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# Résumé

Les mouvements sont un aspect crucial de l'écologie et de l'évolution, car ils déterminent la dynamique des populations et des communautés. Les menaces anthropiques pesant sur ces dynamiques sont donc une préoccupation majeure pour la conservation. Dans cette thèse, j'ai étudié les mouvements de la truite commune (*Salmo trutta*) dans le contexte des rempoissonnements, c'est-à-dire la supplémentation des populations sauvages à partir de souches d'élevage locales et exogènes.

À cette fin, j'ai d'abord développé un nouvel outil génétique utile pour détecter la structure génétique chez la truite commune et l'hybridation avec des souches élevées en captivité. Cet outil a permis un grand succès de génotypage et a permis d'identifier les patrons d'isolement par la distance. Son intérêt réside dans sa facilité de mise en œuvre, et son universalité potentielle pour la génétique des populations de cette espèce dans son aire de répartition. J'ai ensuite développé de nouvelles méthodes d'assignation combinant des données génétiques et isotopiques, afin d'étudier les mouvements à l'échelle spatiale d'un bassin hydrographique, ce qui présente un intérêt pour des questions appliquées telles que la gestion. Cette approche, basée sur le machine learning, a révélé une grande précision et un fort pouvoir discriminant pour assigner des individus à leur population d'origine. J'ai aussi décrit les effets des rempoissonnements sur les patrons de diversité et de différenciation génétiques et constaté que les rempoissonnements ont pour effet d'augmenter la diversité et la différenciation, et que les patrons naturels attendus pouvaient être inversés dans le cas de cette pratique. Ensuite, l'hybridation entre souches sauvages et d'élevage affecte les patrons de dispersion, révélant que les deux souches diffèrent en termes de propension, de distances et de direction dans leurs dispersion, alors que les hybrides présentent des patrons de dispersion moins prononcés. Enfin, j'ai cherché à mieux comprendre l'influence des facteurs individuels, environnementaux et paysagers sur les mouvements des populations naturelles; ici, j'ai trouvé que certains déterminants étaient universels d'un bassin versant à l'autre, par exemple le fait que les gros individus étant plus enclins à se déplacer, ou encore que les sites directement reliés par le flux d'eau, et ceux ayant des similitudes en termes d'altitude et de type de cours d'eau échangeaient plus de migrant que les autres. D'autre part, d'autres facteurs dépendaient du contexte, par exemple, les relations entre patrons de mouvements et position dans les paysages fluviaux et la disponibilité de l'habitat varient entre les rivières.

Cette thèse a contribué à améliorer les méthodes d'étude des mouvements et à identifier les facteurs sous-jacents aux patrons de mouvements à l'échelle du bassin hydrographique. Les implications de ma thèse sont donc à la fois fondamentales et appliquées, car une meilleure compréhension des patrons de mouvement dans le contexte de perturbations humaines est cruciale pour la gestion et la conservation.

# Abstract

Movements are a crucial aspect of ecology and evolution, as they determine population and community dynamics. Threats to these dynamics because of human perturbations are therefore a major concern for conservation. In this thesis, I studied movements in the Brown trout (*Salmo trutta*) in the context of stocking, i.e. the supplementation of wild populations with captive-bred strains from both, native and exogenous origin.

For this purpose, I first developed a new genetic tool useful for detecting genetic structure in the brown trout, as well as hybridization with captive-bred strains, exhibiting high genotyping success and enabling to successfully identify patterns of isolation-by-distance. This tool was shown cost effective, and especially, should be useful for many population genetics studies on this species across its range. Then, I developed novel assignment approaches combining genetic data and stable isotopes, to study movements at the spatial scale of a river basin, which is of interest for applied matters such as management. This approach, based on machine learning, revealed high accuracy and power to discriminate and assign individuals to their population of origin. Further, I described the genetic effects of captive breeding on patterns of genetic diversity and differentiation, and found that captive-bred genotypes increased diversity and differentiation, and that expected natural patterns could be reversed in the case of higher frequency of captive-bred genotypes occurring at the level of populations. Then, I demonstrated that admixture between wild individuals and those carrying captive-bred ancestry affected dispersal patterns, that the two strains displayed different movement patterns in terms of propensity, distances, and direction, and that admixture between strains considerably reduced dispersal. Finally, I aimed at better understanding how individual, environmental and landscape related factors influence movements in natural populations; here I found that some determinants were universal across rivers, with larger individuals being more prone to movement for instance, or sites that are directly connected by the water flow, and those that are similar in terms of elevation and stream order exchanged more migrants. One the other hand, other drivers were context dependent, for instance the relations between movement patterns and position within riverscapes and habitat availability depended on the river basin considered.

This thesis contributed to improve methods for studying movements, and to identify factors underlying patterns of movements at the scale of the river basin. The implications of my thesis are thus both

fundamental and applied as a better understanding of movement patterns in the context of human perturbations is crucial for management and conservation.

# Avant-Propos

Cette thèse a été réalisée au sein de la Station d'Ecologie Théorique et Expérimentale du CNRS à Moulis. Elle a été financée par un contrat de recherche engagé par l'Agence Française pour la Biodiversité (AFB; ex ONEMA), et Électricité de France (EDF).

Cette thèse est présentée en anglais, sous la forme d'une introduction générale, de cinq chapitres (sous forme d'articles scientifiques) et d'une discussion générale.

Le chapitre IV est publié dans Conservation Genetics, le chapitre I est publié dans BMC Genomics. Les chapitres II, III et V sont en préparation pour soumission dans des revues avec comité de lecture.

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# Introduction

## CONTEXT

**"***The ability of individuals to move in space, although highly variable between species, is a general characteristic of all organisms. In fact the very persistence of species on a micro-evolutionary time-scale requires the ability to escape from natural, long-term environmental changes. At shorter time-scales, movement capacities of organisms are universally important to ecological phenomena. For this reason, the study of movement patterns deserves a key position within all disciplines of ecology*" (Ims, 1995)

Studying movements is a challenging yet crucial aspect of life history and behavior, thus of population and community ecology, evolution, but also conservation (Hobson, 1999; Wocher and Rösch, 2006). Movements are key to species persistence and coexistence at various spatial and temporal scales (Ciancio et al., 2008; Hobson and Wassenaar, 1997; Kneitel and Chase, 2004) as they are directly linked to the dynamics of populations (Bowler and Benton, 2005; Clobert, 2001; Kokko, 2006), enabling to reduce risk of extinction for instance (Hanski, 1998). In the last 4 years, I have studied movements of the Brown trout (Salmo trutta) in the context of human induced perturbations, which is the general topic of my PhD thesis. Movements have been studied widely in this iconic species, but generally concern either very local scales (a few hundreds of meters during seasonal migration or dispersal of fry for instance; Acolas et al., 2008; Bagliniere et al., 1989; Bembo et al., 1993; Cuinat and Heland, 1979; Dieterman and Hoxmeier, 2011; Gowan and Fausch, 1996; Klemetsen et al., 2003; Maisse and Bagliniere, 1990; Ovidio et al., 1998), or very large spawning or feeding movement with a majority of studies on migration between freshwater and saltwater habitats (McCarthy and Waldron, 2000). Moreover, these studies have been done mainly using direct estimates of movement (mark-recapture, PIT tagging), genetics, or otoliths isotope analysis. Thus the novelties in my works –and which constitute the main objectives of my thesis- consisted in developing non-lethal indirect methods: a new genetic tool, and novel approaches combining genetic data as well as fin tissue stable isotopes, to study movements in the context of anthropic perturbations, in particular stocking, at spatial scales which are of interest for river managers, i.e. at scales of a river basin (~ few kilometers). Before going deeper in these

objectives, I will first define what the main types of movements consist in, and specify which aspects of movement biology are concerned by my PhD works.

## MOVEMENTS: CONCEPTS AND DEFINITIONS

Movements and connectivity are very complex concepts, and their definitions are highly important to accurately study them. A movement is a spatial displacement of an individual (Ims, 1995), which takes place in three steps: departure, transience and settlement. A movement can therefore have various biological causes and consequences, and take many different forms. Although there is no real consensus, because the type of movement and its definition can highly vary depending on the questions of a study (Table 1; Koehn and Crook, 2013), animal movements can be separated into four different categories: prospecting, foraging, migration and dispersal. These different mechanisms each have different causes and consequences (Ims, 1995), and, depending on the populations, spatial/time scales and species considered, they can be very difficult to disentangle. I hereafter provide a brief definition of each of these movement types:

1) *Prospecting movements* are defined as visits made by individuals to locations where they are not currently breeding, but where they may (or not) settle in the future to reproduce (Reed et al., 1999).

2) *Foraging movements* consist in movements from home habitat to feeding habitat. These movements usually occur at relatively small spatial and temporal scales, although this is not always true (Péron and Grémillet, 2013), and are also known as trivial or daily movements (Johnson, 1969).

3) *Migrations* consist in to-and-from movement of populations (or a large part of the population) between regions where conditions are alternately favorable or unfavorable, and usually including one region in which breeding occurs (Dingle and Drake, 2007), which is therefore temporally predictable (Mehner, 2012). Migration differs from foraging and prospection because it occurs at a much greater scale, and involves movement of much longer duration than those of normal daily activities (Dingle and Drake, 2007).

4) *Dispersal* is defined as the movement of individuals between a location where they were born or bred to a new location where they breed. Technically, dispersal is defined as any movement that

has the potential to lead to gene flow (Clobert, 2001; Ronce, 2007). Dispersal differs from foraging or migration movements as it implies relocation of the natal/breeding site. Another definition of dispersal (literal definition; Begon et al., 1988) would be a movement of individuals which decreases their concentration (and increases the spatial range), for instance movement of fishes from drought refuge after the drought is over. Other definitions exist, for instance a broader one would be movements leading to redistribution within a spatially extended population (Dingle and Drake, 2007; Gatehouse and Woodrow, 1987; Taylor, 1986).

| **Movement scale** | **Description** | **Purpose** |
|---|---|---|
| Micro-scale | Short, non-sustained | Escape from predators, prey capture |
| Meso-scale | Short term but sustained, within normal home range | Feeding, avoidance of local poor conditions, daily movements, spawning |
| Macro-scale | Prolonged, long-term, large scale movements | Migrations, exploration, feeding, spawning, avoidance of larger scale poor conditions, dispersal |

*Table I1: Different scales of movement (Koehn and Crook, 2013).*

These different types of movements have been a vast field of research, in many species, and many studies have aimed at understanding both their causes, and their consequences. Here I did not focus on migratory patterns, but rather on small-scale to medium-scale (within rivers and between main sections of a river network) movements that mainly encompass exploratory movements and dispersal per se, but migrations can also be part of the movements detected here although it is difficult to be certain that they are disentangled.

