

Western Alaska Salmon Stock Identification Program

**Technical
Document:**¹

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Version: 1.0

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Title: Chum Reporting Groups Exploratory Methods
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Introduction

A joint Advisory Panel (AP)/Technical Committee (TC) meeting was held in Anchorage on November 14, 2011, to resolve the designation of reporting groups for chum salmon. The meeting was necessary because there was a lack of concurrence among AP members regarding the *ad hoc* committee’s recommendation that Coastal Western Alaska (CWAK) should be maintained as a single reporting group for WASSIP fishery mixtures, rather than as 4 reporting groups (Technical Document (TD) 15 and addenda). The *ad hoc* committee was established at the September 2011 meeting to examine the feasibility of further dividing the CWAK reporting group into 4 reporting groups for chum salmon.

One of the concerns among some AP members was that the department had not investigated all reasonable methods that might lead to the ability to divide the CWAK reporting group. At the November meeting, the AP asked the TC if there were any other methods that could be implemented that might lead to further resolution within CWAK. Dr. Waples (TC member) suggested 3 approaches that could be explored that might lead to, or provide insights into, better resolution within CWAK. These approaches were included as part of a motion that was approved at the November meeting as follows:

“1) For simulations involving power analysis, implement the algorithm proposed by Anderson et al. (2008), which does not require dropping part of the baseline samples to avoid problems with lack of proper cross validation.

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27 “2) For mixture analyses, implement the method proposed by Koljonen et al. (2005), which they
28 found performed better than standard methods (SPAM, Bayes).

29 “3) To give a rough idea of how much additional resolution can be expected from modest
30 increases in numbers of markers, create baseline datasets for a subset of key populations
31 for which data are already available for 3 different marker types: allozymes, SNPs, and
32 microsats. Using the combined sets of allele frequencies, simulate mixtures from the
33 problem areas and see whether these modest increases in marker number and type
34 substantially improve resolution.”

35 The first 2 approaches might guide the statistical analysis methods for WASSIP mixtures and
36 were therefore time sensitive within the WASSIP timeline. These will be referred to as the
37 “Anderson approach” and the “Koljonen approach” in the remainder of this document. The last
38 approach was designed to provide insights into future methods that might yield the desired
39 resolution within CWAK. The Gene Conservation Laboratory is working on all 3 approaches,
40 but this document describes the methods and results from the first 2 approaches. Results from
41 the last approach will be presented at a later date when these analyses are complete.

42

43 *Anderson Approach*

44 Anderson et al. (2008) introduced an algorithm whereby every individual is pulled out of the
45 baseline, one at a time, and allocated to reporting groups (“leave-one-out”). In their paper, they
46 published a program (ONCOR) that applies the algorithm. The reason why this method may
47 prove to be more appropriate for determining the viability of reporting groups for WASSIP is
48 that this method does not depopulate the baseline as much as the proof tests used in TD 15. In
49 proof tests, all the individuals in the test mixture (for our proof tests, we used 400-fish mixtures)
50 are excluded from the baseline. In contrast, the leave-one-out method excludes only the single
51 fish that is being tested. Therefore, our proof tests may yield excessively conservative
52 performance measures.

53

54 *Koljonen Approach*

55 Koljonen et al. (2005) compared 4 methods for mixed-stock analysis (MSA):

56

57 1) Classical individual assignment (IA);

- 58 2) Proportional assignment using conditional maximum likelihood (CML);
- 59 3) Proportional assignment using a Bayesian method (BAYES); and
- 60 4) Bayesian individual assignment method (BIA).

61

62 The main point of the Koljonen et al. (2005) paper was that proportional assignment (CML or
63 BAYES) performs better than classical individual assignment (IA). The authors also found that
64 the BIA method outpaces the BAYES method slightly. The authors used a flat prior for the
65 Bayesian analyses (as suggested in the BAYES publication; Pella and Masuda 2001; Michele
66 Masuda, pers. com.). Since the TC has recommended that we use an informed prior based on
67 stock compositions of associated strata (TD 13 addendum), we used an informed prior based on
68 the CML (SPAM prior) for the proof tests as a surrogate for the associated-strata priors that we
69 plan to use for the WASSIP mixtures. We know from past experience that informed priors
70 provide less biased BAYES results than flat priors, especially where there is little genetic
71 differentiation among reporting groups. We are not advocating using this SPAM prior method
72 (based on the same mixture) for WASSIP samples as per Pella and Masuda (2001), but we
73 needed a surrogate just for these tests.

74

75 After reviewing Koljonen et al. (2005), we checked in with Dr. Waples to verify that this was the
76 document to which he was referring in the resolution and that our plans for examining the
77 individual assignment methods (BAYES) were in alignment with his suggestion. Dr. Waples
78 confirmed that this was the right document and suggested using the “posterior probability
79 distribution for each individual” from the BAYES output to conduct a test similar to the one
80 described in Koljonen et al. (2005). Dr. Waples further suggested that we contact Michele
81 Masuda or Jerry Pella confirm the methods.

82

83 We examined the methods described in Koljonen et al. (2005) for using the Bayesian individual
84 assignment method and determined that it was similar to the method used in cBAYES, (program
85 in Neaves et al. (2005) and implemented in Beacham et al. (2009)), and we will refer to it as the
86 “roll-up” method. In this method, individuals are assigned to a single population at each
87 iteration and the best estimate is derived from the sum of these assignments divided by the
88 number of iterations times the mixture size, or:

$$\hat{p}_i^{roll-up} = \frac{\sum_{k=1}^K M_i^{(k)}}{KM}.$$

89 Where $M_i^{(k)}$ is the number of individuals in the mixture that are assigned to stock i at the k^{th}
 90 iteration, M is the size of the mixture, and K is the number of iterations. This is similar, but not
 91 quite identical, to a Rao-Blackwellized estimator (Robert and Casella 2004). The Rao-Blackwell
 92 method also incorporates the prior into the estimate and has the form:

$$\hat{p}_i^{RB} = \frac{\sum_{k=1}^K (M_i^{(k)} + \alpha_i)}{K(M + \sum_{i'=1}^C \alpha_{i'})}.$$

93 By incorporating the prior, this estimate has well characterized properties which allow for the
 94 calculation of credibility intervals, among other statistics.

