Effect of riverscape and exotic introgression on the genetic structure and local adaptation of the endangered Mexican golden trout (*Oncorhynchus chrysogaster*).

Marco A. Escalante[1,2,*], Charles Perrier[1], Francisco J. García-De León[2], Arturo Ruiz-Luna[3], Enrique Ortega-Abboud[1] & Stéphanie Manel[1]

1. EPHE, PSL Research University, CNRS, UM, SupAgro, IND, INRA, UMR 5175 CEFE, 1919 route de Mende, 34293 Montpellier, France; escalante@iapg.cas.cz (M.A.E.); charles.perrier@cefe.cnrs.fr (C.P.); enrique.ortega@cefe.cnrs.fr (E.O.); stephanie.manel@cefe.cnrs.fr (S.M.)
2. Laboratorio de Genética para la Conservación, Centro de Investigaciones Biológicas del Noroeste, Instituto Politécnico Nacional, 195, Col. Playa Palo de Santa Rita, 23096 La Paz, BCS, Mexico; fgarciadl@cibnor.mx
3. Laboratorio de Manejo Ambiental, Centro de Investigación en Alimentación y Desarrollo AC, Sábalo-Cerritos s/n, 82100, Mazatlán, Sinaloa, Mexico; arluna@ciad.mx

Corresponding author: García-De León F. J., Laboratorio de Genética para la Conservación, Centro de Investigaciones Biológicas del Noroeste, Instituto Politécnico Nacional, 195, Col. Playa Palo de Santa Rita, 23096 La Paz, BCS, Mexico. Telephone: (+52) (612) 1238484 ext. 3405. Fax: (+52) (612) 1238405. E-mail: fgarciadl@cibnor.mx

[*] Marco A. Escalante current affiliation: Laboratory of Molecular Ecology, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Rumburská 89, 27721 Liběchov, Czech Republic

Abstract

How environmental and anthropogenic factors influence genetic variation and local adaptation is a central issue in evolutionary biology. The Mexican golden trout (*Oncorhynchus chrysogaster*), one of the southernmost native salmonid species in the world, is susceptible to climate change, habitat perturbations and the introduction of exotic rainbow trout (*O. mykiss*) for aquaculture. The present study aimed to explore the effect of both genetic introgression from *O. mykiss* and riverscape variables on the genetic variation of *O. chrysogaster*. Genotyping-by-sequencing (GBS) was applied to generate 9 767 single nucleotide polymorphisms (SNPs), genotyping 270 *O. chrysogaster* and aquaculture trout. Population genomics analyses were combined with riverscape genetics approaches. A small degree of admixture was identified between aquaculture and native trout, and significant effects of riverscape variables on genetic diversity were detected, as well as evidence of isolation by riverscape resistance within a basin. Gene-environment associations identified genes potentially implicated in adaptation to local hydroclimatic variables. This is an integrative study using a non-common multi-scale approach in landscape genetics for the conservation of an endangered riverine species.

Keywords: alien species, climate change, conservation, landscape genomics, riverscape genetics, river, salmonid, Sierra Madre Occidental.

1. INTRODUCTION

Natural and anthropogenic factors influence micro-evolutionary processes (e.g. migration, drift, and selection) generating spatial genetic patterns in endemic populations at complex environments. Thus, disentangling the respective role of environmental and anthropogenic variables on spatial genetic patterns has become a central issue in evolutionary biology and conservation ecology (Baguette et al. 2013; Richardson et al. 2016).

Particularly for lotic environments, landscape genetics and more specifically riverscape genetics can improve knowledge of the effects of riverine landscapes (riverscapes) and anthropogenic

activities (e.g. aquaculture with exotic species) on microevolutionary processes, determining the spatial genetic structure and genetic diversity of populations (Manel and Holderegger 2013; Davis et al. 2018). These processes are influenced by multiscale alterations like landscape fragmentation and water flow changes, which usually reduce habitat size, split environments and create barriers that isolate fish populations (Morita and Yamamoto 2002; Le Pichon et al. 2006). Gene flow and selection may act on different spatial scales (i.e. among basins, and between distant or nearby sites within basins). Therefore, it is necessary to consider multiscale and directionally approaches to analyse these environments. Particularly in complex hydrological networks, where the physical landscape structure, together with reduced pathways, determines patterns of genetic variation (Chaput-Bardy et al. 2008; Kanno et al. 2011). Thus, populations with high genetic structure exhibiting loss of genetic diversity at these complex and fragmented habitats require localized conservation efforts considering both ecological and evolutionary approaches, developing management plans in the face of global change (Tikochinski et al. 2018).

Recently published riverscape genetics studies on the effects of climate patterns and geographical barriers on genetic diversity, used only a small number of neutral molecular markers. Few studies have included large Next Generation Sequencing (NGS) datasets to assess gene-environment interactions (but see Bourret et al. 2013; Brauer et al. 2016; Hand et al. 2016; Hecht et al. 2015). Nevertheless, the use of NGS datasets might be helpful for the genetic monitoring of endangered populations providing valuable information for recovery actions (Faulks et al. 2017). Moreover, the potential to study the interplay of riverscape and exotic invasions in endemic species for conservation purposes has not been considered yet with empirical data (Table 1).

For the case of riverine species the introduction of exotic species for both, recreational and aquaculture purposes, might have detrimental effects in native populations due to predation, competition, introduction of diseases and genetic introgression (Penaluna et al. 2016). When the exotic species is phylogenetically close to the native it can causes genetic introgression, which could have extremely harmful effects since the modification of the native genetic pool might derive in the

loss of selective values (fitness) affecting survival skills in changing environments (Milián-García et al. 2015). Moreover, recent climatic variation have been associated with the spread of genetic introgression at riverine habitats, leading into irreversible evolutionary consequences for endangered species (Muhlfeld et al. 2017).

Both neutral and adaptive components of genetic diversity are the raw material for evolution and should be considered in conservation studies (Kokko et al. 2017). Climate conditions like temperature and precipitation might be drivers of adaptive processes in different salmonid species because they are associated with migration, reproduction, and mortality. Owing to this decisive association with hydroclimatic patterns, upstream salmonids are particularly vulnerable to detrimental effects arising from climate change and genetic introgression, as a consequence of a smaller habitat in comparison to marine and terrestrial environments (Parmesan 2006; Muhlfeld et al. 2017). But also, because of their special habitat requirements (Parmesan 2006; Ruiz-Luna et al. 2017). Yet detecting populations threatened by a decrease in genetic diversity (both neutral and adaptive) is vital for the conservation of endemic species (Hand et al. 2016).

The Mexican golden trout *(Oncorhynchus chrysogaster*; Needham and Gard, 1964) is one of the southernmost native salmonid species, inhabiting three different basins (Río Fuerte, Río Sinaloa, and Río Culiacán) in the highest parts of the Sierra Madre Occidental (SMO) mountains in northwestern Mexico (Hendrickson et al. 2002). Like all salmonids, this species is also susceptible to climate change, habitat perturbations, and the introduction of non-native species (i.e. rainbow trout for aquaculture purposes; Hendrickson et al. 2006). Many aspects of its biology like foraging habits, homing and reproductive behaviour, and dispersal are still unknown (but see Ruiz-Luna and García-De León, 2016). The existing genetic studies on this species have suggested that *O. chrysogaster* is genetically structured by hydrological basins (e.g.; Camarena-Rosales et al. 2008; Escalante et al. 2014). Nevertheless, those studies were conducted with neutral markers, a small number of sample sites, and sample designs that were not adapted to account for riverscape complexity. Thus, microevolutionary processes and their association with the adjacent riverscape are not yet well

understood with the consequent lack of management plans to protect this endangered species. Some studies for this species revealed a genetic substructure and introgression processes in locations near aquaculture facilities (Escalante et al. 2014; Abadía-Cardoso et al. 2015). Moreover, a recent simulation study on *O. chrysogaster* suggested that riverscape acts as a barrier against exotic introgression (Escalante et al. 2018). Thus, strong spatial genetic structure and local adaptation processes in *O. chrysogaster* arising from riverscape patterns are expected, with the consequence that this complex riverscape will prevent introgression processes in populations that are not near aquaculture farms.

This paper studies the effect of anthropogenic and natural variables on the genetic diversity and local adaptation of this endangered endemic salmonid in order to get technical elements to develop management and conservation strategies. Specifically, the following questions are addressed: 1) What is the impact degree of *O. mykiss* aquaculture escapes on the genetic structure of the endemic *O. chrysogaster*? 2) Which riverscape features (i.e. hydroclimatic and topographic variables) affect the extent and distribution of genetic diversity? 3) Are there genomic footprints of local adaptation to heterogeneous hydroclimatic features?

To tackle these questions, this study explored the genetic structure of 270 trout (242 *O. chrysogaster*, 24 aquaculture *O. mykiss*, and 4 lab hybrids) collected in 29 sites in three basins in northwestern Mexico and provided by federal agencies, and genotyped for 9 767 Single Nucleotide Polymorphisms (SNPs). The results were discussed in the context of conservation strategies for endangered riverine species, considering both ecological and evolutionary criteria.

2. MATERIALS AND METHODS

2.1. Study system and data collection

The survey was conducted in the Río Fuerte, Río Sinaloa, and Río Culiacán basins, at altitudes ranging from 1 965 to 2 730 m (Fig. 1). The heterogeneous riverscape of this area offers a diversity of terrestrial and aquatic habitats providing high endemism and biodiversity, with *O. chrysogaster* as one of the

105 main aquatic predators (Hendrickson et al. 2006). However, the introduction of rainbow trout for
106 aquaculture purposes has been reported since the 1860s, and was strongly supported by federal
107 agencies during the 1980s and 1990s (Escalante et al. 2014).

108 Five variables characterizing the riverscape and with high influence on *O. chrysogaster*
109 occurrence and genetic divergence were obtained from a previous study (Escalante et al. 2018):
110 precipitation of the driest month, temperature of the warmest quarter, river length (distance from
111 the lowermost to the uppermost part of the river above 1 500 m), slope, altitude, and stream order.
112 They were generated and/or processed from data available on the worldclim
113 (http://www.worldclim.org/) and the Japanese Space System
114 (http://www.jspacesystems.or.jp/ersdac/GDEM/E/4.html) websites with 1 km and 30 meter
115 resolution, respectively. Worldclim variables were geometrically corrected to 30 meter resolution (for
116 further details see Escalante et al. 2018). The effect of latitude and longitude, recorded with GPS, was
117 also tested.

118 Wild trout were sampled by electrofishing in 25 sample sites in Río Fuerte (10 sites), Río
119 Sinaloa (10 sites), and Río Culiacán (5 sites) basins during the winter and spring seasons of 2013, 2014,
120 and 2015. The sampling was conducted at both, cores and edges of *O. chrysogaster* ecological niche
121 defined in a former work (Escalante et al. 2018), and among multiple locations with high
122 environmental heterogeneity avoiding spatial autocorrelation (Fig. 1). Rainbow trout samples were
123 obtained from two aquaculture farms in Río Sinaloa basin. Additionally, seven farmed *O. chrysogaster*
124 samples and four lab hybrids from *O. chrysogaster* and *O. mykiss*, donated by the Mexican Institute of
125 Fisheries (INAPESCA), were included in the study for further genotyping. Small pieces of tissue (either
126 fin or muscle) were clipped from these samples and preserved in 95% ethanol, for later analysis.

127

128 **2.2. Genotyping by sequencing**

129 Genomic DNA was extracted for each individual following Qiagen DNeasy Blood & Tissue Kit protocol
130 (Qiagen, Hilden, Germany, http://www1.qiagen.com). DNA quality was checked using agarose gel

131 electrophoresis, and quantified using a NanoDrop spectrophotometer (Thermo Scientific) and the
132 Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). Then, DNA libraries were generated by GBS methods
133 (Elshire et al. 2011) at Cornell University in Ithaca, New York, using the ECOT221 enzyme. Finally,
134 single-read 100-bp sequencing was performed on an Illumina HiSeq2500.

135 The quality of raw sequences was controlled; per based sequence averaged quality as well as
136 presence of adapters and over represented sequences were checked with FastQC (Andrews 2010).
137 Reads were processed with Cutadapt (Martin 2011) to remove potential fragments of Illumina
138 adapters, allowing a 10% mismatch rate in the adapter sequence. The bioinformatics
139 software/pipeline Stacks 1.32 (Catchen et al. 2011; Catchen 2013) was used to demultiplex reads,
140 identify restriction site-associated DNA (RAD) loci and call SNPs. Reads with sequencing errors were
141 eliminated, then demultiplexed and trimmed to 85bp using the process_radtags module, where one
142 mismatch in the barcode sequence was allowed. The ustacks module was used, with a minimum stack
143 depth of 4x and a maximum distance allowed between stacks of 4 (6 for secondary reads). The
144 catalogue of loci was built using the cstacks module with n = 4. With the sstacks module, samples were
145 matched against the catalogue of loci. Finally, individuals were genotyped using the population
146 module, with at least 70% of individuals being genotyped, and a minimum read depth of 5x for each
147 locus and individual. Genotypes were exported in VCF format for further filtering. The SNP dataset
148 was filtered using VCFtools (Danecek et al. 2011), achieving a minimum average read depth ranging
149 from 8x to 40x across genotypes and a minor allele frequency of 1%. Loci deviating from Hardy-
150 Weinberg equilibrium (HWE, p-value ≤ 0.05) in at least one population, among the three populations
151 that were exempt from stocking and that had the larger number of individuals were removed.

