

1 **Parallel epigenetic modifications induced by hatchery rearing**
2 **in a Pacific Salmon**

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20 **Highlights**

21 - First study to highlight parallel epigenetic modifications induced by hatchery rearing as a
22 potential explanatory mechanism for rapid change in fitness

23 **Summary**

24 A puzzling question in conservation biology is how to maintain overall fitness of individuals
25 bred in captive environment upon release into the wild, especially for rehabilitating
26 declining or threatened species [1,2]. For salmonid species, a heritable change in fitness
27 related traits and gene expression has been reported to occur in a single generation of
28 captivity in hatchery environment [3–5]. Such rapid changes are congruent with models of
29 inadvertent domestication selection which may lead to maladaptation in the natural
30 environment [4]. Arguably, the underlying mechanism by which captivity may induce
31 fitness difference between wild and captive congeners is still poorly understood. Short-
32 term selection on complex phenotypic traits is expected to induce subtle changes in allele
33 frequency over multiple loci [7–9]. Yet, most studies investigating the molecular basis for
34 rapid change in fitness related traits occurring in hatchery have concentrated their effort on
35 finding evidence for selection at the genome level by identifying loci with large effect.

36 Numerous wild stocks of Pacific anadromous salmon and trout (genus *Oncorhynchus*
37 and *Salmo*) have experienced fluctuating abundance over the past century, with a series of
38 sharp declines [6–8]. With the objectives of preserving ecosystem integrity, enhancing

39 declining populations and sustaining fisheries, conservation hatcheries have been
40 flourishing. This is particularly true along the North American Pacific coast where billions of
41 salmonids, all species included, are released each year. Despite substantial improvement of
42 production management, the beneficial ecological role of hatcheries in enhancing and
43 restoring wild stocks is still debated, mainly because of the reduced fitness and
44 maladaptation of hatchery-fish when released in the wild [3,5,9]. Although previous studies
45 showed that domestication selection was involved in such fitness impairment, they also
46 observed that different environmental conditions (e.g. reduced fish density) significantly
47 modulated the physiological acclimation to hatchery environment [4].

48 Environmental stimuli are especially relevant during early embryonic development,
49 which also correspond to a sensitive methylation reprogramming window in vertebrates
50 [10,11]. It is therefore plausible that differences in rearing environment during early
51 development may result in epigenetic modifications that could in turn impact on fitness.
52 However, the only epigenetic study to date pertaining to captive rearing in salmonids and
53 performed using methylation-sensitive amplified fragments (MSAP) failed to identify
54 significant changes in methylation profile associated with hatchery rearing [12]

55 Here, we used a higher resolution approach to compare the genome-wide pattern of
56 methylation in hatchery-reared juvenile (smolt) Coho Salmon with that of their wild
57 counterparts in two geographically distant rivers in British Columbia, Canada. Using a
58 reduced representation bisulfite sequencing (RRBS) approach covering an average per
59 individual of about 70 million cytosines in CpG context, we identified 100 methylated

60 regions (DMRs) that differed in parallel between hatchery and natural origin salmon in both
61 rivers. The total variance of epigenetic variation among individuals explained by river or
62 origin and rearing environment in a RDA model was 16% (adj.R²=0.16), and both variables
63 equally explained about 8% of the variance after controlling for each other. The gene
64 ontology analysis revealed that regions with different methylation levels between hatchery
65 and natural origin salmon showed enrichment for ion homeostasis, synaptic and
66 neuromuscular regulation, immune and stress response, and control of locomotion
67 functions. We further identified 15,044 SNPs that allowed detection of significant
68 differences between either rivers or sexes. However, no effect of rearing environment was
69 observed, confirming that hatchery and natural origin fish of a given river belong to the
70 same panmictic population, as expected based on the hatchery programs applied in these
71 rivers (see Supplementary experimental procedures). Moreover, neither a standard
72 genome-scan approach nor a polygenic statistical framework allowed detection of selective
73 effects within a single generation between hatchery and natural origin salmon. Therefore,
74 this is the first study to demonstrate that parallel epigenetic modifications induced by
75 hatchery rearing during early development may represent a potential explanatory
76 mechanism for rapid change in fitness-related traits previously reported in salmonids.

77

78 **Results**

79 **Sampling**

80 We collected a total of 40 Coho Salmon from two rivers in British Columbia, Canada; the
81 Capilano and Quinsam Rivers (Table S1, Figure S1). These systems are well suited to test
82 specifically for the effect of rearing environment on patterns of methylation, independent of
83 the genetic background between fish born in the wild (thereafter natural origin) vs. those
84 born in hatchery (see Supplementary experimental procedures). During their downstream
85 migration to the sea, we collected from each river 10 juveniles (smolt stage) reared in
86 captivity in a local hatchery (fin-clipped; hereafter “hatchery origin”) and 10 smolts born in
87 the wild (hereafter “natural origin”). Broodstock for the hatchery fish was collected while
88 returning to spawn in the same year in both rivers. The number of returning adults sampled
89 and used for breeding was 758 and 894 for the Capilano River and Quinsam River
90 populations, respectively. The broodstock included 3 years old females, as well as 2-3 years
91 old males and could represent fish born previously either in hatchery or in the wild.