## DETERMINANTS AND CONSEQUENCES OF ANIMAL MOVEMENT

Although some movements are random, and unlinked to an individual's or species' strategy, movement patterns were widely shown to have strongly been selected for at the individual level (Edelaar and Bolnick, 2012), but also at the population and species level (Shaw and Couzin, 2013). Indeed, species have evolved very particular movement patterns, which are part of their life history, and thus crucial for their persistence (Jones, 1977; Nathan et al., 2008). Movements therefore depend on a wide variety of individual and environmental features (Johnson et al., 2002).

*Foraging movements and exploration*

Food resources are often distributed heterogeneously, and the ability to efficiently exploit them is under natural selection (Barton et al., 1992; Charnov, 1976). Foraging movements thus exhibit a very wide range of strategies and features (O'Brian et al., 1990). These movements have been widely studied, and linked to individual differences in morphology (Armstrong et al., 1997; Riveros and Gronenberg, 2010), behavior (Bacon et al., 2010; Caraco, 1981; Caraco et al., 1990), sex; (Matsumoto et al., 2017), cognitive ability/memory (Rakow et al., 2010), and to environmental features such as resource distribution (Grevstad and Klepetka, 1992; Mercier and Lenoir, 1999), predation (Sweeney et al., 2013), and social interactions (Mehlhorn et al., 2015; Patrick et al., 2017, 2013).

*Migration*

Migratory movements exist at a wide range of spatial and temporal scales (Lucas et al., 2001). Most seasonal migrations have a determinant function in completing the life cycle of species (Runge et al., 2014), because habitat use differs along species' cycle, therefore, each cycle may require a certain habitat type (Aarestrup et al., 2009; Brönmark et al., 2008). Although mainly linked to reproduction (Rakowitz et al., 2008), migrations can also consist in refuge migrations, for instance against predation (Brönmark et al., 2008; Romare and Hansson, 2003; Skov et al., 2013), or adverse environmental conditions (Berthold and Helbig, 2008). Finally, feeding migrations are also observed in many species (Erman, 1981; Rikardsen et al., 2008). Forces that shape the evolution of migratory behavior in animals are thus diverse (Brodersen et al., 2008; Chapman et al., 2011; Olsson et al., 2006; Skov et al., 2013), and migration has shaped physiology and life-history of species (Brönmark et al., 2014). Migration generally concerns a fraction of individuals/populations, therefore, the causes and consequences of migration propensity have been widely studied (Chapman et al., 2011). At the individual level, migration can increase survival (Skov et al., 2013), however its cost is not always affordable and thus sometimes selected against (Plath et al., 2013). For instance, individuals exhibiting poor physiological condition may be forced to adopt a resident strategy (Brodersen et al., 2008). At the population level, intraspecific competition was shown to be a driver of avian migration patterns for instance (Lundberg, 1987). Migration was also widely shown to be environmentally driven (Pulido and Berthold, 2003; van

Noordwijk et al., 2006). Migration can strongly influence both trophic dynamics (Brönmark et al., 2014; Post et al., 2008), for instance on the persistence of plant-herbivore systems (Fryxell and Sinclair, 1988), but also in shaping population structure (Rolshausen et al., 2013; Wood and Foote, 1996).

*Dispersal*

Although random dispersal is a trait selected for species, particularly plants (Hamrick and Loveless, 1986), but also animals (Byers, 2001), dispersal has been widely studied as individual, environment and population driven (Barton et al., 2009; Clobert, 2001; Cote et al., 2010), and depending on the stage of dispersal (Figure 1). At the individual level, one can for instance increase its fitness by dispersing out of changing environments (Jackson and Sax, 2010), or to avoid competition for resource or mates (Gowaty, 1993; Negro et al., 1997). This increase in fitness has thus led evolution to shape some individuals to have improved dispersal traits (Cote et al., 2010; Lowe and McPeek, 2014), also benefiting the species. These traits are diverse, and can be phenotypical or behavioral (Clobert et al., 2009; Cote et al., 2010; Dingemanse et al., 2003). Environments are also crucial in shaping dispersal patterns such as source-sink dynamics. In river ecosystems for instance, the shape of the river network and the water flow create very particular dispersal patterns (downstream sinks due to higher connectivity and downstream water-flow directed dispersal; Paz-Vinas et al., 2015). Finally, another mechanism driving dispersal consists in demo-genetic features such as population density (McPeek and Holt, 1992), kin competition and inbreeding risk avoidance (Branch, 1975; Szulkin and Sheldon, 2008), leading to patterns such as sex biased dispersal for instance (Greenwood, 1980; Li and Kokko, 2019).

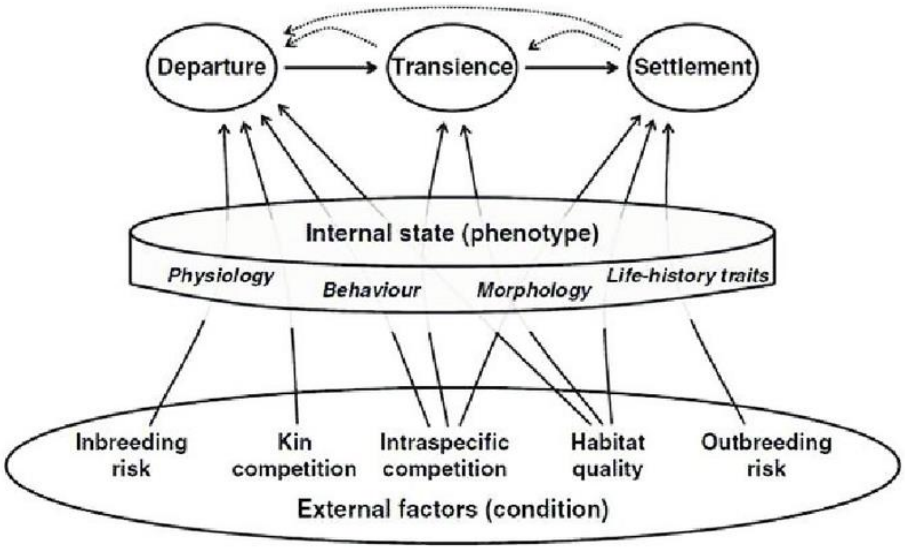


*Figure I1: Relation between the 3 stages of dispersal, phenotype and context* (Clobert et al., 2009).

The biology of dispersal is therefore a fundament for many areas of ecology and evolutionary biology (Bowler and Benton, 2005; Clobert, 2001; Kokko, 2006), as it has a direct link to population dynamics (Figure 2). Indeed, dispersal is a key process for populations as it reduces risk of extinction through counterbalancing genetic drift for instance, and many studies show that impeding dispersal through fragmentation for instance can rapidly lead to collapse of a population/species "...dispersal is probably the most important life history trait involved in both species persistence and evolution..." (Clobert, 2001)

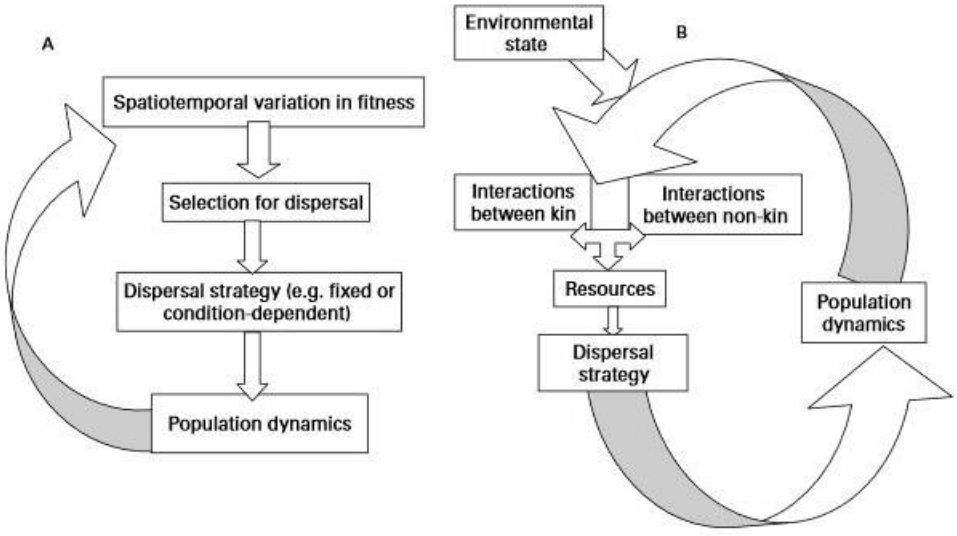


*Figure I2: Evolutionary (A) and ecological (B) loop affecting dispersal (B*owler and Benton 2004)

*Links between types of movements*

Interestingly, all types of movement are closely linked. Although dispersal movements were shown to be clearly distinguishable from foraging/exploratory movements in some cases (Zera and Denno, 1997), other studies have shown that in fact both foraging/exploratory and migratory movements can potentially lead to dispersal. For instance, (Van Dyck and Baguette, 2005) give evidence that in some cases, dispersal can be considered a by-product of routine movements. As for migration leading to dispersal, a well-known example is migratory salmonids: individuals grow at sea, and when returning to their river of origin to breed, approximately 2 to 10% accidentally spawn in another river (straying), a mechanism highly contributing to gene flow (Keefer and Caudill, 2014; Quinn, 1984; Westley et al., 2013). All types of movements are therefore crucial for animals, at all spatial and temporal scales, in particular because they interact. For instance, foraging small scale daily movements are crucial to the direct survival of individuals. At a longer term, migration is determinant for reproduction and therefore for species. Finally, dispersal was also widely shown to be a key mechanism, driving the spatial and temporal evolution of life-history traits of species, influencing populations, but also ecosystems. Thus, understanding the causes and the consequences of movements and the links between types of movements is crucial, in particular in the era of rapid human induced landscape changes. Indeed, identifying habitats used for movements, but also individual traits linked to movement can potentially be a prerequisite for accurate conservation decisions, or at least identification of conservation units at these different scales.

