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## Accurate estimation of Conservation Unit contribution to coho salmon mixed-stock fisheries in British Columbia, Canada using direct DNA sequencing for single nucleotide polymorphisms

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1 Accurate estimation of Conservation Unit contribution to coho salmon mixed-stock fisheries in British  
2 Columbia, Canada using direct DNA sequencing for single nucleotide polymorphisms

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14

## 15 ABSTRACT

16 Determination of population structure and stock identification is a ubiquitous problem in  
17 fisheries assessment and management. Pacific salmon fishery management regimes are evolving  
18 to require higher resolution of stock composition on increasingly smaller reporting units. For  
19 coho salmon (*Oncorhynchus kisutch*), a stock identification baseline comprised of some 57,982  
20 individuals from 332 populations ranging from southeast Russia to California was employed for  
21 genetic stock identification (GSI). GSI analysis based upon variation at up to 480 single  
22 nucleotide polymorphisms (SNPs) was demonstrated to provide accurate estimates of stock  
23 composition for 37 Conservation Units (CU) in British Columbia, 13 reporting groups in the  
24 United States, and one reporting group in Russia. In many instances, accurate population-  
25 specific estimates of stock composition within a CU were possible in fishery samples, as well as  
26 identifying individuals to some specific populations. A genetics-based assessment system  
27 provides an opportunity for conservation-based management of Canadian coho salmon.  
28 Keywords: conservation, coho salmon, genetic stock identification, fisheries management  
29 population structure

30

## 31 Introduction

32           The general objective of genetic stock identification (GSI) for Pacific salmon is to provide the  
33 optimal resolution among stocks or populations present when applied to mixed-stock fishery and  
34 forensic samples at an affordable cost (Beacham et al. 2009). Stock identification of Pacific salmon in  
35 mixed-stock fisheries is important to enable fishery managers to decide on the timing and area of local  
36 salmon fisheries, as well as assess the impact of the fisheries on stocks, particularly those of  
37 conservation concern (Hess et al. 2016). Stock composition information is also important in  
38 determining locations of ocean residence of specific stocks of immature salmon (Farley et al. 2011), and  
39 the migration routes used by immature salmon to reach seasonal rearing areas (Beacham et al. 2014), as  
40 well as the routes used by maturing salmon to return to natal rivers.

41           GSI based on DNA variation has been widely applied in the assessment of mixed-stock  
42 Pacific salmon fisheries. For coho salmon (*Oncorhynchus kisutch*), microsatellites have been  
43 used for many years to evaluate population structure or estimate stock composition in mixed-  
44 stock fisheries (Small et al. 1998a,b; Beacham et al. 2001; Smith et al. 2001; Olsen et al. 2003;  
45 Ford et al. 2004; Bucklin et al. 2007; Johnson and Banks 2008, Beacham et al. 2012a).  
46 Population structure is important to evaluate for stock identification applications. If there is a  
47 regional basis to population structure, individuals in the mixture sample from populations not in  
48 the baseline used for stock composition estimation will generally be assigned to other sampled  
49 populations from the same region (Beacham et al. 2012a). Microsatellites also provided the  
50 basis for estimation of stock composition of juvenile coho salmon sampled off the coasts of  
51 Washington and Oregon (Van Doornik et al. 2007), as well as off coastal British Columbia (BC)  
52 (Beacham et al. 2016). Recent studies in salmonids have indicated that incorporation of several

53 hundred or thousands of SNPs in GSI applications can improve stock assignment accuracy  
54 (Larson et al. 2014; Moore et al. 2014).

55 Direct DNA sequencing is powering a revolution in the application of genetics to  
56 fisheries management and assessment, providing cost-effective genotyping at single nucleotide  
57 polymorphism (SNP) loci (Campbell et al. 2015) or microsatellites (Bradbury et al. 2018).  
58 Incorporating this new technology, Beacham et al. (2019a) provided a brief summary of current  
59 assessment techniques for coho salmon in BC based upon coded-wire tags (CWTs), and  
60 concluded that a combined GSI and parentage-based tagging (PBT) approach can provide critical  
61 information to improve coho salmon assessment and conservation. PBT uses molecular-based  
62 approaches to conduct large-scale parentage assignments and has resulted in the unprecedented  
63 ability to identify genetically millions of hatchery-origin salmonids (Steele et al. 2019).  
64 Assignments are made to parents of known origin, and with that information, it is possible to  
65 determine the origin and age of individuals sampled in fisheries. Application of a PBT-GSI  
66 system of identification of coho salmon in fisheries and escapements in BC provided high-  
67 resolution estimates of stock composition, catch, and exploitation rate by CU or population,  
68 providing an alternate and more effective method in the assessment and management of  
69 Canadian-origin coho salmon relative to CWTs (Beacham et al. 2019a). In addition, PBT can  
70 determine population- and family-specific distributions among fisheries, origins of hatchery  
71 broodstocks and associated stray rates among populations, and productivity of specific  
72 components of some hatchery broodstocks (Beacham et al. 2019b).

73 GSI and PBT applied in combination can provide high-resolution estimates of stock  
74 composition, as assignment of individual coho salmon via PBT is virtually 100% accurate with  
75 respect to hatchery of origin and age of the individual (Beacham et al. 2017; 2019a). However,

76 PBT has not been applied to wild coho salmon populations to date in British Columbia (BC), and  
77 thus accuracy of estimated stock compositions of fisheries where hatchery-produced individuals  
78 comprise only a small portion of the fishery will be dependent mainly upon the accuracy of  
79 estimates generated via GSI. Although initial analyses suggested that accurate fine geographic  
80 scale estimates of stock composition were available through GSI for some coho salmon  
81 populations in southern BC (Beacham et al. 2019a), demonstration of the general applicability of  
82 the accuracy of GSI-derived stock composition estimates for wild and hatchery populations  
83 throughout BC would be desirable.

84 In Canada, the Policy for Conservation of Wild Pacific Salmon (WSP) was established  
85 with the goal of maintaining and restoring healthy and diverse Pacific salmon populations,  
86 making conservation of wild salmon and their habitats the highest priority for resource  
87 management decision-making (Fisheries and Oceans Canada 2005). Fisheries and hatchery  
88 production were to be managed to ensure that wild populations were safeguarded and harvest  
89 benefits sustainable. As a cornerstone of the WSP, wild salmon populations are identified and  
90 maintained in Conservation Units (CUs) that reflect their geographic, ecological, and genetic  
91 diversity. The 43 coho salmon CUs originally identified by Holtby and Ciruna (2007) have been  
92 modified to a current number of 44 CUs (Fisheries and Oceans Canada 2019). Price et al. (2014;  
93 2017) suggested that any suitable assessment technique must provide individual resolution for all  
94 CUs to meet the conservation requirements of Canada's WSP. Accordingly, then this implies  
95 that GSI should provide accurate estimates of stock composition by CU where the majority of  
96 the individuals captured in fisheries are wild in origin. There will be limited opportunity to  
97 enhance GSI-derived estimates of wild-origin coho salmon via PBT analysis because of the  
98 difficulty and expense of complete adult sampling in the wild. Resolution of mixed-stock fishery

99 stock compositions in BC to the CU level would be unprecedented, as GSI has never been  
100 applied to provide such fine-scale resolution of stock composition in BC. Identification of  
101 individual populations within CUs adds to the difficulty of the task, as some mixed-stock fishery  
102 applications may depend on accurate population identification, as well as escapement surveys in  
103 order to estimate fishery-specific population exploitation rates (Beacham et al. 2019a).  
104 Evaluation of the ability to assess accurately the stock composition of mixed-stock coho salmon  
105 fishery samples by CUs and population within CU was the key objective of the current study.

106         The current study is an evaluation of the application of the GSI methodology initially  
107 outlined by Beacham et al. (2017) to determine whether GSI can be used to provide information  
108 on fishery contributions by CU for coho salmon CUs currently identified under the WSP.  
109 Ampliseq was used to amplify hundreds of SNPs in single PCR and, combined with direct DNA  
110 sequencing of the resultant amplicons and automated scoring of the SNP genotypes, resulted in  
111 rapid and cost-effective genotyping. Although the current study was directed towards coho  
112 salmon, similar procedures could be used for other salmonid and non-salmonid species. A stock  
113 identification baseline comprised of some 57,982 individuals from 332 populations ranging from  
114 southeast Russia to California was employed for GSI. Population structure of all populations in  
115 the baseline was evaluated, and genotypes from each population in a CU or geographic region  
116 were simulated and estimated stock composition of the single-population and multi-population  
117 simulated mixtures determined via GSI referencing the 332-population baseline. The baseline  
118 was subsequently used to estimate stock composition in a putatively known-origin sample and  
119 series of 2018 fisheries in BC.

120 Methods and Materials

121 General Methods

122 Evaluation of stock identification capability initially proceeded by development of a  
123 baseline of populations likely to contribute to mixed-stock fishery samples. Once the baseline  
124 was available, a series of tests was conducted in order to evaluate the effectiveness of the  
125 baseline in producing reliable estimates of stock composition in mixed-stock fishery samples.  
126 The initial step included determination of whether population structure was geographically or  
127 regionally based, aiding in definition of reporting units. With potential reporting units and  
128 populations within reporting units determined, an analysis was undertaken whereby simulated  
129 single-population samples were analyzed, and the baseline was used to estimate stock  
130 composition of the simulated samples for both population and reporting group. An estimated  
131 stock composition value of 90% to the reporting group for the 100% single population sample is  
132 generally considered as satisfactory for fishery management applications (Seeb and Crane 1999;  
133 Seeb et al. 2000; Beacham et al. 2012b). The next step was an evaluation of individual self-  
134 assignment accuracy to both population and reporting group. For a specific population, an 80%  
135 self-assignment accuracy has been considered as sufficient for maintaining the population as a  
136 reporting unit (Gilbey et al. 2016). Although not mandatory for evaluation of the reliability of  
137 stock composition estimates, we also evaluated the effect of baseline population sample size for  
138 populations with varying levels of genetic distinctiveness as measured by mean pairwise population  
139  $F_{ST}$ . We next evaluated the baseline by simulating two mixed-stock fishery samples comprised  
140 of eight populations each, with the simulated samples focusing on populations potentially present  
141 in northern BC and southern BC mixed-stock fisheries. The analysis continued with evaluation  
142 of a sample of known origin as represented by 884 individuals recovered from fishery sampling  
143 that had been marked with coded-wire tags. Even if estimated stock compositions of simulated  
144 mixed-stock fishery samples are accurate, there is a potential for biased estimates of stock

145 composition from actual mixed-stock fisheries if a substantial portion of the fishery sample is  
146 derived from reporting groups inadequately represented in the baseline. The final step in the  
147 analysis was estimation of stock composition of actual fishery samples from geographically-  
148 dispersed fisheries in BC as a means to evaluate whether the presence of unsampled populations  
149 in the mixed-stock sample will cause bias in estimated stock compositions.