95

96 We contacted Michele Masuda and asked her to review our proposed analysis methods. She
 97 agreed that these methods would use individual assignment information to come up with stock
 98 composition estimates, but was not convinced that the results would be more accurate or precise.
 99 She suspected that the slightly better results for the BAYES roll-up composition estimates over
 100 the BAYES mixture model estimates, in Table 4 of Koljonen et al. (2005), were somewhat of an
 101 artifact. The true composition was 100%, and proportional assignment is biased at the
 102 boundaries. If they had reported the mode instead of the mean of the posterior distribution, the
 103 results for the 2 methods would likely have been closer. The roll-up composition estimates were
 104 also good because the assignments were good. She said that one would actually not expect the
 105 roll-up composition estimates to be very good in situations where stocks are genetically similar.
 106 The misclassifications between similar stocks will lead to biased composition estimates. It has
 107 been shown that bias can be reduced if fractions of individuals are assigned (proportional
 108 assignment) instead of classifying whole individuals to stocks. She mentioned that there is a
 109 body of published work indicating that using assignments of whole individuals to estimate stock
 110 proportions tends to be more biased than proportionate assignments due to misclassification of
 111 individuals (Pella and Milner 1987; Koljonen et al. 2005; Manel et al. 2005). Proportional
 112 assignment which assigns fractions of fish to stocks allows for direct estimation of stock
 113 proportions and evaluation of precision. Due to time constraints, she was unable to evaluate the
 114 Rao-Blackwell estimator above. Despite these concerns, we moved ahead with these analyses to

115 determine if there were any improvements in correct allocations to the reporting groups within
116 the CWAK area by using individual assignment methods.

117

118 **Methods**

119 *Anderson Approach*

120 We used ONCOR, a Windows-based program available at <http://www.montana.edu/kalinowski>
121 to implement the leave-one-out simulations using 9 (CWAK as a single reporting group) and 12
122 (CWAK divided into 4 reporting groups) reporting groups. This program handles only diploid
123 markers, so we excluded the 2 MHC and 3 mtDNA loci from the analysis. The output from this
124 analysis produces stock proportion point estimates for each population by reporting group for
125 both the 9 and 12 reporting group analyses.

126

127 *Koljonen Approach*

128 The 15 test mixtures assembled under TD 15, and reported in Addendum 2 to TD 15, were re-
129 analyzed in BAYES using the original methods, except that the toggle to export individual
130 assignments at each iteration was turned on. Three independent Markov Chain Monte Carlo
131 chains of 40,000 iterations each were completed with different starting values and information
132 from first ½ of the iterations were discarded to remove influence of initial start values. We
133 defined the starting values for the first chain such that the first 1/3rd of the baseline populations
134 summed to 0.9 and the remaining populations summed to 0.1. Each chain had a different 1/3rd of
135 baseline populations sum to 0.9. We assessed the within- and among-chain convergence of these
136 estimates using the Raftery-Lewis and Gelman-Rubin diagnostics, respectively. If the Gelman-
137 Rubin diagnostic for any stock group estimate was greater than 1.2 and the Raftery-Lewis
138 diagnostic suggested each chain had not converged to stable estimates, we reanalyzed the
139 mixture with 80,000-iteration chains following the same protocol. Iterations were thinned to 1
140 in 100. Output from these assignments was subjected to 2 estimation methods: 1) Rao-
141 Blackwellization, and 2) the roll-up estimator. Point estimates from the Rao-Blackwellization
142 were plotted onto the same plots as presented in Addendum 2 to TD15 for visual comparison of
143 performance. The maximum difference in stock composition between the Rao-Blackwellization
144 results and the roll-up results for all 15 test mixtures for all reporting groups was calculated.

145

146 **Results**

147 *Anderson Approach*

148 ONCOR point estimates by population plotted for each reporting group for both the 9 and 12
149 reporting group analyses are shown in Figures 1 and 2, respectively. Correct proportional
150 allocation of populations in CWAK back to the CWAK reporting group (9-reporting group
151 analysis) was much higher than correct proportional allocations of these populations to their
152 respective sub-regional group (12- reporting group analysis).

153

154 *Koljonen Approach*

155 Gelman-Rubin diagnostic for all stock group estimates was less than 1.2 and the Raftery-Lewis
156 diagnostic suggested each chain had converged to stable estimates. Point estimate proportions
157 for each reporting group derived from Rao-Blackwellization for each hypothetical mixture were
158 plotted onto the same plots as presented in Addendum 2 to TD15 for visual comparison of
159 performance are shown in Figures 3-9.

160

161 The maximum difference in stock composition between the Rao-Blackwell results and the roll-
162 up results for all 15 test mixtures for all reporting groups was 0.0015. This difference was so
163 small that it would be visually indiscernible from the Rao-Blackwell results if plotted in Figures
164 3-9.

165

166

167 **Discussion**

168 Neither the Anderson nor the Koljonen approaches provided results that would indicate that the
169 CWAK reporting group should be separated into 4 reporting groups for WASSIP mixture
170 analyses. The Anderson approach resulted in lower assignment back to the correct reporting
171 group than the original BAYES proportional assignment method. The Koljonen approach
172 provided virtually identical results to the original BAYES proportional assignment method.

173

174 *Anderson Approach*

175 The Anderson et al. (2008) leave-one-out approach was expected to produce better-performing
176 proof tests than the BAYES proof tests originally used to define reporting groups. This

177 improvement was anticipated to come from less depletion of the baseline due to the extraction of
178 mixture individuals from the baseline used in the proof tests. The proof tests removed 400 fish
179 from the baseline, whereas the leave-one-out approach removes only 1 fish from the baseline.

180

181 The results did not follow expectations for better performance. In the proof tests, all 9 reporting
182 groups (CWAK as a single reporting group) exceeded 90% correct allocation (September
183 presentation), whereas in the leave-one-out approach, many populations within reporting groups
184 fell well below this 90% level (Figure 1). Although the overall level dropped for all reporting
185 groups, the order of relative performance remained similar (i.e. Asia and East of Kodiak
186 performed well, and South Peninsula performed least well in both analyses). In the 12 reporting
187 group analysis (CWAK divided into 4 reporting groups), the 4 CWAK reporting groups had the
188 lowest correct assignments (Figure 2).