## CONNECTIVITY: A CRUCIAL CHARACTERISTIC OF LANDSCAPES FOR ALL TYPES OF MOVEMENTS

Because movements are so important for populations, connectivity, i.e. the extent to which a landscape allows biological fluxes (Baguette and Van Dyck, 2007; Taylor et al., 1993; Tischendorf and Fahrig, 2000; Uezu et al., 2005), has been a field of high concern in conservation, but also in fundamental questions on links between movement and landscapes (Fahrig and Merriam, 1985; Taylor et al., 1993; Ziółkowska et al., 2016), in particular because it is a key property for the persistence of spatially structured populations (Metzger and Décamps, 1997; Stevens et al., 2006). There are two facets of landscape connectivity:

- Structural connectivity, which consists in shape, size, and relative location of patches/demes, and is thus a characteristic of the landscape. It therefore depends on physical features such as density and complexity of between-patch corridors (Beier and Noss, 1998), geographical distance between patches, and inter-habitat matrix permeability, i.e. the difficulty for organisms to move between patches ("resistance") (Gascon et al., 1999; Martensen et al., 2008; Metzger and Décamps, 1997).

- Functional connectivity, which is the response of individuals to structural connectivity (movements as well as mechanisms which go with movement such as mortality etc…) as well as subsequent consequences of movement patterns on populations (observable such as gene flow, differentiation etc…). Functional connectivity depends not only on the landscape pattern, but also on the interactions between this pattern and the biological characteristics of the target species, such as their ability to move in areas of non-habitat (Castellón and Sieving, 2006; Greenberg, 1989). Therefore, some species/populations can be less affected by fragmentation and have lower extinction risk than others in the same context of structural connectivity (Sekercioglu, 2002).

Threats to connectivity are therefore a worldwide conundrum for biodiversity (Baguette et al., 2013), and fragmentation of landscapes is recognized as among the main causes of biodiversity loss (Kindlmann and Burel, 2008). Indeed, the probability of extinction of a local population is positively related to its isolation, and negatively related to its size (Baguette et al., 2013; Schnell et al., 2013): small and isolated populations are more vulnerable to genetic, demographic and environmental crashes ("extinction vortex" (Blomqvist et al., 2010; Fagan and Holmes, 2005)). Moreover, these smaller and more isolated populations have a reduced rescue potential (Brown and Kodric-Brown, 1977; Hanski, 1998). Therefore, habitat fragmentation is a direct threat for species' capacity to persist in environments concerned with this phenomenon, thus understanding how human induced modifications of landscapes affect populations through movements is crucial for their conservation (Cote et al., 2017; Fischer and Lindenmayer, 2007; Young et al., 1996).

Habitat fragmentation is now affecting ecosystems worldwide (Haddad et al., 2015). The most famously recognized facet of this problem is probably linked to destruction of tropical primary forests (Giles and Burgoyne, 2008; Williams, 2006), however, even if more insidious, all ecosystems are to a certain degree concerned by habitat fragmentation. In rivers, the construction of dams and weirs is a more recent concern, and its magnitude is also impressive. For instance,

only in France, the estimated number of riverine obstacles is of more than 80000 (ROE: référentiel des obstacles à l'écoulement), and many do not take into consideration connectivity, as they were built way before this was a concern (Figure 3). However, thanks to numerous studies which have proven the importance of connectivity, these issues are already taken into account in landscape management. For example, in 1995, the European Council signed the "Pan European strategy for biological and landscape diversity". In France, this led to the "Trame verte et bleue" (Alphandéry et al., 2012), instored in 2007, with the goal of integrating ecosystem functionality in management projects, notably through preserving "ecological continuity of landscapes" which comprises "biodiversity reservoirs" and "ecological corridors". Information on movements is therefore constantly needed to better take into account specificities of species requirements in terms of connectivity.

In rivers, connectivity is very specific: movements are constrained to a one-dimension pathway in a longitudinal branched network (dendritic network, (Campbell Grant et al., 2007). Moreover, the river flow is unidirectional, directed downstream (Hänfling and Weetman, 2006; Pollux et al., 2009). Therefore, the metapopulation dynamics in rivers has been a wild field of research, showing that upstream populations often serve as sources and downstream populations serve as sinks (Kawecki and Holt, 2002; Morrissey and de Kerckhove, 2009)**.** Additionally, upper demes are generally smaller, and have higher slope, thus exhibit more extinction probability, and less colonization (Gotelli and Taylor, 1999). This is also reinforced by the direction of the flow, which directs gene flow downstream (Kawecki and Holt, 2002). Connectivity between demes in rivers has widely been shown to affect movements, and therefore metapopulation dynamics. For instance, in upstream reaches, populations are generally more differentiated and less genetically diverse than in lower more connected demes, in which confluence patches are a genetic mix of several headwater patches, and hence reservoirs for genetic diversity (Carrara et al., 2014; Paz-Vinas et al., 2015). Thus, headwaters are the sources of diversity in a basin and therefore crucial in terms of conservation (Campbell Grant et al., 2007). Insuring connectivity in such complex systems is thus crucial for preserving their particular dynamics, and a prerequisite for this is to thoroughly understand how they can be affected by natural and/or anthropic perturbations to connectivity (Blanchet et al., 2010; Dudgeon et al., 2006). Indeed, estimates of connectivity in rivers have already been shown as highly valuable tools for conservation, particularly because they

have successfully enabled to prioritize effort on specific obstacles, or on restoring natural corridors essential to metapopulation dynamics (Faulks et al., 2011; Prunier et al., 2018; Raeymaekers et al., 2008).

*Figure I3: ROE map of obstacles in river systems of France. Each dot represents an obstacle, color gradient represents the height (towards red = higher obstacle)*

## DETECTING/MEASURING MOVEMENTS

Because of their importance for species/individuals/populations, measuring movements has therefore been the aim of many studies (Holden, 2006; Nathan et al., 2008; Postlethwaite et al., 2013; Schick et al., 2008), and is quite a challenging task in the wild. Various data types and methods have been applied to this goal. First, direct methods such as telemetry, PIT tagging, or mark recapture (Griesser et al., 2014) which are efficient and straightforward, but present limitations in terms of spatial and temporal scales, number and size of tracked individuals, and feasibility in general (Cooke et al., 2013; Koenig et al., 1996). Then indirect methods were used, mainly using molecular approaches. These methods appear very interesting, in particular in this context for its applicable spatial scale which is coherent with users and managers of these ecosystems. With the technological advancements in genomics (NGS for instance, but even before

with microsatellites) and mass spectrometry for large sample sizes, they are very useful tools in the study of populations and in particular movements. However, they are more efficient when inter-population differences are high, and in some cases only utilizable under this condition (Ward et al., 1994).

*Genetic markers: a key approach for large scale studies*

Genetics were the first data type to be applied to studying movements indirectly, and still remain the most used for this purpose. Indeed, population genetics offer a replicable and evolutionary time-scale data resource which is of very high interest, providing insight into both the ecological and the evolutionary dynamics of wild populations (Elliott, 1994; Pelletier et al., 2009). Wright (1931) first started using genetic differentiation as a proxy for the proportion of individuals moving between populations, through effective sizes (Nem). These methods were shown to have certain limitations in terms of accuracy (Whitlock and McCauley, 1999). Moreover, it requires moving individuals to have reproduced in their destination site, and only enables to investigate gene flow between demes after several generations; therefore real-time movements cannot be detected. Finally, they require a variety of demo-genetic conditions, such as Hardy-Weinberg equilibrium, neutrality of markers, absence of linkage disequilibrium etc…But these population-based methods, along with studies assessing its limitations, opened the door for the development of many other approaches, and in particular, to individual-based approaches.

The main individual-based approaches to detect between population movements are assignment methods which were developed and applied to case studies with great success (Bekkevold et al., 2015; Berry et al., 2004; Bradbury et al., 2015; Karlsson et al., 2011; Manel et al., 2005; Templin et al., 2011). The overall goal of assignment is to identify immigrants/residents (Pritchard et al., 2000; Rannala and Mountain, 1997; Wilson and Rannala, 2003), and individuals' natal populations (Cornuet et al., 1999). These methods are highly interesting because they can be used without the previously required assumptions, and are therefore much more straightforward (Berry et al., 2004). The first assignment test approach was developed by Paetkau et al. (1995), to study movements of polar bears, under the null hypothesis that an individual was born in the population in which it was sampled. This approach consists in calculating the expected frequency of each individual's genotype in each of the sampled populations and subsequent assignment of each

individual to the population where its expected genotype frequency is highest, and is thus the product of expected genotype frequency at each locus, based on the observed distributions of alleles. Subsequently, an individual is assigned to the population for which it has the highest likelihood.

Then, a major challenge is to statistically distinguish real immigrants from Type I error, i.e. residents which by chance have a genotype more expected in a population different than that in which they were sampled. For this, different methods exist:

(i) Methods based on likelihood ratios, for instance comparing the likelihood of the individual genotype within the population where it was sampled versus the highest likelihood value among all available population samples including the population where the individual was sampled (see Paetkau et al. (2004) for other likelihood based ratios). Such likelihood ratios are appropriate when all source populations for immigrants are thought to be sampled.

(ii) Partially Bayesian assignment test (Rannala and Mountain, 1997) that use a Bayesian approach to estimate population allele frequencies and a frequentist approach to compute the statistical significance of individual assignments, which were shown to provide slightly higher assignment accuracy (Cornuet et al., 1999). This test was also extended to include a statistical significance test based on Monte Carlo simulations, that does not require the assumption that the true population of origin has been sampled (i.e., an exclusion test).

(iii) A fully Bayesian method (Pritchard et al., 2000) which provides a coherent framework for incorporating the inherent uncertainty of parameter estimation into the inference procedure. Using this method, the posterior probability value can be interpreted directly as the probability of origin of each individual from each population sampled (assuming the true population of origin has been sampled).

Finally, methods based on discriminant analysis (which defines a model in which genetic variation is partitioned into a between-group and a within-group component, and yields synthetic variables which maximize the first while minimizing the second) were also shown very useful for individual assignment. In particular, Discriminant Analysis on Principal Components (*DAPC* (Jombart et al., 2010)), which relies on data transformation using principal component analysis prior to a

discriminant analysis. This enables to only perform the discriminant analysis on data that is perfectly uncorrelated. Another asset of DAPC is that it allows assessing the contributions of alleles to the structures identified by DAPC, thus making possible to identify regions of the genome driving genetic divergence among groups for instance.