#### 150 Baseline sample collection

151 The initial baseline was outlined by Beacham et al. (2017), and consisted of 20,242  
152 individuals from 117 populations, with the distribution of populations ranging from southeast  
153 Alaska to Puget Sound in Washington State. Beacham et al. (2019a) reported that the baseline  
154 was subsequently expanded to include 40,774 individuals from 267 populations, ranging from  
155 southeast Alaska to Oregon. The primary expansion of the baseline included a survey of  
156 additional populations in southern BC, coastal Washington, the Columbia River drainage, and  
157 Oregon. The baseline in the current study was expanded again to include to include 57,982  
158 individuals from 332 populations, ranging from Russia to California. Prior to 2014, most  
159 samples were collected opportunistically to provide a baseline for the previous microsatellite  
160 analyses concerning population structure and stock identification (Beacham et al. 2011; 2012a).  
161 From 2014 onwards, parentage-based tagging (PBT) was the objective of population sampling  
162 for selected hatcheries in British Columbia, and samples were collected to allow complete  
163 genotyping of the broodstock in a particular year. Fin tissue or operculum punches were  
164 obtained from all individuals sampled.

#### 165 Fishery sample collection

166 General fishery sampling procedures were described by Beacham et al. (2019a).  
167 Summarized briefly, in the northern BC troll fishery, samplers electronically checked adipose  
168 fin-clipped individuals for the presence of a CWT, and heads from those individuals containing a  
169 CWT were subsequently sent to the central laboratory for CWT recovery and tissue sampling for  
170 subsequent genotyping. Tissue samples from clipped individuals with no CWTs were directly  
171 provided for genotyping, as were samples from unclipped individuals. The origin of the samples  
172 from the recreational fishery in BC included voluntary head recoveries of adipose fin-clipped  
173 coho salmon from recreational fisheries in southern BC, and direct creel sampling in some  
174 northern recreational fisheries. Samples from the recreational fishery in southern BC were  
175 derived from clipped individuals, but they may not have been marked with a CWT when  
176 delivered to the CWT head recovery laboratory.

177 We tested accuracy of estimated stock composition for mixed-stock fishery samples by  
178 first estimating stock composition of a known-origin sample. If a coded-wire tag (CWT; Jefferts  
179 et al. 1963) was detected in 2018 sampling of commercial fisheries, the head was sent to a  
180 central CWT head recovery laboratory in Vancouver, BC where the DNA sample was  
181 subsequently taken. A CWT provided a putative known origin of the individual as the number  
182 on the tag is recorded prior to release of the individual from a hatchery or during smolt migration  
183 for a wild population. Heads of adipose-fin clipped individuals from 2018 recreational fisheries  
184 were also sampled for CWTs. If a CWT was successfully recovered and decoded from an  
185 individual head from a fishery, and a genotype was successfully obtained for the individual  
186 sample, then the genotypes of all of these individuals were pooled into a single mixed-stock  
187 sample of known-origin in order to evaluate accuracy of stock compositions by CU and

188 population. The known-origin sample was obtained by genotyping 884 coho salmon from 2018  
189 fisheries that were marked with CWTs.

190 We tested the baseline used in stock composition estimation by analyzing eight 2018  
191 mixed-stock samples of coho salmon with divergent geographic origins. Actual fishery samples  
192 are valuable to investigate as performance of the baseline in estimation of stock composition can  
193 be evaluated relative to expected stock compositions in the fishery, The geographic distribution  
194 of these samples, spanning sites from the northern British Columbia to a freshwater fishery in the  
195 Fraser River, suggested that divergent estimates of stock composition should be obtained when  
196 analyzed with the baseline evaluated in the study. Geographic locations for most of the fisheries  
197 were outlined by Beacham et al. (2019a).

## 198 Genotyping

199 The detailed procedures for DNA extraction, library preparation, and genotyping were  
200 described by Beacham et al. (2017), and a summarized version provided by Beacham et al.  
201 (2019a). The process involved loading amplified DNA from 756 individuals (up to 482  
202 amplicons per individual) on a P1 chip v3 (chip used with the Ion Torrent Proton sequencer) with  
203 an Ion Chef (laboratory instrument used to robotically load DNA libraries on to a sequencing  
204 chip). Two chips were loaded consecutively with a single run of the Ion Chef, both chips were  
205 then subsequently loaded on to an Ion Torrent Proton sequencer, and the genotype of each  
206 individual recorded with automated scoring of the genotype via Proton software Variant Caller®  
207 at one SNP site in each amplicon. Other than the target SNP, additional sequence variation  
208 within the same amplicon was not incorporated in the analysis. Genotypes at all available SNPs  
209 for an individual were assembled to provide a single multi-locus individual genotype for each  
210 individual fish. This multi-locus genotype was the basic input for subsequent analyses. The

211 species identification SNP *OkiOts\_120255-113* and sex identification SNP *Ots\_SEXY3-1* were  
212 omitted from subsequent GSI analyses, leaving up to 480 SNPs included. The species  
213 identification SNP was omitted as it was monomorphic if only coho salmon were sampled and  
214 thus of no value for GSI analyses. It was however of considerable value in eliminating any non-  
215 coho salmon that may have been included in mixed-stock fishery samples. The sex identification  
216 SNP was eliminated because the genotype was either monomorphic (male) or “no call” (female)  
217 and of no value for GSI analyses. The 480 remaining SNPs were derived from a previous SNP  
218 panel outlined by Beacham et al. (2017), supplemented with 209 SNPs derived from the Genome  
219 Canada project Enhancing Production in Coho: Culture, Community, Catch (EPIC4).

220

221 The baseline

222 A listing of populations in the baseline that has been genotyped, along with the  
223 corresponding CU (Canadian populations) or geographic region (Russian and American  
224 populations) is outlined in Supplementary Table 1. Summarized briefly, the baseline survey  
225 consisted of genotyping coho salmon in populations from Russia, Alaska, BC, Washington,  
226 Oregon, and California, with locations of Canadian CUs outlined in Figure 1. The locations for  
227 some of the populations listed in Supplementary Table 1 in each geographic region were  
228 illustrated by Beacham et al. (2017), with most of the samples collected subsequent to 1990.

229 Data analysis

230 Genotypes had to be obtained for at least 150 SNPs for the individual to be retained in the  
231 baseline. In a test where the DNA of the same 382 individuals was genotyped on two occasions,  
232 an average genotyping error rate of 1.07% (1,220 discrepancies in 114,105 comparisons) or an  
233 allele error rate of 0.53% (1,220 discrepancies in 228,210 comparisons) was observed over the

234 302 SNPs scored (Beacham et al. 2017). Expected and observed heterozygosities by locus were  
235 determined with adegenet (Jombart and Ahmed 2011). Population heterozygosity was  
236 determined as the average heterozygosity over all loci. Estimation of  $F_{ST}$  by locus was  
237 conducted with ape (Paradis and Schliep 2018). Amplicon sequences were aligned to the  
238 genome (RefSeq assembly accession GCF\_002021735.1) with BWA mem v0.7.17-r1198 (Li  
239 2013) and filtered using filter\_sam\_file.py in snp-placer (commit 8bd5e72 -  
240 <https://github.com/CNuge/snp-placer>).  $F_{ST}$  by SNP was plotted in R v3.6.0 (R Core Team 2019)  
241 by position along the chromosomes, except for SNPs aligned to unplaced scaffolds which were  
242 plotted based on the average marker spacing observed across the genome. For determination of  
243 population structure,  $F_{ST}$  distance was used to estimate genetic distances among all populations  
244 via StAMPP (Pembleton et al. 2013), with up to 480 SNPs incorporated in the analyses. An  
245 unrooted neighbor-joining tree based upon  $F_{ST}$  distance was generated using phangorn 2.5.5  
246 (Schliep 2011). Bootstrap support for the major nodes in the tree was evaluated based upon  
247 1000 replicate trees.

248 To test the accuracy of identifying the conservation unit and the population of origin, we  
249 performed GSI using Rubias (Moran and Anderson 2019), which employs Bayesian inference  
250 from a conditional genetic stock identification model. In general, the algorithm estimates the  
251 conditional probability distribution for each individual in the mixture, so it is probabilistically  
252 assigned to the closest genetic match from the set of populations in the baseline. To conduct  
253 100% single-population simulations via Rubias, we simulated mixture genotypes from each  
254 population sequentially and determined the allocation to the specific population simulated, as  
255 well as the allocation to the CU (Canada) or reporting region (Russia, United States) to which the  
256 population belonged.

257           Assessment of accuracy of self assignment of individuals was conducted via Rubias,  
258 where each individual in the baseline was evaluated for self-assignment accuracy both to  
259 individual population and to CU or reporting region. Leave-one-out cross validation analysis  
260 provided about 58,000 independent tests of known origin as they were collected from known  
261 specific coho salmon populations, with the assumption of limited straying among populations.

