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8 Fine-scale population structure of rainbow trout (*Oncorhynchus mykiss*) in the

9 Spokane River drainage in relation to hatchery stocking and barriers

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27   Abstract: We examined population structure in rainbow trout (*Oncorhynchus mykiss)*

28   collected from 20 tributaries and three mainstems in the greater Spokane River drainage

29   using 13 microsatellite loci. Populations displayed some excess homozygosity and

30   linkage disequilibrium, which was more pronounced in upper tributary collections and

31   likely the result of small effective population sizes or structuring within tributaries. In

32   general, population structure followed geographic structure: collections from creeks

33   within sub-drainages were most closely related and collections from different tributaries

34   were genetically distinct. Comparisons to cutthroat trout (*O. clarki*) indicated little to no

35   introgression. Comparisons to steelhead, coastal, and inland rainbow trout from

36   hatcheries suggested introgression by hatchery fish into some wild populations.

37   Introgression was suspected in populations from stocked tributaries and where the

38   tributary lacked barriers to escaped hatchery fish. Populations from tributaries above

39   barriers that had not been stocked were genetically distinct from hatchery fish and

40   appeared to be native inland redband rainbow trout.

41

41   Introduction: Effective fisheries management is based upon an understanding of

42   population structure, usually a complex reflection of historical processes, geography and

43   life history (Small *et al.* 1998; McCusker *et al.* 2000; Spidle *et al.* 2003; Spruell *et al.*

44   2003). Historically, tributaries in the Pacific Northwest were recolonized following

45   glacial retreat, with population structure reflecting common founders (Small *et al.* 1998,

46   McCusker *et al.* 2000).   Since salmonids home to their natal stream for breeding, genetic

47   structure is often organized upon geographic structure of drainages (McCusker *et al.*

48   2000, Taylor *et al.* 2003). As some amount of straying naturally occurs within drainages

49   and to a lesser extent among drainages within the same region, population structure

50   generally follows a hierarchy of regional structure with populations more closely related

51   in nearby drainages (Hansen and Mensberg 1998; Spruell *et al.* 2003; Taylor *et al.* 2003).

52   In addition to natural movement, fisheries managers move trout among drainages and

53   among regions, potentially altering genetic structure. Hatchery introductions have mixed

54   impacts upon wild populations: salmonids are regionally adapted (Taylor 1991) and

55   hatchery fish, often of non-local origin, may lack characteristics allowing them to

56   succeed in regions different from their origins or to succeed under natural conditions

57   (Currens *et al.* 1990; Hindar *et al.* 1991; Williams *et al.* 1996; Reisenbichler and Rubin

58   1999; Hansen *et al.* 2001; Weber and Fausch 2003; McGinnity *et al.* 2004).

59   Anthropogenic barriers to fish movement also impact population structure (Neraas and

60   Spruell 2001; Van Houdt *et al.* 2005). Dams, culverts or periodic loss of flow within a

61   waterway from land-use practices that prevent fish from moving throughout drainages

62   may affect gene flow and biodiversity patterns (Taylor *et al.* 2003), and lead to smaller

63   effective population sizes. Natural barriers such as historic dry channels or waterfalls

64   arising from tectonic processes similarly influence fish movement (Currens *et al.* 1990),

65   but might leave a different genetic signature where barriers predated humans. Salmonid

66   management is based partly upon understanding the relative roles of natural and

67   anthropogenic influences on salmonid ecology as reflected in population genetic

68   structure.

69

70   In this study, population genetic structure was investigated in rainbow trout occupying

71   tributaries of the Spokane and Little Spokane rivers in eastern Washington State (Figure

72   1) using microsatellite DNA. We tested the hypothesis that hatchery fish planted in the

73   system over the past century had displaced or introgressed into native populations and

74   examined the influence of natural and anthropogenic barriers on putative native

75   population structure. Native inland "redband" rainbow trout (*Oncorhynchus mykiss*

76   *gairdneri*), both anadromous (steelhead) and resident forms, were once abundant. Dam

77   construction in the early 1900s on the Spokane River (Figure 1) eliminated the

78   anadromous form but native resident populations may still persist in unstocked tributaries

79   isolated by barriers. To mitigate for dam construction, hatchery rainbow trout and

80   hatchery cutthroat trout (*O. clarki*) were stocked throughout the drainage starting in the

81   early 1900s. Hatchery rainbow trout were primarily coastal rainbow trout (*O. mykiss*

82   *irideus*) but also included some inland redband rainbow trout, (inland rainbow trout are

83   distinguished morphologically from coastal rainbow trout by a red lateral stripe and

84   primitive taxonomic structures (Currens *et al.* 1990), genetically by differences at

85   allozyme loci (Currens 1997) and ecologically by adaptations to inland environments

86   (Currens 1997)).   Stocking efforts varied by tributary and were based upon availability

87   of lakes, habitat quality and potential for recreational opportunities.   We found that

88   hatchery fish had introgressed into native populations in tributaries with hatchery

89   stocking and in unstocked tributaries below barriers that were exposed to escaped

90   hatchery fish. Unstocked tributaries above barriers appeared to be native inland redband

91   rainbow trout. Thus, although barriers disrupted connectivity among rainbow trout

92   populations, barriers also served to protect native diversity.

93

94   <u>Materials and methods:</u>

95   Area geography

96   The Spokane River system is a low gradient drainage underlain by a large aquifer

97   maintained by snow-melt and rainwater. Although only upper headwaters were within

98   Pleistocene glacial margins, glacial Lake Spokane covered part of the drainage and part

99   of the area was scoured during the Great Spokane flood around 18,000 years before

100   present (USGS 2006). In addition to waterfalls from tectonic and glacial activity, and

101   man-made barriers (culverts, dams, high sediment load), movement of fish among

102   tributaries is restricted by availability of water since in many creeks some portion is dry

103   for at least part of the year. Although springs throughout the drainage provide high

104   quality water and thermal refugia, water availability and quality in some tributaries has

105   declined dramatically over the past century as riparian plants were removed and road

106   building, mining, timber harvest and agriculture diverted groundwater and increased

107   water temperature, sedimentation and pollution (Bruce Kinkead, tribal biologist, Coeur

108   d'Alene Tribe, pers. comm.).

109

110   Samples and area history

111   Samples of adult fish fin tissue were obtained non-lethally by backpack electrofishing in

112   a stratified random sampling design (10 fish per 100 m blocked section). Rainbow trout

113   samples were collected 2001 through 2004 from 21 tributaries and the mainstem of the

114   Spokane (356 fish) and Little Spokane (940 fish) rivers and Hangman Creek (206

115   rainbow and 108 cutthroat; Figure 1, Table 1). In most tributaries with barriers, samples

116   were collected above barriers (Table 1). Some barriers were complete and one-way (eg.

117   Spokane Falls), others were incomplete (eg. culvert surmountable under high flow) and

118   others were absent under rare conditions (eg. 5.5 km of dry creek bed in each of Coulee

119   and Deep creeks flows only in years of high snowfall). In Dartford Cr., samples were

120   gathered above and below a culvert about 200 m from the creek mouth.

121

122   Historically, inland resident (redband rainbow) and anadromous (steelhead) rainbow trout

123   were throughout the system and cutthroat trout were only above Spokane Falls (Behnke

124   1992). Hatchery rainbow trout have been planted since the early 1900's. Most fish

125   originated at the Spokane Hatchery (Figure 1, Table 2), which maintains a coastal

126   rainbow trout broodstock from McCloud River, CA (Crawford 1979; Busack and Gall

127   1980; Nielsen *et al.* 1999; 96 fish sample included). Other hatchery rainbow trout

128   planted included Trout Lodge Inc. Hatchery (private hatchery, broodstock origin

129   unknown, 49 fish sample), and Phalon Lake Conservation stock (Phalon Lake), an inland

130   redband rainbow stock from Kettle River 100 km north of Spokane River. The Phalon

131   Lake stock was reconstructed in 2002 and we included samples from 2001 and 2002 (200

132   fish). Some Trout Lodge fish were planted by landowners and some escaped from ponds

133   in upper Deep Cr. (Jason McLellan, WDFW, unpublished data). Three coastal rainbow

134   trout hatchery stocks, Goldendale, Eells Springs and South Tacoma (McCloud River

135   origin with additional components), while not planted into the system or part of the study,

136   were included in the cluster analyses to strengthen the examination of relationships

137   between wild-origin inland rainbow trout and hatchery-origin coastal rainbow trout.

138   Some Lyons Ferry Hatchery steelhead (native anadromous inland rainbow trout,

139   broodstock origin mainly in upper Columbia River; Bumgarner *et al.* 2003), were also

140   planted (100 fish included). Introgression by hatchery rainbow trout might be detected as

141   clustering with the hatchery sample rather than with populations from nearby tributaries

142   and by coastal ancestry signals in inland populations.

143

144   Hatchery cutthroat trout, planted less extensively (Table 2), were King's Lake broodstock

145   from the Pend Oreille drainage. Nehchen Cr. received at least unofficial one cutthroat

146   infusion around 20 years ago when a landowner brought in fish following dewatering of

147   the creek (Bruce Kinkead, Coeur d'Alene Tribal Biologist, pers. comm.). Two Nehchen

148   Cr. samples (102 fish) were included to determine if fish were cutthroat trout rather than

149   rainbow trout (redband rainbow have markings similar to cutthroat, Behnke 1992). We

150   compared all samples in the study to three cutthroat trout samples from the Pend Oreille

151   drainage that had been collected for another study, (Sullivan Lake, Sullivan Cr., and Gold

152   Cr.), to identify potential cutthroat trout and possible hybrids. Prior to stocking (and

153   introduction) cutthroat trout were only present above Spokane Falls. Cutthroat and

154   rainbow trout may hybridize, but naturally occupy different portions of a tributary

155   (Allendorf *et al.* 2001; Marshall *et al.* 2006). Fish may be forced into proximity and thus

156   hybridize if hatchery cutthroat trout are introduced into a rainbow-occupied tributary with

157   limited habitat.