92 **Evidence for parallel epigenetic modifications in hatchery environment**

93 We used a Reduced Representation Bisulfite Sequencing (RRBS) approach, with the *MspI*
94 restriction enzyme, to document both genome-wide methylation and genetic variation. In
95 order to avoid the possibility of falsely interpreting existing C-T DNA polymorphism as
96 epigenetic variation, we first masked the genome (GenBank assembly accession:

97 GCA_002021735.1) by removing all C>T polymorphism (1,896,050 SNPs; maf=0.05) found
98 by whole-genome re-sequencing of 20 fish from British Columbia (Supplementary
99 experimental procedures; Figure S1). We used a tiling window approach to quantify the
100 percentage of methylation over 1000-bp regions throughout the masked genome and
101 retained only cytosines in a CpG context for downstream analyses, as these regions
102 represent the responsive methylation context in vertebrates (Supplementary experimental
103 procedures). We used a db-RDA to document methylation variation among hatchery and
104 natural origin fish from both rivers. We first produced a principal coordinate analysis
105 (PCoA) on a Euclidean distance matrix computed using all the raw data and kept axes
106 according to the cumulative broken-stick threshold, which correspond to six axes
107 explaining at least 2.75% of the variance for a total of 42.2% of the variance [13]. A
108 distance-based redundancy analysis (db-RDA) was then produced on the epigenetic
109 variation explained by these PCoA factors (response matrix) with river of origin, rearing
110 environment and sex as explanatory variables. The model was significant with an adjusted
111 R^2 of 0.16 (Figure 1). Both river of origin and rearing environment were significant whereas
112 no significant effect was detected for sex (Figure 1). Partial db-RDAs revealed that the net
113 variation explained by rearing environment (adj. R^2 =0.08; F =4.34; p -value<0.05) was
114 identical to the one explained by the river of origin (adj. R^2 =0.08; F =4.66; p -value<0.01).
115 This shared variation between hatchery origin salmon from both rivers relative to their
116 natural origin congeners provides evidence for similar (parallel) epigenetic modifications
117 induced by hatchery rearing.

118 Moreover, we identified differentially methylated regions (defined as having >15%
119 overall difference; q-value < 0.001; see Supplementary experimental procedures) between
120 rearing environments, using a logistic regression with river of origin as covariates. We
121 identified a total of 100 DMRs that were distributed among 27 chromosomes and 20
122 unmapped scaffolds (Figure 2). The proportion of hypermethylated DMRs was much
123 greater in hatchery origin relative to natural origin salmon (89 vs 11; $\chi^2=60.84$, df=1,
124 $P<0.001$), pointing to a general pattern of downregulation of genes associated with these
125 DMRs in hatchery origin salmon.

126 **Functional annotation and gene ontology of DMRs**

127 We mapped the recently published transcriptome of the Coho Salmon [14] to the species'
128 draft genome (see Supplemental files for details) to infer functional annotation of DMRs.
129 Out of the 100 DMRs, we identified 37 DMRs overlapping 52 unique transcripts and regions
130 comprising 5kb up and downstream of these transcripts. A blastx approach successfully
131 identified 29 unique Uniprot IDs and again revealed an excess of hypermethylation in
132 hatchery relative to wild fish (25 hypermethylated vs. 4 hypomethylated; $\chi^2=15.21$, df=1,
133 $P<0.001$; Figure 2; Table 1). Gene ontology (GO) analysis revealed an over-representation
134 (p-value<0.05 and at least three genes by GO term) of modules associated with ion
135 homeostasis (GO:0055080: cation homeostasis, GO:0042592: homeostatic process,
136 GO:0043167: ion binding, GO:0055065: metal ion homeostasis). It has been shown
137 previously in a closely related species (Rainbow Trout, *Oncorhynchus mykiss*) that hatchery-
138 rearing negatively affects acclimation to seawater as reflected by lower specific activity of