262           The effect of baseline population sample size on accuracy of estimated stock composition  
263 of single-population samples was evaluated for six populations, one with high average  $F_{ST}$  (Noyo  
264 River,  $F_{ST}=0.157$ ), one with intermediate  $F_{ST}$  (Robertson Creek,  $F_{ST}=0.097$ ), and four with lower  
265  $F_{ST}$  (Stave River,  $F_{ST}=0.060$ ; Chilliwack River,  $F_{ST}=0.064$ ; Chehalis River,  $F_{ST}=0.060$ ; and  
266 Qualicum River,  $F_{ST}=0.056$ ). Population sample sizes of 20, 40, 60, 80, 100, 150, 200, 250,  
267 300, 400, 500, 600, 700, 800, and 900 individuals were consecutively evaluated for each  
268 population when available. Baseline sample sizes were constructed by randomly selecting the  
269 desired sample size from previously genotyped individuals in the population, and incorporating  
270 these individuals into the baseline used for stock composition analysis. As only 97 individuals  
271 had been genotyped for the Noyo River (Supplementary Table 1), the analysis was terminated at  
272 a baseline sample size of 80 individuals for this population.

273           The next stage of the evaluation incorporated analyses of two multi-population simulated  
274 mixed-stock fishery samples (200 individuals in each sample) for simulated mixtures as may be  
275 encountered in fishery sampling in northern and southern BC. Eight populations were  
276 incorporated into each simulated mixture at set limits ranging from 5%-20% of the sample.  
277 Rubias was used to estimate stock composition of the resultant mixture, and means and standard  
278 deviations determined for population and CU or reporting region estimates for 100 simulations  
279 of each mixture. Stock composition by CU or reporting group was determined by summation of

280 allocations to all populations in the baseline that belonged to the CU or reporting group under  
281 consideration.

282 For estimation of stock composition in the fishery samples, after an initial burn-in of  
283 25,000 iterations, the last 1,000 iterations from the Monte Carlo Markov Chain from Rubias  
284 were used to estimate the origin of individuals and stock composition, with the mean allocation  
285 to each population in the baseline determined. Standard deviations of estimated stock  
286 compositions were also determined from the last 1,000 iterations from the Monte Carlo Markov  
287 Chain. As with the simulated fishery samples, stock composition by CU or reporting group was  
288 determined by summation of allocations to all populations in the baseline that belonged to the  
289 CU or reporting group under consideration.

290

## 291 Results

### 292 Heterozygosity and $F_{ST}$

293 Expected heterozygosity ranged from 0.00 to 0.50 across the 480 SNPs surveyed  
294 (Supplementary Table 2, excluding species ID and sex ID markers), and observed heterozygosity  
295 ranged from 0.00 to 0.51. Observed heterozygosities were  $> 0.40$  for 42% of the SNPs surveyed  
296 (Figure 2). Global  $F_{ST}$  across SNPs ranged from 0.00 to 0.28, and 58% of the SNPs displayed a  
297  $F_{ST}$  value between 0.05 and 0.10 (Figure 3). SNPs included in the panel included many from a  
298 previous version of the panel (Beacham et al. 2017) and with the new EPIC4 SNPs  
299 (Supplementary Table 2). The EPIC4 SNPs were chosen for their initial stock identification  
300 capability separating BC populations, so heterozygosities and  $F_{ST}$  values may not be  
301 representative of SNPs present in the genome.

### 302 Genomic distribution of SNPs

303           The 480 SNPs surveyed were broadly distributed over the 30 chromosomes present in the  
304 coho salmon genome, ranging from a minimum of four SNPs present on chromosome Okis25 to  
305 32 SNPs present on chromosome Okis6 (Figure 4). Average marker spacing across the  
306 chromosomes was 3.85 Mbp [per chromosome average 2.40 Mbp – 8.7 Mbp]. There were 42  
307 SNPs present in scaffolds unassigned to specific chromosomes. SNPs with higher  $F_{ST}$  values  
308 were also widely distributed across chromosomes, so there was no clustering of these SNP sites  
309 on specific chromosomes.

### 310 Population structure

311           Significant genetic differentiation was observed among coho salmon populations sampled  
312 in the different CUs and geographic regions surveyed. The most distinctive stocks in the survey  
313 included the following: Russia (mean population  $F_{ST}$ =0.130), California (mean  $F_{ST}$ =0.150),  
314 interior Fraser River (CO-47, mean  $F_{ST}$ =0.113), lower Thompson River (CO-7, mean  
315  $F_{ST}$ =0.125), North Thompson River (CO-9, mean  $F_{ST}$ =0.114), and South Thompson River (CO-  
316 8, mean  $F_{ST}$ =0.123) (Supplementary Figure 1). Populations on the islands of Haida Gwaii were  
317 also quite distinct, with those in the Haida Gwaii-Graham Island Lowlands CU (CO-25, mean  
318  $F_{ST}$ =0.103), Haida Gwaii-East CU (CO-23, mean  $F_{ST}$ =0.094), and Haida Gwaii-West CU (CO-  
319 24, mean  $F_{ST}$ =0.097) generally distinct from those on mainland BC. The greatest average  
320 differentiation observed was between populations separated by the greatest geographic distance.  
321 Russian populations were very distinct when compared with California populations, with an  
322 average population pairwise  $F_{ST}$  of 0.200 (SD=0.026).

323           Genetic differentiation was also observed at finer geographic scales. Coho salmon  
324 spawning populations generally clustered together in CUs, river drainages, and in local

325 geographic areas throughout the geographic range surveyed. For example, there was substantial  
326 clustering of populations in the Skeena River and Fraser River drainages (Supplementary Figure  
327 1). Regional clustering was observed in southeast Russian, Alaska, Washington, Columbia  
328 River, Oregon, and California populations.

329

330 Analysis of simulated single-population samples

331 The analysis of population variation indicated that there was a structure based on CUs for  
332 Canadian populations and a geographically-based regional structure for Russian and American  
333 populations. This structure formed the basis to conduct estimation of stock composition for  
334 simulated single-population fishery samples for all populations in the baseline at both the  
335 population and reporting group (CU or region) level. In general, accurate estimates of stock  
336 composition were possible for most populations in the baseline at the CU or reporting group  
337 level (Figure 5), and for many individual populations (Supplementary Figure 2). For example,  
338 overall accuracy for a population estimate to the correct CU was 93.4% for 258 Canadian  
339 populations, 94.8% to the correct geographic region for 65 American populations, 98.8% to the  
340 correct geographic region for nine Russian populations, and an overall accuracy of 93.8% to the  
341 correct CU or geographic region for all 332 populations.

342 Accurate allocations to many individual populations were observed. For example, in the  
343 East Vancouver Island-Georgia Strait CU (CO-13), estimated population stock compositions for  
344 single-population samples resolved with a 332-population baseline were 95.7% for Qualicum  
345 River, 98.6% for Puntledge River, and 99.3% for Quinsam River. These high levels of accuracy  
346 were observed even though there were 17 populations in the CU (Supplementary Figure 2).  
347 Similarly for the Lower Fraser River CU (CO-47), 24 populations were present in the baseline,

348 yet estimates of stock composition for single-population samples were 98.5% for Chehalis River,  
349 99.4% for Chilliwack River, 90.9% for Stave River, 96.6% for Norrish Creek, and 87.3% for  
350 Inch Creek (Supplementary Figure 2).

351

### 352 Assignment of individuals

353 Estimation of stock composition of single-population samples displayed high accuracy  
354 across CUs and populations, and further analyses investigated the accuracy of assignment of  
355 individuals, the most difficult of all stock identification applications. In general, accurate  
356 assignments (>80% accuracy) of individuals were observed across a range of CUs or reporting  
357 regions, corresponding to those CUs or regions that displayed the most genetic distinctiveness  
358 (Figure 6). For example, individuals from North Thompson River populations (CO-9), South  
359 Thompson River populations (CO-8), lower Thompson River populations (CO-7), and interior  
360 Fraser River populations (CO-48) displayed a high level of assignment accuracy to CU, as did  
361 individuals from the Haida Gwaii CUs (CO-23, CO-24, CO-25). Accurate self assignment of  
362 individuals was also observed for a number of populations on Haida Gwaii, Hood Canal,  
363 California, and southeast Alaska (Supplementary Figure 3). Within CUs, high assignment  
364 accuracy of individuals to the correct population (>90%) was also observed for specific  
365 populations such as Conuma River, Maggie River, and Robertson Creek in the WCVI CU (CO-  
366 17), and Sangan River, Tell River, and Yakoun River in the north eastern Haida Gwaii CU (CO-  
367 25). Assignment of individuals could be accurate to both CU and population within CU,  
368 dependent upon the population under evaluation.

369

370 Population sample size in relation to accuracy of estimated stock compositions

371 Accuracy of estimated stock compositions varied among populations, and we  
372 investigated the effect of population sample size on estimation of stock composition accuracy of  
373 single-population samples for populations of varying levels of genetic distinctiveness. The Noyo  
374 River population was considered quite distinct, and only about 40 individuals were required to be  
375 genotyped in order for highly accurate estimates of stock composition to be obtained for the  
376 single-population samples (>99% estimate for a 100% simulated single-population sample)  
377 (Figure 7). Highly accurate estimates of stock composition for the Robertson Creek population  
378 were achieved at a baseline population size of 60 individuals. For less differentiated populations  
379 like Chilliwack River and Chehalis River, accurate estimates of stock composition were  
380 observed at a baseline population sample size of 150 individuals. Accuracy continued to  
381 increase for less differentiated populations Stave River and Qualicum River up to a baseline  
382 sample size of 400 individuals, and marginal increases in accuracy were observed up to a  
383 baseline sample size of 900 individuals. The target sample size for a population to be included in  
384 a SNP GSI baseline varies with the genetic distinctiveness of the population.

