Riverscape genetics in the endangered Mexican golden trout (Oncorhynchus chrysogaster) in Sierra Madre Occidental, Mexico

Marco Alejandro Escalante Sanchez

THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Écologie, Évolution, Ressources Génétiques, Paléobiologie

École doctorale GAIA

Unité de recherche CEFE – UMR 5175

Riverscape genetics in the endangered Mexican golden trout (*Oncorhynchus chrysogaster*) in Sierra Madre Occidental, Mexico

Présentée par Marco Alejandro ESCALANTE SANCHEZ
Le 29 septembre 2017

Sous la direction de Stéphanie MANEL, Francisco Javier GARCIA DE LEON et Pierre COUTERON

Devant le jury composé de

Marie Josée FORTIN, Professor, University of Toronto	Rapporteur
Anne Béatrice DUFOUR, Maître de conférences, Université Claude Bernard Lyon 1	Rapporteur
François BONHOMME, Directeur de Recherche, Centre National de la Recherche Scientifique	Examinateur
Arturo RUIZ LUNA, Principal Investigator, Centro de Investigación en Alimentación y Desarrollo	Examinateur
Stéphanie MANEL, Directrice d'Étude, École Pratique des Hautes Études	Directrice de thèse
Francisco Javier GARCIA DE LEON, Principal Investigator, Centro de Investigaciones Biológicas del Noroeste	Co-directeur de thèse
Pierre COUTERON, Directeur de Recherche, Institute de Recherche pour le Développent	Co-directeur de thèse

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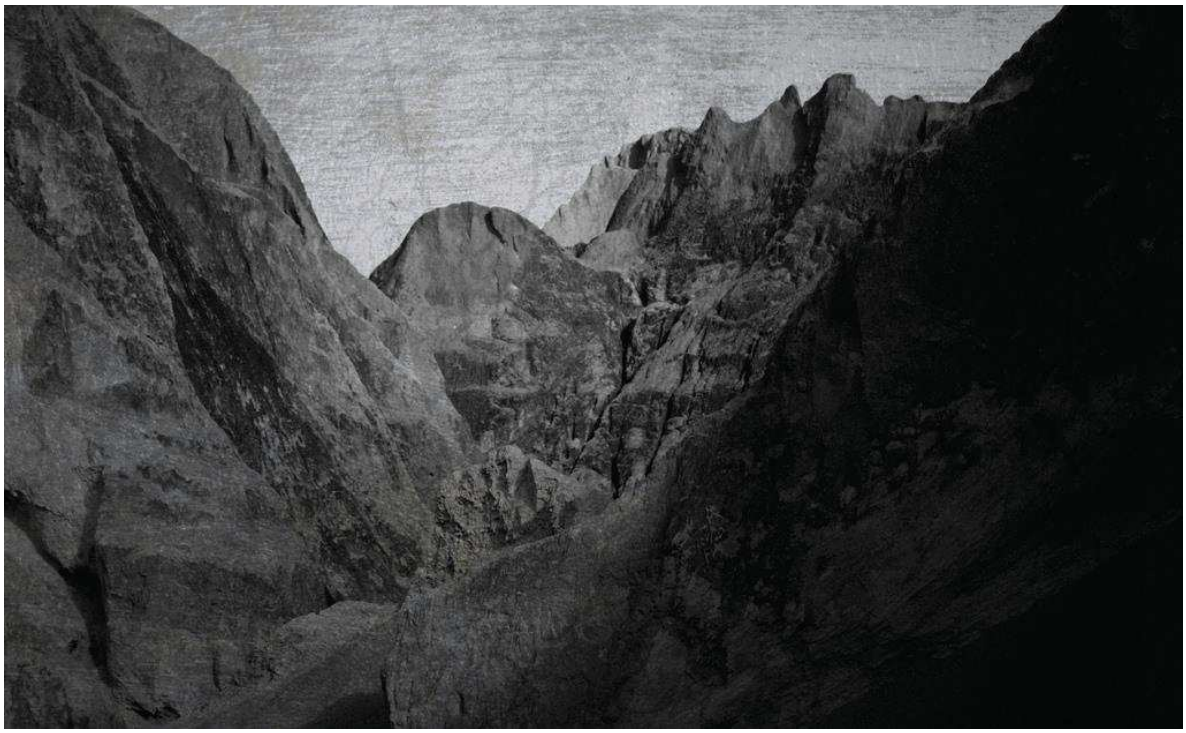
A Papá y Mamá



I could not help feeling that they were evil things -- mountains of madness whose farther slopes looked out over some accursed ultimate abyss.

H. P. Lovecraft

At the Mountains of Madness (1936)

Acknowledgements

This thesis would not have been possible without the support of many people, from both academic and personal sides. Starting from my advisors; I am really grateful for all the time they spent on me, for their valuable contributions to my PhD and for all their advice that have been fundamental on my academic formation. Also, I want to thank to my friends for being always there in both good and bad situations: thanks for being celebrating with me; but also for listening to me when I was sad, for helping me when I had difficulties and also for taking care of my when I had health problems and when I stayed in the hospital.

First of all I want thank to Francisco and Stéphanie for directing this thesis, but also for all their teachings, even long time before I started this PhD project. Paco gracias por introducirme en el mundo de las truchas, pero también por todas tus enseñanzas desde que realicé contigo mi tesis de licenciatura, todo lo aprendido contigo influyó de manera definitiva en que yo decidiera iniciar una carrera en la investigación científica. Stéphanie merci pour toutes tes attentions depuis que je suis allé à Grenoble pendant mes études de master il y a six ans, merci pour ta gentillesse mais aussi pour ta discipline qui m'a forcé à donner le meilleur de moi-même tous les jours depuis que j'ai commencé à travailler avec toi.

I am completely grateful with Arturo Ruiz Luna. Gracias por todo tu apoyo y por siempre confiar en mí, pero sobre todo gracias por tu amistad y por esa calidad humana que tienes con tus estudiantes. Todo lo que aprendí contigo me ha hecho ver mi trabajo de una manera más crítica y analítica, pero también me ha hecho mejorar como persona.

I want to thank Pierre Couteron. Merci pour tout votre aide, car sans elle, cette thèse n'aurait pas été possible. Merci beaucoup.

I am grateful with François Bonhomme and Patrick Berrebi for all their feedbacks and contributions during my thesis committee meetings.

I am especially grateful with Marie Josée Fortin and Anne Béatrice Dufour for reviewing this thesis. Thanks for the time you spent reading this manuscript and for your valuable contributions.

My acknowledgements to CONACYT for providing the scholarship to perform my PhD studies. Additionally, this research was supported under projects founded by CONACYT (Ref. CB-2010-01-152893) and ECOSNORD (M14A04).

Many thanks to Charles Perrier. Merci pout ton aide désintéressée, pour tous tes conseils, mais spécialement pour ton amitié. Merci Carlitos!

Thanks to Erin Landguth for her support and advice that were very important for the development of the Chapter II of this thesis.

I am especially thankful with Marco Andrello. Thanks for all your advices while analyzing my datasets, for this long friendship we have, but especially for your kindness. Thank you for being always there, and for helping in sad and difficult periods. Grande Marco! Grazie mille!

My acknowledgments to Rafael Hernández Guzmán and Alejandro Ramírez Huerta. Gracias por todo su apoyo y arduo trabajo sin el cual este proyecto no hubiera podido llevarse a cabo.

Special thanks to Gorgonio Ruiz Campos. Gracias por la hospitalidad brindada cuando visité tu laboratorio y por todas tus enseñanzas que fueron muy importantes para realizar el trabajo de campo.

I am thankful with Genoveva Ingle, Faustino Camarena, Héctor Espinosa and all ''Truchas Mexicanas'' group.

Many thanks to Brad Shepard, Carter Kruse, Jason Dunham, and others for the donation of an electrofishing equipment.

Thanks to Ricardo Silvas ''El Chalote'', Tomás Durán and all the people who helped us for fieldwork.

Thanks to Guanaceví Municipal Government, PRONATURA and WWF for all their support in field surveys.

Many thanks to Marie Pierre Dubois and all the staff of The Molecular Markers Platform of CEFE.

My acknowledgments to Enrique Ortega Aboud for his aid in gene ontology analysis.

Super dooper thanks to ''The Gang'' for making AMAP such a cool place to work. Also, all of you helped me and stayed with me in difficult periods, I have no words to thank you all. Mathieu, merci pour tout le temps que tu est passé à m'écouter et aussi pour tout ton aide et amabilité; we are not sure there will be not nights of the witch anymore, but I know I count with you to kill the witches. Jimmy merci pour tous ces bières, tous tes conseilles et l'hospitalité de ta famille. Falafel, merci pour les apéros à chez toi, la nourriture asiatique et spécialement ta grande gentillesse. Yogan merci d'être le plus cool jamais collègue de bureau. Jérôme merci de me recevoir pendant tous mes ''homeless periods'', ta gentillesse et ta soutien constant. Diane merci pour ta hospitalité et pour soucier de moi. Luis y Patricia; gracias por toda su amabilidad, ayuda y por estar conmigo en los momentos difíciles. Merlino, merci pour ta compagnie dans les bons et mauvais, et pour mes fleurs et cône d'anniversaire. Emil, thank you for all those funny conversations; however, I'm still thinking Paulo Coelho is awful (I cannot write bad words here). Claudinha, thank you for showing me all those Brazilian songs who changed my life. Pierre Cellier ''Peter Celery-Pedro Apio'' merci pour ton amabilité et les bières. Santiago, gracias por contarnos la historia de ''les petites boulettes''. Lorenzo, thanks for being always able for a beer, pizza or going to the beach, grazie mille ragazzo. Cheers for The Gang! You rock!

Mention honorable à mes colocs français pour tous les moments et leur patience et compréhension pour mes habitudes exotiques. Merci beaucoup Antoine ''Tête de chou-fleur'', Marion ''Mamarion'', Léa ''Oompa Loompa'', Izumi ''Surimi'', Léa ''la non Oompa Loompa'', Lauriane et Verlaine l'échalotte.

Uriel, gracias por tu amistad, por las chelas y por estar conmigo en momentos complicados. Jimena, gracias por toda tu ayuda y por escucharme en todo momento aunque siempre me la hagas de tos. Juan Pablo, gracias por siempre tener tiempo para mí ya sea en cuestiones académicas, problemas personales o simplemente crápula. Sebas, fue un placer tenerte como compañero de oficina y de parrandas. Hellboy! It's really cool to be your friend, also thanks for the hospitality of your family and the kick ass parties. Picka! Jennifer thanks for your kindness; and for that tour across Edinburgh distilleries, beaches and haggis restaurants. Ponchis, muchísimas gracias por tu amabilidad y hospitalidad. Rafa y Elena, gracias por su hospitalidad y esas clases de historia de la Guerra Civil Española, periodo franquista, así como los desplantes de La Condesa del Pirulí y El Conde del Troncho Duro.

Ángel, Ariane y Gábor; gracias por toda su amabilidad, hospitalidad y los múltiples conciertos vividos.

Thanks to Jelena, Nico, Tim, Eva, Ana, Anderson, Jazmín, Mar, Mónica, Xavier and many peple from CEFE; for all the good moments, technical support etc…

Many thanks to Claude, Alicia, Julie, Coline, Emilie, Benjamin, Gaël, Pauline, Pierrick, Olivier, Charlotte, Jean Yves, Amanda, Stan, Roozbeh, Jules, Saliha, Thani, Alessio and all the BEV team.

I also want to thank Yan, Sourabh, Vincent, Ruksan, Patoche, Awaz, Amandine, Hubert, Gilles, Alexis, Valaire, Valerie, Nathelie and many others from AMAP.

Many thanks to Fausto, Criss, Ricardo, Cristina, Leandro, Sarahi, Ilze, Paty from CIBNOR for their support and friendship.

Thanks to Léa, Víctor, Antonio Miguel, Carola, Maria, Ana, Pritha, Fran and many other friends.

I want to thank Alicia Zama for helping me to formalize my scholarship, muchas gracias a donde quiera que estés.

Finally I want to thank my parents. Mamá y Papá, muchas gracias por todo su amor y apoyo.

Abstract

The combined effect of different threats has caused an accelerated loss of biodiversity in endemic species. Then, it is crucial to quantify potential extinction risks as consequence of global change related with human activities, especially in range restricted species. An example of that situation is represented by the native Mexican trout complex inhabiting the highlands of northwest Mexico and representing the group of salmonids with the southernmost distribution in the world, with the Mexican golden trout (*Oncorhynchus chrysogaster*) and the coastal nelson trout (*O. mykiss nelsoni*) as the only described species for this complex.

However, as mountaintop species, these salmonids are highly vulnerable to global change effects, mainly by climate change and the introduction of the exotic rainbow trout for aquaculture purposes. The overall aim of this PhD project is to assess the possible relationships between microevolutionary processes of the Mexican golden trout, as well as the spatial structure of their habitat defining extinction risks derived by global change.

To address those questions, a riverscape genetics approach was applied at different spatial scales and taxonomic levels including population genetics analyses based on neutral microsatellite markers and Next Generation Sequencing (NGS), G.I.S. (Geographic Information Systems; riverscape characterizations), species distribution modeling and demo-genetic simulations.

Initially, population genetics analyses of 11 microsatellite loci revealed a spatial genetic structure for the entire Mexican trout complex as well as genetic introgression for native trout collected in aquaculture farm proximities, these results were corroborated by other using more microsatellite and SNPs markers. Moreover, focusing on *O. chrysogaster*, species distribution models and demogetic simulations defined riverscape as the main factor driving native population genetic structure, and as a boundary against exotic introgression. Additionally, 9,676 SNP's were generated by NGS techniques defining a cryptic genetic structure for *O. chrysogaster*. Finally, landscape genomics approaches revealed a significant influence of riverscape factors on the neutral and adaptive genetic structure of the species.

Keywords: Salmonids, Mexican trout, landscape genetics, riverscape genomics, endangered species, exotic introgression.

Résumé

Les changements globaux provoquent une disparition accélérée des espèces endémiques. Il apparait crucial de quantifier les risques potentiels d'extinction des espèces en relation avec les changements climatiques liés à l'activité anthropique, et particulièrement pour les espèces dont l'aire de répartition est restreinte. Dans ce travail de thèse je me suis intéressé aux effets des changements globaux sur le complexe de la truite du Mexique qui vit dans le nord-ouest du Mexique. Ce complexe représente le groupe des salmonidés avec la distribution la plus méridionale au monde, avec seulement deux taxa décrits : la truite dorée mexicaine (*Oncorhynchus chrysogaster*) et la truite de San Pedro Mártir (*O. mykiss nelsoni*). En tant qu'espèce montagnarde d'altitude, ces salmonidés sont très vulnérables aux effets des changements globaux, et particulièrement au changement climatique et à l'introduction d'espèces exotiques pour l'aquaculture, comme la truite arc en ciel.

L'objectif général de cette thèse a été d'étudier l'impact de la structure spatiale de l'habitat sur les processus micro-évolutifs qui régissent les patrons de variations génétique de la truite mexicaine dorée. En effet cette structure spatiale de l'habitat est un proxy des changements globaux et permet d'appréhender les risques d'extinction futurs des populations.

Afin de répondre à ces questions, j'ai appliqué aux écosystèmes de rivières une approche de génétique du paysage en intégrant différentes échelles spatiales et différents niveaux taxonomiques. J'ai appliqué des analyses de génétique des populations, des analyses de système d'information géographique et de modélisation de la distribution des espèces, ainsi que des simulations démo-génétiques des risques d'extinction engendrés par les changements globaux.

Initialement, les analyses de génétique des populations réalisées sur 11 loci microstallites nous ont permis de mettre en évidence une structure génétique spatialisée pour l'ensemble du complexe des truites mexicaines, ainsi qu'une introgression génétique chez la truite endémique. Ces résultats ont été confirmés par des analyses utilisant un plus grand nombre de microsatellites et de marqueurs SNP. Une étude plus fine, centrée sur *O. chrysogaster,* et combinant simulations génétiques et distribution de l'espèce a permis de définir les caractéristiques des paysages de rivières comme les principaux déterminants de la structure génétique des populations natives, voire comme des barrières aux flux de gènes. Pour cette espèce, j'ai également généré une base de données de 9676 SNP grâce aux techniques de séquençage de nouvelles génération et mis en évidence une structure génétique cryptique chez *O. chrysogaster*. Une approche de génomique du paysage a révélé une influence significative des variables physiques des rivières sur la structuration génétique neutre et adaptative de la truite dorée mexicaine.

Mots clés: Salmonidé, truite mexicaine, génétique du paysage, génomique du paysage, espèces menacées, introgression exotique

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Chapter 1. Synthèse de thèse en français

Introduction générale (A version in English is included in appendix I)

Diversité spécifique et changements globaux

Les niveaux d'organisation biologique sont clairement définis et ordonnés dans un système catégorique. Dans le but de définir des stratégies de gestion pour la conservation, Franklin (1988) a défini dans une étude fondatrice les trois attributs primaires de la biodiversité : sa composition, sa structure et ses fonctions. Ces attributs sont inclus dans un système hiérarchique distribué selon trois niveaux d'organisation spatiale liés à un niveau taxonomique : communautés-écosystèmes, espèces - paysage régional, populations/ génétique - paysage local (Noss 1989). Ainsi, l'étude de la biodiversité nécessite une compréhension des processus prenant place au sein de l'ensemble des niveaux d'organisation.

Récemment, l'effet combiné de différentes menaces (climatique et anthropique) a causé la perte accélérée de biodiversité avec des extinctions d'oiseaux, amphibiens et mammifères endémiques (Pimm et al. 2014). Il apparaît donc crucial de quantifier les risques potentiels d'extinction engendrés par les changements globaux liés aux activités anthropiques qui menacent la dispersion biologique, la connectivité des habitats ainsi que la viabilité des populations (e.g. changements climatiques, introgression exotique, changement d'usage des terres, fragmentation de l'habitat) (Fischer 2007; Tilman et al. 2017; With et al. 2006).

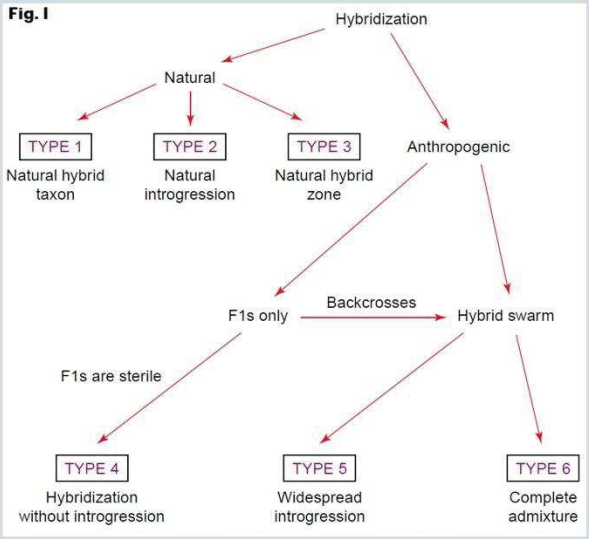
La sensibilité des espèces aux climats actuels et passés augmente la possibilité que les changements climatiques deviennent l'une des causes principales d'extinction dans les prochaines décades, la terre devenant plus chaude que ce qu'elle n'a jamais été depuis le Paléogène, et principalement en raison de l'activité humaine (Thomas & Et 2004; Urban 2015). Les conséquences directes de ces modifications climatiques sont des changements de la phénologie des espèces, et de leur distribution dans tous les environnements soumis aux changements globaux. Les organismes avec des aires de distribution restreintes, comme les espèces polaires et montagnardes, vont être particulièrement touchées et subir de sévères réductions de leurs habitats. Ces organismes ont été identifiés comme les premiers groupes chez lesquels des espèces entières se sont éteintes (Parmesan 2006). Au-delà de l'extinction des espèces et de la réduction des habitats, les changements climatiques pourraient générer un déficit de la productivité des écosystèmes, des invasions par des espèces exotique ainsi que l'émergence de maladies (Peterson et al. 2002).

Les invasions par des espèces exotiques ont été considérées comme une autre menace majeure pour la biodiversité à différents niveaux (i.e. : écosystèmes, espèces) en

raison des risques liés à la prédation et à la compétition entre autres (Goldburg & Triplett, 1997; Tella, et al. 2016). En fait, les impacts négatifs des espèces exotiques vont au-delà des dégâts sur la biodiversité, engendrant des pertes économiques et de sérieux problèmes de santé (Escalante et al. 2016). Aux États-Unis, le coût des dégâts environnementaux causés par les invasions d'espèces exotiques s'élèvent à plus de 120 000 millions de dollars par an (Pimentel et al. 2005). Dans le cas des poissons d'eau douce, les introductions fréquentes d'espèces exotiques à la fois à des fins récréatives et aquacoles ont eu des effets négatifs dans les populations endémiques. Or ces organismes sont plus sensibles que beaucoup d'autres car ils possèdent un habitat plus petit en comparaison des espèces terrestres et marines (Penaluna et al. 2016). Quand l'espèce introduite est phylogénétiquement proche de l'espèce native, elle peut s'hybrider et générer un phénomène appelé introgression génétique (Box 1). L'introgression génétique a un effet négatif qui peut s'avérer extrêmement nocif sur l'espèce endémique car elle entraine modification du pool génétique natif et réduit la valeur sélective (fitness) de l'espèce, ce qui réduit également sa capacité à survivre dans des environnements variables (Milián-García et al. 2015). Quand l'espèce invasive est peu abondante, son taux de migration est faible. Il n'y a alors aucun avantage pour le génotype exotique et l'effet de l'invasion (sur les espèces natives) est quasiment nul (Escalante et al. 2016). Cependant, si le génotype exotique est favorisé, les taux de migration de l'espèce envahissante sont élevés et si en plus la taille de la population native est faible, les effets de l'introgression peuvent être préjudiciables (McGinnity et al. 2003). Allendorf et al. (2001) ont défini 6 catégories d'introgression, parmi lesquelles, ceux qui impliquent l'introgression génétique exotique ont les effets les plus nocifs sur les populations natives (Box 1).

Box 1. Categorization of hybridization (taken from Allendorf *et al.* 2001).

Figure 1 provides a framework with which to categorize hybridization. Each type should be viewed as a general descriptive classification that is used to facilitate discussion rather than as a series of strict, all encompassing divisions. Types 1–3 represent hybridization events that are a natural part of the evolutionary legacy of taxa; these taxa should be eligible for protection. Types 4–6 divide anthropogenic hybridization into three categories that have different consequences from a conservation perspective.



Type 1. Natural hybrid taxon: Virgin River roundtail chub *Gila seminuda* are listed as endangered under the Endangered Species Act of the USA (ESA). It is a hybrid taxon that appears to have originated from hybridization between *G. elegans* and *G. robusta* in the Pleistocene long before human influence in the Colorado River system (DeMarais et al., 1992).

Type 2. Natural introgression: Moorean land snails *Partula tainiata* and *P. suturalis* occur sympatrically on the island of Moorea in French Polynesia. In spite of being markedly different both phenotypically and ecologically, estimates of genetic distance based on molecular markers between some sympatric populations of these species are lower than is typical for conspecific comparisons for these taxa (Clarke et al., 1998). The authors of the study concluded that this apparent paradox was best explained by ''molecular leakage, the convergence of neutral and mutually advantageous genes in two species through occasional hybridization''.

Type 3. Natural hybrid zone: Red- and yellow - shafted northern flickers *Colaptes auratus* hybridize in the Great Plains of North America (Moore and Price, 2002). Their narrow hybrid zone extends from Canada through Texas (USA) and has been remarkably stable historically. The reproductive success of hybrids is equal to that of the parental types, and there is no assortative mating within the hybrid zone. Nevertheless, the parental types are thought to be maintained by sexual selection and by natural selection associated with environmental differences between eastern and western North America.

Type 4. Hybridization without introgression: Bull trout *Salvelinus confluentus* are currently listed as threatened under the ESA. Hybridization with introduced brook trout *S. fontinalis* has been documented throughout much of their range. However, there have been few reports of hybrids beyond the first generation (F1) (Leary *et al.*, 1993). Thus, the major detrimental effect of hybridization in this case is wasted reproductive effort rather than genetic mixing. Removal of the non-native species and F1 hybrids is likely to be beneficial, and restoration of degraded habitat could help decrease hybridization.

Type 5. Widespread introgression: Westslope cutthroat trout *Oncorhynchus clarki lewisi* have suffered from widespread hybridization with introduced rainbow trout *O. mykiss* (Allendorf and Leary, 1998). However, many pure westslope populations remain, especially in isolated headwater areas throughout the range of the subspecies. Hybridized populations are of little conservation value (although they could have other values), and efforts should focus on maintaining and expanding the remaining pure populations.

Type 6. Complete admixture: New Zealand grey duck *Anas superciliosa* have been severely affected by hybridization with introduced mallard ducks *A. platyrhynchos* (Rhymer *et al.*, 1994). Few, if any, pure populations remain and there does not appear to be any selection against the hybrids. Here, conservation of hybrids should be considered, because it is the only available option if we are to avoid the complete loss of the hybridized species.

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Les changements globaux comme les changements climatiques et l'introgression génétique peuvent engendrer des processus d'extinction des espèces dans un futur proche (Figure 1). Dans ce contexte, l'intégration de méthodes multi-échelles et multi-disciplinaires est fondamentale pour une gestion appropriée des espèces en danger (Allendorf & Luikart 2007).

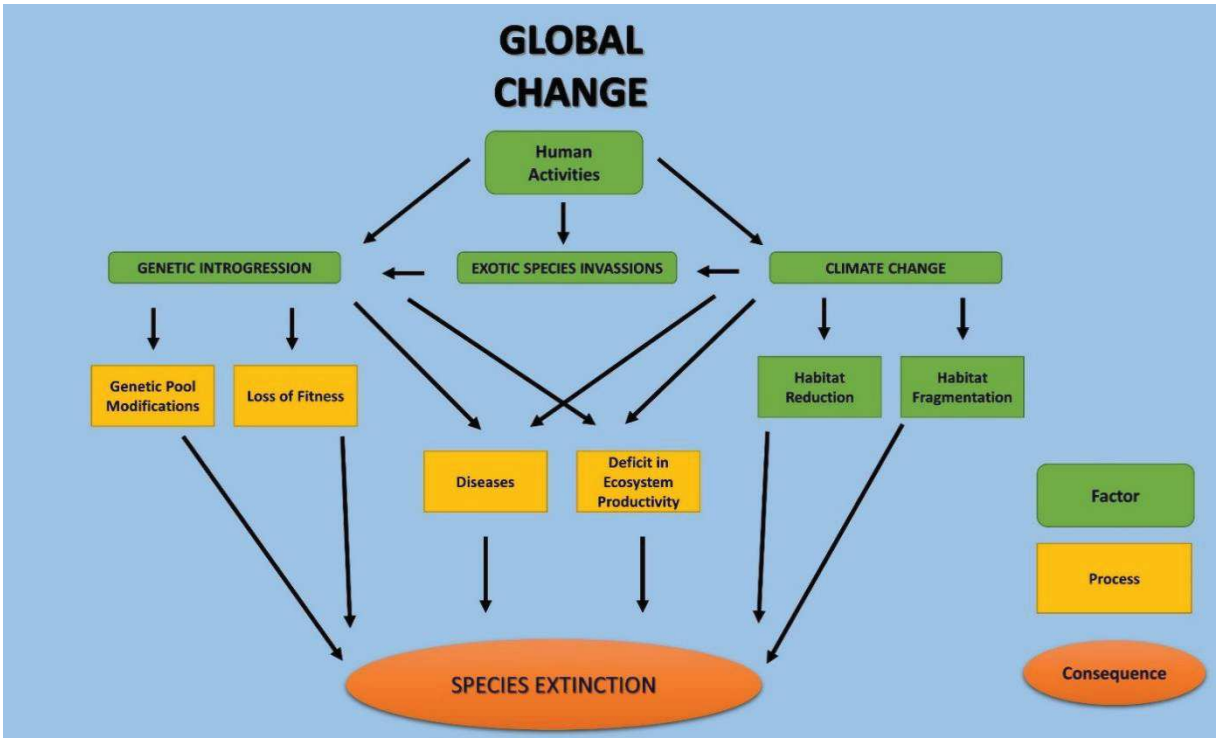

Figure 1. Processus liés aux changements climatiques conduisant à des extinctions d'espèces. Les facteurs sont indiqués en vert, les processus en jaune et la conséquence en orange (Goldburg & Triplett, 1997; Penaluna et al. 2016; Peterson et al. 2002; Thomas & Et, 2004; Urban, 2015).

Approches et échelles spatiales

L'étude des patrons de biodiversité implique des disciplines scientifiques propres à chaque niveau d'organisation (e.g. écosystème/espèces : macroécologie, biogéographie ; intra-spécifique : génétique des populations, biologie moléculaire). Les méthodes développées pour étudier ces patrons ont évoluées de manières différentes, et ne sont habituellement pas utilisées à une échelle différente de celle pour laquelle elles ont été développées, principalement en raison de contraintes méthodologiques (Holling 1998). Les différences d'approche ont tendance à créer une vision étroite et partielle de la réalité, mais malgré ces différences, il est clair qu'elles partagent des points communs qui pourraient rendre possible

une agrégation de différentes disciplines analysant les relations biophysiques entre les interactions multi-échelles, permettant alors la compréhension des systèmes et de leurs processus (Holling 1998; Stein et al. 2014).

Les propriétés des écosystèmes se manifestent à différentes échelles spatiales et temporelles et affectent la structure spatiale des populations ; donc, l'utilisation d'une seule et unique échelle d'analyse peut mener les chercheurs à négliger certains facteurs lors de l'analyse de processus biologiques comme la dispersion, la reproduction, la distribution des espèces, les interactions trophiques et la dynamique vitale (Anderson et al. 2010). Dungan (2002) suggère trois catégories auxquelles les termes en relation avec l'échelle spatiale peuvent-être appliqués : 1) le phénomène associé, par exemple la structure génétique spatiale d'une espèce et le phénomène qui l'affecte; 2) les unités d'échantillonnage ou spatiales utilisées pour acquérir des informations sur le phénomène, par exemple les mesures *in situ* ou les pixels dans une image ; et 3) l'analyse des données utilisée pour les résumer ou faire des inférences. Par conséquent, le phénomène, l'échantillonnage et l'analyse peuvent être considérés comme trois dimensions auxquelles les concepts d'échelle se rapportent (Figure 2).

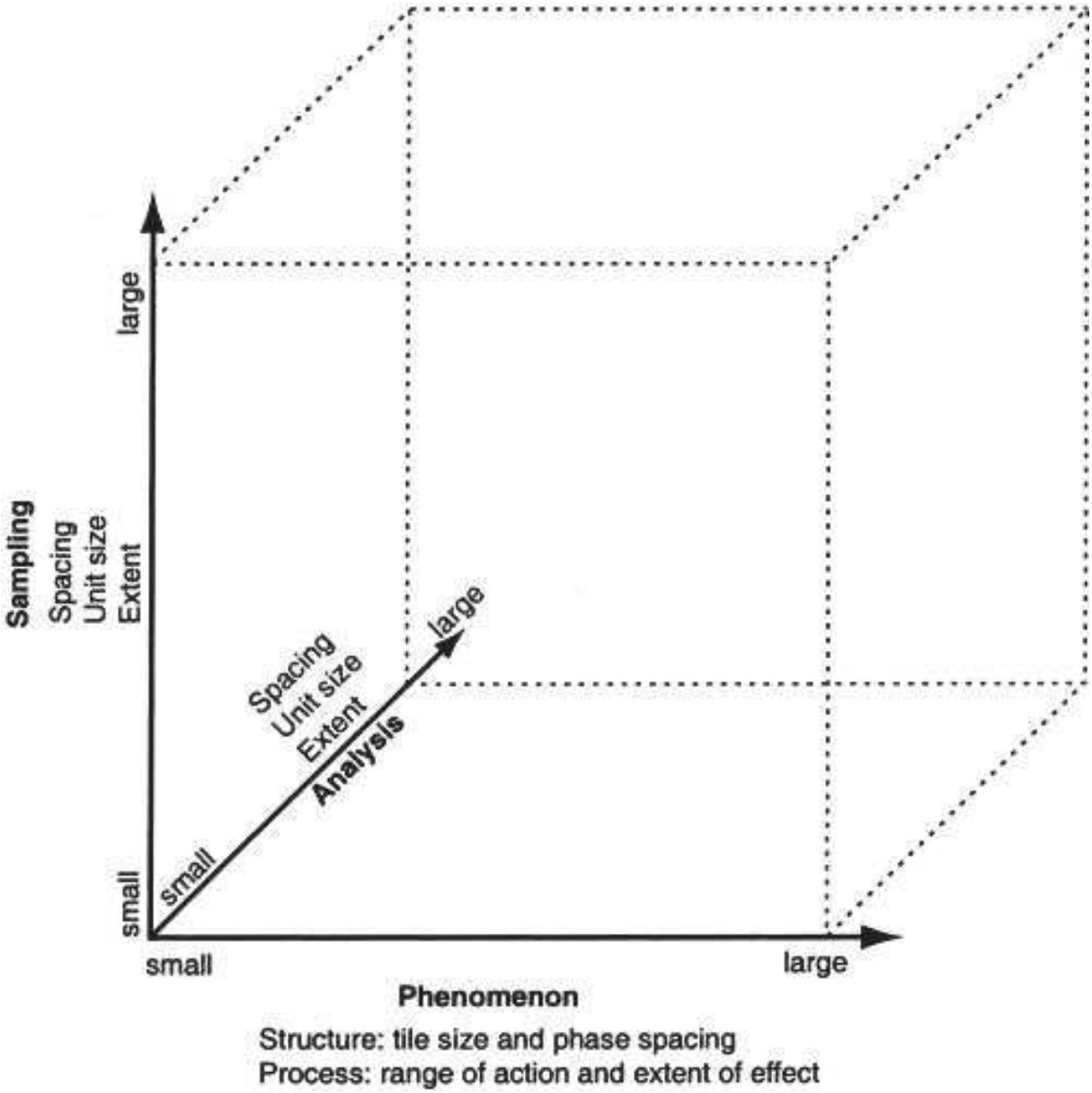

Figure 2 . Les dimensions des concepts d'échelle (source de l'image: Dungan et al. 2002).

Sous les mêmes concepts, les paysages de rivières sont régis par les débits d'eau. En fonction de sa densité et de sa viscosité, l'eau est un vecteur bien plus efficace pour lier les mosaïques paysagères à la fois avec l'échelle et l'espace en fonction de l'air environnant les paysages terrestres (Wiens 2002). De plus, la grande hétérogénéité environnementale et la réduction des habitats des zones de rivière entraînent une réduction de la taille des populations et des taux migratoires par rapport aux écosystèmes marins (Selkoe et al. 2016). Par conséquent, les processus biologiques se produisant aux échelles spécifiques et

intraspécifiques dans les habitats de rivières présentent un intérêt particulier pour l'étude de l'écologie et de la biologie évolutive.

Génétique du paysage

L'interaction entre les génotypes et l'environnement joue un rôle important au sein des processus écologiques et évolutifs. La variation génétique provient des mutations aléatoires des séquences d'ADN, et est augmentée par recombinaison au cours de la reproduction sexuée qui produit des nouvelles combinaisons de gènes. La variation génétique est également créée par l'intégration de nouveaux gènes venus d'autres population grâce à un mécanisme évolutif appelé flux de gènes (Cabrero & Camacho 2000). L'étendue de la variation génétique résulte d'un équilibre entre les différentes forces évolutives (mutation, flux de gènes, dérive, et sélection naturelle ; Box 2) qui crée une différenciation génétique locale au sein des populations (King et al. 2006; Slatkin, 1987). La sélection naturelle est à l'origine de la variabilité adaptative chez les individus, qui va conduire à l'adaptation local ou non des populations. La variabilité adaptative se définit comme la variation phénotypique héritable résultant de la sélection naturelle dans différentes niches écologiques, et qui augmente la valeur sélective d'un génotype/individu (fitness) dans un environnement donné. Ainsi certains génotypes présentent une plus grande capacité à s'adapter en raison de leur meilleure capacité à survivre et à se reproduire pour faire face aux variations environnementales (Hanski et al. 2017; Robinson & Schluter, 2000). Des maladaptations proviennent d'une incompatibilité entre le phénotype et l'environnement et si les populations sont non adaptées localement, elles sont menacées d'extinction (Kokko et al. 2017). De même, si les populations sont constituées d'un faible nombre d'individus, des processus de colonisation récents peuvent entrainer un déficit de diversité génétique (ex : effet fondateur) résultant de l'altération de la fréquence des gènes, avec pour conséquence des variations dans les processus de reproduction et une augmentation de la mortalité (Banks et al. 2013).

Box 2. Evolutionary forces driving genetic variation (King *et al.*, 2006).

Mutation: the random process by which a gene undergoes structural change (Figure 1).

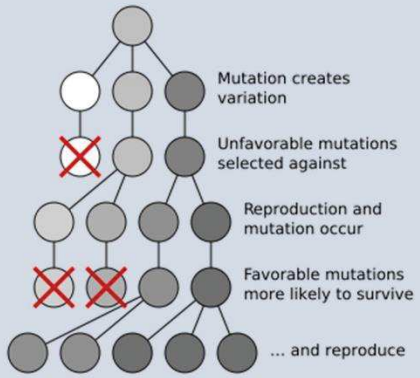

Figure 1. Diagram of mutation and selection in evolution (image source: https://commons.wikimedia.org/wiki/File:Mutation_and_selection_diagram.svg).

Gene flow: the exchange of genes between different populations of the same species produced by migrants, and commonly resulting in simultaneous changes at many loci in the recipient gene pool (Figure 2).

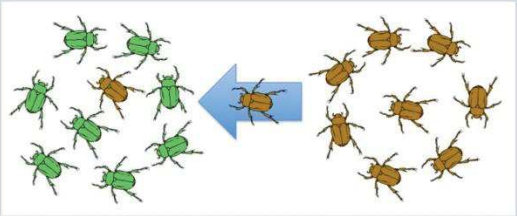

Figure 2. Graphical representation of gene flow (image source: https://upload.wikimedia.org/wikipedia/commons/c/c5/Gene_flow.jpg)

Genetic drift: the random fluctuations of gene frequencies due to sampling errors. While drift occurs in all populations, its effects are most evident in very small populations (Figure 3).

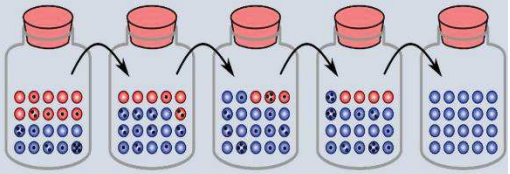

Figure 3. Example describing the effect random sampling has in genetic drift. Dots indicate samples from each generation that are transferred to the next generation. In this population of 20, there is a shift from an allele frequency of 50% for the blue allele to 100% for the blue allele in just 5 generations (image source: https://upload.wikimedia.org/wikipedia/commons/0/0b/Random_sampling_genetic_drift.svg).

Natural Selection: the differential fecundity in nature between members of a species possessing adaptive characters and those without such advantages (Figure 4).

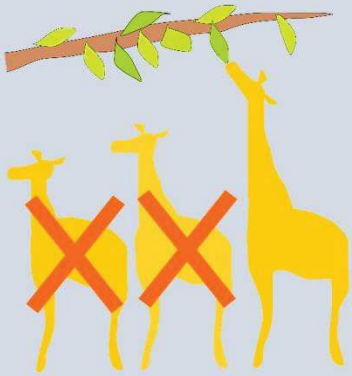

Figure 4. Graphical representation of natural selection (image source: https://upload.wikimedia.org/wikipedia/commons/thumb/8/80/Selection.svg/2000px-Selection.svg.png)

References

King *et al.*, (2006). *A dictionary of genetics*. Oxford University Press.

L'écologie du paysage repose sur l'idée que l'hétérogénéité du paysage influence les systèmes écologiques via par exemple le mouvement des animaux, la persistance des populations, les interactions entre espèces et les fonctions de l'écosystème (Fahrig et al. 2011). Ainsi, les barrières paysagères ont un effet déterminant sur les flux de gènes, les discontinuités génétiques, la structure génétique de la population et l'adaptation locale (Holderegger & Wagner 2006). La compréhension de ces effets pose une grande variété de

questions de recherche fondamentale et appliquée comme: quantifier l'influence des variables du paysage et leur configuration sur la variation génétique; Identifier les barrières au flux des gènes; Identifier les dynamique sources-puits et les corridors de mouvement; Comprendre l'échelle spatiale et temporelle d'un processus écologique; et tester des hypothèses écologiques spécifiques aux espèces (Storfer et al. 2007).

Dans ce contexte, la génétique du paysage, qui combine la génétique des populations et l'écologie du paysage a été introduite afin d'améliorer la compréhension des interactions entre les éléments du paysage et les processus micro-évolutifs (échelle de temps inférieure à la spéciation) comme les flux de gènes et l'adaptation locale (Manel et al. 2003). Cette discipline essaie de comprendre quels sont les processus écologiques ou évolutifs qui influencent et structurent la différenciation génétique entre populations et individus, ainsi que les variations conjointes ente hétérogénéité biologique et environnementale (a Storfer et al. 2007). Dans ce domaine, le développement récent de nouvelles méthodes permettant de générer d'importantes données génomiques (séquençage haut début), comme le polymorphisme des nucléotides (SNP), qui permet de caractériser un individu avec plus de dix mille marqueurs. Associées à une croissance rapide des données des Systèmes d'Information Géographique (SIG) et des méthodes statistiques, ces avancées permettent une meilleure compréhension des processus de connectivité et d'adaptation des populations dans les paysages hétérogènes (Manel & Holderegger 2013; Selkoe et al. 2016). Les paysages de rivières, peu étudiés jusqu'à présent en génétique du paysage (mais voir Table 1), offrent des paysages hétérogènes linéaires idéaux pour tester l'effet de l'environnement sur les patrons micro-évolutifs d'espèces natives (Kanno et al. 2011).

Table 1 Landscape genetics studies testing environmental influence on neutral and adaptive genetic variation at riverine systems.