139 NA⁺ K⁺ ATPase and lower survival following seawater transfer [15]. We also observed a
140 significant enrichment for functions associated with the immune response (GO:0031347:
141 regulation of defense response, GO:0050727: regulation of inflammatory response,
142 GO:0045321: leukocyte activation), as well as synaptic signal modulation and locomotion
143 functions (GO:0099572: postsynaptic specialization, GO:0050885: neuromuscular process
144 controlling balance). The neuromuscular process controlling balance includes the
145 calcium/calmodulin-dependent protein kinase type II subunit beta (CAMK2B),
146 hypermethylated in hatchery fish, which is a main actor of the neuromuscular
147 communication and regulating Ca²⁺ signalling in skeletal muscle tissue [16]. Its activation
148 has also been associated, together with the Ca²⁺ signalling, to sustained and endurance
149 muscle exercise in humans and the control of muscle development and excitation [17,18].
150 Lower critical swimming performance (*Uct*) has been documented in hatchery-reared Coho
151 Salmon compared to their wild counterparts following transfer to seawater, and lower
152 average swimming speed has been documented between wild and F1-hatchery Atlantic
153 Salmon (*Salmo salar*) and Brown trout (*Salmo trutta*) smolts [19,20]. Moreover, the
154 serotonin receptor 2C (HTR2C), which regulates appetite and feeding behavior, was also
155 hypermethylated in hatchery fish [21]. Finally, we observed a GO enrichment for
156 transcription factors (GO:0006357: regulation of transcription from RNA polymerase II
157 promoter) which comprised the TATA-binding protein-associated factor 172, also
158 hypermethylated in hatchery-origin fish, which is involved in the global transcription
159 regulation. Genes under TATA box regulation are more able to respond rapidly (within a

160 single generation) to environmental stress, show more variability in their expression range
161 (phenotypic plasticity) compared to non-TATA regulated genes, and account for the
162 appearance of stress induced phenotypes [22].

163 **No evidence for genome-wide differentiation between hatchery and natural origin salmon**

164 We mapped the trimmed reads to the masked draft Coho Salmon genome assembly and
165 identified 15,044 SNP markers (other than C-T polymorphism) meeting stringent filtering
166 criteria and spread across the genome. The PCoA was produced on a Euclidean distance
167 matrix of the 15,044 markers. Because no axis could be selected according to the broken-
168 stick distribution, we selected all axes explaining at least 2.75% of the variation (10 axes
169 explaining 33.9% of the variance), as previously performed with epigenetic markers [13]. A
170 distance-based redundancy analysis (db-RDA) was produced on the genetic variation
171 explained by these PCoA factors (response matrix) with river of origin, rearing environment
172 and sex as explaining variables. The model was significant with an adjusted R^2 of 0.18
173 (Figure 3). Both river of origin and sex were significant, whereas no significant effect was
174 detected for rearing environment (Figure 3). No significant outlier with a genome-scan
175 approach (Bayescan v2.0 [23]) was detected between sexes (Figure S2). Moreover, an
176 AMOVA revealed no significant genome wide difference between rearing environments
177 within river ($F_{st}=0.005$ and 0.002 , for Capilano River and Quinsam Rivers, respectively; p -
178 value >0.05) while the net difference between rivers was highly significant (mean $F_{st}=0.038$
179 ± 0.003 ; p -value <0.001 ; Table S2) [24]. Additionally, heterozygosity and inbreeding values
180 (G_{IS}) were not significantly different between rivers or between hatchery and natural origin

181 fish (Table S3). Lastly, we used both a standard Bayescan genome-scan method for
182 detecting outliers of large effect [23] and a Random Forest approach accounting for
183 population structure, which allows detecting signals of polygenic selection. This statistical
184 framework recently allowed detection of parallel polygenic selection between habitats
185 within the panmictic North Atlantic eels (*Anguilla sp.*) and associated genetic variation with
186 migration run timing in Chinook salmon (*Oncorhynchus tshawytscha*) populations [25–27].
187 No outlier (FDR>0.05) was detected between hatchery and natural origin fish using
188 Bayescan v2.0 (Figure S3), whereas Random Forest identified 114 covarying markers,
189 distributed over the 30 chromosomes. We used permutations (n = 1000, Supplementary
190 experimental procedures) to assess whether a signal of apparent polygenic selection similar
191 to the one that was detected could be obtained by chance (e.g. due to genetic drift or
192 sampling error). Permutations reveal that similar pattern of apparent polygenic selection
193 according to the distributions of the out-of-bag (OOB) errors could indeed be obtained by
194 chance alone (Figure S4). Altogether population genomics analyses confirmed the
195 prediction that hatchery and natural origin salmon belong to a single panmictic population
196 within a given river. Our results cannot rule out that selection within one generation has
197 caused changes in allele frequency between hatchery and natural origin fish in genome
198 regions that were not screened. Nevertheless, they indicate that such an effect would be
199 modest relative to parallel differences observed at the epigenetic level.