A strong advantage of these methods compared to estimations of gene flow from differentiation is that they allow investigating direct time movements: to be detected as a migrant, an individual does not necessarily have to have reproduced in its new site. This is of high interest because it enables to detect other types of movements such as migration, or even foraging. This is also highly informative because even if they do not (yet) participate to gene flow, these movements can be very important in terms of ecological and populational dynamics. Moreover, these methods are individual-based, therefore, individual data (phenotype, behavior etc…) as well as environmental data can also be used for instance when testing mechanisms linked to movement.

*Isotopic markers: an alternative approach for measuring movement*

The development of assignment methods also enabled a crucial advancement, because they can also use non-genetic data. Although more recently developed than for genetics, stable isotopes have been used for a relatively long time for studying movements of animals.

Stable isotopes are indeed indirect markers of high interest for studying animal movements (Durbec et al., 2010; Hobson and Wassenaar, 2008; Rubenstein and Hobson, 2004). Stable isotopes are non-radioactive forms (variants) of elements, which differ in nuclear mass. On earth, about two-thirds of elements occur naturally in multiple stable isotopes. These isotopes are present in different proportions measured as isotopic differences relative to international standards and reported as ratios in delta (δ) units as parts per thousand (‰). Variation in these proportions depends on various geochemical, and biochemical processes, at variable scales, and this is what can be used to detect movements. The use of this technique to trace the origin or migration of wild life is based on the fact that stable isotope signatures in animal tissues reflect those of local food webs or environments either directly, or indirectly through enrichment or other metabolic factors (Peterson and Fry, 1987): isotopic signatures of food webs or environments can vary spatially and are passed on to consumers feeding in those food webs/individuals living in those environments

(DeNiro and Epstein, 1978). Animals that move between isotopically distinct food webs or environments can retain information of previous location for periods that depend on the elemental turnover rate of the tissue considered (Hobson and Clark, 1992; Tieszen, 1991). This can be used to detect movement provided the spatial and temporal variability in baseline isotopic values are smaller than those between geographic areas (Rubenstein and Hobson, 2004).

There are two main categories of tissues, on which the turnover rates depend: (i) metabolically inert tissues such as bones or keratinous tissues (hair, feathers, or nails for instance) which maintain an isotopic record reflecting the location where the tissue was synthesized (Schell et al., 1989; Thompson and Furness, 1995), and (ii) metabolically active tissues in which the dietary or source signature obtained will be a temporal integration ranging from a few days in the case of liver or blood plasma, to several weeks in the case of muscle or whole blood ("turnover time"; (Hobson and Clark, 1992). In addition to a wide variety of usable tissues, different elements and their different stable isotopes can be used for many different purposes. For the purpose of determining the origin of animal, they first rely on the distinction between environmental or basal source signatures. Therefore having a variable baseline across sites is a crucial prerequisite for the use of isotopes to study movement. Two main types of elements can be used for this purpose (Rubenstein and Hobson, 2004): (i) heavy isotopes such as Strontium (δ86Sr) or lead (δ206,207,208Pb), whose abundances tend to be influenced by geological and biogeochemical processes, and thus vary naturally in soils and bedrock and in some cases as a result of anthropogenic inputs (Durante et al., 2016; Jiang et al., 2001), and (ii) light isotopes whose abundance tend to be influenced by both biological and biogeochemical processes (Hayes, 2001; Kaplan, 1975).

Isotopes have been used successfully in a wide variety of taxa and ecosystems for the purpose of investigating movement. In particular, δ87Sr, δ13C and δ15N values in both metabolically active and inert tissues have been widely used to assign individuals to different foraging locations for a wide variety of taxa. In fish for instance, isotopic ratios of Strontium (δ87Sr) in otoliths which show annual growth rings that directly reflect isotope ratios of the surrounding water without fractionation, and can be used to classify individuals to different habitat along their life (e.g. movements from freshwater to sea, natal stream habitats and the movements of fish from stocked or locations (Kennedy et al., 2002; Martin et al., 2013; Walther and Thorrold, 2006)). Other

examples are numerous, in birds for instance, where isotopes enable to differentiate feeding and breeding locations (Bearhop et al., 2003; Cherel et al., 2006), but also in mammals (Crawford et al., 2008; Kelly, 2000; Newsome et al., 2010).

In my PhD, I used stable isotopes of Carbon and Nitrogen in a metabolic active tissue (fin tissues), which, unlike otoliths, do not require destructive sampling. The turnover rate in these tissues is around a few weeks (3 to 5, Grey, 2001). Stable carbon (δ13C) and nitrogen (δ15N) isotope values are influenced directly by biochemical processes during fixation in plants (Chikaraishi et al., 2004), and particularly during photosynthesis and denitrification processes (Craine et al., 2015). In terms of fractionation along the trophic chain, δ13C values in animal tissues accurately reflect those of their diet, i.e. a consumer will have the same signature as the prey (although there can be enrichment up the trophic chain in some cases, but relatively low 0 to 2‰; (Bocherens and Drucker, 2003)). Therefore, this isotope ratio can give good information on the type of food resource of a consumer. On the other hand, δ15N values in animal tissues show considerable enrichment up the trophic chain (3 to 5‰; (Bocherens and Drucker, 2003)), and can moreover be affected by water and nutritional stress. They thus reflect the trophic position of an organism. Therefore, if individuals forage and assimilate food at a particular location and assume isotopic equilibrium with that diet, then movement patterns between locations with isotopically distinct basal resources can be inferred (Fry et al., 2003), in consideration of turnover time: more concretely, Carbon and Nitrogen isotopes can enable to detect an individual's displacement if: (i) the individual stayed long enough (turnover time) in the location it left, (ii) the individual was sampled recently after its arrival (< turnover time) to its destination location (iii) the isotopic signatures of the origin and destination locations (as well as the outcome of diet on the individual's tissue signature depending on the trophic conditions) are different. In salmonids, this has been used successfully by (Rasmussen et al., 2009) for instance, using Carbon isotopes along a signature gradient in riverscapes: juvenile Atlantic salmon (Salmo salar) exhibited δ13C signatures different from those of their invertebrate prey at their sampling location, suggesting movements of ~20 km.

More generally, stable isotopes have been used to describe spatial patterns of movement at a variety of scales (Hobson, 1999; Hobson and Wassenaar, 2008; Rubenstein and Hobson, 2004). However, the majority of these studies concern large spatial scales and relatively few could be

considered at the fine spatial scale (i.e. a few kilometers; Cunjak et al., 2005; Haas et al., 2009; Litvin and Weinstein, 2004). Moreover, they generally rely on very strong differences between sites which are also linked to longer distances, therefore explain why these studies often concern such large spatial scales. For example, δ15N values have been used to study movement between human imprinted watersheds (high δ15N) and more natural ones (low δ15N: (Cabana and Rasmussen, 1996), which contrast highly in their isotopic signature. Similarly, Harrod et al. (2005) also used a salinity gradient to assess site fidelity in eels (*Anguilla Anguilla*) with δ13C and δ15N. Within a catchment, isotopic differences may naturally exist between the main river channel and its tributaries (Douglas et al., 2002), or within a river between reaches (Hershey et al., 1993; Rasmussen et al., 2009), however, to our knowledge, no studies use stable isotopes of carbon and nitrogen to study movements within these types of network at fine scales (of a few tens of kilometers), and in which between site isotopic differences are certainly lower than in the examples cited previously.

*Toward a combined approach*

As both genetics and stable isotopes are very valuable tools for detecting individual movements, using both data types (as well as others potentially) appears as a promising way of improving detections of movements. Indeed, both data types can capture movements which concern different time scales, and differences in "uniqueness" of a site / discrimination between sites can vary between these markers.

Therefore, according to scales considered, combining these tools has the potential for great insight into movements compared to when only considering one marker. Combining genetics and stable isotopes has already been done to study movement. For instance in fish, a good example is in Perrier et al. (2013), where they determine that the progeny of hatchery-born juveniles of Atlantic salmon released into the wild, which have pure hatchery pedigrees can be determined as river-born with otoliths isotope analysis, whereas they would be mistaken for hatchery-raised if only genetics had been considered. In particular, in some cases the spatial discriminant power of a given marker can be low, notably when the resolution of spatially distinct biological units across fine spatial scales can be difficult as weak genetic divergence may limit the accuracy of assignment tests (Larson et al., 2014). Therefore, in these cases, combining datasets can potentially be a highly

valuable input across both large and small geographic scales. Some statistical tools developed at first for genetics such as DAPC (Jombart, 2008; Jombart et al., 2010) can allow using different types of data to investigate movement, however without easily allowing integration of genetic and non-genetic data or allowing for the separation of PCs between data types for instance. One of the latest developments in assignment, which enables to use different data types in combination is supervised machine learning (Kotsiantis, 2007), which has been raising in terms of interest for assignment purposes, particularly through an increasing popularity for Random Forests (Chen et al., 2018; Cutler et al., 2007)**.**

The principle of random forest is very clearly explained by Touw et al. (2013). Briefly, random forests are "bagged decision tree models" (Breiman, 2001). A decision tree splits data into smaller data groups based on features of the data until attaining a small enough set that only has data points under one label. In a decision tree model, these splits are chosen according to a purity measure (residual sum of squares for regression, Gini index or entropy for classification). Bagging (or bootstrap aggregating) signifies that a number of decision trees are trained on bootstrapped training sets. The final predicted value is the average value of all decision trees, or in other words, the "majority vote". Random forest is an improvement of bootstrap aggregating as it decorrelates the trees by splitting on a random subset of features: at each split of the tree, the model considers only a small subset of features rather than all of the features of the model. Therefore, if some predictors are very strong, trees would be highly correlated, thus random forest enables to go beyond this limitation. Therefore, predictors can also be ranked by importance based on the change in classification error affected by the presence or absence of a predictor in a subset, while considering various combinations of predictor subsets. For the purpose of assigning an individual to populations, the use of the implemented classification algorithm, where an individual is assigned to a class (e.g., population) is highly interesting. Random forests are highly promising because instead of starting with a data model, they use an algorithm to learn the relationship between the response and its predictors (Hastie et al., 2009).