Species	Location	Number of molecular markers/approach	Main findings	Reference
Freshwater mussel (*Cumbrelandia monodonta*)	Mississippi River Basin, U.S.A	Simulation study	Climate change will significantly reduce population connectivity and genetic diversity	Inoue & Berg, 2017
Rainbow/steelhead trout (*Oncorhynchus mykiss*)	Columbia River Basin, U.S.A.	180 SNP's	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 SNP's	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
Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	Akokala Creek, U.S.A.	Simulation study	Inability of exotic populations to recolonize patches as well as lower	Landguth et al. 2016

			genetic exchange when barriers are added	
Chinook salmon (*Oncorhynchus tshawytscha*)	Northeastern Pacific Coast, U.S.A. and Canada	19,703 SNP's	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	Both 310 AFLP loci (empirical data) and simulation study	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	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	Genetic diversity deficit related with waterfalls and forest road culverts	Torterotot et al. 2014

Bull trout (*Salvelinus confluentus*)	Upper Flathead River, Canada and U.S.A.	Simulation study	Suitable habitat fragmentation generating loss of genetic diversity	Landguth et al. 2014
Atlantic salmon (*Salmo salar*)	Southeastern Canada	5,500 SNP's	Temperature, precipitation and geological characteristics related to both potentially adaptive and neutral genetic divergence	Bourret et al. 2013
Two castastomidae species (*Catostomus discobolus Discobolus*) and (*Catostomus discobolus yarrow*)	Western U.S.A.	16 microsatellite loci	Stream hierarchy defining gene flow	Hopken et al. 2013
Atlantic salmon (*Salmo salar*)	Western Russia	14 microsatellite loci	Genetic diversity associated with carrying capacity and stream gradient	Ozerov et al. 2012
Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	Akokala Creek, U.S.A.	Simulation study	Barriers placed at headwater areas caused loss of genetic diversity	Muhlfeld et al. 2012

Atlantic salmon (*Salmo salar*)	Western France	17 microsatellite loci	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	Gene flow mitigated by riverscape barriers	Kanno et al. 2011
Bull trout (*Salvelinus confluentus*)	Glacier National Park, Montana U.S.A.	11 microsatellite loci	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	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	Heterozygosity negatively correlated with elevation, precipitation and upstream distance, while positively	Narum et al. 2008

			correlated with temperature. Additionally, geographical barriers drive genetic structure of life history types	
Three-spined stickleback (*Gasterosteus aculeatus*)	Scheldt River, Flanders Belgium	6 microsatellite loci	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	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	Within populations expected heterozygosity negatively correlated with altitude, while within lakes with habitat size	Castric et al. 2001

Les paysages de rivières (riverscape) sont les variables physiques et chimiques qui constituent les bassins versants, et influencent de manière particulière les processus microévolutifs. Les flux de gènes sont en effet contraints par la composition dendritique du réseau fluvial et la variation adaptative est affectée par les paramètres environnementaux du cours d'eau et des zones environnantes (Chaput-Bardy et al. 2008; Wiens, 2002). Ainsi, la génétique du paysage appliquée aux paysages de rivières été utilisée à l'origine avec un nombre limité de marqueurs neutres pour évaluer l'influence des paramètres hydroclimatiques et les conditions de l'habitat en termes de diversité génétique, ainsi que les effets des barrières géographiques sur les flux de gènes (e.g. Castric et al. 2001; Dionne et al. 2008; Osborne et al. 2014; Ozerov et al. 2012). Avec l'apparition des nouvelles techniques de séquençage (NGS), la génomique des paysages de rivières a permis de définir l'influence des facteurs environnementaux sur la variation adaptative à l'aide de grands jeux de données génomiques (Bourret et al. 2013; Brauer et al. 2016; Hand et al. 2016; Hecht et al. 2015). De surcroit, des simulations génétiques ont été réalisées afin de tester les hypothèses sur les flux de gènes dans des paysages fragmentés lorsque les données manquaient (Cooke et al. 2014; Inoue & Berg, 2017; Landguth et al. 2014; Muhlfeld et al. 2012).

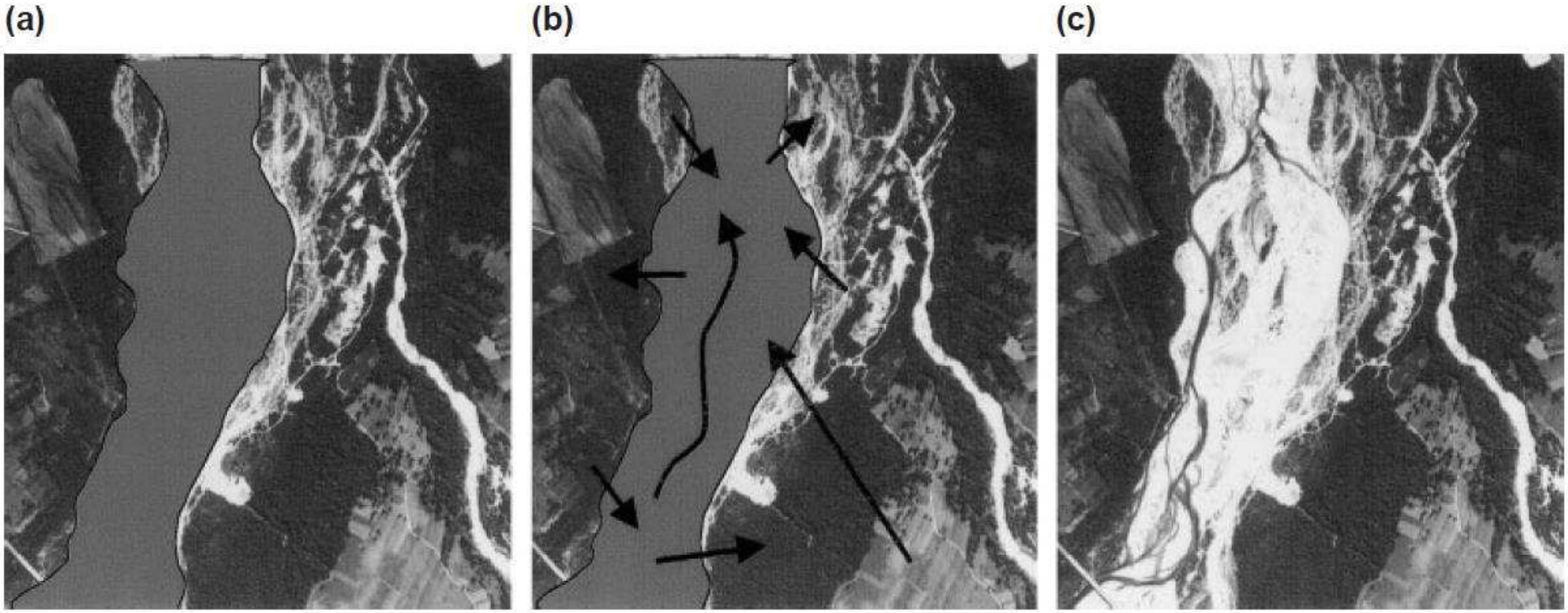

Figure 3. Structure des paysages de rivières et processus micro-évolutifs. (A) Une conception erronée du paysage de rivière en tant qu'élément homogène contenu dans un paysage terrestre plus large. (B) La rivière est reliée au paysage environnant par une série d'écoulements en bordure terrestre, ou longitudinalement dans le corridor de de la rivière. Par conséquent, les processus microévolutifs sont affectés par des patterns internes et externes. (C) La rivière fait partie d'un paysage dendritique qui est hétérogène en interne, et il existe un «paysage» dans le système de la rivière. Ainsi, les populations isolées dans les paysages de rivières peuvent subir différents processus micro-évolutifs (source d'image: Wiens, 2002).

Mon travail de thèse en se focalisant sur l'influence des effets de l'habitats des rivières sur la structure géntique d'un complexe de truite de rivière à permettre des avancées dans ce domaine.

Système d'étude

La Sierra Madre Occidental située au nord-ouest du Mexique présente une structure paysagère hétérogène, avec une géologie complexe comprenant des pentes abruptes et de fortes variations d'altitude (environ 3300m) et plusieurs bassins qui s'écoulent vers le golfe de Californie (Hendrickson et al 2006). Cette zone présente des températures extrêmes allant de -30°C à 40°C et un régime de précipitations oscillant entre 250 et 1600mm par an (Rzedowski, 2006); Servicio Meteorológico Nacional (http://www.smn.cna.gob.mx/). Ce paysage complexe présente une grande diversité d'habitats terrestres et aquatiques de laquelle découlent un fort taux d'endémisme biotique et une grande biodiversité. Pour ces raisons, La Sierra Madre Occidental est considérée comme un point chaud de biodiversité prioritaire (Mittermeier et al. 2002).

Les organismes d'eau douce et plus particulièrement les poissons autochtones sont parmi les groupes les plus menacés de cette région. Au sein de ce groupe, le complexe des truites autochtones de cette zone représente le groupe de salmonidés ayant la distribution la plus méridionale au monde. La truite côtière arc en ciel (*Oncorhynchus mykiss nelsoni*) de Sierra de San Pedro Mátir au Baja California Péninsule et la truite dorée méxicaine (*O. chrysogaster*) au Sierra Madre Occidental sont les seules espèces décrites de ce groupe (Hendrickson et al. 2002 ; Figure 4 et 5). Malgré leur importance potentielle pour l'industrie aquacole internationale en tant que salmonidé, ce complexe n'a fait l'objet que de rares études (Ruiz-Luna & García-De León, 2016; Ruiz-Luna et al. 2017). L'étude du complexe des truites autochtones mexicaines revêt donc une importance capitale du point de vue taxonomique, génétique, et écologique. Il est également nécessaire de comprendre les facteurs géographiques et environnementaux qui régissent leur distribution ainsi que les conditions critiques de leur habitat dans un objectif de conservation dans un contexte de changements globaux.

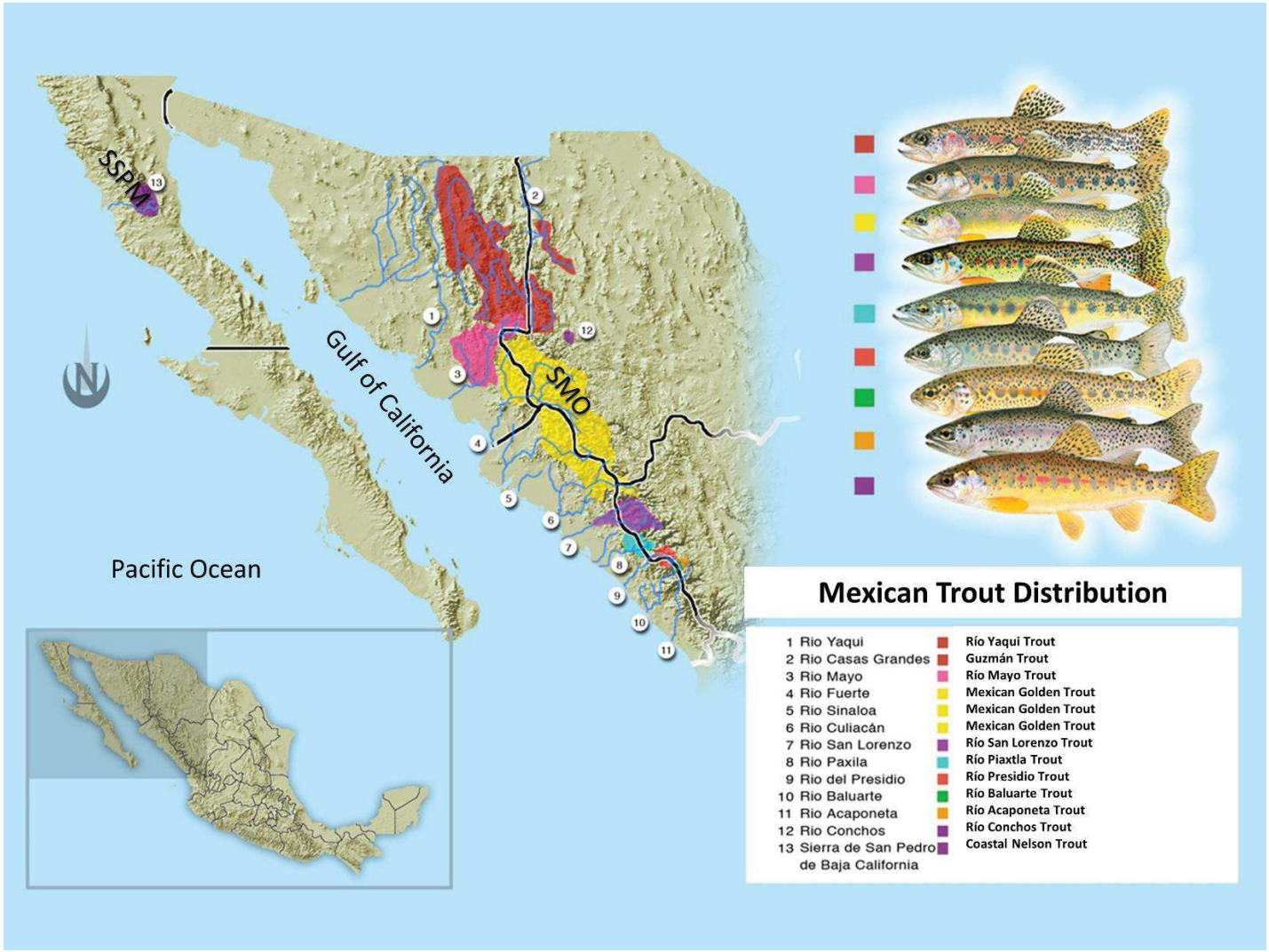

Figure 4. Distribution du complexe des truites mexicaines au nord-ouest du Mexique à Sierra de San Pedro Mártir (SSPM) et Sierra Madre Occidental (SMO). Source d'image: Espinosa et al. 2007

Figure 5. Truite dorée mexicaine (*Oncorhynchus chrysogaster*, Image fournie par Arturo Ruiz Luna).

La distribution d'*O. chrysogaster* au nord-ouest du Mexique comprend les altitudes les plus élevées de la Sierra Madre Occidental dans les bassins de Rio Fuerte, Sinaloa et Culiacan basins (Hendrickson et al. 2002). Plusieurs aspects de la biologie de cette espèce restent inconnus (mais voir Ruiz-Luna & García-De León, 2016) et bien qu'il existe des études sur la structure génétique de l'espèce, les résultats varient en fonction de la méthode utilisée et des caractéristiques des bases de données. Nielsen & Sage (2001) ont analysé 11 loci microsatellites de 28 truites récoltées sur deux sites d'échantillonnage et ont trouvé un seul groupe génétique pour *O. chrysogaster.* Camarena-Rosales et al. (2008) ont également défini un seul groupe génétique à partir de l'utilisation d'ADN mitochondrial de 58 truites échantillonnées dans quatre sites d'études. En utilisant 98 SNP et 18 microsatellites provenant de 207 truites collectées dans neuf sites d'échantillonnage, Abadia-Cardoso et al. (2015) ont défini 2 groupes génétiques principaux comprenant quatre sous-groupes composés par des affluents géographiquement adjacents de différents bassins. Escalante et al. (2016) ont utilisé 11 marqueurs microsatellites génotypés pour 206 individus prélevés dans neuf sites d'études, et ont mis en évidence quatre groupes génétiques avec la même composition géographique

qu'Abadia-Cardoso et al. (2015) ; pour plus d'informations sur les sites considérés dans les études précédentes et actuelles voir la Figure 6. Les différences entre les études peuvent s'expliquer en partie à cause de l'utilisation de différents marqueurs moléculaires, mais aussi des zones d'études restreintes. Ces différences mettent en évidence la nécessité de créer de nouvelles données impliquant un effort d'échantillonnage accru ainsi que des marqueurs moléculaires plus polymorphes afin de résoudre la structure génétique de cette espèce.

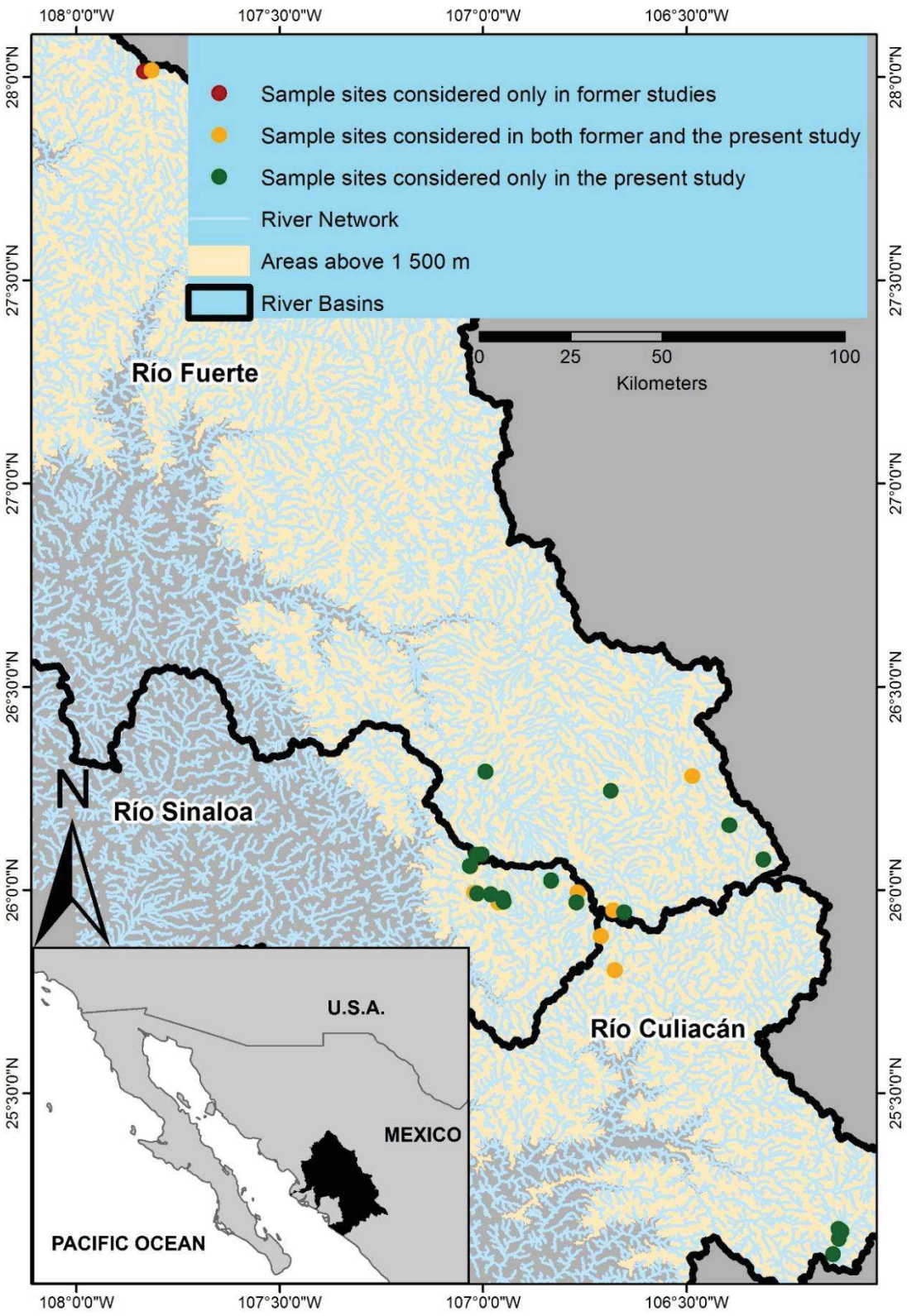

Figure 6. Area de distribution d'*Oncorhynchus chrysogaster* défini par Hendrickson et al. (2002) à Río Fuerte, Río Sinaloa et Río Culiacán (au-dessus de 1,500 m) et sites d'échantillonnage considérés à la fois pour les études précédents et la présente.

En dehors des menaces liées à la réduction et à la fragmentation de l'habitat, le réchauffement global devrait également diminuer l'aire de distribution d'O. *chrysogatser* (Ruiz-Luna et al. 2017). En effet, il existe des preuves d'Europe jusqu'en Amérique du nord, que le réchauffement global est responsable des variations dans l'amplitude de la distribution d'un certain nombre de taxons, les déplaçant vers les pôles, et dans les cas des espèces montagnardes vers des altitudes plus élevées (Penaluna, et al. 2016), ce qui est le cas d'*O. chrysogaster*. De plus, l'introduction de la truite exotique arc-en-ciel (*O. mykiss*) à des fins aquacoles, peut impacter le pool génétique de la truite dorée mexicaine, mais avoir également d'autres impacts préjudiciables causés par la compétition et l'introduction de maladies, d'autant plus que la truite arc-en-ciel est reconnue comme étant une espèce exotique envahissante dangereuse (Lowe et al. 2000). Il a été montré que la truite arc-en-ciel peut s'hybrider avec les truites autochtones dans le nord du Mexique (Escalante et al. 2016; Abadía-Cardoso et al. 2015). Malgré cette connaissance, les décisions économiques et sociales prises par les gouvernements fédéraux et locaux ont encouragé les exploitations introduisant *O. mykiss* depuis le XIXème siècle jusqu'à nos jours (Escalante et al. 2016; Hendrickson et al. 2006). Les conséquences probables de cette activité pourraient être l'hybridation ou le remplacement d'*O. chrysogaster* par l'espèce exotique entrainant des pertes de biodiversité autochtone.

Malgré le peu de connaissances disponibles au sujet de la biologie de la truite dorée du Mexique, il a toutefois été prédit que les gradients des variables constituants les paysages de rivières (*i.e.* augmentation de la température, variations dans les régimes de précipitation ainsi que des changements hydrologiques et topologiques) qui séparent les populations d'*O. chrysogaster* entravent les flux de gènes et entraineront une forte structuration génétique spatialisée, de la même manière que ce qui se produit avec les autres espèces de salmonidés (Frank et al. 2011). La diversité génétique et la variation adaptative pourront également être affectées par ces gradients environnementaux de rivière et l'introduction d'espèces exotiques, de la même manière que ce qui se produit avec les autres espèces de truites (Hand et al. 2016; Penaluna et al. 2016).

Objectifs

L'objectif principal de cette thèse est de déterminer l'impact de l'habitat - pris comme témoin du changement global - sur les processus microévolutifs des populations de truites mexicaines dorées. Pour répondre à cet objectif, j'ai échantillonné des jeux de données empiriques de truites, pour lequels j'ai développé à la fois des marqueurs neutres et adaptatifs, et j'ai

développé des données simulées avec des systèmes d'information géographique. La thèse est structurée en 3 chapitres pour répondre à cet objectif.

Chapitre II : Introgression génétique de la truite arc-en-ciel cultivée dans le complexe des truites autochtones mexicaines.

L'objectif de ce chapitre était d'évaluer la structure génétique spatiale de l'intégralité du complexe des truites mexicaines et l'introgression génétique liée à la culture de la truite arc-en-ciel. J'ai utilisé une base de données constituée de 1 017 individus génotypés pour 11 marqueurs microsatellites couvrant l'ensemble du complexe de truites mexicaines et distribués dans 13 bassins fluviaux dans le nord-ouest du Mexique, ainsi que des truites arc-en-ciel autochtones de Californie (Etats-Unis) et des truites arc-en-ciel cultivées issues de trois fermes aquacoles. A partir de ces données, j'ai appliqué des analyses de la structure et la diversité génétique, ainsi que de phylogéographie. J'ai mis en évidence un patron de structure génétique spatiale pour le complexe de truites mexicaines ainsi que de l'introgression génétique de la truite arc-en-ciel cultivée dans les populations proches des fermes aquacoles.

Chapitre III : Impacts du paysage et l'introgression par la truite exotique sur la structure génétique de la truite dorée du Mexique (Ochorynchus chrysogaster).

Ce chapitre se focalise exclusivement sur l'espèce *O. chrysogaster.* J'ai combiné des approches de niche écologique et de génétique des populations afin de simuler l'introgression de la truite arc-en-ciel sur les réponses démographiques et génétiques de la truite endémique, en considérant différentes hypothèses de mouvements des individus dans la rivière (distance euclidienne ; résistance de la rivière). Dans ce contexte, J'ai également analysé les variables du paysage les plus influentes sur la distribution d'*O. chrysogaster*. J'ai ensuite comparé les résultats issus des simulations avec les résultats de l'étude empirique réalisée dans le chapitre 2 dans le but d'identifier le scénario le plus réaliste. J'ai mis en évidence que les barrières physiques constituées par l'environnement des rivières étaient suffisamment fortes pour structurer génétiquement les populations de la truite endémique et empêcher l'introgression par les truites exotiques.

Chapitre IV : Génétique des paysages de rivières chez la truite dorée du Mexique endémique.

L'objectif de cette partie est de définir les échelles spatiales de la structure et de la diversité génétique des populations d'*O. chrysogaster* ainsi que l'influence des paramètres des paysages de rivières sur la diversité génétique neutre et adaptative.

J'ai collecté des spécimens d'*O. chrysogaster* et de truite arc-en-ciel dans les bassins de Rio Fuerte, Sinoloa et Culiacán pour générer une base de données de 9 676 SNP. Ces analyses de génomique des populations ont révélé une structure génétique cryptique pour *O. chrysogaster* et ont permis de définir de manière plus précise que les études précédentes le degré d'introgression génétique. De plus, les approches de génétique des paysages de rivière nous ont permis de définir avec précision l'influence des patrons des paysages de rivières sur la diversité et la structure génétique des populations. Enfin, nous avons pu identifier avec des méthodes d'associations les loci soumis à une pression de sélection d'origine climatique, tandis que les approches ontologiques nous ont permis de caractériser les fonctions biologiques de ces loci.

Chapitre V : Discussion générale.
Ce chapitre discute les principaux résultats de cette étude et ouvre des perspectives pour la conservation du complexe de truites au Mexique.

Résultats principaux

Chapitre II : Introgression génétique de la truite arc-en-ciel cultivée dans le complexe des truites autochtones mexicaines.

Les estimations de diversité génétique ont été réalisées uniquement pour les truites de pisciculture (n = 76) provenant de quatre écloseries génotypes pour 11 loci microsatellites. Le nombre maximum, minimum et la moyenne d'allèles étaient respectivement de 9.8, 5.5 et 7.2. Par ailleurs, la valeur moyenne de l'hétérozygotie observée était de 0.67 (intervalle de variation de 0.5 à 0.75), tandis la valeur attendue était de 0.7 (intervalle de variation de 0.67 à 0.75). Globalement, les truites provenant de l'écloserie de Río San Lorenzo ont montré la plus haute valeur de diversité génétique (Tableau 2, Chapitre II)

Les analyses des 1017 individus par des algorithmes de classification non supervisée bayésien ont permis de définir 18 groupes, parmi lesquels 16 étaient constitués de truites mexicaines autochtones, une de truites arc-en-ciel californiennes et une de truite arc-en-ciel exotique provenant de pisciculture (Figure 3, Chapitre II). De plus, les analyses de classifications couplées avec les index d'hybridation ont montré une introgression moyenne

élevée provenant de truites de pisciculture dans les truites autochtones collectées dans l'Arroyo Aparique à Río Fuerte, l'Arroyo Agua Blanca à Río Culiacán; Río San Lorenzo; Río Piaxtla; Río Presidio; Río Baluarte; and Río Acaponeta (Figure 3 et Table 4, Chapitre II).

Chapitre III: Impacts du paysage et de l'introgression par la truite exotique sur la structure génétique de la truite dorée du Mexique (Ochorynchus chrysogaster).

Le modèle de distribution d'espèces a détecté trois variables corrélées à la présence de la truite dorée du Mexique : l'altitude, les précipitations du mois le plus sec, et la température de la saison plus chaud. Les probabilités de présences les plus élevées pour *O. chrysogaster* étaient situées à l'est et au sud de Río Fuerte, à l'est de Río Culiacán, et dans les limites de trois bassins de distribution d'*O. chrysogaster* (Río Fuerte, Río Sinaloa et Río Culiacán), de façon prépondérante dans les cours d'eau supérieurs (Figure 3, Chapitre III). En me basant sur ces résultats, j'ai pu estimer les meilleures conditions pour l'occurrence de l'espèce: il s'agit des sites avec des précipitations lors du mois le plus chaud proches de 22 mm, à une altitude d'environ 2600 mètres et à une température du quart le plus chaud inférieure à 15°C (Figure 4, Chapitre III).

Parmi les différents scénarios simulés, le plus plausible était celui caractérisé par une résistance du paysage de rivière sans introgression exotique (Scénario II). Les analyses sPCA montrent que ce scénario détecte les trois mêmes groupes génétiques (est, ouest et centre) que ceux détectés par les données empiriques (Figure 5, Chapitre III). De plus le Scénario II atteint un F_{ST} global (0.190) similaire à celui des données empiriques (0.20) (Table 3, Chapitre III). Le scénario simulé avec une introgression exotique et une résistance du paysage (IV) était le deuxième plus proche des données empiriques, montrant les trois mêmes groupes génétiques et un F_{ST} global de 0.088 (Figure 5 et Table 3, Chapitre III). Ces résultats suggèrent une influence déterminante du paysage sur la structure génétique et un effet barrière contre les introgressions exotiques.

Chapitre 4 : Génétique des paysages de rivières chez la truite dorée du Mexique endémique.

Le filtrate bioinformatique des SNP a permis de retenir 270 individus génotypés pour 9,767 SNP's. Les taux d'hétérozygoties attendus varient de 0,01 dans trois sites d'échantillonnage à Río Culiacán (CED, CER et CER2) à 0.16 dans les fermes de pisciculture (AQEB). En outre, le plus grande taille effective de population (145,7) a été trouvée dans le site d'échantillonnage SBA et la plus petite (0,4) dans le site d'échantillonnage SMA, toutes les deux à Río Sinaloa (Table 2, Chapitre IV). L'approche de classification bayésienne non supervisée a identifié 6

groupes génétiques distincts se superposant à la géographie et un groupe correspondant à de la pisciculture (Figure 3 et 4, Chapitre 4).

Les analyses visant à connaître l'influence du paysage de rivière comme facteur de la diversité génétique neutre ont montré une corrélation significative à différentes échelles spatiales. Pour les analyses prenant en compte les populations localisées au centre de l'aire d'étude, des corrélations de l´hétérozygotie attendue avec l'altitude, les précipitations du mois le plus sec ainsi que l'altitude ont été trouvées. En outre, l'hétérozygotie attendue pour le bassin de Río Fuerte est corrélée avec la latitude, la longitude, la température du mois le plus chaud, la longueur de la rivière ainsi que le sens du courant (Tableau 3, Chapitre IV). Les tests de Mantel effectués afin de tester l'effet du paysage sur la structure de la diversité neutre ont montré des corrélations significatives au niveau du bassin. Pour les populations de Río Fuerte et de Río Sinaloa, les variables distances linéaires des rivières et résistance du paysage étaient significativement corrélées avec les F_{st} ($r \geq 0.49$ et $p \leq 0.01$; Tableau 4, Chapitre IV).

Les analyses de loci outliers ont détecté 566 SNP sous sélection divergente, parmi lesquels 388 étaient corrélés avec au moins une des variables hydroclimatiques testées : la température de la saison la plus chaude et les précipitations du mois le plus sec (Figure 5, Chapitre IV). De plus, l'application de filtres de qualité dans l'analyse, j'ai retenu 21 loci SNP sous sélection divergente avec des annotations protéiques (Tableau 6, Chapitre IV). La plupart de ces annotations sont associées à des fonctions biologiques qui peuvent être liées à des facteurs hydroclimatiques. Ces résultats suggèrent potentiellement une adaptation d'*O. chrysogaster* aux conditions locales.

Discussion générale

Synthèse

L'objectif principal de cette thèse était d'évaluer les effets des caractéristiques des paysages de rivière et de l'introgression génétique sur les processus microévolutifs (structure génétique et adaptation locale) de la truite dorée Méxicaine. Dans une analyse préliminaire du complexe des espèces de truites Méxicaines j'ai montré que la structure génétique était composée de 16 groupes génétiques natifs, et caractérisée par un haut degré d'admixture avec les populations de truite d'aquaculture au Sud du Sierra Madre Occidental. J'ai ensuite utilisé des simulations démo-génétiques qui ont montré que la résistance des paysages de rivières est le principal facteur qui structure la diversité génétique spatiale chez *O. chrysogaster*, et constitue une barrière à l'introgression de gènes exotiques. Finalement, à travers l'analyse d'un large panel de SNP provenant de 270 individus, nous avons confirmé qu'il y a peu d'admixture entre *O. chrysogaster* et les truites d'élevage, et encore une fois une influence des paysages de

rivières sur la structure génétique neutre. Des analyses de type gène-environnement ont suggéré une influence des patrons hydroclimatiques sur la variation génétique adaptative d'*O. chrysogaster*.

Chapitre II : Introgression génétique de la truite arc-en-ciel cultivée dans le complexe des truites autochtones mexicaines.

L'analyse de 11 loci microsatellites a montré que la diversité génétique des truites d'élevage est plus élevée que celle de la truite native mexicaine montré dans d'autres études (Abadía-Cardoso et al. 2015; De los Santos-Camarillo 2008; Escalante et al. 2016), mais également pour des salmonidae de plus hautes latitudes (Bohling et al. 2016; Ozerov et al. 2012; Torterotot et al. 2014). Les goulots d'étranglement peuvent expliquer la perte de diversité génétique, en particulier pour des populations de petite taille avec un faible nombre de colonisateurs et des faibles taux d'immigration (Nei et al. 1975). Par conséquent, l'hypothèse la plus vraisemblable pour expliquer la présence de salmonidae dans le Mexique du Nord est celle d'un processus de colonisation à partir de salmonidae californiens au cours du Pleistocene, quand un groupe de truite tête d'acier a migré vers le golf de Californie en réponse à des températures froides. Successivement, quand la température globale a augmenté à la fin du Pléistocène, ces truites ont colonisé les parties les plus hautes des rivières du SMO, puis ont été isolées en plusieurs populations fragmentées et soumises alors à plusieurs processus microévolutifs (Behnke et al. 2002; Hendrickson et al. 2006). Ce processus de colonisation peut avoir engendré des goulots d'étranglement génétiques qui expliqueraient le déficit de diversité génétique du complexe de truites mexicaines. De plus, notre étude a démontré l'existence d'une structure génétique spatiale pour le complexe des truites mexicaines entre bassins-versants et au sein des bassins. Plus particulièrement, l'analyse de 1017 individus a permis d'identifier 16 groupes génétiques endémiques dans 13 bassins dans le Nord du Mexique. Des patrons de structure génétiques similaires ont été détectés par d'autres auteurs (Abadía-Cardoso et al. 2015; De los Santos-Camarillo, 2008) .

De plus, nous avons observé de forts niveaux d'admixture entre les truites natives et d'élevage dans le Sud du SMO, en particulier dans les Río San Lorenzo, Río Presidio, Río Baluarte et Río Acaponeta. Dans le Río San Lorenzo, j'ai observé de l'introgression seulement pour les truites d'Arroyo La Sidra capturées à proximité des élevages. Cependant, pour for Río Presidio, Río Baluarte et Río Acaponeta, toutes les truites ont montré des signaux d'admixture avec les truites d'élevage, et dans certains cas la proportion de gènes exotiques était supérieure à celle des gènes natifs. Puisque les élevages de truites sont abondants dans ces bassins-versants, les truites de Río Presidio, Río Baluarte et Río Acapanoneta sont les plus

exposées à l'introgression génétique (Camarena-Rosales et al. 2008; Héctor Espinosa personal comunication). Nous avons observé de faibles niveaux d'admixture génétique entre les truites d'élevage et *O. chrysogaster* dans peu de sites. Dans le cas particulier d'Arroyo Aparique (Río Fuerte), quand nous avons analysé les truites d'un élevage abandonné et des truites sauvages échantillonnées à proximité, nous avons observé de l'admixture dans les deux sens (depuis l'aquaculture vers les natives et vis versa). Ce type d'admixture génétique pourrait être une conséquence de la proximité entre les deux sites, mais une explication alternative pourrait être l'utilisation de truites natives pour l'aquaculture dans cette ferme aquacole. En conclusion, ces résultats et ceux d'autres études suggèrent que les truites d'élevage ne s'étendent pas beaucoup au-delà de l'immédiat voisinage des fermes aquacoles.

Chapitre III: Impacts du paysage et de l'introgression par la truite exotique sur la structure génétique de la truite dorée du Mexique (Ochorynchus chrysogaster).

L'analyse de 11 loci microsatellite présentée dans le Chapitre II a montré l'existence d'une structure génétique spatiale chez *O. chrysogaster* avec un faible niveau d'introgression. J'ai créé un modèle de simulation qui combine un modèle de distribution d'espèce et un modèle démo-génétique pour clarifier quels facteurs parmi la structure du paysage et l'admixture génétique avec les truites d'aquaculture déterminent la structure génétique actuelle d'*O. chrysogaster*.

Parmi les scénarios simulés avec le modèle démo-génétique, le scénario qui incluait la structure fluviale sans introgression a donné les résultats les plus proches de ceux des données génétiques empiriques, en termes de structure génétique spatiale (trois groupes), indice de différenciation génétique globale F_{st} et diversité génétique.
Ces résultats montrent que la structure du paysage est le facteur principal qui détermine la structure génétique d'*O. chrysogaster* en créant des barrières à la dispersion es individus. Les résultats montrent aussi que l'introgression est faible. Les trois groupes génétiques étaient séparés par des gradients climatiques, des ruptures topographiques caractérisées par la température, l'ordre des cours d'eau, l'augmentation de la pente et la diminution de l'altitude. Des effets de la structure du paysage ont aussi été observées dans d'autres études, à travers des simulations (Muhlfeld et al. 2012; Landguth et al. 2014; Inoue & Berg 2017) ou des analyse de données empiriques (Hopken et al. 2013; Osborne, Perkin et al. 2014; Perrier et al. 2011).

Le modèle de distribution d'espèce a montré que l'altitude, la température de la saison la plus chaude et les précipitations de la saison la plus sèche ont une forte influence sur l'occurrence d'*O. chrysogaster*. Les mêmes variables ont été trouvées dans une autre étude sur la truite dorée Méxicaine dans le Río Sinaloa et le Río Culiacán (Ruiz-Luna et al. 2017) et

dans d'autres études sur le complexe des truites Mexicaines et des truites des États-Unis (Hendrickson et al. 2006; Wenger et al. 2011). Ces variables sont en fait essentielles pour la survie des truites dans leur habitat (Roberts et al. 2013; Ruiz-Luna et al. 2017). De façon générale, les résultats de ce chapitre montrent que la structure des paysages de rivières, et non seulement la simple distance euclidienne spatiale, est le facteur principal qui règit la dispersion de la truite dorée Mexicaine et les processus qui en dépendent (flux de gènes et introgression de gènes exotiques).

Chapitre IV : Génétique des paysages de rivières chez la truite dorée du Mexique endémique

Les résultats obtenus dans le Chapitre III de cette thèse suggèrent que la structure génétique spatiale d'*O. chrysogaster* est influencée par la structure de l'habitat, et non pas par l'introgression avec les truites d'aquaculture. Nous avons ensuite fait des nouvelles analyses génétiques en incluant plus de sites d'échantillonnage que dans toutes les études antérieures sur *O. chrysogaster*, afin de i) valider les effets de la structure du paysage sur la structure génétique neutre observées dans les simulations, ii) définir les effets de variables hydroclimatiques sur les processus adaptatifs et iii) évaluer avec précision le niveau d'admixture génétique avec les truites exotiques. Pour cette nouvelle étude, j'ai développé 9767 marqueurs de type SNPs génotypés sur 270 individus en utilisant des techniques GBS.

Les analyses de génétique des populations faites sur ce large jeu de données génomique ont confirmé l'absence d'admixture génétique avec les truites d'aquaculture suggérée dans le Chapitre III. Les analyses ont aussi permis de détecter six groupes génétiques coïncidant avec la géographie (Figure 4 dans le Chapitre IV). Cependant, les sites échantillonnés avaient de faibles effectifs efficaces (20.96 en moyenne). Les plus basses valeurs de diversité génétique ont été observées dans les populations localisées dans les marges de l'aire de distribution de l'espèce. Ceci suggère que ces populations sont isolées et ont une faible diversité génétique à cause d'un faible nombre d'individus reproducteurs qui pourraient être dus à des effets de goulot d'étranglement génétique et au faible taux d'immigration en raison de la fragmentation de l'habitat (Eckert et al. 2008; Hartl & Clark, 1997).

L'influence des paysages de rivières sur la divergence génétique neutre a été observée à plusieurs échelles spatiales. L'hétérozygotie était corrélée de façon significative et positivement avec l'altitude, la longueur des rivières et l'ordre des rivières; et négativement avec la latitude, la longitude, la température de la saison la plus chaude et les précipitations du mois le plus sec. Ces résultats montrent que les patrons de paysage de rivière mentionnés plus haut contrôlent le flux de gènes, en empêchant l'arrivée d'immigrants dans certaines

populations et engendrant un déficit d'hétérozygotie; un effet du paysage sur l'hétérozygotie a été aussi montré pour d'autres salmonidés dans les zones les plus au nord de l'Amérique du Nord et de l'Eurasie (Mcphee et al. 2014; Ozerov et al. 2012; Torterotot et al. 2014). De plus, ces résultats confirment l'effet de la résistance du paysage sur la structure génétique observée dans les simulations. Cet effet était plus fort quand l'analyse était faite séparément par bassins versants, comme dans les simulations. Les groupes génétiques observés au sein des bassins versants étaient organisés en fonction des « ruptures » paysagères et des gradients environnementaux. Cet effet des structures paysagères est comparable aux résultats obtenus pour *Salvelinus confluentus* et Salvelinus fontinalis (Kanno et al. 2011; Meeuwig et al. 2010), qui montre aussi un effet de barrières des habitats de rivières qui empêchent le flux de gènes entre populations.

Les tests d'association gène-environnement ont identifié des outliers potentiels associés à la température de la saison la plus chaude et aux précipitations du mois le plus sec. L'effet de ces variables hydro-climatiques sur la variation génétique adaptative peut être expliqué à travers leur influence sur la survie, la reproduction et la migration des salmonides (Bourret et al. 2013; Hand et al. 2016; Hecht et al. 2015). Finalement, les analyses d'ontologie génétique ont trouvé des fonctions dans des gènes en lien avec le régime hydroclimatique. Ces fonctions sont liées à l'acclimatation à la température, au comportement reproducteur et à la mortalité des œufs et des larves (Crockett, 1998; Hale et al. 2011; Milano et al. 2014; Salem et al. 2010), et constituent une preuve additionnelle de l'effet des fluctuations hydroclimatiques sur la variation génétique adaptative.

Conclusions

Cette thèse représente la plus grande étude de génétique des populations chez *O. chrysogaster* jusqu'à présent. Les résultats obtenus ici ont montré un effet déterminant des paysages de rivières sur la diversité génétique neutre d'*O. chrysogaster* et suggèrent aussi un effet des facteurs hydroclimatiques dans l'adaptation locale. Même si l'effet de l'introgression exotique était presque nul chez *O. chrysogaster*, j'ai observé des forts niveaux d'introgression dans des espèces natives non décrites dans le Sud de la Sierra Madre Occidental. Par conséquent, les activités d'aquaculture devraient être règlementées de façon stricte dans cette région. Les résultats de cette étude peuvent aider dans la définition de stratégies de conservation qui considèrent à la fois les aspects écologiques et évolutifs pour préserver les populations de truite dorée Mexicaine.

Chapter 2. Genetic introgression of cultured rainbow trout in the Mexican native trout complex.

Conserv Genet
DOI 10.1007/s10592-014-0599-7

RESEARCH ARTICLE

Genetic introgression of cultured rainbow trout in the Mexican native trout complex

Marco A. Escalante · Francisco J. García-De-León · Casey B. Dillman · Anabelia de los Santos Camarillo · Anna George · Irene de los A. Barriga-Sosa · Arturo Ruiz-Luna · Richard L. Mayden · Stéphanie Manel

Received: 16 November 2013 / Accepted: 18 March 2014

Abstract The Mexican native trout complex is the group of salmonids that naturally has the southernmost distribution in the world. Despite its unique status and evidence of more than 13 distinct lineages, there are only two described species (*Oncorhynchus mykiss nelsoni and O. chrysogaster*). These fishes are threatened by environmental and anthropogenic factors, most notably the introduction of the exotic species *O. mykiss* (rainbow trout) for aquaculture. Here we applied population genetics analysis in 1,017 wild and cultured trout to understand the extent of genetic introgression of rainbow trout in Mexican native trout. Present results indicate a high degree of introgression and genetic admixture among introduced rainbow trout and some populations of Mexican native trout, exposing them to loss of genetic diversity. Thus, introduction of exotic trout for aquaculture purposes must be strictly regulated or avoided and we advise the use of native trout for aquaculture.