200

201 **Discussion**

202 The decline of many wild stocks of Pacific salmon encouraged the development of
203 conservation hatcheries for enhancement. However, the hatchery environment during early
204 life stages induces significant differences in the biology, physiology and behaviour, and
205 ultimately in the fitness of hatchery-born fish [28]. Hatchery fish show higher reproductive
206 success than their wild counterparts in hatchery conditions but lower success when
207 released in the wild with an accumulative impact over a generation, advocating for
208 inadvertent domestication effects occurring after a single generation of hatchery rearing
209 [4,5,28]. Recent work provided evidence for a pronounced difference in gene expression
210 between wild and hatchery fish after one year of captivity despite no significant differences
211 at the genome level [3], as also reported between recently domesticated (five generations)
212 Atlantic Salmon and their wild congeners [29]. However, epigenetic variation was recently
213 associated with rapid adaptation to different natural environments (salt- vs fresh-water) in
214 the Threespine Stickleback (*Gasterus aculeatus*) [30]. Here, for the first time, our results
215 support the hypothesis that epigenetic modifications induced by hatchery rearing may
216 represent a potential explanatory mechanism for rapid change in fitness related traits
217 previously reported in salmonids. These similar epigenetic modifications were induced
218 independently in two genetically distinct populations and in apparent absence of overall
219 neutral and adaptive variation between hatchery and natural origin fish in these systems.
220 This demonstrates that rapid epigenetic modifications are induced every generation during
221 early development in the hatchery environment.

222 Indeed, combining a canonical multivariate approach (db-RDA) and pairwise F_{st}
223 estimates, we found no significant evidence for genetic differentiation between hatchery
224 and natural origin salmon, whereas genetic differentiation was highly significant between
225 rivers systems. These results confirm that hatchery and natural origin fish belong to a single
226 panmictic population, as predicted based on the hatchery programs applied in these rivers.
227 These “integrated programs” are based on local populations and involve spawning in
228 hatchery and natural environments. Hatchery and natural origin fish in each river are not
229 kept separate, thus hatchery origin fish spawn in both the hatchery and the natural habitat
230 as do natural origin fish, which can maintain high gene flow in the whole system.

231 Furthermore, no difference in genetic diversity (heterozygosity or inbreeding
232 coefficient) was observed between hatchery and natural origin salmon, hence not
233 supporting the hypothesis of increased probability of inbreeding depression in hatchery
234 fish for the populations we studied. Finally, we found no evidence of either large effect or
235 polygenic selection acting between hatchery and wild samples when using either a standard
236 genome scan approach or statistical framework appropriate for investigating effect of weak
237 selection in multiple regions of the genome. Therefore, our work corroborates recent
238 findings on juvenile Steelhead Trout (*Oncorhynchus mykiss*) showing that only a single
239 generation in captivity induced differences on the expression of hundreds of genes in
240 offspring reared in identical environments, without a noticeable overall genetic difference
241 ($F_{st}=0.008$; [3]).

242

243 In contrast to the apparent absence of significant genetic differences, our results
244 revealed highly significant differences in methylation profiles between hatchery and natural
245 origin salmon that were as pronounced as those observed between populations from
246 different rivers. Our results differ from a study that compared hatchery-born and wild
247 Steelhead Trout where no significant difference in methylation profiles was observed [12].
248 It may be that the impact of rearing environment on epigenetic modifications differs among
249 species. It also may be that the negative result for trout was due to the lower resolution of
250 the method available at that time, and indeed, the authors suggested that limited epigenetic
251 differences between hatchery and wild fish could not be ruled out [12]. With a different
252 approach offering substantial increase in genomic resolution, we found evidence for a
253 highly significant effect of hatchery-rearing on DNA methylation profiles in many regions of
254 the Coho Salmon epigenome, when controlling for population structure. Moreover, our
255 results revealed that the same epigenetic modifications developed in parallel between the
256 two independent study systems.

257 In Coho Salmon, it has been shown that hatchery fish are not as efficient as wild fish
258 for rapid seawater acclimation[15]. In addition, acclimation to seawater induces profound,
259 yet transient, changes in methylation levels in Brown Trout (*Salmo trutta* L.)[31]. We
260 showed that genomic regions demonstrating differential methylation profiles between
261 hatchery and wild salmon in both rivers were enriched for ion homeostasis and control of
262 body fluid levels functions, adding growing evidence that hatchery rearing may affect the
263 osmoregulatory process during smoltification. For instance, the serine/threonine-protein