158

159   Sample processing

160   DNA from 1502 wild-origin rainbow trout, 445 hatchery rainbow trout and 102 wild-

161   origin cutthroat trout was extracted using a chelex protocol (Small *et al.* 1998).

162   Microsatellite alleles at 14 loci were PCR-amplified using fluorescently labeled primers

163   (see Table 3 for detailed PCR information). PCR's were conducted on an MJResearch

164   PTC-200 thermocycler in 96 well plates in 5 μl volumes employing 1 μl template with

165   final concentrations of 1.5 mM $MgCl_2$, 200μM of each dNTP, 1X Promega PCR buffer,

166   and 0.01U Taq polymerase. After initial three minute denature at 92°, 33 cycles

167   consisting of 92° for 15 seconds, annealing (temperature in Table 3) for 30 seconds,

168   extension at 72° for 60 seconds were followed by a 30 minute extension at 72°. Samples

169   were run on ABI 3100 and 3730 automated sequencers and alleles were sized (to base

170   pairs) and binned using an internal lane size standard (GS500Liz from Applied

171   Biosystems) and Genemapper software (Applied Biosystems). A subsample was run on

172   both sequencer platforms to standardize allele mobilities.

173

174   Within collection data analysis

175   Statistical tests were conducted on loci and samples from each collection site to assess

176   conformation to Hardy Weinberg expectations (Hardy Weinberg equilibrium, HWE), and

177   genotypic heterogeneity using GENEPOP version 3.3 (Raymond and Rousset 1995) with

178   100 batches and 2,000 iterations.   Loci were examined for large allele drop-out, null

179   alleles and scoring errors using MICROCHECKER (Van Oosterhout *et al.* 2004). We

180   used FSTAT 2.9.3 (Goudet 2001) to calculate F_{IS} values and their significance for each

181   locus in each collection (350,000 randomizations), and calculate Weir and Cockerham's

182   (1984) estimators of Wright's *F* statistics (F, Θ, and *f*, hereafter referred to as F_{IS}, F_{IT},

183   and F_{ST}) and their jackknife intervals over all loci. Linkage disequilibrium was assessed

184   using GENEPOP with 200 batches and 3,000 iterations. Allelic richness was calculated

185   using rarefaction as implemented in HP-RARE v1.4 (Kalinowski 2004), and based upon

186   permutation of 50 alleles. Populations were tested for population bottleneck signals

187   using BOTTLENECK (Piry *et al.* 1998). Gene diversity (Nei 1987) was calculated using

188   FSTAT.

189

190   Between collection data analysis

191   Partitioning of molecular variance was explored using AMOVA tests (analysis of

192   molecular variance, Excoffier *et al.* 1992) implemented in ARLEQUIN 2.000 (Schneider

193   *et al.* 2001) with collections organized by tributary. We conducted an assignment test

194   using GENECLASS2 (Cornuet *et al.* 1999) to examine the likelihood that, based upon

195   the genotype of the fish and allele frequencies in collections, an individual fish originated

196   in the population or tributary where it was sampled. We used the Rannula and Mountain

197   (1997) option, with collections grouped by tributary. A Bayesian analysis implemented

198   in STRUCTURE 2.1 (Pritchard *et al.* 2000) was used to assess hybridization between

199   rainbow and cutthroat trout, to examine introgression by hatchery rainbow trout into

200   native populations, and to estimate individual ancestry. In initial analyses, all collections

201   (cutthroat, hatchery and wild-origin rainbow trout) were included with K (number of

202   clusters or possible populations) set from 2 to 30. With K = 2, we hypothesized that the

203   data set would divide into cutthroat and rainbow trout, with possible hybrid individuals

204   sharing ancestry in both groups. With K = 3, we hypothesized that the rainbow trout

205   cluster would divide into coastal and inland redband rainbow trout, also with hybrid

206   individuals sharing ancestry in both groups. With K > 3 we expected population

207   structure to emerge. Initial analyses with K = 2 to 5 were conducted in 10 independent

208   runs allowing admixture with 50,000 burn-ins and 1,000,000 iterations. Due to

209   computational intensity, only single runs (50,000 burn-ins and 1,000,000 iterations) were

210   conducted for K = 6 to 30. After identifying collections with possible hatchery

211   introgression (> 25% average ancestry in the cluster occupied by Spokane Hatchery

212   collection), we conducted pairwise tests with introgressed collections and the Spokane

213   Hatchery collection with K = 2, to look more closely at introgression (10 runs, 50,000

214   burn-ins, 1,000,000 iterations). Other pairwise tests were employed to examine

215   relationships among collections: collections were tested for heterogeneity in genotypic

216   distributions at each locus and across all loci using GENEPOP with 300 batches and

217   3,000 iterations. To examine the magnitude of differentiation between populations and

218   between tributary groups, pairwise F_{ST} values and their significance were evaluated using

219   FSTAT with 100,000 permutations. All test results were adjusted for multiple

220   comparisons using sequential Bonferroni corrections.

221

222   Genetic relationships among collections were also explored with cluster analyses.

223   Population allele frequencies were generated from genotypic data using CONVERT 1.3

224   (Glaubitz 2004). Pairwise chord distances (Cavalli-Sforza and Edwards, 1967) among

225   collections were calculated from allele frequencies using GENDIST in PHYLIP 3.5c

226   (Felsenstein 1993). We used geometric distance since fluctuation in population sizes

227   should increase the impact of drift relative to mutation. Chord distances were plotted in a

228   neighbor-joining (NJ) tree using PHYLIP. To test the repeatability of NJ tree branching,

229   the allele frequency file was bootstrapped 10,000 times across loci using SEQBOOT.

230   Tree topologies were created for replicates with NEIGHBOR, and a consensus tree was

231   generated using CONSENSE and plotted with TREEVIEW 1.6.6 (Page 2001). Chord

232   distances were also plotted in a multidimensional scaling analysis using NTSYSpc

233   version 2.02j (Rohlf 1993) to view relationships among collections in the absence of

234   dendrogram architecture.

235

236   Results:

237

238   PCR amplifications at some loci were less successful for cutthroat trout than rainbow

239   trout (average missing loci per individual genotype = 4 vs 2, respectively), particularly at

240   *One*-102, *One*-108, *Ots*-100, *Ots*-1, *Omm*-1130, *Omy*-1001, and *Omy*-1011. Cutthroat

241   trout generally had significant homozygosity at these loci (Table 4). In individual locus

242   tests, 39 out of 462 (14 loci x 33 collections, excluding cutthroat) total F_{IS} tests were

243   significant for excess homozygosity (Table 4, adjusted alpha, 0.05/462 = 0.0001).

244   Twelve of the significant excesses occurred at *One*-108 and MICROCHECKER

245   suggested a null allele, so this locus was excluded from further analyses.

246   MICROCHECKER indicated a possible null allele at *Omm*-1130, but since there were

247   only five significant homozygote excesses at this locus and population HWE significance

248   values were unchanged when *Omm*-1130 was removed (data not shown), we left this

249   locus in the analysis.   In global tests, one third of collections (after removing *One*-108,

250   Table 1) were out of HWE for homozygote excess, and F_{IT} values (Table 3) indicated

251   significant deficits of heterozygotes at all loci. Average F_{IS} values were higher in above

252   barrier populations than below barrier populations (0.061 and 0.038, respectively), but

253   were not significantly different ($P = 0.06$, FSTAT group comparison, 1000

254   permutations). F_{ST} values indicated significant variance among populations at all loci

255   (Table 3).

256

257   Positive F_{IS} values and disequilibrium suggested that collections contained offspring

258   from matings among close relatives (inbreeding) within populations or that collections

259   contained admixtures of trout from different breeding groups. To study this further, wild-

260   origin rainbow trout collections were examined for family groups and relationships with

261   IDENTIX (Belkhir *et al.* 2002). In the Deadman Cr. collection, the first nine individuals

262   collected appeared to be siblings (all Q values > 0.35): Queller and Goodnight's (1989)

263   relatedness value Q = 0.25 for half-sibs and 0.5 for full-sibs. In the California Cr.

264   collection, five individuals appeared to be full sibs. Mean relatedness values for

265   Nehchen, Coulee, and Dartford creeks (above barrier) were in the top 5% of a random

266   distribution of 500 permuted values. However, all but the Buck Cr. sample had

267   significantly high variance in pairwise relatedness values among individuals, suggesting

268   related groups within most samples (Belkhir *et al.* 2002). Further, some pairwise

269   relatedness values suggested that half-sib relationships (offspring of mating among

270   relatives) increased in collections from further up drainages (e.g. Lower Spokane = 2%

271 half sibs, Middle Spokane = 3.8% half sibs and Upper Spokane = 5.2% half sibs).

272 Relatedness values were higher in above barrier collections than in below barrier

273 collections (-0.079 and –0.129, respectively), and the differences were on the edge of

274 significance ($P = 0.05$, FSTAT group test, 1000 permutations).   In collections from

275 headwaters and regions isolated above barriers, population sizes may have been restricted

276 by available habitat, fostering mating among relatives and decreasing heterozygosity

277 through inbreeding (Castric *et al.* 2002). In streams with intermittent good habitat,

278 collections may have included breeding groups associated with habitat patches, which

279 would decrease overall heterozygosity in a Wahlund-type effect (Castric *et al.* 2002).

280 Thus, although sampling followed a randomized design, some samples contained non-

281 random components or related individuals.

282

283 In other examinations, loci were tested for linkage in pairwise genotypic disequilibrium

284 tests. Several collections had one to three pairs of loci (out of 78 possible pairs per

285 population after *One*-108 was removed) in linkage disequilibrium, with a high of 9 in

286 California Cr. (Table 1). After removing four of five siblings in the California Cr.

287 collection, only one locus pair was linked. No locus pair was linked in more than three

288 populations. *Omm*-1130 was involved in 11/29 linkages (8/21 after California Cr.