Keywords Genetic introgression · Genetic hybridization · Mexican trout · Rainbow trout · Northwestern Mexico · Sierra Madre Occidental

Introduction

The salmonids (Pisces: Salmonidae) are naturally distributed in cold water systems in northern latitudes of America and Eurasia, this fishes range in North America from the Mackenzie River basin in Canada, to the highlands of Acaponeta River in Sierra Madre Occidental (SMO), northwest Mexico (Behnke 1992; Hendrickson et al. 2002). The SMO is the natural worldwide southernmost distribution limit for salmonids (Behnke 1992). Within the Mexican territory there are two described trout of the genus *Oncorhynchus*, besides the introduced rainbow trout (*O. mykiss*) that is used for aquaculture purposes; the

M. A. Escalante · S. Manel
UMR Botanique et BioinforMatique de l'Architecture des Plantes, Bd de la Lironde TA A51/PS2, 3439811 Montpellier Cedex 5, France

M. A. Escalante · A. Ruiz-Luna
Laboratorio de Manejo Ambiental, Centro de Investigación en Alimentación y Desarrollo AC, Sábalo-Cerritos s/n, 82100 Mazatlán, Sinaloa, Mexico

F. J. García-De-León (✉) · A. de los Santos Camarillo
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
e-mail: fgarciadl@cibnor.mx

C. B. Dillman
Virginia Institute of Marine Science, Gloucester Point, VA, USA

A. George
Tennessee Aquarium Conservation Institute, Chattanooga, TN, USA

I. de los A. Barriga-Sosa
Laboratorio de Genética y Biología Molecular-PExPA, Departamento de Hidrobiología, Universidad Autónoma Metropolitana Iztapalapa, Av. San Rafael Atlixco No. 186. Col. Vicentina. Del, 09340 Iztapalapa, DF, Mexico

R. L. Mayden
Department of Biology, Saint Louis University, St. Louis, MO, USA

S. Manel
Laboratoire Population Environnement Développement, Université Aix-Marseille, 3 place Victor Hugo, 13331 Marseille, France

Published online: 27 March 2014

coastal rainbow or Nelson's trout *O. mykiss nelsoni*, which inhabits streams and rivers of Sierra de San Pedro Mártir (SSPM) in Baja California, Mexico, and the Mexican golden trout *O. chrysogaster*, living at high altitudes in the Fuerte, Culiacán and Sinaloa river basins, in the SMO (Ruiz-Campos et al. 2003; Hendrickson et al. 2006; Mayden et al. 2010). The undescribed trout from the SMO occur north and south of *O. chrysogaster* range, e.g. endorheic Guzmán basin and Yaqui and Mayo rivers to the north, and those found in San Lorenzo, Piaxtla, Presidio, Baluarte and Acaponeta rivers to the south (Ruiz-Campos et al. 2003; Hendrickson et al. 2006). These species are now threatened because of diverse environmental and anthropogenic factors, highlighting global climate change, pollution, land use and land cover changes and most importantly, the introduction of exotic species (Hendrickson et al. 2002). Although extinction risks affect the whole species complex, Mexican regulations only protect *O. mykiss nelsoni* and *O. chrysogaster* because of their status as recognized species (Camarena-Rosales et al. 2008).

Concerning genetic and taxonomic studies on native trout species distributed within Mexican territory, analyzing microsatellites markers and DNA control region different authors conclude that this group has a distinct genetic structure when compared with trout from the USA. Nielsen (1997) described the trout from Yaqui and Mayo rivers as a separated genetic group from the Pacific coastal trout of USA, also genetic differences were found between Yaqui River trout and trout from California, Nevada, Arizona and New Mexico (Nielsen et al. 1998, 1999), as well as studies performed in Mexican golden trout from Sinaloa and Culiacán rivers highlight the genetic differences between this species and the salmonids from northern latitudes (Nielsen and Sage 2001). Furthermore, Hendrickson et al. (2006) study comparing mitochondrial DNA region (D-loop) sequence data from USA trout, SSPM trout and SMO samples revealed genetic separation between *O. chrysogaster*, several unrecognized forms of the SMO trout and rainbow (*O. mykiss*), apache (*O. apache*) and gila (*O. gilae*) trout. Camarena-Rosales et al. (2008) found similar results using mitochondrial DNA haplotypes. They found two different genetic groups in addition to *O. mykiss* and *O. chrysogaster* belonging to specimens from Yaqui and Mayo basins to the north and from Piaxtla River to the south. More recent analyses, based on gene flow, F_{ST} values, phenograms, and Bayesian analysis suggest a total of 13 trout species in northwest Mexico river basins (García-De-León et al., unpublished manuscript).

Evidence suggests that hybridization between salmonids could have irreversible impacts through the introduction of genes from organisms not adapted to local conditions with the consequent loss of selective value (fitness) in the local populations (McGinnity et al. 2003). Specifically, there are reports on the negative impacts of rainbow trout in salmonids of North America (Rinne 1990; Weigel et al. 2003; Cordes et al. 2006). Rainbow trout is currently one of the most introduced fish in the world (Fuller et al. 1999), and it is among the 100 most invasive species at a worldwide level (Lowe et al. 2000). The intensification of rainbow trout aquaculture has become a major obstacle for the correct management of the native stocks in North America because of hybridization, substitution and their effect on the environment (Hitt et al. 2003), and possess a threat in Mexico considering that rainbow trout was introduced over 100 years ago and its aquaculture has been supported by governmental agencies, particularly during the 1980s and 1990s in almost all highland aquatic systems in Mexico (Contreras-Balderas and Escalante-Cavazos 1984; Hendrickson et al. 2006).

This study aims to analyze to what extent the genome of native Mexican trout has been introgressed by hybridization with *O. mykiss* either accidentally or intentionally released in the local water courses.

Materials and methods

Tissue collection and DNA extraction

Muscle and fin tissue samples were collected from 1,017 trout specimens (Table 1, for a more thorough description of the native samples see García-De-León et al., unpublished manuscript). Mexican native trout were collected from 55 different sites across 12 watersheds distributed along the SMO (Guzmán, Yaqui, Mayo, Conchos, Fuerte, Sinaloa, Culiacán, San

Table 1 Surveyed sites, sample size (*n*) and collection dates at Sierra Madre Occidental (SMO), Sierra de San Pedro Mártir (SSPM) and California (CA, USA)

Survey locality (Basin)	*n*	Sampling years
Guzmán, SMO	47	1996 and 2005
Yaqui, SMO	257	1996, 2005 and 2007
Mayo, SMO	15	1997 and 2005
Conchos, SMO	45	2005–2008
Fuerte, SMO	77	1997, 2000, 2005 and 2008
Sinaloa, SMO	88	2007 and 2008
Culiacán, SMO	16	1997 and 2007
San Lorenzo, SMO	179	2000 and 2004
Piaxtla, SMO	60	2004
Presidio, SMO	29	2000 and 2004
Baluarte, SMO	36	2004
Acaponeta, SMO	26	2004
Santo Domingo, SSPM	40	1990 and 1994
Fork Cosumnes, CA, USA	26	2004
Cultured trout	76	2000 and 2004

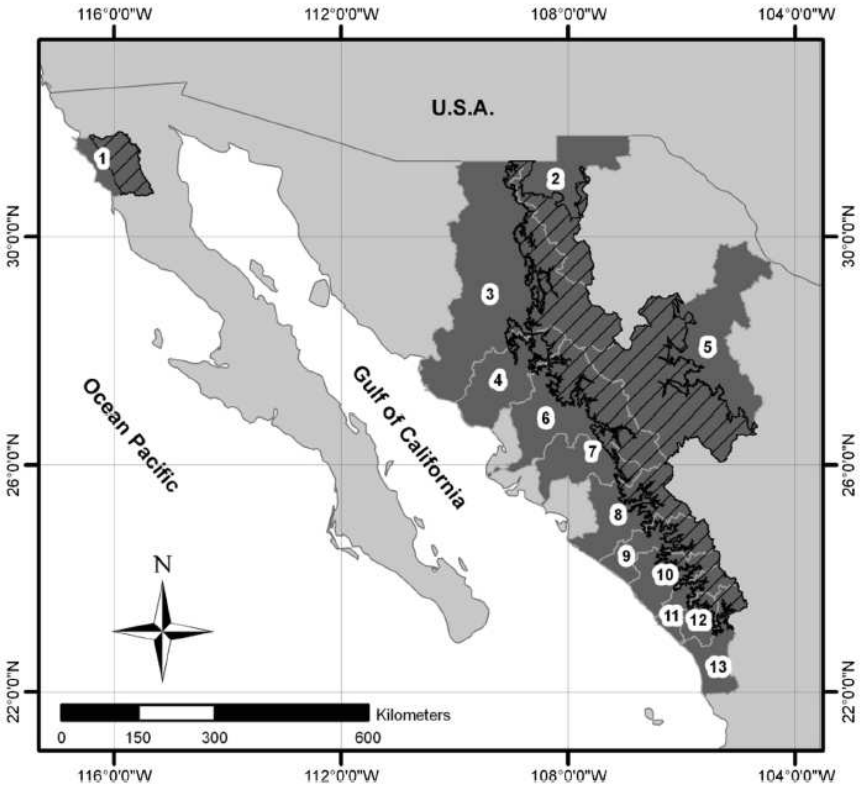

Fig. 1 Habitat basins of Mexican trout: *1* Santo Domingo in SSPM, *2*. Guzmán, *3* Yaqui, *4* Mayo, *5* Conchos, *6* Fuerte, *7* Sinaloa, *8* Culiacán, *9* San Lorenzo, *10* Piaxtla, *11* Presidio, *12* Baluarte and *13* Acaponeta. *Lines* represent the potential area of distribution for Mexican trout, above 1,500 m above sea level in SMO and 500 m above sea level in SSPM

Lorenzo, Piaxtla, Presidio, Baluarte and Acaponeta), and from Santo Domingo Basin in SSPM (Fig. 1). Samples of wild rainbow trout from Middle Fork Cosumnes (California, USA). Cultured trout from three hatcheries in the SMO basins of Fuerte, San Lorenzo and Presidio and one from San Baltazar Altimeyaya (Puebla, Mexico) were also included for comparison. Total genomic DNA was extracted using phenol/chloroform (Hillis et al. 1996).

Microsatellite standardization and genotyping

Making use of their high polymorphism, eleven microsatellite were selected from rainbow trout (*O. mykiss*), sockeye salmon (*O. nerka*); chinook salmon (*O. tschawytscha*), brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*). The selected microsatellites are Omy2DU, Omy27DU (O'Connell, Marine Gene Probe Laboratory, Dalhousie Uni., personal com.), Omy77DU (Morris et al. 1996), Omy207UoG, Omy325UoG (O'Connell et al. 1997), One8ASC, One11ASC (Scribner et al. 1996), Ots1BML (Banks et al. 1999), Sfo8LAV (Angers et al. 1995), Ssa14DU and Ssa289DU (McConnell et al. 1995). Microsatellite genotyping were analyzed according to protocols established by Nielsen et al. (1999) and Nielsen and Sage (2001) except that we used a Beckman Coulter CEQ 8000 automatic sequencer and Fragment Analysis software V6.0.75 to visualize and document size of the microsatellite alleles.

Genetic diversity

Genetic diversity was estimated only with specimens from the aquaculture hatcheries ($n = 76$), since results for wild trout are already reported by García-de León et al. (unpublished manuscript). GENALEX 6.5 (Peakall and Smouse 2006) was used to calculate number of alleles (N_A), number of effective alleles (N_E), observed heterozygosity (H_o), unbiased expected heterozygosity ($_UH_E$) and fixation index (F).

Allelic richness (A_R) values were obtained by the software FSTAT 2.9.3 (Goudet 1995) using the individuals collected in the four hatcheries. For this analysis we discarded 11 individuals with null alleles.

Population structure and genetic introgression

The hierarchical structure of the sampled specimens was analyzed using the program POPULATIONS 1.2.32 (Langella 1999), which initially determines genetic distances with the Cavalli-Sforza chord distance method for

all pairs of sampling sites (Cavalli-Sforza and Edwards 1967) and subsequently constructs a phenogram with bootstrapping permutations over loci (5,000 permutations in this study). The phenogram was visualized with the software program TREEVIEW (Page 1996).

We then applied a spatial Bayesian clustering algorithm to detect genetic clusters from the analysis of individual genotypes sampled at different geographical locations without assuming predefined populations (Safner et al. 2011) using TESS 2.3.1 (François et al. 2006; Chen et al. 2007). The analysis was run with 25 as the maximum number of clusters (K_{max}), 10 as the number of runs for each cluster, 20,000 as the total number of sweeps for each run, and 1,500 as a burn in for the number of sweeps. Subsequently, the run that best represents the number of genetic groups was selected by plotting the deviance information criterion (DIC) against K_{max} for each run performed, in search of the one with the lowest DIC value on the plateau of the curve, as recommended by François and Durand (2010). Afterwards, the admixture coefficient estimated by TESS 2.3.1 was used to define the individuals and genetic clusters with introgression from rainbow trout genome.

Genetic introgression was then assessed too by a hybrid index (HI) obtained in the software INTROGRESS (Gompert and Buerkle 2009; 2010), HI is the proportion of alleles inherited from each of two parental populations. HI was estimated only for the genetic clusters that showed a considerable degree of genetic introgression in TESS 2.3.2 analysis, as is recommended by Gompert and Buerkle (2009). HI was estimated to define genetic introgression from cultured rainbow trout in native trout as well as genetic introgression of native trout in other native trout. HI of native trout in cultured trout was calculated only for the samples collected in Fuerte basin hatchery in 2005 due to were the only rainbow trout samples that showed genetic admixture in TESS 2.3.2 analysis.

Results

Genetic diversity

The highest genetic diversity measures were associated with trout harvested from a hatchery in the San Lorenzo basin, located in the middle of the SMO. The mean number of alleles N_A and effective alleles N_E for the individuals sampled in the four aquaculture hatcheries were 7.2 and 4.1, respectively. The mean values for heterozygosity were $H_O = 0.66$ and ${}_UH_E = 0.73$. The F index, had the highest value for trout from the hatchery located in the Puebla (0.010) indicating a deficit of heterozygotes (inbreeding) in this location; while the lowest values for the F index, indicating an excess of heterozygotes (outbreeding), was observed for trout from the hatchery located in the San Lorenzo basin (−0.013). Values ranges for every index are displayed in Table 2.

Table 2 Genetic diversity values obtained in GENALEX 6.5 for cultured trout in Sierra Madre Occidental (SMO)

Hatchery location	N_A	N_E	H_O	H_E	${}_UH_E$	F
Puebla	5.545	3.777	0.679	0.691	0.726	0.010
Fuerte Basin	7.727	4.093	0.505	0.704	0.718	0.277
San Lorenzo Basin	9.818	5.312	0.753	0.747	0.761	−0.013
Presidio Basin	5.636	3.285	0.711	0.669	0.701	−0.076
Mean	7.182	4.117	0.662	0.703	0.726	0.049

N_A number of alleles, N_E number of effective alleles, H_o observed heterozygosity, H_E expected heterozygosity, ${}_UH_E$ unbiased expected heterozygosity, F fixation index

Table 3 Allelic richness (A_R) values obtained in FSTAT 2.9.3 for cultured trout in Sierra Madre Occidental (SMO)

Hatchery Locus	Puebla	Fuerte Basin	San Lorenzo Basin	Presidio Basin	Total
Omy2DU	6	11	18	8	8.26
Omy27DU	3	5	3	4	3.13
Omy77DU	3	8	6	6	5.10
Omy207UoG	9	9	13	6	7.70
Omy325UoG	6	10	11	7	6.48
Onu8ASC	3	5	6	3	3.92
One11ASC	2	3	3	3	2.44
Ots1BML	6	8	14	6	6.79
Sfo8LAV	5	8	14	4	6.17
Ssa14DU	6	9	13	8	7.31
Ssa289DU	5	7	7	7	5.74

The allele richness A_R values obtained for hatchery samples ranged from 2.44 (locus One11ASC) to 8.26 (locus Omy2DU) with an average of 5.73. When hatcheries were analyzed separately, the lowest A_R value (2) was found for samples from the Puebla's hatchery in the locus One11ASC, while the highest (18) was found for trout collected in the hatchery located in San Lorenzo basin in locus Omy2DU (Table 3).

Population structure and genetic introgression

The neighbor joining phenogram detected 18 well supported genetic clusters or clades (≥60 % of bootstrapping value) in all cases composed by nearby sampling sites (Fig. 2). The genetic cluster formed by trout harvested from Puebla and San Lorenzo hatcheries were joined in the same group (60 %), and interestingly native trout collected from Aparique creek (Fuerte basin) were joined with the Fuerte basin hatchery (100 %).

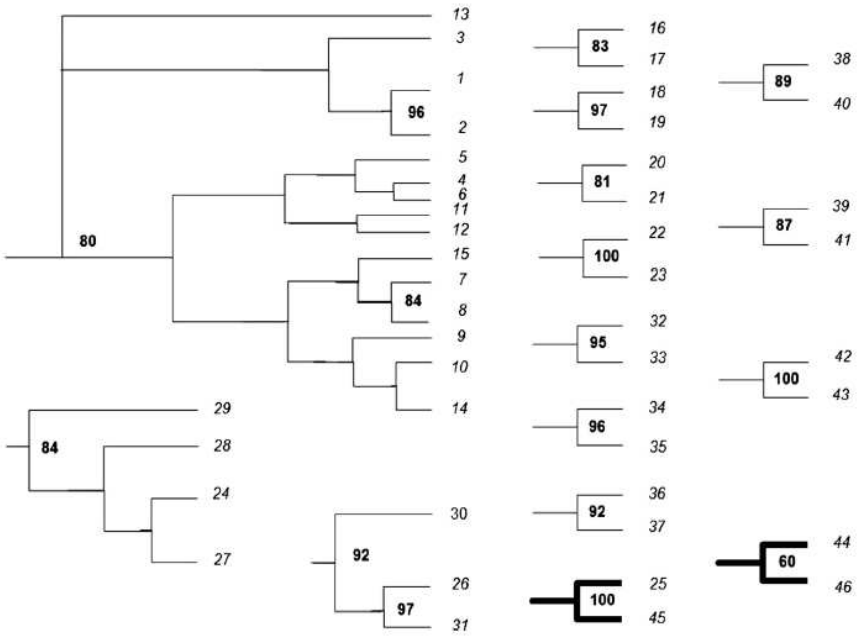

Fig. 2 Well supported genetic clusters (≥60 % of bootstrapping value) of the neighbor joining phenogram performed in POPULATIONS 1.2.32. The clusters related to aquaculture hatcheries are shown in *bold*, *numbers in bold* represent bootstrappring values for each well supported cluster and the sample sites are represented with *numbers in italics: 1* Escalariado creek in Guzmán basin sampled in 1996, *2* Escalariado creek in Guzmán basin sampled in 2005, *3* La Playa creek in Guzmán basin, *4* La Presita creek in Yaqui Bavispe sub-basin sampled in 1996, *5* La Presita creek in Yaqui Bavispe sub-basin sampled in 2005, *6* La Barca creek in Yaqui Bavispe sub-basin, *7* Las Guacamayas creek in Yaqui Bavispe sub-basin sampled in 1996, *8* Las Guacamayas creek in Yaqui Bavispe sub-basin sampled in 2007, *9* El Cuartel creek in Yaqui Bavispe sub-basin sampled in 1996, *10* El Cuartel creek in Yaqui Bavispe sub-basin sampled in 2007, *11* Las Nutrias creek in Yaqui Bavispe sub-basin, *12* Pedernal creek in Yaqui Bavispe sub-basin, *13* Largo creek in Yaqui Bavispe sub-basin, *14* El Cocoño creek in Yaqui Bavispe sub-basin, *15* Segundo de Mayo creek in Yaqui Bavispe sub-basin, *16* Tutuaca creek in Yaqui Sirupa sub-basin sampled in 1996, *17* Tutuaca creek in Yaqui Sirupa sub-basin sampled in 2005, *18* El Salto creek in Yaqui Sirupa sub-basin sampled in 1996, *19* El Salto creek in Yaqui Sirupa sub-basin sampled in 2007, *20* Rituchi creek in Conchos basin, *21* Ureyna creek in Conchos basin, *22* El Molino creek in Conchos basin sampled in 2007, *23* El Molino creek in Conchos basin sampled in 2008, *24* La Onza creek in Fuerte basin, *25* Aparique creek in Fuerte basin, *26* Las Truchas creek in Fuerte basin, *27* Agua Blanca creek in Culiacán basin, *28* Santa Rosa creek in Culiacán basin, *29* El Medio creek in Sinaloa basin, *30* El Soldado creek in Sinaloa basin, *31* El Potrero creek in Sinaloa basin, *32* La Sidra Above creek in San Lorenzo basin sampled in 2000, *33* La Sidra Above creek in San Lorenzo basin sampled in 2004, *34* La Sidra Below creek in San Lorenzo basin sampled in 2000, *35* La Sidra Below creek in San Lorenzo basin sampled in 2004, *36* El Granizo creek in Piaxtla basin, *37* La Cruz Larga creek in Piaxtla basin, *38* Quebrada de Vega creek in Presidio basin, *39* Nogales creek in Presidio basin, *40* Santa Barbara creek in Baluarte basin, *41* Tanquecitos creek in Acaponeta basin, *42* San Antonio creek in Santo Domingo basin, *43* Las Grullas creek in Santo Domingo basin, *44* Hatchery of Puebla State, *45* Hatchery of Fuerte basin sampled in 2005, *46* Hatchery of San Lorenzo basin

TESS also defined 18 groups with respect to the lowest value of DIC 48835 on the curve's plateau. Trout belonged to the genetic clusters collected in Guzmán basin and Yaqui Bavispe sub-basin; Mayo basin; Aparique creek in Fuerte basin; La Onza creek in Fuerte basin, El Medio creek in Sinaloa basin and Culiacán basin; La Sidra creek in San Lorenzo basin; Piaxtla basin as well as Presidio, Baluarte and Acaponeta basins showed a considerable degree of genetic admixture with cultured rainbow trout. On the other hand, trout belonged to the genetic clusters collected in Guzmán basin and Yaqui Bavispe sub-basin; Mayo basin; Tutuaca creek in Yaqui Sirupa sub-basin; Banderella creek in Yaqui Sirupa sub-basin; La Sidra creek in San Lorenzo basin as well as Piaxtla basin showed a considerable degree of genetic admixture with native trout from other genetic clusters. Also, rainbow trout collected in the hatchery located in Fuerte basin in 2005 showed genetic admixture native trout belonged to Aparique creek genetic cluster (Fig. 3).

Finally, the analysis performed in INTROGRESS highlighted high degrees of genetic introgression (HI ≥ 0.5) by cultured rainbow trout in native trout collected in Aparique creek, La Sidra creek as well as Presidio Baluarte and Acaponeta basins (Table 4).

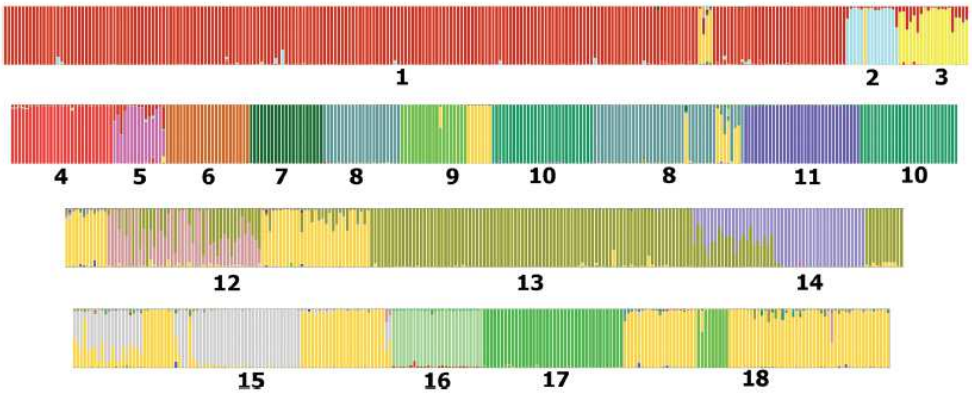

Fig. 3 Assignment probabilities and genetic clusters obtained in TESS 2.3.1, each individual analyzed is represented by a *vertical line* and each *color* represent a distinct genetic cluster: *1* Trout from Guzmán basin and Yaqui Bavispe Sub-basin, *2* Trout from Mayo basin, *3* Trout from Tutuaca creek in Yaqui-Sirupa sub-basin, *4* Trout from El Salto creek in Yaqui Sirupa sub-basin, *5* Trout from Banderella creek in Yaqui-Sirupa sub basin, *6* Trout from Rituchi and Ureyna creeks in Conchos basin, *7* Trout from El Molino creek in Conchos basin, *8* Trout from Culiacán basin, La Onza creek in Fuerte basin and El Medio creek in Sinaloa basin, *9* Trout from Aparique creek in Fuerte basin, *10* Trout from Las Truchas creek in Fuerte basin and El Potrero creek in Sinaloa basin, *11* Trout from El Soldado creek in Sinaloa basin, *12* Trout from La Sidra creek in San Lorenzo basin, *13* Trout from San Lorenzo basin, *14* Trout from Piaxtla basin, *15* Trout from Presidio, Baluarte and Acaponeta basins, *16* Trout from California, *17* Trout from Santo Domigo basin in SSPM, *18* Trout from Aquaculture. (Color figure online)

Discussion

This is the first attempt to evaluate hypotheses on genetic introgression from exotic rainbow trout into the Mexican native trout gene pool. This study had considerable sampling from which to draw conclusions, 55 different sample sites in 13 watersheds/basins in northwest Mexico, with additional samples for genetic information from four commercial trout hatcheries and wild trout from California, USA. Besides the geographic extent, along with the studies performed by García-De-León et al. (unpublished manuscript) this is the most comprehensive study to date for Mexican native trout. Here for the first time we have included aquaculture samples that were not previously analyzed. The main contribution of this work is the quantification of the degree of introgression of cultured trout into the Mexican native trout genome, which is very high for some populations. Therefore, it is necessary to take immediate measures to conserve Mexican native trout.

In the present study 11 microsatellite loci were analyzed. Most of these loci were also utilized by Nielsen and Sage (2001) and García-De-León et al. (unpublished manuscript). Trout collected from hatcheries in this study have lower genetic diversity when compared with some salmonids from North America such as *S. salar* (Dionne et al. 2008) and *O. mykiss* (Narum et al., 2010), but these hatchery fish have higher genetic diversity than reported for *Salvelinus confluentus* (Meeuwig et al. 2010), *O. tshawytscha* (Banks et al. 2000) and *O. mykiss* (Nielsen et al. 1999).

Bottleneck processes can trigger deficit of genetic diversity, when the size of natural populations is low due to reduced number of founding colonizers and low rates of immigration with the consequent loss of heterozigosity and allelic richness, imposing changes in reproduction processes and increasing mortality (sensu Nei et al. 1974; Banks et al. 2013). Tout from the SMO derived from the spreading of California salmonids to the Gulf of California during the last glaciation at the Pleistocene. Afterwards, at the end of the Pleistocene those salmonids colonized the highest parts of SMO rivers and became segregated when the global temperature increased, and were isolated in different watersheds having separated microevolutionary processes in parallel (Behnke 1992; Hendrickson et al. 2006). Therefore, the low genetic diversity of these Mexican native trout could be explained by bottleneck events. Also, this could explain in part that trout from most of the studied basins showed a high degree of genetic homogeneity with a proportion larger than 50 % and up to 100 % of the sampled individuals belonging to homogeneous clusters (11 of 13). However, it is worrying that the golden trout *O. chrysogaster*, the only recognized Mexican trout species in SMO, as well as the other native Mexican trout clusters associated with lower latitudes are being impacted by hybridization as reflected in the high values of the introgression indicators obtained here.

It is important to note that results in taxonomic analysis could vary depending on the analysis method used and the size of the database. Even with the same loci but without

Table 4 HI obtained in INTROGRESS

Samples	LCI	HIrt	HCI	LCI	HIn	HCI
Trout from Guzmán basin and Yaqui Bavispe sub-basin	0.05	0.16	0.37	0.07	0.24	0.50
Trout from Mayo basin	0.07	0.12	0.27	0.01	0.06	0.35
Trout from Tutuaca creek in Yaqui Sirupa sub-basin	–	–	–	0.10	0.31	0.59
Trout from Banderella creek in Yaqui Sirupa sub-basin	–	–	–	0.04	0.16	0.46
Trout from Culiacán basin, La Onza creek in Fuerte basin and El medio creek in Sinaloa basin	0.24	0.49	0.76	–	–	–
Trout from Aparique creek in Fuerte basin	0.43	0.57	0.71	–	–	–
Trout from La Sidra creek in San Lorenzo basin	0.42	0.54	0.76	0.09	0.32	0.65
Trout from San Lorenzo basin	0.03	0.10	0.29	–	–	–
Trout from Piaxtla basin	0.13	0.27	0.47	0.24	0.40	0.57
Trout from Presidio, Baluarte and Acaponeta basins	0.49	0.70	0.84	–	–	–
Rainbow trout from Fuerte basin hatchery (2005)	–	–	–	0.40	0.49	0.62

HIrt hybrid index from cultured rainbow trout, *LCI* low confidence interval for HI from cultured rainbow trout, *HCI* high confidence interval for HI from cultured rainbow trout, *HIn* hybrid index from other native genetic cluster, *LCI* low confidence interval for HI from other native genetic cluster, *HCI* high confidence interval for HI from other native genetic cluster

aquaculture samples, García-de León et al. (unpublished manuscript) found some differences in the output set of trout clusters when applying a non-spatial assignment of individuals test as proposed by Pritchard et al. (2000). Also, the results from Ruiz-Campos et al. (2003), using standardized lineal morphological characters (non genetic data) slightly vary assigning subsets to one homogenous group found here. Despite minor differences, previous studies on native trout are complementary to our present findings, this is particularly true in the case of La Sidra creek trout. García-De-León et al. (unpublished manuscript) found a homogeneous group in analysis of microsatellite markers. While Ruiz-Campos et al. (2003) suggested from the observation of morphological characters like abundant presence of black spots above and below the lateral line as well as large longitude and heavy weight of some individuals, that trout from La Sidra creek has probable hybridization with exotic trout. We reconcile these results here with the finding that specimens from La Sidra created a single cluster but had the lowest proportion of individuals assigned to a homogeneous genetic cluster showing high genetic admixture with trout from San Lorenzo cluster, and at the same time is among the clusters with the highest probabilities of genetic introgression with cultured rainbow trout.

The golden trout inhabiting rivers from the Fuerte, Sinaloa and Culiacán basins appear to be exposed to genetic contamination from aquaculture sources. The Neighbor Joining phenogram included trout collected in Aparique creek (Fuerte basin) in 2005 with trout from the hatchery located in the same basin, while similar results were produced by the TESS and INTROGRESS analysis, which outputs an exchange of individuals from the hatchery into the wild and vice versa, as well as a recent admixture process between native and cultured trout in both senses due to the individuals collected in the same sites in 2000 does not showed introgression. This is likely a consequence of the proximity of aquaculture facilities to the native trout sampling sites.

Official information on trout hatcheries number and location in the study area is not available. Nevertheless, the introduction of rainbow trout for aquaculture purposes in Mexico has been documented in former studies (Contreras-Balderas and Escalante-Cavazos 1984; Hendrickson et al. 2006; Camarena-Rosales et al. 2008), and just for the Fuerte basin in the study area, it was possible to detect around 50 hatcheries, recorded during the field surveys and with a Google Earth examination during 2013 (results not shown). However the status of these hatcheries as active or inactive and knowledge on the cultured rainbow trout species in their facilities have not been assessed yet, thereby limiting our knowledge on the real impact of hybridization. Nonetheless, Camarena-Rosales et al. (2008) mention hatcheries widespread in the Presidio, Baluarte and Acaponeta basins, which together with San Lorenzo basin showed high presence of genes from rainbow trout for the native individuals sampled in this study, even exceeding in proportion the native genes. Situations like these have been reported for other salmonids at northern latitudes (Heggberget et al. 1993; Fauschf 2007; Gunnell et al. 2008; Caudron et al. 2011, Marie et al. 2012). Also Heggberget et al. (1993) remarked that wild populations of Atlantic salmon in Norway, characterized by a small number of individuals, declined when cultured salmon was introduced due the fast expansion of the aquaculture industry, in addition the monogean parasite *Gyrodactylus solaris* and *G. forunculosis* were also introduced, resulting in high mortality in wild populations.

Pressure on native trout is increasing as a consequence of mining, land cover changes and the introduction of exotic trout species for aquaculture purposes (Hendrickson et al. 2006). The infrastructure and technology that supports rainbow trout aquaculture facilitate its trade and use in local farms, so that the efforts to cultivate and to conserve the Mexican native trout have been limited.

The genetic introgression caused by interbreeding or by selection forces due to reduction of effective population size, genetic drift and inbreeding, which erode the genetic pool of native salmonids, could be seen as the most detrimental for native trout (Krueger and Menzel 1979; Campton 1987; Bartley and Gall 1990; Krueger and May 1991; Hindar et al. 1991; Campton 1995; Naish et al. 2008; Corsi et al. 2013). Because of their geographic distribution, Mexican native trout have genes adapted to conditions warmer than those for other salmonids form northern latitudes, which have been isolated at least since the Pleistocene (Behnke 1992). These native lineages are at high risk, highlighting the need to preserve this unique gene pool. This particularity also opens a window opportunity for aquaculture developments in the region, based on the responsible and proper use of the Mexican native trout, which could support a local aquaculture industry replacing rainbow trout, allowing the conservation of the genetic pool of the Mexican native trout.

Acknowledgments This research was supported by SAGARPA-CONACYT (Ref. 12147) and PDCBS (Ref. 14707.03), the U.S. National Science Foundation (Ref. DEB-0240184, DEB-0817027) and SAGARPA-FIRCO 2012 (Ref. RGA-BCS-12-000003). Special thanks to the binational group Truchas Mexicanas, especially Joseph R. Tomelleri, Bernard R. Kuhajda, Hector Espinosa, David A. Neely, James E. Brooks, David L. Propst, Gorgonio Ruiz-Campos, Dean Hendrickson and Lloyd Findley, for their extensive assistance in collecting and their collaborative efforts. Marco A. Escalante was the recipient of CONACYT fellowships to obtain his MsC degree in the CIAD Postgraduate Program and now during his PhD studies in AMAP Lab, under a project funded by CONACYT (Ref. CB-2010-01-152893). Collection permits were issued by DGVS/SEMARNAT (FAUT 0117, issued to H. Espinosa Pérez) and Fishing promotion licenses by SAGARPA (DGOPA 21205/0765; 250406/1606; 030506/1677; 240800 213-03/2366; 300801-613-03/1607).

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In Chapter 2 the assessment of the spatial genetic structure for the entire Mexican trout complex highlighted a cryptic genetic structure among and within basins. Moreover, population genetics analyses revealed strong genetic introgression from cultured rainbow trout for some native populations. That introgression could have very detrimental effects with the loss of genetic pool, among other negative consequences leading in high extinction risks for native

populations. Nevertheless, to define how native populations are genetically divergent as well as their potential extinction risks, is necessary to understand the effect of the natural environment and anthropogenic activities (i. e. introduction of exotic species) on the spatial genetic structure. This understanding requires, population genetics analyses considering reduced spatial scales and a large amount of sample sites, but also the integration of landscape ecology approaches.

In Chapter 3, I aimed to explain which factors between riverscape features and exotic introgression of cultured rainbow trout determinate the current genetic structure of the endemic Mexican golden trout. Thus, I combined riverscape characterizations, species distribution modeling, demo-genetic simulations and population genetics analyses.

Chapter 3. The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (*Oncorhynchus chysogaster*), a simulation approach.

Original article in revision for Journal of Biogeography

The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (*Oncorhynchus chysogaster)*.

M.A. Escalante[1,2], F.J. García-De León[2], A. Ruiz-Luna[3], E. Landguth[4] and S. Manel[1]

1-PSL Research University, CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, Biogéographie et Ecologie des Vertébrés, 1919 route de Mende, 34293 Montpellier, France

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

3-Laboratorio de Manejo Ambiental, Centro de Investigación en Alimentación y Desarrollo AC, Sábalo-Cerritos s/n, 82100 Mazatlan, Sinaloa, Mexico[3]

4-Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA

Corresponding author: Francisco J. García-De León; fgarciadl@cibnor.mx

Word count inclusive of abstract, main text and references: 6072

Abstract

Aim Using simulations, we explore which factors between riverscape features and exotic introgression of cultured rainbow trout (*Oncorhynchus mykiss*) drive the current genetic structure of the endemic Mexican golden trout (*Oncorhynchus chrysogaster*).

Location Sierra Madre Occidental, Northwest Mexico, North America.

Methods We created a system model for Mexican golden trout in order to test various scenarios of rainbow trout introgression on the demographic and genetic response. We initialized Mexican golden trout simulated individuals into three genetically spatial distinct groups based on the empirical genetic data and spatially using a species distribution model. We included the hypotheses for movement of riverine distance and riverscape resistance. We evaluated model performance by performing population and landscape genetics analyses on the simulation outputs and compared simulated results with the observed genetic structure.

Results The scenario that best represents the empirical genetic data included riverscape resistance without introgression. Additionally, temperature, precipitation, hydrology, and topography were the most important variables involving species occurrence and dispersal.

Main conclusion This study presents one of the first empirically-derived simulation study of introgression. It highlights the importance of considering riverscape resistance (i.e., hydroclimatic and topographical gradient effects) in models of spatial genetic structure and introgression. Moreover, we found evidence that what we characterize as riverscape resistance is acting as a boundary against exotic introgression.

Keywords

Endemic species, genetic introgression, rainbow trout, riverscape genetics, simulations, species distribution modelling

Introduction

Understanding how landscapes affect the processes defining genetic structure and the mechanisms of local adaptation is a main objective of landscape genetics (Manel & Hodleregger 2013). This understanding is crucial, not only to improve the biological and ecological knowledge, but also to promote conservation of genetic legacy and proper management of the genetic diversity of endangered populations (Allendorf *et al*. 2010).

Aquatic organisms inhabiting riverine environments depend on hierarchical systems influenced by climate, topography and land use at multiple scales, which drive processes that divide those environments in several microhabitats (Allen & Star 1982; Montgomery 1999). In addition, those aquatic systems are connected in a dendritic pattern defining distances between sites by the length of the riverine path and not by simple linear distance; this geometrical arrangement also determines species distribution and distance between populations (Chaput-Bardy *et al*. 2009). Thus, genetic structure is expected to be influenced by the complexity of riverine systems (physical barriers and riverine distance), as well as changing climatic conditions and past demographic events (Wenger *et al*. 2011). Similarly, anthropogenic factors, mainly land use changes, often increase fragmentation of aquatic habitats, transforming a metapopulation into several isolated peripheral populations and increasing chances of local and system-wide extinction (Kelson *et al*. 2015).

Furthermore, the introduction of exotic species could have detrimental effects in native populations by interspecific competition, predation, diseases, parasites transfer and genetic introgression (Penaluna *et al*. 2016). One of the most negative impacts of genetic introgression is the corruption of native genomes that have evolved over thousands of years with the consequent loss of evolutionary potential of local populations (Muhlfeld *et al*. 2009).

The endemic Mexican golden trout (*Oncorhynchus chrysogaster*; MGT) inhabits the ríos Fuerte, Sinaloa and Culiacán basins in the SMO, representing one of the southernmost distributed salmonid species and the only one protected by the Mexican regulations (Needham and Gard 1964; Hendrickson *et al*. 2006). MGT habitat is reduced to the highest parts of the SMO mountains. Thus, these populations are endangered by anthropogenic and environmental factors such as, changing land use and climate, as well as the introduction of the exotic rainbow trout (*O. mykiss*; RT) with aquaculture purposes (Hendrickson *et al*. 2006). Recent studies found cryptic genetic structure in MGT divided in different genetic clusters with some populations introgressed by cultured RT

(Escalante *et al*. 2014; Abadía-Cardoso *et al*. 2015). However, all previously published studies have used data with sample designs not adjusted to environmental gradients. Consequently, the knowledge of the effect of landscape on the genetic structure of MGT is still missing.

The genetic processes occurring in riverine landscapes (riverscapes) are highly influenced by the landscape through which they flow and their understanding requires a multi-scale approach due to all biological processes occurring in streams (Fausch *et al*. 2002). Combining species distribution models and landscape genetics simulation can improve our understanding of the complex effect of riverscapes on genetic processes (Scoble & Lowe 2010; Erős *et al*. 2012). Further, simulation methods in landscape genetics have successfully explained processes like migration, selection, mutation, recombination and drift in heterogeneous habitats (Neuenschwander *et al*. 2008; Landguth and Cushman 2010; Hoban *et al*. 2013; Andrello & Manel 2015). However, few simulation studies have been conducted until now in riverscape genetics (Table 1), while such studies can help to better disentangle process behind patterns (Cushman 2015). In this work, we aim to investigate how simulations in riverscape genetics can help our understanding of the effect of riverscape complexity and RT introgression on population structure of MGT, that is, (1) do riverscapes drive MGT genetic structure? And (2) is genetic introgression influencing current MGT genetic structure? To address these questions, we used an integrative approach combining species distribution modeling, empirically-derived riverscape genetics analyzes, and simulations. We then compared simulated data with empirical data of MGT genetic structure (Escalante *et al*. 2016). Data simulated under different demographic and genetic (herein defined as demogenetic; Landguth *et al*. 2014) scenarios will help for a more realistic interpretation of the process behind the observed spatial genetic structure of the species.

Table 1. Examples of studies applying simulations on riverscape genetics.

Species	Location	Simulated Processes	Main Results	Reference
Westslope cutthroat trout *Oncorhynchus clarkii lewisi*	Akokala Creek in U.S.A.	Barriers driving demo-genetic connectivity	Barriers placed at headwater streams caused loss of genetic diversity	Muhlfeld *et al*. 2012
Electric fish *Steatogenys elegans*	Amazon and Negro rivers in South America	Genetic structure and spatial selection	Difference in neutral and selection-driven genetic structure influenced by the spatial selection gradient	Cooke *et al*. 2014
Bull trout *Salvelinus confluentus*	Upper Flathead River in Canada and U.S.A.	Stream resistance driving demo-genetic connectivity	Riverscape resistance causing suitable habitat fragmentation and loss of genetic diversity	Landguth *et al.* 2014
Freshwater mussel *Cumbrelandia monodonta*	Mississippi River in U.S.A	Effects of climate change in demo-genetic connectivity	Climate change will significantly reduce population connectivity and genetic diversity	Inoue &Berg *et al.* 2017
Westslope cutthroat trout *Oncorhynchus clarkii lewisi*	Washington U.S.A.	Managed removal of nonnative species and influence of barriers in genetic exchange	Inability of exotic populations to recolonize patches and lower genetic exchange when barriers are added	Landguth *et al.* 2016

Materials and methods

Study area

This study focused on the MGT distribution area described by Hendrickson *et al*. (2002), located at upstream waters in altitudes higher than 1500 m at ríos Fuerte, Sinaloa and Culiacán basins. This zone has a river network mostly corresponding to 1, 2 and 3 stream order values, an average altitude

of 2107 m, an annual mean temperature of 14.4 °C, and an annual precipitation mean of 911 mm (Fig. 1). It is worth noting that the invasive RT, was introduced to this area (with aquaculture purposes) in the 1860's (Contreras-Balderas & Escalante-Cavazos 1984).