Random forest is a very advantageous method. Indeed, it exhibits high versatility, handling with ease either regression or classification, can handle both binary, categorical and numerical features, and requires no pre-processing such as scaling or transforming the data. As it functions with subsets of data, random forest is very appropriate for high dimensionality, with impressive

prediction speed. Statistically, random forest is a very robust procedure, very efficient at handling outliers and non-linear data. By averaging trees, variance is also averaged, therefore, random forest exhibit very low bias. One of most interesting assets of random forest is that it handles unbalanced data very well, by balancing error according to population size. In empirical datasets, this is highly interesting because sampling can often be a difficult task, for instance during field sessions for my PhD, although I aimed at sampling the same number of individuals at each location, it was in some cases impossible due to sampling conditions or fish density. Another interesting use of random forests is identification of informative SNPs (Sylvester et al., 2018), or relative contributions of groups of variables (e.g. genetics vs. isotopes) to explaining the response variable. However, there are some limitations which must be accounted for: first, random forest models are relatively intricate, and thus sometimes difficultly interpretable. Another main drawback is that they can tend to over fit, therefore, choosing the right parameters can be tricky because of the intricacy. Finally, in the case of very large data sets, the size of the trees can be quite demanding in terms of computational power.

Overall, random forests appear highly suitable for assignment in the aim of studying movements, with high sample sizes and high numbers of markers. They have already been used successfully for assignment and determining population structure, and although it appears intricate in terms of use, this tool is very promising (Guinand, 2002; Ning and Beiko, 2015; Schrider and Kern, 2018; Sheehan and Song, 2016; Sylvester et al., 2018)**.** Combining different data types can for instance enable to answer questions about movements which concern different spatial and time scales than genetics alone. For instance, when using tissue isotopes, for which turnover time is short (a few weeks;  Busst and Britton, 2018; Franssen et al., 2017), movements will not be accounted for in the same way than for markers which take several generations to change at the population level (genetics). Therefore, these markers may also better reflect real time changes in movement during a recent environmental change. Indeed, there is a recognized time lag between an event causing a change in genetic structure, and the actual change in structure (Landguth et al., 2010), which can be misleading when investigating a vicariance object for instance (Cayuela et al., 2018; Prunier et al., 2014). Assignment methods, using other markers such as isotopes or morphometrics are thus a promising tool for inferring different types of movements (Chen et al., 2018; Kelly et al., 2005;

Rundel et al., 2013), and investigating how these markers and their combinations can improve assignment tests was one of the aims of this thesis.

## THE BROWN TROUT, A FASCINATING SPECIES TO STUDY MOVEMENTS USING MOLECULAR MARKERS

In river systems, movement is a very important and specific question (Fourcade et al., 2013; Paz-Vinas et al., 2013). For instance, the European Water Framework Directive (DCE, 2000) defines the objectives of the European Union to improve ecological status of water bodies. The free circulation of aquatic organisms and their ability to access to important zones for spawning, growth, feeding and shelter was reminded to be a key element of a good ecological status. Habitat connectivity was also law-enforced for natural transport of sediment. The french water legislation, the LEMA (loi sur l'eau et les milieux aquatiques Art. L214-17, 2006) even protects some rivers from new obstacle constructions and some existing ones must be adapted to fish and/or sediment circulation. As connectivity is a great concern in these environments, and laws are being written to protect it, it appears essential to deepen knowledge on this mechanism in order to optimize its enforcement. Fish have in fact been the main trigger for enforcing these new laws on riverine connectivity. Indeed, the most obvious species concerned are diadromous migratory fish. Salmon for instance, need to circulate between rivers and sea to complete their life cycle. Therefore, fragmentation has a direct effect on these species. However, concern was also raised for resident (or partially migrating) organisms, as the effects of fragmentation are now better understood.

Among the numerous species inhabiting rivers, the brown trout (*Salmo trutta*) is one of the most widespread freshwater fish species in Eurasia, and it has been widely introduced in both the southern and northern hemispheres (Lobón-Cervía and Sanz, 2018; Baglinière and Maisse, 1999; Rahel, 2007). As all Salmonidae, it is a scientifically interesting species because of its diversity in terms of ecology, life history strategies and habitat use (Rahel and Nibbelink, 1999; Klemetsen et al., 2003). The brown trout was shown to have evolved mainly because of the multiple Pleistocene glaciations (isolation by glaciers and subsequent dispersal and secondary contact between lineages (Sanz et al., 2000)**)**: during the last 1.7 million years, there were 17 episodes of glaciation-deglaciation, with two periods of freshwater fish isolation in each glacial cycle. This gave many opportunities for geographical isolation and divergent evolution (Hamilton et al., 1989), because

glaciation cycles affected climate, habitats and the potential for dispersal, translating into marked patterns of genetic diversity and structure. Seven main mitochondrial DNA lineages were shaped (although more may be to be discovered): Atlantic (AT), Mediterranean (ME), Danubian (DA), Adriatic (AD), Marmoratus (MA), Duero (DU) in Europe, Tigris (TI) in Turkey (Bernatchez, 2001; Susnik et al., 2005; Vera et al., 2010), and Dades in Morocco (Snoj et al., 2011) and have remained relatively allopatric since then for both biological reasons and physical isolation (Figure 4).

However, some sub-lineages have been identified throughout Europe, making the situation far more complex. For example, Northern Africa harbors one AT matriarchal lineages (Tougard et al., 2018) that diverged significantly from the main AT lineage identified by Bernatchez (2001). Among these lineages, there is also high divergence. For instance among the Atlantic group, several sub-lineages were discovered (Cortey and Garcia-Marin, 2002; Krieg and Guyomard, 1985; Vera et al., 2015). Similarly, the Western Mediterranean regions host divergent sub-lineages of the AD and ME evolutionary lineage between the Iberian Peninsula (Cortey et al., 2004), and Italy (Splendiani et al., 2016). Finally, island sub-lineages have also been described as divergent from the continental populations, for instance the Corsican populations (Berrebi et al., 2019).

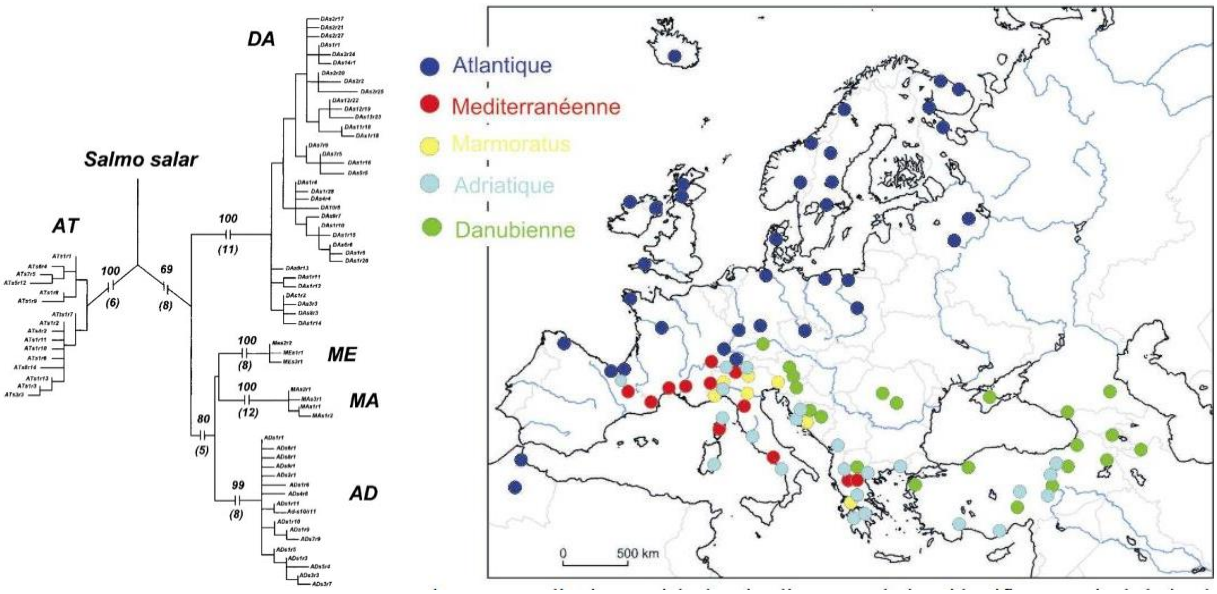


*Figure I4: phylogeny and range of the 5 main brown trout lineages*

At more local scales, the brown trout exhibits very high levels of variability in terms of genetic diversity and population structure, but also in terms of ecology (Swatdipong et al., 2010; Vøllestad et al., 2012; Berrebi, 2015). This species thus appears as the perfect model to study dispersal in a human affected landscape. Indeed, inhabiting higher river stretches, among those most concerned by fragmentation, this species is often the one for which connectivity efforts must be made, in particular because of its mobility (Gouraud et al. 2008) and its high association to human interests (Butler et al., 2009; Ferguson, 1989; Mills, 1989).

## STOCKING PRACTICES IN EUROPE, AND EFFECT ON MOVEMENT INFERENCE

A strong bias to the study of movements in natural systems is human-induced displacements or introductions in these systems. Because the brown trout is strongly associated to human interests, wild brown trout populations are widely managed and this species has been domesticated for more than a century, and spread worldwide (Laikre et al., 1999). Hatchery strains have been largely used to sustain wild populations worldwide (Elliott, 1994; Berrebi et al., 2000; Bohling, 2016), mainly from the Atlantic strain (majority of Danish origin; Laikre et al., 1999), even in rivers naturally inhabited by Mediterranean strains (Krieg and Guyomard, 1985). Therefore, these massive introductions in river systems make the study of movement, especially using genetics, very difficult. Thus, identifying introduced vs. natural strains is an indispensable step, and measuring to what extent the natural spatial patterns are affected by this practice is a prerequisite for understanding natural populations processes.

This is a very difficult task, indeed, although stocking started with mainly Atlantic trout, (Hansen, 2002; Araki and Schmid, 2010; Christie et al., 2012; Leitwein et al., 2016), its negative effects (Largiader and Scholl, 1996) led managers to change stocking practices (Arlinghaus and Mehner, 2005; Laikre et al., 1999) either by stopping it, or by using strains closer to the ones naturally found in the rivers stocked (Caudron et al., 2006). There has thus been such a long period of stocking, with many strains from different origins that they are now part of the natural dynamics of populations (Saint-Pé et al., 2018). Captive-breeding and the selection pressures associated to it generally make these strains very different from wild ones (Blanchet et al., 2008; Christie et al., 2016; Frankham et al., 1986), thus enabling to distinguish wild from non-wild individuals.