385

386 Analysis of simulated multi-population fishery mixtures

387 The accuracy and precision of two multi-population simulated fishery samples were  
388 estimated for both population and CU/regional components. The average error of estimated  
389 stock compositions of a simulated mixed-stock fishery sample from northern BC containing  
390 individuals from eight populations was 1.0% for population and 0.4% for CU or region (Table  
391 1). For the southern BC simulated mixture, the average error was 0.5% for population and 0.2%  
392 for CU or region. Accurate estimates of stock composition were obtained for simulated mixed-  
393 stock fishery samples if all populations present in the mixed-stock sample were present in the

394 baseline, which indicated a successful completion in this step of the evaluation of the baseline for  
395 mixed-stock fishery analysis.

#### 396 Analysis of known-origin mixture

397 Estimations of stock compositions of simulated single-population and multi-population  
398 samples suggested that accurate estimates of stock composition by reporting group and in some  
399 cases by population should be possible when applied to mixed-stock fishery samples of unknown  
400 origin. Assessment of this potential capability was tested by estimation of stock composition of  
401 a known-origin sample of 884 genotyped individuals from both Canadian and American  
402 populations which had been previously marked with coded-wire tags. There were 525 tags from  
403 Canadian populations across 13 CUs. With only Canadian-origin tags considered, the average  
404 error of estimation was 0.4% across the 13 CUs. With the addition of 359 American-origin tags,  
405 the average error of estimation was 0.5% across the 13 CUs (Figure 8). The increase in error of  
406 stock composition was largely a result of the estimate for the lower Stikine River CU, with an  
407 error of 0.2% (actual 2.1%, estimated 2.3%) when only Canadian-origin tags were considered,  
408 but 3.0% (actual 1.4%, estimated 4.4%) when both Canadian-origin and American-origin tags  
409 were considered. Only one population (Scud River) was genotyped in the CU, and self-  
410 assignment of individuals to the population was relatively accurate (93.6%, Supplementary  
411 Figure 3). The Klawock Lake population, not in the current baseline, contributed 35.7% (46 of  
412 129 tags) of Alaskan-origin tags, and there was likely some misallocation of these individuals to  
413 the lower Stikine River CU.

414 American-origin tags originated from populations in 10 reporting groups. The average  
415 error of estimation was 1.8% across the 10 reporting groups, but this result was heavily  
416 influenced by the southeast Alaska reporting group error (actual 14.6%, estimated 6.8%) (Figure

417 8). The average error of estimation across the nine US West Coast reporting groups was 1.0%.  
418 Coded-wire tags originating from populations in Washington and the Columbia River constituted  
419 26.0% of the tags in the sample, and total estimated stock contribution of the nine US West  
420 Coast reporting groups was 25.6%, indicating that errors in estimation were largely distributed  
421 among US West coast reporting groups, rather than between Canadian CUs and US West Coast  
422 reporting groups.

423 The results from estimation of stock composition of the simulated single-population  
424 fishery samples suggested that accurate estimates of contributions of specific populations may be  
425 possible. The Canadian-origin tags previously noted were recovered from 16 populations. The  
426 average error of estimation across the 16 populations was 0.4% (Figure 9). The capability of  
427 estimating accurate stock composition of specific populations of coho salmon has been verified  
428 for those populations where a high accuracy of identification in the simulated single-population  
429 samples was observed.

430

## 431 Discussion

432 In Canada, the adoption of Conservation Units (CUs) under the Wild Salmon Policy  
433 (WSP) presented a fisheries management and assessment challenge. There are currently 44 CUs  
434 identified for coho salmon in British Columbia, and successful implementation of the WSP will  
435 likely require identification of fishery impacts by CU. At the time of the adoption of the WSP,  
436 there were no known methods available to enable resolution of fishery impacts across all CUs.  
437 Some fishery managers were doubtful that such resolution of mixed-stock fishery stock  
438 compositions in BC to the CU level would be possible, as GSI had never been applied to provide  
439 such fine-scale resolution of stock composition over wide geographic areas. The current study

440 has provided accurate identification of Canadian coho salmon sampled from mixed-stock  
441 fisheries to the CU level, and has enabled assessment of fishery impacts that is sufficiently  
442 informative for conservation-based management as envisaged in WSP. Coho salmon harvested  
443 in Canadian commercial and recreational fisheries were identified to Canadian CUs and  
444 American geographic regions from southeast Alaska to California, confirming the utility of a  
445 GSI approach for conservation-based assessment of mixed-stock harvest on a wide geographic  
446 scale.

447         The adoption of the WSP required that populations of all five main species of Pacific  
448 salmon in BC be classified into CUs for assessment purposes, even though at the time of  
449 adoption there was no method to identify fishery impacts by CU. Direct DNA sequencing,  
450 coupled with Ampliseq technology which allowed genotyping at hundreds of SNPs through a  
451 single PCR, provided the advance in applied genetics which allowed identification of fishery  
452 impacts by CU in coho salmon. This paradigm-shifting advance can be applied to other species,  
453 and we are in the process of applying it to Chinook salmon (*O. tshawytscha*), chum salmon (*O.*  
454 *keta*), and sockeye salmon (*O. nerka*), and coupling it with PBT technology for Chinook salmon  
455 (Beacham et al. 2018). High-resolution stock identification, which may combine both GSI and  
456 PBT, can be achieved and will provide increased accuracy in estimation of stock composition for  
457 those populations of potential conservation concern, typically comprising < 5% of a fishery  
458 sample.

459

460 Population structure

461 A regionally-based population structure is generally required in the application of GSI in  
462 Pacific salmon, as an important assumption in the application is that the portion of the mixed-  
463 stock sample derived from populations not in the baseline is allocated to sampled populations  
464 from the same region. GSI works well when this assumption is met, as the cost and complexity  
465 of developing a baseline for stock composition analysis is reduced when not all populations  
466 potentially contributing to a mixed-stock sample are included in the baseline. Thus, a study of  
467 population structure can yield valuable insights as to how GSI will perform in mixed-stock  
468 fishery application. Previous microsatellite-based population structure studies indicated that a  
469 regional geographically-based structure was apparent (Olsen et al. 2003; Bucklin et al. 2007;  
470 Johnson and Banks 2008; Beacham et al. 2011). Population structure of coho salmon  
471 populations surveyed in the current study displayed a pattern of CU or regionally-based  
472 population structure. Therefore, coho salmon population structure thus meets the important  
473 condition that unsampled populations contributing to mixed fishery samples will likely be  
474 allocated to sampled populations in the same region. In applications where errors in population  
475 estimation are considered to be too large for satisfactory use, then it is necessary to increase the  
476 sample size of key existing baseline populations or sample additional baseline populations in the  
477 CU or region in order to enhance the reliability of regional estimates of stock composition.

478 Population samples were available from 37 of the 44 CUs defined for BC coho salmon,  
479 with the unrepresented CUs restricted to northern BC where the remoteness of the locations has  
480 precluded sample collection to date. Planning is underway for collection of samples in some of  
481 these CUs, and we expect that population structure within these unsampled CUs will reflect a CU  
482 basis. If not, re-evaluation of defined CUs may be required.

483 Population sample size in relation to accuracy of estimated stock compositions

484           The target baseline population sample size is dependent upon population genetic  
485 differentiation. Through simulation of SNP variation, Morin et al. (2009) reported that  
486 increasing sample size up to 100 individuals for detection of population structure was beneficial.  
487 However, for populations with  $F_{ST}=0.10$ , little increase in detection power was observed above  
488 60 sampled individuals, similar to the results for the Robertson Creek population in the current  
489 study. Beacham et al. (2011) reported that less genetically distinct populations required larger  
490 population sample sizes to achieve a given level of accuracy in estimated stock compositions,  
491 similar to the results observed in the current study. When increased accuracy of estimated stock  
492 compositions is required for a particular population in the baseline, the most direct route to  
493 follow is to increase sample size for the target population. For SNP-based baseline development,  
494 100 genotyped individuals per population is a reasonable initial target. However, for populations  
495 more difficult to discriminate, sample sizes of up to 500 individuals may be required. In the  
496 current baseline, 25.9% of the populations contained at least 100 genotyped individuals, 50.9%  
497 of the populations contained 40-99 genotyped individuals, 15.7% of the populations contained  
498 20-39 individuals, and 7.5% of the populations contained 11-19 individuals. Many of the poorly  
499 identified populations were associated with population sample sizes of < 40 genotyped  
500 individuals.

501

502 Analysis of known-origin mixture

503           Accurate estimation of stock composition via GSI relies on a baseline that includes all  
504 major populations potentially contributing to a mixed-stock fishery sample. Our study provided  
505 an illustration of the misallocation that may occur when this assumption is violated. The  
506 Klawock Lake population comprised 35.7% of the Alaskan-origin CWTs and 5.2% of the total

507 CWT sample, but the population was not in the baseline. The resultant misallocation was  
508 observed across northern CUs, the most notable was the lower Stikine River CU, where the  
509 contribution of the CU to the known-origin CWT sample was overestimated by 3.0%, some 58%  
510 of the Klawock Lake contribution to the total sample. The Stikine River originates in northern  
511 BC, and flows across southeast Alaska to enter the Pacific Ocean proximal to other southeast  
512 Alaska populations, with geographic proximity accounting for the majority of the misallocation  
513 of the Klawock Lake population component. The addition of the Klawock Lake population to  
514 the baseline would likely remediate the observed misallocation.

515 In general, analysis of the known-origin CWT sample confirmed the ability of the  
516 baseline to provide reliable estimates of stock composition for CUs when applied to analysis of  
517 mixed-stock fishery samples, a result which had been suggested by single-population  
518 simulations. Furthermore, reliability of estimates of contributions for specific populations was  
519 generally confirmed, provided that high accuracy had been observed in the single-population  
520 sample simulations. For those populations such as Inch Creek where underestimation of actual  
521 contributions was observed and would be expected as illustrated by the single-population  
522 simulations, parentage-based tagging (PBT) may provide a method to enhance estimates of stock  
523 composition for specific populations (Beacham et al. 2019a). For example, the Inch Creek  
524 contribution to the total CWT sample was underestimated by 2.7% by GSI alone (actual 14.4%,  
525 estimated 11.7%), whereas when both GSI and PBT were applied to estimate the Inch Creek  
526 contribution to the CWT sample, the estimate was 14.7%, an error of 0.3%.