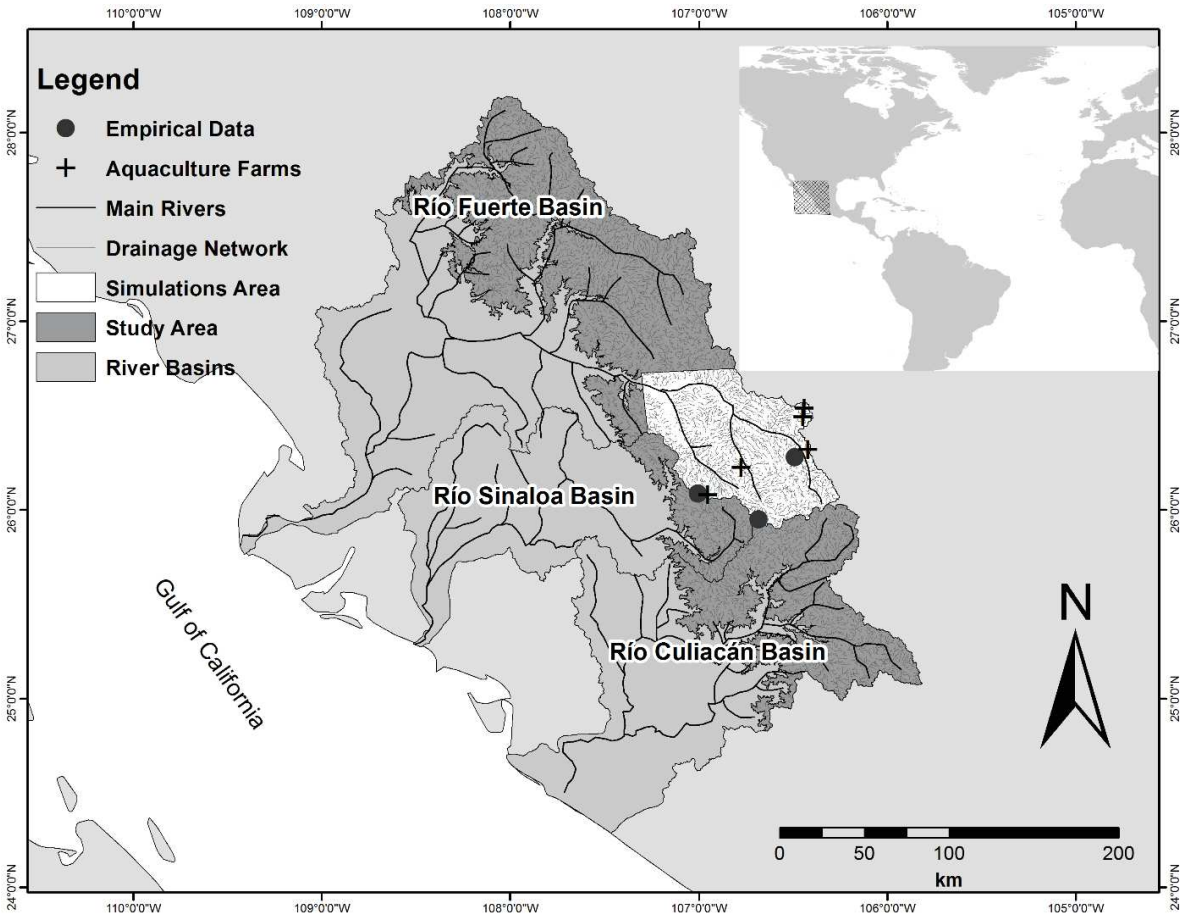

Figure 1 Study Area: Ríos Fuerte, Culiacán and Sinaloa basins over 1 500 meters above the sea level in northwestern Mexico. Dots indicate the location of empirical data sampling sites, crosses indicate the location of aquaculture farms and the simulated study system is shown in the white area.

Riverscape data

Initially, slope and aspect models were produce from Aster Digital Elevation Models (DEM) with 30 m resolution, downloaded from the Japanese Space Systems website (http://gdem.ersdac.jspacesystems.or.jp/). From this DEM, we defined the study area based on a basin and sub-basin delineation. Then, a hydrologic model including the drainage network, stream orders, drainage density and stream length was created. Geological data (rock types) at 1: 250,000 scale were downloaded from the Mexican Institute of Statistics and Geography (http://www.inegi.gob.mx). We used 19 bioclimatic variables at 1 km resolution from the worldclim website (http://www.worldclim.org). Lastly, for subsequent analyses we included 26 riverscape variables (see Appendix 2 in supporting information) which were geometrically corrected to 30 m to fit with the DEM resolution. All spatial analyses were conducted in ArcGIS v 10.2 (ESRI 2013) (Appendix 2).

Species distribution modeling

MGT distribution area was modeled using MAXENT (MaxEnt 3.33k software; Phillips *et al*. 2006). We calculated a correlation matrix for each raster layer (Appendix 2) and riverscape variables were discarded based on a correlation cutoff of $r \geq 0.5$ to avoid redundancy. With the selected environmental information, MAXENT predicts the occurrence of the species using a maximum entropy algorithm that recognizes the properties of the reference locations (occurrence records). For this study, 45 occurrences from 1952 to 2014 (all the historical records for MGT till 2014) were employed as presence data, and the 26 aforementioned riverscape variables were used to run an initial MAXENT model. Then, variables with weak influence (AUC with jackknife test < 0.7) on MGT distribution were discarded. The resulting reduced model allowed to initialize starting locations for the simulation program, as well as to help inform riverscape resistance surface creation (Milanesi et al. 2016; see below).

Riverscape demogenetic program

Riverscape influence on genetic exchanges and introgression processes in MGT populations were simulated using CDMETAPOP (Landguth *et al*. 2016). This approach models demogenetic processes

between individuals located at different patches. In order to distinguish between our two species, we initialize patches with individuals that had allele frequencies matching each species (see more detail below in the genetic section). Inside patches, individuals undergo migration, reproduction and mortality, and the subsequent genetic processes over a user-specified given time step at the individual level. A further explanation about the theoretical life cycle is found in the software's manual (http://www.github.com/computationalecologylab/CDMetaPOP/). Our simulation model required parameterization of a number of species-specific processes. Following, we describe some of the major parameters and processes as it relates to our research questions and further details can be found in Appendix 3.

Patches and carrying capacity

Due to the large study area, we focused our simulations on the highest parts of the south of Río Fuerte Basin (Fig. 1). This area also has RT aquaculture farm locations. Fig. 2 shows the patch locations delineated for natal and migratory (i.e., overwintering/foraging). We applied a MGT species distribution model probability of occurrence cutoff of 0.8 to initialize 249 patches considered as natal spawning ground locations, and 1692 sites were considered for migratory patches. We defined the starting source locations for RT from the geographic location of five aquaculture farms obtained in field surveys and through government and academic agencies.

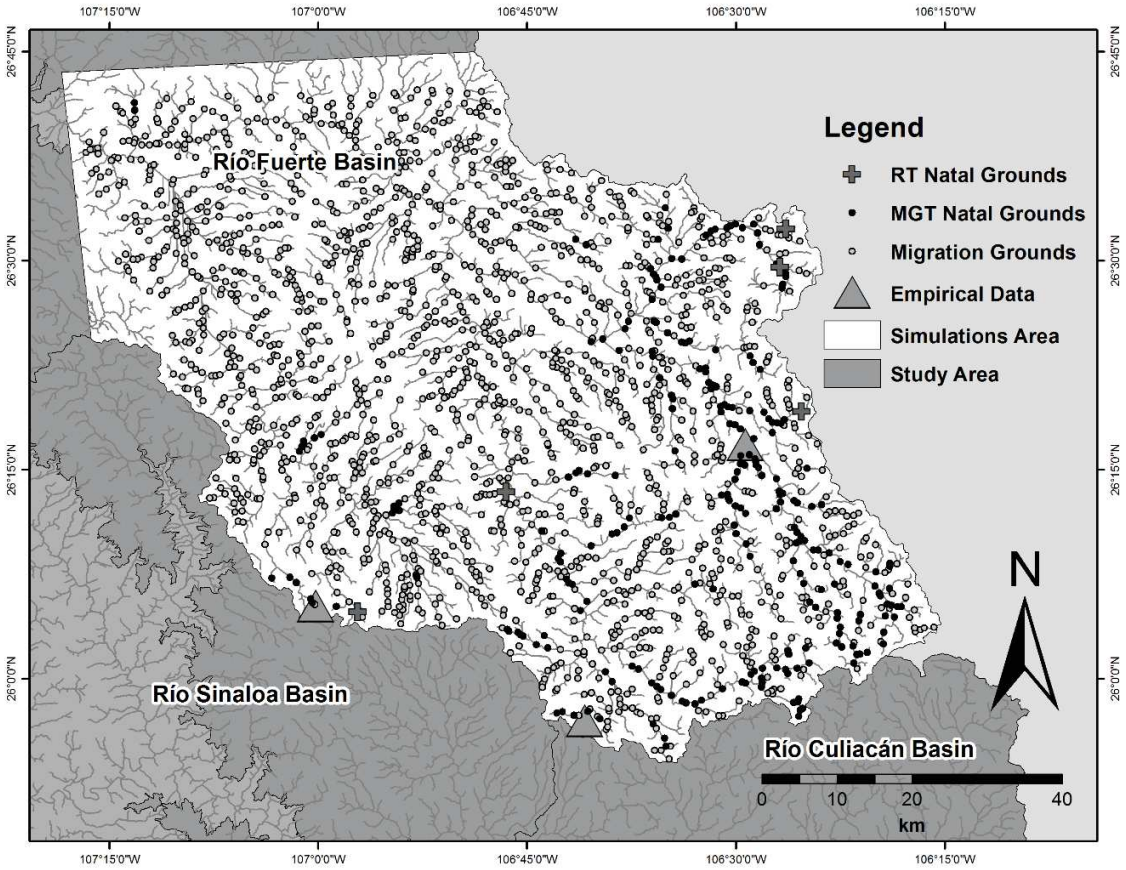

Figure 2 Initial simulated patches: triangles indicate the location of empirical data sampling sites, black dots indicate Mexican golden trout (MGT) natal grounds, crosses indicate cultured rainbow trout (RT) natal grounds, gray dots indicate migration ground and the simulated study system is shown in the white area.

For MGT patches (both natal and migratory), carrying capacities ranged from 69 to 255 individuals and initial number of individuals ranged from 31 to 137. These values were designated based on native coastal Nelson trout (Ruiz-Campos & Pister 1995). For RT source locations, we fixed a carrying capacity of 10,000 with 9,200 starting individuals (adults). See Appendix 3 for more details on patch initiation.

Genetic data

The genotype for each individual was initialized by allele frequencies defined from 11 neutral microsatellite loci for three genetic clusters of MGT with a sample size (n) of 57 individuals and one genetic cluster of RT (n = 26) (Escalante *et al.* 2016). We discarded allele frequencies of the admixed individuals either with MGT or RT in order to test our hypothesis with non introgressed genetic clusters. MGT allele frequencies were used to simulate native trout at three different subregions depending of their geographical location and their proximity to the predefined genetic clusters. The specific allele frequencies for RT were used to mimic RT at aquaculture farms (Appendix 3). The simulation program then simulates genetic exchange following Mendelian inheritance and specified species-specific movement and life history strategies described following.

Age classes, migration, maturation and fecundity

Since we lacked the necessary empirical data to parameterize a number of model parameters, we defined parameters based on a similar system, westlope cutthroat trout (i.e., WCTM model; Landguth et al. 2016, Appendix 3). The model was initialized at time = 0 with eight age classes. Migration rates started from 0 at age 0 with an annual increment of 0.1 until age 4, and a straying probability of 0.05 was assigned to all age classes. Maturation rates and the fecundity model were taken from the WCTM with the exception of RT individuals, where maturation rates were slightly modified based on data published for RT farming (Secretaría de Pesca 1982) to create earlier maturing individuals for RT (Appendix 3).

Dispersal

Resistance surfaces quantified the influence of landscape features on functional population connectivity (Spear *et al*. 2010). In our study, a riverscape resistance surface was created where each pixel represents the cost of crossing each location. Literature-based and expert opinion-based resistance modeling approach was combined to generate this resistance surface from altitude, slope, mean temperature of the warmest quarter (TWQ) and stream order. Then, these variables were scaled and processed in ArcGIS v 10.2 (Appendix 4). These variables were chosen based on field surveys (M.A.E. personal observations) and also, related to the response curves at the MGT species distribution model (Fig. 4). In addition, these variables have been involved in survival

requirements for MGT and connectivity patterns reported for other similar salmonid species from North America (Hendrickson *et al*. 2006; Meeuwig *et al*. 2010). In those published studies higher temperature values, abrupt slopes, altitudinal gradient and increase of stream order were suggested as inducing riverscape resistance for trout movement.

To quantify landscape fragmentation and connectivity between MGT populations, riverine least-cost distance and riverscape resistance matrices of connectivity values among all patches were calculated using 'gdistance' package (van Etten 2012) in R 3.2.5 (R Development Core Team 2016). This method simulates potential movement for species in a spatially structured landscape, linking different dispersal functions and connectivity thresholds by the Djikstra's shortest path algorithm. In this study, we generated four matrices under different hypothesis of movement: (Matrix I) hypothesis of movement between native patches with isolation by riverine distance, using only the length of the river network, (Matrix II) hypothesis of movement between native patches by riverscape resistance, using the resistance surface to subsequently analyze with 33% of maximum resistance threshold, (Matrix III) hypothesis of movement between native and aquaculture patches with isolation by riverine distance, using only the length of the river network and (Matrix IV) hypothesis of movement between native and aquaculture patches by riverscape resistance, using the resistance surface to subsequently analyze with 33% of maximum resistance threshold (see Appendix 3 and 4).

Riverscape genetic simulation scenarios

Given the approximate date of introduction of RT into the area, we performed simulations for 150 years. Thus, four different demographic and riverscape scenarios were considered: Isolation by riverine distance without exotic introgression (I); Isolation by riverscape resistance without exotic introgression (II); Isolation by riverine distance with exotic introgression (III) and Isolation by riverscape resistance with exotic introgression (IV) (see Table 2 and Appendix 3). We ran three replicates in order to optimize computational time.

Genetic diversity

Genetic diversity for the four scenarios at the last year (150) was estimated using GENALEX 6.5 (Peakall & Smouse 2006). This analysis calculated the number of different alleles (N_a), observed heterozygocity (H_o), unbiased expected heterozygocity (H_e) and deviations from Hardy-Weinberg equilibrium. Furthermore, to validate significant differences between scenarios, Student's t-test (Student 1908) were performed for N_a, H_o and H_e using the Excel Macro XLstat v 2017.1 (Adinsoft 2017).

Table 2. Main parameters for each simulated scenario.

Scenario	Scenario's Name	Age Classes, Migration, Maturation and Fecundity	Movement/Resistance Matrix	Initial Number of Individuals per Patch	Carrying Capacity per Patch	Dispersal Movement Thresholds of Riverscape Distance/Resistance
I	Isolation by riverine distance without exotic introgression	WCTM	Matrix I	From 31 to 137	From 69 to 255	-
II	Isolation by riverscape resistance without exotic introgression	WCTM	Matrix II	From 31 to 137	From 69 to 255	-
III	Isolation by riverine distance with exotic introgression	WCTM/ Secretaria de Pesca (1982)	Matrix III	From 31 to 137 for native patches 9200 for exotic patches	From 69 to 255 for native patches 10000 for exotic patches	33%
IV	Isolation by riverscape resistance with exotic introgression	WCTM/ Secretaria de Pesca (1982)	Matrix IV	From 31 to 137 for native patches 9200 for exotic patches	From 69 to 255 for native patches 10000 for exotic patches	33%

Population structure and analyzes

The spatial genetic structure for year 150 of all scenarios was assessed using Spatial Principal Component Analyses (sPCA) available in the R package 'adegenet' (Jombart 2008; Jombart *et al*. 2010). This method combines variability and spatial autocorrelation of allele frequencies to generate synthetic principal components describing spatial genetic structure.

To analyze the effect of exotic introgression on population genetic structure, Principal Component Analysis (PCA) included in 'adegenet' were used to compare genetic variability on allele frequencies at the first year (before migration started; generation = 1) and year 150 across the riverscape resistance simulation scenarios (II and IV).

To assess the spatial influence of the two riverscape surfaces (riverine distance versus riverscape resistance), genetic distances were first estimated for year one among all patches using pairwise F_{ST} coefficients for all scenarios. Those coefficients were calculated in GENALEX 6.5 software. Then, Mantel tests (Mantel 1967) were implemented using XLstat on each riverine/riverscape surface scenario and corresponding genetic distance matrix. Mantel tests were applied between F_{ST} coefficients generated in GENALEX 6.5 for the last year (150) of all scenarios. All tests were performed under 10,000 permutations assuming no correlation.

Results

Potential species distribution modeling

The MAXENT based species distribution model detected three low correlated variables with robust influence in MGT presence (Appendix 2): Altitude, precipitation of the driest month (PDM) and TWQ. The highest probabilities of MGT occurrence located at the east and south of Río Fuerte Basin, east of Río Culiacán Basin and the limits between ríos Fuerte and Sinaloa basins and ríos Culiacán and Sinaloa basins, predominantly in headwaters (Fig. 3). The AUC score was highly significant (0.87) and the model successfully predicted the 95.3% of the species records. Based on these results, we estimated the best conditions for the species occurrence at places with a PDM close to 22 mm, altitudes around 2 600 meters and a TWQ lower than 15° C (Fig. 3; Fig. 4). The three subregions for the simulations were separated by ruptures in the MAXENT model represented by low species occurrence probabilities, having similar environmental conditions, but isolated by the heterogeneous riverscape.

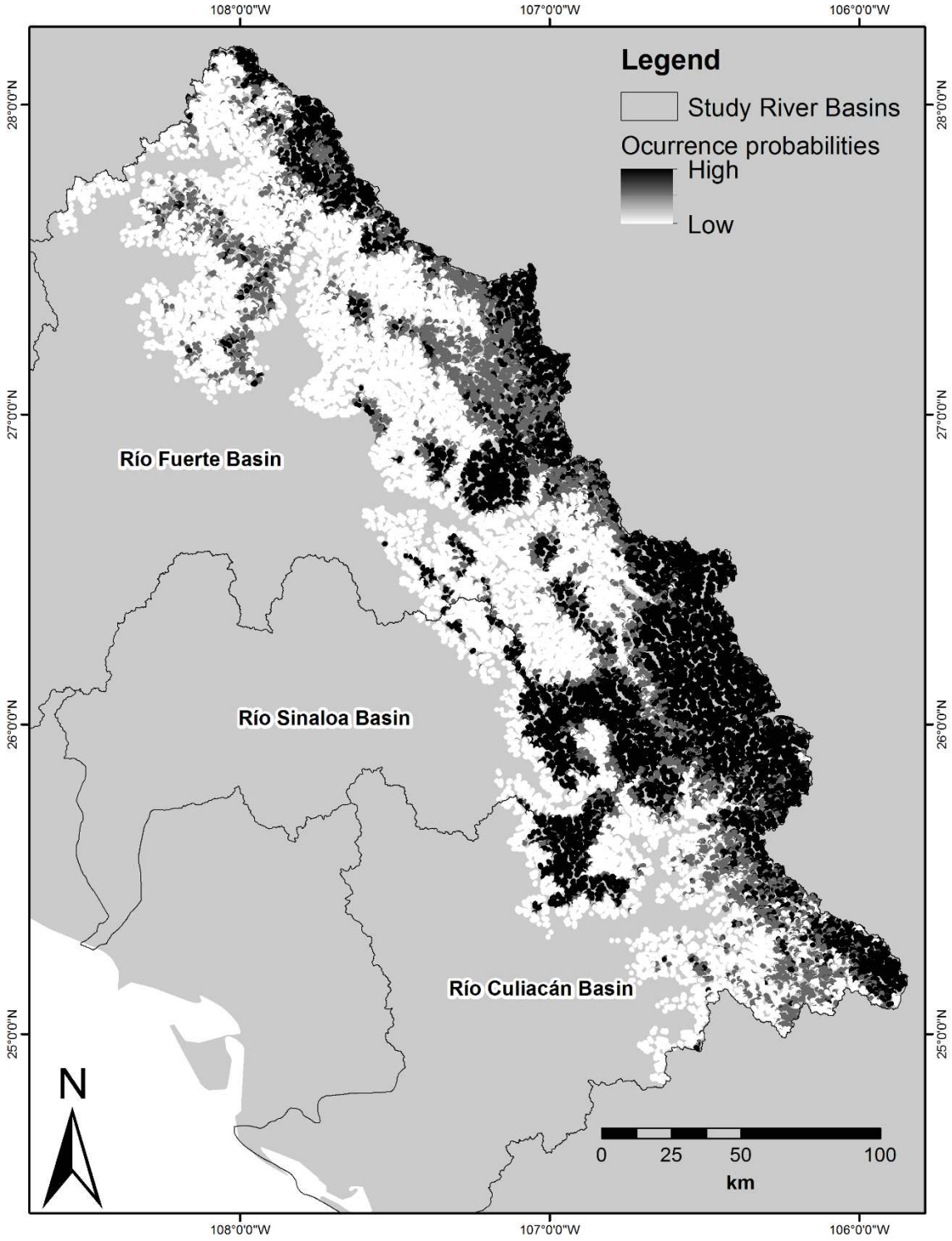

Figure 3 *Oncorhynchus chrysogaster* occurrence probabilities derived from the species distribution modeling in Maxent.

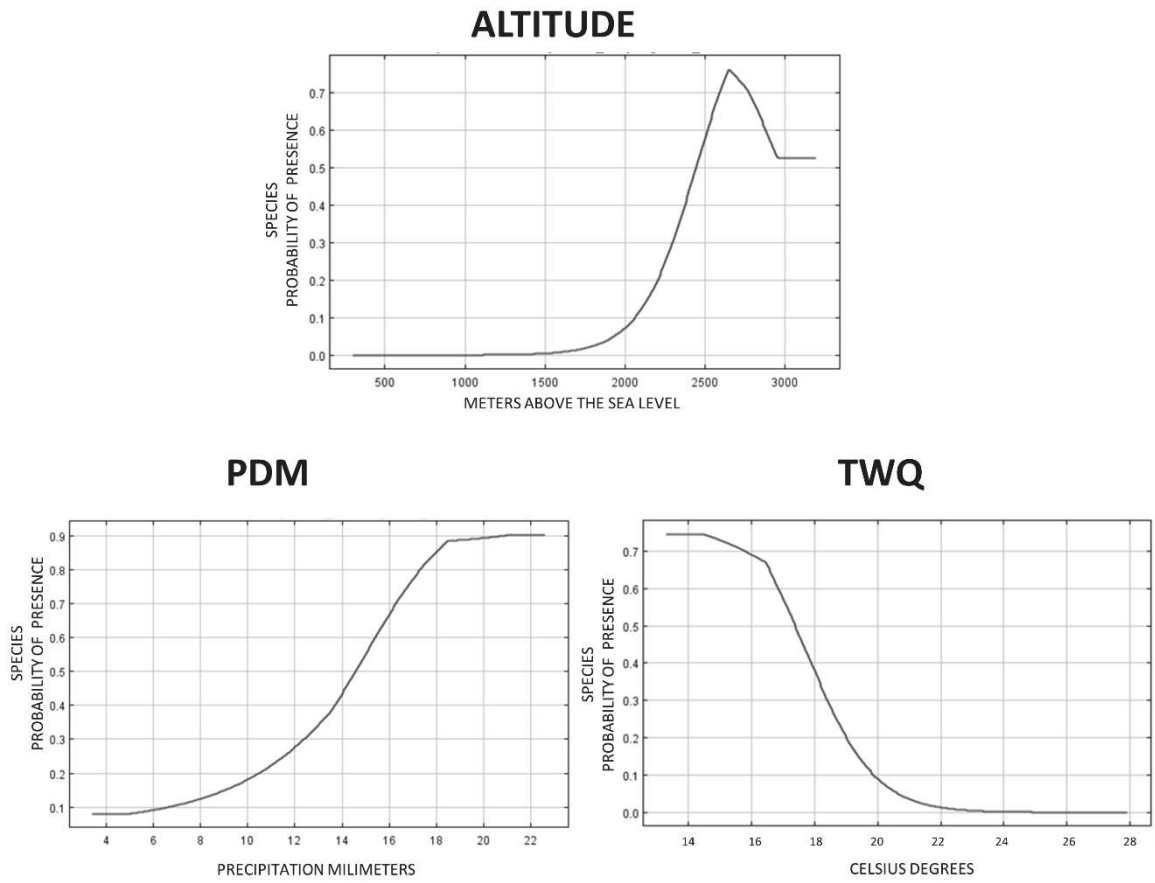

Figure 4 Environmental functions of the species distribution modeling in MAXENT for *Oncorhynchus chrysogaster*. The curves predict the changes of species distribution as a function of each environmental variable: altitude, precipitation of the driest month (PDM) and mean temperature of the warmer quarter (TWQ).

Genetic diversity

Based on the empirical data, it is expected that scenarios under exotic introgression will reach the highest genetic diversity values, therefore, most realistic scenarios will obtain closer values in comparison with empirical data. The highest genetic diversity values for year 150 among all scenarios were reached by individuals simulated under Scenario III (Isolation by riverine distance with exotic introgression) with means of N_a = 10.352, H_o = 0.788 and H_e = 0.746. The lowest mean genetic diversity values but the most similar to empirical data were found at Scenario II (Isolation by riverscape resistance without exotic introgression): N_a =6.487, $H_{0=}$ 0.634 and H_E = 0.605. In addition, the higher proportion of loci under Hardy Weinberg disequilibrium (P <0.05) was observed in Scenario III with 80.9% while Scenario II reached the lowest proportion (61.7%) (Table 3). Furthermore, Student's t-test

revealed significant differences (P < 0.0001) for all genetic diversity values among scenarios. Scenarios III and IV including exotic introgression exhibited the higher genetic diversity values, among them, the one including just riverine distance showed higher scores, indicating a stronger introgression pattern when riverscape resistance is excluded.

Table 3. Results of the analyses performed in GENALEX for the 150th year of data simulated under the four scenarios and empirical data (Escalante et al. 2016) (ED): Sample size (n), number of different alleles (N_a), observed heterozygosity (H_o), unbiased expected heterozygocity (H_e), global F_{ST} between all populations (F_{ST}) and proportion of locci under Hardy Weinberg disequilibrium (%HW).

Scenario	n	N_a	H_o	H_e	F_{ST}	%HW
I	39490	8.111	0.726	0.682	0.067	75.10%
II	31350	6.487	0.634	0.605	0.190	61.70%
III	61426	10.352	0.788	0.746	0.060	80.90%
IV	39927	9.375	0.773	0.731	0.088	77.50%
ED	179	6.48	0.466	0.566	0.020	-

Population structure and analyzes

The sPCA performed for scenarios I and III did not detect genetic clusters (results not shown). However, for scenarios II and IV both sPCA's detected three different clusters separated in three subregions corresponding to the main river branches (west, center and east) and separated with high riverscape resistance agreeing with empirical data. For scenarios II and IV, the first two eigenvalues of the sPCA's explained almost the 100% of the variance and were retained (Fig. 5).

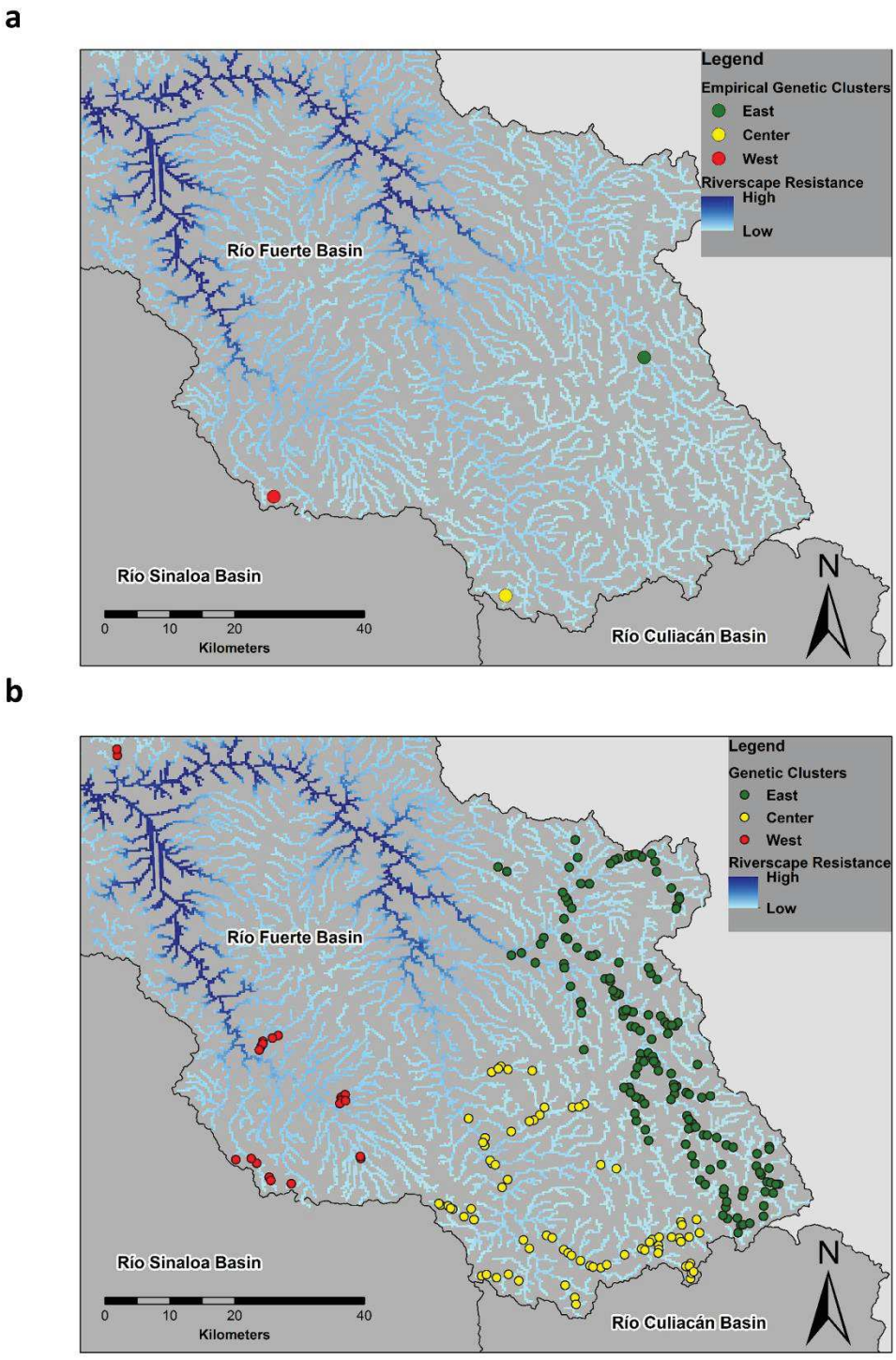

Figure 5 Geographic distribution of the empirical genetic clusters from 11 microsatellite loci of 57 individuals (a) and Spatial Principal Component Analysis (sPCA) outputted genetic clusters for scenarios II and IV (b): east in green, center in yellow and west in red.

PCA results to examine introgression for year 1 of Scenario II detected three genetic clusters of MGT corresponding to the same subregions found in the sPCA (west, center and east) (Fig. 6a). This also

mimicked the genetic structure and introgression results found in the empirical data (Fig. 5a). For the 1st generation of Scenario IV, we identified the same three genetic clusters of MGT clearly separated from RT (Fig. 6b). For year 150 of Scenario II, the three MGT clusters were still present, with west cluster clearly separated from the rest and east and center clusters sharing some admixed individuals (Fig. 6c). Furthermore, for year 150 of Scenario IV, individuals from aquaculture origins (represented by crosses) were dispersed in the three MGT clusters (Fig. 6d). Additionally, MGT clusters were less separated in comparison with analysis without introgression, this proximity could be an effect of genetic admixture with the RT located at the three subregions (Fig. 6d). This pattern is less consistent with the empirical data results and in relation with the other outputs.

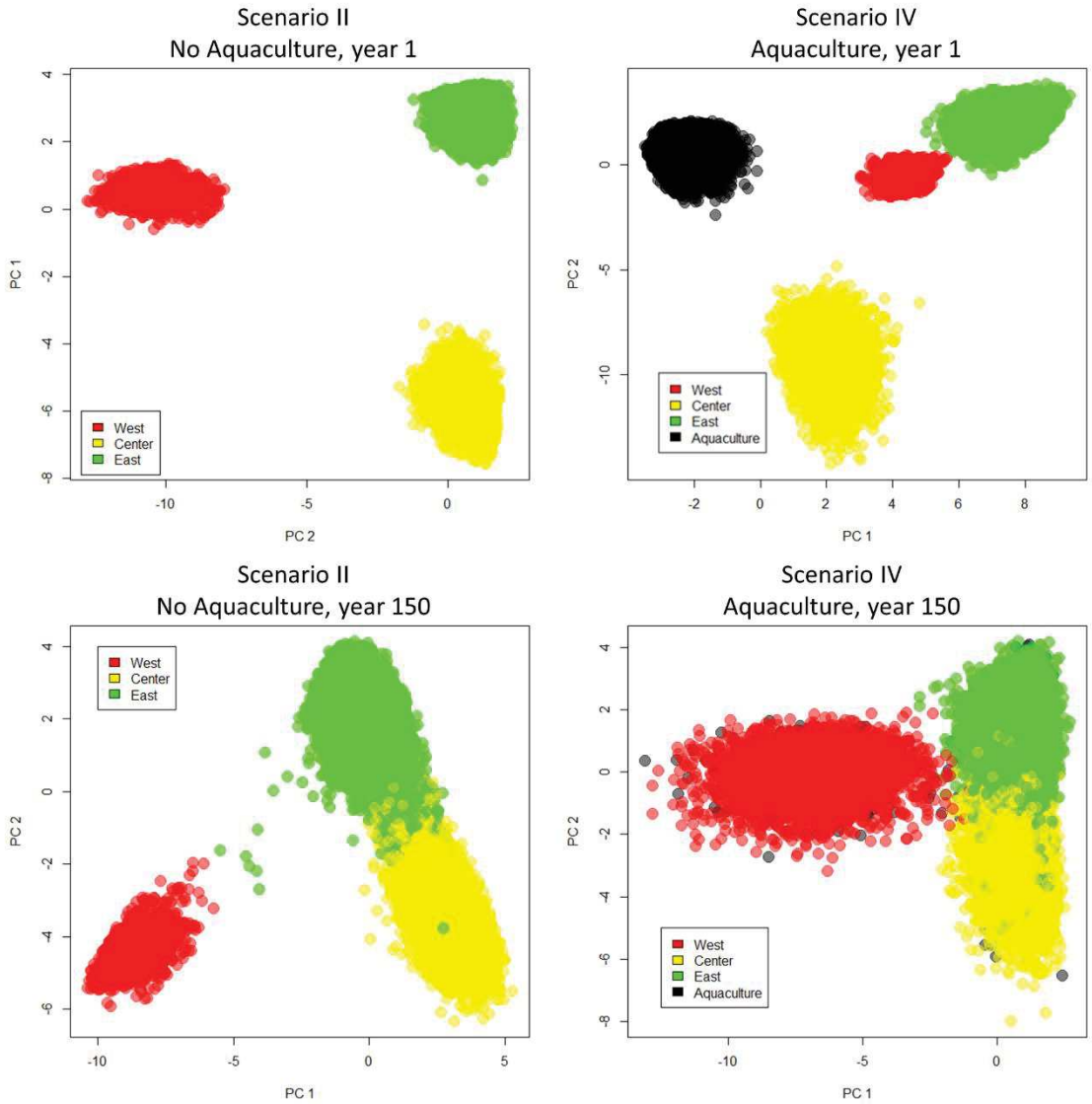

Figure 6 Genetic clusters obtained from Principal Component Analysis (PCA) on simulated data for Scenario II at generation 1 (a), Scenario IV at generation 1 (b), Scenario II at generation 150 (c) and Scenario IV at generation 150 (d). The origin of each individual is represented by different figures: east in light gray triangles, center in gray circles, west in dark gray squares and aquaculture in black crosses.

In all analyzes, scenarios simulated under riverscape resistance showed higher degrees of genetic differentiation. The highest global F_{ST} values between populations were observed in Scenario II (0.190) and were the most similar to empirical data (0.20), while Scenario III obtained the lowest values (0.060) (Table 3).

All Mantel tests were highly statistically significant ($p < 0.0001$). Moreover, r-values were substantial only for scenarios II (0.906) and IV (0.675) simulated under riverscape resistance, while

scenarios considering just riverine distance (I and III) obtained values near zero. Those results are consistent with the sPCA's, corroborating the strong influence of the riverscape structure characterize by long riverine distances, abrupt slopes, stream order changes as well as elevation and temperature gradients on the genetic differentiation of MGT.

Discussion

The integration of both spatial and biological data in pattern-process modeling, allows researchers to define the influence of landscape structure in species genetic variation (Epperson *et al*. 2010). We found that the simulated scenario with riverscape resistance without exotic introgression matched our empirical data results the best. This comparison suggests that there is a strong effect of a riverscape resistance on the genetic structure of the native MGT. Moreover, we found evidence that riverscape resistance is acting as a boundary against exotic introgression at basin level.

Our most plausible scenario (Scenario II), highlighted the same three different genetic clusters in comparison to empirical data and reached similar global F_{ST} values, as well as mean genetic diversity. The three well defined genetic clusters separated by strong riverscape resistance corresponds to the main river branches at the south of Río Fuerte Basin. The scenario simulated with exotic introgression and riverscape resistance (IV) was the second closest to the empirical data confirming the importance of riverscape to explain the genetic variation. Where genetic introgression was considerably less strong, indicating that a complex riverscape could act as a boundary against introgression. These results suggest that riverscape features are the main driver on MGT genetic structure, most likely due to riverscape features influence on dispersal movements. Additionally, Mantel tests detected a strong significant correlation among riverscape resistance and genetic distances. In more details, the genetic discontinuities matched riverscape with higher resistance represented by temperature increase, altitude decrease, sharped slopes and increase in stream order. Thus, the genetic structure of MGT is shaped by the complex structure of the riverscape and not only by simple riverine distance. These findings illustrate the need to consider landscape characterization as an important explanatory variable for spatial genetic structure in riverscape systems (Meeuwig *et al*. 2010; Hand *et al.* 2016; Landguth *et al.* 2016).

Riverscape variables used in the MAXENT model were also included in the resistance surfaces, with two additional variables related with dispersal (slope and stream order). Thus, we found that similar riverscape conditions drive both species occurrence, as well as demographic and genetic processes, which is not always the case (Araújo & Luoto 2007; Scoble & Lowe 2010; Dellicour *et al*.

2016). The strong influence of TWQ, as well as PDM and altitude, on MGT occurrence in the MAXENT modeling was also found in other studies with trout species from North America (Hendrickson *et al.* 2006; Wenger *et al*. 2011). Those studies showed a positive effect of temperature decrease, as well as altitude and precipitation increase, on trout occurrence, while the simulations confirmed that those variables are also shaping the genetic structure of MGT.

Additionally, the comparison of genetic variation among scenarios, independently from empirical data, allows to evaluate the potential effects of introgression and riverscape on native populations. The highest genetic diversity values among scenarios and lowest F_{ST} values were found for data simulated under exotic introgression. Such patterns can be explained as a consequence of genetic admixture between exotic and native individuals, as are expected in native populations with frequent introgressions (Berrebi *et al*. 2000). This phenomenon was observed on previous studies on Atlantic salmon, where populations subjected to high genetic introgression obtained lower F_{ST} values compared with slightly or non introgressed populations (Perrier *et al*. 2011; Le Cam *et al*. 2015). Similarly, a simulation study on *Salmo salar* found less genetic introgression from aquaculture stocks when populations were exposed to lower dispersal levels (Perrier *et al*. 2013). Additional observations including MGT and undescribed forms from SMO (Escalante *et al*. 2014; Abadía-Cardoso *et al.* 2015) detected the highest genetic diversity values for introgressed populations located near to aquaculture farms at ríos San Lorenzo, Presidio, Baluarte and Acaponeta basins.

Furthermore, among both scenarios with exotic introgression, the one with riverscape resistance (Scenario IV) showed lower genetic diversity values and higher F_{ST} values. This finding supports the hypothesis of riverscape structure of MGT habitat, characterized by abrupt hydroclimatic and topographic gradients mitigating introgression processes as consequence of reduced connectivity between RT sources and some native populations.

Although Scenario II without exotic introgression was detected as the more realistic to describe empirical data, we need to be cautious with this assumptions since other studies based on distinct molecular markers and sampling locations in MGT at the south of Río Fuerte Basin detected different number of genetic clusters ranging from one to three (Camarena–Rosales *et al.* 2008; Abadía-Cardoso *et al*. 2015; Escalante *et al*. 2016). In this sense, the lack of information about reproductive and migration habits for MGT as well as strength of aquaculture activities and scape rates from farms makes awkward to simulate accurate introgression conditions. Thus, native populations at aquaculture proximities are still in risk. It is reported that invasions of salmonids from aquaculture origins could have harmful effects in native populations putting them in risk of extinction (Dunham *et al*. 2004; Penaluna *et al.* 2016). This issue highlight the necessity of generate new data with larger collection

efforts, wide sampling geographic distribution and a higher number of polymorphic molecular markers to study MGT. Next generation sequencing opens new perspectives to generate tens of thousands of high resolution molecular markers (Elshire *et al.* 2011; Peterson *et al*. 2012) and the application of that methods could aid to understand better the respective influence of landscape and exotic tout on the genetic structure of MGT.

ACKNOWLEDGEMENTS

This research was supported under projects founded by CONACYT (Ref. CB-2010-01-152893) and ECOSNORD (M14A04). Marco A. Escalante is recipient of CONACYT fellowships to perform his PhD studies. We thank M. Andrello from CEFE in Montpellier France for his help on Fig. 5. We are grateful with G. Ingle from the Mexican Fisheries Institute (INAPESCA) and H. Espinosa from UNAM Biology Institute for the facilitation of aquaculture farm locations. E. Landguth funding for this research comes from Seattle City Light and NASA grant NNX14AC91G.

BIOSKETCH

Marco A. Escalante is broadly interested in landscape ecology, landscape genetics, G.I.S. and conservation of endangered species. He performed this study as part of his PhD project at CEFE CNRS and CIBNOR.

Author contributions: M.A.E. performed data analyses. F.G.D.L., A.R.L., E.L. and S.M. advised data analyses. All the coauthors were involved in the study design and the manuscript writing. The final version of the manuscript was approved by all authors.