However, tracking the stocking histories is extremely complex, and some populations used to stock are no longer available for sample, therefore, information can be lacking in the case of multiple hatchery stocks.

## MOVEMENT PATTERNS IN BROWN TROUT POPULATIONS

In a river system, all fish are not mobile, and movements are highly variable among individuals, populations and depend on the species considered (Radinger and Wolter, 2014; Skalski and Gilliam, 2000). Moreover in river systems, the characteristics of the network as well as hydrological features (slope, flow, speed of flow, obstacles etc…) shape very particular patterns of movement (Carrara et al., 2014; Hitt and Angermeier, 2008; Labonne et al., 2008; Paz-Vinas et al., 2015). For instance, upstream reaches, which are more isolated, and smaller tend to have less immigrants than more connected ones, with strong genetic implications such as strong isolation by distance (IBD, i.e. pairwise genetic differentiation increase with riparian distance between populations) and downstream increase in genetic diversity (Paz-Vinas and Blanchet, 2015).

The brown trout exhibits very complex movement patterns which strongly depend on spatial, and time contexts (Baglinière, 1999; Elliott, 1994; Jonsson et al., 1991). Aquatic ecosystems inhabited by brown trout present a continuous gradient of physical conditions (river continuum concept; Vannote et al., 1980), brown trout can thus exhibit a continuum in time and space of life history tactics to optimize individual fitness and population persistence (Cucherousset et al., 2005). Three main ecological forms of brown trout have however been described (Hindar et al., 1991; Klemetsen et al., 2003), and although they are genetically undistinguishable, the strategies were shown to be heritable (Ferguson, 2007): resident river trout, sea trout (which migrate to sea for growth), and lake trout (which migrate to lakes for growth), all forms spawning in rivers. This thesis only concerns the first of these forms, as the rivers studied do not have access to the sea or to lakes. Cucherousset et al. (2005) showed that depending on a combination of individual (age, growth) and environmental factors, movement patterns are highly variable even at a very small spatial scale, and can take all possible forms. Investigating movement patterns in this species at a broader scale is thus a challenge and disentangling the different types of movement is very difficult.

*Life cycle of the brown trout*

In the brown trout, sexual maturity is attained around 2 years old (Maisse and Bagliniere, 1990). Reproduction generally occurs from September to January (Gouraud 1999) after a spawning migration, generally upstream towards tributaries by adults (Elliott, 1994; Höjesjö et al., 2007; Whoriskey, 2005). The number of eggs depends on the size of the female, for instance a 500g will ley around 800 eggs. Then, depending on conditions, between 4 and 80% of these eggs will hatch, generally around February, after a duration depending on water temperature: 60 days at 7.8°C, and 97 days at 4.7°C. Then the alevins live in the gravel and feed off the remaining yolk for two to four weeks also depending on temperature. Once the yolk has been consumed, the alevins become fry: they emerge from the gravel and start feeding on small invertebrates in slow flowing shallow water (1 – 40cm). After their first year ("parr" stage), and as they grow, they chose deeper and faster water, gradually dispersing downstream (Bagliniere et al., 1989; Northcote, 1992; Solomon and Templeton, 1976; Olsen and Vollestad, 2005; Schager et al., 2007), although rare upstream movements of juvenile trout have been observed on very small distances (Heland, 1980; Lucas et al., 2001). Mortality rates at these vulnerable young stages are high. In terms of population dynamics, this cycle therefore generally translates in tributaries acting as sources (reproduction of adults, and departure for juveniles), while mainstreams act as nurseries and feeding/growth zones (Figure 3), although this simplified model is very context dependent (Acolas et al., 2008; Bagliniere et al., 1989; Bembo et al., 1993; Cuinat and Heland, 1979; Dieterman and Hoxmeier, 2011; Gowan and Fausch, 1996; Klemetsen et al., 2003; Maisse and Bagliniere, 1990; Ovidio et al., 1998).

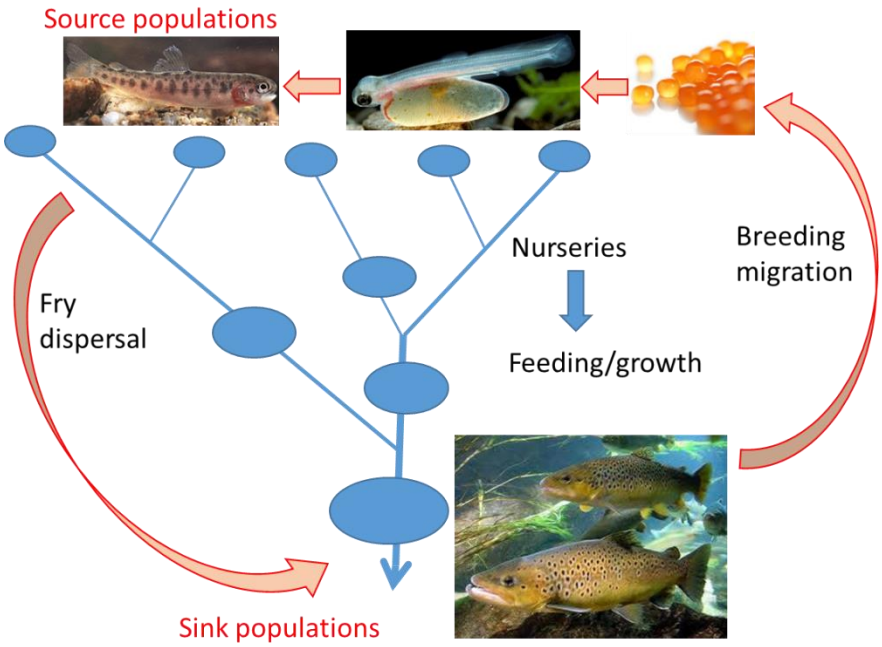


*Figure I5: Simplified life cycle of resident Brown trout. Adults living in lower stretches of the mainstream spawn in tributaries after an upstream migration. After the hatch, the juveniles leave the nursery areas and start dispersing downstream.*

*Movement determinants in the brown trout*

Movement in this species is highly variable, and appears very context dependent as some studies support that movements are very limited (Bagliniere et al., 1989; Harcup et al., 1984; Knouft and Spotila, 2002; Rodríguez, 2002) whereas others show high mobility (Gowan and Fausch, 1996; Höjesjö et al., 2007). The types of movements can also exhibit high variability, for instance foraging movements generally concern small areas (~15 $m^2$**;** Bachman, 1984) although they can also take place on very long distances (>30 Km; Clapp et al., 1990), while dispersal of young of the year can extend on several kilometers although it generally occurs on a few hundred meters (Aparicio et al., 2018; Knouft and Spotila, 2002; Vøllestad et al., 2012). Spawning migrations are also variable, and depend on the context, but generally concern a few hundred meters to a few kilometers (Burrell et al., 2000). Finally, some extreme movement events (more than 120 Km) of which the cause was not identified have been reported (Linløkken, 1993). Determinants of movement in the brown trout are complex, and strongly interact with the developmental stage, as the different spatial uses in a river basin depend on these stages (Roussel and Bardonnet, 2002;

Acolas et al., 2008; Cucherousset et al., 2005; Forseth et al., 1999; Greenberg and Giller, 2001; Heggenes and Traaen, 1988; Olsen and Vollestad, 2005; Vollestad et al., 2002) in which tributaries act as nurseries for juveniles, while mainstreams mostly host larger adults (Bembo et al., 1993; Kelly-Quinn and Bracken, 1989; Maisse and Bagliniere, 1990).

From the first stage following emergence (fry), brown trout already become territorial and a hierarchy is put in place, through competition for habitat: fry emerging later, or not able to rapidly find suitable habitat were shown to drift farther downstream for instance (Cuinat, 1971; Heland, 1980). Later in the life cycle, dominance relationships are also shaped by aggressive behavior and individual size, as individuals defend a well-defined home range forcing subordinates fish to move (Elliott, 1994; Höjesjö et al., 2007). Larger and more dominant fish also tend to have larger home ranges than smaller and subordinate ones, and feed during the most beneficial times of the day (Alanärä et al., 2001). These relations therefore occur among the different size/age classes in the population (Skalski and Gilliam, 2000) and also between males and females (Hutchings and Gerber, 2002; Pirhonen and Forsman, 1998). Metabolism is also very determinant at all stages, for instance it was shown individuals with a faster metabolism, and therefore faster growing, emigrate earlier downstream at the fry and parr stages (Cucherousset et al., 2005; Forseth et al., 1999). Moreover, in adults large and faster growing fish move more, which is congruent with the fact they can more easily afford the costs of movement (Bonte et al., 2012). However, metabolism is closely linked to growth and thus size, and therefore, Heland (1980) found that fry with lower growth rate tended to move more, as smaller individuals. Once again, movement patterns in this species are highly variable and context-dependent, and different determinants of movements can strongly interact.