264 kinase (SGK2) is a potent stimulator of epithelial Na⁺ channels [32]. Similarly, seawater
265 acclimation is associated with the level of SGK1 expression (no SGK2 or SGK3 ortholog
266 present in the killifish genome) in the killifish (*Fundulus heteroclistus*) [33]. Considering the
267 fundamental role of these biological functions during the smoltification (physiological
268 adaptation to seawater), and migration of parr salmonids to the ocean [34], we propose
269 that hatchery-induced epigenetic modifications during early developmental stages could be
270 partly responsible for the saltwater acclimation deficiency reported in previous studies
271 [35]. Moreover, neuromuscular communication, through regulation of Ca²⁺ levels, was
272 among the biological functions showing the most pronounced differences between hatchery
273 and natural origin salmon in both rivers. The enrichment was generally associated with a
274 hypermethylation in hatchery fish, notably of a major regulator of motoneuron signal
275 transmission through Ca²⁺ levels (CAMK2). This observation strongly suggests an alteration
276 of the neuromuscular communication that could reduce swimming performance as
277 previously reported in hatchery-reared Coho Salmon [19]. Finally, although these results
278 should be interpreted cautiously because they were limited to muscle tissue only, the
279 enrichment for overall synaptic signal control functions raise the hypothesis that hatchery
280 environment causes epigenetic modifications that may advocate a wealth of physiological
281 and endocrinal differences. For instance, epigenetic differences we observed at some major
282 neurological regulators such as HTR2C may play a role in the commonly observed
283 behavioural differences between captive-reared and wild fish, such as increased
284 aggressiveness, foraging, and boldness [30, 45–50]. This hypothesis could be tested by

285 comparing methylation profiles in the brain between fish with different aggressiveness,
286 foraging and boldness characteristics [41].

287 **Conclusions and implication for conservation and management**

288 The reduced genome representation method used here and the fact that we could
289 investigate only one tissue resulted in only a partial coverage of all possible epigenetic
290 differences that may exist between hatchery and natural origin salmon. As such, our results
291 should be interpreted as being conservative in reflecting the scale of epigenetic
292 modifications incurred in the hatchery environment. Nevertheless, our results suggest that
293 hatchery-rearing induces epigenetic variations that may alter the physiological (i.e. parr-to-
294 smolt) transformation as well as the locomotor capacity that may result in reduced smolt
295 fitness during juvenile seawater migration and ultimately, survival at sea. Whether or not
296 the observed epigenetic modifications are inherited and be acted upon by natural selection
297 cannot be answered from our results. Based on previous studies, it is reasonable to
298 hypothesize that hatchery-induced epigenetic modifications during early developmental
299 stages (post-fertilization and germ cell differentiation) almost certainly impact on lifelong
300 phenotypic changes [42]. For conservation purposes, different practices in hatchery rearing
301 are currently evaluated in order to circumvent the general observation that captive rearing
302 reduces fitness in the wild. Alternative rearing practices may differ in environmental
303 conditions (e.g. hatchery facilities or open lake), age at release (young fry or parr fish) or
304 nutrition (supplemented or not by commercial food). For instance, previous work reported
305 that salt-enriched food impact epigenetics and was correlated with a higher survival during
15

306 sea transfer [31]. Clearly, improving our understanding of the dual role of genetic and
307 epigenetic variation induced by captive rearing will contribute to development of the best
308 practices for the management and conservation of salmonid fishes and possibly numerous
309 other species that are managed through supplementation worldwide [43].

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319 **Data accessibility**

320 Sequences data are deposited on NCBI Short Read Archive database and will be made
321 available upon acceptance (NCBI Bioproject: PRJNA389610).

322 **Authors contribution**

323 J.LL, L.B designed the experiment. J.LL and M.L. analyzed the data and wrote the paper with
324 L.B. J.LL performed the laboratory work. T.B, R.W, K.K designed and conducted the
325 sampling. B.F.K, J.S.L and E.R provided the prepublication reference genome and re-
16

326 sequencing data. All co-authors contributed substantially to revisions.