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In Chapter 3 demo-genetics simulations suggested that riverscape is the main factor shaping *O. chrysogaster* genetic structure and also acts as a barrier against exotic introgression. Additionally, species distribution modeling indicated a strong influence of altitude and hydroclimatic variables in *O. chrysogaser* occurrence. However, to validate what was found in Chapter 3, wide sampling efforts are needed to define with accuracy gene flow processes and exotic introgression. On the other hand, hydroclimatic variables with high influence on the species occurrence may be related with local adaptation, still high resolution molecular markers are necessary to define that. Consequently, those assessments might be of high relevance in order to develop conservation strategies for native salmonids.

To define the influence of riverscape patterns in both neutral and adaptive microevolutionary processes, and the current degree of exotic introgression from aquaculture trout. In Chapter 4 sampling efforts were conducted across *O. chrysogaster* distribution area and a database of 9,767 SNP's was generated by GBS techniques. Then, exotic introgression was assess by population genetics analyses including both native and aquaculture trout. Subsequently, the influence of rivercape patterns in neutral genetic structure and the effect of hydroclimatic factors in adaptive variation was tested by riverscape genomics approach.

Chapter 4. Riverscape genetics of the endemic Mexican golden trout, a conservation genomics approach.

Original article submitted to Molecular Ecology

Riverscape genetics of the endemic Mexican golden trout, a conservation genomics approach.

Escalante M.A.[1,2], Perrier C.[1], García-De León F.J.[2], Ruiz-Luna A.[3], Ortega-Abboud E.[1] & Manel S.[1]

1- PSL Research University, CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, Biogéographie et Ecologie des Vertébrés, 1919 route de Mende, 34293 Montpellier, France

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

3- Laboratorio de Manejo Ambiental, Centro de Investigación en Alimentación y Desarrollo AC, Sábalo-Cerritos s/n, 82100 Mazatlan, Sinaloa, Mexico

Keywords: riverscape genomics, salmonids, introgression, endangered species, management strategies.

Corresponding autor: 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. Fax: (+52) (612) 1238405. Email: fgarciadl@cibnor.mx

Abstract

How environmental factors influence neutral and adaptive genetic variation is a central issue in evolutionary biology and conservation ecology. Mexican golden trout (*Oncorhynchus chrysogaster*) is one of the salmonid species with the southernmost distribution in the world, inhabiting three different basins at Sierra Madre Occidental (SMO) mountains in northwest Mexico. Like all salmonids this species is susceptible to climate change, habitat perturbations and the introduction of rainbow trout (*O. mykiss*) with aquaculture purposes. We aimed to investigate the effect of genetic introgression from *O. mykiss* and riverscape variables on the neutral and adaptive genetic variation of this endemic salmonid. To accomplish that, we applied Genotyping by Sequencing (GBS) methods to generate 9,767 Single Nucleotide Polymorphisms (SNP's) and genotype 270 *O. chrysogaster* and aquaculture trout. Then, population genomics analyses were conducted together with riverscape genetics approaches, outlier loci detections and gene ontology analyses. We found nearly null admixture among aquaculture and native trout. We detected a significant effect of riverscape variables on genetic diversity, and a significant isolation by riverine distance and riverscape resistance within basins. Outlier detection and gene ontology analyses identified genes that could be implicated in adaptation to local climate heterogeneity, suggesting an influence of hydroclimatic factors in adaptive variation. We discussed our results in the context of conservation strategies for endangered riverine species.

Introduction

How environmental factors influence neutral (migration, drift) and adaptive (selection) genetic variations and how spatial genetic patterns emerge in endemic population are central issues in evolutionary biology and conservation ecology (Baguette et al., 2013; Gagnaire & Gaggiotti, 2016; Richardson et al., 2016). The recent progress in landscape genetics (Manel & Holderegger, 2013) facilitated by the development of Next Generation Sequencing tools (NGS), and combined with advances in geomatics can help to better address those questions. Hence, today is possible to combine tens of thousands of molecular markers with high resolution environmental data, to better understand the influence of landscape on fine scale patterns of neutral genetic structure as well as local adaptation (Ellegren, 2014).

Particularly for lotic environments, riverscape genetics improves knowledge about the effects of the riverine landscapes (riverscapes) on microevolutionary processes (e.g. selection, drift, mutation, gene flow), that determine the spatial genetic structure of populations. These processes are influenced by multiscale alterations like landscape fragmentation and water flow

changes (Le Pichon et al., 2006), that usually reduce habitat size, split environments and generate barriers that isolate fish populations (Morita & Yamamoto, 2002). Moreover, distinct microevolutionary processes could act at different spatial scales (i.e. among basins, and distant or nearby sites within basins) remarking the need of considering multiscale approaches while analyzing these environments, particularly in complex hydrological networks where the physic landscape structure together with reduced pathways determinate genetic variation patterns (Chaput-Bardy et al., 2008; Kanno et al., 2011). Recently, riverscape genetics appeared studying the effects of climatic patterns and geographical barriers in neutral genetic diversity using a small number of molecular markers (Table 1). However, for riverine landscapes only few studies has included large NGS datasets to assess gene environment interactions in riverine species (but see Bourret et al., 2013; Brauer et al., 2016; Hand et al., 2016; Hecht et al., 2015), and still the potential to study endemic species with conservation purposes has been poorly considered (Table 1). Among riverine fish species, endemic salmonids tend to show basin scale variation in demographic and genetic traits (Frank et al., 2011; Landguth et al., 2014). These species undergo variable movement dynamics that are strongly influenced by the adjacent riverscapes, due to the fact that dispersal, mating and mortality are directly related with the heterogeneity of the environment (Neville et al., 2006).

Table 2 Landscape genetics studies testing environmental influence on neutral and adaptive genetic variation at riverine systems.

Species	Location	Number of molecular markers	Main findings	Reference
Rainbow/steelhead trout (*Oncorhynchus mykiss*)	Columbia River Basin, U.S.A.	180 SNP's	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 SNP's	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 SNP's	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	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	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	Genetic diversity deficit related with waterfalls and forest road culverts	Torterotot et al., 2014
Atlantic salmon (*Salmo salar*)	Southeastern Canada	5,500 SNP's	Temperature, precipitation and	Bourret et al., 2013

			geological characteristics related to both potentially adaptive and neutral genetic divergence	
Two castastomidae species (*Catostomus discobolus Discobolus*) and (*Catostomus discobolus yarrow*)	Western U.S.A.	16 microsatellite loci	Stream hierarchy defining gene flow	Hopken et al., 2013
Atlantic salmon (*Salmo salar*)	Western Russia	14 microsatellite loci	Genetic diversity associated with carrying capacity and stream gradient	Ozerov et al., 2012
Atlantic salmon (*Salmo salar*)	Western France	17 microsatellite loci	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	Gene flow mitigated by riverscape barriers	Kanno et al., 2011
Bull trout (*Salvelinus confluentus*)	Glacier National Park, Montana U.S.A.	11 microsatellite loci	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	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	Heterozygosity negatively correlated with elevation, precipitation and upstream distance, while positively correlated with	Narum et al., 2008

			temperature. Additionally, geographical barriers drive genetic structure of life history types	
Three-spined stickleback (*Gasterosteus aculeatus*)	Scheldt River, Flanders Belgium	6 microsatellite loci	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	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	Within populations expected heterozygosity negatively correlated with altitude, while within lakes with habitat size	Castric et al., 2001

Climatic, topographical and hydrological changes tend to segregate salmonid populations provoking gene flow alteration and spatial genetic structure (Landguth et al., 2016; Timm et al., 2016). Moreover, genetic diversity fluctuation is determined by hydrological and topographic gradients at local riverscapes, but also by hydroclimatic fluctuations (e.g. variations in temperature, precipitation and stream flow) affecting survival and reproductive behavior (Castric et al., 2001; Narum et al., 2008; Ozerov et al., 2012). In consequence, the detection of populations threatened by neutral or adaptive genetic diversity loss is vital for the conservation of endemic species (Hand et al., 2016). Upstream salmonids often show small population sizes, which may be subject to strong drift effects that produce low levels of genetic diversity, and eventually leads to inbreeding depression, allowing deleterious genes to be expressed and reducing the ability of adaptation to changing environments (Abadía-Cardoso et al., 2015; Wilson, 2016).

In addition to the study of neutral genetic approaches, climate conditions like temperature and precipitation might be drivers of adaptive processes on different salmonid species, because they are related with migration, reproduction and mortality (Bourret et al., 2013; Perrier et al., 2011). Owing to this determinant relation with hydroclimatic patterns, riverine salmonids are particularly vulnerable to detrimental effects derived from climate change and the introduction of exotic species, as consequence of a smaller habitat in relation to marine and terrestrial environments as well as their special habitat requirements (Parmesan, 2006; Ruiz-Luna et al., 2017).

Mexican golden trout (*Oncorhynchus chrysogaster*) is one of the salmonid species with the southernmost distribution in the world, inhabiting three different basins (Río Fuerte; Río Sinaloa and Río Culiacán) at Sierra Madre Occidental (SMO) mountains in northwest Mexico (Hendrickson et al., 2002). Like all salmonids this species is also susceptible to climate change, habitat perturbations and the introduction of rainbow trout with aquaculture purposes (Escalante et al., 2014).

Although there are some studies on this species (Ruiz-Luna and García-De León 2016), many aspects of its biology are still unknown. However, despite the focus and characteristics of the data sets, previous genetic studies on the species agree that these trout are genetically structured by hydrological basins (Abadía-Cardoso et al., 2015; Camarena-Rosales et al., 2008; Escalante et al., 2014; Nielsen & Sage, 2001). Those studies have been developed with neutral markers, small number of sample sites and sample designs not adjusted to riverscape genetics approach, in consequence microevolutionary processes and their relation with the adjacent riverscape are not yet well understood.

Recent studies for this species revealed a cryptic genetic structure and introgression processes in locations nearby aquaculture facilities (Escalante et al., 2014, Abadía-Cardoso et al., 2015). Moreover, an empirically-derived simulation study on *O. chrysogaster* suggested that riverscape derives spatial genetic structure and acts as a barrier against exotic introgression (Escalante et al. revision). Thus, strong spatial genetic structure and local adaptation processes on *O. chrysogaster* derived by riverscape patterns are expected. Furthermore, it is predicted that riverscape will prevent introgression processes in populations that are not nearby aquaculture farms.

Here, we investigated the effect of anthropogenic and natural variables on the neutral and adaptive genetic diversity of an endemic salmonid. We specifically addressed the following questions: 1) Does aquaculture escapes of exotic *O. mykiss* generates genetic admixture with the endemic *O. chrysogaster*? 2) Which riverscape features affect the extent and distribution of genetic diversity? 3) Are there genomic footprints of local adaptation to heterogeneous hydroclimatic features? To tackle the aforementioned questions, we applied Genotyping by Sequencing (GBS) methods to generate 9,767 Single Nucleotide Polymorphisms (SNP's) and genotyped 270 O. chrysogaster and aquaculture *O. mykiss* from 29 sample sites at three basins in Northwest Mexico. We discussed our results in the context of anthropogenic pressure (i.e. climate change and aquaculture activities) and conservation strategies for endangered species.

Materials and methods

Study system and data collection

In this study, the sampling was conducted in Río Fuerte, Río Sinaloa and Río Culiacán at altitudes ranging from 1965 to 2730 m (Fig. 1). The heterogeneous riverscape of this area offers a great diversity of terrestrial and aquatic habitats providing high endemism and biodiversity (Hendrickson et al., 2006), with *O. chrysogaster* as one of the principal predators inhabiting this riverine ecosystems. However, the introduction of rainbow trout with aquaculture purposes is reported since the 1860's, being strongly supported by federal agencies during the 1980's and 1990's (Escalante et al., 2014).

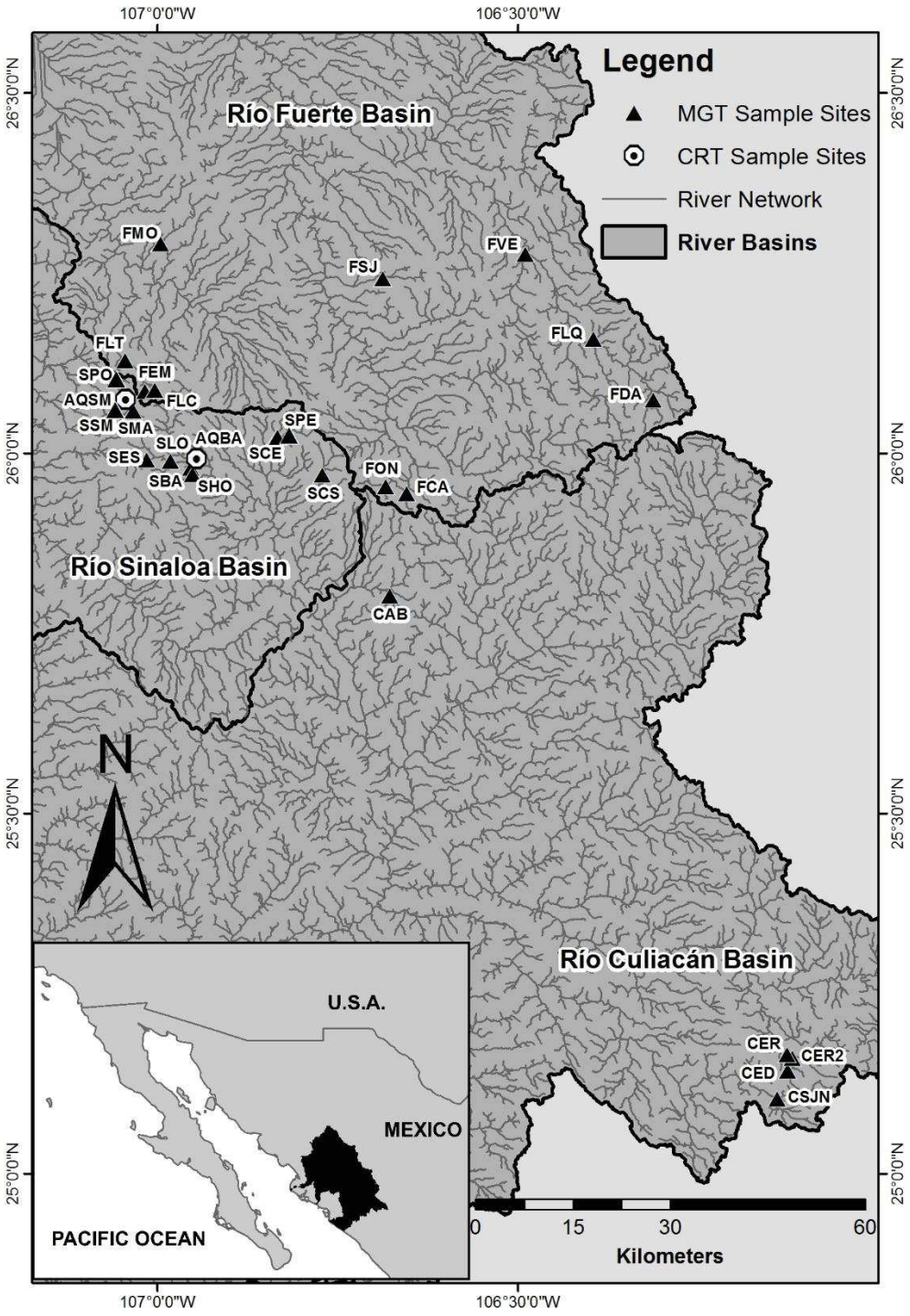

Fig. 1 Study area. Mexican golden trout (MGT) and cultured rainbow trout (CRT) sample sites at Río Fuerte, Río Sinaloa and Río Culiacán. See table 2 for explanation of the sample site codes.

Based on previous study (Escalante et al., revision), five variables were considered to characterize riverscape: precipitation of the driest month, temperature of the warmest quarter, river length, slope, altitude and stream order. They were generated in that study from data available at worldclim database (http://www.worldclim.org/) and the Japanese Space System (http://www.jspacesystems.or.jp/ersdac/GDEM/E/4.html) websites. Moreover, the effect of latitude and longitude was also tested.

Wild trout were collected by electrofishing in 26 sample sites at Río Fuerte (10 sites), Río Sinaloa (11 sites) and Río Culiacán (5 sites) during winter and spring season of 2013, 2014 and 2015 (Fig. 1). Additionally, rainbow trout samples obtained from two aquaculture farms at Río Sinaloa; as well as seven farmed *O. chrysogaster* and four lab hybrids from *O. chrysogaster* and *O. mykiss* donated by the Mexican Institute of Fisheries (INAPESCA), were included in the study for further genotyping. From them, small pieces of tissue (either fin or muscle) were clipped and preserved in 95% ethanol, for posterior analyses.

Genotyping by sequencing

Genomic DNA of each individual was extracted using Qiagen DNeasy Blood & Tissue Kit protocol (Qiagen, Hilden, Germany, http://www1.qiagen.com). DNA quality was checked using agarose gel electrophoresis, and quantified using Nano-Drop spectrophotometer (Thermo Scientific) and QuantiT Picogreen dsDNA Assay Kit (Invitrogen). Then, DNA libraries were generated by GBS methods (Elshire et al., 2011) at Cornell University in Ithaca New York using ECOT221 enzyme. Finally, single-read 100-bp sequencing was performed on Illumina HiSeq2500.

The quality of raw sequences was controlled with FastQC (Andrews et al., 2010). Reads were treated with Cutadapt (Martin, 2011) to remove potential fragments of Illumina adapters, allowing only 10% mismatch in the adapter sequence. The bioinformatics software/pipeline Stacks 1.32 (Catchen, 2013; Catchen et al., 2011) was used to demultiplex reads, identify Restriction site Associated DNA (RAD) loci and call SNP's. Reads were filtered for overall quality, demultiplexed and trimmed to 85bp using process_radtags module, where one mismatch in the bar code sequence was allowed. We used the ustacks module of Stack, with a minimum stack depth of 4x, a maximum distance allowed between stacks of 4 (6 for secondary reads, which could not be used to call SNP's). Using cstacks module, the catalogue of loci using n = 4 was built. With sstacks module, samples were matched against the catalog of loci. Finally, individuals were genotyped using populations module with at least 70% of the individuals being genotyped and a minimum read depth of 5x for each loci. Genotypes were

exported in VCF format for further filtering. The SNP's dataset was filtered using VCFtools (Danecek et al., 2011) for a minimum average read depth ranging from 8 to 40x and a minor allele frequency of 1%. We constituted a blacklist of loci deviating from Hardy-Weinberg equilibrium (HWE, p-value ≥ 0.05) in 1 or more populations among 3 populations exempt from stocking and with relatively large number of individuals.

We constituted six datasets at four different spatial scales after quality filters: Dataset A for population genetic analysis including all the genotyped individuals, Dataset B for landscape genetics analyses across all the study area including native trout, Dataset C for landscape genetic analysis with central populations, Dataset D for landscape genetic analyses at Río Fuerte, Dataset E for landscape genetics analyses at Río Sinaloa, and Dataset F for gene environment associations across all the study area with native trout. Further information about datasets is included in Appendix 5.

Population genomics analyses

Trout genetic diversity (Dataset A) was estimated from private polymorphisms (PP), expected heterozygosity (H_E) and observed heterozygosity (H_O) using adegenet (R package; Jombart, 2008). Effective population (NE) size was calculated applying a molecular co-ancestry method (Nomura, 2008) implemented in the R package NeEstimator (Do et al., 2014) .

Genetic distances among all sample sites (Dataset A) were assessed with two different approaches using the adegenet package. Initially, pairwise F_{ST} coefficients among sample sites were calculated. Moreover, a phenogram was built from all individuals using Nei genetic distance (Tamura & Nei, 1993) by the neighbor joining algorithm (Saitou & Nei, 1987). Confidence intervals based on bootstrap values were estimated from 10,000 permutations.

We then applied fastStructure (Raj et al., 2014), to assess the genetic structure of *O. chrysogaste*r and genetic admixture with aquaculture trout. Based on a Bayesian framework, this approach infers population genetic structure for a large amount of SNP's datasets without assuming predefined populations. We ran fastStructure using Dataset A including all SNP's from all the genotyped individuals. Based on the number of sample sites, we considered K =30 as maximum value.

Riverscape genetics analyses

Riverscape drivers on genetic diversity

To investigate the influence of riverscape on neutral genetic diversity, we applied a multiple linear regression between expected heterozygosity and riverscape variables. A bidirectional stepwise selection procedure was performed (R package MASS; Ripley, 2002) and the environmental variables with significant influence on expected heterozygosity were selected based on AKAIKE criteria. We ran four analyses with different datasets (Dataset B, Dataset C, Dataset D and Dataset E) testing each time seven predictors: latitude, longitude, precipitation of the driest month, temperature of the warmest quarter, river length, altitude and stream order.

Riverscape drivers on genetic divergence

To investigate the effect of riverscape on neutral genetic differentiation, we first derived a resistance surface from four riverscape features using ArcGIS v10.2 (ESRI, 2013). This surface was defined from the rasters of temperature of the warmest quarter, slope, stream order, and altitude. We assigned values to the pixels at each raster representing the degree to obstruct movement according to survival and dispersal requirements for distinct trout species (Hendrickson et al., 2006; Meeuwig et al., 2010). Further information about parameterization is included in Appendix 6.

Then, gdistance R package (van Etten, 2012) was applied to calculate riverine least cost distance and riverscape resistance matrices among sample sites at different spatial scales. This method simulates potential movement for species in a spatially structured landscape, linking different dispersal functions and connectivity thresholds by the Djikstra's shortest path algorithm. Therefore, eight matrices were generated under different hypothesis of movement of both Isolation by Riverine Distance (IBD) or Riverscape Resistance (RR): IBD for all populations (Matrix I), RR for all populations (Matrix II), IBD for central populations (Matrix III), RR for central populations (Matrix IV), IBD for Río Fuerte (Matrix V), RR for Río Fuerte (Matrix VI), IBD for Río Sinaloa (Matrix VII) and RR for Río Sinaloa (Matrix VIII). For further details, see Appendix 6.

In order to define the influence of both riverine distance and riverscape resistance on genetic distances, we tested the movement hypothesis aforementioned using ecodist R package (Goslee & Urban, 2007). Thus, Mantel tests were performed among the regression of $F_{ST}/(1-F_{ST})$ and their corresponding riverine distance/riverscape resistance matrices, under the eight movement hypothesis (Appendix 6). All tests were performed under 10,000 permutations assuming no correlation.

Riverscape adaptive genomics

Detection of SNP's under divergent selection

To detect *O. chrysogaster* SNP's potentially under selection we analyzed Dataset F using three different software considering two different approaches: population (i.e. sampled sites) outlier detection approach (1) and association tests between genotypes and continuous climatic variables (i.e. riverscape adaptive genomics) (2).

First, we applied PCAdapt R package (Luu et al., 2016) to detect SNP's under selection by the approach 1. Combining principal component analysis and Mahalanobis distances, this method assumes that molecular markers excessively associated with population structure are candidates for local adaptation. Based on the vector of z-scores, loci not following the distribution of the main bulk of points are considered outliers. The analysis was run with a threshold of 10% and K=6 based on fastStructure results.

For approach 2, we used two gene environment association software, testing two environmental explanatory variables (temperature of the warmest quarter and precipitation of the driest month) on each method. Those variables were previously suggested as important adaptation drivers in salmonids (Hand et al., 2016; Hecht et al., 2015). Using mixed models, both methods detect outlier loci by allele frequencies exhibiting strong statistical correlations with environmental variables. Initially, we applied BAYEN2 (Günther & Coop, 2013), using an average of five independent runs (100,000 iterations). Also, Latent Factor Mixed Models (LFMM) algorithm included in the R package LEA (Frichot & François, 2015; Frichot et al., 2013) was run with five repetitions, 10,000 cycles, 5,000 burn in and K=6 (based on fastStruture outputs). For LFFM and BBAYENV2, we defined a threshold of 1% of the total SNPs to select the outlier SNP's with the highest posterior probabilities.

Gene ontology analyses

We conducted a gene ontology analysis on the nucleotide sequences (80 bp) containing all SNP's obtained from *O. chrysogaster*, using Dataset F. This analysis is based on a BLAST query (blastn) of the sequences against peptides from the rainbow trout genome database (Berthelot et al., 2014). Functional categorization by Gene Ontology terms (GO; http://www.geneontology.org) was carried out using Blast2GO software (version 4.1, http://www.blast2go.com/). Subsequently, protein annotations were filtered, retaining those from loci detected to be under divergent selection with an e-Value ≤ 10-6 cutoff.

Results

Genotyping by sequencing

The total number of raw sequences obtained was 722,836,026 with an average of 1,300,000 reads per individual. After SNP calling a total of 270 individuals and 9,767 SNP's were retained for subsequent analyses.

Population genetic analysis

The highest private polymorphisms values (53) were found at FVE sample site (see Table 2 for abbreviations) in Río Fuerte while the lowest (0) were at SMA and SPE sample sites at Río Sinaloa and CED at Río Culiacán. For observed heterozygosity, *O. chrysogaster* and rainbow trout lab hybrids obtained the highest values (0.22) whereas wild *O. chrysogaster* collected at CED, CER and CER2 in Río Culiacán obtained the lowest (0.01). Expected heterozygosity highest scores (0.16) were found in aquaculture trout collected at AQEB in Río Sinaloa while the lowest (0.01) were observed at CED, CER and CER2 in Río Culiacán (Table 2). Focusing on wild *O. chrysogaster* only, the highest heterozygosity was found at the center of the study area in the limits between the three basins, while the lowest in the peripheral areas. Private polymorphisms values showed an opposite pattern (Fig. 2). Effective population sizes were successfully estimated for 19 sample sites, the highest value (145.7) was found at SBA and the lowest (0.4) in SMA both in Río Sinaloa (Table 2)

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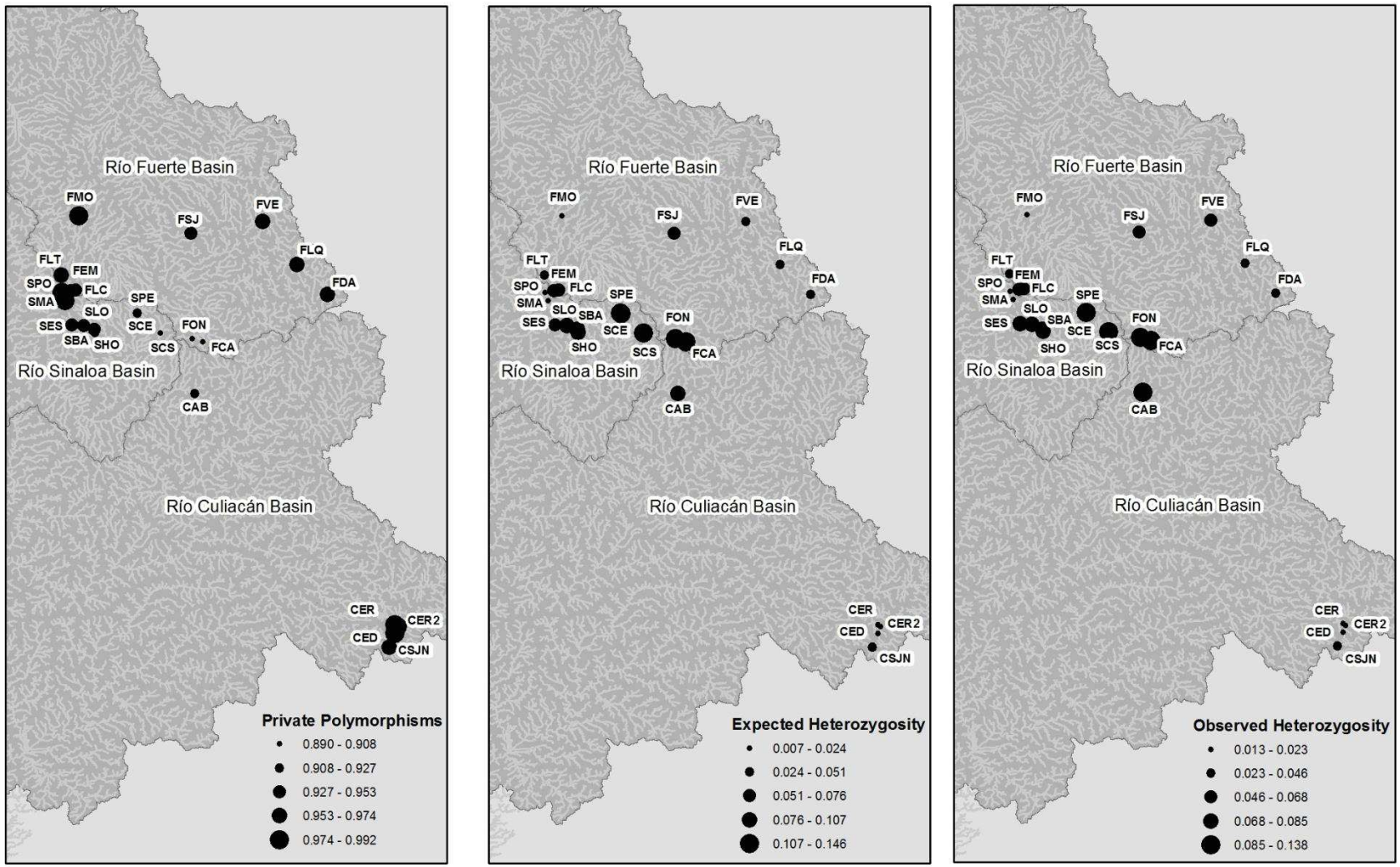

Fig. 2 Geographical distribution of genetic diversity values for native *O. chrysogaster*. See table2 for explanation of the sample site codes.

Table 2 Genetic diversity values for Mexican golden trout, aquaculture rainbow trout and lab hybrids. Number of samples (N), private polymorphisms (*PP*), expected heterozygosity (H_E), observed heterozygosity (H_O) and effective population size (N_E).

River Basin	CODE	Latitude	Longitude	Location	Description	N	PP	H_O	H_E	N_E
Río Fuerte										
	FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10	7	0.03	0.04	44.2
	FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8	5	0.06	0.06	35.3
	FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10	4	0.06	0.07	33.7
	FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12	18	0.04	0.05	116.8
	FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8	1	0.04	0.04	-
	FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5	2	0.02	0.02	-
	FSJ	26.24	-106.69	Arroyo San José	Wild trout	12	1	0.07	0.07	14.7
	FCA	25.94	-106.65	Arroyo Calera	Wild trout	9	2	0.14	0.13	8.2
	FON	25.95	-106.68	Arroyo La Onza	Wild trout	6	1	0.14	0.12	-
	FVE	26.28	-106.49	Río Verde	Wild trout	12	53	0.05	0.05	11
	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
	SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9	4	0.08	0.08	5.9
	SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11	2	0.09	0.11	2.4
	SLO	25.99	-106.98	Arroyo La Osera	Wild trout	10	6	0.08	0.09	1.5
	SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10	0	0.02	0.02	0.4
	SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11	3	0.02	0.02	-
	SSM	26.07	-107.04	Arroyo San Miguel	Trout in aquaculture proximities	4	13	0.17	0.15	-
	SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8	5	0.11	0.15	1.4
	SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7	1	0.09	0.12	1.8
	SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7	0	0.12	0.12	7.5
	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	-
	CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12	1	0.01	0.01	-
	CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12	1	0.01	0.01	-
	CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	12	6	0.05	0.04	8
	CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10	7	0.11	0.1	42.7
	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	-
	REP			INAPESCA Cultive Center	Domestic Mexican golden trout	7	7	0.08	0.08	-
	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
	AQSM	26.07	-107.04	San Miguel Aquaculture Farm	Aquaculture rainbow trout	10	7	0.13	0.15	22.1
	Totals and means	**-**	**-**	**-**	**-**	**24**	**22**	**0.14**	**0.16**	**20.6**

Pairwise F_{ST} was the highest (0.964) among SPO and CED sample sites belonging to two different basins (Río Culiacán and Río Sinaloa), while the lowest (0.008) was between CER and CER2 (both in Río Culiacán). Among basins the highest FST averages (0.83) were observed between sample sites at Río Fuerte and Río Culiacán (for further information see Appendix 7).

The Nei phenogram detected 26 well supported clades (>97 % of bootstrapping value), in all cases composed by nearby geographically sampling sites. Interestingly, aquaculture trout (AQEB and AQSM) were joined in a clade with lab hybrids (H) and trout collected at SSM in aquaculture proximities. Within this clade, trout from SSM was joined in a subclade with aquaculture trout (Fig. 3).

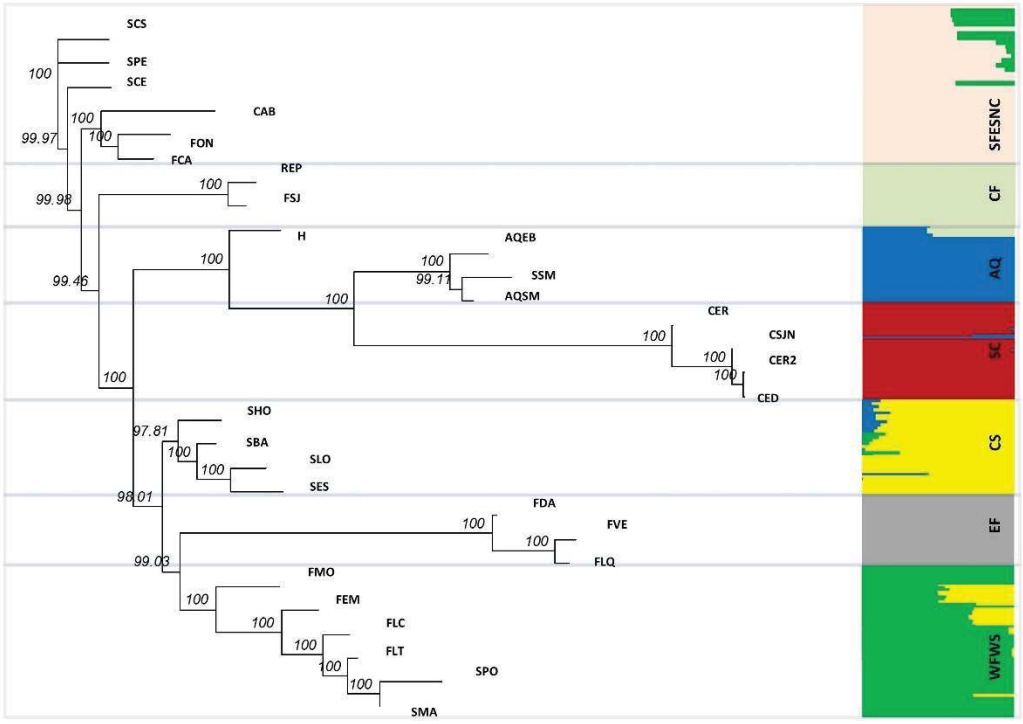

Fig. 3 Genetic structure of *O. chrysogaster* and aquaculture rainbow trout defined by Nei distances (Tamura & Nei, 1993) in adegenet and a Bayesian assignment test in fastStructure. (a) Nei phenogram, the bootstrapping value for each clade is represented by numbers, see table 1 for explanation of the 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).

The unsupervised Bayesian clustering approach (fastStructure) identified six wild distinct genetic clusters that overlay the geography and one aquaculture cluster: Eastern Fuerte; Central Fuerte; Southern Fuerte, Eastern Sinaloa and Northern Culiacán; Western Fuerte and Western Sinaloa; Central Sinaloa; Southern Culiacán; and Aquaculture (Fig. 3b and 4). Trout from Western Fuerte collected at FEM, FLO and FLT showed admixture with trout from Central Sinaloa. Trout from Central Sinaloa collected at SBA showed genetic admixture with trout from Western Fuerte, while the trout collected at SHO and one individual at SLO showed admixture with aquaculture trout. Trout from Eastern Sinaloa collected at SCE, SCS and SPE presented genetic admixture with Western Fuerte. Some trout from Southern Culiacán collected at CSJN presented genetic admixture with aquaculture trout and one individual that entirely belonged to aquaculture cluster. Trout collected at SSM nearby San Miguel aquaculture farm belonged to aquaculture cluster, identified as aquaculture trout perhaps as a product of aquaculture escapes. Finally, for lab hybrids half of their genome belonged to native *O. chrysogaster* from Central Fuerte cluster and the other half to aquaculture cluster. Comparing different K values, there was a similar genetic structure (among K=6, K=7 and K=8) with slight differences for K=6, where trout from FSJ (Central Fuerte in K=7 and 8) were joined with trout from FCA, FON, CAB, SCE, SPE and SCS (Appendix 8). In general, the genetic admixture among native and culture trout was very slight.

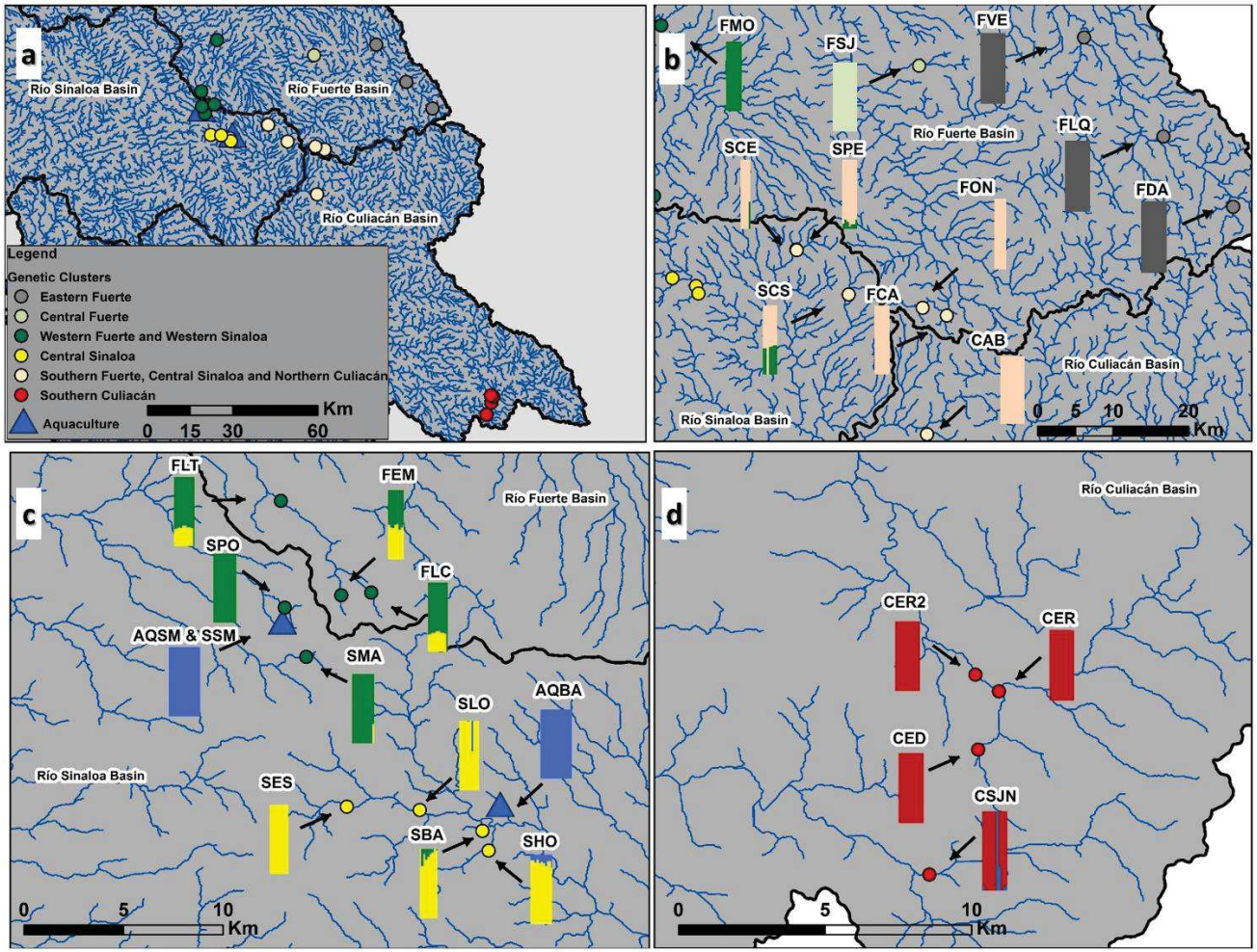

Fig. 4 Spatial distribution of native *O. chrysogaster* and aquaculture rainbow trout genetic clusters defined in FastStructure. a) Spatial genetic structure across all the study area. b) Trout from Eastern Fuerte; Central Fuerte; Southern Fuerte, Central Sinaloa and Northern Culiacán genetic clusters; 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.

Landscape genetics analyses

Riverscape drivers on genetic diversity

Only, the analyses performed with Central populations (Dataset C) and Río Fuerte populations (Dataset D) showed significant correlations among expected heterozygosity and riverscape predictors. For Dataset C we found correlations between expected heterozygosity and latitude, precipitation of the driest month and altitude (Table 3). Moreover, for Dataset D expected heterozygosity showed correlations with latitude, longitude, temperature of the warmest quarter, precipitation of the driest month, river length and stream order (Table 3).

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
Dataset D	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

Riverscape drivers on genetic divergence

All Mantel tests between pairwise $F_{ST}/(1-F_{ST})$ and riverine distance/riverscape resistance matrices were highly statistically significant with p values ≤ 0.038. However, r coefficients substantially increased at the lowest spatial scale while analyzing Dataset D (Río Fuerte) and Dataset F (Río Sinaloa), indicating that the influence of riverine distance and riverscape resistance increases within basins (Table 4).

Table 4 Mantel test performed under 10,000 permutations between $(F_{ST}/(1-F_{ST})$ and the hypothesis of movement stated at the riverine distance/riverscape resistance matrices.

Dataset	Hypothesis of movement	r(AB)	p-value
Dataset B (All native populations)	Isolation of riverine distance (Matrix I)	0.2947597	0.0003000
	Riverscape resistance (Matrix II)	0.2949066	0.0002000
Dataset C (Central populations)	Isolation by riverine distance (Matrix III)	0.2045295	0.0339000
	Riverscape resistance (Matrix IV)	0.2034526	0.0380000
Dataset D (Río Fuerte populations)	Isolation by riverine distance (Matrix V)	0.4939394	0.0128000
	Riverscape resistance (Matrix VI)	0.4985507	0.0115000
Dataset E (Río Sinaloa populations)	Isolation by riverine distance (Matrix VII)	0.8419562	0.0007000
	Riverscape resistance (Matrix VIII)	0.8296010	0.0003000

Riverscape adaptive genomics

Detection of SNP's under divergent selection

A total of 566 outlier loci were detected with PCAdapt, BAYENV2 and LFMM (Fig. 5). PCAdapt identified a total of 278 outliers, among which 260 where only selected by this method. Moreover, for association tests between genotypes and continuous environmental variables BAYENV2 and LFMM identified 97 outliers for each analysis with each environmental variable. These two methods together summarized 388 outlier detections, among which 306 were unique in relation to PCAdapt. On the one hand, BAYENV2 has 151 unique outlier selections, 39 of them were identified with temperature of the warmest quarter, 58 with precipitation of the driest month, and 54 by both environmental variables. On the other hand, LFMM detected 153

unique outliers, among them, temperature of the warmer quarter determined 75 auto-selections, precipitation of the driest month 59, whereas 19 outliers were identified by both environmental variables.