152 Subsequently, six datasets were created including all loci after SNP calling at four different
153 spatial scales: Dataset A for population genetic analyses including all the genotyped individuals,
154 Dataset B for landscape genetics analyses across the entire study area discarding highly introgressed
155 native trout, Dataset C for landscape genetics analyses with central non-introgressed native
156 populations (at the centre of the study area), Dataset D for landscape genetics analyses of non-

157 introgressed native populations in Río Fuerte, Dataset E for landscape genetics analyses of native non-
158 introgressed populations in Río Sinaloa, and Dataset F for gene-environment associations across the
159 entire study area with non-introgressed native trout. Further information about datasets is included
160 in appendix S1.
161
162 **2.3. Population genomics analyses**
163 The genetic diversity of trout (Dataset A) was estimated from private polymorphisms (*PP*), expected
164 heterozygosity (H_E), and observed heterozygosity (H_O) using adegenet R package (Jombart, 2008).
165 Effective population size (N_E) was calculated by applying a molecular co-ancestry method (Nomura
166 2008) implemented in NeEstimator (Do et al. 2014).
167 The genetic differentiation between all sample sites (Dataset A) was assessed considering two
168 different approaches. First, pairwise genetic differentiation coefficients (F_{ST}) were calculated between
169 all sample sites in Genodive 3.0 (Meirmans and Van Tienderen 2004); this method uses an analysis of
170 molecular variance, performed between each pair of populations. Second, a phenogram was built in
171 adegenet from all individuals using the neighbour-joining algorithm (Saitou and Nei 1987) based on
172 Nei's genetic distance (Tamura and Nei 1993). Bootstrap confidence intervals were estimated from 10
173 000 permutations.
174 The unsupervised Bayesian clustering approach implemented in the package fastStructure
175 (Raj et al. 2014) was used to assess the genetic structure of *O. chrysogaster* and genetic admixture
176 with aquaculture trout. This approach infers population genetic structure for a large number of SNP
177 datasets without assuming predefined populations. Thus, fastStructure was run using Dataset A
178 including all SNPs from all the genotyped individuals. Based on an approximation of the number of
179 sample sites (total number of sampling sites + 1), K = 30 was considered the maximum value to avoid
180 underestimations in the number of clusters.
181
182 **2.4. Riverscape genetics analyses**

183 To explore the influence of riverscape on genetic diversity, a multiple linear regression was applied
184 between H_E and riverscape variables. A bidirectional stepwise selection procedure was performed (R
185 package MASS; Ripley, 2002) and the environmental variables with a significant influence on H_E were
186 selected based on Akaike criteria. Four analyses were run with different datasets (Dataset B, Dataset
187 C, Dataset D, and Dataset E), testing seven predictors each time: latitude, longitude, precipitation of
188 the driest month, temperature of the warmest quarter, river length, altitude, and stream order.

189 To examine the effect of riverscape on genetic differentiation, first a resistance surface was
190 derived from four riverscape features using ArcGIS v10.2 (ESRI, 2013). This surface was defined from
191 temperature of the warmest quarter, slope, stream order, and altitude raster files. Values were
192 assigned to the pixels at each raster representing the degree at which movement is obstructed. The
193 criteria reported by Escalante et al. (2018) was considered, as the species and the study area fit with
194 the current study. Then, resistance values from 0 to 10 were assign to the raster pixels (30 m
195 resolution) for each variable independently (see appendix S2). Thus, a riverscape resistance raster was
196 generated averaging the resistance values for the four environmental variables at each pixel. For
197 subsequent analyses in gdistance package (van Etten, 2012), resistance values were rescaled from 1
198 to 2, with 1 representing absence of riverscape resistance and 2 maximum resistance. Further
199 information about parameterisation is included in appendix S2.

200 Then, the gdistance R package (van Etten 2012) was used to calculate linear distance and
201 riverscape resistance matrices among sample sites within basins. This method simulates potential
202 movement for species in a spatially structured landscape, linking different dispersal functions and
203 connectivity thresholds using Djikstra's shortest path algorithm (Dijkstra 1959). Thus, two matrices
204 were generated under the hypothesis of Isolation by Riverscape Resistance (RR) at intra-basin scale:
205 RR for Río Fuerte populations (Matrix I) and RR for Río Sinaloa populations (Matrix II). Additionally,
206 two linear distance matrices were generated for Río Fuerte populations (Matrix III) and Río Sinaloa
207 populations (Matrix IV) in order to control differences in distance in subsequent analyses (for further
208 details, see appendix S2). To define the influence of isolation by riverscape resistance on genetic

209 distances, Partial Mantel tests were applied using PASSaGE package (Rosenberg and Anderson 2011).
210 Partial Mantel tests were performed between the regression of $F_{ST}/(1-F_{ST})$ and corresponding
211 riverscape resistance matrices (either Matrix I, either Matrix II), using geographical linear distance as
212 constant matrices (either Matrix III, either Matrix IV) allowing the control for differences in distance
213 (appendix S2). All tests were performed under 10 000 permutations and assuming no correlation.
214

215 **2.5. Detection of SNPs under divergent selection**

216 To detect *O. chrysogaster* SNPs potentially under selection, Dataset F was analysed using three
217 different software programs and considering two different approaches: a population (i.e. sampled
218 sites) outlier detection approach (1) and association tests between genotypes and continuous
219 climatic variables (i.e. riverscape adaptive genomics) (2).

220 First, the PCAdapt R package (Luu et al. 2016) was used to detect SNPs under selection in
221 approach 1. This method combines principal component analysis and Mahalanobis distances and
222 assumes that molecular markers excessively associated with population structure are candidates for
223 local adaptation. Based on the vector of z-scores, loci not following the distribution of the main cluster
224 of points are considered outliers. The analysis was run with a threshold of 10% and K=6 based on
225 fastStructure results (observed spatial genetic structure).

226 For approach 2, two gene-environment association software were used to test the effect of
227 temperature of the warmest quarter and precipitation of the driest month. These variables had
228 previously been suggested as significant adaptation drivers in salmonids (Hecht et al. 2015; Hand et
229 al. 2016). Using mixed models, both methods detect outlier loci through allele frequencies exhibiting
230 strong statistical correlations with environmental variables. Initially, Bayenv2 was applied (Günther
231 and Coop 2013), using an average of five independent runs (100 000 iterations). Also, the latent factor
232 mixed models (LFMM) algorithm implemented in the R package LEA (Frichot et al. 2013; Frichot and
233 François 2015) was run with five repetitions, 10 000 cycles, 5 000 burn-in, and K=6 (based on
234 fastStructure outputs of the spatial genetic structure) as a random factor in the regression analysis.

235 For both LFFM and Bayenv2, a threshold of 1% of total SNPs was defined to select the outlying SNPs
236 with the highest posterior probabilities.
237 A gene ontology analysis was conducted on the nucleotide sequences (80 bp) containing all
238 SNPs obtained from *O. chrysogaster*, using Dataset F. This analysis is based on a BLAST query (blastn)
239 of the sequences against peptides from the rainbow trout genome database (Berthelot et al. 2014).
240 Functional categorization by gene ontology terms (GO; http://www.geneontology.org) was carried out
241 using Blast2GO software (version 4.1, http://www.blast2go.com/). Subsequently, protein annotations
242 were filtered, retaining those from loci detected to be under divergent selection with an e-value cutoff
243 of $\leq 10^{-6}$.
244

245 **3. RESULTS**

246 **3.1. Genotyping by sequencing**

247 The total number of raw sequences obtained was 722 836 026 with an average of 1 300 000 reads per
248 individual. After SNP calling, a total of 270 individuals and 9 767 SNPs were retained for subsequent
249 analysis.
250

251 **3.2. Population genomics analyses**

252 The highest private polymorphism values (53) were found at the FVE sample site (see Table 2 for
253 abbreviations) in Río Fuerte while the lowest (0) were at the SMA and SPE sample sites in Río Sinaloa
254 and CED in Río Culiacán. For H_O, *O. chrysogaster* and rainbow trout lab hybrids obtained the highest
255 values (0.22) whereas wild *O. chrysogaster* collected at CED, CER, and CER2 in Río Culiacán obtained
256 the lowest (0.01). For H_E, the highest scores (0.16) were found in aquaculture trout collected at AQEB
257 in Río Sinaloa, while the lowest (0.01) were observed at CED, CER, and CER2 in Río Culiacán (Table 2).
258 Focusing on wild *O. chrysogaster* only, the highest heterozygosity (both H_E and H_O) was found in the
259 center of the study area at the limits between the three basins, while the lowest was located in the
260 periphery of the study area. *PP* values showed the opposite pattern (Fig. 2). N_E were successfully

261 estimated for 19 sample sites; the highest value (145.7) was found at SBA and the lowest (0.4) at SMA,
262 both in Río Sinaloa (Table 2).

263 Pairwise F_{ST} was highest (0.964) at the SPO and CED sample sites, which belonged to two
264 different basins (Río Culiacán and Río Sinaloa), while the lowest (0.008) was between CER and CER2
265 (both in Río Culiacán). Among basins the highest F_{ST} averages (0.83) were observed between sample
266 sites in Río Fuerte and Río Culiacán (for further information, see appendix S3). The high F_{ST} values
267 among some populations reflect high spatial genetic structure and are normally observed in trout
268 inhabiting heterogeneous landscapes (e.g. Abadía-Cardoso et al. 2015; Perrier et al. 2017).

269 The Nei phenogram detected 27 well-supported clades (>97 % of bootstrapping value), in all
270 cases made up of geographically close sampling sites. Interestingly, aquaculture trout (AQEB and
271 AQSM) showed greater genetic similarity (given its lower branch length) with lab hybrids (H, clearly a
272 heterogeneous group where it is known that hybrids have been artificially obtained). Also, the trout
273 collected in SSM fall within the aquaculture clade, suggesting that they are rainbow trout that escaped
274 from the farming facilities (see below, Fig. 3).

275 The unsupervised Bayesian clustering approach (fastStructure) identified six distinct wild
276 genetic clusters defined by geography, and one aquaculture cluster: Eastern Fuerte; Central Fuerte;
277 Southern Fuerte, Eastern Sinaloa, and Northern Culiacán; Western Fuerte and Western Sinaloa;
278 Central Sinaloa; Southern Culiacán; and Aquaculture (Fig. 3b and 4). Trout from Western Fuerte
279 collected at FEM, FLC, and FLT showed admixture with trout from Central Sinaloa. Trout from Central
280 Sinaloa collected at SBA showed genetic admixture with trout from Western Fuerte, while the trout
281 collected at SHO and one individual from SLO showed admixture with aquaculture trout. Trout from
282 Eastern Sinaloa collected at SCE, SCS, and SPE exhibited genetic admixture with Western Fuerte. Some
283 trout from Southern Culiacán collected at CSJN exhibited genetic admixture with aquaculture trout,
284 and one individual belonged entirely to the aquaculture cluster. Trout collected at SSM near the San
285 Miguel aquaculture farm belonged to the aquaculture cluster and were identified as aquaculture
286 trout, perhaps as a product of aquaculture escapes. Finally, for lab hybrids, half of their genome

287 belonged to native *O. chrysogaster* from the Central Fuerte cluster (parental population), and the
288 other half to the aquaculture cluster. A comparison of different K values shows a similar genetic
289 structure (across K=6, K=7, and K=8) with slight differences for K=6, where trout from FSJ (Central
290 Fuerte in K=7 and 8) were joined with trout from FCA, FON, CAB, SCE, SPE, and SCS (appendix S4). In
291 general, the genetic admixture between native and farmed trout was very slight. Moreover, for two
292 genetic clusters (i.e. Southern Fuerte, Eastern Sinaloa, and Northern Culiacán; and Western Fuerte
293 and Western Sinaloa; Fig. 3b) the observed spatial genetic structure do not follow a basin delineating,
294 suggesting human translocations of native trout or potential connectivity among basins during flood
295 events, or both. Interestingly, while analysing the dataset excluding outliers (with only neutral SNPs)
296 the same genetic structure was outputted (results not shown).
297
298 **3.3. Riverscape genetics analyses**
299 Only the analyses performed with the Central populations (Dataset C) and Río Fuerte populations
300 (Dataset D) showed significant correlations between H_E and riverscape predictors. For Dataset C,
301 correlations between H_E and latitude, precipitation of the driest month, and altitude were found
302 (Table 3). Moreover, for Dataset D, H_E showed correlations with latitude, longitude, temperature of
303 the warmest quarter, precipitation of the driest month, river length, and stream order (Table 3).
304 The Partial Mantel test between pairwise $F_{ST}/(1-F_{ST})$ and riverscape resistance at Río Sinaloa
305 was highly statistically significant ($p = 0.0002$ and $r = 0.87$), suggesting a strong influence of riverscape
306 resistance on the genetic divergence within this basin. Interestingly the effect of riverscape resistance
307 was not observed at Río Fuerte, probably due to the sampling strategy (Table 4). Is worth mentioning
308 that at Río Sinaloa the sampling sites distribution is restricted to a small area (few streams) following
309 the river length, but that is not the case for Río Fuerte.
310
311 **3.4. Detection of SNPs under divergent selection**

312 A total of 566 outlier loci were detected with PCAdapt, Bayenv2, and LFMM (Fig. 5). PCAdapt identified
313 a total of 278 outliers. On the other hand, Bayenv2 and LFMM identified a total of 306 outliers
314 correlated with environmental variables (temperature of the warmest quarter and precipitation of the
315 driest month).

316 After quality filters in the gene ontology analysis, 21 SNP loci under divergent selection, which
317 had protein annotations, were retained (Table 5). Most of these annotations are associated with
318 biological functions (e.g. growth, reproduction and thermal tolerance), which in turn could be
319 associated with hydroclimatic variables.