289 siblings removed).

290

291 Among wild collections, allelic richness was highest in lower Little Spokane River and

292 lowest in Coulee Cr. (Table 1). Allelic diversity was lower but not significantly different

293 in collections from upper portions and smaller branches of tributaries (9.2 in above

294 barrier collections vs 10.2 in below barrier collections, $P = 0.056$ from FSTAT group

295 comparison, 1000 permutations) suggesting smaller effective population sizes or fewer

296 founders in above barrier collections. Bottleneck tests conducted under the infinite allele

297 model also supported recent reduction in population sizes in several collections (Table 1).

298 Gene diversity generally concurred with other diversity measures: collections with higher

299 allelic richness had higher gene diversity. Six alleles at various loci were absent from

300 coastal hatchery collections (Spokane and Trout Lodge hatcheries) and present at

301 intermediate frequency (4-9%) in suspected introgressed collections and present at high

302 frequency (15 - 28%) in suspected pure redband rainbow trout collections. Sixteen

303 alleles at various loci were found at high frequencies in coastal hatchery collections,

304 intermediate frequencies in suspected introgressed collections, and low frequencies in

305 suspected pure redband rainbow trout collections. Coastal hatcheries had no private

306 alleles, Phalon Lake had 16 private alleles, and Lyons Ferry Hatchery had 10 private

307 alleles. All alleles in coastal hatchery collections were found in wild-origin collections,

308 although sometimes at very low frequencies (eg. 26% in Spokane Hatchery vs 0.4% in

309 wild). Two hundred and sixteen alleles present in redband rainbow trout collections

310 (wild or hatchery) were absent in coastal hatchery collections.

311

312 Pairwise (not shown) and global F_{ST} values (overall $F_{ST} = 0.080$, excluding cutthroat

313 trout and hatchery collections) were all significantly different from 0 (pairwise test

314 adjusted alpha $= 0.05/300 = 0.000167$) except within the following tributaries: Deer and

315 Little Deer creeks, upper and lower Deep Creek, Dartford Cr. above and below barrier,

316 2002 and 2003 middle Spokane River, and 2003 Indian and 2004 Indian from upper

317   Hangman (other Hangman collections were too small for statistical tests). Pairwise

318   genotypic test results (not shown) were similar to pairwise F_{ST} test with one additional

319   non-significant comparison, Little Deep and South Fork Little Deep creeks. Populations

320   occupying tributaries within the greater Spokane drainage were thus genetically

321   differentiated from each other and distinct from hatchery rainbow trout and steelhead.

322

323   AMOVA results supported pooling samples by tributary. Although significant variation

324   was partitioned within tributaries (3.21%, $P < 0.001$), more variation was found among

325   tributaries (5.51%, $P < 0.001$). In pairwise F_{ST} tests with collections grouped by

326   tributary, all values were significantly different from 0 (Table 5). Coulee Cr., unstocked

327   and isolated above a dry creek bed, was highly differentiated from other collections (see

328   average and individual values in Table 5). Deep Cr., also isolated by a dry creek bed but

329   exposed to hatchery escapees from ponds in upper Deep Cr. and limited stocking, was

330   genetically closer to other collections impacted by hatchery fish through stocking or

331   escapees: Buck and Dartford creeks and lower Little Spokane River.

332

333   The topology of the consensus neighbor-joining tree and bootstrap support illustrated

334   associations among collections within tributaries (Figure 2). Tributary collections

335   formed branches with at least 98% bootstrap support. Collections from lower portions of

336   tributaries (eg. middle Spokane River) had shorter branch lengths indicating less

337   distinction, possibly due to one-way gene flow from upper tributaries and straying from

338   other tributaries. Cutthroat trout and Nehchen Cr. collections formed a branch with

339   100% bootstrap support, indicating that Nehchen Cr. fish were cutthroat trout. Coastal

340   hatchery collections occupied a branch, which included Trout Lodge Hatchery, Marshall

341   Cr., and Deep Cr. collections with 64% bootstrap support (Figure 2), indicating a coastal

342   origin for Trout Lodge broodstock and possible hatchery introgression in Marshall and

343   Deep creek populations. In a consensus tree with combined collections (not shown), the

344   Buck and Dartford creek collections also joined the hatchery branch with 75% bootstrap

345   support, also suggesting hatchery introgression into these collections. In a

346   multidimensional scaling plot employing combined collections (Figure 3), the first axis

347   was defined by Trout Lodge and Spokane Hatchery on the far left and Lyons Ferry

348   Hatchery on the far right, with the Marshall, Dartford, Buck and Deep creek collections

349   on the coastal hatchery side and putative native inland redband rainbow trout collections

350   and Phalon Lake on the Lyons Ferry Hatchery side. The first axis appears to show a

351   genetic gradient from coastal to introgressed inland rainbow to inland rainbow and

352   steelhead collections (Figure 3).

353

354   Hybridization with cutthroat trout and introgression by hatchery fish were estimated with

355   a Bayesian analysis. When the number of hypothetical populations (K) was set at 2,

356   cutthroat trout and Nehchen Cr. occupied one cluster and coastal and inland rainbow trout

357   collections occupied the other cluster, with an average 3% or less cutthroat ancestry in

358   each rainbow trout collection (Table 6). Three individual fish (individual data not

359   shown) from upper Spokane appeared to be cutthroat-rainbow hybrids (10 - 30% ancestry

360   in cutthroat trout cluster) and one was a possible cutthroat trout (90% ancestry in

361   cutthroat trout cluster). With K = 3 (Table 6), rainbow trout collections divided: inland

362   redband rainbow trout collections occupied a cluster that included Lyons Ferry Hatchery

363   and Phalon Lake, and coastal hatchery groups occupied a cluster that included substantial

364   portions (> 25%) of some inland redband rainbow trout collections that had been stocked

365   with hatchery fish over several years (lower Little Spokane River, Buck, Deep (additional

366   pond escapes), Dragoon, and Marshall creeks). Collections from above barriers in

367   tributaries with single or few stockings (Otter, Deer, Coulee creeks, and Upper Little

368   Spokane River) shared modest ancestry (6 – 18%) with coastal hatchery fish. Collections

369   from below barriers that were stocked once or possibly exposed to hatchery strays

370   (California, Little Deep, West Branch Dragoon and Upper Dragoon creeks) also shared

371   modest ancestry (13 – 18%) with the coastal hatchery cluster. The mouth of Dartford Cr.

372   was a kilometer below the Spokane Hatchery outfall, and collections from below and

373   above the culvert (semi-permeable barrier) shared around 50% ancestry with coastal

374   hatchery fish. Collections from unstocked tributaries above barriers (all Hangman

375   tributaries, South Fork Deadman, North and South Fork Little Deep, and Little Deer

376   creeks) had less than 3% ancestry in the coastal hatchery cluster. The 2001 and 2002

377   Phalon Lake collections shared 30% and 2% ancestry with coastal hatchery fish,

378   respectively: Phalon Lake broodstock was suspected of contamination with coastal

379   hatchery fish and was reconstructed in 2002. In analyses with K = 4 (all collections

380   included), Spokane Hatchery and coastal hatchery collections moved into their own

381   cluster (data not shown). With increased K, tributary groups and then single collections

382   eventually occupied predominantly single clusters, and some collections subdivided

383   among multiple clusters (data not shown).

384

385   We conducted further STRUCTURE analyses with suspected introgressed collections (>

386   25% coastal ancestry) paired with the Spokane Hatchery collection with K = 2 (Table 7).

387   In these analyses inland collections shared less than 2% Spokane Hatchery ancestry.

388   Individual fish admixture values indicated that 1% of wild-origin inland redband rainbow

389   individuals shared more than 10% ancestry with the Spokane Hatchery collection (7/642

390   fish, average coastal ancestry within these 7 fish = 19%, highest = 37%).

391

392   In maximum likelihood assignment tests, assignments were classified as positive and

393   unambiguous if the assignment was 100 times more likely than the next most likely

394   assignment, and positive but ambiguous when the assignment was less than 100 times

395   more likely. Most fish were assigned unambiguously to their tributary of origin (Table

396   8). Misassigned fish (fish not assigning to collection of origin) may be strays coming

397   down out of tributaries into the Spokane and Little Spokane rivers, or fish moving among

398   tributaries in Deadman, Little Deep and Dragoon creeks. A few fish were ambiguously

399   misassigned between 01Phalon Lake and wild-origin rainbow collections. Since few

400   Phalon Lake fish were planted in the system, this may reflect shared common ancestry

401   rather than introgression. Spokane and Trout Lodge hatcheries had 100% correct

402   assignment and no wild-origin fish were assigned to hatchery collections, suggesting low

403   impact on native populations.

404

404   Discussion:

405   Introgression by hatchery rainbow trout

406   This study explored the population structure of wild rainbow trout populations in the

407   greater Spokane River drainage and assessed whether hatchery fish planted throughout

408   the system over the past 100 years had introgressed into native populations. Since we

409   lacked genetic data for native populations prior to hatchery supplementation, we

410   compared gene pools of wild spawning populations to gene pools maintained in

411   hatcheries and introduced into the system within the past 60 years. A coastal strain of

412   rainbow trout was the most extensively planted hatchery fish. Introgression by coastal

413   hatchery rainbow trout was indicated in collections exposed to hatchery fish through

414   escapees and planting (lower Little Spokane River, Buck, Marshall, Deep, and Dartford

415   creeks). We suspected hatchery introgression because these collections were on the

416   coastal branch in the neighbor-joining tree and the coastal side of the first axis in the

417   multidimensional scaling analysis and appeared to share coastal ancestry in the large-

418   scale Bayesian analysis. However, in contrast to other studies where some wild-origin

419   fish assigned to hatcheries after several years of stocking (Fritzner *et al.* 2001; Hansen *et*

420   *al.* 2001; Spidle *et al.* 2003), assignment tests suggested that hatchery impact was not as

421   strong as might be expected. Further, other hatchery introgression studies demonstrated

422   admixture in wild-origin individuals (Hansen 2002, Sušnik *et al.* 2004), whereas our

423   pairwise analyses with hatchery and introgressed wild-origin indicated that wild

424   collections shared little ancestry with hatchery collections and few individuals appeared

425   admixed. We hypothesized that in inland redband rainbow trout populations exposed to

426   coastal-origin hatchery rainbow, introgression shifted these gene pools away from the

427   native inland redband rainbow trout gene pool but not to the point where the individual

428   identity of a population was compromised.