189

190 There are a couple of possible reasons why the leave-one-out tests performed worse than the
191 original BAYES proof tests: 1) No mixture information is used in assigning stock proportion to
192 individuals; and 2) Individuals with incomplete genotypes were excluded from the analysis. By
193 definition, the leave-one-out approach cannot use information from the rest of the mixture to
194 inform allocation of the individual (the mixture is made up of 1 fish). This information from
195 other fish in the mixture may be particularly informative when the mixture is made up of
196 individuals from a single reporting group, as is true in the proof tests. Only a small number of
197 individuals with incomplete genotypes were excluded from the leave-one-out analysis, but
198 included in the original analysis (<5%), so this effect was likely small.

199

200 *Koljonen Approach*

201 The Koljonen et al. (2005) roll-up method was anticipated to provide better estimates for the
202 less-distinguishable reporting groups (such as the 4 reporting groups within CWAK) based on
203 previous work with Atlantic salmon. Those results were based on proof tests where the known
204 mixture was made up of a single reporting group. One of the limitations to the roll-up method is
205 that it does not provide any measure of variation (i.e. no standard deviation or error or no
206 confidence or credibility intervals). This limitation may be why Koljonen et al. (2005) did not
207 use the roll-up when estimating stock compositions from unknown mixtures, instead using the

208 proportional assignment method. By using the Rao-Blackwell method on the individual
209 assignments we were able to derive statistics that included both a point estimate and a credibility
210 interval. However, since all 3 methods (individual assignment with the Rao-Blackwell,
211 individual assignment with the roll-up, and proportional assignment) yielded almost identical
212 point estimates, there is no reason to move to the individual assignment with Rao-Blackwell
213 method. These results follow Michael Masuda's expectations that this method was unlikely to
214 yield better estimates (see introduction).

215 216 **References** 217 218 219

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Questions for Technical Committee

- 1) Do these explorations provide confidence that the department should continue to analyze the WASSIP mixtures using the original methods (proportional assignment BAYES with an informative prior)?

- 2) Do these explorations support using CWAK as a single reporting group for WASSIP mixtures?

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Responses from Technical Committee

251 *[Excerpt from email to Bill Templin, with cc to AP and TC members from Robin Waples dated 1/11/12]*

252

253 ... Sorry I can't be there next week, but I had a quick look at this document. You, Chris, and
254 others have done a lot of work in a short time. I would have been surprised if these alternative
255 methods had produced radically different results, but I am a bit surprised that there is essentially
256 no improvement in resolution. Still, you have accomplished an important objective, which is
257 demonstrating that the methods used in your analyses are truly state of the art. Although the
258 existence of these other methods was noted during the course of this project, it was only in the
259 last few months when the limitations to resolution for chum salmon in CWAK became evident
260 that they seemed worth pursuing. Given the considerable disappointment by many in the
261 resolution attainable with the current baseline and methods, it seemed important to evaluate any
262 reasonable alternative that might produce a more useful result. You have now done that, so the
263 Advisory Panel has a firmer foundation for making decisions about future options. The third
264 analysis still might prove informative, but in any case it could not be implemented within the
265 time frame for this project, so it is not as time sensitive.

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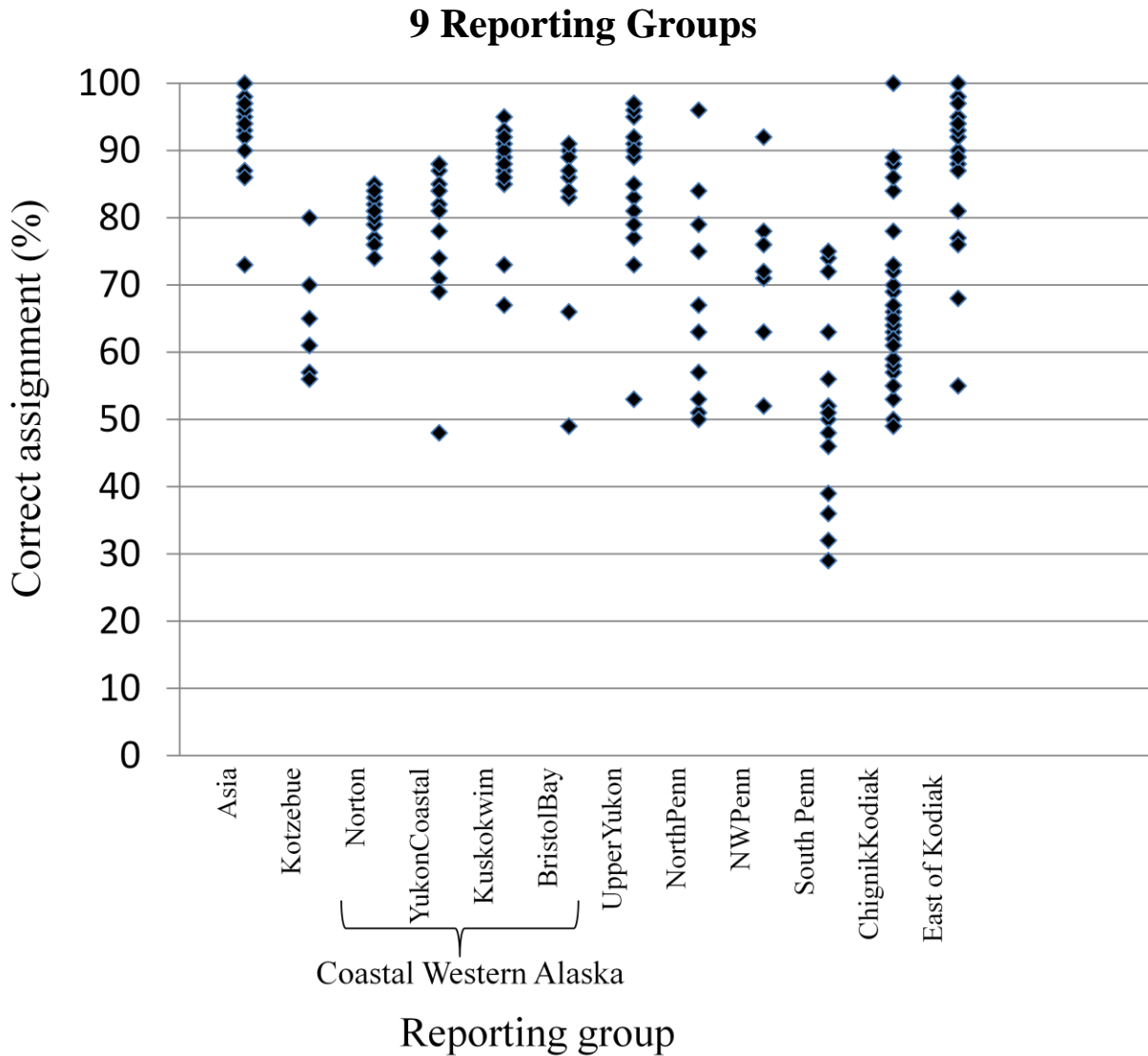
267 For the record, I would answer 'yes' to both the questions posed to the TC at the end of the
268 document.