320

321 **4. DISCUSSION**

322 Our main objective was to explore the effect of both riverscape and cultured rainbow trout farm
323 escapes, on the genetic diversity and local adaptation of an endemic salmonid. Low genetic admixture
324 between aquaculture *O. mykiss* and native trout was found for the populations sampled. A significant
325 influence of riverscape variables on genetic diversity was detected, in addition to evidence of isolation
326 by riverscape resistance within a basin. Outlier detection and gene ontology analyses identified genes
327 that could be implicated in adaptation to local climate heterogeneity. This integrative approach
328 reveals that riverscape influence acts on different microevolutionary processes depending on the
329 spatial scale: *i) local scale:* genetic divergence might be defined by hydrologic and topographic
330 gradients and ruptures at intra-basin level. *ii) regional scale:* the effect of temperature and
331 precipitation on genetic diversity is observed within basins, but also among non-distant places at
332 different basins. iii) *broad scale:* temperature and precipitation gradients could be implicated in local
333 adaptation at large scales. These findings, in addition to shedding light on riverscape genomics, are
334 discussed in the context of the development of management strategies for endangered riverine
335 species in order to preserve both genetic diversity and adaptive potential in the face of global change.

336

337 **4.1. Absence of strong admixture between *O. chrysogaster* and aquaculture *O. mykiss***

The low genetic admixture (introgression) between native and exotic trout found in this study can be explained by the lack of proximity to aquaculture sites, as intensive aquaculture activities were not detected in the study area (authors' field observations). Other studies at broader spatial scales found evidence of high genetic admixture, but only for undescribed Mexican trout forms in southernmost SMO areas, characterised by intensive aquaculture activities (Abadía-Cardoso et al. 2015; Escalante et al. 2014). In addition to the overall geographic remoteness of aquaculture activities, the low levels of exotic introgression found in this study might be explained by the local riverscape acting as a boundary against exotic introgression. This barrier effect caused by riverscape (i.e. altitude decrease; slope, stream order and temperature increase) was suggested in recent simulation studies for this same species and *O. clarkii lewisi* (Landguth et al. 2016), but also by empirical data for *O. clarkii bouvieri* (Gunnell et al. 2008). The extensive introduction of exotic trout has been documented as one the greatest threats to the entire endemic Pacific trout complex (Miller et al. 1989; Bahls 1992; Penaluna et al. 2016). Also, the harmful effects of exotic salmonid invasions have been recognised in native salmonid populations all over the world through parasites, native food web alterations, competition, and replacement of the native gene pool (Heggberget et al. 1993; Fausch 2007; Muhlfeld et al. 2009; Marie et al. 2012; Vera et al. 2017). In the SMO, particularly, due to economic interests, it is expected that aquaculture activities will increase in coming years, while their impact on native trout populations is still little known, making more challenging the application for management plans with conservation purposes (Hendrickson et al. 2002).

However, the low admixture between native and exotic trout detected in this study highlights the beneficial effect of environmental heterogeneity and low aquaculture activity, but can inevitably be reversed if such activity increases. Therefore, the proper use of native trout for aquaculture activities in the area considering only closely related populations and respecting the ecological niches, may offer an alternative that makes it possible to avoid the harmful effects of exotic trout invasions. The low exotic admixture with aquaculture trout observed in *O. chrysogaster* in previous works supports our results. Indeed, in the specific case of Arroyo Agua Blanca in Río Culiacán, former studies

364 reported high levels of genetic admixture with *O. mykiss* in an analysis of wild samples collected in
365 1997 (Escalante et al. 2014), while samples collected in 2015 used in this study did not display genetic
366 admixture. These results are encouraging and revealed that the genetic pool of riverine species could
367 still be preserved despite the existence of aquaculture practices in their distribution area. Thus,
368 conservation strategies may be considered to avoid the probable harmful effects of exotic
369 introgression in *O. chrysogaster* populations, banning or strongly regulating rainbow trout
370 aquaculture.
371

372 **4.2. Riverscape drivers of genetic diversity**

373 The higher values of expected heterozygosity were associated with altitude increase. While low levels
374 of heterozygosity were correlated with an increase in latitude, longitude, temperature of the warmest
375 quarter, stream order, and precipitation of the driest month. Is worth mentioning that this effect was
376 observed at regional and intra-basin scales (Dataset C for central populations and Dataset D for Río
377 Fuerte populations); but not at the largest scale (Dataset B for all native populations) neither with the
378 smallest dataset (Dataset E for Río Sinaloa). The samples continuously distributed along wide
379 environmental gradients could explain the effect of these environmental variables on genetic diversity
380 (Schoville et al. 2012). This is not the case for Dataset B where populations from the southernmost
381 part of Río Culiacán are isolated from the vast majority of sampling sites. Neither for Dataset E, where
382 sampling locations are concentrated in a small area at Río Sinaloa with low hydroclimatic variation
383 among sampling sites.

384 The genetic diversity values observed here are lower than those reported for trout from
385 northern latitudes (e.g. Linløkken et al. 2016; Perreault-Payette et al. 2017; Wenne et al. 2016). From
386 an ecological point of view, the main sources of variation in genetic diversity are*: i) variation in*
387 *effective size*: populations with large effective sizes are expected to have higher heterozygosity than
388 populations with smaller effective sizes, as they have a larger number of breeders that keep allele
389 frequencies stable (Höglund 2009). *ii) space, mainly through variation in the environment and*

habitats: isolated populations with low immigration rates may exhibit inbreeding and reduced gene exchange (gene flow) with closely related populations, as they are exposed to genetic drift (Riginos and Liggins 2013). iii) *contemporary and historical events, and human disturbances*: the fragmentation or connection of populations with the consequent variations in genetic diversity due to microevolutionary processes (drift, migration, mutation, etc.) (Hewitt 2000; Banks et al. 2013).

Negative influences of temperature and stream order, as well as a positive influence of altitude on H_E were detected. The optimal niche conditions for *O. chrysogaster* are located at the coldest parts of the SMO, but also low stream orders at high altitudes are associated with high quality habitats for this species (Escalante et al., 2018; Ruiz-Luna et al., 2017). Thus, populations inhabiting places with non-optimal habitat/niche conditions might be expose to higher mortality rates. Therefore, exhibiting lower population sizes that is reflected in a generalised lack of heterozygosity, which in turn results in endogamy due to population substructure and a lower effective size (Hartl and Clark 1997). Consequently, those populations may have a small number of breeders suffering from a loss of genetic diversity due to genetic drift.

The occurrence of extreme hydroclimatic events during embryo incubation may have catastrophic consequences in trout populations, causing strong variations in population sizes (Hand et al. 2016). In this study, a negative influence was found from precipitation of the driest month on H_E. This correlation may be explained by the fact that *O. chrysogaster* mates during the dry season (December – March; García-De León et al. 2016). Thus, flash flood events during the reproductive period of *O. chrysogaster* may be causing embryo mortality and, consequently, dramatic reductions in population size that translate into a decline in heterozygosity. A negative influence from precipitation on heterozygosity has also been observed in native steelhead trout in the USA, with similar life history traits and habitat conditions to *O. chrysogaster* (Narum et al. 2008).

The decline in heterozygosity is greater in the periphery of the study area (i.e. east of Río Fuerte, southwest of Río Fuerte, northwest of Río Sinaloa, and south of Río Culiacán) (Fig. 3). Those zones are defined in former studies at the limits of the species ecological niche (Ruiz-Luna et al. 2017;

416 Escalante et al. 2018). This pattern of genetic diversity deficit at peripheries has been broadly observed
417 in a wide number of species (de Lafontaine et al. 2018). Additionally, the small N_E found in our study
418 (≤146) fall far short of what is needed (≥ 500) to preserve long-term viability in salmonids, which raises
419 the question of the long-term survival of the populations under study (Koskinen et al. 2002; Rieman
420 and Allendorf, 2001). It is possible that the low values of N_E (≥ 500) are causing a lack of a positive
421 correlation between the N_E and H_E reported in this study. Certainly the effects of drift are shown in
422 sites with high values of N_E and low values of H_E and vice versa, for example FLQ had a N_E of 116.8 and
423 a H_E of 0.05, while FCA had a N_E of 8.2 and a H_E of 0.13 (Table 2). However, generally low N_E and H_E
424 values are common in species that experience rapid expansions after glaciation periods because of
425 habitat shifts and isolation (Hewitt 2000). Thus, this decline in genetic diversity may be due to a
426 combination of several factors: bottlenecks occurring after the colonization of the SMO long ago
427 (Behnke et al. 2002), and/or habitat fragmentation due to riverscape resistance impeding immigrant
428 exchange among populations (Channell and Lomolino 2000; Behnke et al. 2002; Eckert et al. 2008;
429 Ruzzante et al. 2016).

430 Under any past or present scenarios that may explain the low levels of N_E, the continuous loss
431 of genetic diversity could be avoided by the translocation of individuals between small and isolated
432 populations that contain the same gene pool, ensuring the preservation of genes involved in local
433 adaptation (Sato and Harada, 2008). Translocation is a conservation strategy that can be useful to
434 avoid genetic diversity reduction, and could be achieved by ensuring founding populations including
435 as many individuals as possible, augmenting those relocated populations after establishment with new
436 trout from existing populations (Faulks et al. 2017). However, this strategy requires extensive genetic
437 monitoring of both existing and relocated populations. The definition of natural protected areas at the
438 highest parts of SMO where *O. chrysogaster* inhabits strictly regulating land use and land change
439 activities there, can also help to preserve dispersal corridors among populations maintaining gene
440 flow but also the habitat quality, which is necessary to ensure the native genetic diversity (Olsen et al.
441 2017).

4.3. Riverscape drivers of genetic divergence

The influence of riverscape structure on genetic divergence has already been tested in salmonids, with riverscape resistance having been defined as a fundamental driver of gene flow (Kanno et al. 2011; Landguth et al. 2016). It is documented that partial Mantel tests are very robust in their ability to detect the driving processes that are responsible for genetic variation patterns across landscapes (Cushman and Landguth 2010). In this study, partial Mantel tests suggests an influence of riverscape resistance in genetic variation only at Río Sinaloa. This effect might be due to sampling strategy, which was more adequate to a least cost path approach in Río Sinaloa with the sampling sites homogeneously distributed along few streams in a small spatial scale, compared with Río Fuerte where sampling sites are distributed in a larger spatial extent at several streams. Nevertheless, demo-genetic simulations suggested a strong influence of riverscape resistance in genetic divergence of *O. chrysogaster* at Río Fuerte (Escalante et al. 2018). That effect of riverscape at Río Fuerte might be evidenced with a more continuous sampling strategy along the stream lengths. Actually, by linking what is already suggested by simulated data at Río Fuerte with an appropriated sampling of empirical data it is possible to detect with a good signal the effect of riverscape in genetic divergence (Cushman and Landguth 2010). The effect of riverscape resistance on gene flow may determine adaptation to local environments, due to the fact that gene flow allows the entry of new genes, and then, through recombination during sexual reproduction, a new combination of genes is produced and populations gain genetic advantages to cope with changing environments (Kokko et al. 2017). For endangered species living in restricted heterogeneous habitats, it is vital to understand the relationship between riverscape and gene flow in order to define management units based on populations with historical gene flow, and which occupy similar ecological niches (Crandall et al. 2000; Olsen et al. 2017; Schmidt et al. 2017). It is important to use large genomic datasets to define the influence of landscape patterns on gene flow processes among populations at local scales (within basins), to then understand adaptive variation across wider scales (along different basins). Thus, the development of riverscape resistance

468 surfaces could help for the conservation of native trout, defining and preserving dispersal corridors to
469 maintain gene flow among closely related populations with low genetic diversity. However, additional
470 sampling strategies are necessary to increase our understanding about the effect of riverscape
471 resistance in the genetic variation of *O. chrysogaster*, particularly at Río Fuerte.
472

473 **4.4. Detection of SNPs under divergent selection**

474 The 306 SNPs correlated with hydroclimatic variables (i.e. precipitation of the driest month and
475 temperature of the warmest quarter), suggest that these environmental variables may act as selective
476 factors in *O. chrysogaster*. The influence of temperature and precipitation on adaptive genetic
477 variation has also been suggested for steelhead trout from the Inner Columbia River Basin (Hand et
478 al. 2016). Temperature has been defined as a potential driver in adaptive processes in salmonids
479 mainly because ectothermic body temperature is closely associated with the environment (Hecht et
480 al. 2015; Hand et al. 2016). Moreover, variations in temperature and precipitation affect phenomena
481 such as changes in dispersal and reproduction timing, age at maturity, growth, fecundity, and survival
482 (Crozier & Hutchings, 2014; Hecht et al., 2015).

483 Among the annotated gene functions detected by a gene ontology analysis in outlier loci,
484 actin-binding proteins might be playing an important role in water flow and temperature acclimation,
485 as it is reported in other species like the European hake (Milano et al. 2014). Thus, adaptive genetic
486 variation across the study area may increase among populations exposed to different temperature
487 and precipitation regimes. However, analyses of adaptive variation, through the use of population
488 genetics analyses of outlier loci, are needed to confirm this. A study performed on European hake
489 across the Mediterranean Sea revealed that outlier loci associated with actin-binding proteins are
490 driving local adaptation to water temperature (Milano et al. 2014). Moreover, protein binding is also
491 associated with trout growth and flesh quality, which in turn may be associated with stream flow and
492 water temperature (Salem et al. 2010). Even though our results suggest potential adaptation of *O.*
493 *chrysogaster* to local conditions, these findings should be interpreted with caution. It is acknowledged

494 that the sample size is small and the averaged genetic differentiation is high, and may result in false
495 positives in gene-environment associations (Hoban et al. 2016).