429

430   Hatchery fish have not replaced wild fish in any collection examined. All wild-origin

431   gene pools had alleles absent from the hatchery gene pool and wild fish remain

432   genetically distinct from hatchery fish (Table 5). All alleles in coastal-origin hatchery

433   fish were found in inland collections, supporting introgression into inland populations:

434   several alleles at high frequency in coastal hatchery fish were at intermediate frequency

435   in suspected introgressed populations and at low frequency in populations unexposed to

436   hatchery fish. Genetic drift may have eliminated some coastal-type allelic diversity

437   during 60 years of hatchery rearing. It is unlikely that associations between wild and

438   hatchery fish arose from native inland redband rainbow trout entering hatchery gene

439   pools since other coastal hatchery collections clustered with Spokane and Trout Lodge

440   hatchery collections. We suspect that, similar to other species (Williams *et al.* 1997;

441   Nielsen *et al.* 2001; Weber and Fausch 2003; McGinnity *et al.* 2004), hatchery-adapted

442   rainbow trout of coastal ancestry did not replace native fish because they may not have

443   survived as well or been as successful on wild spawning grounds as regionally adapted

444   native inland redband rainbow trout, and that hybrid offspring may have suffered reduced

445   survival and fitness (Utter 2001).

446

447   The mainstem of the Spokane and Little Spokane rivers were heavily planted (Table 2)

448   but showed little impact from hatchery fish. In the middle Spokane River, below

449   Spokane Falls, hatchery fish tended to move down the river into the reservoir associated

450   with Nine Mile Falls dam (McLellan 2005a), and thus may not have used upstream

451   mainstem and tributary spawning grounds. Similarly, the Little Spokane River enters

452   Long Lake dam reservoir and hatchery fish may have moved down into the reservoir.

453   Dragoon and Deadman creeks also received a fair number of hatchery fish but showed

454   little relationship to hatchery collections. Dragoon Cr. encompassed some of the most

455   degraded habitat in the system, although a spring in the middle of the system provided a

456   refuge, and in Deadman Cr., good habitat was intermittent between large stretches of

457   poor habitat (McLellan 2005a, 2005b). In both tributaries, hatchery fish may have

458   survived poorly or were unable to compete with native fish inhabiting habitat patches.

459

460   Two inland stocks from Phalon Lake and Lyons Ferry Hatchery were also planted

461   minimally into the Spokane River system. Assignment tests suggested that a few

462   Deadman Cr., and Little Spokane River fish looked genetically like Phalon Lake fish

463   (Table 8). Since Phalon Lake fish were planted in the Little Spokane River (Table 2),

464   and fish from the Little Spokane River appeared to stray into Deadman Cr. (Table 8),

465   these misassigned fish could have originated at Phalon Lake.   However, three Phalon

466   Lake fish looked genetically like wild rainbow in lower Little Spokane River. Reciprocal

467   misassignments could also reflect recent shared ancestry: although mouths of the

468   Spokane and Kettle rivers are around 100 km apart, prior to 1911 no barriers prevented

469   straying. The relationship may also reflect common ancestry if colonists from the same

470   refuge repopulated inland drainages following the retreat of glaciers and glacial lakes

471   (Currens 1997, McCusker *et al.* 2000, Docker and Heath 2002).

472

473   Stocking efforts were lower for cutthroat trout and we saw no evidence of genetic impact

474   in collections from stocked regions. Four possible hybrids were collected in the upper

475   Spokane River, which had not been stocked, but was the only region where cutthroat

476   trout had occurred naturally prior to stocking. However, our results indicated that

477   cutthroat trout were successfully introduced into Nehchen Cr. in the upper Hangman Cr.

478   drainage following dewatering 20 years ago. The dewatering may have eliminated native

479   rainbow trout, allowing the cutthroat trout unchallenged colonization. Further, these

480   cutthroat trout were from Benewah Cr. in the Coeur d'Alene drainage across the ridge

481   from Nehchen Cr. Ecological conditions may have been similar such that fish were

482   adapted to conditions prevailing in Nehchen Cr.

483

484   Impact of barriers

485   The impact of hatchery supplementation was complexed with habitat quality and the

486   presence, permeability and duration of barriers. Habitat in the Spokane drainage was

487   affected by anthropogenic activities throughout the 1900's. In addition to hatchery

488   supplementation and decreases in water quality and availability, dam and road building

489   decreased physical connectivity throughout the drainage. Most collections exhibited

490   genetic signals suggesting that populations had experienced genetic bottlenecks that

491   might be associated with disrupted gene flow. The foremost effect of barriers upon

492   populations depended upon whether the barrier could be traversed. All barriers allowed

493   fish to move downstream, generating one-way gene flow between formerly connected

494   groups of fish.

495

496   Some barriers permitted upstream movement. In Dartford Cr., the barrier was a culvert

497   surmountable by larger fish under certain flow conditions. Although allelic richness was

498   lower above the barrier, the collections were undifferentiated indicating that the

499   population remained connected and possibly effectively larger, countering drift and

500   differentiation. The semi-permeable barrier also allowed hatchery introgression into the

501   portion of the population above the barrier.

502

503   In Deadman Cr., barriers included an unsurmountable dam as well two possibly

504   surmountable culverts.   Although genetically closest to each other, the population in SF

505   Deadman Cr. that was isolated above the barriers differed significantly from the

506   Deadman Cr. population below the barriers. While their genetic relationship prior to

507   barriers is unknown, the longer branch length in the dendrogram, decreased allelic

508   richness, and positive bottleneck signal suggested that the isolated SF Deadman Cr.

509   population experienced enhanced genetic drift.

510

511   Within Deep Cr., a waterfall barrier predated human activity. Although the tributary was

512   further isolated by a dry creek bed (discussed below), hatchery fish influenced collections

513   above and below the waterfall, likely bringing the gene pools closer to each other and to

514   other gene pools influenced by hatchery fish (Waples 1995). In the Little Spokane River,

515   a waterfall also divided the river into upper and lower regions. While documented

516   hatchery stocking was mild in the upper river and intense in the lower river, hatchery fish

517   were free to move down-river into Long Lake, a preferred habitat, possibly decreasing

518   impact on the populations. The pairwise F_{ST} value indicated that the upper and lower

519   river collections had more variance between them than all other above and below barrier

520   comparisons, possibly because of longer isolation, fewer founders in the upper river

521   population, and lower hatchery impact.

522

523   Habitat quality and availability also likely influenced population sizes and rates of

524   genetic drift. Where habitat was poor or patchy and limited by a barrier, as in Dragoon

525   Creek, drift would be stronger in smaller populations, increasing divergence seen in

526   longer branch lengths in the dendrogram. In collections from upper drainages positive

527   F_{IS} values, higher relatedness and lower allelic richness indicated smaller population

528   sizes. In contrast, populations below barriers where habitat was less restricted, such as

529   the lower Spokane River and Deadman Cr., received allelic infusions from strays,

530   increasing their allelic richness and genetic connections to other populations (where

531   strays originated), illustrated by short branch lengths in the dendrogram and lower

532   average pairwise F_{ST} values.

533

534   The Coulee Cr. collection was the most divergent in the study, and although habitat is

535   good above the barrier and supported a large population, the dry creek bed isolated the

536   tributary long before humans altered connectivity in the system. Given the episodic

537   nature of flow throughout the full creek length, divergence may have been enhanced by a

538   small founder group. Deep Cr. shared the barrier (and likely founders) with Coulee Cr.

539   However, as discussed above, exposure to hatchery fish ponded above the barrier and

540   stocked into Deep Cr. shifted the Deep Cr. gene pool closer to other hatchery-influenced

541   collections as well as increasing allelic richness in Deep Cr.

542

543   We found that, similar to brown trout (Van Houdt *et al.* 2005), barriers protected some

544   populations from hatchery escapees, thus preserving some native genetic diversity. In

545   unstocked tributaries, collections from above barriers, had little to no coastal hatchery

546   genetic signal and collections from below barriers appeared to be introgressed. The data

547   suggested that hatchery introgression in wild populations depended upon the magnitude

548   of exposure to hatchery fish through stocking or hatchery escapees.

549

550

560

561

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753

753 Table 1. Statistical information for collections: the number of locus pairs in genotypic
754 linkage disequilibria (link), gene diversity (Gene Div), allelic richness (Rich), positive
755 bottleneck signal (BNeck) and F_{IS} and associated P value (bold values significant after
756 corrections). Combined abbreviation (Comb) indicates groups for assignment and
757 pairwise F_{ST} tests. "Hatch" indicates hatchery and na = sample too small. Collections
758 above barriers indicated by * and stocked collections indicated by @.

