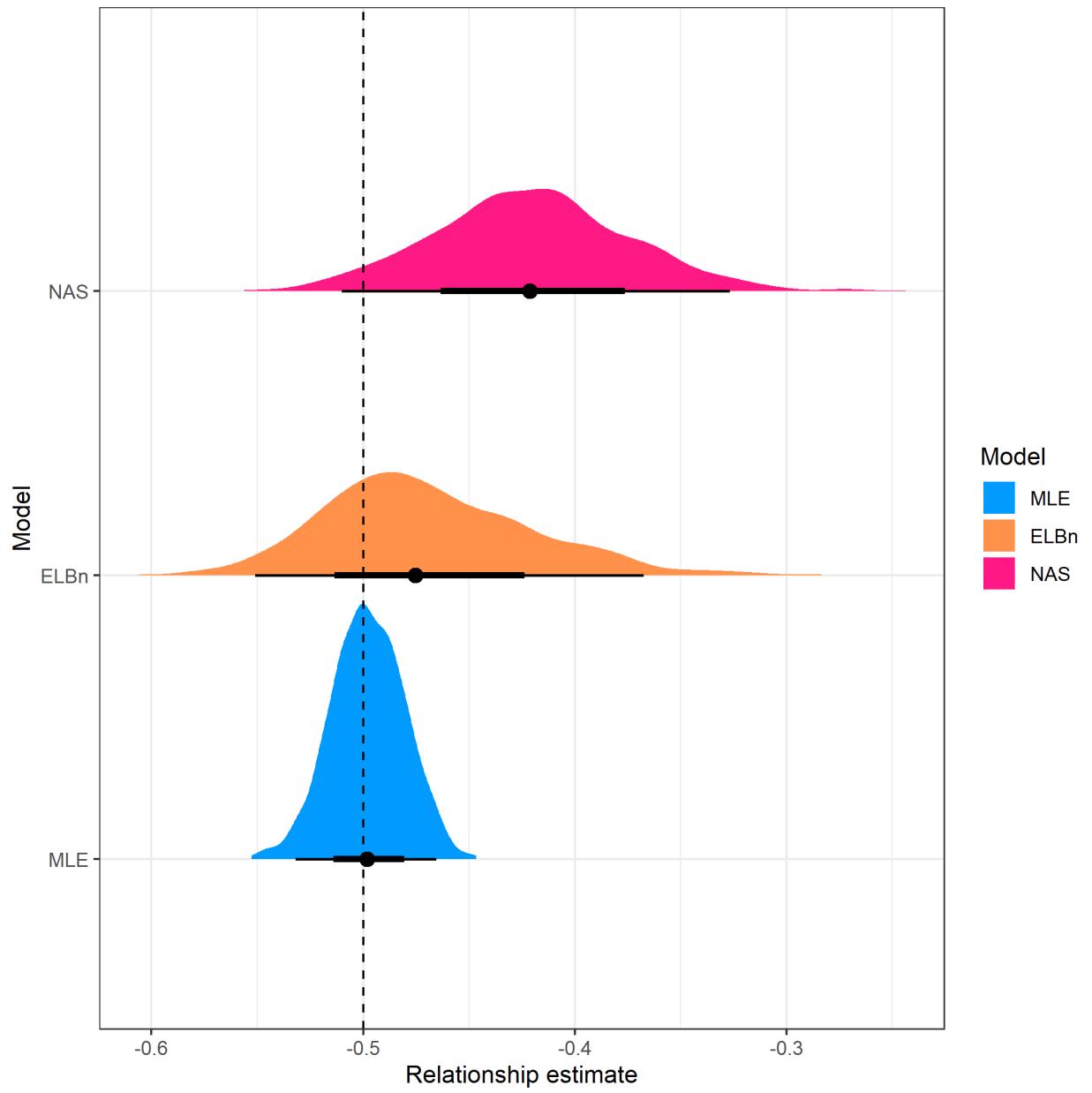


Figure S18: Relationship estimate

325 **Environmental Gradient**

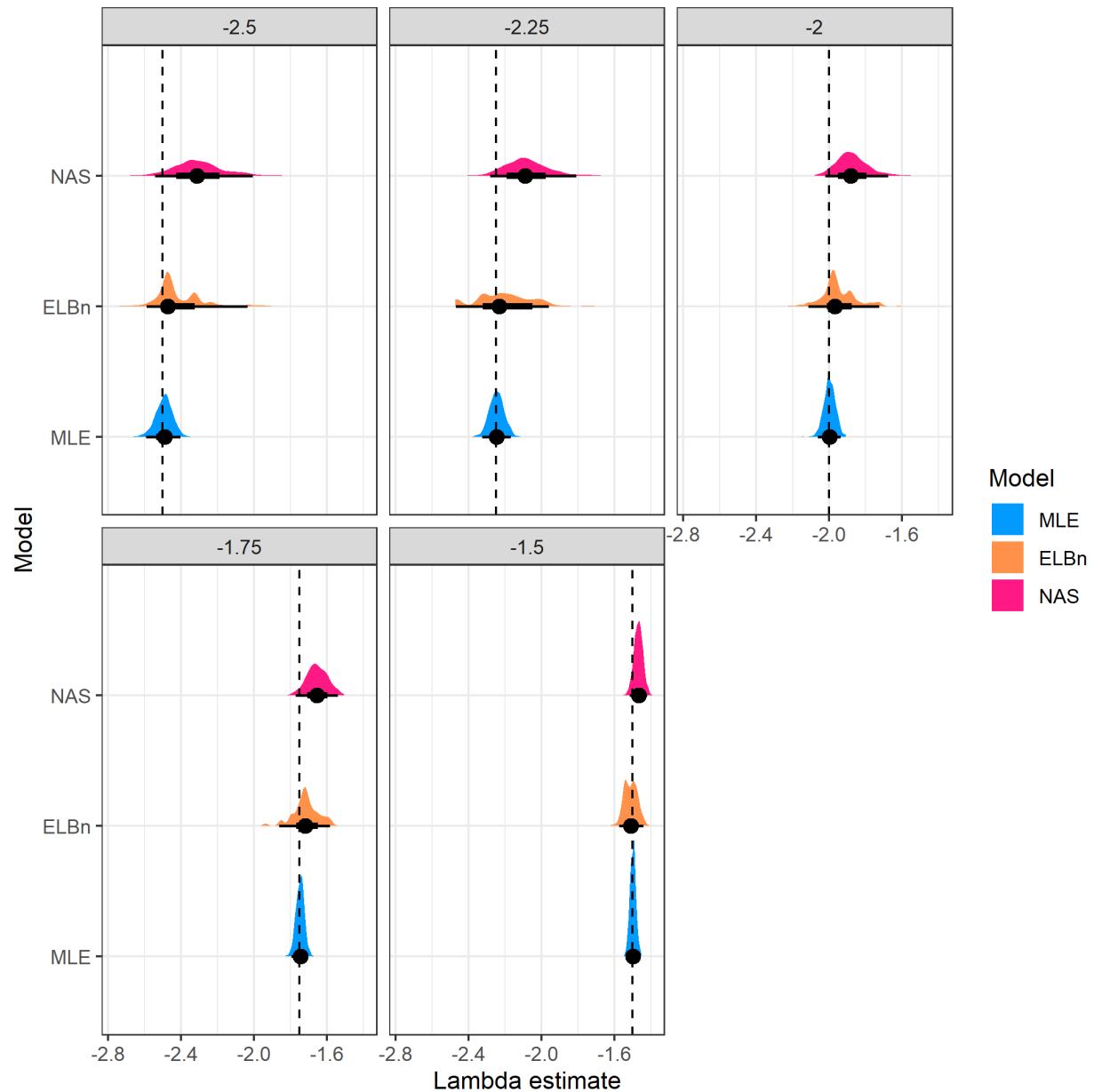


Figure S19: Distribution of estimated λ coefficient for five sites across a hypothetical gradient with known values (dashed line). The range of the environmental gradient was increased (-100 to 100).