496 Our findings, together with previous work on related species, indicate that salmonids develop
497 adaptations to cope with changing climatic conditions (Hecht et al. 2015; Bourret et al. 2013).
498 However, faced with the threat of man-made global warming, habitat fragmentation and exotic
499 introduction the species' risk of extinction is high, especially for small populations with putative fitness
500 loss. Indeed, actions at local scales may not be enough to ensure the survival of the species (Rahel et
501 al. 2008; Lawler et al. 2010). On this basis, federal agencies should consider climate change in all their
502 management plans, integrating multidisciplinary approaches to understand the effect of climate
503 fluctuations on native species at different scales. The landscape genomics approach considered in this
504 study may be helpful in the development of these management strategies. Finally, predictive models
505 that associate climate change with genomic diversity in order to maintain local adaptation skills are
506 urgent to design the best conservation strategies for all endangered salmonid species in North
507 America.

508

509 **4.5. Conclusions**

510 The integrative study presented here described the effect of both landscape and anthropogenic
511 factors on microevolutionary processes at different spatial scales, and gave information about possible
512 effects of hydroclimatic variables on local adaptation. This kind of approach has not been considered
513 in conservation genomics studies, as generally, they only focus on a single spatial scale, and not
514 accounting that genomic data could answer microevolutionary questions at multi-scale level. Our
515 findings suggested very low genetic introgression, but also, that riverscape patterns have an effect on
516 genetic diversity and adaptive genetic variation. The approach presented here may be useful in
517 developing conservation strategies for endangered riverine fish species that consider both ecological
518 and evolutionary aspects.

519

ACKNOWLEDGEMENTS

This research was supported under projects funded by CONACYT (Ref. CB-2010-01-152893) and ECOSNORD (M14A04). Marco A. Escalante was a recipient of CONACYT fellowships to perform his PhD studies. We are especially grateful to R. Hernández G., L. A. Ramirez H., and other members of CIAD staff for all their support in the planning, logistics and performing of field surveys. We are grateful to G. Ingle from the Mexican Fisheries Institute (INAPESCA) for facilitating trout samples. Thanks also to T. Durán for his support during field surveys. We want to thank all the local guides who helped us during trout sampling, especially Ricardo Silvas ''El Chalote''. Also, we are grateful to the municipal government of Guanaceví, PRONATURA, and WWF for their assistance with field surveys. Thanks to all members of the ''Truchas Mexicanas'' binational group, and especially G. Ruiz C. and F. Camarena R. Thanks to B. Shepard, C. Kruse, J. Dunham, and others for donating electrofishing equipment. We also wish to thank F. Valenzuela Q., C. A. Rivera, A. Delongeville and the staff of the Molecular Markers Platform at the CEFE and the Laboratorio de Genética para la Conservación at CIBNOR for their support in molecular biology work.

REFERENCES

Abadía-Cardoso, A., Garza, J.C., Mayden, R.L., and García-De León, F.J. 2015. Genetic Structure of Pacific Trout at the Extreme Southern End of Their Native Range. PLoS One **10**(10): e0141775. doi:10.1371/journal.pone.0141775.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V.M., and Turlure, C. 2013. Individual dispersal, landscape connectivity and ecological networks. Biol. Rev. **88**(2): 310–326. doi:10.1111/brv.12000.

Bahls, P. 1992. The status of fish populations and management of high mountain lakes in the western United States. Northwest Sci. **66**(1): 183–193. doi:10.1016/0006-3207(94)90036-1.

Banks, S.C., Cary, G.J., Smith, A.L., Davies, I.D., Driscoll, D.A., Gill, A.M., Lindenmayer, D.B., and Peakall, R. 2013. How does ecological disturbance influence genetic diversity? Trends Ecol. Evol. **28**(11): 670–679. Elsevier.

Behnke, R.L., Tomelleri, J.R., and McGuane, T. 2002. Trout and Salmon of North America. Free Press, Chicago. Available from https://books.google.es/books?id=3WlHElmgQVgC.

Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noël, B., Bento, P., Da Silva, C.,

Labadie, K., Alberti, A., Aury, J.-M., Louis, A., Dehais, P., Bardou, P., Montfort, J., Klopp, C., Cabau, C., Gaspin, C., Thorgaard, G.H., Boussaha, M., Quillet, E., Guyomard, R., Galiana, D., Bobe, J., Volff, J.-N., Genêt, C., Wincker, P., Jaillon, O., Roest Crollius, H., and Guiguen, Y. 2014. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat. Commun. **5**: 3657. doi:10.1038/ncomms4657.

Blanchet, S., Rey, O., Etienne, R., Lek, S., and Loot, G. 2010. Species-specific responses to landscape fragmentation: Implications for management strategies. Evol. Appl. **3**(3): 291–304. doi:10.1111/j.1752-4571.2009.00110.x.

Bourret, V., Dionne, M., and Bernatchez, L. 2014. Detecting genotypic changes associated with selective mortality at sea in Atlantic salmon: Polygenic multilocus analysis surpasses genome scan. Mol. Ecol. **23**(18): 4444–4457. doi:10.1111/mec.12798.

Bourret, V., Dionne, M., Kent, M.P., Lien, S., and Bernatchez, L. 2013. Landscape genomics in Atlantic salmon (Salmo salar): searching for gene-environment interactions driving local adaptation. Evolution **67**(12): 3469–87. doi:10.1111/evo.12139.

Bradbury, I.R., Hamilton, L.C., Robertson, M.J., Bourgeois, C.E., Mansour, A., and Dempson, J.B. 2014. Landscape structure and climatic variation determine Atlantic salmon genetic connectivity in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. **71**(2): 246–258. doi:10.1139/cjfas-2013-0240.

Brauer, C.J., Hammer, M.P., and Beheregaray, L.B. 2016. Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. Mol. Ecol. **25**(20): 5093–5113. doi:10.1111/mec.13830.

Camarena-Rosales, F., Ruiz-Campos, G., De La Rosa-Vélez, J., Mayden, R.L., Hendrickson, D.A., Varela-Romero, A., and García-De León, F.J. 2008. Mitochondrial haplotype variation in wild trout populations (Teleostei: Salmonidae) from northwestern Mexico. Rev. Fish Biol. Fish. **18**(1): 33–45. doi:10.1007/s11160-007-9060-z.

Castric, V., Bonney, F., and Bernatchez, L. 2001. Landscape Structure and Hierarchical Genetic Diversity in the Brook Charr, Salvelinus Fontinalis. Evolution (N. Y). **55**(5): 1016–1028. doi:10.1111/j.0014-3820.2001.tb00618.x.

Catchen, J.M. 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. **22**(11): 3124–3140. doi:10.1111/mec.12354.Stacks.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J.H., and De Koning, D.-J. 2011. Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. Genes|Genomes|Genetics **1**(3): 171–182. doi:10.1534/g3.111.000240.

Channell, R., and Lomolino, M. V. 2000. Dynamic biogeography and conservation of endangered species. Nature **403**(6765): 84–86. doi:10.1038/47487.

Chaput-Bardy, A., Lemaire, C., Picard, D., and Secondi, J. 2008. In-stream and overland dispersal across a river network influences gene flow in a freshwater insect, Calopteryx splendens. Mol. Ecol. **17**(15): 3496–3505. doi:10.1111/j.1365-294X.2008.03856.x.

Cooke, G.M., Landguth, E.L., and Beheregaray, L.B. 2014. Riverscape genetics identifies replicated ecological divergence across an Amazonian ecotone. Evolution (N. Y). **68**(7): 1947–1960. doi:10.1111/evo.12410.

Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., and Wayne, R.K. 2000. Considering evolutionary processes in conservation biology. Trends Ecol. Evol. **15**(7): 290–295. doi:10.1016/S0169-5347(00)01876-0.

Crockett, E.L. 1998. Cholesterol Function in Plasma Membranes from Ectotherms: Membrane-Specific Roles in Adaptation to Temperature. Am. Zool. **38**(2): 291–304. doi:10.1093/icb/38.2.291.

Crozier, L.G., and Hutchings, J.A. 2014. Plastic and evolutionary responses to climate change in fish. Evol. Appl. **7**(1): 68–87. doi:10.1111/eva.12135.

Cushman, S.A., and Landguth, E.L. 2010. Spurious correlations and inference in landscape genetics. Mol. Ecol. **19**(17): 3592–3602. doi:10.1111/j.1365-294X.2010.04656.x.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., and Durbin, R. 2011. The variant call format and VCFtools. Bioinformatics **27**(15): 2156–2158. doi:10.1093/bioinformatics/btr330.

Davis, C.D., Epps, C.W., Flitcroft, R.L., and Banks, M.A. 2018. Refining and defining riverscape genetics: How rivers influence population genetic structure. Wiley Interdiscip. Rev. Water **5**(2): e1269. John Wiley & Sons, Inc. doi:10.1002/wat2.1269.

Dijkstra, E.W. 1959. A Note on Two Problems in Connexion with Graphs. Numer. Math. **1**: 269–27. Available from http://www-m3.ma.tum.de/foswiki/pub/MN0506/WebHome/dijkstra.pdf [accessed 20 May 2018].

Dionne, M., Caron, F., Dodson, J.J., and Bernatchez, L. 2008. Landscape genetics and hierarchical genetic structure in Atlantic salmon: The interaction of gene flow and local adaptation. Mol. Ecol. **17**(10): 2382–2396. doi:10.1111/j.1365-294X.2008.03771.x.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. **14**(1): 209–214. doi:10.1111/1755-0998.12157.

Eckert, C.G., Samis, K.E., and Lougheed, S.C. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. Mol. Ecol. **17**(5): 1170–1188. doi:10.1111/j.1365-294X.2007.03659.x.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., and Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One **6**(5): 1–10. doi:10.1371/journal.pone.0019379.

Escalante, M.A., García-De-León, F.J., Dillman, C.B., de los Santos Camarillo, A., George, A., de los A. Barriga-Sosa, I., Ruiz-Luna, A., Mayden, R.L., and Manel, S. 2014. Genetic introgression of cultured rainbow trout in the Mexican native trout complex. Conserv. Genet. **15**(5): 1063–1071. doi:10.1007/s10592-014-0599-7.

Escalante, M.A., García-De León, F.J., Dillman, C.B., De los Santos-Camarillo, A., George, A., and de los A. Barriga-Sosa, I. 2016. Introgresión genética de la trucha arcoíris exótica en poblaciones de trucha dorada mexicana. *In* La Trucha Dorada Mexicana. *Edited by* A. Ruiz-Luna and F.J. García-De León. CIAD-CIBNOR, Mexico. pp. 125–136.

Escalante, M.A., García-De León, F.J., Ruiz-Luna, A., Landguth, E., and Manel, S. 2018. The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (Oncorhynchus chrysogaster), a simulation approach. J. Biogeogr. **45**(7). doi:10.1111/jbi.13246.

van Etten, J. 2012. gdistance: Distances and routes on geographical grids. R package version 1.1--4. Available a t CRAN. R-project. org/package= gdistance.

Faulks, L.K., Kerezsy, A., Unmack, P.J., Johnson, J.B., and Hughes, J.M. 2017. Going, going, gone? Loss

of genetic diversity in two critically endangered Australian freshwater fishes, Scaturiginichthys vermeilipinnis and Chlamydogobius squamigenus, from Great Artesian Basin springs at Edgbaston, Queensland, Australia. Aquat. Conserv. Mar. Freshw. Ecosyst. **27**(1): 39–50. doi:10.1002/aqc.2684.

Fausch, K.D. 2007. Introduction, establishment and effects of non-native salmonids: Considering the risk of rainbow trout invasion in the United Kingdom. J. Fish Biol. **71**(SUPPL. D): 1–32. doi:10.1111/j.1095-8649.2007.01682.x.

Frichot, E., and François, O. 2015. LEA: An R package for landscape and ecological association studies. Methods Ecol. Evol. **6**(8): 925–929. doi:10.1111/2041-210X.12382.

Frichot, E., Schoville, S.D., Bouchard, G., and François, O. 2013. Testing for associations between loci and environmental gradients using latent factor mixed models. Mol. Biol. Evol. **30**(7): 1687–1699. doi:10.1093/molbev/mst063.

Gagnaire, P.-A., and Gaggiotti, O.E. 2016. Detecting polygenic selection in marine populations by combining population genomics and quantitative genetics approaches. Curr. Zool.: 1–14. doi:10.1093/cz/zow088.

García-De León, F.J., Getino-Mamet, L.N., Rodríguez-Jaramillo, C., Sánchez-González, S., Márquez, F., and Ruíz-Luna, A. 2016. Dimorfismo sexual y periodo reproductivo de la trucha dorada mexicana, Oncorhynchus chrysogaster en los ríos Fuerte, Sinaloa y Culiacán. *In* La Trucha Dorada Mexicana. *Edited by* A. Ruíz-Luna and F.J. García-De León. CIAD-CIBNOR. pp. 53–72.

Gunnell, K., Tada, M.K., Hawthorne, F.A., Keeley, E.R., and Ptacek, M.B. 2008. Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (Oncorhynchus clarkii bouvieri) and introduced rainbow trout (O. mykiss) in the South Fork of the Snake River watershed, Idaho. Conserv. Genet. **9**(1): 49–64. doi:10.1007/s10592-007-9302-6.

Günther, T., and Coop, G. 2013. Robust identification of local adaptation from allele frequencies. Genetics **195**(1): 205–220. doi:10.1534/genetics.113.152462.

Hale, M.C., Xu, P., Scardina, J., Wheeler, P.A., Thorgaard, G.H., and Nichols, K.M. 2011. Differential gene expression in male and female rainbow trout embryos prior to the onset of gross morphological differentiation of the gonads. BMC Genomics **12**(1): 404. doi:10.1186/1471-2164-12-404.

Hand, B.K., Muhlfeld, C.C., Wade, A.A., Kovach, R.P., Whited, D.C., Narum, S.R., Matala, A.P., Ackerman, M.W., Garner, B.A., Kimball, J.S., Stanford, J.A., and Luikart, G. 2016. Climate variables explain neutral and adaptive variation within salmonid metapopulations: the importance of replication in landscape genetics. Mol. Ecol. **25**(3): 689–705. doi:10.1111/mec.13517.