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270 I am a bit curious about the results for the Anderson et al. method, but don't have time to work
271 on that at the moment. The suggested explanation could be correct, but when I get time I will try
272 to discuss this with Eric and see if he has any ideas. I would not, however, suggest holding up
273 your project for those discussions.

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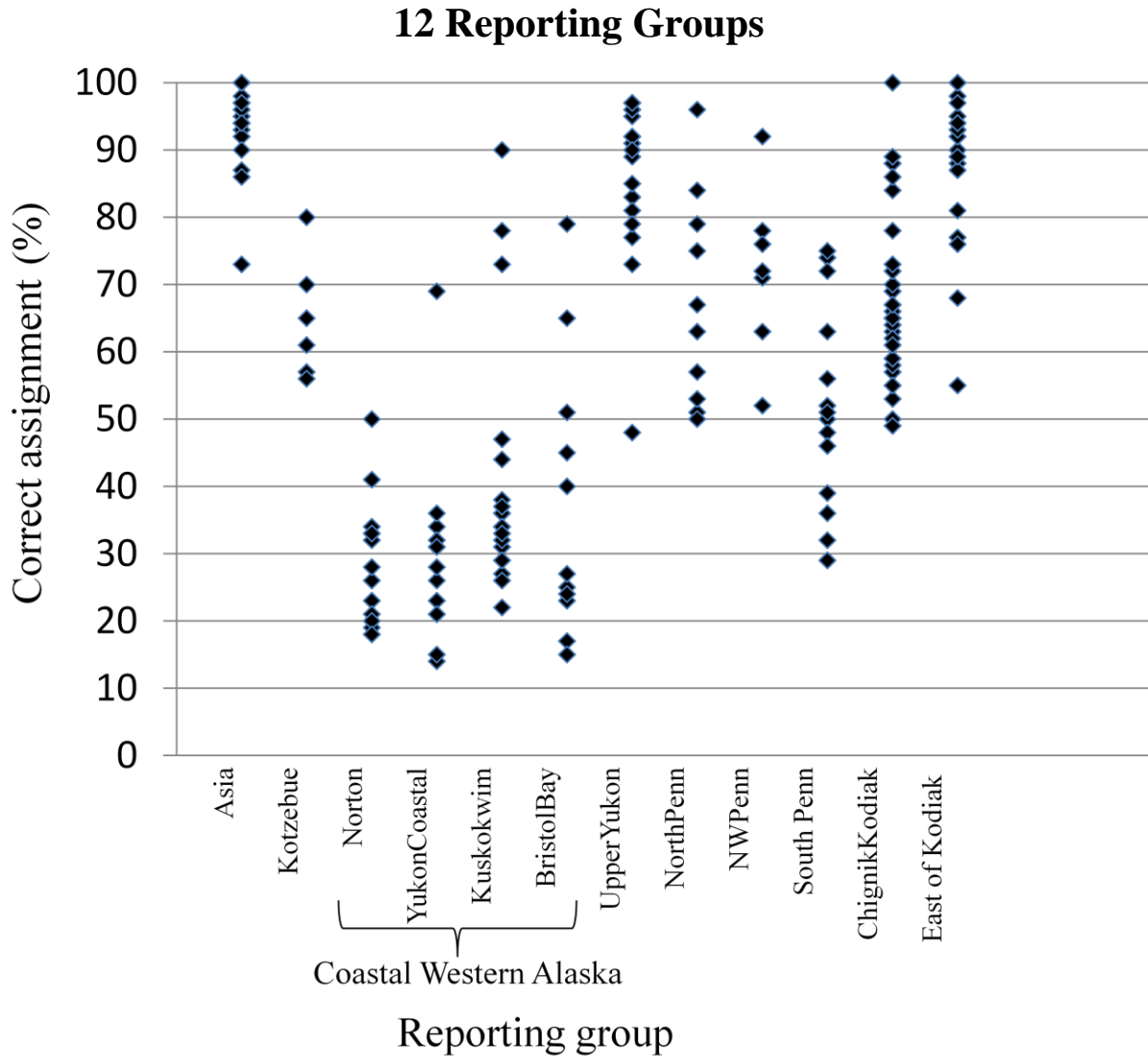
Figures



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Figure 1. Percent of correctly assigned individuals to 9 reporting groups using the leave-one-out method from Anderson et al. (2008). These 9 reporting groups include a single Coastal Western Alaska reporting group composed of Norton, YukonCoastal, Kuskokwim, and Bristol Bay. Each diamond represents the average correct assignment for each population within each reporting group.