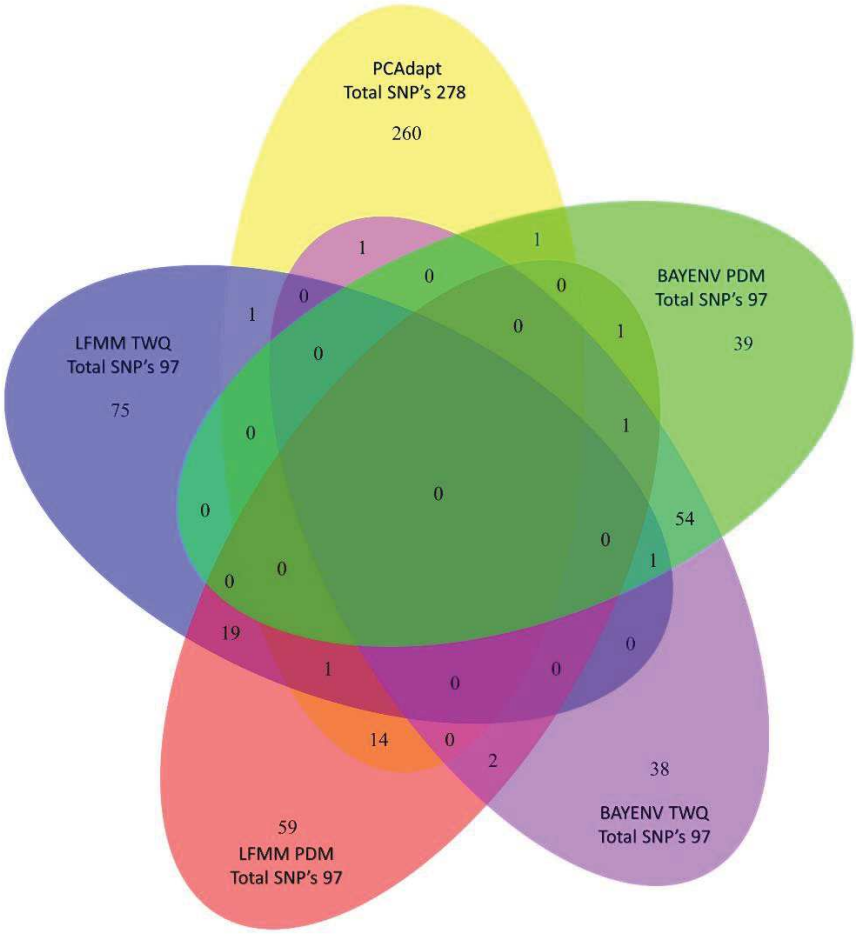

Fig. 5 Venn diagram of outliers detected for *O. chrysogaster* among PCAdapt, LFMM using temperature of the warmest quarter (TWQ), LFMM using precipitation precipitation of the driest month (PDM), BAYENV using temperature of the warmest quarter (TWQ) and BAYENV using precipitation of the driest month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

Gene ontology analyses

After quality filters in the gene ontology analysis, we retained 21 SNP's loci under divergent selection, which had protein annotations (Table 5). Most of those annotations are associated with biological functions (e.g. growth, reproduction, chemistry composition and thermal tolerance) that could be related with hydroclimatic factors.

Table 5 Blast hits from sequences containing a SNP found to be putatively under selection by PCAdapt, BAYENV and LFMM. SNP's 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
CLocus_63824	GSONMP00076296001	1.93E-08	F:GO:0003676	F:nucleic acid binding
CLocus_17128	GSONMP00061816001	0.0000145	F:GO:0008270; P:GO:0030163	F:zinc ion binding; P:protein catabolic process
CLocus_24893	GSONMP00073281001	0.000209	F:GO:0003676	F:nucleic acid binding
CLocus_34692	GSONMP00068395001	0.00000574	F:GO:0005524; F:GO:0004672; P:GO:0006468	F:ATP binding; F:protein kinase activity; P:protein phosphorylation
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
CLocus_40408	GSONMP00028710001	0.000684	P:GO:0055085; C:GO:0016021	P:transmembrane transport; C:integral component of membrane
CLocus_40780	GSONMP00020503001	0.000225	F:GO:0005515	F:protein binding
CLocus_41412	GSONMP00076296001	0.000311	F:GO:0003676	F:nucleic acid binding
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
CLocus_54656	GSONMP00051628001	0.00000314	F:GO:0005515	F:protein binding
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
CLocus_5644	GSONMP00023521001	0.000247	F:GO:0004725; P:GO:0006470	P:tyrosine metabolic

				process; F:protein tyrosine phosphatase activity; P:protein dephosphorylation
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
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
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
CLocus_6876	GSONMP00019151001	0.00052	F:GO:0003676	F:nucleic acid binding
CLocus_69641	GSONMP00059101001	0.0000108	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
CLocus_71994	GSONMP00069497001	0.0000141	F:GO:0005515; F:GO:0005509	F:protein binding; F:calcium ion binding
CLocus_77569	GSONMP00045823001	0.0000424	F:GO:0003676	F:nucleic acid binding

Discussion

Our main objective was to investigate the effect of riverscape and farm escapes on the neutral and adaptive genetic diversity of an endemic salmonid. We found nearly null admixture among aquaculture *O. mykiss* and native trout for the populations sampled. We detected a significant influence of riverscape variables on genetic diversity, and a significant isolation by riverine

distance and riverscape resistance within basins, demonstrating a strong effect of riverscape on the species neutral genetic variation, depending on the spatial scale. Outlier detection and gene ontology analyses identified genes that could be implicated in adaptation to local climate heterogeneity. This is a pioneer study in riverscape genomics, analyzing the influence of riverscape on microevolutionary processes at different spatial scales with conservation purposes. These findings, together with shedding light on riverscape genetics and putative local adaptation in a native trout species are discussed in the context of the development of management strategies for endangered riverine species.

Absence of strong admixture between O. chrysogaster and O. mykiss

The absence of genetic admixture (introgression) among native and exotic trout found in this study can be explained by the lack of proximity with aquaculture sites, as we were not able to detect intensive aquaculture activities (Author's field observations) in the study area. Other studies found evidence of high genetic admixture but only for undescribed Mexican trout forms at southernmost SMO zones characterized by strong aquaculture activities (Abadía-Cardoso et al., 2015; Escalante et al., 2014). Our results show that, in addition to the overall geographic remoteness of aquaculture activities, the local riverscape acts as a boundary against exotic introgression. This barrier effect of riverscape was also suggested in recent simulation studies for this species (Escalante et al. revision) and *O. clarkii lewisi* (Landguth et al., 2016), and with empirical data for *O. clarkii bouvieri* (Gunnell et al., 2008). The extensive introduction of exotic trout is documented as one the highest threats for all the endemic Pacific trout complex (Bahls, 1992; Miller et al., 1989; Penaluna et al., 2016). Also the harmful effects of exotic salmonid invasions have been recognized in native salmonid populations all over the world due to parasites, native food webs alterations, competition and replacement of native genetic pool (Fausch, 2007; Heggberget et al., 1993; Marie et al., 2012; Muhlfeld et al., 2009). Particularly for the SMO, due to economic interests, it is expected that aquaculture activities will increase in the next years while their impacts on native trout populations are difficult to assess (Hendrickson et al., 2002).

However, the low admixture among native and exotic trout detected in this study, highlight the beneficial effect of environmental heterogeneity and low aquaculture activity, but can inevitably be reversed if such activity increases. The low exotic admixture with aquaculture trout observed in previous works in *O. chrysogaster* support our results. Indeed for the particular case of Arroyo Agua Blanca in Río Culiacán, former studies reported high levels of genetic admixture with *O. mykiss* analyzing wild samples collected in 1997 (Escalante et al.,

2014), while samples collected in 2015 keeping without genetic admixture. A hypothesis to explain this pattern could be reproductive biases or sterility in hybrids (Bettles et al., 2005). However, additional studies are needed to confirm this assumption. Still, these results are encouraging and revealed that the genetic pool of riverine species could still be preserved despite existing aquaculture practices in their distribution zone.

Riverscape drivers on genetic diversity

The higher values of expected heterozygosity were associated to high altitudes and long river lengths. While low levels of heterozygosity were correlated with the increase of latitude, longitude, temperature of the warmest quarter, stream order and precipitation of the driest month.

From an ecological point of view the main sources of genetic diversity variation are: i) variation of effective size: populations with large effective sizes are expected to have higher heterozygosity than populations with smaller effective sizes, as they have larger amount of breeders that maintain allele frequencies stable (Höglund, 2009). ii) space mainly through environment and habitat variation: as isolated populations with low immigration rates may have inbreeding and reduced gene exchange (gene flow) with closely related populations, being expose to genetic drift (Riginos & Liggins, 2013). iii) contemporary and historical events, and human disturbances: that fragment or connect populations with the consequent variations of genetic diversity due to microevolutionary processes (drift, migration and mutation) (Banks et al., 2013; Hewitt, 2000).

The riverscape resistance due the temperature, slope and stream order increase; as well as altitude decrease could acts as a barrier against gene flow generating isolation of populations that were reflected in a generalized deficit of heterozygosity, originating endogamy due to population substructure and lower effective size (Hartl & Clark, 1997). Then *O. chrysogaster* populations isolated by topographic, hrydrological and thermal barriers may have a small amount of breeders suffering genetic diversity loss 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). We found a negative influence of precipitation of the driest month in expected heterozygosity. That correlation could 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 at *O. chrysogaster* reproductive period may be causing embryo mortality and

consequently, dramatic population size reductions that translate into heterozygosity loss. A negative influence of precipitation in heterozygosity have also been observed in the native steelhead trout in U.S.A, with similar life history traits and habitat conditions in relation to *O. chrysogaster* (Narum et al., 2008).

The deficit of heterozygosity is stronger in peripheral areas (i.e. east of Río Fuerte, northwest of Río Fuerte and Río Sinaloa, and South of Río Culiacán). Additionally, the small effective population sizes detected in our study (≤146) are far from the one (NE ≥ 500) needed to preserve salmonid long term viability, asking the question of the long term survival of the populations under study (Koskinen et al., 2002; Perrier et al., 2014). Low effective population sizes and heterozygosity values are common for species that have rapid expansions after glaciation periods as consequence of habitat shifts and isolation (Hewitt, 2000). Thus, this genetic diversity deficit could be due to the combination of several factors: bottlenecks occurring after the colonization long time ago and/or habitat fragmentation due to riverscape resistance but also to anthropogenic factors like deforestation and dams' construction impeding immigrants exchange among populations (Behnke et al., 2002; Channell & Lomolino, 2000; Eckert et al., 2008; Ruzzante et al., 2016). Under the assumption that endangered species had a recent origin, which can be the case for *O. chrysogaster* in the SMO (≤ 12,000 years; Behnke et al., 2002), the continuous loss of genetic diversity could be avoid by the translocation of individuals between small and isolated populations but with the same genetic pool, ensuring the preservation of genes involved in local adaptation (Sato & Harada, 2008). But also, strictly regulating land use and land change activities can help to preserve dispersal corridors among populations and maintain genetic diversity.

Riverscape drivers on genetic divergence

The influence of riverscape structure on genetic divergence has been already tested in salmonids, defining riverscape resistance as a fundamental driver of gene flow (Kanno et al., 2011; Meeuwig et al., 2010). In this study, when considering scales larger than intra-basins, the strength of the isolation by riverine distance and riverscape resistance decreased (lower r values of Mantel test); and the sample sites among basins sharing ancestry coefficients were not genetically related in the neighbor joining phenogram (i. e. FLT, FEM, FLC, SBA, SCE, SPE, SCS; see Fig. 3), suggesting not recent connectivity among some populations with genetic backgrounds in common (Castric & Bernatchez, 2003; Dionne et al., 2008; Harris, et al., 2013). This degree of divergence has been interpreted by some authors as cryptic species

(Abadía-Cardoso et al., 2015). However, divergence times should be estimate to better understand these genetic divergences in *O. chrysogater*.

Riverscape resistance is strongly affecting gene flow within basins, acting as a barrier for dispersal also at local scales. Thus, the effect of riverscape on gene flow may determinates the adaptation to local environments, due to gene flow allows the entrance of new genes; then, through recombination during sexual reproduction new combination of genes are produced and populations have 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 among riverscape and gene flow, in other to define management units based on populations with historical gene flow occupying similar ecologic niches (Crandall et al., 2000). This study in particular highlights the importance of using large genomic datasets to define the influence of landscape patterns in gene flow processes among populations at local scales (within basins), to then understand adaptive variation across wider scales (along different basins).

Detection of SNP's under divergent selection

The 306 SNPs correlated with hydroclimatic variables (i.e. precipitation of the driest month and temperature of the warmest quarter), suggest that these environmental variables could act as selective factors in *O. chrysogaster*. The influence of temperature and precipitation in adaptive genetic variation has also been suggested for the steelhead trout from the inner Columbia River Basin (Hand et al., 2016). Temperature has been defined as a potential driver in salmonid's adaptive processes mainly because ectothermic body temperature is closely related with the environment (Hand et al., 2016; Hecht et al., 2015). Moreover, temperature and precipitation variations are involved in phenomena such as changes of dispersal and reproduction timing, age at maturity, growth, fecundity, and survival (Crozier & Hutchings, 2014; Hand et al., 2016; Hecht et al., 2015).

At the moment it is difficult to determine the relationship of gene function with environmental variables in *O. chrysogaster*. However, among the annotated functions detected by gene ontology analyses in outlier loci, protein acting binding might be playing an important role in water flow and temperature acclimation (Milano et al., 2014). Thus, the adaptive genetic variation across the study area may increase among populations exposed to different temperature and precipitation regimes. However, genetic analyses of adaptive variation, through the use of population genetics analyses on outlier loci are needed to confirm it. A study performed in the European hake across the Mediterranean Sea revealed that outlier loci

related with protein acting binding are driving local adaptation to water temperature (Milano et al., 2014). Moreover, protein binding is also associated with trout growth and flesh quality that may be related with stream flow and water temperature (Salem et al., 2010). Even that our results suggest potential adaptation of *O. chrysogaster* to local conditions, these findings should be taken with caution acknowledging that sample size is small and could result in false positives in gene environmental associations.

Our findings together with what is reported for related species indicate that salmonids develop adaptive skills to cope changing climatic conditions (Bourret et al., 2013; Hand et al., 2016; Hecht et al., 2015). However, there is a high risk species of extinction in the face of the threat of global warming and fragmentation of the habitat by the man, especially for small populations with putative fitness loss. Then actions at local scales could not be enough to ensure species survival (Lawler et al., 2010; Rahel et al., 2008). According to the above, federal agencies should consider climate change in all their management plans, integrating multidisciplinary approaches to understand the effect of climate fluctuations in native species at different scales. The landscape genomics approach considered in this study may be helpful in the development of such management strategies. Finally, predictive models that take into account climate change with genomic diversity are urgent to design the best conservation strategies in North American endangered salmonid species.

Conclusions

Here a novel and integrative study defined the effect of both landscape and anthropogenic factors in neutral microevolutionary processes at different scales, and gave information about possible effects of hydroclimatic factors in local adaptation. Such kind of approach is not common in conservation studies. Our findings defined an effect of riverscape patterns on neutral microevolutionary processes at different spatial scales, and suggested an effect of hydroclimatic factors in adaptive variation. The approach presented here may be useful in the development of conservation strategies for endangered riverine fish species.

Acknowledgements

This research was supported under projects founded by CONACYT (Ref. CB-2010-01-152893) and ECOSNORD (M14A04). Marco A. Escalante is recipient of a CONACYT fellowship to perform his PhD studies. We are especially grateful with Rafael Hernández

Guzmán, Luis Alejandro Ramirez Huerta and other members of CIAD staff for all their support on the planning, logistic and performing of field surveys. We are grateful with Genoveva Ingle from the Mexican Fisheries Institute (INAPESCA) for the facilitation of trout samples. Thanks to Tomás Durán Moreno for his support during field surveys. We want to thank all the local guides who helped us for trout sampling especially to Ricardo Silvas González ‘’EL Chalote’’. Also, we are grateful with Guanaceví Municipal Government, PRONATURA and WWF for their aid on field surveys. Thanks to all ‘’Truchas Mexicanas’’ binational group especially to Gorgonio Ruiz Campos and Faustino Camarena Rosales. Thanks to Brad Shepard, Carter Kruse, Jason Dunham, and others for the donation of electrofishing equipment. We want to thank the staffs of Molecular Markers Platform of CEFE and Laboratorio de Genética de la Conservación of CIBNOR for their support on molecular biology work.

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Author contributions

M.A.E., F.G.D.L., A.R.L, C.P. and S.M. design the study. M.A.E., F.G.D.L. and A.R.L. performed fieldwork. M.A.E. performed molecular biology laboratory work. M.A.E., C.P. and E.O.A. conducted data analyses. M.A.E., C.P., F.G.D.L., A.R.L. and S.M. wrote the manuscript. All authors revised and approved the final manuscript.

Chapter 5. General Discussion

Thesis synthesis

The main aim of this thesis was to assess the influence of riverscape features and genetic introgression on microevolutionary processes (i.e. neutral gene flow and local adaptation) of the Mexican golden trout. A preliminary analysis of the entire Mexican trout complex detected a spatial genetic structure composed by 16 genetic clusters, and a high degree of genetic admixture with aquaculture trout for populations at the south of Sierra Madre Occidental. Then, focusing on *O. chrysogaster*, demo-genetic simulations suggested that riverscape resistance is the main factor driving the spatial genetic structure, and preventing exotic introgression due to their effect on dispersal. Lastly, the analysis of a wide SNP's datasets of 270 individuals sampled in 25 wild sites, two rainbow trout hatcheries and a breeding farm of *O. chrysogater* confirms low genetic admixture among *O. chrysogaster* and aquaculture trout, as well as the influence of riverscape on the neutral genetic structure. Moreover, gene environment association analyses suggested an influence of the hydroclimatic variables on *O. chrysogaster* adaptive genetic variation.

Genetic introgression of cultured rainbow trout in the Mexican native trout complex

Here the analyses of 11 microsatellite loci revealed that aquaculture trout genetic diversity values are higher than the native Mexican trout genetic diversity reported in other studies (De los Santos-Camarillo 2008; Abadía-Cardoso et al. 2015; Escalante et al. 2016). The native Mexican trout genetic diversity values were also lower than those observed in salmonids from northern latitudes (Bohling et al. 2016; Ozerov et al. 2012; Torterotot et al. 2014).

The most accepted hypothesis to explain the presence of salmonids in northwest Mexico is a recent colonization by the California salmonids in the Pleistocene, when a group of steelhead trout migrated to the Gulf of California favored by the cold temperatures (Behnke al. 2002). Afterwards, when the global temperature increased at the end of the Pleistocene, those trout colonized the highest parts of the Sierra Madre Occidental rivers. The fragmented structure of this riverscapes generated isolated populations (Behnke et al., 2002; Hendrickson et al., 2006). This colonization could have generated bottleneck events, and might explain the genetic diversity deficit of the Mexican trout complex. The reduction of effective population size in the native trout due to bottleneck can generate genetic diversity loss. Such bottlenecks are expected in native trout because of a reduced amount of colonizers and low immigration rates (Nei et al. 1975; Peery et al. 2012).

Furthermore, results of Chapter II revealed a spatial genetic structure for the entire Mexican trout complex among and within basins. In more details, the analysis of 1017 individuals defined 16 endemic genetic clusters at 13 different basins in northwest Mexico. A similar spatial genetic structure pattern for the Mexican trout complex have been reported by other authors (De los Santos-Camarillo, 2008; Abadía-Cardoso et al., 2015).

Moreover, in Chapter II a strong genetic admixture between native and aquaculture trout was observed at the South of Sierra Madre Occidental; particularly, at Río San Lorenzo, Río Presidio, Río Baluarte and Río Acaponeta basins. At Río San Larenzo, introgression was only detected in trout from Arroyo La Sidra collected in aquaculture proximities. However, for Río Presidio, Río Baluarte and Río Acaponeta, trout from all sample sites exhibited genetic admixture with aquaculture trout, and in some cases exotic genetic signal exceeded in proportion the native genes pool. It is known that aquaculture farms are widespread in those basins. Therefore, trout at Río Presidio, Río Baluarte and Río Acaponeta are the most exposed to genetic introgression within the Mexican trout complex (Camarena-Rosales et al. 2008; Espinosa-Pérez et al. 2016; Figure 1). We also found moderated genetic admixture within aquaculture trout and *O. chrysogaster* in few sample sites. For the particular case of Arroyo Aparique at Río Fuerte, when trout from an abandoned farm and wild trout collected nearby were analyzed, introgression in both senses was found (from aquaculture trout into the native trout and *vice versa*). This kind of genetic admixture may be a consequence of the geographic proximity between both sites, but another explanation could be the use of native trout for aquaculture proposal in this hatchery. Overall, this findings together with other studies suggest that aquaculture trout not extend their range much beyond the immediate vicinity of the farms (De los Santos-Camarillo, 2008: Abadía-Cardoso et al., 2015).

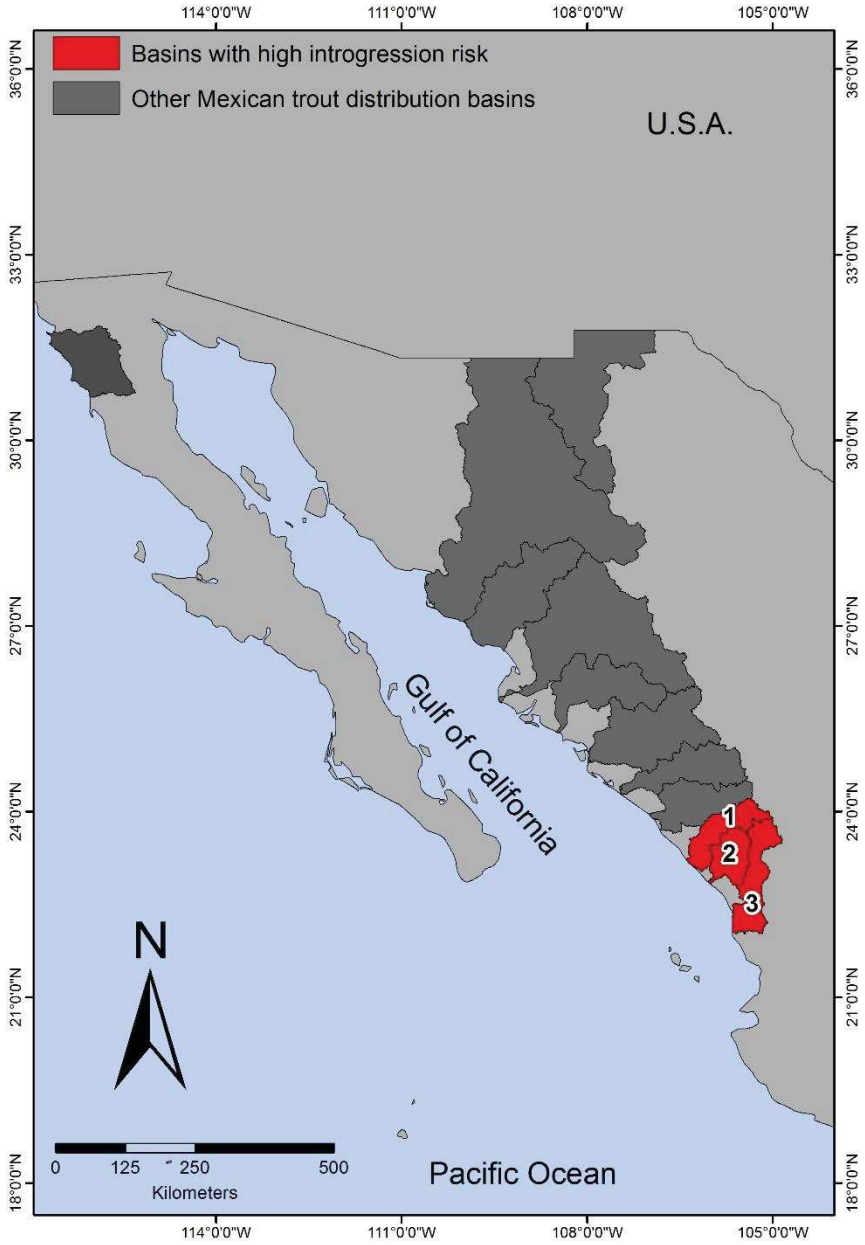

Figure 1. Mexican trout distribution basins with high risk of genetic introgression from aquaculture rainbow trout: Río Presidio (1), Río Baluarte (2) and Río Acaponeta (3).

The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (Oncorhynchus chysogaster)

To better interpret the empirical analysis of 11 microsatellite loci in the Chapter II of this thesis detecting spatial genetic structure for *O. chrysogaster* with a slight degree of genetic introgression, we combined species distribution models and demo-genetic simulations to explore which factors among riverscape and genetic admixture with aquaculture trout determinate the current genetic structure of *O. chrysogaster*.

Among the demo-genetic simulated scenarios, the one including riverscape without introgression was the most congruent with the empirical data in predicting the genetic structure of this species: this scenario exhibited a similar spatial genetic structure composed by three groups, as well as alike global F_{st} and genetic diversity values. These findings suggest that riverscape features such as altitude, temperature, stream order and slope are the main determinants of the genetic structure of this species because they are acting as barriers to dispersal. The three observed genetic clusters were separated by hydroclimatic gradients and topographic ruptures characterized by temperature, stream order and slope increase, as well as altitude decrease. This barrier effect of riverscape have been also reported in riverscape genetics studies on related species, either through simulations (Muhlfeld et al. 2012; Landguth et al. 2014; Inoue & Berg 2017) or empirical data analyses (Hopken et al. 2013; Osborne et al. 2014; Perrier et al. 2011).

Riverscape variables used in the species distribution model were also included in the resistance surfaces, with two additional variables related with dispersal (slope and stream order). Thus, we found that similar riverscape conditions drive both species occurrence, as well as demographic and genetic processes, which is not always the case (Araújo & Luoto 2007; Dellicour et al. 2017; Scoble & Lowe 2010). Species distribution modeling detected strong influence of altitude, temperature of the warmest quarter and precipitation of the driest month in *O. chrysogaster* occurrence. Those variables were recently suggested to influence Mexican golden trout occurrence in a study restricted to Río Sinaloa and Río Culiacán (Ruiz-Luna et al. 2017), but also in studies for all the Mexican trout complex and trout from the U.S.A. (Hendrickson et al., 2006; Wenger et al., 2011). These variables are indeed essential in trout habitat for survival (Roberts et al., 2013; Ruiz-Luna et al., 2017).

The main contribution of this chapter is that the spatial genetic structure of Mexican trout cannot be explained by a classical model of isolation by distance. Instead riverscape strongly influence gene flow and exotic introgression.

Riverscape genetics of the endemic Mexican golden trout, a conservation genomics approach

To validate the findings observed in Chapter III, that are, the influence of riverscape features on neutral genetic structure and the low degree of genetic admixture with exotic trout, but also to define the effect of hydroclimatic variables on adaptive processes, we conducted new analyses including more sampling sites than any former *O. chrysogater* study. For this new study, 9,767 SNP's genotyped for 270 individual using GBS techniques were developed.

Population genomics analyses of this extensive dataset confirmed the lack of genetic admixture with aquaculture trout suggested in Chapter III. They also detected six genetic groups that overlay the geography (see Figure 4 in Chapter IV). In general the lowest genetic diversity values were observed in the populations located in the periphery of the distribution of the species (east and northwest of Río Fuerte, northwest of Río Sinaloa, and South of Río Culiacán). The habitat fragmentation and decreasing population size caused by anthropogenic factors like deforestation and dams' construction could be the causes of the low genetic diversity values for trout inhabiting that areas. It is documented that peripheral populations have smaller sizes, and usually occupy more fragmented habitats receiving less immigrants in relation to core populations caused by both natural and anthropogenic factors (Channell & Lomolino 2000; Sexton et al. 2016). Consequently, isolation of peripheral populations may induce founder effects that together with genetic drift could generate genetic diversity loss increasing extinction risks (Johansson et al. 2006; Sexton et al. 2016). Likewise, *O. chrysogaster* populations exhibited low effective population sizes, ranging from 0.4 to 147, while studies in the *Salmo salar* suggested sizes over 500 in order to guarantee long term viability for native populations (Perrier et al., 2014). For species with northernmost distributions, it is reported that low effective population sizes are consequence of habitat shifts and isolation by environment after rapid postglacial colonization (Hewitt 2000), as is the case for *O. chrysogaster*. In addition to past demographic events, life history traits (e. g. inbreeding, reproductive success, and number of reproductive cycles); environmental features (e. g. habitat size and redds availability) as well as anthropogenic factors fragmenting habitats (e. g. dams' construction and deforestation) may have an influence on effective population size specifically in salmonids with low populations sizes inhabiting small streams (Hare et al. 2011; Ruzzante et al. 2016; Araki et al. 2007).

The influence of riverscape features (resistance) on neutral genetic divergence was also observed at different spatial scales. Heterozygosity was significantly positively correlated with altitude, river length and stream order; and negatively with latitude, longitude, temperature of the warmest quarter and precipitation of the driest month. These results indicate that the afore mentioned riverscape resistance keep populations isolated by reducing gene flow among some populations and generating a deficit of heterozygosity due to population genetic substructure. This effect of riverscape in heterozygosity have been also reported for other salmonids at northernmost parts of North America and Eurasia (Mcphee et al., 2014; Ozerov et al., 2012; Torterotot et al., 2014). Furthermore, the influence of riverscape resistance on genetic structure was also confirmed with simulations, being of greater amplitude within basins where the barriers and gradients were determinant to prevent the gene flow. Riverscape discontinuities preventing gene flow among populations has been found in the brook charr and

the bull trout (Kanno et al., 2011; Meeuwig et al., 2010), who also live in headwater heterogeneous habitats isolated by an abrupt topography.

From the point of view of adaptive variation, genes with adaptive potential in endemic populations give advantages to cope with changing environments (Kokko et al. 2017). Gene environment association analysis identified 306 potential outlier loci (e.g. CLocus_40780, CLocus_5089, CLocus_54656, CLocus_55309, CLocus_69641, CLocus_71994) associated to temperature of the warmest quarter and precipitation of the driest month. These hydroclimatic variables have been reported as drivers of survival, reproduction and migration of salmonid (Bourret et al., 2013; Hand et al., 2016; Hecht et al., 2015). Lastly, gene ontology analyses found functions in outlier loci that are involved in temperature acclimation, reproductive behavior, as well as egg and larvae mortality (Crockett, 1998; Hale et al., 2011; Milano et al., 2014; Salem et al., 2010). Thus, it is necessary to reanalyze the population genetic structure only with these outlier loci to define if the adaptive variation generates a spatial genetic differentiation in relation to hydroclimatic factors.

Implications for conservation

Abadía-Cardoso et al. (2015) suggested at least four different species within the Mexican trout complex in SMO. Nevertheless, only the coastal Nelson trout from Río Santo Domingo in Baja California Peninsula (*O. mykiss nelsoni*) and the Mexican golden trout are already taxonomically described (Hendrickson et al., 2002). Unfortunately, the existing regulatory laws in Mexico only protect *O. mykiss nelsoni* and *O. chrysogaster* due their condition of described species. Then, the taxonomic description for all the Mexican trout forms is of high relevance in order to establish management strategies to preserve their genetic legacy.

The present study revealed genetic introgression from aquaculture *O. mykiss* in native trout populations inhabiting aquaculture proximities. Genetic introgression could have catastrophic effects in local populations with low effective population sizes and high genetic drift. Mainly genetic introgression erodes the genetic pool inducing a consequent loss of selective values (fitness) (Frankham et al. 2002). This fitness loss usually leads to maladaptation, due to the low skills of the affected populations to cope the selective regimes, leading in high risk of extinction as consequence of climate change (Karlsson et al. 2016; McGinnity et al. 2003). Moreover, other ecological and physiological threats are related to exotic invasions as consequence of aquaculture escapes or intentional releases: such as competition, introduction of parasites and diseases, as well as habitat perturbations; which have caused high mortality in native populations (Muhlfeld et al. 2009; Penaluna et al. 2016).

Hence, there is an urgent need to establish conservation strategies avoiding or strictly regulating aquaculture activities with rainbow trout in the Sierra Madre Occidental.

From an applied point of view, given its geographical distribution, the Mexican trout forms could have genes adapted to higher temperatures in relation to salmonid from northern distributions (Escalante et al. 2016). Then, it appears of high importance to look for such genes combining genome wide association studies and functional analysis. The proper use of those genes respecting the niches occupied by other species could generate developments in aquaculture biotechnology (Hendrickson et al. 2006) by also considering the economic development of the region. One strategy to achieve a sustainable development in the SMO could be to use the native trout instead of *O. mykiss* for the aquaculture activities in the zone (Escalante et al. 2016). Such strategy has already been adopted in this zone by Barriga-Sosa et al. (2016), and their findings indicate that farmed *O. chrysogaster* could reach similar sizes compared to rainbow trout from commercial farm. However, more studies are necessary in order to know *O. chrysogater* adaptation to aquaculture conditions, even so this opens a window opportunity for aquaculture developments in the region.

Besides, SNP's analysis detected low genetic diversity patterns for populations at peripheral areas located at the east and northwest of Río Fuerte, northwest of Río Sinaloa and south of Río Culiacán (Figure 2, Chapter IV). Therefore, a strategy to increase the effective population size and to preserve the native genetic diversity, could be the translocation of individuals among closely related populations from the most isolated areas (Sato & Harada 2008). However, further field surveys are required to determine whether these low genetic diversity values are a normal consequence of the colonization from a Pacific coastal trout ancestor in the SMO basins or a consequence of the anthropogenic impacts. The latter condition deserves a recovery strategy.

Local adaptation to changing environments might be also disadvantaged by low effective population and neutral genetic diversity levels (Cabrero & Camacho 2000). I found a strong effect of hydroclimatic factors on *O. chrysogaster* adaptive genetic variation. It is predicted that hydroclimatic conditions will fluctuate in the next years due to climate change (Parmesan 2006). Then, it is necessary to maintain the genetic diversity of local populations, avoiding the loss of genes adapted to hydroclimatic variations.

To define management units in relation to changing environments, Crandall et al. (2000) suggested integrative criteria considering genetic and ecological exchangeability. Here, the detected neutral genetic structure helped to define closely related populations and the riverscape resistance analysis characterized the connectivity pattern among populations, which may aid to preserve dispersal networks that maintain the adaptive diversity. Likewise,

gene flow could be artificially maintained in fragmented populations by translocations. Also, if recent genetic divergence is derived by anthropogenic factors, the restoration of natural conditions of ecological and genetic interchange should be attempted by habitat restoration (Crandall et al. 2000; Lawler et al. 2010; Rahel et al. 2008).

This study used a multidisciplinary approach, identifying the influence of several riverscape factors on microevolutionary processes at different scales. Such approach is not usually applied in conservation studies. Thus, despite management strategies at local scales, federal agencies must consider global change in all their management plans, integrating climate change into planning exercises, improving inter-agency regional coordination, increasing and maintaining monitoring programs, considering multidisciplinary studies to develop management plans, among other strategies (Heller & Zavaleta 2009).

Limitations of the study

The biology of the Mexican trout complex has been poorly studied. Some reasons to explain this lack of knowledge are the little interest of Mexican federal agencies in biodiversity studies till the near past, making difficult the development of research projects for endangered species, until few years ago. Also, the abrupt topography of the Sierra Madre Occidental together with the deficiency of roads complicate the access to zones where trout inhabits. Thus, due to the conjunction of the aforementioned reasons, there was a lack of information about the biology and distribution of the Mexican golden trout to start this project. Therefore, several times field work was conducted in streams with trout absence, being necessary to increase the number of field trips to the study area. Moreover, illegal and bandit activities mostly related with drug traffic prevented the access to some *O. chrysogaster* potential distribution areas, specifically at northernmost parts of Río Fuerte, south of Río Sinaloa, as well as north and center of Río Culiacán. Consequently the desired amount and distribution of sample sites per river basin was not fulfilled.

It was not possible to infer the effective population size in eight native populations. It can be due in part to the small number of sampled individuals in these sampling sites. This small number of sampled individuals could also result in false positives in gene environmental associations with a risk of type I error in the outlier loci detections (Narum & Hess 2011).

The gene environmental associations tested the two hydroclimatic variables who has been reported as the most influential in salmonids adaptive variation (Bourret et al., 2014; Hand et al., 2016; Hecht et al., 2015). However, there might be unidentified outliers related with other environmental predictors that have not been tested yet; thus, additional studies are

needed to detect if there are another riverscape variables influencing trout adaptive variation. It is also reported that outlier detection methods are susceptible to commit type II errors, due to their limited accuracy to identify outlier loci when directional selection is weak or patterns of neutral and adaptive variation differs (Milano et al. 2014). In consequence, these results should be considered as suggestive information rather than definitive conclusions.

Perspectives

This thesis represents the larger landscape genetics study performed in *O. chrysogaster* to the date, defining relationships among landscape patterns and microevolutonary processes; and also confirming the spatial genetic structure of the entire Mexican trout complex reported by other authors. However, the findings of this thesis could be complemented by additional studies including larger sampling efforts, population genetics analyses using different datasets and approaches, as well as spatial analyses at finer scales.

Future studies should consider additional sampling efforts in areas not included in this work, as there are non-sampled areas where species distribution models predicted high probabilities for trout occurrence. Thus sampling efforts should be conducted there; specifically along the east of Río Fuerte, Río Sinaloa and Río Culiacán, and in the limits between Río Fuerte and Río Culiacán (Figure 2). This could provide a more accurate approximation about *O. chrysogaster* distribution and spatial genetic structure composition, but also better estimations of the species adaptive genetic variation and their environmental drivers. Moreover, the inclusion of additional specimens from sites already sampled will improve effective population size estimations. In addition of extending the spatial sampling, it can be very useful to conduct a temporal sampling in the same site, helping to assess genetic diversity fluctuations and the variation of genetic introgression across the time, assessments that are determinant for the definition of management strategies.

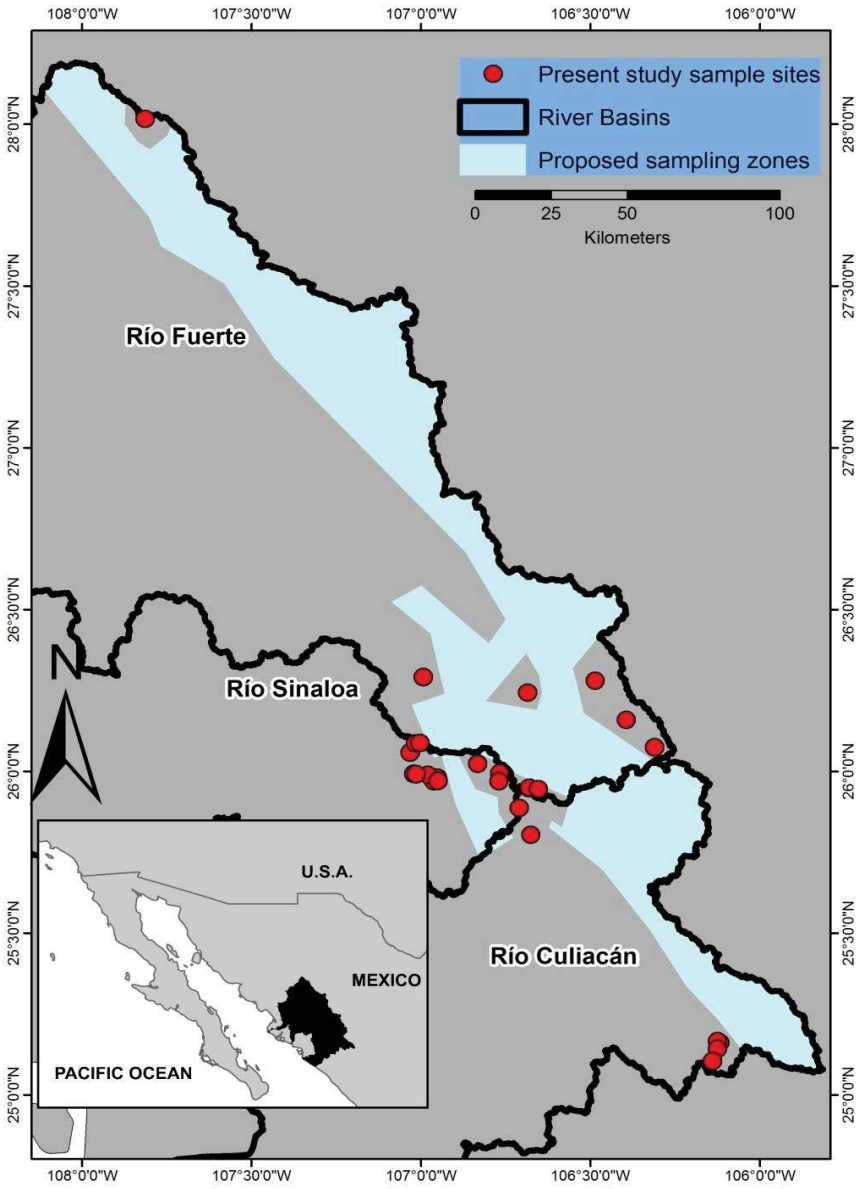

Figure 2. Proposed sampling zones for future *O. chrysogaster* studies, defined by high occurrence probabilities in the species distribution models.

The assessment of habitat quality and fragmentation is necessary to define with high accuracy gene flow (dispersal) among populations and suitable habitat conditions. Thus, satellite imagery classifications from datasets at different spatial scales (e.g. MODIS, LANDSAT, SPOT) and *in situ* observations could be included to define with precision suitable habitat patches and habitat fragmentation. Then, this information may be used to define gene flow (effective migration) through suitable habitat patches according to cost and benefits of dispersal, but also to test migration selection models with outlier loci (Baguette et al., 2013);

giving a better approximation about gene flow barriers affecting neutral genetic structure, and suitable habitat conditions related to local adaptation.

To define future suitable habitat areas in relation to climate change, species distribution modeling could be performed with future climate predictions (e.g. worldclim future climate datasets). These approximations about future potential distribution areas may give estimations about *O. chrysogaster* response to changing environments. Therefore, the definition of future dispersal corridors and areas with suitable habitats, would help to estimate extinction risks as has been done in other species considering distinct climate change scenarios (Hoffmann & Sgrò, 2011; Peterson et al. 2002; Sinclair et al. 2010; Wang et al. 2016; Wright et al. 2016).

Given *O. chrysogaster* cryptic genetic structure, Isolation by Migration Models (Pinho & Hey 2010; Hey & Nielsen 2007; Hey 2010; Hey & Nielsen 2004) could be applied in neutral loci to assess divergence time among predefined genetic clusters. Such approach based on coalescent events estimate both past and current gene flow. Thus, an estimation of current gene flow processes among closely related populations might provide new insights to understand the influence of riverscape in neutral and adaptive microevolutionary processes among and within populations.

Since outlier loci have been defined in this study for Mexican golden trout, a review of other gene environment analyses in salmonid species could help to understand in detail the mechanisms of *O. chrysogaster* local adaptation to riverscape factors. That review may identify the outliers defined for *O. chrysogaster* in other salmonids, as well as the driving patterns and consequent processes reported for other species.

As their condition of one of the salmonid with the southernmost distribution in the world *O. chrysogater* is an important species from an evolutionary point of view, but also considering aquaculture biotechnology development. Therefore, conservation strategies considering both evolutionary and ecological aspects should be conducted on this endangered species in order to preserve their native genetic legacy.

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Supporting information

Appendix 1. General Introduction (English vesion).