Hartl, D.L., and Clark, A.G. 1997. Principles of population genetics. Sinauer Associates Incorporated, Sunderland, US. Available from https://www.cabdirect.org/cabdirect/abstract/19980108046.

Hecht, B.C., Matala, A.P., Hess, J.E., and Narum, S.R. 2015. Environmental adaptation in Chinook salmon (Oncorhynchus tshawytscha) throughout their North American range. Mol. Ecol. **24**(22): 5573–5595. doi:10.1111/mec.13409.

Heggberget, T.G., Johnsen, B.O., Hindar, K., Jonsson, B., Hansen, L.P., Hvidsten, N.A., and Jensen, A.J. 1993. Interactions between wild and cultured Atlantic salmon: a review of the Norwegian experience. Fish. Res. **18**(1–2): 123–146. doi:10.1016/0165-7836(93)90044-8.

Hendrickson, D.A., Neely, D.A., Mayden, R.L., Anderson, K., Brooks, J.E., Camarena-Rosales, F., Cutter, R., Cutter, L., los Santos, A.B., Ernsting, G.W., and others. 2006. Conservation of

Mexican native trout and the discovery, status. Protection and recovery of the Conchos trout, the first native. Stud. North Am. desert fishes Honor EP Pist. Conserv. Univ. Autónoma Nuevo León, Monterrey: 162–201.

Hendrickson, D.A., Pérez, H.E., Findley, L.T., Forbes, W., Tomelleri, J.R., Mayden, R.L., Nielsen, J.L., Jensen, B., Campos, G.R., Romero, A.V., van der Heiden, A., Camarena, F., and García-De-León, F.J. 2002. Mexican native trouts: A review of their history and current systematic and conservation status. Rev. Fish Biol. Fish. **12**(2–3): 273–316. doi:10.1023/A:1025062415188.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**(6789): 907–913. doi:10.1038/35016000.

Hoban, S., Kelley, J.L., Lotterhos, K.E., Antolin, M.F., Bradburd, G., Lowry, D.B., Poss, M.L., Reed, L.K., Storfer, A., and Whitlock, M.C. 2016. Finding the Genomic Basis of Local Adaptation: Pitfalls, Practical Solutions, and Future Directions. Am. Nat. **188**(4): 379–97. University of Chicago PressChicago, IL. doi:10.1086/688018.

Höglund, J. 2009. Evolutionary conservation genetics. Oxford University Press. Available from https://books.google.es/books?hl=es&lr=&id=JkIVDAAAQBAJ&oi=fnd&pg=PR5&dq=hoglund+2009+population+size&ots=MYjMCFTG8q&sig=u0JdopyZ3TnzVTzkoVaebIe_PFY#v=onepage&q&f=false [accessed 14 July 2017].

Hogstrand, C., Wilson, R.W., Polgar, D., and Wood, C.M. 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol. **186**: 55–73. Available from http://www.ncbi.nlm.nih.gov/pubmed/7525831.

Hopken, M.W., Douglas, M.R., and Douglas, M.E. 2013. Stream hierarchy defines riverscape genetics of a North American desert fish. Mol. Ecol. **22**(4): 956–971. doi:10.1111/mec.12156.

Jombart, T. 2008. Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics **24**(11): 1403–1405. doi:10.1093/bioinformatics/btn129.

Kanno, Y., Vokoun, J.C., and Letcher, B.H. 2011. Fine-scale population structure and riverscape genetics of brook trout (Salvelinus fontinalis) distributed continuously along headwater channel networks. Mol. Ecol. **20**(18): 3711–3729. doi:10.1111/j.1365-294X.2011.05210.x.

Kokko, H., Chaturvedi, A., Croll, D., Fischer, M.C., Guillaume, F., Karrenberg, S., Kerr, B., Rolshausen, G., and Stapley, J. 2017. Can Evolution Supply What Ecology Demands? Trends Ecol. Evol. **32**(3): 187–197. doi:10.1016/j.tree.2016.12.005.

Koskinen, M.T., Haugen, T.O., and Primmer, C.R. 2002. Contemporary fisherian life-history evolution in small salmonid populations. Nature **419**(6909): 826–830. doi:10.1038/nature01029.

de Lafontaine, G., Napier, J.D., Petit, R.J., and Hu, F.S. 2018. Invoking adaptation to decipher the genetic legacy of past climate change. Ecology **99**(7): 1530–1546. Wiley-Blackwell. doi:10.1002/ecy.2382.

Landguth, E.L., Bearlin, A., Day, C.C., and Dunham, J. 2016. CDMetaPOP: an individual-based, eco-evolutionary model for spatially explicit simulation of landscape demogenetics. Methods Ecol. Evol. **8**(1): 4–11. doi:10.1111/2041-210X.12608.

Lawler, J.J., Tear, T.H., Pyke, C., Shaw, R.M., Gonzalez, P., Kareiva, P., Hansen, L., Hannah, L., Klausmeyer, K., Aldous, A., Bienz, C., and Pearsall, S. 2010. Resource management in a changing and uncertain climate. Front. Ecol. Environ. **8**(1): 35–43. doi:10.1890/070146.

Linløkken, A.N., Haugen, T.O., Mathew, P.K., Johansen, W., and Lien, S. 2016. Comparing estimates of number of breeders Nb based on microsatellites and single nucleotide polymorphism of

three groups of brown trout (Salmo trutta L.). Fish. Manag. Ecol. **23**(2): 152–160. doi:10.1111/fme.12169.

Luu, K., Bazin, E., and Blum, M.G.B. 2016. *pcadapt* : an R package to perform genome scans for selection based on principal component analysis. Mol. Ecol. Resour. doi:10.1111/1755-0998.12592.

Manel, S., and Holderegger, R. 2013. Ten years of landscape genetics. Trends Ecol. Evol. **28**(10): 614–621. doi:10.1016/j.tree.2013.05.012.

Marie, A.D., Bernatchez, L., Garant, D., and Taylor, E. 2012. Environmental factors correlate with hybridization in stocked brook charr (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **69**(5): 884–893. doi:10.1139/f2012-027.

Martin, M. 2011. Sequencing Reads. EMBnet.journal **17**(1): 10–12.

Meeuwig, M.H., Guy, C.S., Kalinowski, S.T., and Fredenberg, W.A. 2010. Landscape influences on genetic differentiation among bull trout populations in a stream-lake network. Mol. Ecol. **19**(17): 3620–3633. doi:10.1111/j.1365-294X.2010.04655.x.

Meirmans, P.G., and Van Tienderen, P.H. 2004. GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes **4**(4): 792–794. Wiley/Blackwell (10.1111). doi:10.1111/j.1471-8286.2004.00770.x.

Milano, I., Babbucci, M., Cariani, A., Atanassova, M., Bekkevold, D., Carvalho, G.R., Espiñeira, M., Fiorentino, F., Garofalo, G., Geffen, A.J., Hansen, J.H., Helyar, S.J., Nielsen, E.E., Ogden, R., Patarnello, T., Stagioni, M., Tinti, F., and Bargelloni, L. 2014. Outlier SNP markers reveal fine-scale genetic structuring across European hake populations (Merluccius merluccius). Mol. Ecol. **23**(1): 118–135. doi:10.1111/mec.12568.

Milián-García, Y., Ramos-Targarona, R., Pérez-Fleitas, E., Sosa-Rodríguez, G., Guerra-Manchena, L., Alonso-Tabet, M., Espinosa-López, G., and Russello, M.A. 2015. Genetic evidence of hybridization between the critically endangered Cuban crocodile and the American crocodile: implications for population history and in situ/ex situ conservation. Heredity (Edinb). **114**(3): 272–280. doi:10.1038/hdy.2014.96.

Miller, R.R., Williams, J.D., and Williams, J.E. 1989. Extinctions of North American Fishes During the Past Century. Fisheries **14**(6): 22–38. doi:10.2307/1104578.

Morita, K., and Yamamoto, S. 2002. Effects of habitat fragmentation by damming on the persistence of a stream - dwelling charr. Conserv. Biol. **16**(5): 1318–1323. doi:10.1046/j.1523-1739.2002.01476.x.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. Available from http://rsbl.royalsocietypublishing.org/content/early/2009/03/13/rsbl.2009.0033.abstract.

Muhlfeld, C.C., Kovach, R.P., Al-Chokhachy, R., Amish, S.J., Kershner, J.L., Leary, R.F., Lowe, W.H., Luikart, Matson, G.P., Schmetterling, D.A., Bradley, Shepard, B.B., Westley, P.A.H., Whited, D., Whiteley, A., and Allendorf, F.W. 2017. Legacy introductions and climatic variation explain spatiotemporal patterns of invasive hybridization in a native trout. Glob Chang. Biol **23**: 4663–4674. doi:10.1111/gcb.13681.

Narum, S.R., Zendt, J.S., Graves, D., and Sharp, W.R. 2008. Influence of landscape on resident and anadromous life history types of *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. **65**(6): 1013–1023. doi:10.1139/F08-025.

Needham, P.R., and Gard, R. 1964. A New Trout from Central Mexico: Salmo chrysogaster, the Mexican Golden Trout. Copeia **1964**(1): 169. doi:10.2307/1440847.

Nielsen, J.L., and Sage, G.K. 2001. Microsatellite analyses of the trout of northwest Mexico. Genetica **111**(1–3): 269–278. doi:10.1023/A:1013777701213.

Nomura, T. 2008. Estimation of effective number of breeders from molecular coancestry of single cohort sample. Evol. Appl. **1**(3): 462–474. doi:10.1111/j.1752-4571.2008.00015.x.

Olsen, M.T., Islas, V., Graves, J.A., Vincent, A.O.C., Brasseur, S., Frie, A.K., and Hall, A.J. 2017. Genetic population structure of harbour seals in the United Kingdom and neighbouring waters. doi:10.1002/aqc.2760.

Osborne, M., Perkin, J., Gido, K., and Turner, T. 2014. diversity for conservation and restoration of Great Plains fishes. Mol. Ecol. **23**(23): 5663–5679. doi:10.5061/dryad.v7d6t.

Ozerov, M.Y., Veselov, A.E., Lumme, J., Primmer, C.R., and Moran, P. 2012. "Riverscape" genetics: river characteristics influence the genetic structure and diversity of anadromous and freshwater Atlantic salmon (Salmo salar) populations in northwest Russia. Can. J. Fish. Aquat. Sci. **69**(12): 1947–1958. doi:10.1139/f2012-114.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annu. Rev. Ecol. Evol. Syst. **37**(1): 637–669. doi:10.1146/annurev.ecolsys.37.091305.110100.

Pavlova, A., Beheregaray, L.B., Coleman, R., Gilligan, D., Harrisson, K.A., Ingram, B.A., Kearns, J., Lamb, A.M., Lintermans, M., Lyon, J., Nguyen, T.T.T., Sasaki, M., Tonkin, Z., Yen, J.D.L., and Sunnucks, P. 2017. Severe consequences of habitat fragmentation on genetic diversity of an endangered Australian freshwater fish: A call for assisted gene flow. Evol. Appl. **10**(6): 531–550. doi:10.1111/eva.12484.

Penaluna, B.E., Abadía-Cardoso, A., Dunham, J.B., García-De León, F.J., Gresswell, R.E., Luna, A.R., Taylor, E.B., Shepard, B.B., Al-Chokhachy, R., Muhlfeld, C.C., Bestgen, K.R., Rogers, K., Escalante, M.A., Keeley, E.R., Temple, G.M., Williams, J.E., Matthews, K.R., Pierce, R., Mayden, R.L., Kovach, R.P., Garza, J.C., and Fausch, K.D. 2016. Conservation of Native Pacific Trout Diversity in Western North America. Fisheries **41**(6): 286–300. doi:10.1080/03632415.2016.1175888.

Perreault-Payette, A., Muir, A.M., Goetz, F., Perrier, C., Normandeau, E., Sirois, P., and Bernatchez, L. 2017. Investigating the extent of parallelism in morphological and genomic divergence among lake trout ecotypes in Lake Superior. Mol. Ecol. **26**(6): 1477–1497. doi:10.1111/mec.14018.

Perrier, C., Ferchaud, A.-L., Sirois, P., Thibault, I., and Bernatchez, L. 2017. Do genetic drift and accumulation of deleterious mutations preclude adaptation? Empirical investigation using RADseq in a northern lacustrine fish. Mol. Ecol. **26**(22): 6317–6335. Wiley/Blackwell (10.1111). doi:10.1111/mec.14361.

Perrier, C., Guyomard, R., Bagliniere, J.L., and Evanno, G. 2011. Determinants of hierarchical genetic structure in Atlantic salmon populations: Environmental factors vs. anthropogenic influences. Mol. Ecol. **20**(20): 4231–4245. doi:10.1111/j.1365-294X.2011.05266.x.

Le Pichon, C.L.E., Gorges, G., Boët, P., Baudry, J., Goreaud, F., and Faure, T. 2006. A spatially explicit resource-based approach for managing stream fishes in riverscapes. Environ. Manage. **37**(3): 322–335. doi:10.1007/s00267-005-0027-3.

Raeymaekers, J.A.M., Maes, G.E., Geldof, S., Hontis, I., Nackaerts, K., and Volckaert, F.A.M. 2008. Modeling genetic connectivity in sticklebacks as a guideline for river restoration. Evol. Appl. **1**(3): 475–488. doi:10.1111/j.1752-4571.2008.00019.x.

Rahel, F.J., Bierwagen, B., and Taniguchi, Y. 2008. Managing aquatic species of conservation concern in the face of climate change and invasive species. Conserv. Biol. **22**(3): 551–561. doi:10.1111/j.1523-1739.2008.00953.x.