527 The CWT sample consisted of tags recovered from both wild- and hatchery-origin  
528 individuals. The GSI baseline can be applied to fisheries where both hatchery-origin and wild-  
529 origin coho are caught. In BC, there are many integrated hatcheries resulting in very similar or

530 undifferentiated hatchery and wild populations (Le Luyer et al. 2017). As long as the population  
531 is represented in the baseline, no difference in accuracy or precision of estimated stock  
532 compositions is expected between wild- and hatchery-origin individuals in a mixed-stock fishery  
533 sample.

534

535 Mixed-stock fisheries

536 In our study, we estimated stock composition of eight mixed-stock samples of unknown  
537 origin of coho salmon with a 332-population baseline arranged by CU for Canadian populations  
538 and geographic reporting group for American populations (Supplementary Results). The  
539 rationale was that given the geographic locations and timing of the fisheries, it should be possible  
540 to evaluate estimated stock compositions of actual fishery samples against expectations to  
541 measure performance of the baseline for stock identification applications. In general, stock  
542 composition results met expectations based upon the geographic location of the fishery. The  
543 highly-mixed stock troll fishery off Haida Gwaii in northern BC displayed contributions from  
544 southeast Alaska, Haida Gwaii, northern and central BC, southern BC, Washington, and the  
545 Columbia River, as would be expected (Beacham et al. 2012a). In southern BC fisheries, all  
546 individuals sampled were adipose fin clipped and indicative of their hatchery origin. Little if any  
547 contributions from northern or central coast (CUs north of CO-12) would be expected, and these  
548 were the exact results observed in the fisheries. For example, the August WCVI recreational  
549 fishery was expected to be comprised primarily of individuals from the WCVI and lower Fraser  
550 River CUs, along with Washington reporting regions, and these were indeed the observed results.  
551 The September sample from Barkley Sound and Alberni Inlet is from a much more inshore  
552 fishery than the WCVI fishery of which it is a part. The later timing, coupled with a restricted

553 geographic location led to virtually all of the individuals originating from the WCVI CU, again  
554 meeting expectations. There are only two populations (Robertson Creek, Conuma River) in the  
555 CU for which adipose fin clipping is conducted, and given the location of the fishery, virtually  
556 all adipose fin-clipped individuals should be of Robertson Creek origin. Virtually all WCVI CU  
557 individuals (n=119) were identified as originating from Robertson Creek (n=118), with one  
558 individual identified from Conuma River, confirming the reliability of the stock composition  
559 estimates to a specific population. The freshwater fishery investigated was in Nicomen Slough,  
560 and estimated stock composition of the fishery was comprised almost entirely of individuals of  
561 lower Fraser River origin. The one non-Fraser River-origin individual in the October 2018  
562 fishery sample was identified as Robertson Creek origin with a 100% probability level,  
563 presumably indicative of a stray. Inch Creek individuals were estimated to comprise about 60%  
564 of the sample, and Norrish Creek about 34% of the sample. Norrish Creek is a later-returning  
565 population than Inch Creek, and this was illustrated by Norrish Creek comprising 91.5% of the  
566 November fishery sample (n=59), and Inch Creek 8.5%. In summary, estimated stock  
567 compositions of the fishery samples corresponded very well to those expected based upon  
568 fishery location and timing.

## 569 Summary

570 Current and historical assessment of coho salmon fisheries impacts in BC has been  
571 conducted with the application of CWTs, but CWTs are not applied to releases from some of the  
572 largest hatcheries in southern BC due to funding limitations, and thus their specific contributions  
573 to mixed-stock ocean fisheries are unknown in a CWT-based assessment system. The current  
574 study has not only demonstrated that it was possible to identify coho salmon mixed-stock fishery  
575 contributions by CU, but also possible in many instances to identify specific populations within a

576 CU in the fishery samples, as well as identifying individuals to some specific populations. Coho  
577 salmon are mass marked (juvenile adipose fin clipped) upon hatchery release in many hatcheries  
578 in BC, and distinguishing between hatchery-origin and natural-origin individuals can be done  
579 visually. A genetics-based assessment regime benefits from the mass marking of hatchery-  
580 produced salmon, thereby facilitating improved monitoring of wild-enhanced fish interactions,  
581 and the evaluation of hatchery contributions to harvest. We have demonstrated that a genetics-  
582 based assessment system can overcome the deficiencies present in the current CWT-based  
583 assessment regime (Beacham et al. 2019a), and also provides an opportunity for conservation-  
584 based management of Canadian coho salmon.

585 The ability to provide reliable estimates of stock composition by CU was facilitated by  
586 the switch from a microsatellite-based baseline to a SNP-based baseline with the SNPs  
587 genotyped via direct DNA sequencing of amplicons. Ampliseq allowed hundreds of SNPs to be  
588 amplified in single PCR, and direct DNA sequencing of the resultant amplicons, coupled with  
589 automated scoring of the genotypes, resulted in cost-effective genotyping and unprecedented  
590 ability to provide accurate estimates of stock composition to very discrete geographic regions or  
591 CUs. Similar results can be expected when applied to other non-salmonid species, and a new era  
592 in salmonid stock identification is dawning.

593

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620

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762 analyses provide insight into the early ocean stock distribution and survival of juvenile  
763 coho salmon off the coasts of Washington and Oregon. *N. Am. J. Fish. Manage.* 27: 220-  
764 237.

765 Table 1. Estimated percentage stock composition of simulated mixed-stock samples of coho  
 766 salmon (n=200) as may be encountered in northern and southern British Columbia (BC). The  
 767 expected CU/Regional compositions were obtained by adding the true population components  
 768 for each CU/Region. Standard deviation is in parentheses.

CU/Region	Population	True	Population	CU/Region
Northern BC				
Southeast Alaska	Ford Arm Lake	15.0	14.9 (2.7)	14.9 (2.7)
CO-25	Yakoun River	5.0	5.1 (1.7)	5.2 (1.7)
CO-23	Pallant Creek	10.0	9.7 (1.9)	9.7 (1.9)
CO-36	Meziadin River	20.0	19.0 (2.3)	19.0 (2.3)
CO-34	Motase River	10.0	10.2 (2.3)	10.3 (2.3)
CO-27	Tyler Creek	15.0	13.4 (2.7)	13.8 (2.8)
CO-29	Kitimat River	10.0	12.9 (2.7)	25.9 (3.3)
CO-29	Gilttoyes Creek	15.0	13.0 (2.4)	
Southern BC				
CO-17	Conuma River	15.0	15.0 (2.7)	25.1 (3.4)
CO-17	Robertson Creek	10.0	10.0 (2.0)	
CO-13	Cowichan River	10.0	9.1 (2.2)	20.0 (3.1)
CO-13	Quinsam River	10.0	10.8 (2.1)	
CO-10	Capilano River	15.0	14.5 (2.8)	14.8 (2.8)
CO-47	Chilliwack River	10.0	9.9 (2.1)	10.0 (2.1)
Northern Puget Sound	Snohomish River	15.0	14.0 (2.1)	14.0 (2.1)
Columbia River	Clackamus River	15.0	14.3 (2.6)	15.2 (2.7)

770 Table 2. Percentage stock composition by geographic region or CU of 2018 Stikine River commercial and test, North Coast troll, Johnstone Strait  
 771 sport, west coast Vancouver Island sport, Barkley Sound sport, Juan de Fuca Strait sport, northern Strait of Georgia sport, and Nicomen Slough  
 772 sport fisheries. Standard deviation is in parentheses.