River	Collection	Abbreviation	Comb	n	link	Gene Div	Rich	BNeck	F_{IS}	P value
Upper Hangman Cr.	2003 Mission Cr.*	MSS	Hang	9	na	na	na	na	na	na
	2004 Mission Cr.*	MSS	Hang	10	na	na	na	na	na	na
	2003 Sheep Cr.*	MSS	Hang	3	na	na	na	na	na	na
	2004 Sheep Cr.*	MSS	Hang	5	na	na	na	na	na	na
	2003 Martin Cr.*	03Martin	Hang	13	na	na	na	na	na	na
	2004 Hangman Cr.*@	04Hang	Hang	9	na	na	na	na	na	na
	2003 Indian Cr.*	03Indian	Hang	37		0.75	9.05	x	0.053	0.004
	2004 Indian Cr.*	04Indian	Hang	21		0.77	na	na	0.091	**<0.001**
(cutthroat)	2003 Nehchen Cr.*	03Nehc		65		0.60	8.51	x	0.162	**<0.001**
(cutthroat)	2004 Nehchen Cr.*	04Nehc		37		0.60	8.51	x	0.092	0.005
Lower Hangman Cr.	2004 Marshall Cr.@	04Marsh		50		0.71	6.57	x	0.060	0.003
	2004 California Cr.@	04Calif		49	9	0.76	8.79	x	0.056	0.002
Spokane R.	2002 Middle Spokane R.*@	02MidSpok	Spok	47		0.77	10.49		0.056	0.002
	2003 Middle Spokane R.*@	03MidSpok	Spok	92		0.74	9.55		0.072	**<0.001**
	2003 Upper Spokane R.*@	03UpSpok	Spok	67		0.67	7.62		0.071	**<0.001**
	2004 Coulee Cr.*@	04Coulee		50		0.64	6.36		0.011	0.323
	2004 Deep Cr.*@	04Deep	Deep	50		0.73	8.17	x	0.035	0.031
	2004 Upper Deep Cr.*(@pond escape)	04UpDeep	Deep	50		0.70	6.71	x	0.018	0.209
Little Spokane R.	2003 Upper Little Spokane R.*@	03UpLSpok		39		0.75	9.07	x	0.043	0.023
	2003 Lower Little Spokane R.@	03LoLSpok		62		0.80	12.17		0.153	**<0.001**
	2003 Dartford Cr. (above barrier)*	03DartAB	Dart	51		0.79	9.59	x	0.042	0.010
	2003 Dartford Cr. (below barrier)	03DartBB	Dart	29	3	0.78	10.39		0.001	0.477
	2003 Deadman Cr. @	03Dead	Dead	100	2	0.78	11.84		0.111	**<0.001**
	2003 South Fork Deadman Cr. *	03SFDead	Dead	49	2	0.74	9.99	x	0.037	0.027
	2003 Little Deep Cr.	03LDeep	LDeep	50	1	0.75	11.00		0.030	0.040
	2003 North Fork Little Deep Cr.	03NFLDeep	LDeep	50	1	0.69	8.94		0.034	0.044
	2003 South Fork Little Deep Cr.	03SFLDeep	LDeep	60		0.71	9.46		0.062	**<0.001**
	2001 Otter Cr.*@	01Otter		50	3	0.76	9.32	x	0.052	0.003
	2001 Deer Cr.*@	01DeerCr	Deer	100		0.77	11.86		0.033	0.003
	2002 Little Deer Cr.*	02LilDeer	Deer	50	1	0.73	10.34		0.037	0.028
	2001 Buck Cr.*@	01Buck		50		0.80	11.19		0.014	0.199
	2002 Lower Dragoon Cr. @	02LoDrag	Drag	100		0.79	11.56	x	0.047	**<0.001**
	2002 West Branch Dragoon Cr.	02WBDrag	Drag	50	2	0.77	9.53	x	-0.005	0.603
	2002 Upper Dragoon Cr.*	02UpDrag	Drag	50		0.77	10.16	x	0.022	0.112
Hatch (Redband)	2001 Phalon Lake Hatch	01PhaH	PhaH	100	2	0.83	13.38	x	0.021	0.030
	2002 Phalon Lake Hatch	02PhaH	PhaH	100	1	0.79	11.57	x	0.081	**<0.001**
Hatch (Uncertain)	2004 Trout Lodge Hatch	04TroutH		49		0.65	5.64	x	0.022	0.182
Hatch (Coastal)	2000 Spokane Hatch	00SpHat		96		0.70	6.35	x	-0.007	0.663
Hatch (Upper Columbia)	2002 Lyons Ferry Hatch steelhead	02LFH		100	2	0.74	11.70	x	0.065	**<0.001**

759

1 Table 2. Sources and numbers of hatchery trout stocked into tributaries and lakes of the
2 Spokane drainage (from Brodie Cox, WDFW, unpublished data). Lakes under Buck
3 Creek were within the Buck Creek system.
4
5

Tributary	# fish	Year planted	# Years planted	Source
Rainbow				
Coulee Creek	7,000	1936	1	Spokane Hatchery
Upper Little Spokane River	49,160	1940-1944	5	Spokane Hatchery
Little Spokane River	922,240	1936 - 2002	36	Spokane Hatchery
Little Spokane River	500	2002	1	Phalon Lake Hatchery
Dragoon Creek	388,556	1934 - 1985	20	Spokane Hatchery
Buck Creek	781,387	1941 - 1980	4	Spokane Hatchery
Diamond Lake	5,006,950	1933 - 1994	41	Spokane Hatchery
Diamond Lake	69,768	1997	1	Lyons Ferry Hatchery
Sasheen Lake	1,963,661	1939 - 1994	35	Spokane Hatchery
Deadman Creek	78,419	1934 - 1955	13	Spokane Hatchery
Deer Creek	10,000	1936	1	Spokane Hatchery
Otter Creek	10,000	1936	1	Spokane Hatchery
Spokane River	1,348,134	1935 - 2002	34	Spokane Hatchery
Spokane River	22,296	1996 - 1998	3	Phalon Lake Hatchery
Spokane River	7,500	1995	1	Trout Lodge Hatchery
Spokane River	16,886	1995	1	Lyons Ferry Hatchery
Marshall Creek	628,911	1936-1983	25	Spokane Hatchery
Deep Creek	24,448	1936-1944	4	Spokane Hatchery
California Creek	10,000	1936	1	Spokane Hatchery
Hangman Creek	3,573	1979 -1987	8	Spokane Hatchery
Cutthroat				
Deep Creek	4,000	1939	1	Spokane Hatchery
Dragoon Creek	19,000	1936 - 1937	2	Spokane Hatchery
Little Spokane River and Buck Cr. area	5,116,802	1933 - 1992	36	Spokane Hatchery
Marshall Creek	82,000	1957	1	Spokane Hatchery

6

1   Table 3. Information for multiplexes and loci including primer concentration, number of alleles in this study, size range (in
2   basepairs), observed heterozygosity (Ho), repeat unit size (in basepairs), F_{ST}, F_{IS}, and F_{IT} values (calculated without cutthroat trout, all
3   bold values significantly different from zero). References for primer sequences are under Source.
4

Multiplex	Anneal T	cycles	Locus	conc [uM]	#alleles	range	Ho	repeat	F_{ST}	F_{IS}	F_{IT}	Source
Omy-B2	55	26	*One*-102	0.05	34	182-305	0.789	4	**0.059**	**0.049**	**0.197**	Olsen *et al.* 2000
			One-114	0.05	26	177-280	0.828	4	**0.078**	0.024	**0.192**	Olsen *et al.* 2000
			Ots-100	0.04	26	168-298	0.826	2	**0.090**	-0.042	**0.096**	Nelson and Beacham 1999
Omy-C2	55	28	*One*-101	0.02	25	119-243	0.444	4	**0.115**	**0.098**	0.395	Olsen *et al.* 2000
			One-108	0.02	35	161-337	0.714	4	**0.088**	**0.181**	0.298	Olsen *et al.* 2000
			Ots-103	0.015	9	56-90	0.217	4	**0.099**	0.029	**0.100**	Small *et al.* 1998
Omy-D2	49	25	*Omy*-77	0.03	22	97-147	0.835	2	**0.068**	0.056	**0.246**	Morris *et al.* 1996
			Ots-1	0.03	22	158-266	0.727	2	**0.098**	0.014	**0.172**	Banks *et al.* 1999
			Ots-3M	0.02	11	132-156	0.573	2	**0.088**	**0.084**	**0.237**	Banks *et al.* 1999
Omy-E2	62	26	*Omm*-1070	0.025	42	164-374	0.797	4	**0.064**	**0.127**	**0.239**	Rexroad *et al.* 2001
			Omm-1130	0.05	57	185-399	0.800	4	**0.073**	**0.042**	**0.152**	Rexroad *et al.* 2001
			Omy-1011	0.045	21	134-245	0.798	4	**0.075**	**0.056**	0.219	Spies *et al.* 2005
Omy-F2	52	25	*Oki*-10	0.02	18	92-151	0.796	2	**0.095**	-0.060	**0.123**	Smith *et al.* 1998
			Omy-1001	0.03	30	167-242	0.810	2	**0.120**	-0.012	**0.147**	Spies *et al.* 2005

5

1 Table 4. Loci information for each collection: F_{IS} value at each locus, underlined values
2 were significant before Bonferroni correction and bold values significant after correction.
3