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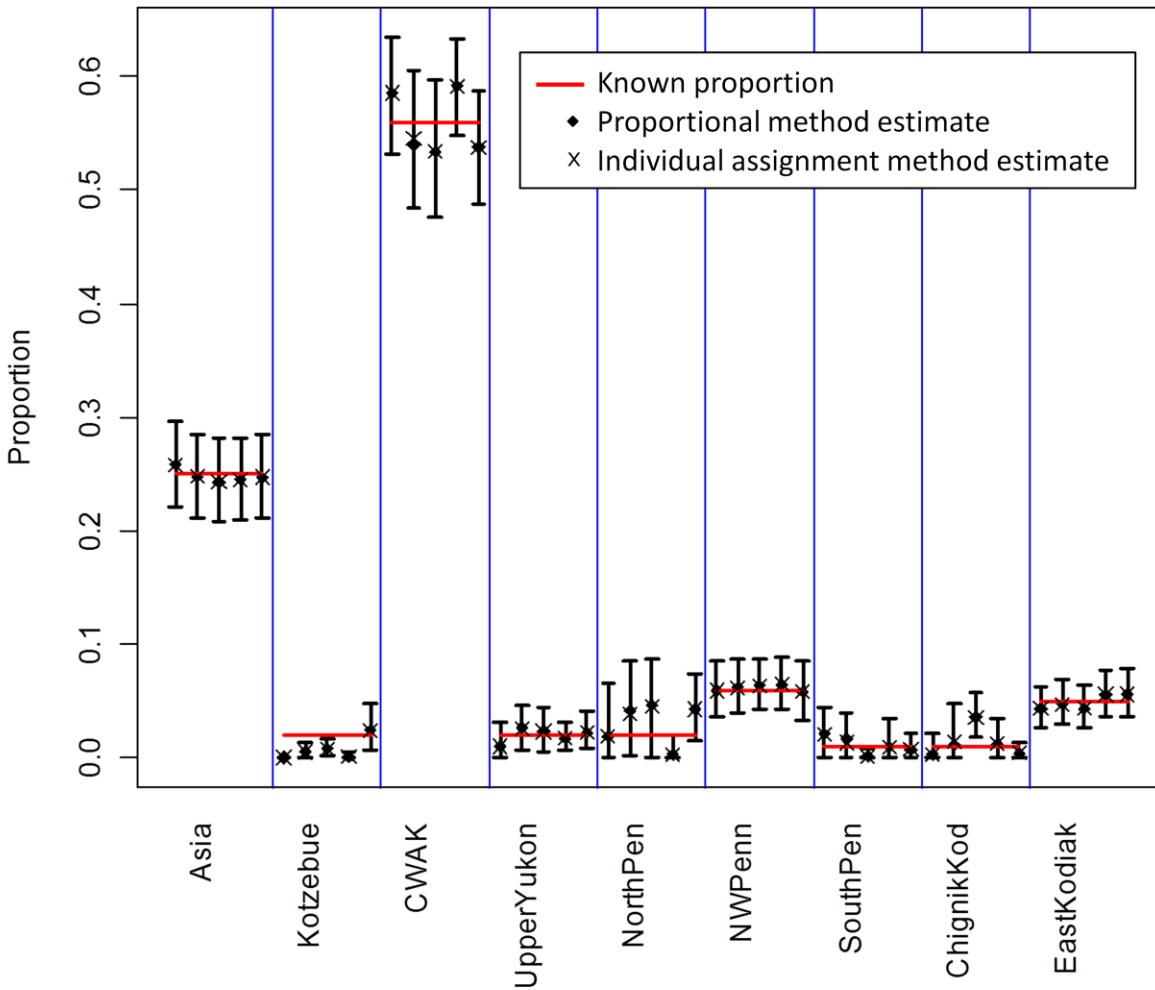
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288 Figure 2. Percent of correctly assignments of individuals to 12 reporting groups using the leave-
 289 one-out method from Anderson et al. (2008). These 12 reporting groups include the separation
 290 of Coastal Western Alaska into 4 reporting groups. Each diamond represents the average correct
 291 assignment for each population within each reporting group.

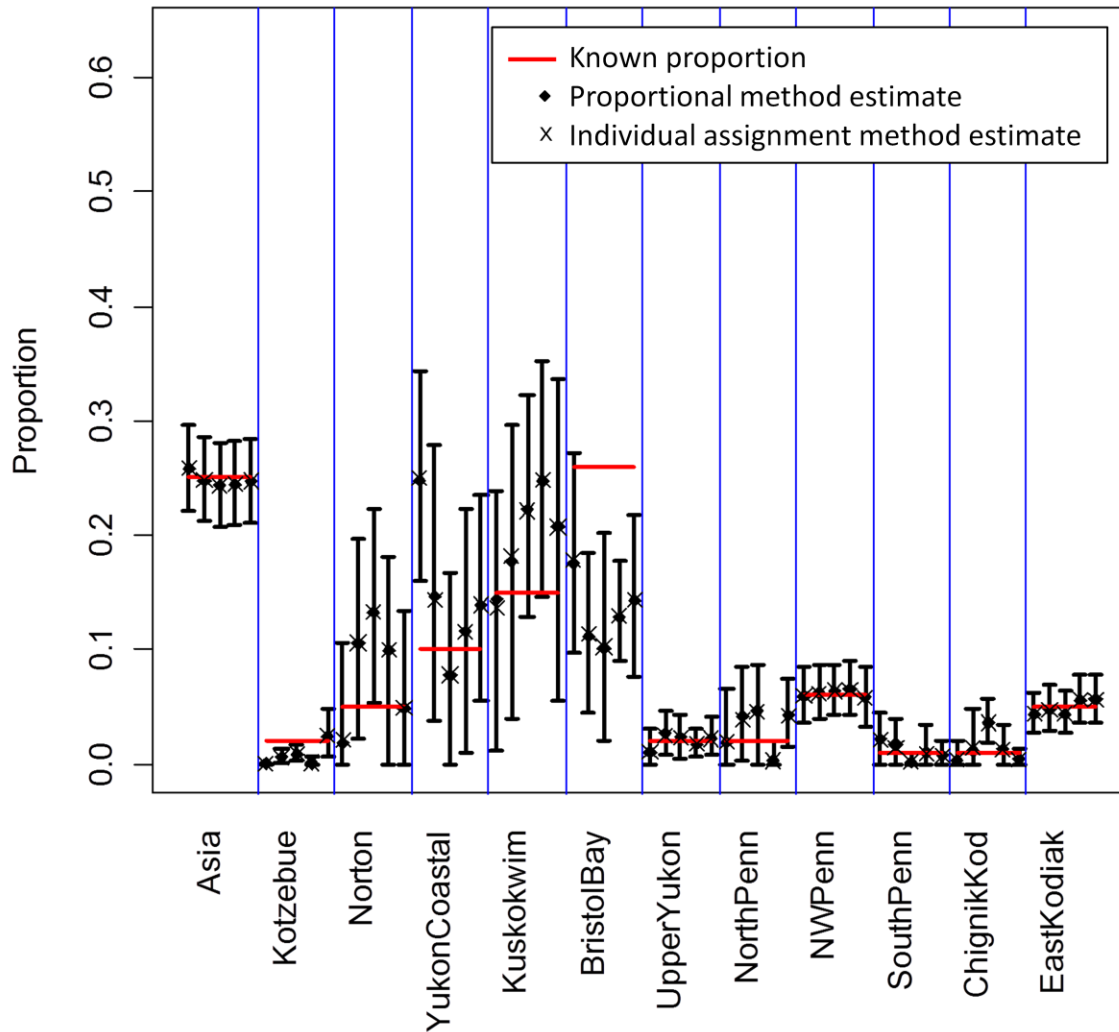
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9 Reporting Groups