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451 **Supporting Material**

452 Appendix 1: Supplementary experimental procedures, figures S1-S4 and tables S1-S3

453 **Figure legends**

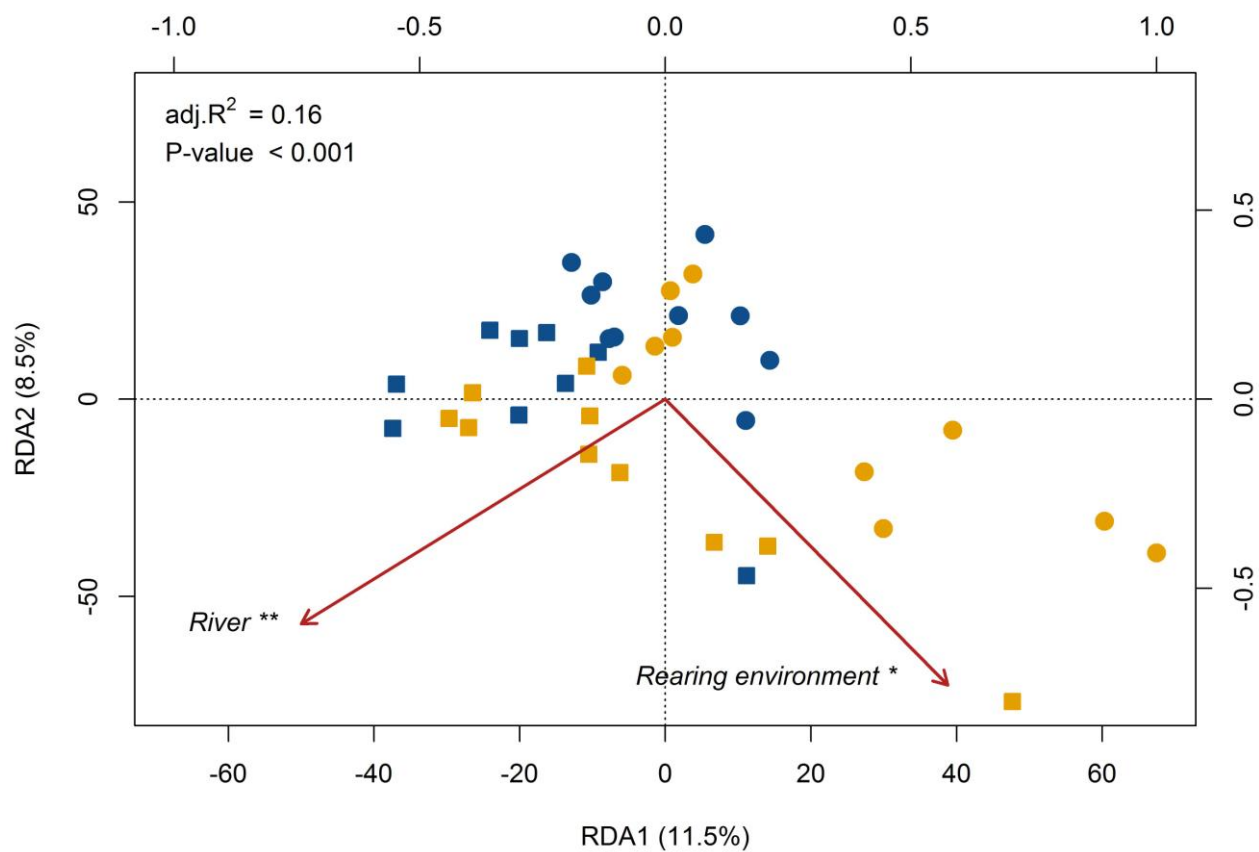
454 **Figure 1: Distance-base redundancy analysis (db-RDA) performed on the DNA**
455 **methylation levels of 131,807 1000-bp sliding window regions for each individual**
456 **(n=39).** Symbols represent rivers: Capilano (circle) and Quinsam (square). Colors
457 represent rearing environment: hatchery (blue) and wild (yellow). The db-RDA was
458 globally significant and explained 16% of all DNA methylation regions variation
459 (adj.R²=0.16). River of origin and rearing environment both significantly explained 8% of
460 the variation after controlling for each other with subsequent partial db-RDAs. Asterisks
461 represent p-value < 0.01 (**) and p-value < 0.05 (*) related to the explanatory factors.

462 **Figure 2: Circos plot of differentially methylated regions between hatchery and wild**
463 **fish.** Only the chromosomes (n = 27) and scaffolds (n =20) containing differentially
464 methylated regions are plotted. Barplots represent hypermethylated regions (red) and
465 hypomethylated regions (blue) in hatchery fish. Only annotated regions (blastx e-value <
466 10⁻⁶) are represented.

467 **Figure 3: Distance-base redundancy analysis (db-RDA) performed on the total**
468 **filtered 15,044 SNPs identified.** Symbols represent rivers: Capilano (circle) and Quinsam
469 (square). Colors represent captivity treatment: hatchery (blue) and wild (yellow). The db-
470 RDA was globally significant and explained 18% of all SNPs variation (adj.R²=0.18). River of
21

471 origin and sex explained significantly 16% and 2% of the variation after controlling for each
472 other with subsequent partial db-RDAs. Asterisks represent p-value<0.001 (***) and p-
473 value<0.05 (*) related to the explanatory factors.