Gr. Spokane	One-102	One-114	Ots-100	One-101	One-108	Ots-103	Omy-77	Ots-1	Ots-3M	Omm-1070	Omm-1130	Omy-1011	Oki-10	Omy-1001
02MidSpok	0.096	0.078	0.032	0.182	0.026	0.076	0.018	0.08	-0.042	0.039	0.072	<u>0.125</u>	-0.003	-0.039
03UpSpok	0.056	0.049	0.012	<u>0.189</u>	0.089	-0.013	-0.053	<u>0.213</u>	-0.008	0.057	0.091	**<u>0.316</u>**	0.027	-0.015
03MidSpok	0.072	<u>0.113</u>	0.045	-0.126	0.059	-0.013	<u>0.093</u>	0.012	0.044	**0.176**	0.046	<u>0.149</u>	0.011	0.061
04Coulee	-0.020	-0.027	-0.031	-0.012	0.046	0.010	-0.072	-0.100	-0.114	**0.320**	0.020	0.101	-0.035	-0.031
04Deep	0.041	<u>0.213</u>	-0.001	0.150	0.106	-0.141	0.018	-0.013	0.004	<u>0.111</u>	-0.023	0.055	-0.017	0.006
04UpDeep	0.013	-0.034	-0.178	<u>0.351</u>	**0.436**	-0.044	-0.048	0.077	0.099	<u>0.196</u>	0.020	0.053	-0.075	-0.115
04Marshall	<u>0.206</u>	0.057	0.102	0.024	**0.268**	0.230	**0.342**	-0.010	<u>0.271</u>	0.013	-0.047	-0.095	-0.108	-0.080
04Calif	0.056	0.046	-0.014	-0.119	<u>0.205</u>	0.110	0.016	<u>0.159</u>	0.019	0.051	0.077	<u>0.143</u>	-0.041	<u>0.171</u>
MSS	<u>0.205</u>	<u>0.337</u>	0.106	-0.048	<u>0.175</u>	-0.034	0.211	<u>0.383</u>	0.222	-0.115	-0.119	0.153	-0.052	0.009
03Indian	-0.033	-0.058	-0.003	<u>0.383</u>	0.059	0.063	0.022	**0.560**	-0.093	<u>0.197</u>	**0.256**	0.029	0.055	-0.017
04Indian	<u>0.468</u>	0.042	-0.116	-0.250	0.029	<u>0.508</u>	-0.108	<u>0.416</u>	0.141	**0.337**	<u>0.207</u>	-0.116	-0.021	-0.054
04Hang	0.118	0.127	-0.032	0.289	0.097	0.000	-0.134	0.119	-0.347	-0.161	0.015	0.111	<u>0.453</u>	-0.134
03UpLSpok	-0.085	0.062	0.030	0.131	<u>0.177</u>	-0.118	0.082	0.052	0.011	0.061	0.093	0.111	0.044	-0.022
03LoLSpok	-0.027	<u>0.109</u>	0.047	0.068	<u>0.064</u>	0.148	0.012	<u>0.100</u>	-0.122	**0.726**	**0.544**	0.079	0.089	-0.010
03DartAB	0.014	0.095	-0.101	0.062	<u>0.153</u>	-0.130	0.017	-0.042	0.065	**0.231**	<u>0.129</u>	0.028	0.081	0.033
03DartBB	0.072	-0.022	-0.032	0.029	<u>0.289</u>	0.118	-0.106	-0.006	0.074	-0.042	0.103	-0.033	-0.092	-0.003
03Dead	<u>0.089</u>	<u>0.076</u>	<u>0.081</u>	0.068	**0.195**	-0.058	0.062	**0.194**	0.108	**0.256**	**0.196**	<u>0.072</u>	0.065	0.023
03SFDead	-0.049	-0.020	0.093	0.105	**0.361**	-0.055	-0.139	0.029	0.085	<u>0.154</u>	0.023	0.088	0.078	0.108
03LDeep	-0.130	0.012	0.028	-0.026	<u>0.193</u>	0.310	-0.025	0.102	0.036	0.004	0.087	<u>0.132</u>	<u>0.131</u>	0.028
03NFLDeep	0.079	-0.044	<u>0.218</u>	-0.028	**0.434**	-0.014	-0.045	0.099	-0.005	0.040	-0.010	0.027	0.006	-0.015
03SFLDeep	0.101	0.082	0.000	-0.152	<u>0.111</u>	-0.009	0.094	0.080	0.039	0.069	0.061	0.052	-0.014	<u>0.117</u>
01Otter	0.081	-0.004	0.047	-0.102	**0.273**	0.073	0.094	-0.020	**0.395**	0.054	0.087	-0.094	0.003	0.064
01DeerCr	0.042	0.019	0.038	0.084	**0.280**	0.198	0.037	-0.059	<u>0.198</u>	0.025	**0.110**	-0.063	0.032	-0.041
02LilDeer	-0.006	0.023	0.028	0.043	**0.371**	-0.088	0.064	0.053	0.031	<u>0.185</u>	<u>0.118</u>	-0.084	-0.042	-0.030
01Buck	0.045	-0.049	-0.003	0.042	<u>0.106</u>	-0.065	0.017	-0.061	0.043	<u>0.150</u>	-0.057	0.037	-0.016	0.024
02LoDrag	<u>0.105</u>	0.011	-0.033	0.019	**0.172**	-0.048	0.060	0.120	-0.036	<u>0.052</u>	<u>0.070</u>	0.062	-0.036	<u>0.082</u>
02WBDrag	-0.150	0.016	-0.029	0.133	<u>0.194</u>	0.045	-0.083	0.029	0.056	<u>0.139</u>	0.040	-0.008	-0.034	-0.153
02UpDrag	-0.077	-0.023	-0.003	-0.017	<u>0.165</u>	0.021	<u>0.191</u>	-0.026	-0.117	0.040	<u>0.123</u>	<u>0.128</u>	-0.114	-0.027
Hatchery														
01PhaHat	0.019	0.013	0.055	0.050	<u>0.084</u>	-0.004	-0.006	0.005	0.040	0.045	<u>0.054</u>	-0.026	-0.036	0.005
02PhaHat	<u>0.078</u>	0.048	0.020	-0.086	0.059	-0.126	0.037	**0.296**	0.012	**0.228**	<u>0.098</u>	0.030	-0.033	<u>0.070</u>
04TroutH	-0.013	-0.148	-0.155	0.124	0.115	0.000	0.080	-0.005	0.071	0.074	<u>0.267</u>	0.123	-0.129	-0.043
00SpHat	-0.034	0.043	-0.014	0.021	0.006	0.124	0.004	-0.006	0.020	0.004	-0.062	-0.018	-0.162	-0.106
02LFH	<u>0.061</u>	<u>0.058</u>	0.053	0.032	<u>0.113</u>	-0.085	0.052	**0.195**	0.007	**0.140**	**0.150**	-0.031	NA	0.010
Cutthroat														
03Nehc	-0.206	0.135	0.667	0.047	0.060	0.014	-0.242	0.108	0.064	<u>0.367</u>	<u>0.172</u>	<u>0.692</u>	-0.075	0.103
04Nehc	-0.289	-0.059	<u>0.365</u>	<u>0.774</u>	0.042	0.137	0.074	0.003	0.114	0.211	-0.016	0.407	0.021	-0.070

4

Table 5. Pairwise F_{ST} values with collections combined by tributary (see Table 1). Average pairwise F_{ST} values for wild collections
compared to other wild collections, and hatchery collections compared to only wild collections are under "Avg". Highest average
value is in bold type. All pairwise values were significantly different from 0 ($P < 0.0001$).

	Avg	Hang	Spok	04Coulee	Deep	04Marsh	04Calif	03UpLSpok	03LoLSpok	Dart	Dead	LDeep	01Otter	Deer	01Buck	Drag	PhaH	02LFH	00SpHat	TroutH
Hang	0.0912																			
Spok	0.0962	0.0566																		
04Coulee	**0.1647**	0.1493	0.1516																	
Deep	0.1102	0.1441	0.1275	0.1753																
04Marsh	0.1459	0.1815	0.1674	0.2387	0.1065															
04Calif	0.1000	0.0606	0.0739	0.1531	0.1480	0.1908														
03UpLSpok	0.1044	0.0899	0.1192	0.1972	0.1248	0.1702	0.1218													
03LoLSpok	0.0591	0.0629	0.0692	0.1439	0.0725	0.1075	0.0622	0.0664												
Dart	0.0859	0.1100	0.1145	0.1844	0.0817	0.1073	0.1110	0.0910	0.0475											
Dead	0.0628	0.0444	0.0580	0.1448	0.0973	0.1391	0.0614	0.0636	0.0279	0.0630										
LDeep	0.0816	0.0686	0.0851	0.1622	0.1164	0.1423	0.0930	0.0955	0.0499	0.0752	0.0360									
01Otter	0.0820	0.0871	0.0940	0.1705	0.0976	0.1459	0.0908	0.0878	0.0415	0.0664	0.0441	0.0676								
Deer	0.0695	0.0516	0.0654	0.1523	0.1156	0.1476	0.0560	0.0853	0.0224	0.0673	0.0194	0.0394	0.0512							
01Buck	0.0740	0.1013	0.0915	0.1673	0.0550	0.0867	0.0996	0.0821	0.0312	0.0364	0.0532	0.0676	0.0609	0.0668						
Drag	0.0623	0.0692	0.0725	0.1448	0.0802	0.1109	0.0774	0.0667	0.0228	0.0464	0.0271	0.0437	0.0423	0.0320	0.0362					
PhaH	0.0590	0.0566	0.0718	0.1371	0.0695	0.1048	0.0655	0.0608	0.0218	0.0523	0.0309	0.0556	0.0491	0.0397	0.0392	0.0301				
02LFH	0.0734	0.0546	0.0890	0.1460	0.1052	0.1431	0.0738	0.0693	0.0431	0.0742	0.0360	0.0613	0.0576	0.0405	0.0662	0.0417	0.0336			
00SpHat	0.1440	0.1868	0.1879	0.2336	0.1258	0.1616	0.1811	0.1357	0.1078	0.0982	0.1280	0.1436	0.1288	0.1436	0.0951	0.1019	0.1084	0.1465		
TroutH	0.1573	0.2011	0.1791	0.2668	0.1128	0.1388	0.2048	0.1796	0.1191	0.1163	0.1492	0.1628	0.1580	0.1663	0.0853	0.1247	0.1277	0.1623	0.1769	

1
Table 6. Bayesian analysis of cutthroat-rainbow hybridization (clusters set at 2), and
redband-coastal hatchery introgression (clusters set at 3). Values above 25% are in bold
type.