Appendix 2. Riverscape variables and correlation matrix (Chapter 3).

Appendix 3. Main parameters for CDMetaPOP (Chapter 3).

Appendix 4. Riverscape resistance surface (Chapter 3).

Appendix 5. Six datasets at four different spatial scales (Chapter 4).

Appendix 6. Riverine/Riverscape surfaces and matrices (Chapter 4).

Appendix 7. F_{ST} coefficients obtained in adegenet among sample sites of Mexican golden trout, aquaculture rainbow trout and lab hybrids (Chapter 4).

Appendix 8. Assignment probabilities obtained in fastStructure for different K values (Chapter 4).

Appendix 1. General Introduction

Species biodiversity and global change

The levels of biological organization are clearly defined and ordered in a categorized system. In order to define management strategies for conservation, in a seminal work Franklin (1988) defined three primary attributes in biodiversity: composition, structure and function. Those attributes are included in a nested hierarchy that fits and incorporates their elements in three levels of spatial organization in relation to taxonomy: community – ecosystems, species – regional landscape, and genetic – local landscape (Noss 1989). Thus, the study of biodiversity requires an understanding about processes occurring among all their elements at different organization levels.

Recently, the combined effect of different threats (climatic and anthropogenic) has caused an accelerated loss of biodiversity with the extinction of endemic birds, amphibians and mammals (Pimm et al. 2014). Nonetheless, it is crucial to quantify potential extinction risks as consequence of global change related with human activities (e.g. climate change, exotic introgression, land use change, and habitat fragmentation). As they have strong effect in biological dispersion, habitat connectivity and viability of populations, among other process threatening species biodiversity (Fischer, 2007; Tilman et al., 2017; With et al., 2006).

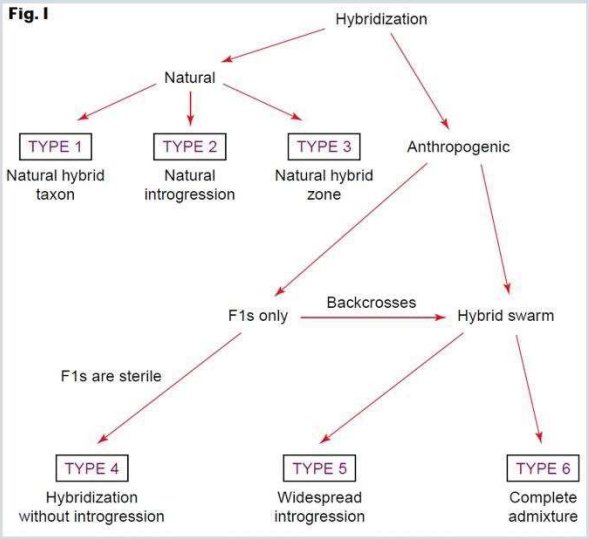
The sensitivity of species to current and past climate change raises the possibility that climate change could became one of the main causes of extinctions in the next decades, with the Earth becoming warmer than any other period since the Paleogene, mostly due to human activities (Thomas & Et 2004; Urban 2015). A direct consequence of this is that ecological changes in species phenology and distribution will occur in all the environments sensitive to scenarios of global warming. Thus, range restricted organisms such as polar or mountaintop species suffer severe habitat reductions and are reported as the first groups on which entire species have been extinct (Parmesan 2006). Besides species extinctions and habitat reductions, it is also predicted that climate change could generate a deficit in ecosystems productivity, emerging diseases and exotic species invasions (Peterson et al. 2002).

Exotic species invasions have been considered as other major risk for the conservation of biodiversity at different levels (i.e. ecosystem, species) through predation, competition, among other factors (Goldburg & Triplett, 1997; Tella et al., 2016). Nevertheless, the negative impacts of exotic species exceed the damage to biodiversity, originating economic losses and severe health problems (Escalante et al. 2016). Just in the U.S.A., it has been estimated that the cost of environmental damages caused by exotic invasions amounts to more than 120,000 million of dollars per year (Pimentel et al., 2005). For the case of freshwater fishes, it is reported

that the frequent introduction of exotic species for both recreational and aquaculture purposes has had negative effects in endemic populations, is worth mentioning that those organisms are more sensitive in comparison to others due to their habitat is smaller as compared to terrestrial or marine species (Penaluna et al. 2016). When the introduced species is phylogenetically close to the native, they can hybridize generating a phenomenon known as genetic introgression, which has a negative effect that could be extremely harmful since the modification of the native genetic pool might derive in the loss of selective values (fitness) affecting the survival abilities in changing environments (Milián-García et al. 2015). When the invasive species is not abundant, their migration rates are low and there is not advantage for the exotic genotype, the effect of the invasion is likely to be null (Escalante et al. 2016). However, if the exotic genotype is favored, there are high migration rates from the invasive species and the native population size is low; the effects of the introgression could be quite detrimental (McGinnity et al. 2003). Allendorf et al. (2001) have defined six categories of hybridization; among them, those involving exotic genetic introgression have the more harmful effects in native populations (Box 1).

Box 1. Categorization of hybridization (taken from Allendorf *et al.* 2001).

Figure 1 provides a framework with which to categorize hybridization. Each type should be viewed as a general descriptive classification that is used to facilitate discussion rather than as a series of strict, all encompassing divisions. Types 1–3 represent hybridization events that are a natural part of the evolutionary legacy of taxa; these taxa should be eligible for protection. Types 4–6 divide anthropogenic hybridization into three categories that have different consequences from a conservation perspective.



Type 1. Natural hybrid taxon: Virgin River roundtail chub *Gila seminuda* are listed as endangered under the Endangered Species Act of the USA (ESA). It is a hybrid taxon that appears to have originated from hybridization between *G. elegans* and *G. robusta* in the Pleistocene long before human influence in the Colorado River system (DeMarais et al., 1992).

Type 2. Natural introgression: Moorean land snails *Partula tainiata* and *P. suturalis* occur sympatrically on the island of Moorea in French Polynesia. In spite of being markedly different both phenotypically and ecologically, estimates of genetic distance based on molecular markers between some sympatric populations of these species are lower than is typical for conspecific comparisons for these taxa (Clarke et al., 1998). The authors of the study concluded that this apparent paradox was best explained by ''molecular leakage, the convergence of neutral and mutually advantageous genes in two species through occasional hybridization''.

Type 3. Natural hybrid zone: Red- and yellow - shafted northern flickers *Colaptes auratus* hybridize in the Great Plains of North America (Moore and Price, 2002). Their narrow hybrid zone extends from Canada through Texas (USA) and has been remarkably stable historically. The reproductive success of hybrids is equal to that of the parental types, and there is no assortative mating within the hybrid zone. Nevertheless, the parental types are thought to be maintained by sexual selection and by natural selection associated with environmental differences between eastern and western North America.

Type 4. Hybridization without introgression: Bull trout *Salvelinus confluentus* are currently listed as threatened under the ESA. Hybridization with introduced brook trout *S. fontinalis* has been documented throughout much of their range. However, there have been few reports of hybrids beyond the first generation (F1) (Leary *et al.*, 1993). Thus, the major detrimental effect of hybridization in this case is wasted reproductive effort rather than genetic mixing. Removal of the non-native species and F1 hybrids is likely to be beneficial, and restoration of degraded habitat could help decrease hybridization.

Type 5. Widespread introgression: Westslope cutthroat trout *Oncorhynchus clarki lewisi* have suffered from widespread hybridization with introduced rainbow trout *O. mykiss* (Allendorf and Leary, 1998). However, many pure westslope populations remain, especially in isolated headwater areas throughout the range of the subspecies. Hybridized populations are of little conservation value (although they could have other values), and efforts should focus on maintaining and expanding the remaining pure populations.

Type 6. Complete admixture: New Zealand grey duck *Anas superciliosa* have been severely affected by hybridization with introduced mallard ducks *A. platyrhynchos* (Rhymer *et al.*, 1994). Few, if any, pure populations remain and there does not appear to be any selection against the hybrids. Here, conservation of hybrids should be considered, because it is the only available option if we are to avoid the complete loss of the hybridized species.

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Global change through both climate change and genetic introgression could generate extinction processes in the near future (Figure 1). Thus, the integration of multiscale and multidisciplinary methods is fundamental for an appropriated management of endangered species; addressing the causes both phenotypic and genetic variation, and with the assessment and application thereof in conservation (Allendorf & Luikart, 2007).

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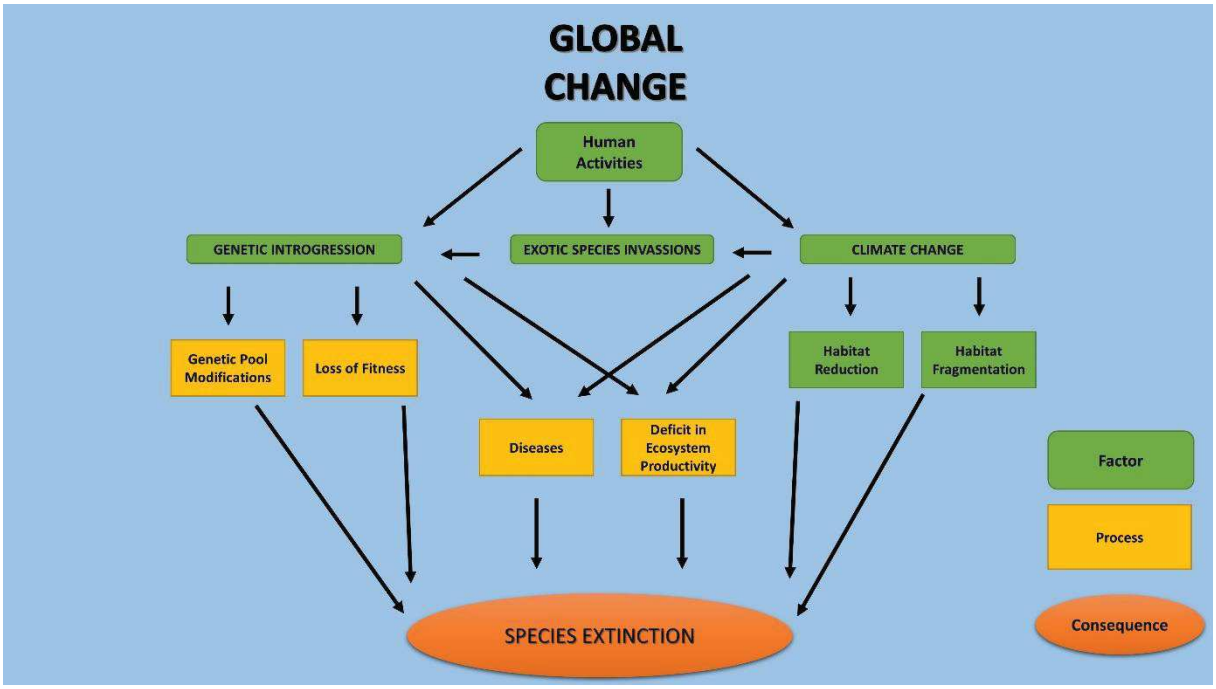

Figure 1 Global change factors and derived processes leading species extinction. Factors are shown in green, processes in yellow and the consequence in orange (Thomas & Et 2004; Urban 2015; Peterson et al. 2002; Goldburg & Triplett 1997; Penaluna et al. 2016).

Approaches and spatial scales

The study of biodiversity patterns at each level of organization requires different disciplines (e. g. ecosystem/species: macroecology, biogeography; intra specific: population genetics, molecular biology). However, the methods developed to study each of those patterns have advanced in different ways and are not usually applied at scales for which they were not developed; mostly due to approach, space, data and research questions constraints (Holling 1998). Consequently, the omission of multidisciplinary assessments while analyzing ecological processes tends to create a partial and narrow vision of the reality. Thus, the amalgamation of different disciplines analyzing biophysical relations with multiscale interactions, allows the understanding of systems and processes involving populations, species, ecosystems, and landscape structures (Holling, 1998; Stein et al., 2014).

Landscape features exist at multiple spatial and temporal scales affecting population spatial structure; thus, the use of only one analysis scale may lead researchers to overlook important factors in the case of analyzing biological processes like dispersal, mating, species distribution, trophic interactions, and vital dynamics (Anderson et al. 2010). Dungan (2002) suggested three different categories to which spatial scale-related terms may be applied: 1) the phenomenon being studied, for example the spatial genetic structure of a species and the processes that affect it; 2) the spatial or sampling units used to acquire information about the phenomenon, for example in situ measures or pixels in an image; and 3) the analysis of the data, used to summarize them or make inferences. Therefore, the phenomenon, sampling and analysis categories can be thought of as three dimensions to which scale concepts pertain (Figure 2).

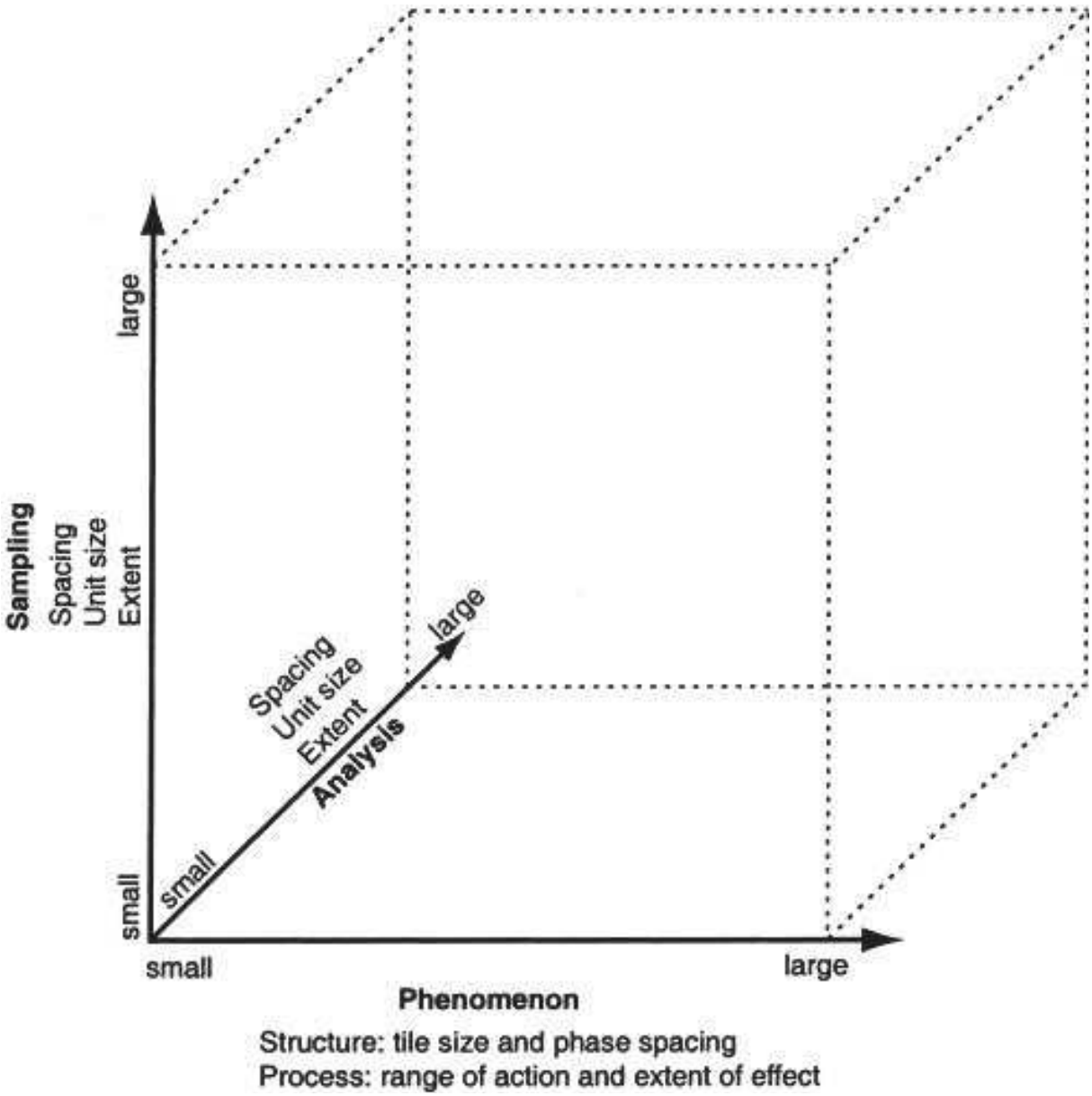

Figure 2 Dimensions of scale concepts in ecology: phenomenon, sampling and analysis (image source: Dunhan et al., 2002).

Under these same concepts, riverine landscapes (riverscapes) are ruled by water flows, and due to its density and viscosity, water is a much more effective agent linking landscape patterns both in scale and space in relation to air surrounding terrestrial landscapes (Wiens 2002). Moreover, the high environmental heterogeneity and reduced habitats at riverscapes derive in lower population sizes and migration rates in relation to marine ecosystems (Selkoe et al.

2016). Hence, biological processes occurring among species or at intraspecific levels in riverine ecosystems are of particular interest in ecology and evolutionary biology.

Landscape genetics

The interaction between genotypes and environment plays an important role in ecological and evolutionary processes. Genetic variation is derived by the random mutation of DNA sequences, increasing through recombination during sexual reproduction that produces new combination of genes, but also by gene flow, through the entrance of new genes from other populations (Cabrero & Camacho 2000). The extent of spatial genetic variation results from a balance of evolutionary forces (mutation, gene flow, genetic drift and natural selection; Box 2) tending to produce local genetic differentiation among populations (King et al., 2006; Slatkin, 1987). One of these forces is natural selection that might derived in adaptive variation. Adaptive variation is defined as the heritable phenotypic variation that is sorted by natural selection into different environmental niches, enhancing selective values (fitness) in specific environments; therefore, it is assumed that some genotypes have more advantages for adaptation, due to their greater survival and reproductive skills to face environmental changes (Hanski et al., 2017; Robinson & Schluter, 2000). Thus, maladaptation often results from phenotype-environment incompatibility and if populations are not locally adapted this carries a great risk of survival and therefore extinction (Kokko et al. 2017). Also, if populations are established with a small number of individuals, recent colonization processes can result in deficit of genetic diversity values (e.g. founder effect) as consequence of alterations on gene frequencies, imposing changes in reproduction processes and increasing mortality (Banks et al. 2013).

Box 2. Evolutionary forces driving genetic variation (King *et al.*, 2006).

Mutation: the random process by which a gene undergoes structural change (Figure 1).

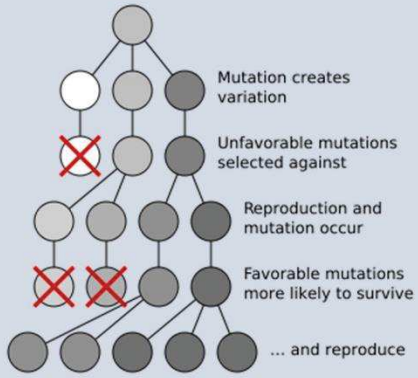

Figure 1. Diagram of mutation and selection in evolution (image source: https://commons.wikimedia.org/wiki/File:Mutation_and_selection_diagram.svg).

Gene flow: the exchange of genes between different populations of the same species produced by migrants, and commonly resulting in simultaneous changes at many loci in the recipient gene pool (Figure 2).

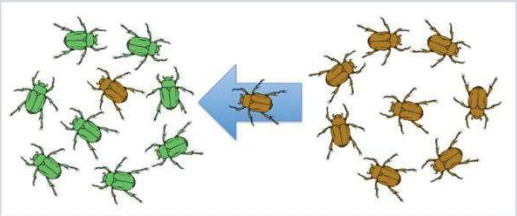

Figure 2. Graphical representation of gene flow (image source: https://upload.wikimedia.org/wikipedia/commons/c/c5/Gene_flow.jpg)

Genetic drift: the random fluctuations of gene frequencies due to sampling errors. While drift occurs in all populations, its effects are most evident in very small populations (Figure 3).

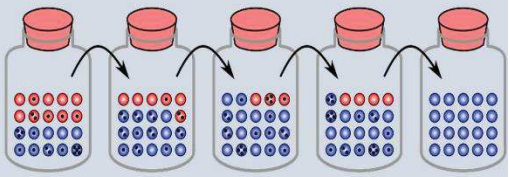

Figure 3. Example describing the effect random sampling has in genetic drift. Dots indicate samples from each generation that are transferred to the next generation. In this population of 20, there is a shift from an allele frequency of 50% for the blue allele to 100% for the blue allele in just 5 generations (image source: https://upload.wikimedia.org/wikipedia/commons/0/0b/Random_sampling_genetic_drift.svg).

Natural Selection: the differential fecundity in nature between members of a species possessing adaptive characters and those without such advantages (Figure 4).

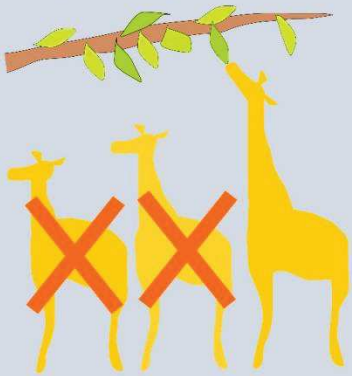

Figure 4. Graphical representation of natural selection (image source: https://upload.wikimedia.org/wikipedia/commons/thumb/8/80/Selection.svg/2000px-Selection.svg.png)

References

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A fundamental concept in landscape ecology is that landscape heterogeneity determinates ecological systems; influencing animal movement, population persistence, species interactions, and ecosystem function, among other processes (Fahrig et al. 2011). Thus, landscape discontinuities have a determinant effect on gene flow, genetic discontinuities, genetic population structure and local adaptation (Holderegger & Wagner 2006). The

understanding of those effects requires a wide variety of basic and applied research questions like: quantifying influence of landscape variables and configuration on genetic variation; identifying barriers to gene flow; identifying source-sink dynamics and movement corridors; understanding the spatial and temporal scale of an ecological process; and testing species-specific ecological hypotheses (Storfer et al. 2007).

In order to tackle those research questions, landscape genetics has emerged as the amalgamation of molecular population genetics and landscape ecology, aiming to provide information about the interaction between landscape features and microevolutionary processes like gene flow and local adaptation (Manel et al., 2003). More recently, the development of landscape genomics was aided by the emergence of methods to generate large genomic data, such as single polymorphism nucleotides (SNP), providing tens of thousands of markers genotyped per individual, that together with the rapid growth of GIS data and the advances in statistical methods offer more accurate assessments about processes of population connectivity and adaptation (Manel & Holderegger, 2013; Selkoe et al., 2016). In the same way, riverine landscapes are an extension of the previous concepts that allows to analyze a type of ecological system, providing linear and heterogeneous habitats that are ideal to test the effect of environmental factors on spatial microevolutionary patterns of native species (Kanno et al., 2011). However, organisms inhabiting riverscapes have been poorly studied in a landscape genetics context (but see Table 1).

Table 3 Landscape genetics studies testing environmental influence on neutral and adaptive genetic variation at riverine systems.

Species	Location	Number of molecular markers/approach	Main findings	Reference
Freshwater mussel (*Cumbrelandia monodonta*)	Mississippi River Basin, U.S.A	Simulation study	Climate change will significantly reduce population connectivity and genetic diversity	Inoue & Berg, 2017
Rainbow/steelhead trout (*Oncorhynchus mykiss*)	Columbia River Basin, U.S.A.	180 SNP's	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 SNP's	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
Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	Akokala Creek, U.S.A.	Simulation study	Inability of exotic populations to recolonize patches as well as lower genetic exchange when barriers are added	Landguth et al., 2016
Chinook salmon (*Oncorhynchus tshawytscha*)	Northeastern Pacific Coast, U.S.A. and Canada	19,703 SNP's	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	Both 310 AFLP loci (empirical data) and simulation study	Analyses of population structure suggest a strong correlation between water color and genotype	Cooke et al., 2014
Plains minnow (*Hybognathus placitus*), emerald	Great Plains, U.S.A.	8 microsatellite loci	Historical signature of past climates and geology shaping contemporary	Osborne et al., 2014

shiner (*Notropis atherinoides*) and red shiner (*Cyprinella lutrensis*)			landscape scale patterns of genetic diversity in *C. lutrensis* and *H. placitus*	
Brook Charr (*Salvelinus fontinalis*)	Saint Louis River, Quebec Canada	16 microsatellite loci	Genetic diversity deficit related with waterfalls and forest road culverts	Torterotot et al., 2014
Bull trout (*Salvelinus confluentus*)	Upper Flathead River, Canada and U.S.A.	Simulation study	Suitable habitat fragmentation generating loss of genetic diversity	Landguth et al., 2014
Atlantic salmon (*Salmo salar*)	Southeastern Canada	5,500 SNP's	Temperature, precipitation and geological characteristics related to both potentially adaptive and neutral genetic divergence	Bourret et al., 2013
Two castastomidae species (*Catostomus discobolus Discobolus*) and (*Catostomus discobolus yarrow*)	Western U.S.A.	16 microsatellite loci	Stream hierarchy defining gene flow	Hopken et al., 2013
Atlantic salmon (*Salmo salar*)	Western Russia	14 microsatellite loci	Genetic diversity associated with carrying capacity and stream gradient	Ozerov et al., 2012
Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)	Akokala Creek, U.S.A.	Simulation study	Barriers placed at headwater areas caused loss of genetic diversity	Muhlfeld et al., 2012
Atlantic salmon (*Salmo salar*)	Western France	17 microsatellite loci	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	Gene flow mitigated by riverscape barriers	Kanno et al., 2011
Bull trout (*Salvelinus confluentus*)	Glacier National Park, Montana U.S.A.	11 microsatellite loci	Genetic differentiation between populations was	Meeuwig et al., 2010

			greater when barriers were present than when absent	
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	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	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	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	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	Within populations expected heterozygosity negatively correlated with altitude, while within lakes with habitat size	Castric et al., 2001

Often, landscape ecologists erroneously consider rivers as a simple element of a landscape mosaic. However, riverscapes are complex environments driving in a particular way microevolutionary processes in relation to other habitats, since gene flow is constrained by the dendritic composition of the river network as well as topographic changes, and adaptive variation is affected by the environmental characteristics within the river and surrounding it (Chaput-Bardy et al., 2008). Some examples of that are waterfalls or stream order changes obstructing gene flow within the river; as well as air temperature conducting water temperature and precipitation driving stream flow patterns, originating local adaptation (Chaput-Bardy et al., 2008; Wiens, 2002; Figure 3). Riverscape genetics approach have been initially applied using few neutral markers to assess the influence of hydroclimatic features and habitat conditions in genetic diversity, as well as the effect of geographical barriers in gene flow (e.g. Castric et al., 2001; Dionne et al., 2008; Osborne et al., 2014; Ozerov et al., 2012). Then, with the development of NGS techniques, riverscape genomics have defined the effect of hydroclimatic factors in adaptive variation using large genomic datasets (Bourret et al., 2013; Brauer et al., 2016; Hand et al., 2016; Hecht et al., 2015). Moreover, genetic simulations have been applied to test hypothesis of gene flow in fragmented landscapes when empirical data is not available (Cooke et al., 2014; Inoue & Berg, 2017; Landguth et al., 2014; Muhlfeld et al., 2012). Therefore, the integration of multidisciplinary approaches like G.I.S. techniques, ecological modeling and population genetics; may facilitate the development of riverscape genetics studies aiming to define the influence of heterogeneous environments in processes like gene flow, local adaptation and genetic introgression.

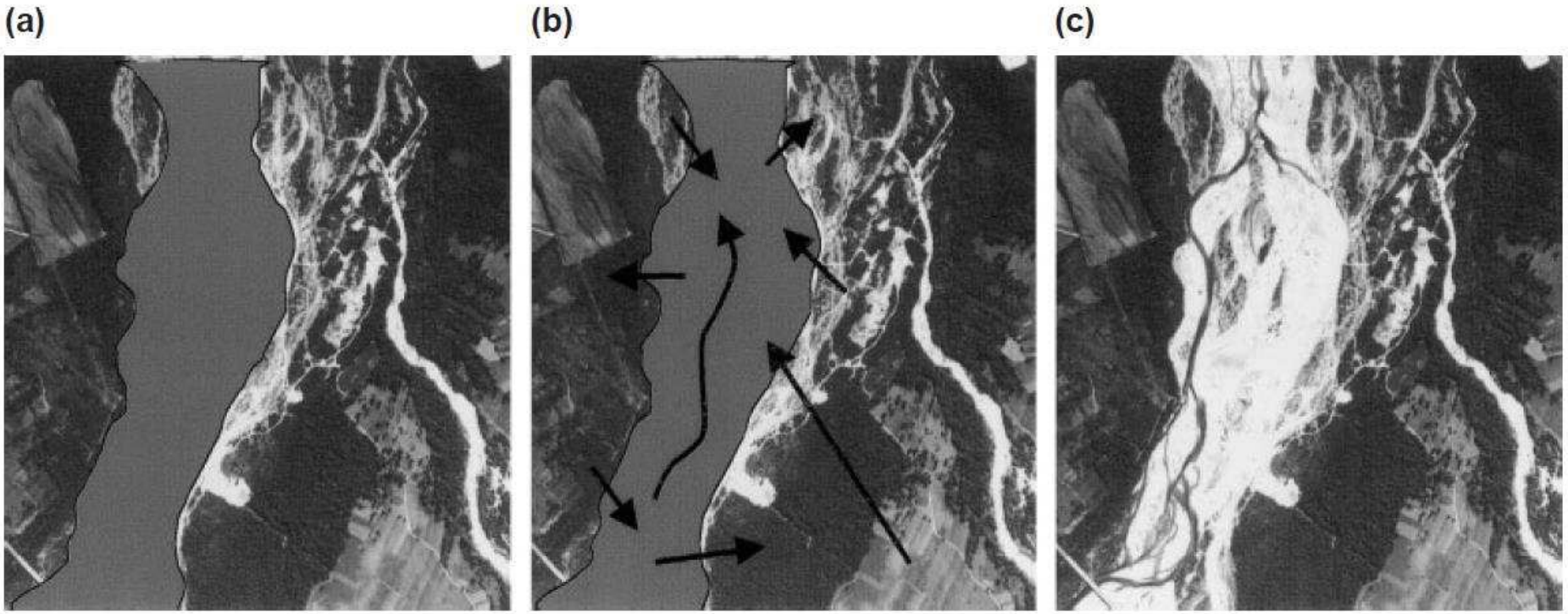

Figure 3. Riverscape structure and microevolutionary processes. (a) Erroneous conception of riverscape as an internally homogeneous element contained within a broader terrestrial landscape. (b) The river is connected with the surrounding landscape by a series of flows across the land–water boundary, or longitudinally down the river corridor. Therefore, microevolutionary processes are affected by both internal and external patterns. (c) The river is a part of a dendritic landscape that is internally heterogeneous, and there is a 'landscape' within the river system as well. Thus, isolated populations within riverscapes may have different drivers on microevolutionary processes (image source: Wiens, 2002).

Study system

The Sierra Madre Occidental, northwest Mexico, has a heterogeneous landscape structure with complex geology, including abrupt slopes and elevation changes of 3,300 meters and several basins draining into the Gulf of California (Hendrickson et al., 2006). In combination with these landscape features, extreme temperature records between -30 and 40 °C and a precipitation range between 250 and 1600 mm per year have been historically recorded (Rzedowski, 2006; Servicio Meteorológico Nacional (http://www.smn.cna.gob.mx/)). This complex landscape provides great diversity of terrestrial and aquatic habitats, resulting in high biotic endemism and biodiversity. Thus, the Sierra Madre Occidental is considered a high priority hot spot due to its great endemic biodiversity (Mittermeier et al., 2002).

Considering the threatened groups in the region, the freshwater biota and particularly native fishes are among the most endangered. Between them, the native trout complex found in this area represents the salmonids with the southernmost distribution in the world, with the Coastal Nelson Trout (*Oncorhynchus mykiss nelsoni*) from Sierra de San Pedro Mártir at Baja California Peninsula and the Mexican golden trout (*O. chrysogaster*) from Sierra Madre Occidental as the only described species of this group (Hendrickson et al., 2002; Figure 3 and 4). However, in spite of being salmonids with potential importance in the worldwide aquaculture industry, they have been poorly studied (but to see: Ruiz-Luna & García-De León, 2016; Ruiz-Luna et al., 2017). So, is of high relevance to study this fish complex, from a taxonomic, genetic, and ecological view; but also it is necessary to understand the environmental and geographic factors that define their distribution and critical habitat conditions for conservation purposes in the context of global change.

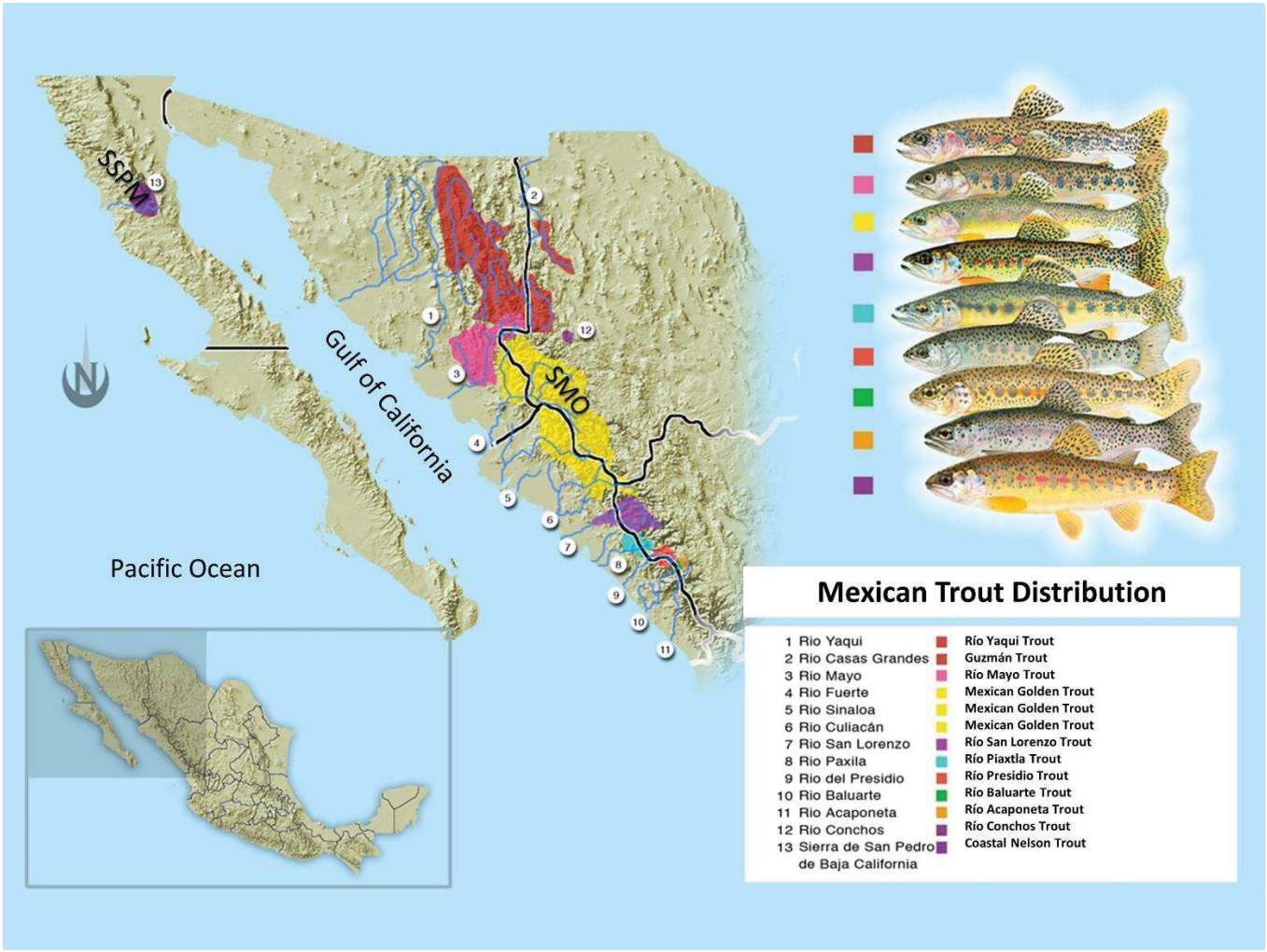

Figure 3. Mexican trout complex distribution in northwest Mexico at Sierra de San Pedro Mártir (SSPM) and Sierra Madre Occidental (SMO). Image source: Espinosa et al., 2007.

Figure 4. Mexican golden trout (*Oncorhynchus chrysogaster*, image provided by Arturo Ruiz Luna).

O. chrysogaster distribution in northwest Mexico includes elevations above 1,500 m in the Sierra Madre Occidental at the Río Fuerte, Río Sinaloa and Río Culiacan basins (Hendrickson et al., 2002); covering a total area of 27,460 km^2 with a stream network length of 18,610 km (Figure 5). The biology of this species is unknown in many aspects (but see Ruiz-Luna & García-De León 2016) and even there are some studies about the genetic structure, those results vary depending on the methods used and the characteristics of the databases. Nielsen & Sage (2001) analyzing 11 microstellite loci from 28 trout collected at two sample sites found one cluster for *O. chrysogaster*. Camarena-Rosales et al. (2008) using mitochondrial DNA from 58 trout collected at four sample sites also defined one genetic cluster. Abadía–Cardoso et al. (2015) using 98 SNP's and 18 microsatellites from 207 trout collected at nine sample sites, defined two main genetic groups including four subgroups composed by geographically adjacent tributaries of different basins. Whereas Escalante et al. (2016) analyzing 11 microsatellite loci from 206 trout collected at nine sample sites reported four genetic clusters with the same geographical composition in relation to Abadía-Cardoso et al. (2015) (for further information about the sample sites considered in both former and the present study see Figure 5). Thus, the differences above mentioned together with the lack of sampling efforts at *O.*

chrysogaster potential distribution areas, highlight the necessity to generate new data with larger collection efforts and novel molecular markers to study this species.

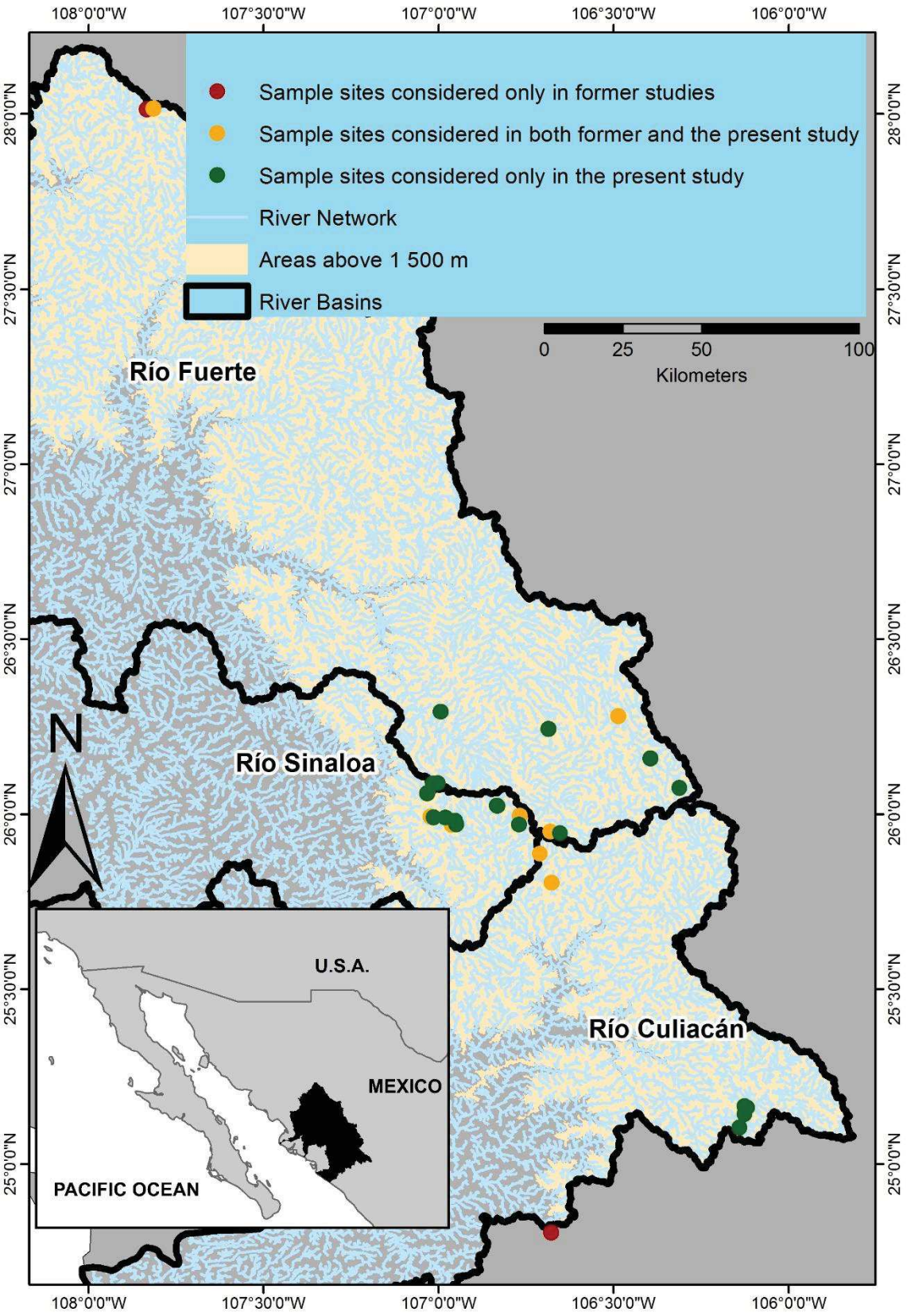

Figure 5. Mexican golden trout distribution area defined by Hendrickson et al. (2002) in zones at Río Fuerte, Río Sinaloa and Río Culiacán above 1,500 m; and sample sites considered for both former and the present study.

Besides threats due to habitat reduction and fragmentation, it is expected that global warming will have negative effects on *O. chrysogaster* distribution range (Ruiz-Luna et al. 2017). In effect, from Europe to North America there are evidences that global warming is causing changes in the range of distribution of several taxa, moving them in poles direction and in the case of mountain organism to higher elevations (Penaluna et al. 2016), making *O. chrysogaster* susceptible to global warming effects. Moreover, the introduction of the exotic rainbow trout (*Oncorhynchus mikyss*) for aquaculture purposes, may cause loses or disruption of the genetic pool of Mexican golden trout, but also other detrimental factors due to competition and introduction of diseases, since rainbow trout is recognized as one of the most harmful exotic invaders in all the world (Lowe et al., 2000). Rainbow trout has been proven to hybridize with the native trout in northwest Mexico (Escalante et al. 2016; Abadía-Cardoso et al. 2015). Even so, economic and social rather than ecological decisions of federal and local governments have promoted trout aquaculture introducing *O. mikyss* since the XIX century up today (Escalante et al., 2016; Hendrickson et al., 2006). Among others, possible consequences of this activity could be the hybridization or substitution of *O. chrysogaster* by the exotic species with the subsequent loss of native biodiversity.

In spite of the low knowledge about Mexican golden trout biology, it is predicted that *O. chrysogaster* populations separated by riverscape gradients (i.e. temperature increase, variation in precipitation ranges as well as hydrological and topographical changes) that prevent gene flow will show high spatial genetic structuration, as it is documented in other salmonids (Frank et al., 2011). Also, genetic diversity and adaptive variation could be affected by those riverscape gradients and the introduction of exotic species, as it occurs with other trout species (Penaluna et al. 2016; Hand et al. 2016).