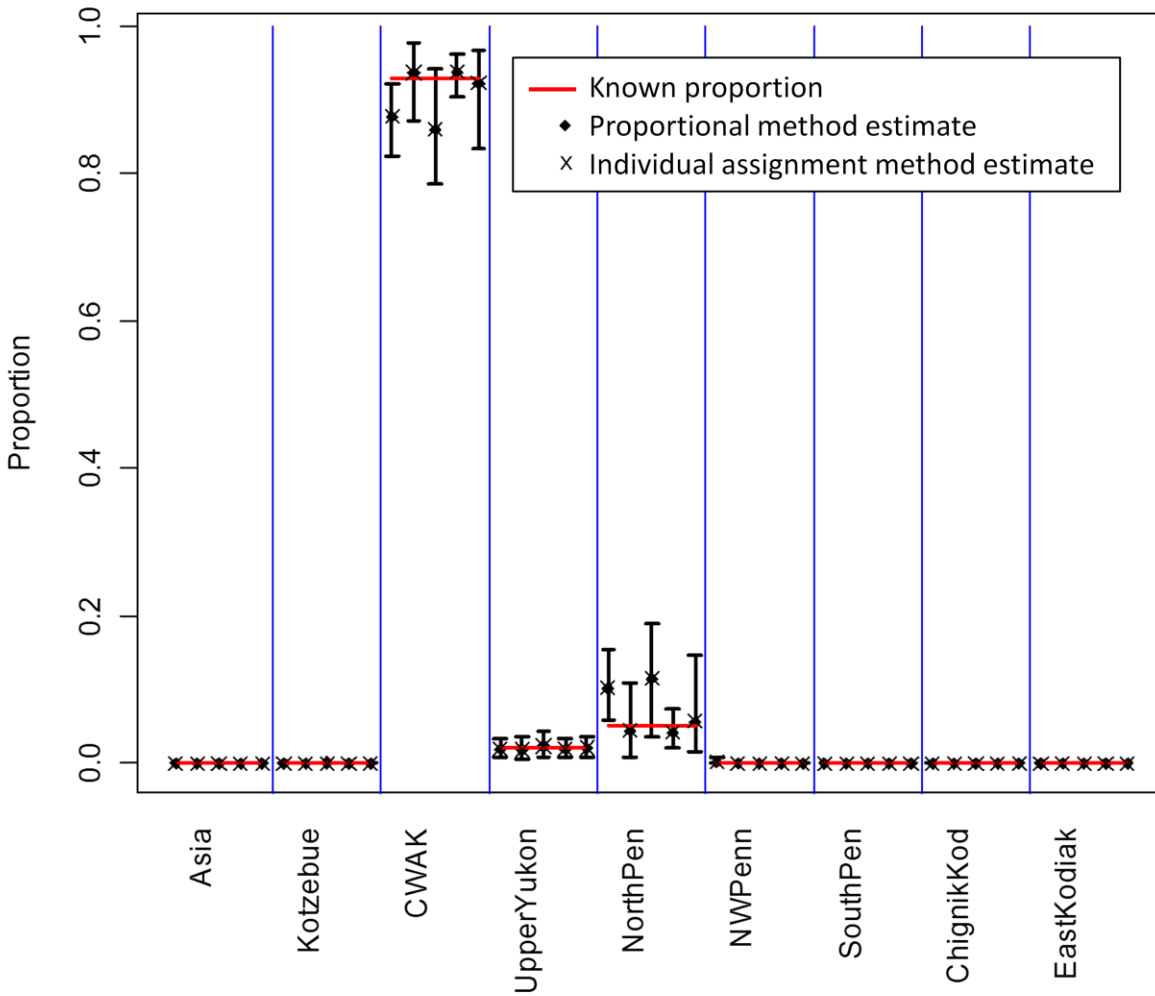
293
 294 Figure 3. BAYES estimates for 5 replicate samples for the fishery-based proof test “South Pen June
 295 (b) as run” (see Figure 1 in addendum 2 of TD 15) for 9 reporting groups where coastal western
 296 Alaska (CWAK) is a single reporting group. The actual stock composition of the replicate samples is
 297 shown as a red horizontal line. For each replicate sample, the proportional method estimate
 298 (diamond), and the individual assignment (Rao-Blackwell) method estimate (X), and lower and
 299 upper 90% credibility interval (vertical line) are provided.
 300