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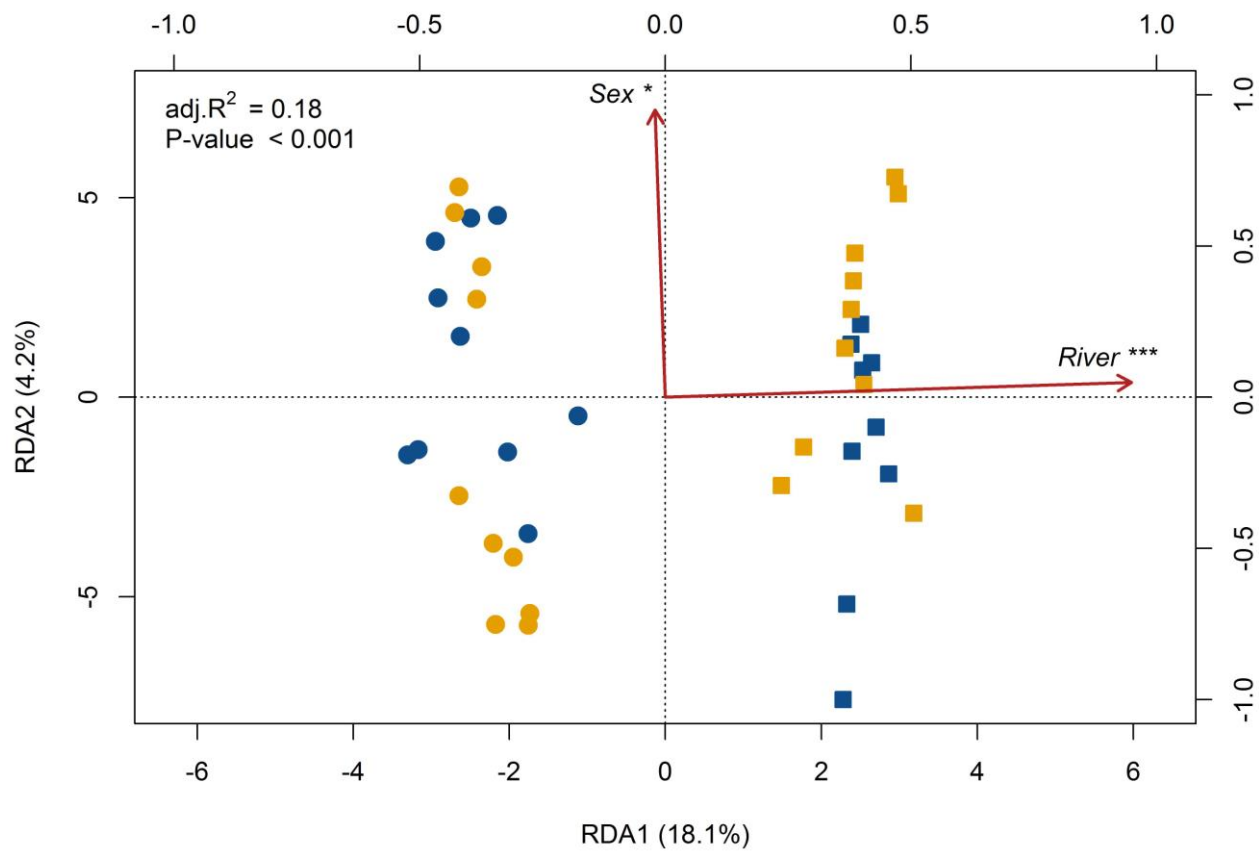


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480 Table 1: Differentially methylated regions (DMRs) and their association with Uniprot
 481 entries between hatchery and wild smolt Coho Salmon. Annotation was based on a blastx
 482 approach against Uniprot-Swissprot database (e-value<10⁻⁶). Only significant DMRs were
 483 included (methylation difference between hatchery and wild (Meth. diff.) >15%; q-value<
 484 0.01). Positive values are associated with hypermethylation relative to natural origin
 485 salmon. Transcript IDs correspond to the multi-tissue reference transcriptome for the Coho
 486 Salmon [14]. Each region represents a 1000 bp portion of one of the 30 chromosomes
 487 (Okis; Chr.) or additional scaffolds (scaffold; Scaff.) from the draft Coho Salmon genome
 488 assembly (GenBank assembly accession: GCA_002021735.1).

Symbol	Uniprot ID	Transcript ID	Gene name	Chr. / Scaff.	Met. diff.
5HT2C	Q5IS66	GDQG01000256.1	5-hydroxytryptamine receptor 2C	scaffold04777	23.3
ANK1	Q02357	GDQG01029546.1	Ankyrin-1	Okis08	21.1
AT2A2	Q03669	GDQG01041157.1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	Okis23	15.7
BCR	P11274	GDQG01010189.1	Breakpoint cluster region protein	Okis23	15.7
BCR	Q6PAJ1	GDQG01010373.1	Breakpoint cluster region protein	Okis23	15.7
BEGIN	Q9BUH8	GDQG01032671.1	Brain-enriched guanylate kinase-associated protein	Okis21	17.3
BTAF1	O14981	GDQG01038303.1	TATA-binding protein-associated factor 172	Okis11	15.6
BTAF1	O14981	GDQG01038304.1	TATA-binding protein-associated factor 172	Okis11	15.6
CHKA	P35790	GDQG01024040.1	Choline kinase alpha	Okis04	15.6
CSK21	Q60737	GDQG01021514.1	Casein kinase II subunit alpha	Okis17	19.6
DDX53	Q86TM3	GDQG01005021.1	Probable ATP-dependent RNA helicase DDX53	Okis04	16.1
DJC17	Q91WT4	GDQG01018295.1	DnaJ homolog subfamily C member 17	Okis14	17.3
DUS12	Q9UNI6	GDQG01021214.1	Dual specificity protein phosphatase 12	Okis28	-18.9