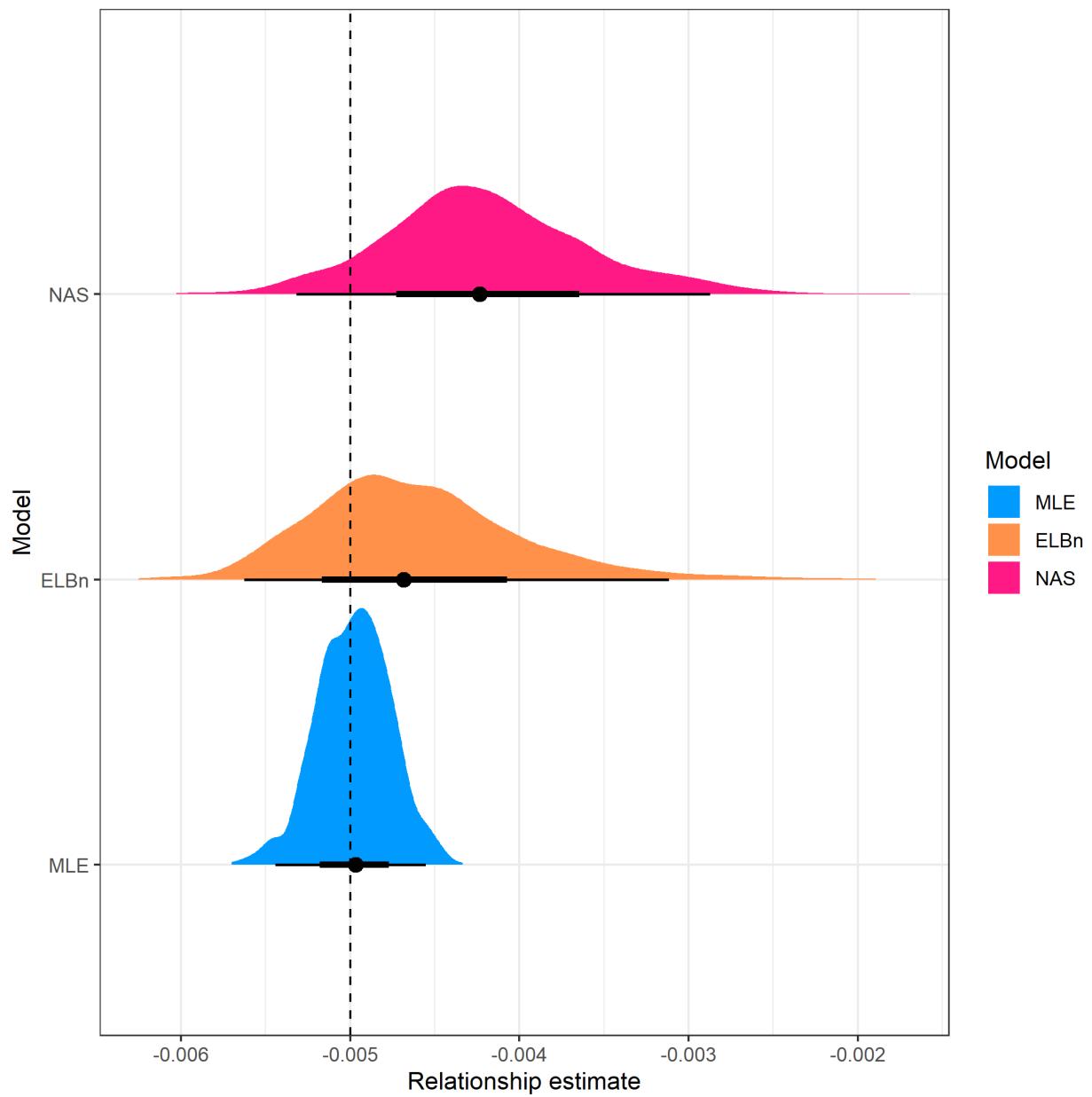


Figure S20: Relationship estimate

326 Range of body sizes = 1 to 100

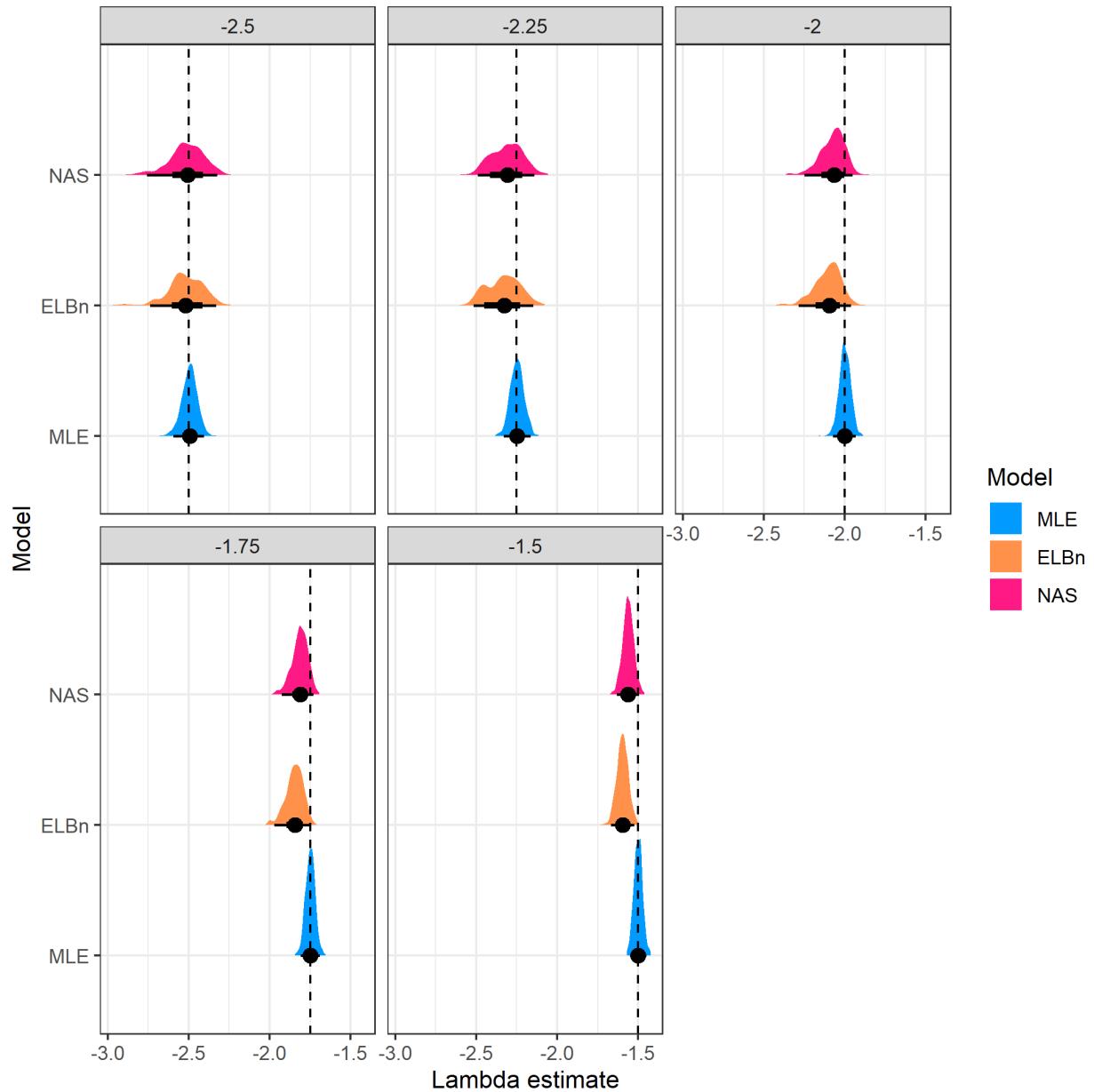


Figure S21: Distribution of estimated λ coefficient for five sites across a hypothetical gradient with known values(dashed line). Range of body sizes is smaller than main analysis and ranges from 1, to 100.