12 Reporting Groups

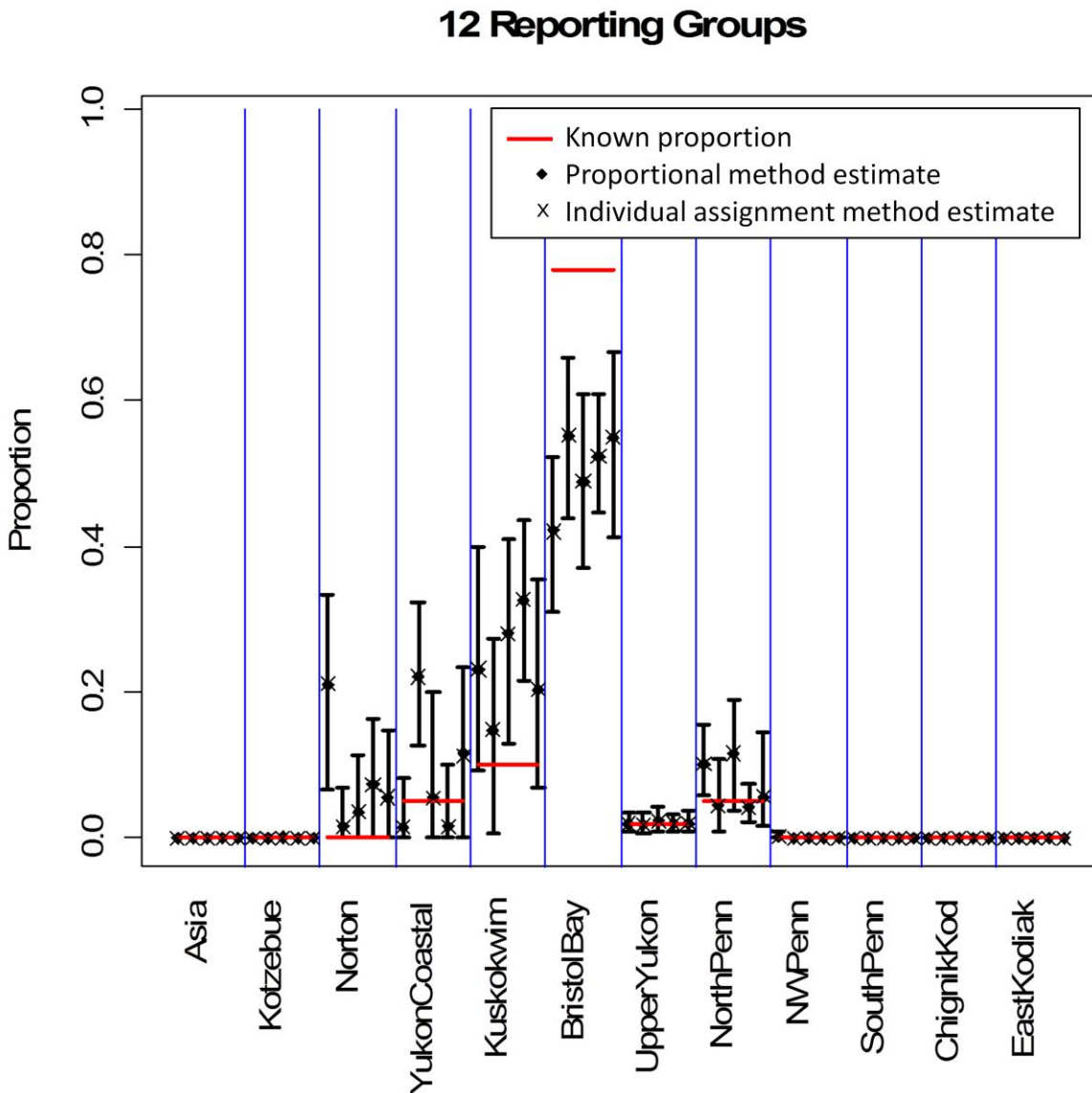


301
 302 Figure 4. BAYES estimates for 5 replicate samples for the fishery-based proof test “South Pen June
 303 (b) as run” (see Figure 2 in addendum 2 of TD 15) for 12 reporting groups where coastal western
 304 Alaska (CWAK) is divided into 4 reporting groups. The actual stock composition of the replicate
 305 samples is shown as a red horizontal line. For each replicate sample, the proportional method
 306 estimate (diamond), and the individual assignment (Rao-Blackwell) method estimate (X), and lower
 307 and upper 90% credibility interval (vertical line) are provided.
 308