Region/Conservation Unit	Stikine River	North Coast	Johnstone Strait	WCVI	Barkley Sound	Juan de Fuca Str	Georgia Str. (N)	Nicomen Slough
	September	August	July	August	September	September	July	October
Sample size	12	189	172	368	120	242	105	96
Southeast Alaska	0.0 (0.8)	20.5 (3.6)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)
Alsek River CO-45	0.0 (1.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)
Lower Stikine CO-39	100.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Lower Nass CO-35	0.0 (6.5)	2.9 (1.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Upper Nass CO-36	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Portland Sound-Observatory Inlet-Portland Canal CO-37	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Skeena Estuary CO-31	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)
Lower Skeena CO-32	0.0 (0.4)	1.1 (1.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)
Middle Skeena CO-33	0.0 (0.1)	0.3 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Upper Skeena CO-34	0.0 (0.1)	2.2 (1.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
Haida Gwaii-Graham Island								
Lowlands CO-25	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)
Haida Gwaii-East CO-23	0.0 (0.2)	14.8 (2.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.3)
Haida Gwaii-West CO-24	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Northern Coastal Streams CO-30	0.0 (0.0)	0.2 (0.3)	0.0 (0.2)	0.0 (0.1)	0.0 (0.2)	0.0 (0.1)	0.0 (0.0)	0.0 (0.2)
Hecate Strait Mainland CO-27	0.0 (0.6)	3.6 (1.8)	0.0 (0.1)	0.3 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.4)
Mussel-Kynoch CO-26	0.0 (0.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Douglas Channel-Kitimat Arm CO-29	0.0 (0.1)	0.1 (0.5)	0.0 (0.0)	0.1 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Bella Coola-Dean Rivers CO-22	0.0 (0.0)	0.4 (0.5)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)
Rivers Inlet CO-21	0.0 (2.2)	0.3 (1.0)	0.0 (0.2)	0.0 (0.1)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)
Smith Inlet CO-20	0.0 (1.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Southern Coastal Streams-Queen Charlotte Strait-Johnstone Strait-								
Southern Fjords CO-12	0.0 (0.1)	0.0 (0.1)	0.2 (0.4)	0.1 (0.2)	0.0 (0.2)	0.0 (0.4)	0.0 (0.0)	0.0 (0.0)
Homathko-Klinaklini Rivers CO-19	0.0 (1.3)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Georgia Strait Mainland CO-11	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Howe Sound-Burrard Inlet CO-10	0.0 (0.0)	0.0 (0.2)	9.3 (2.2)	2.4 (0.7)	0.8 (0.7)	12.3 (1.9)	22.6 (3.7)	0.0 (0.1)
East Vancouver Island-Georgia Strait CO-13	0.0 (1.5)	10.1 (1.9)	39.8 (3.7)	2.9 (1.3)	0.0 (0.1)	7.6 (1.6)	8.8 (2.9)	0.0 (0.2)
East Vancouver Island-Johnstone Strait-Southern Fjords CO-14	0.0 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Nahwitti Lowland CO-15	0.0 (0.0)	2.3 (1.2)	2.3 (1.2)	1.4 (0.6)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.3)
West Vancouver Island CO-17	0.0 (1.1)	4.8 (1.3)	3.6 (1.5)	18.6 (2.2)	99.1 (0.8)	0.0 (0.4)	0.0 (0.0)	1.0 (0.9)
Clayoquot CO-18	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Juan de Fuca-Pachena CO-16	0.0 (0.4)	0.0 (0.4)	1.6 (0.9)	2.5 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Lower Fraser CO-47	0.0 (0.2)	2.2 (0.9)	32.0 (3.4)	15.0 (1.7)	0.0 (0.1)	26.9 (3.4)	43.1 (4.0)	99.0 (1.2)
Lillooet CO-4	0.0 (1.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)
Fraser Canyon CO-5	0.0 (0.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Interior Fraser CO-48	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Lower Thompson CO-7	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.2 (0.7)	0.0 (0.0)	0.0 (0.0)
North Thompson CO-9	0.0 (1.3)	0.0 (0.1)	0.0 (0.4)	0.0 (0.1)	0.0 (0.3)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)
South Thompson CO-8	0.0 (1.8)	0.0 (0.1)	0.6 (0.6)	0.0 (0.1)	0.0 (0.1)	0.4 (0.4)	0.0 (0.1)	0.0 (0.0)
Boundary Bay CO-1	0.0 (1.7)	0.0 (0.1)	0.0 (0.1)	0.9 (0.7)	0.0 (0.0)	2.8 (1.1)	1.0 (1.4)	0.0 (0.0)
Nooksack River	0.0 (0.2)	0.0 (0.0)	0.3 (0.8)	10.0 (2.0)	0.0 (0.0)	0.9 (1.0)	7.6 (3.0)	0.0 (0.0)
Skagit River	0.0 (0.1)	5.5 (1.8)	0.3 (0.3)	10.0 (2.2)	0.0 (0.0)	19.0 (3.1)	5.2 (2.4)	0.0 (0.0)
Northern Puget Sound	0.0 (0.2)	8.6 (1.8)	2.4 (1.4)	15.1 (2.1)	0.0 (0.0)	16.7 (3.4)	5.7 (2.5)	0.0 (0.0)
Mid-Puget Sound	0.0 (0.0)	0.7 (0.7)	0.5 (0.6)	8.8 (1.9)	0.0 (0.1)	9.1 (2.1)	3.8 (2.3)	0.0 (0.0)
Southern Puget Sound	0.0 (0.3)	0.3 (0.8)	0.0 (0.0)	0.2 (0.5)	0.0 (0.0)	0.4 (0.6)	2.2 (2.0)	0.0 (0.1)
Juan de Fuca Strait	0.0 (1.0)	0.9 (0.7)	0.0 (0.0)	0.9 (0.5)	0.0 (0.0)	1.4 (0.7)	0.0 (0.0)	0.0 (0.0)
Hood Canal	0.0 (0.0)	12.5 (2.5)	0.0 (0.0)	9.3 (1.7)	0.0 (0.1)	0.1 (0.1)	0.0 (0.2)	0.0 (0.0)
Coastal Washington	0.0 (0.3)	5.8 (1.7)	1.2 (0.8)	1.6 (0.5)	0.0 (0.1)	0.1 (0.3)	0.0 (0.0)	0.0 (0.0)
Columbia River	0.0 (0.3)	0.0 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.0)
Oregon	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.1)	0.0 (0.0)

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Northern California	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
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Draft

774 List of Figures

775 Figure 1. Boundaries of Conservation Units defined for coho salmon in British Columbia. The  
776 map was prepared with Arc Gis with the shape files located at Open Government at  
777 [https://search.open.canada.ca/en/od/?sort=last\\_modified\\_tdt%20desc&page=1&od-search-](https://search.open.canada.ca/en/od/?sort=last_modified_tdt%20desc&page=1&od-search-portal=Open%20Data&search_text=NuSEDS)  
778 [portal=Open%20Data&search\\_text=NuSEDS](https://search.open.canada.ca/en/od/?sort=last_modified_tdt%20desc&page=1&od-search-portal=Open%20Data&search_text=NuSEDS)

779 Figure 2. Distribution of observed heterozygosity for 480 SNPs surveyed in coho salmon  
780 populations ranging from Russia to California.

781 Figure 3. Distribution of  $F_{ST}$  for 480 SNPs surveyed in coho salmon populations ranging from  
782 Russia to California.

783 Figure 4. Distribution of  $F_{ST}$  across 30 coho salmon chromosomes and unassigned scaffolds for  
784 the 480 SNPs. The number of SNPs originating from each of the chromosomes is listed at the  
785 top of the figure. Position of the points along the X-axis reflects the position of the SNP within  
786 each chromosome. For unassigned scaffolds, the points are separated by even spacing. The plot  
787 does not include Ots\_SEXY3\_1 or OkiOts\_120255\_113 used for Sex ID and Species ID,  
788 respectively.

789 Figure 5. For simulated single-population samples, average % accuracy for estimated stock  
790 compositions for individual populations in a CU or reporting group back to population (open  
791 portion of bar) and to CU (black portion of bar) for all populations in a CU or reporting group,  
792 with the number of populations in the CU reported to the right of the bar. The 90% accuracy  
793 level is indicated in the figure.

794 Figure 6. Average % accuracy for self assignment for individual populations in a CU or  
795 reporting group back to population (open portion of bar) and to CU (grey portion of bar) for all

796 populations in a CU or reporting group, with the number of populations in the CU reported to the  
797 right of the bar. The 80% accuracy level is indicated in the figure.

798 Figure 7. Average % accuracy for estimated stock compositions for simulated single-population  
799 samples of 200 individuals for Noyo River (○), Robertson Creek (+), Stave River (●),  
800 Chilliwack River (◆), Chehalis River (X), and Qualicum River (▽) populations for actual  
801 baseline sample sizes varying from 20 to 400 individuals where available.

802 Figure 8. Accuracy of regional (United States) and conservation unit (Canada) estimated stock  
803 composition (%) for a mixed-stock sample of 884 coded-wire tagged individuals sampled in  
804 2018 fisheries in BC estimated with GSI. Actual percentage is the black bar, estimated % is the  
805 clear bar. Standard deviation is indicated for the estimate.

806 Figure 9. Accuracy of estimated Canadian population-specific stock composition (%) for a  
807 mixed-stock sample of 884 coded-wire tagged individuals sampled in 2018 fisheries in BC  
808 estimated with only GSI. Actual percentage is the black bar, estimated % is the clear bar. Only  
809 Canadian populations for which CWTs were recovered and GSI analysis was conducted are  
810 illustrated, along with standard deviation of the estimate.