F172A	Q7T297	GDQG01019163.1	Protein FAM172A	Okis08	20.3
HXB3A	O42368	GDQG01036924.1	Homeobox protein Hox-B3a	Okis10	15.2
HYAL2	Q12891	GDQG01026988.1	Hyaluronidase-2	Okis05	-17.6
HYAL2	Q12891	GDQG01026990.1	Hyaluronidase-2	Okis05	-17.6
HYAL2	Q12891	GDQG01026993.1	Hyaluronidase-2	Okis05	-17.6
HYAL2	Q12891	GDQG01027002.1	Hyaluronidase-2	Okis05	-17.6
KCC1A	Q63450	GDQG01026516.1	Calcium/calmodulin-dependent protein kinase type 1	Okis17	17.9
KCC2B	P28652	GDQG01028157.1	Calcium/calmodulin-dependent protein kinase type II subunit beta	Okis29	18.9
LRC47	Q505F5	GDQG01001482.1	Leucine-rich repeat-containing protein 47	Okis17	15.6
OARD1	Q9Y530	GDQG01002423.1	O-acetyl-ADP-ribose deacetylase 1	Okis17	-17.1
P73	Q9XSK8	GDQG01009063.1	Tumor protein p73	Okis17	17.5
PCDH8	O95206	GDQG01028291.1	Protocadherin-8	Okis26	-15.9
PHB2	Q5XIH7	GDQG01016094.1	Prohibitin-2	Okis30	15.5
PKHA1	Q9HB21	GDQG01013238.1	Pleckstrin homology domain-containing family A member 1	Okis11	27.7
SAM12	Q0VE29	GDQG01021692.1	Sterile alpha motif domain-containing protein 12	Okis17	15.1
SGK2	Q9HBY8	GDQG01021555.1	Serine/threonine-protein kinase Sgk2	Okis17	18.8
SRSF9	Q5PPI1	GDQG01030164.1	Serine/arginine-rich splicing factor 9	Okis23	19.2
SRSF9	Q5PPI1	GDQG01030165.1	Serine/arginine-rich splicing factor 9	Okis23	19.2
STX16	Q8BVI5	GDQG01021767.1	Syntaxin-16	Okis01	15.3
TMC5	Q6UXY8	GDQG01032265.1	Transmembrane channel-like protein 5	scaffold04350	27.4
TSH2	Q9NRE2	GDQG01039096.1	Teashirt homolog 2	Okis03	22.2
UBE2K	P61087	GDQG01019710.1	Ubiquitin-conjugating enzyme E2 K	Okis19	15.3
Unknown	Unknown	GDQG01000757.1	Unknown	Okis07	15.6
Unknown	Unknown	GDQG01002050.1	Unknown	scaffold07390	15.2
Unknown	Unknown	GDQG01003870.1	Unknown	Okis21	17.3
Unknown	Unknown	GDQG01005352.1	Unknown	Okis19	16.5
Unknown	Unknown	GDQG01007903.1	Unknown	Okis04	16.1
Unknown	Unknown	GDQG01008658.1	Unknown	scaffold04821	18.0
Unknown	Unknown	GDQG01009276.1	Unknown	Okis13	15.6
Unknown	Unknown	GDQG01009277.1	Unknown	Okis13	15.6

Unknown	Unknown	GDQG01020612.1	Unknown	scaffold03114	15.0
Unknown	Unknown	GDQG01023154.1	Unknown	Okis04	16.1
Unknown	Unknown	GDQG01023155.1	Unknown	Okis04	16.1
Unknown	Unknown	GDQG01023157.1	Unknown	Okis04	16.1
Unknown	Unknown	GDQG01025110.1	Unknown	Okis17	19.6
Unknown	Unknown	GDQG01026416.1	Unknown	scaffold04821	18.0
Unknown	Unknown	GDQG01027613.1	Unknown	scaffold00446	17.9
Unknown	Unknown	GDQG01033116.1	Unknown	scaffold04821	18.0
Unknown	Unknown	GDQG01042549.1	Unknown	scaffold02804	19.2

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