Environmental conditions have been shown to affect all components of movements in trout. In foraging movements for instance, lateral within “home range” movements depend mostly on nychtemeral patterns (Roussel and Bardonnet, 2002; Heland 1980). Environmental conditions appear to be relatively context dependent, for instance, habitat characteristics such as depth, cover and water velocity were shown to affect movement, but differently according to the river considered (Aparicio et al., 2018). Water flow is also a crucial determinant for the dispersal of juveniles (Gouraud et al., 2008; Holmes et al., 2014), particularly because the downstream

dispersal of fry is in large part due to their low swimming ability, and is therefore passive (Crisp and Hurley, 1991; Cuinat and Heland, 1979; Heggenes and Traaen, 1988). Moreover, the type of stream is also determinant in these movements, as they generally consist in movements from tributaries to the mainstream (Bembo et al., 1993; Dieterman and Hoxmeier, 2011), and additionally, they were also shown to be determined by higher habitat diversity and food availability in these downstream destination reaches (Aparicio et al., 2018; Northcote, 1992). Indeed, as fish grow larger, preferences in cover, water depth and velocity and substrate size may change, leading to niche shifts (Heggenes et al., 1999; Ovidio et al., 1998). Finally, spawning migrations were also show to be driven by environmental factors (Cucherousset et al. 2005). These migrations are the most important movement in this species (García-Vega et al., 2017). They are determined by multiple factors such as solar radiation, flow and water: in particular, low temperatures and low and stable flows were shown to trigger these migrations (Slavik and Bartos, 2004). Finally, food resource also plays an important role in these migrations as migrating towards productive feeding habitats was shown to increase fitness, notably in females because it allows for higher fecundity (Northcote 1992)

Populational parameters are also determinants of movements; indeed, both population density and kinship between individuals were shown to act on dispersal in particular. First, population density was shown to be an important trigger of movement. In particular, during the fry and parr stages, dispersal was shown to be density-dependent in many studies as individuals flee over populated locations in which competition for space and resource is too high (Bagliniere et al., 1989; Cuinat and Heland, 1979; Einum et al., 2006; Baglinière and Maisse 2002). Second, kin interactions and inbreeding were also shown to modulate movements (Sanz et al. 2011), for instance, in poor environmental conditions, dispersal of related fish was shown to be low thus resulting in high relatedness among neighboring juveniles (Hansen et al., 1997; Vera et al., 2010). This is unexpected as dispersal was generally shown to be a way of avoiding kin-interaction via kin-avoidance (Kasuya, 2000; Perrin and Mazalov, 1999). However, kin-biased distribution can also present many benefits such as increasing the chances that at least one or a few relatives spawn, and has been observed in several studies on salmonids (Carlsson et al., 2004).

*The importance of connectivity in trout movements*

These complex movement patterns, necessary to the species' life cycle, are however affected by habitat fragmentation. The impacts of dams/weirs were widely studied, and shown to affect different types of movements all across the life cycle. First, and most obviously, all upstream movements can be impeded or at least made very difficult when an obstacle is present. Therefore, this affects mainly adult migration (Gosset et al., 2006; Ovidio et al., 1998). Moreover, subsequent flow regulation by dams also affect migration patterns by providing non-natural high flows and colder water during summer (García-Vega et al., 2017). However, studies have shown that downstream movements are also affected by dams, in particular downstream dispersal of juveniles, as dams were shown to increase mortality at this development stage (Larinier and Travade, 2002). Therefore, dam users are increasingly taking these issues into account, equipping them with systems enabling upstream passage, but also downstream (Larinier and Travade, 2002; Schilt, 2007), which have already shown to be successful (Tomanova et al., 2018).

## AIMS OF THE PHD

The overall aim of this thesis is to develop new methodological approaches to study movement at the scale of a river basin, and to bring new perspectives on movement patterns and their underlying causes. This thesis therefore has a double aim: a methodological aim which consists in developing novel tools to study movement, and a more biological aim to bring new insight in the biology of movement.

The first aim was answered with both the development of a universal genetic tool, and then the use of recent statistical developments to assess how combining different markers to study movement can improve accuracy and power. Genetics and isotopes notably are very valuable tools to study movements; however, they are for now mainly used separately, and on spatial scales which require a high differentiation between sample locations to make movement inference possible. Therefore, using new methods of machine learning to combine them is a very promising approach, and at the scales of the rivers studied in this PhD, these novel methods appear as an optimal way of investigating movements. This is notably of high interest for conservation, as in general, managers focus on rather local spatial scales. Indeed, users and managers of river systems, such as land owners, dams and weirs operators, and actors of fish/river management (AFB, DDT, Dreal, Angling departments and associations, and environmental agencies) are generally interested in what is happening at the scale they are managing. This methodological aim is developed in Chapters 1 and 2:

- In chapter 1, I developed a tool useful for detecting genetic structure in the brown trout, as well as hybridization with captive-bred strains. Although many tools exist for population genetics in this species, in particular microsatellites and mitochondrial markers, they appear costly and time consuming in the era of new generation sequencing, and especially lack replicability between studies. We therefore developed Single Nucleotide Polymorphism markers (SNPs), by first identifying a set of 12204 RADs markers mapped on the brown trout linkage map and then used this novel resource to develop a cost-effective array of 192 SNPs evenly spread on the brown trout linkage map.

- In the second chapter, we use recent statistical developments to assess how combining different markers to study movement can improve accuracy and power. More precisely, we assessed the

contribution of combining genetics, carbon and nitrogen stable isotopes, and morphometrics in assigning individuals to their population of origin in two independent river catchments. For this, we used a machine learning framework, based on random forest classification to determine individual probabilities of originating from each sampling site, using every combination of our data (genetics, isotopes, morphometrics, genetics + isotopes, genetics + morphometrics, isotopes + morphometrics, and genetics + isotopes + morphometrics), and implemented a permutation procedure which enabled to obtain a threshold above which assignment can be considered robust. This chapter also highlights which types of data and combinations can be of use at such spatial scales, at which differentiation between sampling locations can be relatively low with one data type, but better resolution can be obtained by combinations.

The second goal is biological, and consists in improving knowledge on the brown trout, particularly in the context of human-induced perturbations: the final aim being to identify factors underlying movement, we were first confronted to the effects of stocking wild populations with captive-bred strains. Indeed, this practice was shown to modify spatial patterns of genetic diversity and differentiation, which are the base of movement detection, therefore, stocking can strongly interfere with movement inference. Therefore, before studying movements and their determinants, we first described the genetic effects of captive breeding. Movements have been widely studied at extremely small scales for instance with direct measurements (in particular with many studies on daily movements using PIT tags, downstream dispersal of fry after hatching from tributaries to mainstream), or on the contrary at very wide scales using genetics (phylogeny of lineages for instance) or isotopes but for very contrasted environments and long distances (anadromous-freshwater migration for instance), but much less at “river basin scales”, which, as discussed above, is the scale of interest for many organizations managing rivers, in particular those managing brown trout populations. This part therefore aims at better understanding how factors influence movements, which, in term, aims at better quantifying what is needed for improving connectivity in this species, and to prioritize conservation efforts. Indeed, for preserving facets of trout life history linked to movements, such as “ecological continuity”, information on movements at the scale of interest is constantly needed. These aims are developed in chapters 3, 4 and 5:

- In chapter 3, we first investigated how stocking affects spatial patterns of genetic diversity and differentiation. Understanding how stocking rivers with captive-bred fish can affect these natural

patterns at the scale of a river basin is therefore a crucial question. Using the previously developed 192 SNP panel (Chapter 1), we aimed at quantifying and described the spatial distribution of captive-bred ancestry in four independent watersheds having been stocked using Bayesian clustering methods, and tested the effect of captive-bred ancestry on the distribution of genetic diversity and differentiation.

- In Chapter 4, we investigated how patterns of admixture between wild and captive-bred individuals affect patterns of dispersal in a brown trout population from a small watershed that was stocked until 1999. We inferred first generation migrants to identify dispersal events and test whether genetic admixture (in addition we tested the effect of sex and body length) affected dispersal probability, distance of dispersal and direction of the dispersal event.

- Finally, in chapter 5, we investigated the individual, environmental, and populational determinants of movements in three riverscapes. Understanding patterns of connectivity/dispersal is of crucial importance in evolution, ecology and conservation biology, and assessing how different components (individual, environments, populations) interact in shaping dispersal patterns is thus of high interest for both fundamental and applied issues. Using assignment methods based on random forests, with genetics (SNPs developed in chapter 1 and stable isotopes of Carbon and Nitrogen) as developed in chapter 2, we investigated the effect of individual phenotypes, environmental characteristics, and populational indicators in shaping movement patterns in three independent river basins. More precisely, we tested whether individual characteristics are linked to dispersal propensity and movement characteristics. Then, we investigated which populations are more likely to receive immigrants or contrary to be emigrated from, linking these percentages to environmental or/and populational drivers. Finally, using a pairwise approach, we tested whether individual exchanges between two sites are linked to connectivity and to environmental similarity.

# Chapter I. Development of a large SNPs resource and a low-density SNP array for brown trout (*Salmo trutta*) population genetics

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## Abstract

*Background* The brown trout (*Salmo trutta*) is an economically and ecologically important species for which population genetic monitoring is frequently performed. The most commonly used genetic markers for this species are microsatellites and mitochondrial markers that lack replicability among laboratories, and a large genome coverage. An alternative that may be particularly efficient and universal is the development of small to large panels of Single Nucleotide Polymorphism markers (SNPs). Here, we used Restriction site Associated DNA sequences (RADs) markers to identify a set of 12,204 informative SNPs positioned on the brown trout linkage map and suitable for population genetics studies. Then, we used this novel resource to develop a cost-effective array of 192 SNPs (96 x 2) evenly spread on this map. This array was tested for genotyping success in five independent rivers occupied by two main brown trout evolutionary lineages (Atlantic -AT- and Mediterranean -ME-) on a total of 1862 individuals. Moreover, inference of admixture rate with domestic strains and population differentiation were assessed for a small river system (the Taurion River, 190 individuals) and results were compared to a panel of 13 microsatellites.

*Results* A high genotyping success was observed for all rivers (<1 % of non-genotyped loci per individual), although some initially used SNP failed to be amplified, probably because of mutations in primers, and were replaced. These SNPs permitted to identify patterns of isolation-by-distance for some rivers. Finally, we found that microsatellite and SNP markers yielded very similar patterns for population differentiation and admixture assessments, with SNPs having better ability to detect introgression and differentiation.

*Conclusions* The novel resources provided here opens new perspectives for universality and genome-wide studies in brown trout populations.

## Background

The brown trout (*Salmo trutta*) is one of the most widespread freshwater fish species in Eurasia, and it has been widely introduced in both the southern and northern hemispheres (Lobón-Cervía and Sanz, 2018). As part of the Salmonidae family, it is a scientifically interesting species because of its diversity in terms of ecology, life history strategies and habitat use (Klemetsen et al., 2003; Rahel and Nibbelink, 1999). Thanks to its wide ecological variability and excellent ability to spread and colonize new watersheds, the species is found both in fresh and salt waters over most of its range. The brown trout is also an economically major species in terms of farming, net fishing (for the sea-run form), and expenditure in recreational angling (Butler et al., 2009; Mills, 1989), partly explaining its worldwide intentional introduction (Rahel, 2007). Because this species is strongly associated to human interests, wild brown trout populations are widely managed, either to sustain attractive leisure activities such as recreational angling or to conserve declining and/or emblematic populations. Moreover, the brown trout has been domesticated since the 19$^{th}$ century (Laikre et al., 1999), and hatchery strains have been largely used to sustain wild populations worldwide (Elliott, 1994; Berrebi et al., 2000; Bohling, 2016). Instead of positive expected effects of these stocking activities, most have proven to have negative long term effects on wild brown trout populations in part because of the reduced fitness brought by hatchery fish in wild populations, and the loss of local genetic heritage caused by the replacement of local wild populations with genetically homogeneous hatchery strains (Hansen, 2002; Araki and Schmid, 2010; Christie et al., 2012; Leitwein et al., 2016).