Raj, A., Stephens, M., and Pritchard, J.K. 2014. FastSTRUCTURE: Variational inference of population structure in large SNP data sets. Genetics **197**(2): 573–589. doi:10.1534/genetics.114.164350.

Richardson, J.L., Brady, S.P., Wang, I.J., and Spear, S.F. 2016. Navigating the pitfalls and promise of landscape genetics. Mol. Ecol. **25**(4): 849–863. doi:10.1111/mec.13527.

Rieman, B.E., and Allendorf, F.W. 2001. Effective population size and genetic conservation criteria for bull trout. North Am. J. Fish. Manag. **21**(4): 756–764. doi:10.1577/1548-8675(2001)021<0756:EPSAGC>2.0.CO;2.

Riginos, C., and Liggins, L. 2013. Seascape Genetics: Populations, Individuals, and Genes Marooned and Adrift. Geogr. Compass **7**(3): 197–216. doi:10.1111/gec3.12032.

Ripley, W.N.V. and B.D. 2002. Package ‘ MASS .’ Mod. Appl. Stat. with S.

Rosenberg, M.S., and Anderson, C.D. 2011. PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2. Methods Ecol. Evol. **2**(3): 229–232. Wiley/Blackwell (10.1111). doi:10.1111/j.2041-210X.2010.00081.x.

Ruiz-Luna, A., Hernández-Guzmán, R., García-De León, F.J., and Ramírez-Huerta, A.L. 2017. Potential distribution of endangered Mexican golden trout (Oncorhynchus chrysogaster) in the Rio Sinaloa and Rio Culiacan basins (Sierra Madre Occidental) based on landscape characterization and species distribution models. Environ. Biol. Fishes: 1–28. doi:10.1007/s10641-017-0624-z.

Ruzzante, D.E., McCracken, G.R., Parmelee, S., Hill, K., Corrigan, A., MacMillan, J., and Walde, S.J. 2016. Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, Salvelinus fontinalis. Proc. R. Soc. B **283**(Ld): 20152601. doi:10.1098/rspb.2015.2601.

Saitou, N., and Nei, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evo **4**(4): 406–425. doi:citeulike-article-id:93683.

Salem, M., Kenney, P.B., Rexroad, C.E., and Yao, J. 2010. Proteomic signature of muscle atrophy in rainbow trout. J. Proteomics **73**(4): 778–789. Elsevier B.V. doi:10.1016/j.jprot.2009.10.014.

Schmidt, D.J., Espinoza, T., Connell, M., and Hughes, J.M. 2017. Conservation genetics of the Mary River turtle (Elusor macrurus) in natural and captive populations. doi:10.1002/aqc.2851.

Schoville, S.D., Bonin, A., François, O., Lobreaux, S., Melodelima, C., and Manel, S. 2012. Adaptive Genetic Variation on the Landscape: Methods and Cases. Annu. Rev. Ecol. Evol. Syst. **43**(1): 23–43. Annual Reviews . doi:10.1146/annurev-ecolsys-110411-160248.

Taggart, J.B., Bron, J.E., Martin, S.A.M., Seear, P.J., Høyheim, B., Talbot, R., Carmichael, S.N., Villeneuve, L.A.N., Sweeney, G.E., Houlihan, D.F., Secombes, C.J., Tocher, D.R., and Teale, A.J. 2008. A description of the origins, design and performance of the TRAITS-SGP Atlantic salmon Salmo salar L. cDNA microarray. J. Fish Biol. **72**(9): 2071–2094. Wiley-Blackwell. doi:10.1111/j.1095-8649.2008.01876.x.

Tamura, K., and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. **10**(3): 512–26. doi:10.1093/molbev/msl149.

Tikochinski, Y., Bradshaw, P., Mastrogiacomo, A., Broderick, A., Daya, A., Demetropoulos, A., Demetropoulos, S., Eliades, N.-G., Fuller, W., Godley, B., Kaska, Y., Levy, Y., Snape, R., Wright, L.,

and Carreras, C. 2018. Mitochondrial DNA short tandem repeats unveil hidden population structuring and migration routes of an endangered marine turtle. Aquat. Conserv. Mar. Freshw. Ecosyst. Wiley-Blackwell. doi:10.1002/aqc.2908.

Torterotot, J.-B., Perrier, C., Bergeron, N.E., and Bernatchez, L. 2014. Influence of forest road culverts and waterfalls on the fine-scale distribution of brook trout genetic diversity in a boreal watershed. Trans. Am. Fish. Soc. **143**(6): 1577–1591. doi:10.1080/00028487.2014.952449.

Vera, M., Martinez, P., and Bouza, C. 2017. Stocking impact, population structure and conservation of wild brown trout populations in inner Galicia (NW Spain), an unstable hydrologic region. Aquat. Conserv. Mar. Freshw. Ecosyst. doi:10.1002/aqc.2856.

Wenne, R., Bernaś, R., Poćwierz-Kotus, A., Drywa, A., and Wąs, A. 2016. Recent genetic changes in enhanced populations of sea trout (Salmo trutta m . trutta) in the southern Baltic rivers revealed with SNP analysis. Aquat. Living Resour. **103**: 29. doi:10.1051/alr/2016012.

TABLE 1 Examples of landscape genetics studies testing environmental influence on neutral and adaptive genetic variation at riverine systems with empirical data.

Species	Location	Number of molecular markers	Studied microevolutionary process	Main findings	Reference
Macquarie perch (*Macquaria australasica*)	Southeastern Australia	19 microsatellite loci	Neutral	Maximum distances from barriers were associated with higher genetic diversity while minimum temperature of the coldest month was related to lower genetic diversity	(Pavlova et al. 2017)
Rainbow/steelhead trout (*Oncorhynchus mykiss*)	Columbia River Basin, U.S.A.	180 SNPs	Neutral and adaptive	Climate-related variables explaining neutral and adaptive patterns of genetic differentiation within metapopulations	(Hand et al. 2016)
Southern pigmy perch (*Nannoperca australis*)	Murray–Darling River Basin, Australia	5 162 SNPs	Adaptive	Environmental variables related to temperature and precipitation influencing adaptive variation at regional and local scales, while human disturbance only at local scales	(Brauer et al. 2016)
Chinook salmon (*Oncorhynchus tshawytscha*)	Northeastern Pacific Coast, U.S.A. and Canada	19 703 SNPs	Adaptive	Temperature and precipitation defined as strong drivers of adaptive genomic divergence of the species	(Hecht et al. 2015)
Electric fish (*Steatogenys elegans*)	Amazon River Basin, Brazil	310 AFLP loci	Adaptive	Analyses of population structure suggest a strong correlation between water color and genotype	(Cooke et al. 2014)

Plains minnow (*Hybognathus placitus*), emerald shiner (*Notropis atherinoides*) and red shiner (*Cyprinella lutrensis*)	Great Plains, U.S.A.	8 microsatellite loci	Neutral	Historical signature of past climates and geology shaping contemporary landscape scale patterns of genetic diversity in *C. lutrensis* and *H. placitus*	(Osborne et al. 2014)
Brook Charr (*Salvelinus fontinalis*)	Saint Louis River, Quebec Canada	16 microsatellite loci	Neutral	Genetic diversity deficit related with waterfalls and forest road culverts	(Torterotot et al. 2014)
Atlantic salmon (*Salmo salar*)	Southeastern Canada	5 500 SNPs	Neutral and adaptive	Temperature, precipitation and geological characteristics related to both potentially adaptive and neutral genetic divergence	(Bourret et al. 2014)
Atlantic salmon (*Salmo salar*)	Easter Canada	15 microsatellite loci	Neutral	Freshwater habitat explaining the majority of the spatial genetic variance	(Bradbury et al. 2014)
Two castastomidae species *Catostomus discobolus* *Discobolus* and *Catostomus discobolus yarrow*	Western U.S.A.	16 microsatellite loci	Neutral	Stream hierarchy defining gene flow	(Hopken et al. 2013)
Atlantic salmon (*Salmo salar*)	Western Russia	14 microsatellite loci	Neutral	Genetic diversity associated with carrying capacity and stream gradient	(Ozerov et al. 2012)
Atlantic salmon (*Salmo salar*)	Western France	17 microsatellite loci	Neutral	Coastal distance, geological substrate and river length predicting population genetic structure	(Perrier et al. 2011)

Brook Charr (*Salvelinus fontinalis*)	Connecticut, U.S.A.	8 microsatellite loci	Neutral	Gene flow mitigated by riverscape barriers	(Kanno et al. 2011)
Bull trout (*Salvelinus confluentus*)	Glacier National Park, Montana U.S.A.	11 microsatellite loci	Neutral	Genetic differentiation between populations was greater when barriers were present than when absent	(Meeuwig et al. 2010)
European chub (*Leuciscus cephalus*), rostrum dace (*Leuciscus leuciscus*), gudgeon (*Gobio gobio*) and European minnow (*Phoxinus phoxinus*)	Adour-Garonne River Basin, France	8 - 15 microsatellites loci	Neutral	Significant differences between fragmented and continuous landscapes, both for genetic diversity and the genetic structure	(Blanchet et al. 2010)
Rainbow/steelhead trout (*Oncorhynchus mykiss*)	Klickitat River Basin, Washington U.S.A.	13 microsatellite loci	Neutral	Heterozygosity negatively correlated with elevation, precipitation and upstream distance, while positively correlated with temperature. Additionally, geographical barriers drive genetic structure of life history types	(Narum et al. 2008)
Three-spined stickleback (*Gasterosteus aculeatus*)	Scheldt River, Flanders Belgium	6 microsatellite loci	Neutral	Geographical barriers affecting genetic diversity and controlling the balance between gene flow and genetic drift	(Raeymaekers et al. 2008)
Atlantic salmon (*Salmo salar*)	Southeastern Canada	13 microsatellite loci	Neutral	Both coastal distance and temperature regime influencing the observed genetic structure	(Dionne et al. 2008)
Brook Charr (*Salvelinus fontinalis*)	Maine, U.S.A.	6 microsatellite loci	Neutral	Within populations expected heterozygosity negatively correlated with altitude, while within lakes with habitat size	(Castric et al. 2001)

TABLE 2 Genetic diversity values for Mexican golden trout, aquaculture rainbow trout and lab hybrids.

River Basin	CODE	Latitude	Longitude	Location	Description	N[a]	*PP*[b]	H_O[c]	H_E[d]	N_E[e]	*95% CI* N_E[f]
Río Fuerte											
	FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10	7	0.03	0.04	44.2	38.3–51.9
	FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8	5	0.06	0.06	35.3	32.4-38.7
	FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10	4	0.06	0.07	33.7	32.2–35.3
	FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12	18	0.04	0.05	116.8	110.3-139.6
	FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8	1	0.04	0.04	-	Infinite
	FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5	2	0.02	0.02	-	Infinite
	FSJ	26.24	-106.69	Arroyo San José	Wild trout	12	1	0.07	0.07	14.7	14.4-15
	FCA	25.94	-106.65	Arroyo Calera	Wild trout	9	2	0.14	0.13	8.2	8.1-8.2
	FON	25.95	-106.68	Arroyo La Onza	Wild trout	6	1	0.14	0.12	-	Infinite
	FVE	26.28	-106.49	Río Verde	Wild trout	12	53	0.05	0.05	11	10.8-11.2
	Totals and means	-	-	-	-	**92**	**9.4**	**0.065**	**0.065**	**37.7**	
Río Sinaloa	SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9	4	0.07	0.09	145.7	114.8-198.7
	SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9	4	0.08	0.08	5.9	5.8-6
	SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11	2	0.09	0.11	2.4	2.3-2.4
	SLO	25.99	-106.98	Arroyo La Osera	Wild trout	10	6	0.08	0.09	1.5	1.5-1.5
	SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10	0	0.02	0.02	0.4	0.4-0.4
	SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11	3	0.02	0.02	-	Infinite

	SSM	26.07	-107.04	Arroyo San Miguel	Trout in aquaculture proximities	4	13	0.17	0.15	-	Infinite
	SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8	5	0.11	0.15	1.4	1.4-1.4
	SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7	1	0.09	0.12	1.8	1.8-1.8
	SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7	0	0.12	0.12	7.5	7.3-7.6
	Totals and means	**-**	**-**	**-**	**-**	**86**	**3.8**	**0.085**	**0.095**	**20.83**	
Río Culiacán	CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12	0	0.01	0.01	-	Infinite
	CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12	1	0.01	0.01	-	Infinite
	CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12	1	0.01	0.01	-	Infinite
	CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	12	6	0.05	0.04	8	7.9-8.2
	CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10	7	0.11	0.1	42.7	41.4-44
	Totals and means	**-**	**-**	**-**	**-**	**57**	**3**	**0.038**	**0.034**	**25.35**	
Lab Trout	H			INAPESCA Cultive Center	Mexican golden trout and aquaculture rainbow trout lab hybrids	4	4	0.22	0.15	-	Infinite
	REP			INAPESCA Cultive Center	Domestic Mexican golden trout	7	7	0.08	0.08	-	Infinite
	Totals and means	**-**	**-**	**-**	**-**	**11**	**5.5**	**0.15**	**0.12**		
Aquaculture Farms	AQEB	25.99	-106.95	El Barro Aquaculture Farm	Aquaculture rainbow trout	14	37	0.15	0.16	19.1	18.9-19.2
	AQSM	26.07	-107.04	San Miguel Aquaculture Farm	Aquaculture rainbow trout	10	7	0.13	0.15	22.1	21.7-22.5
	Totals and means	**-**	**-**	**-**	**-**	**24**	**22**	**0.14**	**0.16**	**20.6**	

a-Number of samples (N), *b*-private polymorphisms (*PP*), *c*-expected heterozygosity (H_E), *d*-observed heterozygosity (H_O), *e*-effective population size (N_E) and *f*-95% confidence intervals for effective population size (95% CI N_E).