811

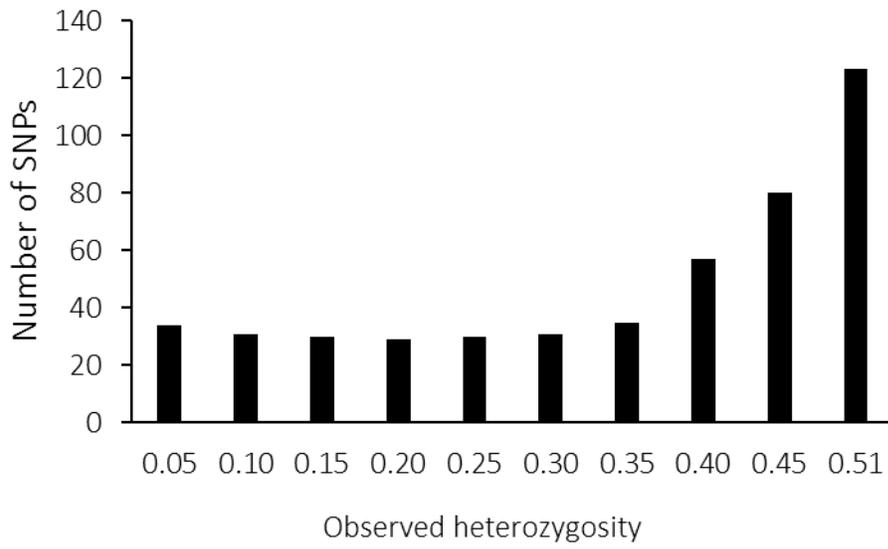
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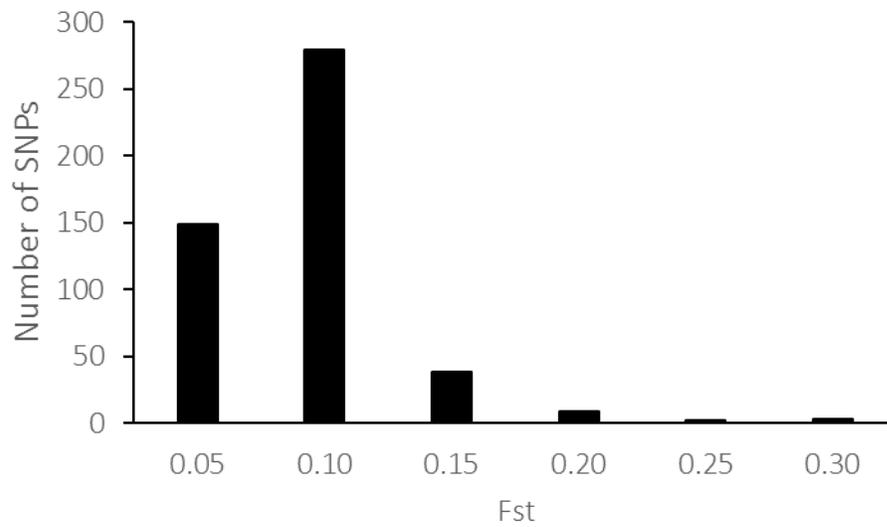
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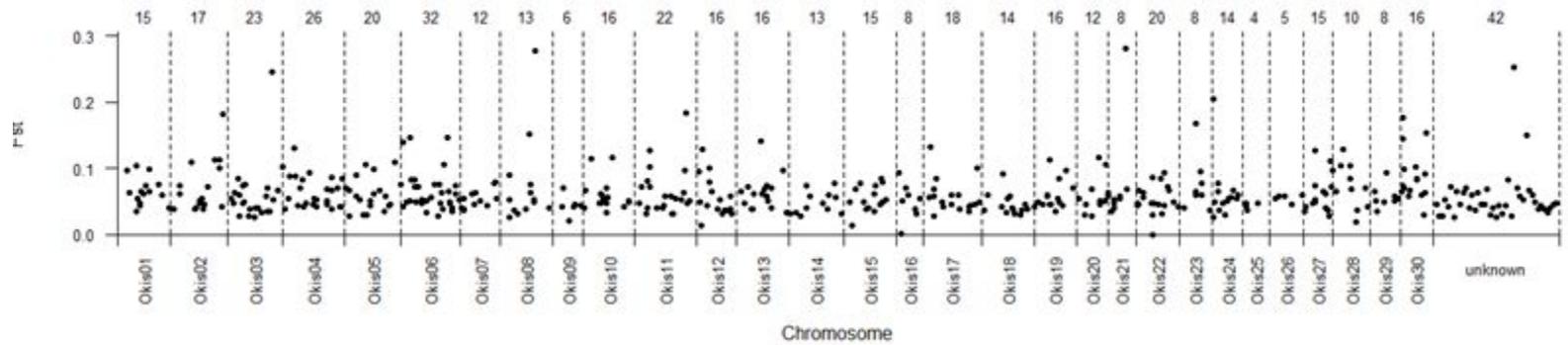
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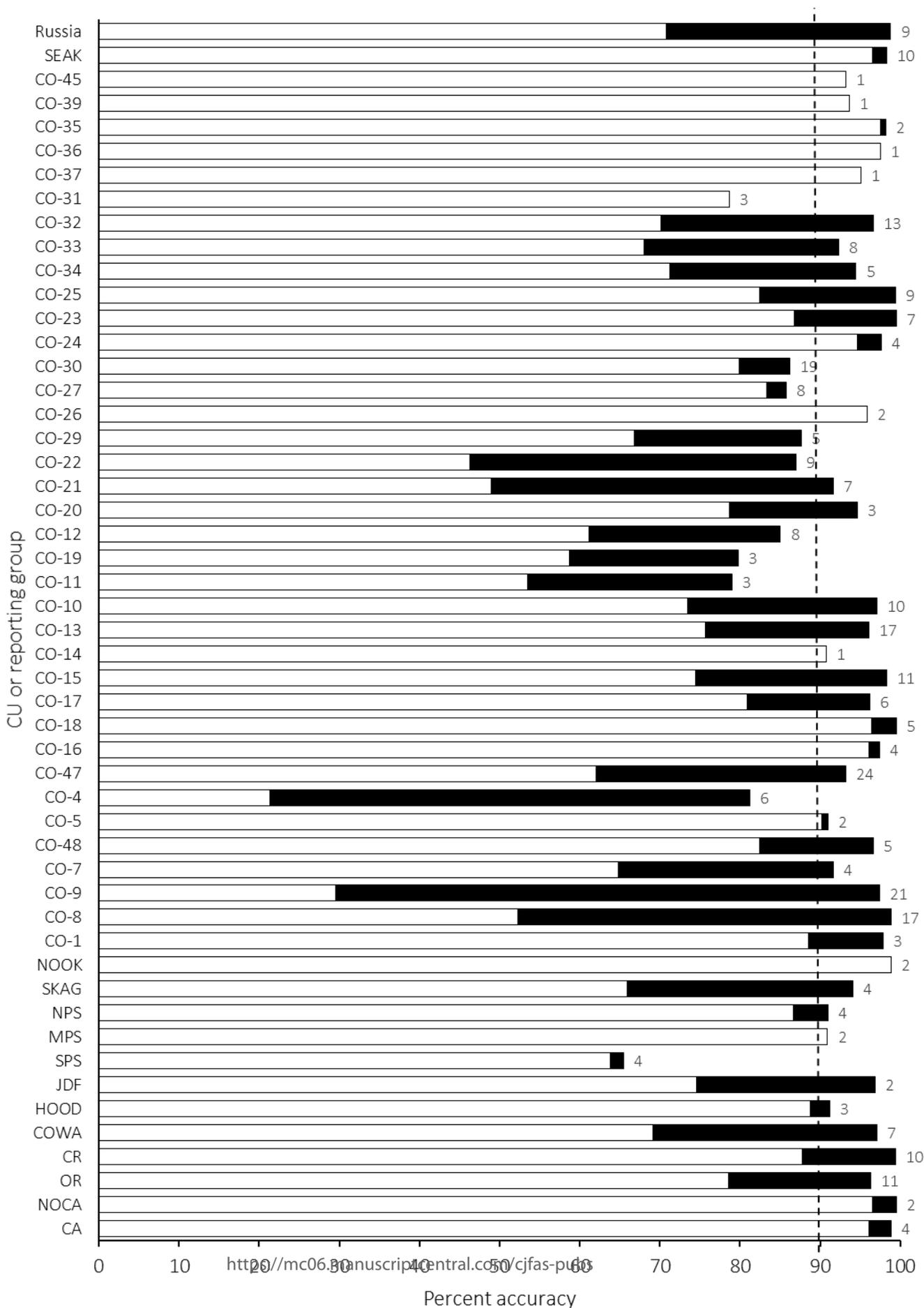
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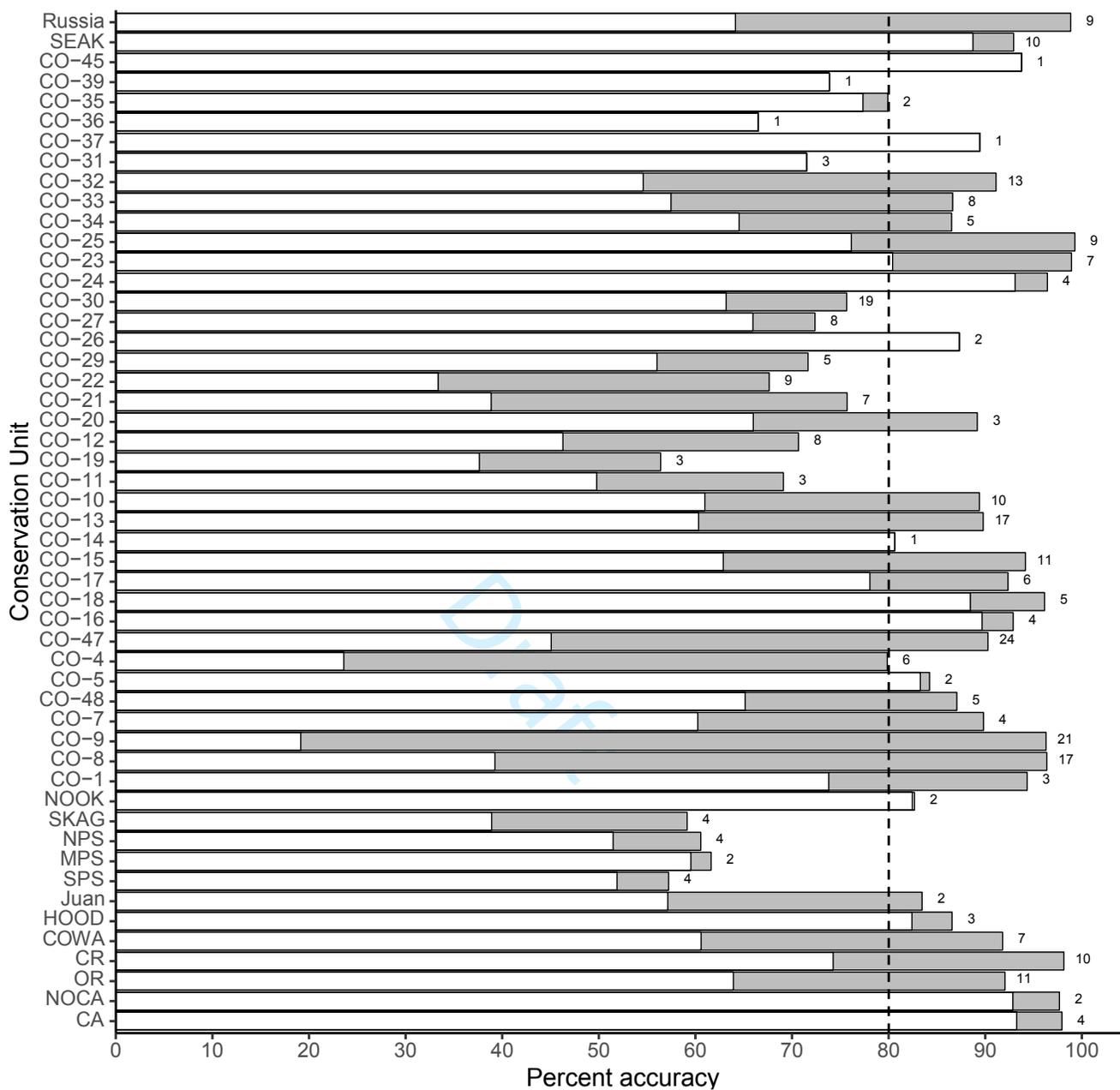
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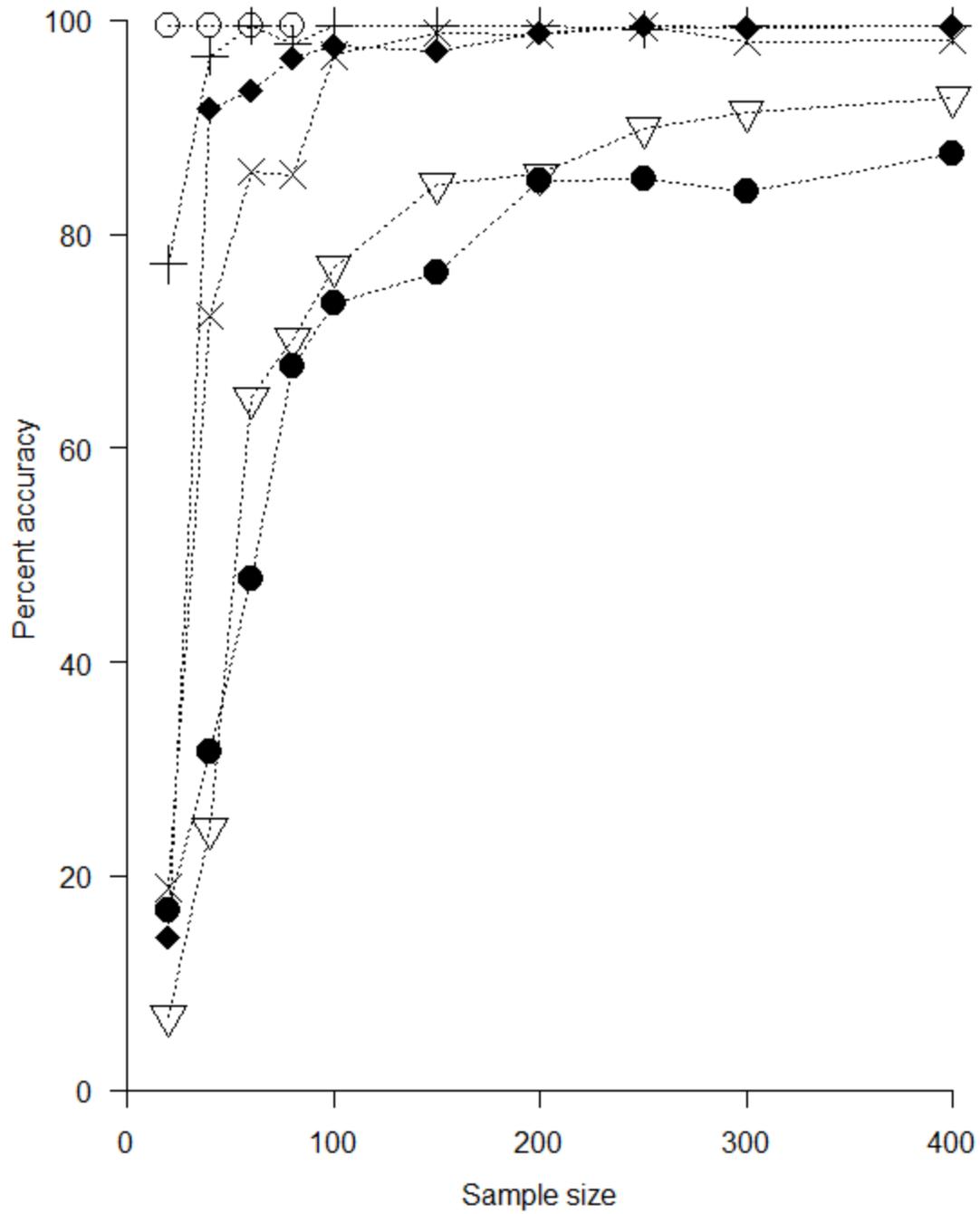