	1	2		1	2	3	n
03UpSpok	**0.972**	0.028	03UpSpok	0.011	0.027	**0.963**	67
03MidSpok	**0.996**	0.004	03MidSpok	0.037	0.005	**0.959**	92
02MidSpok	**0.996**	0.004	02MidSpok	0.171	0.004	**0.825**	47
03UpLSpok	**0.996**	0.004	03UpLSpok	0.183	0.005	**0.813**	39
03LoLSpok	**0.996**	0.004	03LoLSpok	**0.387**	0.005	**0.608**	62
03DartAB	**0.998**	0.002	03DartAB	**0.450**	0.002	**0.548**	51
03DartBB	**0.998**	0.002	03DartBB	**0.508**	0.002	**0.489**	29
03Dead	**0.998**	0.002	03Dead	0.138	0.003	**0.859**	100
03SFDead	**0.997**	0.003	03SFDead	0.026	0.002	**0.971**	49
03LDeep	**0.998**	0.002	03LDeep	0.144	0.003	**0.853**	50
03NFLDeep	**0.998**	0.002	03NFLDeep	0.016	0.002	**0.982**	50
03SFLDeep	**0.998**	0.002	03SFLDeep	0.023	0.003	**0.975**	60
01Otter	**0.988**	0.012	01Otter	0.059	0.009	**0.932**	50
01DeerCr	**0.991**	0.009	01DeerCr	0.061	0.009	**0.930**	100
02LilDeer	**0.996**	0.004	02LilDeer	0.023	0.004	**0.973**	50
01Buck	**0.996**	0.004	01Buck	**0.750**	0.005	0.245	50
02LoDrag	**0.996**	0.004	02LoDrag	**0.267**	0.004	**0.729**	100
02WBDrag	**0.996**	0.004	02WBDrag	0.178	0.004	**0.818**	50
02UpDrag	**0.997**	0.003	02UpDrag	0.129	0.003	**0.868**	50
04Deep	**0.997**	0.003	04Deep	**0.981**	0.003	0.016	50
04UpDeep	**0.995**	0.005	04UpDeep	**0.983**	0.004	0.014	50
04Coulee	**0.998**	0.002	04Coulee	0.143	0.003	**0.855**	50
04Calif	**0.998**	0.002	04Calif	0.075	0.003	**0.923**	49
04Marsh	**0.998**	0.002	04Marsh	**0.970**	0.002	0.028	50
AllIndian	**0.989**	0.011	AllIndian	0.005	0.009	**0.985**	67
03Nehchen	0.025	**0.975**	03Nehchen	0.004	**0.971**	0.025	61
04Nehchen	0.003	**0.997**	04Nehchen	0.004	**0.991**	0.005	37
MSS	**0.998**	0.002	MSS	0.003	0.002	**0.995**	33
03Martin	**0.996**	0.004	03Martin	0.009	0.004	**0.987**	13
00SpHat	**0.997**	0.003	00SpHat	**0.993**	0.003	0.005	100
01Gold	**0.997**	0.003	01Gold	**0.987**	0.003	0.010	48
01Eell	**0.997**	0.003	01Eell	**0.993**	0.002	0.005	89
02STac	**0.997**	0.003	02STac	**0.988**	0.002	0.010	50
TroutH	**0.997**	0.003	TroutH	**0.991**	0.004	0.005	49
02LFH	**0.994**	0.006	02LFH	0.012	0.005	**0.982**	100
01PhaHat	**0.996**	0.004	01PhaHat	**0.295**	0.005	**0.700**	100
02PhaHat	**0.995**	0.005	02PhaHat	0.020	0.006	**0.973**	100
02SullCr	0.007	**0.993**	02SullCr	0.004	**0.990**	0.006	96
03SullLk	0.118	**0.882**	03SullLk	0.059	**0.871**	0.070	34
03GoldCr	0.019	**0.981**	03GoldCr	0.022	**0.973**	0.005	42

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8

1   Table 7. Bayesian analysis of shared ancestry in pairwise tests comparing a subset of
2   wild-origin rainbow trout collections from the Spokane River and Spokane Hatchery
3   rainbow trout.
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5
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	1	2	n
00SpHat	0.005	**0.995**	100
04Marsh	**0.995**	0.005	50
00SpHat	0.014	**0.986**	100
01Buck	**0.988**	0.012	50
00SpHat	0.008	**0.992**	100
04Deep	**0.991**	0.009	50
04UpDeep	**0.995**	0.005	50
00SpHat	0.007	**0.993**	100
Drag	**0.988**	0.012	200
00SpHat	0.008	**0.992**	100
03DartAB	**0.994**	0.006	51
03DartBB	**0.987**	0.013	29
Spok	0.014	**0.986**	100
03LoLSpok	**0.991**	0.009	62
00SpHat	0.004	**0.996**	100
01Otter	**0.995**	0.005	50
00SpHat	0.003	**0.997**	100
04Coulee	**0.993**	0.007	50

8

1 Table 8. Maximum likelihood assignment test results with collections combined by tributary (see Table 1). Columns show the
2 number of fish from a single collection assigned to baseline collections. Positive, unambiguous assignments (100 times more likely
3 than assignment to another collection, no bracket around number) are followed by positive, ambiguous assignments (highest
4 assignment likelihood but less than 100 times more likely) in brackets. Fish assigned back to origin are along the diagonal. Total
5 number of fish in the baseline collection is in "total" row, number of correct assignments (unambiguous combined with ambiguous) is
6 in "correct" row, and percentage of correct assignments is in "% correct" row. Abbreviations follow Table 1.
7

	Hang	Spok	04Coulee	04Deep	04MarCr	04Calif	03UpLSpok	03LoLSpok	Dart	03Dead	03LDeep	01Otter	01DeerCr	01Buck	Drag	00SpHat	01PhaHat	02LFH	TroutH
Hang	105 (7)																		
Spok		188 (1)				(1)		(1)											
04Coulee		1	49	1					(1)										
04Deep		7 (2)	(1)	99							1 (1)								
04MarCr					50														
04Calif		(1)				45(2)													
03UpLSpok							39											(1)	
03LoLSpok		(2)						38 (10)		3 (4)	(3)		(2)		(2)			(2)	
Dart								2	76 (2)										
03Dead										104 (20)	(3)		(1)						
03LDeep	(1)							1 (1)		4 (5)	129 (21)				(2)				
01Otter												49			(1)				
01DeerCr		(1)						2 (5)		(1)			114 (31)		6 (3)				
01Buck								(1)		(2)	(1)		(1)	47 (3)	(1)				
Drag		(3)				(1)			(1)	(3)	(1)	(1)	(1)		169 (16)				
00SpHat																100			
01PhaHat								(1)			(3)						90 (7)		
02LFH																		99 (1)	
TroutH																			49
total	113	206	50	100	50	49	39	62	80	149	160	49	150	50	200	100	100	100	49
correct	112	189	49	99	50	47	39	48	78	124	150	50	145	50	185	100	97	100	49
% correct	99.12	91.75	98.00	99.00	100.00	95.92	100.00	77.42	97.50	83.22	93.75	102.04	96.67	100.00	92.50	100.00	97.00	100.00	100.00

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1   Figure 1. Map of Spokane River drainage showing tributaries where collections

2   originated. Waterfalls are indicated by river kilometers in boxes. Man-made barriers and

3   dry creek bed locations are designated approximately with triangles (Dartford Cr. barrier

4   not shown). Filled circle marks the location of Spokane Hatchery. Map was constructed

5   by Jim Shaklee (WDFW). City of Spokane (118°40'W; 47°50'N) is shaded with grey

6   color.

7

8   Figure 2. Consensus neighbor joining tree of Cavalli-Sforza and Edwards genetic chord

9   distances (1967) among rainbow and cutthroat collections from Spokane and Pend

10   Oreille drainages and hatcheries. Numbers at the nodes indicate the percentage (greater

11   than 60%) of 10,000 trees in which collections beyond the nodes grouped together.
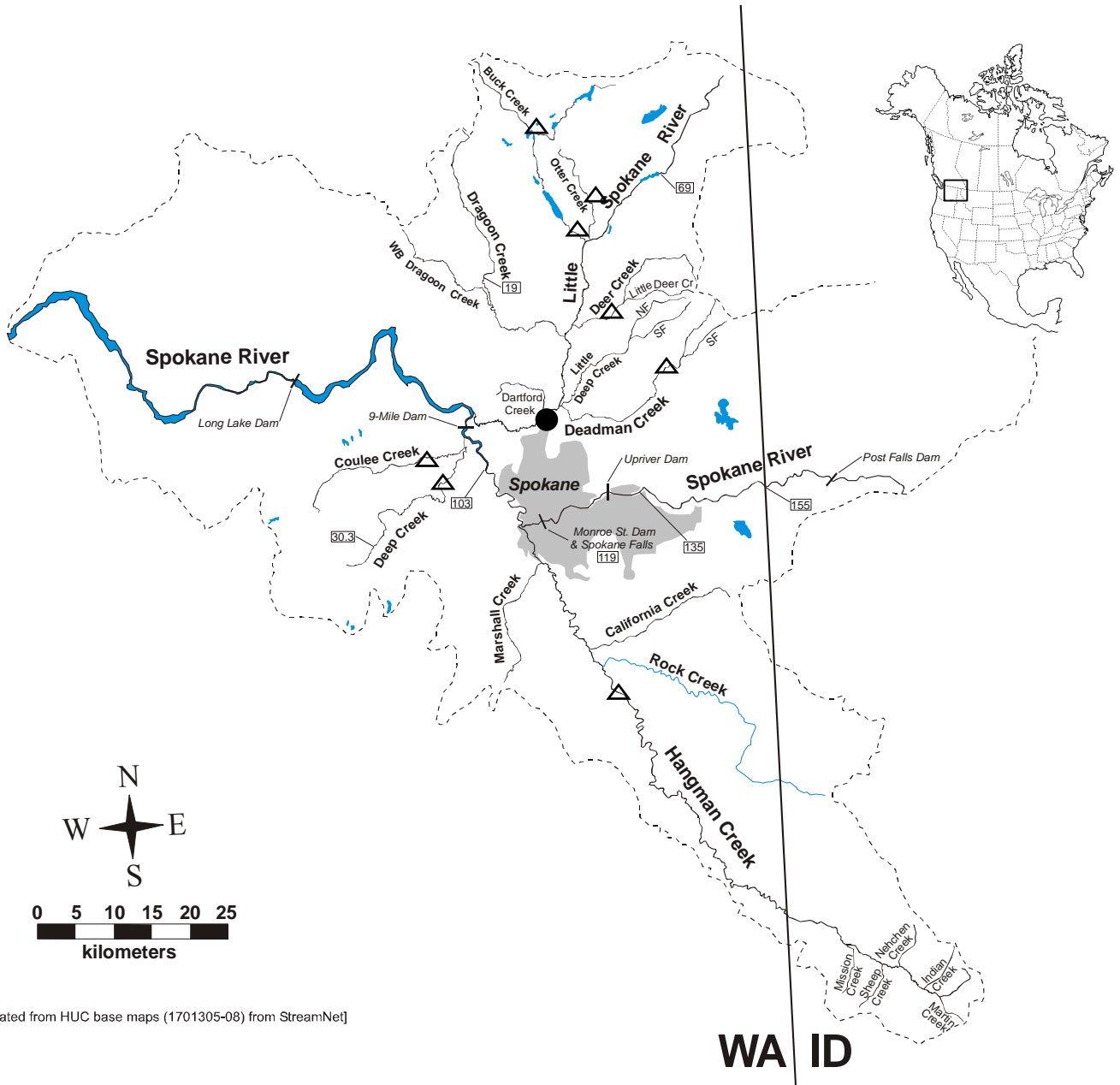
12