TABLE 3 Stepwise selection for the most accurate riverscape model explaining expected heterozygosity (H_E) for Central populations (Dataset C) and Río Fuerte populations (Dataset D).

Dataset	Variable	Estimate	Std. Error	t value	Pr(>|t|)
Dataset C	Intercept	5.6609847	1.8405004	3.076	0.00961
	Latitude	-0.2232689	0.0681720	-3.275	0.00664
	Precipitation of the driest month	-0.0292422	0.0091961	-3.180	0.00792
	Altitude	0.0002844	0.0001075	2.646	0.02135
	AIC: -67.542				
Dataset D	Intercept	-1.649e+01	2.514e+00	-6.561	0.02245
	Latitude	-3.547e-01	2.399e-02	-14.783	0.00454
	longitude	-2.546e-01	2.819e-02	-9.031	0.01204
	Temperature of the warmest quarter	-5.679e-02	8.080e-03	-7.028	0.01965
	Precipitation of the driest month	-4.589e-02	4.616e-03	-9.941	0.00997
	River length	2.774e-07	6.342e-08	4.374	0.04850
	Altitude	8.418e-05	5.178e-05	1.626	0.24553
	Stream order	-1.183e-02	3.206e-03	-3.690	0.06622·
	AIC : -74.982				

TABLE 4 Partial Mantel test performed under 9,000 permutations between (F_{ST}/(1-F_{ST}) and riverscape resistance matrices.

Dataset	r	p-value
Dataset D (Río Fuerte populations)	-0.05	0.73
Dataset E (Río Sinaloa populations)	0.872	0.0002

Table 5 Blast hits from sequences containing a SNP found to be putatively under selection by PCAdapt, BAYENV and LFMM. SNPs are identified by locus ID. Sequence names are identified as in Berthelot *et al.* (2014). Statistical significance of the hits is represented by the e-Value. Functional characterizations by gene ontology terms obtained in Blast2GO are identified by GO ID's.

Locus ID	Sequence Name	e-Value	GO ID's	Known functions	Associated functions
CLocus_63824	GSONMP00076296001	1.93E-08	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_17128	GSONMP00061816001	0.0000145	F:GO:0008270; P:GO:0030163	F:zinc ion binding; P:protein catabolic process	Adaptation to elevated concentrations of waterborne (Hogstrand et al. 1994)
CLocus_24893	GSONMP00073281001	0.000209	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_34692	GSONMP00068395001	0.00000574	F:GO:0005524; F:GO:0004672; P:GO:0006468	F:ATP binding; F:protein kinase activity; P:protein phosphorylation	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_39212	GSONMP00076439001	0.00000039	P:GO:0097264; C:GO:0005887; F:GO:0005515; P:GO:0007165; C:GO:0016021; F:GO:0005102	C:integral component of plasma membrane; P:self proteolysis; P:signal transduction; F:receptor binding	Temperature adaptation (Crockett 1998)
CLocus_40408	GSONMP00028710001	0.000684	P:GO:0055085; C:GO:0016021	P:transmembrane transport; C:integral component of membrane	Temperature adaptation (Crockett 1998)
CLocus_40780	GSONMP00020503001	0.000225	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_41412	GSONMP00076296001	0.000311	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_45731	GSONMP00008827001	0.000766	F:GO:0008641	F:small protein activating enzyme activity	
CLocus_48136	GSONMP00021876001	0.000665	C:GO:0005615; P:GO:0010506	C:extracellular space; P:regulation of autophagy	
CLocus_5089	GSONMP00082580001	0.000619	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_54656	GSONMP00051628001	0.00000314	F:GO:0005515	F:protein binding	Water flow and temperature acclimation (Milano et al. 2014)

CLocus_55309	GSONMP00044696001	0.000544	F:GO:0003677; C:GO:0005634; F:GO:0008270; F:GO:0003707; P:GO:0006355; P:GO:0043401	C:nucleus; F:DNA binding; F:zinc ion binding; F:steroid hormone receptor activity; P:regulation of transcription, DNA-templated; P:steroid hormone mediated signaling pathway	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_5644	GSONMP00023521001	0.000247	F:GO:0004725; P:GO:0006470	P:tyrosine metabolic process; F:protein tyrosine phosphatase activity; P:protein dephosphorylation	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_58838	GSONMP00030033001	0.00000662	P:GO:0008360; F:GO:0003779; P:GO:0007010; P:GO:0016043; F:GO:0017048; F:GO:0005488; P:GO:0030036	P:regulation of cell shape; F:actin binding; F:Rho GTPase binding; P:actin cytoskeleton organization	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_59529	GSONMP00030319001	0.0000646	P:GO:0016337; F:GO:0005515; P:GO:0007155; F:GO:0005488; P:GO:0032467; P:GO:0043547	P:single organismal cell-cell adhesion; F:protein binding; P:positive regulation of cytokinesis; P:positive regulation of GTPase activity	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_63824	GSONMP00059101001	6.73E-07	F:GO:0003676; F:GO:0005524; C:GO:0005634; F:GO:0003677; P:GO:0015074; P:GO:0006355; P:GO:0006313	C:nucleus; F:DNA binding; F:ATP binding; P:DNA integration; P:regulation of transcription, DNA-templated; P:transposition, DNA-mediated	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_6876	GSONMP00019151001	0.00052	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)
CLocus_69641	GSONMP00059101001	0.0000108	F:GO:0003676; F:GO:0005524; C:GO:0005634; F:GO:0003677;	C:nucleus; F:DNA binding; F:ATP binding; P:DNA integration; P:regulation of transcription,	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)

			P:GO:0015074; P:GO:0006355; P:GO:0006313	DNA-templated; P:transposition, DNA-mediated	
CLocus_71994	GSONMP00069497001	0.0000141	F:GO:0005515; F:GO:0005509	F:protein binding; F:calcium ion binding	Water flow and temperature acclimation (Milano et al. 2014)
CLocus_77569	GSONMP00045823001	0.0000424	F:GO:0003676	F:nucleic acid binding	Sex expression, lipid metabolism (Taggart et al. 2008; Hale et al. 2011)

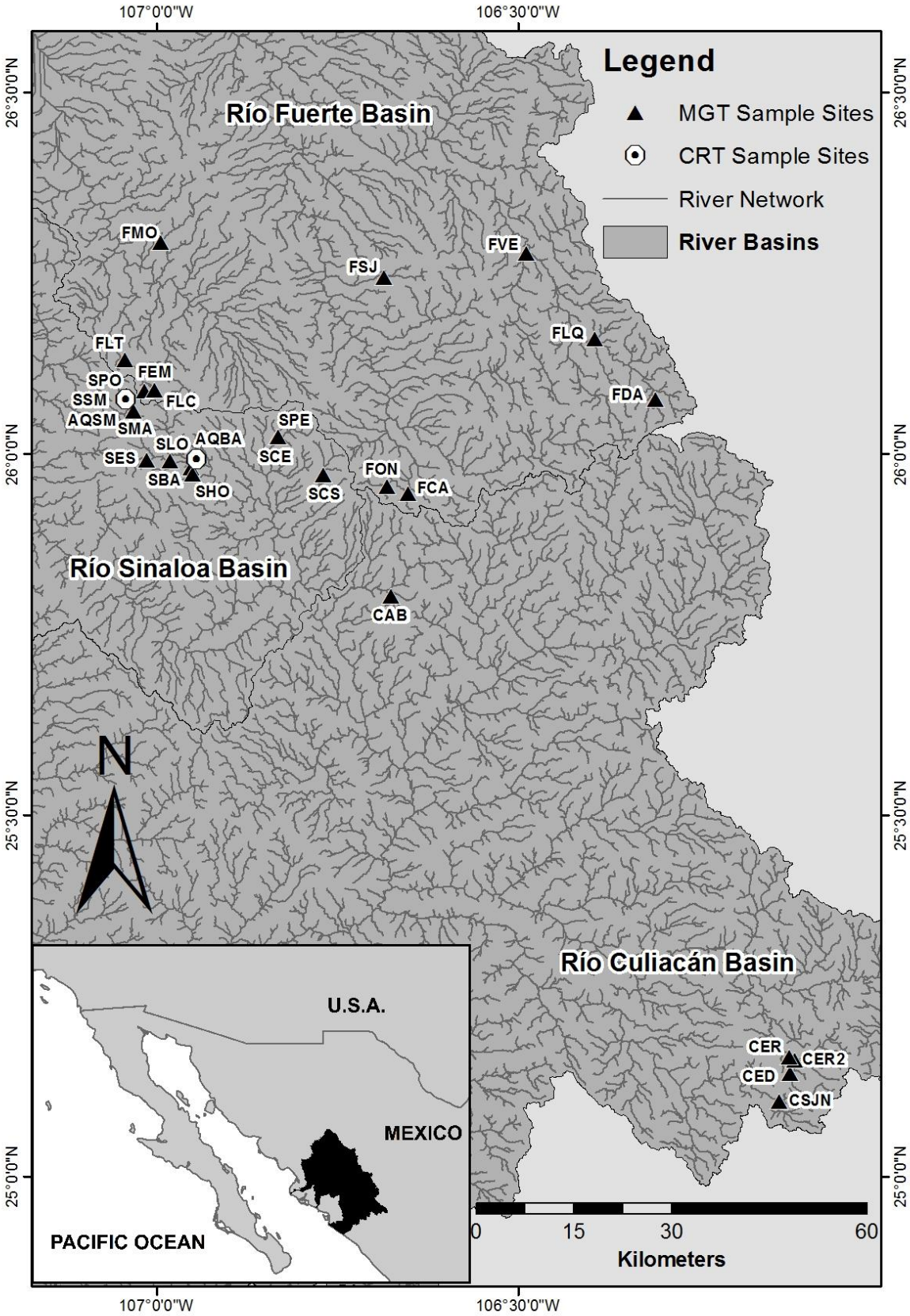

Figure 1 Study area. Mexican golden trout (MGT) and cultured rainbow trout (CRT) sample sites at Río Fuerte, Río Sinaloa and Río Culiacán basins. See table 2 for explanation of the sample site codes.

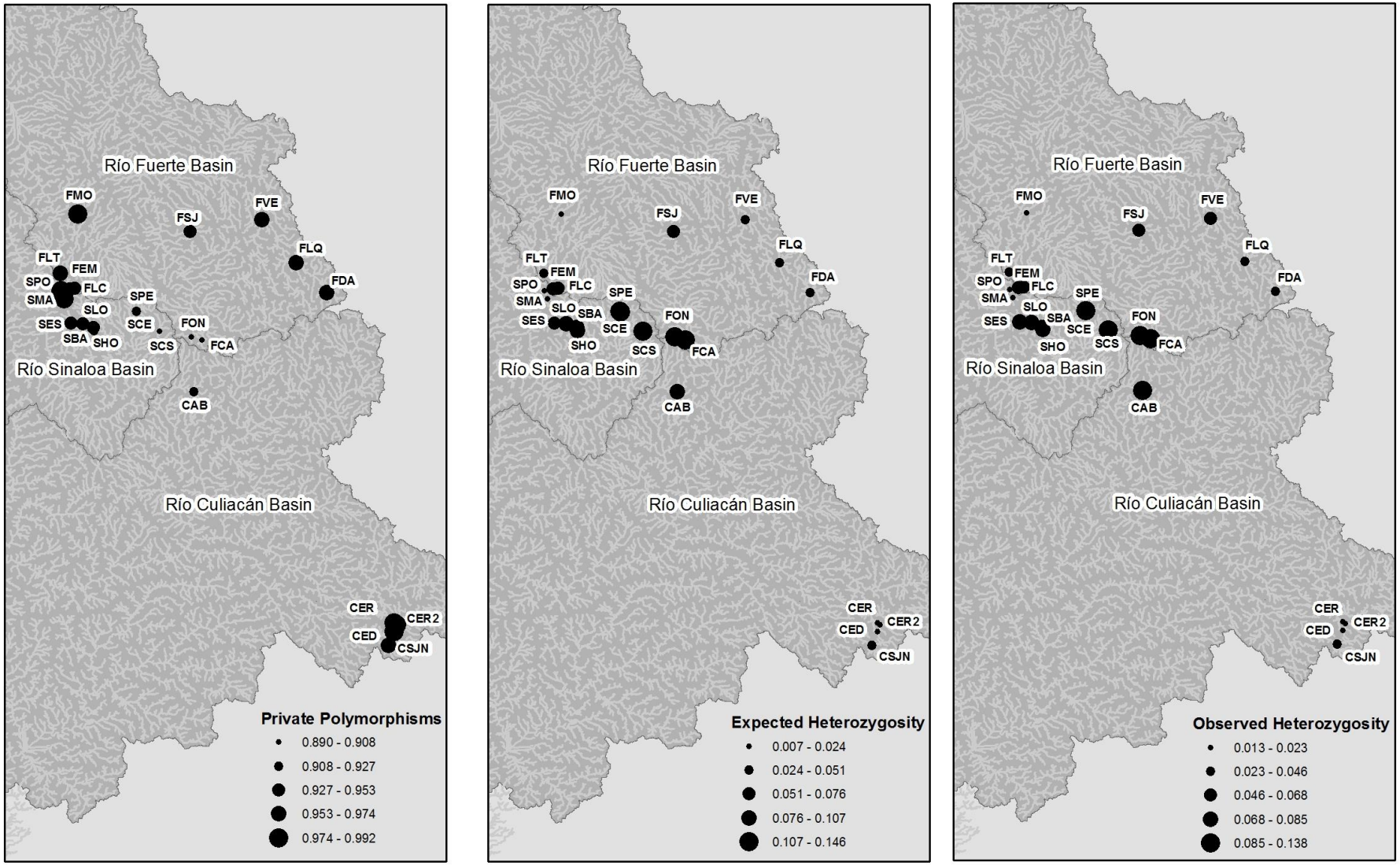

Figure 2 Geographical distribution of genetic diversity values for native O. chrysogaster. See table 2 for explanation of the sample site codes.