Objectives

The overall aim of this PhD project is to determinate the possible relationships among microevolutionary processes of Mexican golden trout populations and the spatial structure of their habitat defining the effects of global change factors. To accomplish that, native trout were collected to develop both neutral and adaptive markers. Moreover, I generated riverscape datasets by G.I.S to develop species distribution and demo-genetic models. Finally, riverscape genetics/genomics analyses were conducted to investigate the neutral and adaptive genetic variation. This thesis is structured in three chapters in order to accomplish the main objective:

Chapter II: Genetic introgression of cultured rainbow trout in the Mexican native trout complex.

The objective of this chapter was to investigate the genetic structure of the entire Mexican trout complex and the genetic introgression from cultured rainbow trout. Then, specimens from the entire Mexican trout complex distributed at 13 river basins in Northwestern Mexico, along with native rainbow trout from California U.S.A. and cultured rainbow trout from three farms were considered to generate a database of 11 microsatellite loci from 1,017 individuals. Next; genetic approaches were performed through genetic diversity measures, phylogenetic analyses, Bayesian clustering algorithms and hybrid indexes. Results indicated a spatial genetic structure pattern for the Mexican trout complex; moreover, populations nearby aquaculture farms presented high levels of genetic introgression from cultured rainbow trout.

Chapter III: The interplay of riverscape features and exotic introgression on the genetic structure of the Mexican golden trout (Oncorhynchus chysogaster).

The objective of this chapter was to focus exclusively in *O. chrysogaster* species, to create a system model integrating niche modeling and simulations in order to test various scenarios of rainbow trout introgression on demographic and genetic response, including the hypotheses of movement by both riverine distance and riverscape resistance. With this in mind, the riverscape variables with large influence in *O. chrysogaster* distribution were analyzed. Then, riverscape genetics approaches and the comparison with empirical data generated in the chapter 2, allowed to identify the most realistic scenario and the influence of riverscape in introgression processes. The results of this chapter indicated that riverscape is the main factor shaping *O. chrysogaster* current genetic structure and also acts as a barrier against exotic introgression.

Chapter IV: Riverscape genetics of the endemic Mexican golden trout, a conservation genomics approach.

The objective of this chapter was to define *O. chrysogater* genetic structure and diversity at different spatial scales, assess the influence of riverscape patterns in both neutral and adaptive microevolutive processes, and to evaluate genetic introgression derived from aquaculture activities. To accomplish that, *O. chrysogaster* and cultured rainbow trout specimens were collected at Río Fuerte, Río Sinaloa and Río Culiacán to generate a database of 9,767 SNP's from 270 individuals. Subsequently, population genomics analyses were conducted together with riverscape genetics approaches and gene environment associations. Therefore, results

revealed a spatial genetic structure for *O. chrysogaster* and nearly null genetic introgression from cultured trout. Moreover, I found that riverscape has a strong influence in neutral genetic diversity at different spatial scales. Finally, I identified loci under selective pressure derived from climatic drivers, and biological functions related with annotations in those genes were revealed.

Chapter 5: General Discussion.

In this chapter, the overall approach and the key findings of this study were discussed. Also, perspectives to complement the findings obtained in this thesis and implications for the conservation of the Mexican trout complex were presented

Appendix 2. Riverscape variables and correlation matrix

Table 1. Riverscape variables, the variables considered in the final MAXENT model are shown in bold.

ID	Variable	Source
1	Topographic Aspect	Japanese Space Systems post-processed in ArcGIS
2	Annual Mean Temperature	Bioclim
3	Mean Diurnal Range	Bioclim
4	Isothermality	Bioclim
5	Temperature Seasonality	Bioclim
6	Maximum Temperature of Warmest Month	Bioclim
7	Minimum Temperature of Coldest Month	Bioclim
8	Temperature Annual Range	Bioclim
9	Mean Temperature of Wettest Quarter	Bioclim
10	Mean Temperature of Driest Quarter	Bioclim
11	**Mean Temperature of Warmest Quarter**	**Bioclim**
12	Mean Temperature of Coldest Quarter	Bioclim
13	Annual Precipitation	Bioclim
14	Precipitation of Wettest Month	Bioclim
15	**Precipitation of Driest Month**	**Bioclim**
16	Precipitation Seasonality	Bioclim
17	Precipitation of Wettest Quarter	Bioclim
18	Precipitation of Driest Quarter	Bioclim
19	Precipitation of Warmest Quarter	Bioclim
20	Precipitation of Coldest Quarter	Bioclim
21	Drainage Density	Japanese Space Systems post-processed in ArcGIS
22	River length	Japanese Space Systems post-processed in ArcGIS
23	**Altitude**	**Japanese Space Systems**
24	Geology	Mexican Institute of Statistics and Geography
25	Topographic Slope	Japanese Space Systems post-processed in ArcGIS
26	Stream order	Japanese Space Systems post-processed in ArcGIS

Table 2. Riverscape variables correlation matrix: 1.Topographic Aspect, 2.Annual Mean Temperature, 3.Mean Diurnal Range, 4.Isothermality, 5.Temperature Seasonality, 6.Maximum Temperature of Warmest Month, 7.Minimum Temperature of Coldest Month, 8.Temperature Annual Range, 9.Mean Temperature of Wettest Quarter, 10.Mean Temperature of Driest Quarter, 11.Mean Temperature of Warmest Quarter, 12.Mean Temperature of Coldest Quarter, 13.Annual Precipitation, 14.Precipitation of Wettest Month, 15.Precipitation of Driest Month, 16. Precipitation Seasonality, 17.Precipitation of Wettest Quarter, 18.Precipitation of Driest Quarter, 19. Precipitation of Warmest Quarter, 20.Precipitation of Coldest Quarter, 21.Drainage Density, 22.River length, 23.Altitude, 24.Geology, 25.Topographic Slope and 26.Stream order.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.0	0.0	0.0	0.0	0.1	0.1	0.1	-0.1	0.1	0.1	0.0	0.1	0.0	0.0	-0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	-0.1
2	0.0	1.0	1.0	1.0	-0.2	0.1	-0.8	0.7	0.1	-0.8	-0.1	-0.4	0.0	-0.4	0.3	1.0	1.0	0.2	1.0	1.0	0.1	-0.9	-1.0	0.1	-0.1	0.2
3	0.0	1.0	1.0	1.0	-0.3	0.0	-0.8	0.8	0.0	-0.8	-0.1	-0.4	0.1	-0.5	0.4	1.0	0.9	0.3	1.0	1.0	0.1	-0.8	-1.0	0.0	-0.1	0.2
4	0.0	1.0	1.0	1.0	-0.1	0.2	-0.8	0.7	0.2	-0.7	-0.1	-0.3	-0.1	-0.4	0.2	0.9	1.0	0.1	1.0	1.0	0.1	-0.8	-1.0	0.1	-0.1	0.2
5	0.1	-0.2	-0.3	-0.1	1.0	0.9	0.5	-0.4	0.9	0.6	0.7	0.8	-0.6	0.2	-0.7	-0.3	0.0	-0.6	-0.3	-0.1	0.0	0.1	0.2	0.1	0.2	-0.1
6	0.1	0.1	0.0	0.2	0.9	1.0	0.2	0.0	1.0	0.3	0.8	0.5	-0.4	0.1	-0.5	0.0	0.2	-0.4	0.0	0.1	0.0	-0.2	-0.1	0.1	0.2	-0.1
7	0.1	-0.8	-0.8	-0.8	0.5	0.2	1.0	-0.8	0.2	0.9	0.2	0.6	-0.2	0.4	-0.5	-0.8	-0.7	-0.3	-0.8	-0.8	-0.1	0.7	0.8	0.0	0.2	-0.2
8	-0.1	0.7	0.8	0.7	-0.4	0.0	-0.8	1.0	0.0	-0.9	0.0	-0.7	0.3	-0.2	0.5	0.8	0.6	0.4	0.8	0.7	0.0	-0.7	-0.8	0.0	-0.2	0.1
9	0.1	0.1	0.0	0.2	0.9	1.0	0.2	0.0	1.0	0.2	0.8	0.5	-0.5	0.1	-0.5	0.0	0.2	-0.5	0.0	0.1	0.0	-0.2	0.0	0.1	0.1	-0.1
10	0.1	-0.8	-0.8	-0.7	0.6	0.3	0.9	-0.9	0.2	1.0	0.2	0.7	-0.3	0.3	-0.5	-0.8	-0.7	-0.4	-0.8	-0.7	-0.1	0.6	0.8	0.0	0.2	-0.2
11	0.0	-0.1	-0.1	-0.1	0.7	0.8	0.2	0.0	0.8	0.2	1.0	0.3	-0.1	0.2	-0.3	-0.1	0.0	-0.2	-0.1	-0.1	0.0	0.0	0.1	0.1	0.1	-0.1
12	0.1	-0.4	-0.4	-0.3	0.8	0.5	0.6	-0.7	0.5	0.7	0.3	1.0	-0.7	0.0	-0.7	-0.5	-0.2	-0.6	-0.5	-0.3	0.0	0.2	0.4	0.1	0.3	-0.1
13	0.0	0.0	0.1	-0.1	-0.6	-0.4	-0.2	0.3	-0.5	-0.3	-0.1	-0.7	1.0	0.0	0.8	0.2	-0.2	0.9	0.1	-0.1	0.0	0.0	-0.1	-0.1	-0.1	0.0
14	0.0	-0.4	-0.5	-0.4	0.2	0.1	0.4	-0.2	0.1	0.3	0.2	0.0	0.0	1.0	-0.5	-0.5	-0.4	-0.4	-0.5	-0.4	0.0	0.4	0.5	0.0	-0.1	-0.1
15	-0.1	0.3	0.4	0.2	-0.7	-0.5	-0.5	0.5	-0.5	-0.5	-0.3	-0.7	0.8	-0.5	1.0	0.5	0.1	0.9	0.5	0.3	0.0	-0.3	-0.4	-0.1	-0.2	0.1
16	0.0	1.0	1.0	0.9	-0.3	0.0	-0.8	0.8	0.0	-0.8	-0.1	-0.5	0.2	-0.5	0.5	1.0	0.9	0.4	1.0	0.9	0.1	-0.8	-1.0	0.0	-0.1	0.2
17	0.0	1.0	0.9	1.0	0.0	0.2	-0.7	0.6	0.2	-0.7	0.0	-0.2	-0.2	-0.4	0.1	0.9	1.0	-0.1	0.9	1.0	0.1	-0.8	-0.9	0.1	-0.1	0.2
18	0.0	0.2	0.3	0.1	-0.6	-0.4	-0.3	0.4	-0.5	-0.4	-0.2	-0.6	0.9	-0.4	0.9	0.4	-0.1	1.0	0.3	0.1	0.0	-0.1	-0.2	-0.1	-0.1	0.1
19	0.0	1.0	1.0	1.0	-0.3	0.0	-0.8	0.8	0.0	-0.8	-0.1	-0.5	0.1	-0.5	0.5	1.0	0.9	0.3	1.0	1.0	0.1	-0.9	-1.0	0.0	-0.2	0.2
20	0.0	1.0	1.0	1.0	-0.1	0.1	-0.8	0.7	0.1	-0.7	-0.1	-0.3	-0.1	-0.4	0.3	0.9	1.0	0.1	1.0	1.0	0.1	-0.8	-1.0	0.1	-0.1	0.2
21	0.0	0.1	0.1	0.1	0.0	0.0	-0.1	0.0	0.0	-0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.1	1.0	0.0	-0.1	0.0	0.0	0.1
22	0.0	-0.9	-0.8	-0.8	0.1	-0.2	0.7	-0.7	-0.2	0.6	0.0	0.2	0.0	0.4	-0.3	-0.8	-0.8	-0.1	-0.9	-0.8	0.0	1.0	0.9	0.0	0.1	-0.1
23	0.0	-1.0	-1.0	-1.0	0.2	-0.1	0.8	-0.8	0.0	0.8	0.1	0.4	-0.1	0.5	-0.4	-1.0	-0.9	-0.2	-1.0	-1.0	-0.1	0.9	1.0	0.0	0.1	-0.2
24	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.1	-0.1	0.0	-0.1	0.0	0.1	-0.1	0.0	0.1	0.0	0.0	0.0	1.0	0.0	0.0
25	0.2	-0.1	-0.1	-0.1	0.2	0.2	0.2	-0.2	0.1	0.2	0.1	0.3	-0.1	-0.1	-0.2	-0.1	-0.1	-0.1	-0.2	-0.1	0.0	0.1	0.1	0.0	1.0	-0.2
26	-0.1	0.2	0.2	0.2	-0.1	-0.1	-0.2	0.1	-0.1	-0.2	-0.1	-0.1	0.0	-0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.1	-0.1	-0.2	0.0	-0.2	1.0

Appendix 3. Main parameters for CDMetaPOP

A further explanation about parametrization and the theoretical life cycle is found in the software's manual (http://www.github.com/computationalecologylab/CDMetaPOP/)

Table 1. Main parameters for Mexican golden trout (MGT) and Rainbow trout at four different demogenetic scenarios in CDMetaPOP: Isolation by riverine distance without introgression (SI); Isolation by riverscape resistance without introgression (SII); Isolation by riverine distance with exotic introgression (SIII) and Isolation by riverscape resistance with exotic introgression (SIV).

Input parameter	Description	Values	References
Patch-level controls			
Patches, (X,Y)	Number of patches and location	249 MGT natal grounds and migration grounds placed in 0.8 or higher probability of MGT occurrence (all scenarios). 5 RT natal grounds placed in real geographical position of aquaculture farms (SII and SIV). 1692 migration grounds (all scenarios).	-
K	Carrying capacity	130, 255, 236, 69, 143, 119 and 158 for MGT (all scenarios). 10 000 for RT (SIII and SIV)	Ruiz-Campos & Pister (1995)
N	Initial abundance	68, 137, 123, 31, 62, 63 and 82 for MGT (all scenarios). 9 200 for RT (SIII and SIV)	Ruiz-Campos & Pister (1995)
Genetics	Initial allele frequency	3 Subregions for MGT (east, center west) for all scenarios Farmed aquaculture trout for RT (SIII and SIV).	Escalante *et al.* (2016)
Mortality	Patch-level mortality	0	-
Migration probability	Emigration probability	1	-
Local dispersal probability	Straying probability	1	-
Temperature Winter	Temperature values used to grow individuals	Ranging from 6 to 9.8 °C for MGT natal grounds (all scenarios). N for RT (SIII and SIV).	www.worldclim.org
Grow Days Winter	Grow days during this period	121	-
Temperature Summer	Temperature values used to grow individuals	Ranging from 12.6 to 25.85 °C for MGT natal grounds (all scenarios). N for RT (SIII and SIV).	www.worldclim.org
Grow Days Summer	Grow days during this period	244	-
Class-level controls			
Age Class	Number of age classes	8	-
Body Size	Size of each age class	Age 0 = 31; Age 1 = 83; Age 2 = 130; Age 3 = 162; Age 4 = 181; Age 5 = 192; Age 6 = 199 and Age7 = 206 mm for MGT (all scenarios).	Landguth *et al.* 2016; Secretaría de Pesca *et al.* 1982

		Age 0 = 60, Age 1 = 240, from Age 2 = 330 mm for RT (SIII and SIV).	
Distribution	Distribution within each class	1	Landguth *et al.* 2016
Mortality	Class-specific mortality	0	Landguth *et al.* 2016
Migration Probability	Emigration probability	Age 0 = 0, Age 1 = 0.1, Age 2 = 0.2, Age 3 = 0.3, from Age 4 = 0.4.	Landguth *et al.* 2016
Local Dispersal Probability	Straying probability	0.05	Landguth *et al.* 2016
Male Maturation	The probability of being a reproducing individual.	Age 0 = 0.02; Age 1 = 0.04; Age 2 = 0.43; Age 3 = 0.84; Age 4 = 0.94; Age 5 = 0.97; and from Age 6 = 0.98 for MGT (all scenarios). Age 0 = 0.02; Age 1 = 0.43; Age 2 = 0.84; Age 3 = 0.94; Age 4 = 0.97; and from Age 7 = 0.98 for RT (SII and SIV).	Landguth *et al.* 2016; Secretaría de Pesca *et al.* 1982
Female Maturation	The probability of being a reproducing individual.	Age 0 = 0; Age 1 = 0; Age 2 = 0.03.; Age 3 = 0.69; Age 4 = 0.94 and from Age 5 = 1 for MGT (all scenarios). Age 0 = 0; Age 1 = 0.03.; Age 2 = 0.69; Age 3 = 0.94 and from Age 4 = 1 for RT (SII and SIV).	Landguth *et al.* 2016
Size Control	Processes can either operate based on size or age relationships	Age control	-
Run parameters and output			
MCruns	Replicate runs	3	-
Runtime	Total years	150	-
Start Genes	Year at which genetic exchange begins	1	-
Output Years	Years individual data produced	0,15,30,45,60,75,90,105,120,135,150	-
Output Format	Format genetic output	STRUCTURE	-
Population Model	Population growth model choice	Density dependent class	-
Landscapes and movement rules			
Trout Surface and Movement Rules	Resistance to movement surface	Probability = (1 – (1/Threshold) * Effective Distance): SI = movement between native patches with isolation by riverine distance, using a riverine distance matrix. SII = movement between native patches by riverscape resistance, using a riverscape resistance matrix with 33% of maximum resistance threshold.	

		SIII = movement between native and aquaculture patches with isolation by riverine distance, using a riverine distance matrix. SIV = movement between native and aquaculture patches by riverscape resistance, using a riverscape resistance matrix with 33% of maximum resistance threshold.	
Home Attempt	Possibility that a migrant that did not become a strayer attempts to immigrate back to its original natal patch but cannot	'stray_emiPop'	Landguth *et al*. 2016
Reproduction options			
Sexual Reproduction	Heterosexual or mononecious reproduction	Heterosexual	-
Selfing	Consider selfing	No	-
Freplace, Mreplace	Determine the mating structure	Polygamy	
Mature Length	Use a set length (for size control) or set age (for age control) to specify when a female and male becomes mature.	Age	-
Offspring options			
Offno	Draw choice for eggs produced	Poisson	-
Equal clutch Size		Yes	-
Egg Frequency	The frequency (count per year) in which the female will lay eggs.	Every year	-
Egg mortality		62%	Landguth *et al*. 2016
Egg female percent	Percent females born in litter	50%	
Genetic options			
Loci/alleles	Number of loci/alleles	11/2	Escalante *et al*. (2016)
Mutation rate and model		0	-
Spatial selection options			
Type of selection	Selection model	Selection applied to mature individuals only	-

Implement selection	The time of year to apply spatial selection	See time flow diagram	-
Growth options			
Growth option	Model for incremental growth for each individuals	Temperature	Landguth *et al*. 2016

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Appendix 4. Riverscape resistance surface

Resistance surfaces help to understand the influence of landscape features in functional population connectivity analysis. Those surfaces are calculated with GIS methods and are defined as spatial layers that assign a value to each landscape feature that represents the degree to which that feature obstructs or allows movement for an organism of interest (Spear *et al.* 2010). To analyze connectivity in Mexican golden trout populations, a riverscape resistance surface was created in a raster where each pixel represents the unit cost of crossing each location. Based on previous biological knowledge for Mexican golden trout obtained during field observations and the model generated in MAXENT, four variables were selected to generate this surface (altitude, slope, temperature of the warmest quarter and stream order). Those variables have been also shown to be involved in survival requirements for *O. chrysogaster* (Hendrickson *et al*. 2002; Hendrickson *et al*. 2006; Escalante *et al*. 2014) as well as dispersal patterns reported for other trout from North America (Narum *et al*. 2008; Meewig *et al*. 2010).

Based on the response curves of the Maxent model as well as North American trout and connectivity patterns, resistance values from 0 to 10 were assign to each variable independently (Table 1). Thus, a riverscape resistance raster was generated averaging the resistance values for the four environmental variables. For subsequent analyses in gdistance package (van Etten 2012) resistance values were rescaled form 1 to 2, where 1 represents absence of riverscape resistance and 2 maximum resistance. Thus, to test the hypothesis of Isolation by riverine distance a riverine distance surface was generated assigning absence of resistance to each pixel (All pixels equal to 1). All the aforementioned analyses were conducted in ArcGIS v 10.2 (ESRI 2013).

Table 1. Resistance values for riverscape variable values: altitude, slope, temperature of the warmest quarter (TWQ) and stream order (ST).

Altitude (m)	Altitude Resistance Values
3009 - 1950	0
1949 - 1900	1
1899 - 1850	2
1849 - 1800	3
1799 - 1750	4
1749 - 1700	5
1699 - 1650	6
1649 - 1600	7
1599 - 1550	8
1549 - 1500	9
≤ 1499	10
Slope (° Slope)	**Slope Resistance Values**
0 - 6.74	0
6.75 - 13.48	1
13.49 - 20.22	2
20. 23 - 26.96	3
26.97 - 33.70	4
33.71 - 40.44	5
40.45 - 47.18	6
47.19 - 53.92	7
53.93 - 60.66	8
60.67 - 67.40	9
67.41 - 74.14	10
TWQ (°C)	**TWQ Resistance Values**
≤ 14	0
15	1
16	2
17	3
18	4
19	5
20	6

21	7
22	8
23	9
≥ 24	10
ST (Strahler Order)	**ST Resistance Values**
1 - 3	0
4	3
5	6
6	8
7	10

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Appendix 5. Six datasets at four different spatial scales

We constituted six datasets at four different spatial scales, after quality filters (Table 1). Dataset A: to perform population genetics analysis we keep all samples after bioinformatics filtering (native, aquaculture and hybrids), including 270 genotyped individuals (Table 2). Dataset B: To conduct landscape genetics analyses, we discarded the trout collected at Arroyo San Miguel (SSM) since it is close to aquaculture facilities, farmed *O. chrysogaster*, lab hybrids and aquaculture trout; we also discarded one individual from Arroyo La Osera (SLO) and two individuals from Arroyo San Juan del Negro (CSJN) who presented high aquaculture ancestry coefficients in fastStructure output (Table 3). Dataset C: due to most of the sample sites are located at the center of the study area in the limits of the three river basins, we considered another scale of analysis including those central sample sites (Table 4). Additionally, we performed riverscape genetics analyses at basin scale, using two datasets. Dataset D: including native trout from the ten sample sites at Río Fuerte (Table 5). Dataset E: including native trout from nine sites at Río Sinaloa (Table 6). Native trout from Río Culiacán was not analyzed independently at basin scale due to the small amount of sample sites. Dataset F: to conduct gene environment association and gene ontology analyses, we discarded from Dataset B SLO sample site and two individuals of CSJN since they presented slight aquaculture ancestry coefficients in fastStructure output (Table 7).

Table 1 Characteristics of the six genetic datasets.

Dataset	**Non introgressed wild Mexican golden trout**	**Wild Mexican golden with high aquaculture ancestry coefficients**	**Wild Mexican golden with slight aquaculture ancestry coefficients**	**Aquaculture trout**	**Lab Mexican golden trout and aquaculture trout hybrids**	**Farmed Mexican trout**
Dataset A (all study area)	X	X	X	X	X	X
Dataset B (all study area)	X		X			
Dataset C (center of the study area)	X		X			
Dataset D (Río Fuerte)	X		X			
Dataset E (Río Sinaloa)	X		X			
Dataset F (all study area)	X					

Table 2. Dataset A.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	10
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SSM	26.07	-107.04	Arroyo San Miguel	Trout in aquaculture proximities	4
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**86**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12
CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12
CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12
CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	12
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Total	-	-	-	-	**57**
Lab Trout					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
H			INAPESCA Cultive Center	Mexican golden trout and aquaculture	4

				rainbow trout lab hybrids	
REP			INAPESCA Cultive Center	Farmed Mexican golden trout	7
Total	-	-	-	-	**11**
Aquaculture Farms					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
AQEB	25.99	-106.95	El Barro Aquaculture Farm	Aquaculture rainbow trout	14
AQSM	26.07	-107.04	San Miguel Aquaculture Farm	Aquaculture rainbow trout	10
Total	-	-	-	-	**24**
Dataset total					**270**

Table 3. Dataset B.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**81**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12
CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12
CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12
CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	10
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Totals	-	-	-	-	**55**
Dataset total					**228**

Table 4. Dataset C.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**62**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**81**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Total	-	-	-	-	**10**
Dataset total					**153**

Table 5. Dataset D.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Dataset total	-	-	-	-	**62**

Table 6. Dataset E.

Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SLO	25.99	-106.98	Arroyo La Osera	Wild trout	9
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Dataset total	-	-	-	-	**81**

Table 7. Dataset F.

Río Fuerte					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
FDA	26.07	-106.31	Arroyo del Agua	Wild trout	10
FEM	26.09	-107.02	Arroyo El Manzano	Wild trout	8
FLC	26.09	-107.00	Arroyo Las Cuevas	Wild trout	10
FLQ	26.16	-106.40	Arroyo La Quebrada	Wild trout	12
FLT	26.13	-107.04	Arroyo Las Truchas	Wild trout	8
FMO	26.29	-106.99	Arroyo Momorita	Wild trout	5
FSJ	26.24	-106.69	Arroyo San José	Wild trout	12
FCA	25.94	-106.65	Arroyo Calera	Wild trout	9
FON	25.95	-106.68	Arroyo La Onza	Wild trout	6
FVE	26.28	-106.49	Río Verde	Wild trout	12
Total	-	-	-	-	**92**
Río Sinaloa					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
SBA	25.98	-106.95	Arroyo Baluarte	Wild trout	9
SES	25.99	-107.01	Arroyo El Soldado	Wild trout	9
SHO	25.97	-106.95	Arroyo Hondo	Wild trout	11
SMA	26.06	-107.03	Arroyo Macheras	Wild trout	10
SPO	26.08	-107.04	Arroyo Potrero	Wild trout	11
SCS	25.97	-106.77	Arroyo Cerro Solo	Wild trout	8
SCE	26.02	-106.83	Arroyo Cebollín	Wild trout	7
SPE	26.02	-106.83	Arroyo Pericos	Wild trout	7
Total	-	-	-	-	**72**
Río Culiacán					
CODE	**Latitude**	**Longitude**	**Location**	**Description**	**N**
CED	25.14	-106.13	Arroyo El Desecho	Wild trout	12
CER	25.16	-106.12	Arroyo El Río 1	Wild trout	12
CER2	25.17	-106.13	Arroyo El Río 2	Wild trout	12
CSJN	25.10	-106.14	Arroyo San Juan del Negro	Wild trout	8
CAB	25.80	-106.68	Arroyo Agua Blanca	Wild trout	10
Totals	-	-	-	-	**53**
Dataset total					**217**

Appendix 6. Riverine/Riverscape surfaces and matrices

To analyze connectivity among Mexican golden trout populations, a riverscape resistance and surface was created. To accomplish that, rasters of four variables were selected to generate this surface: altitude, slope, temperature of the warmest quarter and stream order.

Based on the criteria reported in Chapter 3 and Appendix 3, resistance values from 0 to 10 were assign to the pixels at the raters for each variable independently (Table 1). Thus, a riverscape resistance raster was generated averaging the resistance values for the four environmental variables at each pixel. For subsequent analyses in gdistance package (van Etten 2012) resistance values were rescaled from 1 to 2, whit 1 representing absence of riverscape resistance and 2 maximum resistance.

Additionally, to test the hypothesis of Isolation by riverine distance, a riverine distance surface was generated assigning absence of resistance to each pixel (All pixels equal to 1). Both riverscape resistance and riverine distance surfaces were produced in ArcGIS v 10.2 (ESRI 2013).

Table 3 Resistance values for riverscape variables: altitude, slope, temperature of the warmest quarter (TWQ) and stream order (ST).

Altitude (m)	**Altitude Resistance Values**
3009 - 1950	0
1949 - 1900	1
1899 - 1850	2
1849 - 1800	3
1799 - 1750	4
1749 - 1700	5
1699 - 1650	6
1649 - 1600	7
1599 - 1550	8
1549 - 1500	9
≤ 1499	10
Slope (° Slope)	**Slope Resistance Values**
0 - 6.74	0

6.75 - 13.48	1
13.49 - 20.22	2
20. 23 - 26.96	3
26.97 - 33.70	4
33.71 - 40.44	5
40.45 - 47.18	6
47.19 - 53.92	7
53.93 - 60.66	8
60.67 - 67.40	9
67.41 - 74.14	10
TWQ (°C)	**TWQ Resistance Values**
≤ 14	0
15	1
16	2
17	3
18	4
19	5
20	6
21	7
22	8
23	9
≥ 24	10
ST	**ST Resistance Values**
1 - 3	0
4	3
5	6
6	8
7	10

To calculate riverine least cost distance and riverscape resistance matrices among sample sites at different spatial scales, gdistance R package (van Etten 2012) was applied. Therefore, eight matrices were generated under different hypothesis of movement: Hypothesis of

movement by isolation of riverine distance among Dataset B for all native populations (Matrix I), Hypothesis of movement by riverscape resistance among Dataset B for all native populations (Matrix II), Hypothesis of movement by isolation of riverine distance among Dataset C for central populations (Matrix III), Hypothesis of movement by riverscape resistance among Dataset C for central populations (Matrix IV), Hypothesis of movement by isolation of riverine distance among Dataset D for Río Fuerte populations (Matrix V), Hypothesis of movement by riverscape resistance among Dataset D for Río Fuerte populations (Matrix VI), Hypothesis of movement by isolation of riverine distance among Dataset E for Río Sinaloa populations (Matrix VII) and Hypothesis of movement by riverscape resistance among Dataset E for Río Sinaloa populations (Matrix VIII); (Table 2).

Table 2. Riverine distance and riverscape resistance matrices.

Matrix	Dataset	Hypothesis of movement	Spatial scale
Matrix I	Dataset A	Isolation by riverine distance	All study area
Matrix II	Dataset A	Isolation by riverscape resistance	All study area
Matrix III	Dataset B	Isolation by riverine distance	Central populations
Matrix IV	Dataset B	Isolation by riverscape resistance	Central populations
Matrix V	Dataset C	Isolation by riverine distance	Río Fuerte
Matrix VI	Dataset C	Isolation by riverscape resistance	Río Fuerte
Matrix VII	Dataset D	Isolation by riverine distance	Río Sinaloa
Matrix VIII	Dataset D	Isolation by riverscape resistance	Río Sinaloa

References

ESRI. (2013). *ArcGIS, version 10.2 for Desktop*. ESRI, Redlands, California.

van Etten, J. (2012). *gdistance: Distances and routes on geographical grids.* R package. Available at: http://www.CRAN.R-project.org/package=gdistance.

Appendix 7. F_{ST} coefficients obtain in adegenet among sample sites of Mexican golden trout

Table 1. F_{ST} coefficients obtain in adegenet among sample sites of Mexican golden trout, aquaculture rainbow trout and lab hybrids: Arroyo del Agua in Río Fuerte (FDA), Arroyo El Manzano in Río Fuerte (FEM), Arroyo Las Cuevas in Río Fuerte (FLC), Arroyo La Quebrada in Río Fuerte (FLQ), Arroyo Las Truchas in Río Fuerte (FLT), Arroyo Momorita in Río Fuerte (FMO), Arroyo San José in Río Fuerte (FSJ), Arroyo Caleras in Río Fuerte (FCA), Arroyo La Onza in Río Fuerte (FON), Arroyo Río Verde in Río Fuerte (FVE), Arroyo Baluarte in Río Sinaloa (SBA), Arroyo El Salto in Río Sinaloa (SES), Arroyo Hondo in Río Sinaloa (SHO), Arroyo La Osera in Río Sinaloa (SLO), Arroyo Macheras in Río Sinaloa (SMA), Arroyo El Potrero in Río Sinaloa (SPO), Arroyo San Miguel in Río Sinaloa (SSM), Arroyo Cerro Solo in Río Sinaloa (SCS), Arroyo Cebollín in Río Sinaloa (SCE), Arroyo Pericos in Río Sinaloa (SPE), Arroyo El Desecho in Río Culiacán (CED), Arroyo El Río in Río Culiacán (CER), Arroyo El Río 2 in Río Culiacán (CER2), Arroyo San Juan del Negro in Río Culiacán (CSJN) and Arroyo Agua Blanca in Río Culiacán (CAB), El Barro Aquaculture Farm (AQBA), San Miguel Aquaculture Farm (AQSM),farmed Mexican golden trout (REP), and Mexican golden trout and rainbow trout lab hybrids (H). The F_{ST} coefficients among sample sites at the same basin are shown in colors: Río Fuerte in yellow, Río Sinaloa in green, Río Culiacán in red, Lab trout in orange and aquaculture trout in blue.

Fst	FDA	FEM	FLC	FLQ	FLT	FMO	FSJ	FCA	FON	FVE	SBA	SES	SHO	SLO	SMA	SPO	SSM	SCS	SCE	SPE	CED	CER	CER2	CSJN	CAB	H	REP	AQEB	AQSM
FDA	0.00	0.78	0.76	0.07	0.83	0.84	0.78	0.66	0.74	0.10	0.71	0.75	0.68	0.71	0.86	0.89	0.76	0.62	0.69	0.70	0.95	0.94	0.95	0.90	0.74	0.73	0.78	0.67	0.71
FEM		0.00	0.24	0.78	0.41	0.61	0.67	0.51	0.56	0.77	0.27	0.43	0.38	0.33	0.51	0.61	0.67	0.39	0.52	0.51	0.91	0.91	0.91	0.85	0.60	0.59	0.65	0.60	0.64
FLC			0.00	0.76	0.21	0.47	0.67	0.54	0.58	0.75	0.35	0.51	0.46	0.43	0.27	0.39	0.68	0.39	0.53	0.52	0.89	0.88	0.89	0.84	0.61	0.60	0.65	0.62	0.65
FLQ				0.00	0.81	0.81	0.78	0.67	0.74	0.07	0.71	0.76	0.69	0.72	0.84	0.87	0.77	0.63	0.70	0.70	0.93	0.93	0.93	0.89	0.74	0.73	0.78	0.69	0.73
FLT					0.00	0.52	0.73	0.59	0.66	0.80	0.48	0.63	0.53	0.56	0.13	0.25	0.72	0.43	0.59	0.58	0.94	0.94	0.94	0.88	0.67	0.66	0.72	0.63	0.67
FMO						0.00	0.74	0.59	0.67	0.80	0.55	0.66	0.55	0.60	0.64	0.75	0.72	0.39	0.57	0.55	0.96	0.96	0.96	0.89	0.67	0.66	0.74	0.61	0.64
FSJ							0.00	0.40	0.46	0.77	0.57	0.61	0.56	0.58	0.77	0.81	0.67	0.42	0.42	0.44	0.88	0.88	0.88	0.83	0.52	0.40	0.06	0.60	0.64
FCA								0.00	0.10	0.67	0.41	0.45	0.40	0.42	0.64	0.69	0.51	0.22	0.18	0.21	0.78	0.78	0.78	0.73	0.28	0.35	0.37	0.50	0.51
FON									0.00	0.73	0.43	0.50	0.41	0.44	0.73	0.79	0.50	0.20	0.17	0.21	0.87	0.87	0.87	0.79	0.31	0.35	0.42	0.48	0.50
FVE										0.00	0.70	0.75	0.68	0.71	0.83	0.86	0.76	0.63	0.69	0.69	0.92	0.92	0.92	0.88	0.73	0.72	0.77	0.68	0.72
SBA											0.00	0.13	0.18	0.06	0.56	0.64	0.56	0.32	0.40	0.41	0.86	0.86	0.86	0.80	0.51	0.47	0.54	0.51	0.54
SES												0.00	0.24	0.12	0.69	0.75	0.62	0.40	0.46	0.47	0.88	0.87	0.88	0.82	0.55	0.52	0.59	0.56	0.59
SHO													0.00	0.20	0.60	0.66	0.45	0.35	0.39	0.39	0.80	0.79	0.80	0.73	0.49	0.39	0.53	0.41	0.43
SLO														0.00	0.62	0.69	0.58	0.36	0.41	0.42	0.85	0.85	0.85	0.79	0.52	0.48	0.55	0.53	0.56
SMA															0.00	0.10	0.77	0.49	0.66	0.64	0.95	0.95	0.95	0.90	0.71	0.73	0.78	0.66	0.71
SPO																0.00	0.82	0.56	0.72	0.71	0.96	0.96	0.96	0.92	0.76	0.79	0.82	0.70	0.75
SSM																	0.00	0.47	0.51	0.51	0.80	0.78	0.79	0.68	0.59	0.28	0.63	0.04	0.05
SCS																		0.00	0.11	0.12	0.78	0.76	0.78	0.72	0.33	0.32	0.38	0.47	0.48
SCE																			0.00	0.13	0.84	0.83	0.84	0.77	0.31	0.34	0.38	0.49	0.51
SPE																				0.00	0.83	0.82	0.83	0.76	0.33	0.35	0.40	0.49	0.51
CED																					0.00	0.00	0.01	0.11	0.84	0.83	0.89	0.64	0.69
CER																					0.00	0.00	0.00	0.11	0.83	0.82	0.89	0.63	0.68
CER2																							0.00	0.11	0.83	0.83	0.89	0.64	0.69
CSJN																								0.00	0.78	0.73	0.83	0.57	0.61
CAB																									0.00	0.45	0.49	0.54	0.57
H																										0.00	0.35	0.29	0.29
REP																											0.00	0.58	0.61

AQEB																											0.00	0.04
AQSM																												0.00

Table 2. Avergae F_{ST} coefficients within and among basins, lab trout and aquaculture farms.

Fst	Río Fuerte	Río Sinaloa	Río Culiacán	Lab Trout	Aquaculture
Río Fuerte	0.60	0.56	0.83	0.59	0.62
Río Sinaloa		0.45	0.77	0.51	0.50
Río Culiacán			0.36	0.77	0.63
Lab Trout				0.35	0.44
Aquaculture					0.04

Appendix 8. Ancestry coefficients obtained in faststructure for different K values (6, 7 and 8: Figure 1)

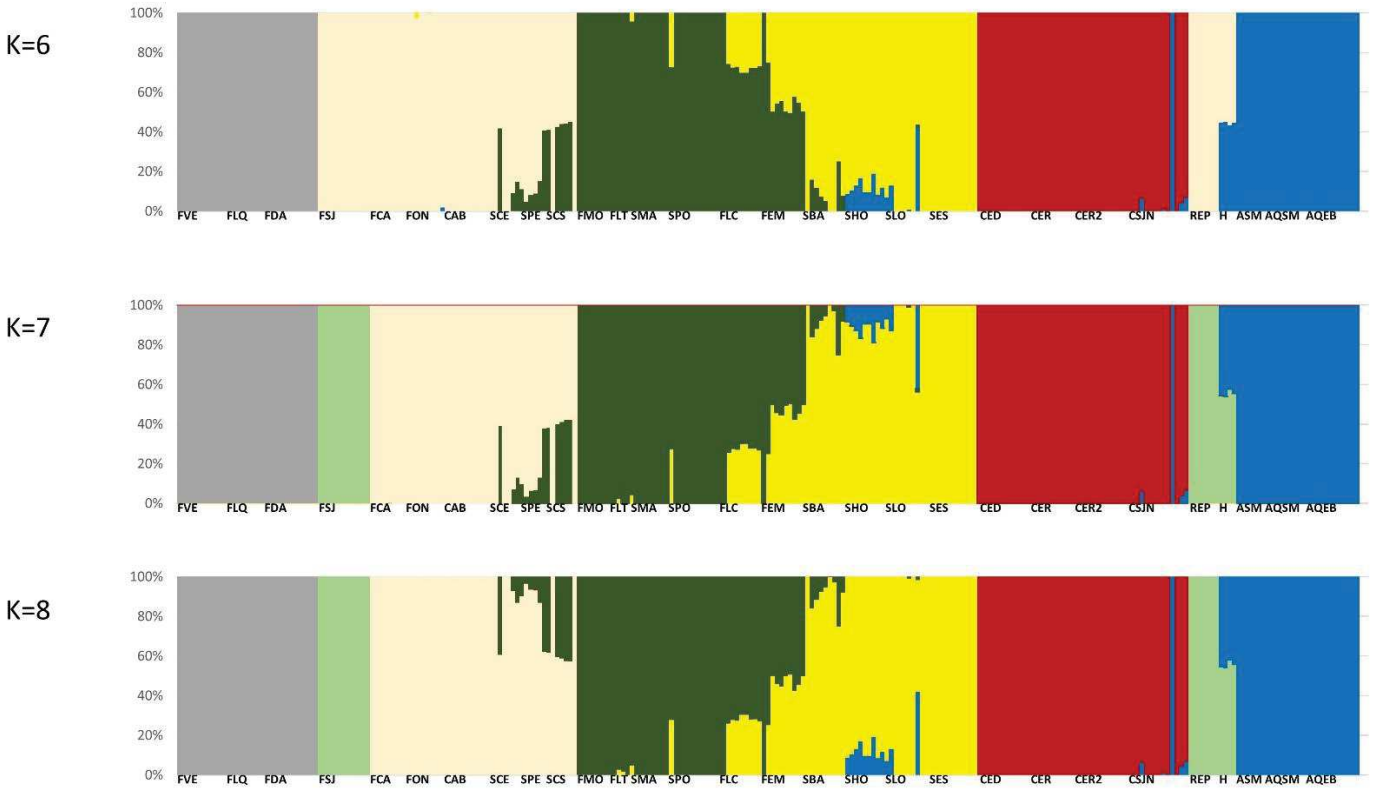

Figure 1. Ancestry coefficients obtained in faststructure for different K values (6, 7 and 8). Each color represents a different genetic cluster, the genome of each individual is represented by a vertical line and each sample site is represented by code: Arroyo del Agua in Río Fuerte (FDA), Arroyo El Manzano in Río Fuerte (FEM), Arroyo Las Cuevas in Río Fuerte (FLC), Arroyo La Quebrada in Río Fuerte (FLQ), Arroyo Las Truchas in Río Fuerte (FLT), Arroyo Momorita in Río Fuerte (FMO), Arroyo San José in Río Fuerte (FSJ), Arroyo Caleras in Río Fuerte (FCA), Arroyo La Onza in Río Fuerte (FON), Arroyo Río Verde in Río Fuerte (FVE), Arroyo Baluarte in Río Sinaloa (SBA), Arroyo El Salto in Río Sinaloa (SES), Arroyo Hondo in Río Sinaloa (SHO), Arroyo La Osera in Río Sinaloa (SLO), Arroyo Macheras in Río Sinaloa (SMA), Arroyo El Potrero in Río Sinaloa (SPO), Arroyo San Miguel in Río Sinaloa (SSM), Arroyo Cerro Solo in Río Sinaloa (SCS), Arroyo Cebollín in Río Sinaloa (SCE), Arroyo Pericos in Río Sinaloa (SPE), Arroyo El Desecho in Río Culiacán (CED), Arroyo El Río in Río Culiacán (CER), Arroyo El Río 2 in Río Culiacán (CER2), Arroyo San Juan del Negro in Río Culiacán (CSJN) and Arroyo Agua Blanca in Río Culiacán (CAB), El Barro Aquaculture Farm (AQBA), San Miguel Aquaculture Farm (AQSM), domestic Mexican golden trout (REP), and Mexican golden trout and rainbow trout lab hybrids.