9 Reporting Groups

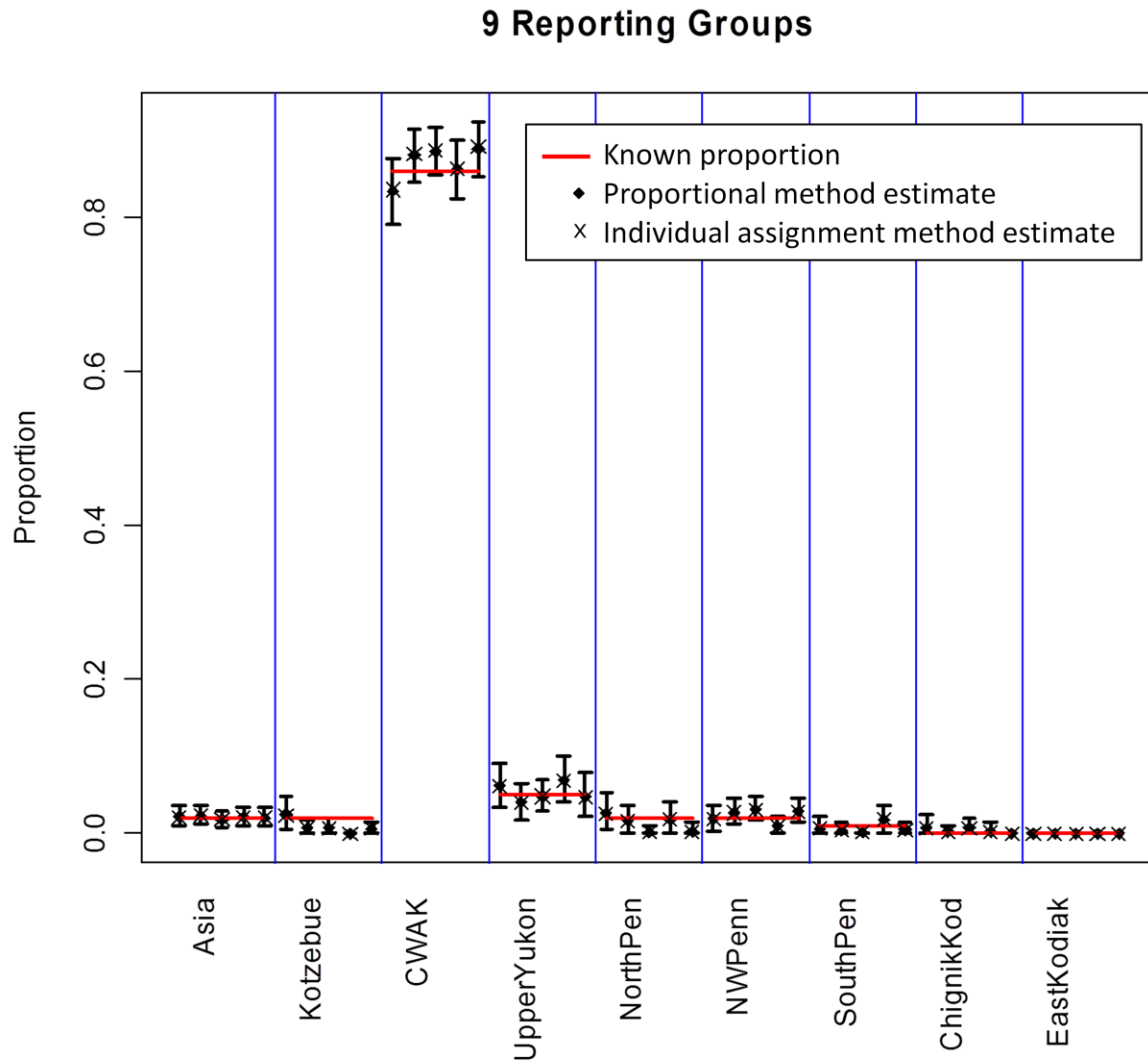


309
 310 Figure 5. BAYES estimates for 5 replicate samples for the fishery-based proof test “Bristol Bay”
 311 (see Figure 3 in addendum 2 of TD 15) for 9 reporting groups where coastal western Alaska
 312 (CWAK) is a single reporting group. The actual stock composition of the replicate samples is shown
 313 as a red horizontal line. For each replicate sample, the proportional method estimate (diamond), and
 314 the individual assignment (Rao-Blackwell) method estimate (X), and lower and upper 90%
 315 credibility interval (vertical line) are provided.
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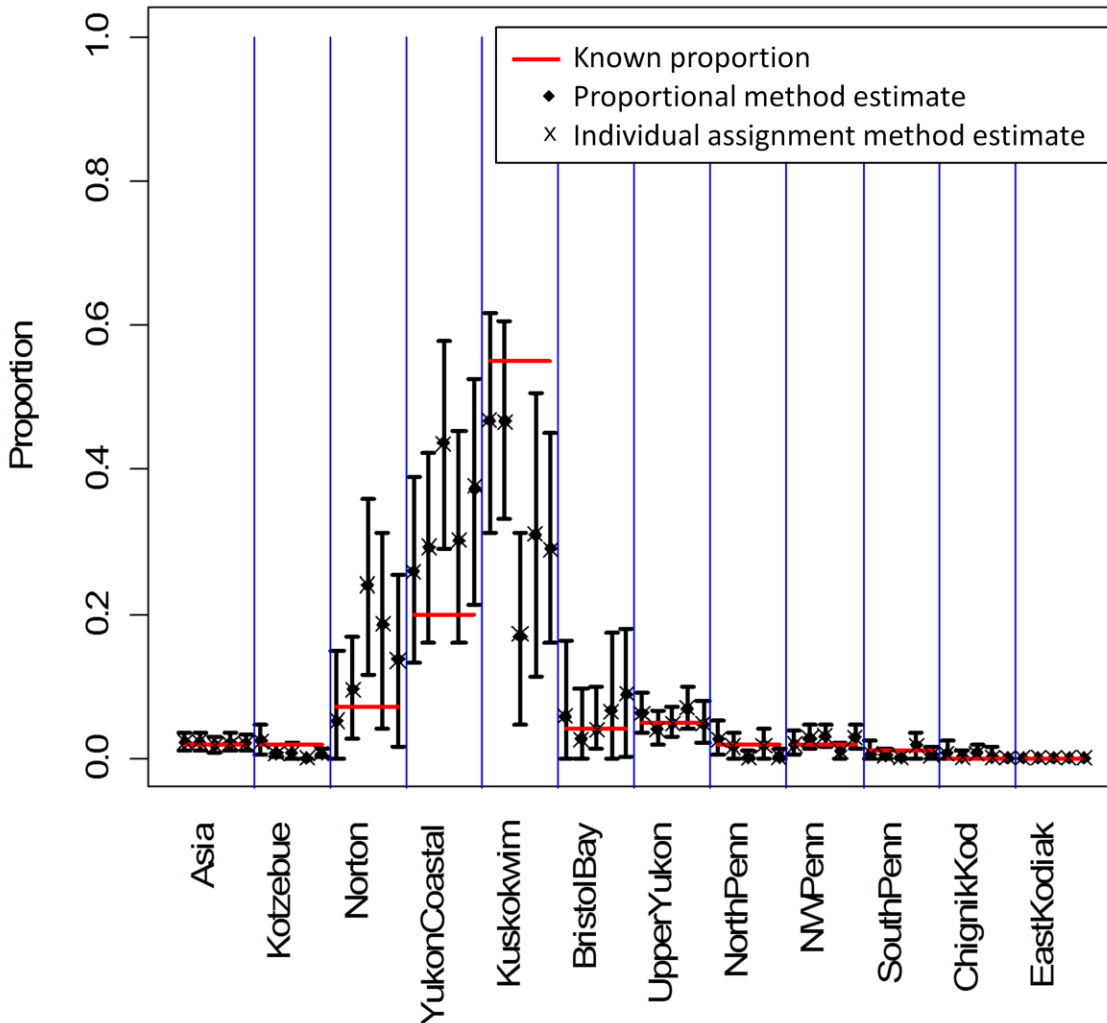
317
 318 Figure 6. BAYES estimates for 5 replicate samples for the fishery-based proof test “Bristol Bay”
 319 (see Figure 4 in addendum 2 of TD 15) for 12 reporting groups where coastal western Alaska
 320 (CWAK) is divided into 4 reporting groups. The actual stock composition of the replicate samples is
 321 shown as a red horizontal line. For each replicate sample, the proportional method estimate
 322 (diamond), and the individual assignment (Rao-Blackwell) method estimate (X), and lower and
 323 upper 90% credibility interval (vertical line) are provided.
 324

325



326
 327 Figure 7. BAYES estimates for 5 replicate samples for the fishery-based proof test “Kusko Bay”
 328 (see Figure 5 in addendum 2 of TD 15) for 9 reporting groups where coastal western Alaska
 329 (CWAK) is a single reporting group. The actual stock composition of the replicate samples is shown
 330 as a red horizontal line. For each replicate sample, the proportional method estimate (diamond), and
 331 the individual assignment (Rao-Blackwell) method estimate (X), and lower and upper 90%
 332 credibility interval (vertical line) are provided.
 333

12 Reporting Groups



334
 335 Figure 8. BAYES estimates for 5 replicate samples for the fishery-based proof test “Kusko Bay”
 336 (see Figure 6 in addendum 2 of TD 15) for 12 reporting groups where coastal western Alaska
 337 (CWAK) is divided into 4 reporting groups. The actual stock composition of the replicate samples is
 338 shown as a red horizontal line. For each replicate sample, the proportional method estimate
 339 (diamond), and the individual assignment (Rao-Blackwell) method estimate (X), and lower and
 340 upper 90% credibility interval (vertical line) are provided.