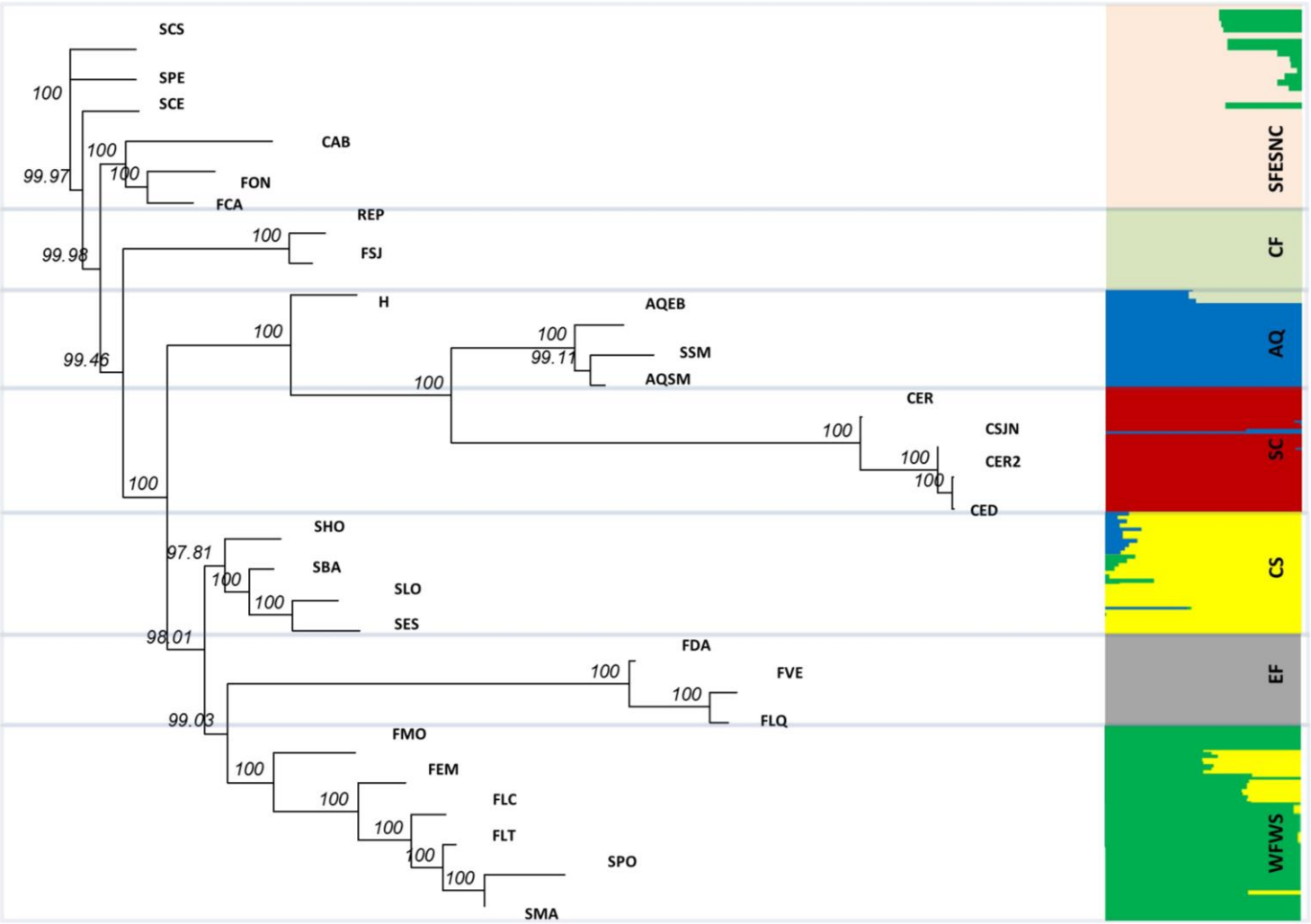

Figure 3 Genetic structure of *O. chrysogaster* and aquaculture rainbow trout defined by Nei distances (Tamura and Nei, 1993) in adegenet and a Bayesian assignment test in fastStructure. (a) Nei phenogram, the bootstrapping values for each clade are represented by numbers, see table 1 for explanation of the sampling site codes. (b) Bayesian assignment test barplot, each color represents a different genetic cluster and the genome of each individual is represented by a vertical line: Eastern Fuerte (EF); Central Fuerte (CF); Southern Fuerte, Eastern Sinaloa and Northern Culiacán (SFESNC); Western Fuerte and Western Sinaloa (WFWS); Central Sinaloa (CS); Southern Culiacán (SC); Aquaculture (AQ).

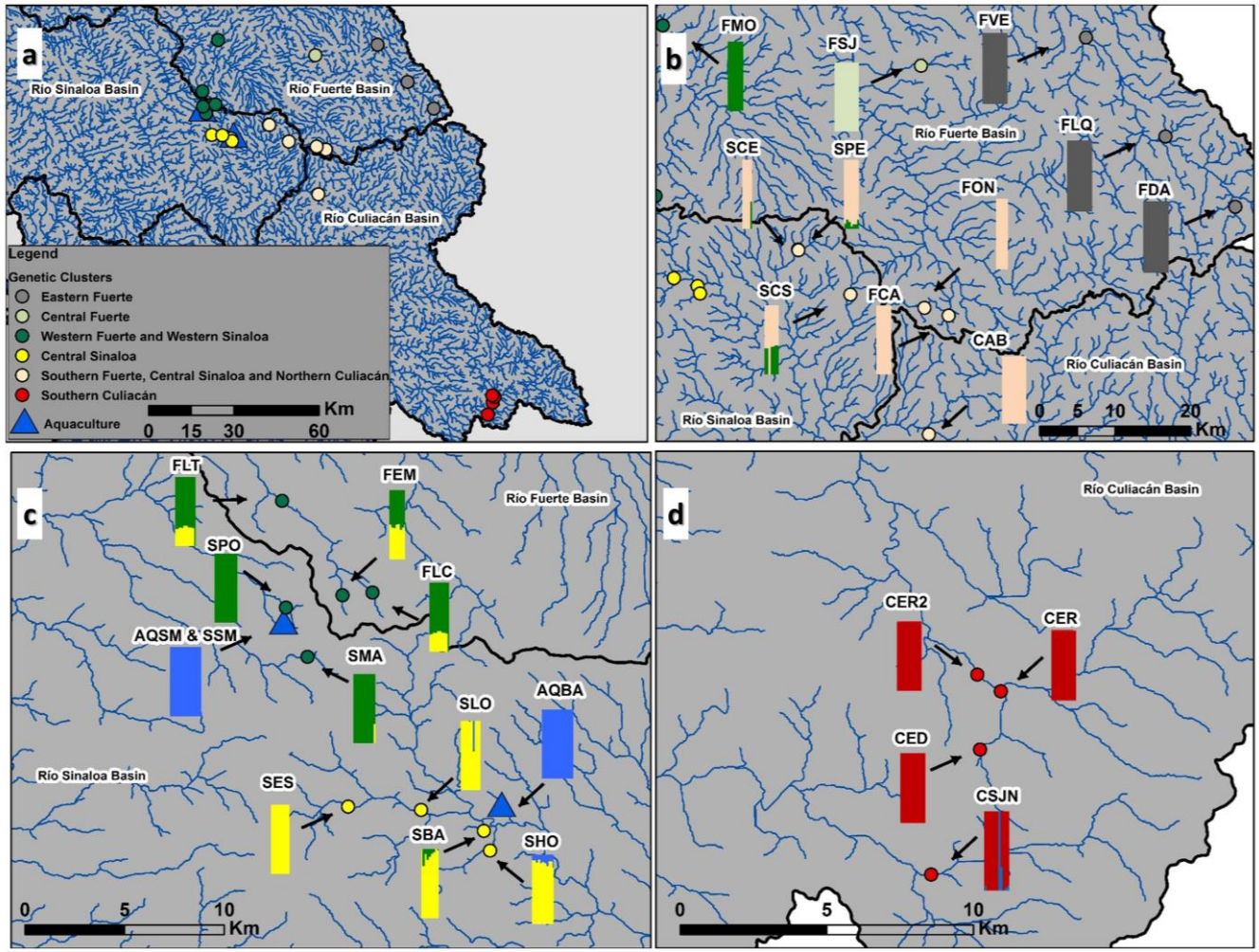

Figure 4 Spatial distribution of native *O. chrysogaster* and aquaculture rainbow trout genetic clusters defined in FastStructure. a) Spatial genetic structure across all the study area. b) Trout from Eastern Fuerte; Central Fuerte; Southern Fuerte, Central Sinaloa and Northern Culiacán genetic clusters; and FMO sample site from Western Fuerte and Western Sinaloa genetic cluster. c) Trout from Western Fuerte and Western Sinaloa; Central Sinaloa; and Aquaculture genetic clusters. d) Trout from Southern Culiacán genetic cluster. See table 1 for explanation of the sample site codes

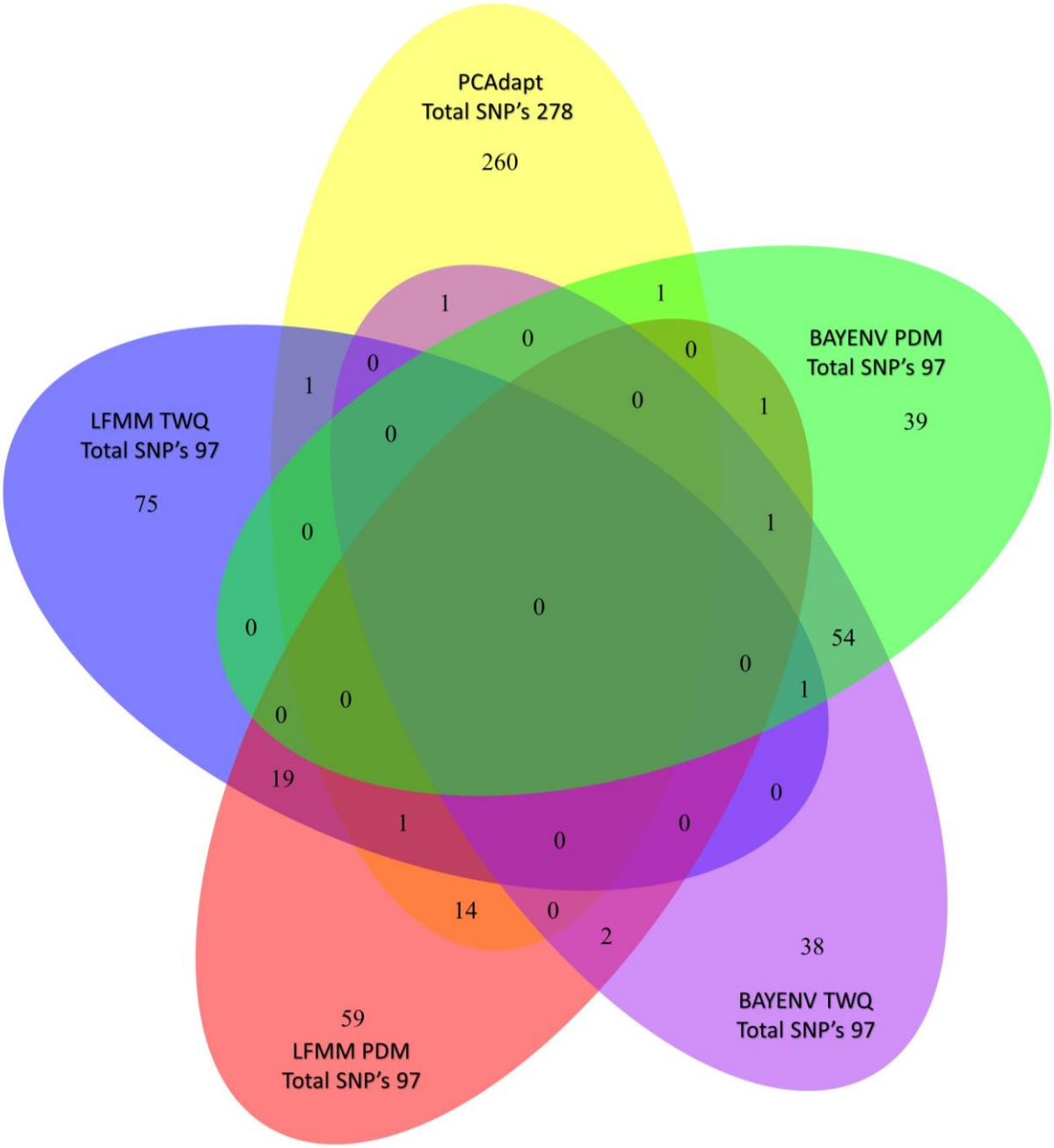

Figure 5 Venn diagram of outliers detected for O. chrysogaster among PCAdapt, LFMM using temperature of the warmest quarter (LFMM TWQ), LFMM using precipitation of the driest month (LFMM PDM), Bayenv2 using temperature of the warmest quarter (BAYENV TWQ), and Bayenv2 using precipitation of the driest month (BAYENV TWQ).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Six datasets at four different spatial scales.

Appendix S2. Riverscape resistance surfaces and matrices.

Appendix S3. F_{ST} coefficients among sample sites of Mexican golden trout, aquaculture rainbow trout and lab hybrids.

Appendix S4. Assignment probabilities obtained in fastStructure for different K values.