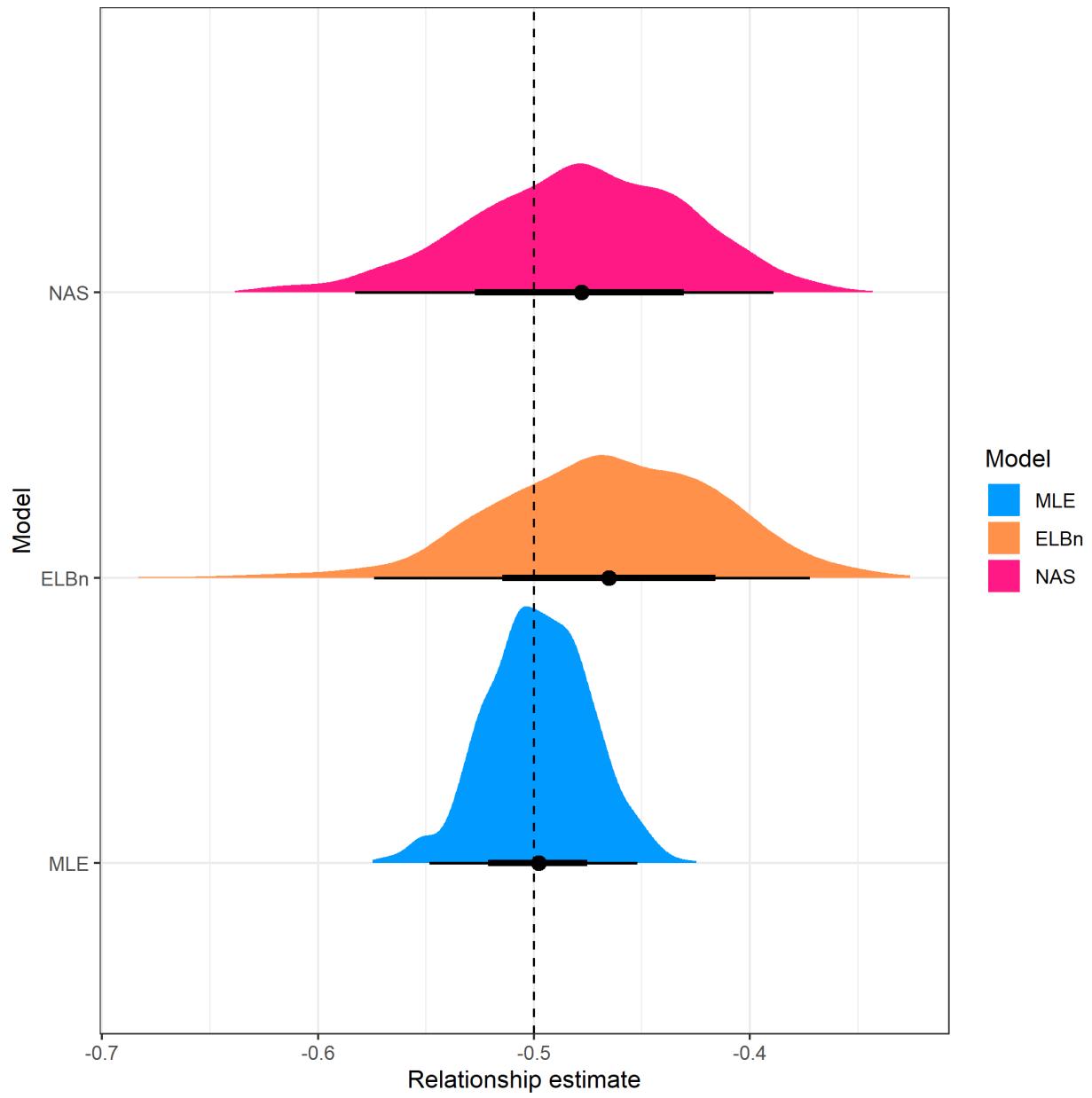


Figure S22: Relationship estimate