13   Figure 3. Multidimensional scaling analysis plot of Cavalli-Sforza and Edwards

14   distances (1967) among hatchery collections and combined rainbow trout collections
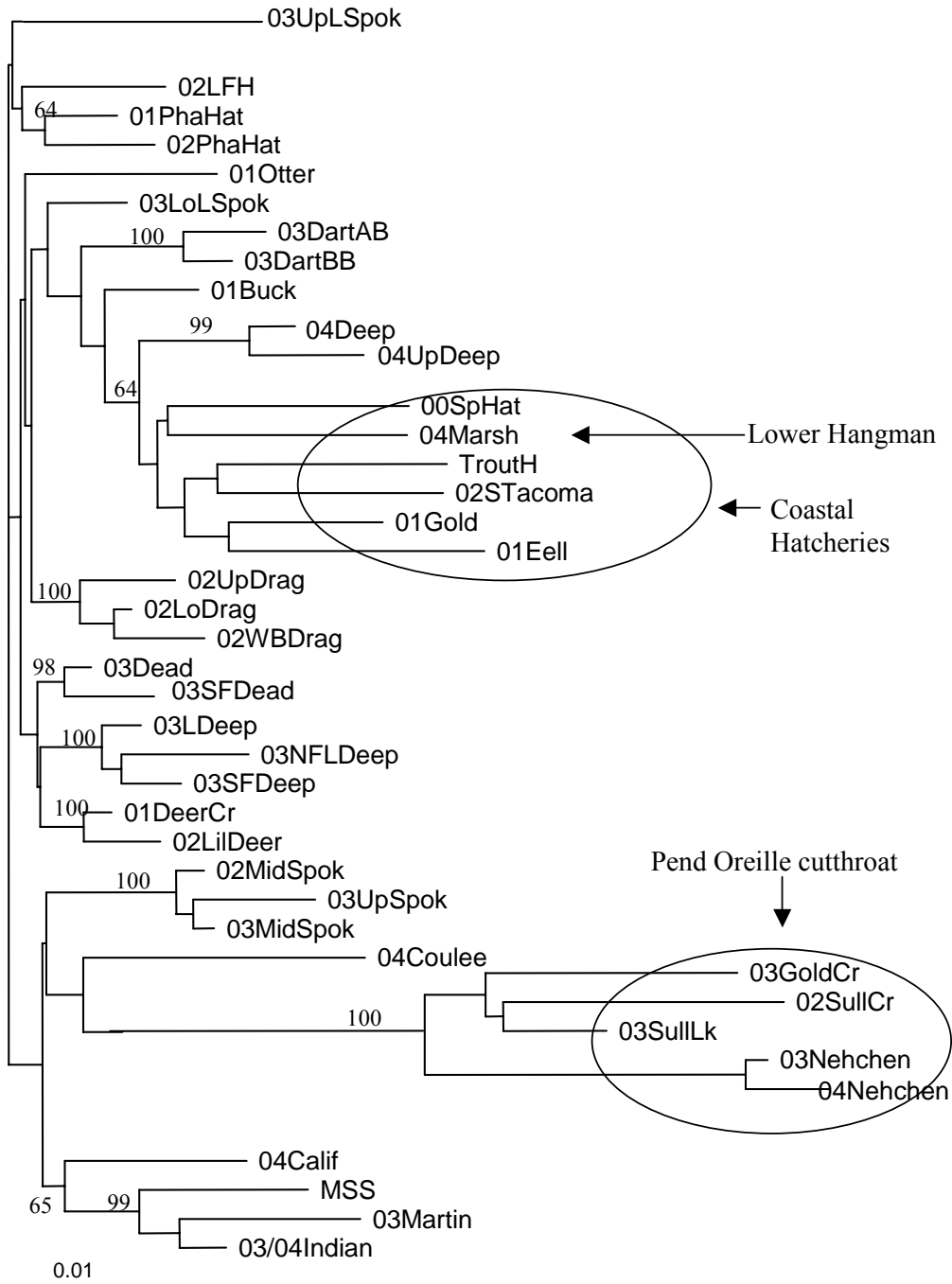
15   from the Spokane drainage.
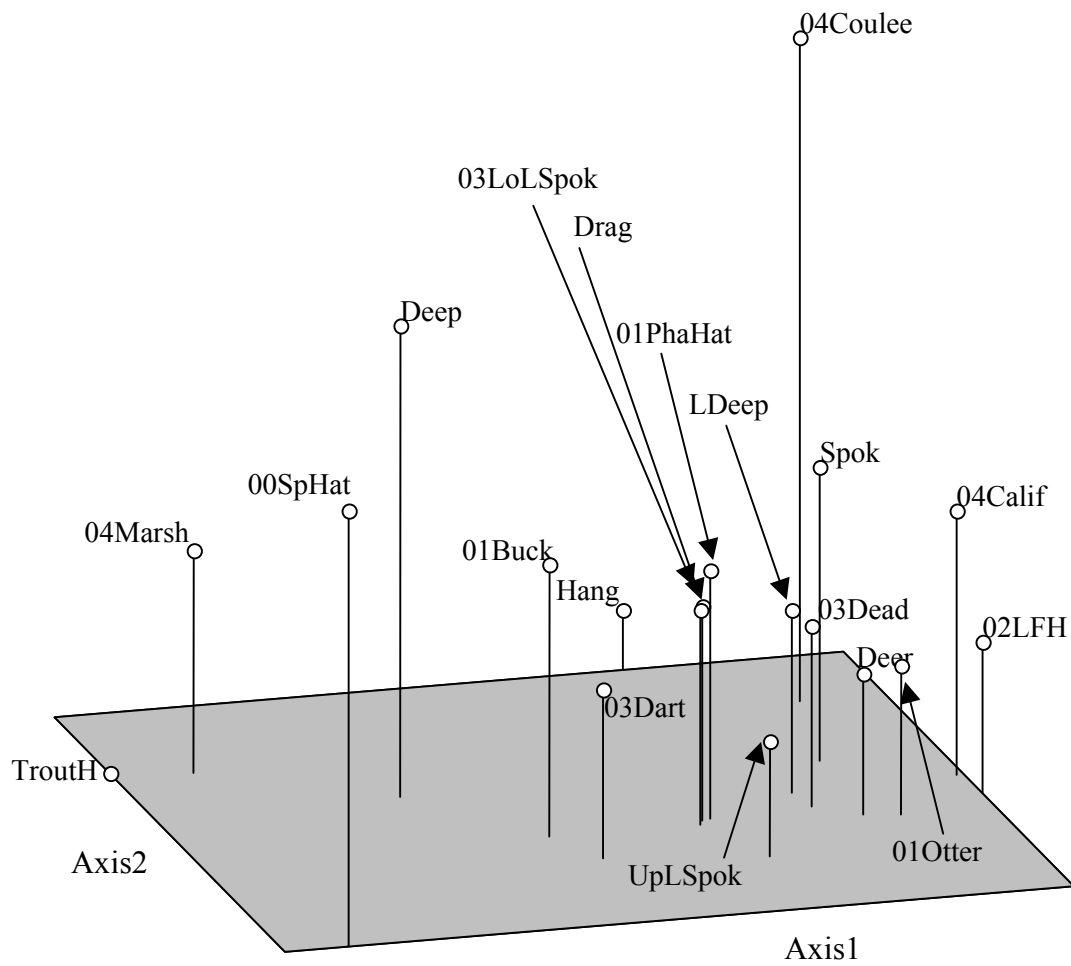
16

1   Figure 1.

2
3

[map created from HUC base maps (1701305-08) from StreamNet]

1   Figure 2.

2

03UpLSpok
02LFH
64 01PhaHat
02PhaHat
01Otter
03LoLSpok
100 03DartAB
03DartBB
01Buck
99 04Deep
04UpDeep
64 00SpHat
04Marsh   Lower Hangman
TroutH
02STacoma
01Gold   Coastal
01Eell   Hatcheries
100 02UpDrag
02LoDrag
02WBDrag
98 03Dead
03SFDead
100 03LDeep
03NFLDeep
03SFDeep
100 01DeerCr
02LilDeer
Pend Oreille cutthroat
100 02MidSpok
03UpSpok
03MidSpok
04Coulee
03GoldCr
02SullCr
100 03SullLk
03Nehchen
04Nehchen
04Calif
MSS
65 99 03Martin
03/04Indian
0.01

3

1   Figure 3.
2

3