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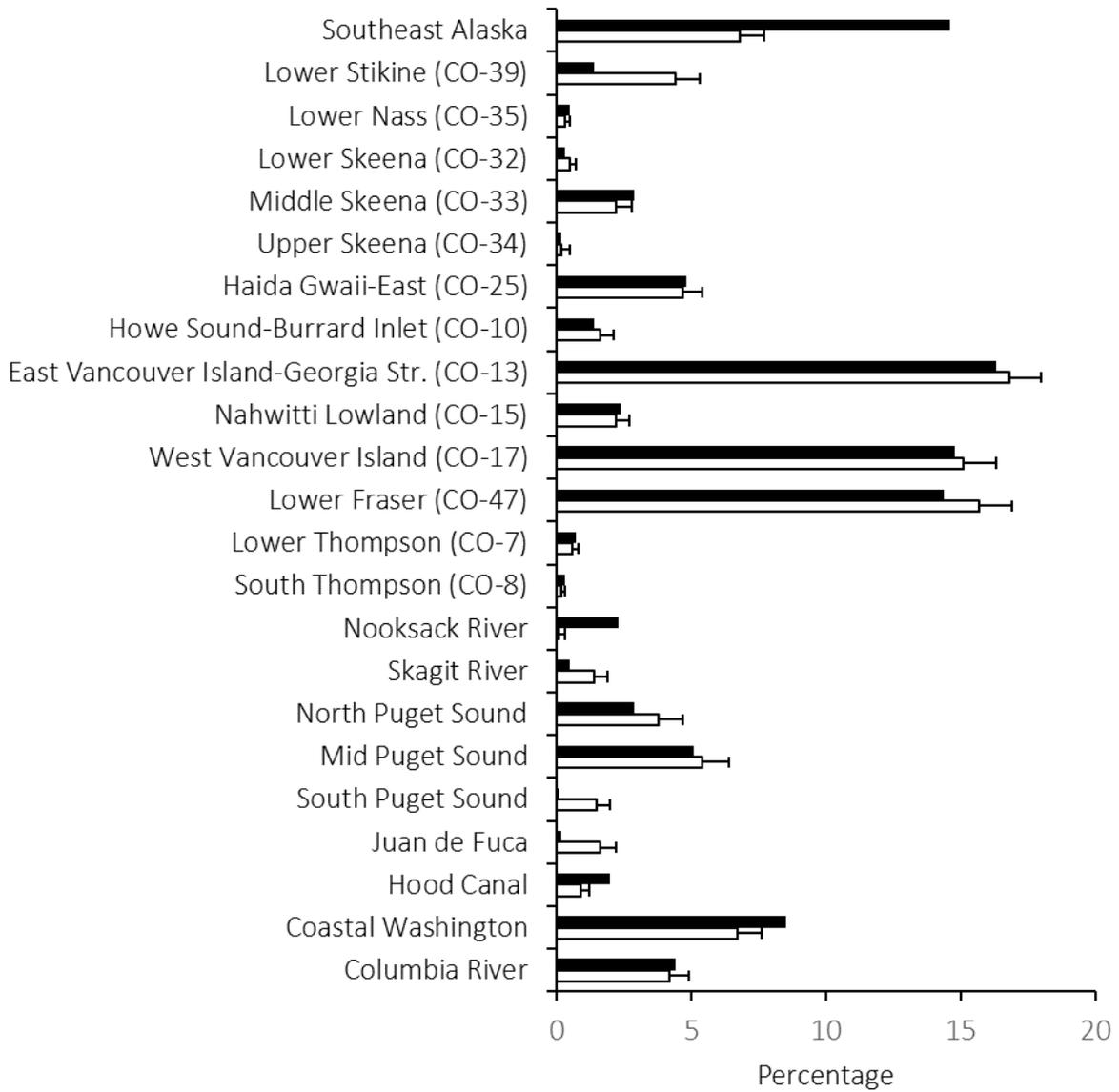


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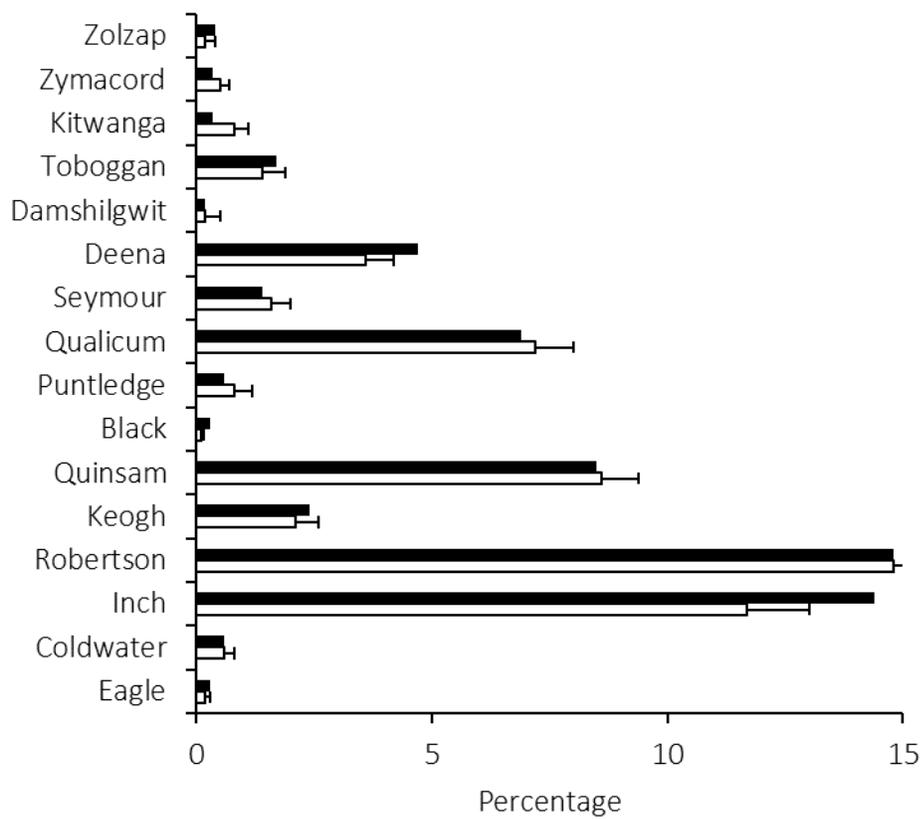
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