The brown trout presents high levels of phenotypic and genetic polymorphism, with seven main mitochondrial (mtDNA) lineages with various geographical extents being generally recognized. These consist of (i) four sub-continental lineages: the Atlantic (AT), Mediterranean (ME), Danubian (DA) and Adriatic (AD) lineages, (ii) two regional lineages: Marmorata (MA) in the north of the Adriatic Sea and North African (NA) in Morocco, Algeria and Sicily (Bernatchez, 2001; Susnik et al., 2005; Tougard et al., 2018) and (iii) two local lineages limited to one river basin: the Duero (DU) in Europe, and Tigris (TI) in Turkey (Bardakci et al., 2006; Suárez et al., 2001; Vera et al., 2015, 2010). Within these extent lineages that cover the whole range of *S. trutta*, high levels of genetic and phenotypic polymorphism are also observed at more local spatial scales within lineages (e.g. population scale; Berrebi, 2015; Swatdipong et al., 2010; Vøllestad et al.,

2012). However, diversity patterns in brown trout have also been locally influenced by stocking practices that mostly relied on European hatchery strains of AT origin (Cortey et al., 2004) to supplement local populations, with the exception of a few local strains stemming from local populations (Bohling et al., 2016; Caudron et al., 2006; Cortey and Garcia-Marin, 2002).

Genetic tools appeared as a key approach for scientists and local managers to optimize conservation efforts (Altukhov et al., 2000; Sato and Harada, 2008) because they provide insight into both the ecological and the evolutionary dynamics of wild populations (Elliott, 1994; Pelletier et al., 2009). For instance, assignment tests, fine-scale population structure, kinship analyses and genome-wide surveys (Araki et al., 2007; Stinchcombe and Hoekstra, 2008; Bourret et al., 2013) enable to monitor populations effectively, and have high potential applications for conservation and management in salmonids, including the brown trout (Caudron et al., 2006; Hansen, 2002; Waples and Hendry, 2008).

Molecular studies on trout populations first used allozymes, mitochondrial markers, and then microsatellite loci (Apostolidis et al., 1997; Carlsson et al., 1999; Laikre et al., 1999). These markers (notably microsatellites) are useful and adequate to answer many biological questions, but their genome coverage is generally weak, and replicability and universality are relatively low since each research group generally uses its own panel of markers. Single nucleotide polymorphisms (SNP) markers have been shown to potentially reduce these limitations (Davey et al., 2011; Etter et al., 2011; Helyar et al., 2011). They allow to uncover a relatively high number of annotated and mapped markers with low scoring-error rates (Brumfield et al., 2003; Morin et al., 2004). Also, SNPs markers can easily be chosen to represent both neutral genomic regions and regions under selection, at a genome-wide scale and across large samples (Morin and Mccarthy, 2007). Despite being biallelic markers, SNPs can be highly informative for most analyses used in population genetics, as far as the number of loci is sufficiently high and evenly spread across the genome (>50; Bradbury et al., 2015; Freamo et al., 2011; Kaiser et al., 2017; Karlsson et al., 2011; Paschou et al., 2007). Genome coverage is an important aspect in the choice of markers for population genetics: first, markers evenly spread across the genome are less likely to exhibit linkage disequilibrium (Scheet and Stephens, 2008), and second, it was shown that many population events, such as introgressive hybridization can only concern certain genomic blocks, a full coverage thus enables to capture these events (Lamaze et al., 2012; Leitwein et al., 2018).

SNP arrays are commonly used for conservation purposes in salmonids (Dominik et al., 2010;

Kochzius et al., 2008; Lien et al., 2011), although rarely in brown trout, which is probably because only a handful of SNP markers were available for this species (Pustovrh et al., 2012; Drywa et al., 2013; Sušnik Bajec et al., 2015). However, higher density resources were more recently developed. In particular, Linløkken (Linløkken et al., 2017) developed 3781 SNPs to analyse genetic differences between wild and hatchery brown trout in a tributary of Lake Savalen in central Norway. Moreover, a new lineage-specific high density linkage map for *S. trutta* comprising ancestry informative SNPs for both Atlantic (AT) and Mediterranean (ME) evolutionary lineages from Western Europe was also developed (Leitwein et al., 2017, 2016). This latter resource provides a novel baseline for the development of mapped SNPs that may be of prime interest for studies on brown trout population genetics across a wide spatial range.

The aim of this study was to develop a genome-wide, mapped and universal set of SNPs for the brown trout for both Atlantic and Mediterranean lineages (AT and ME lineages; sensu Bernatchez, 2001), which would be a useful and affordable tool for both scientists and environmental managers. By taking advantage of the new genomic resource available for brown trout (Leitwein et al., 2018, 2017, 2016), we developed a panel of 12,204 RADs containing 1 or 2 polymorphic SNPs evenly spread across the genome structured in 40 chromosomes, and ancestry informative for at least two of the main brown trout lineages, the Atlantic and the Mediterranean lineages. Among these, a sub-panel of 192 SNPs describing the whole genome was included in a low-density SNP array. The validity of this low-density SNP array was tested by quantifying genotyping success and number of polymorphic loci in five independent populations from the two lineages at a large spatial scale. Finally, the power of this array was compared to a panel of 13 microsatellites for answering classical population genetics questions: admixture with stocked domestic individuals and genetic differentiation among wild populations. All resources are made freely available for future users (Appendices S1 and S2).

## Methods

*Development of a genome-wide reference SNP panel*

The panel of SNPs markers identified is this study was filtered from the variants described in Leitwein (Leitwein et al., 2018) using double-digest RAD sequencing. restriction enzymes *Eco*RI-

HF and *Msp*I were used to digest individual genomic DNA and create the dd-RAD library, which was submitted to size selection in order to retain fragments of 200 to 700 bp using CleanPCR beads. The library was then amplified by PCR and sequenced with Illumina HiSeq2500, producing 125-bp paired-end reads. The initial set contained 75,000 SNPs discovered from 82 wild Mediterranean *S. trutta* from tributaries of the Orb River catchment in southern France, and 102 captive-bred individuals from farms formerly used for stocking in this region (41 and 61 hatchery fish from the ME and AT lineages respectively; see Leitwein (Leitwein et al., 2018) for details). These SNPs were anchored to the-high density *S. trutta* linkage map using an intermediate step of physical mapping to the Atlantic salmon reference genome: using their relative positions on the Atlantic salmon reference genome, it was possible to determine the relative mapping positions of a large number of additional RAD loci that were not present on the brown trout linkage map (Leitwein et al., 2017).

We applied a series of filters which allowed for selecting a panel of SNP markers that were (i) likely to be highly polymorphic, (ii) mapped on the linkage map, and (iii) present in the two brown trout lineages. To do so, we removed from the initial database all SNPs with a minimum allele frequency (MAF) of 5% or less using *vcftools* (Danecek et al., 2011) based on all individuals (AT + ME). Then, in order to have clean sequences, straightforward to be genotyped, RAD sequences with more than two SNPs, and SNP positions falling in the first or last 30 bp of the RAD sequences were also removed, as well as sequences with undetermined (N) nucleotides. Finally, we kept only RAD sequences for which mapping positions on the *S. trutta* linkage map were determined (see above).

*Development of the low-density SNP array*

We used this large SNPs resource to develop a low-density SNP array containing 192 SNPs. The goal was to propose a cost-effective tool which holds on two 96-wells genotyping plates for analyzing a large number of individuals. Moreover, SNPs were selected to be informative for population genetics analyses of brown trout populations from at least the AT and ME lineages (sensu Bernatchez (Bernatchez, 2001)) from Western Europe. The array was genotyped using the KASPAR technology ® (Smith and Maughan, 2015) that allows a rapid and cost-effective genotyping service for such a number of markers. We hence applied further filters to fulfill KASPAR genotyping constraints. In particular, RADs with more than one SNP between the

primer designing zones (50bp at the two extremities) were excluded. To ensure a good representativeness of the genome, we selected SNPs evenly spread and spaced by at least 3.5 cM across the 1453 cM estimated length of the *S. trutta* linkage map (Leitwein et al., 2017). This resulted in 245 SNPs (average 1453/245=5.9 cM) among which, in order to retain 182 SNPs, we randomly removed 63 SNPs using the *sample()* R function. Then, we added five ancestry informative SNPs developed by the Institut National de Recherche en Agronomie and the University of Savoie (UMR-0042 CARRTEL, France) that were used in previous studies to distinguish individuals from the AT and ME lineages (OMM1164, OMM1105, OMM1154, Str541INRA, Str591INRA; Harrang et al., 2015; Caudron et al., 2012, 2006; Estoup et al., 2000).Finally, we added five mitochondrial SNPs previously used to differentiate among the five main brown trout lineages (mitoDA10Proline, mitoDA10ProlineB, mitoCytoB, mitoATPaseIVA, mitoATPaseIVB; (Bernatchez et al., 1992; Giuffra et al., 1994); Appendix S1). These numbers of five ancestry informative nuclear or mtDNA markers were chosen to represent approx. 5% of the markers present on the SNP array. This resulted in a low-density array of 192 SNPs markers. Information and sequences are available on Figshare, DOI: 10.6084/m9.figshare.8174708

*Genotyping success of the low-density SNP array*

This low-density SNP array was first evaluated for genotyping success using individuals from five independent French river basins: two from the Pyrénées mountains (the Ône and the Aude Rivers), two from the Alps mountains (the Roya and the Doron de Bozel Rivers) and one from the Massif Central mountains (the Seuge River) (Figure 1). Three of these rivers belong to the Mediterranean lineage (ME; the Aude, the Doron and the Roya Rivers), whereas the two others belong to the Atlantic lineage (AT; the One and the Seuge Rivers) (see Figure 1).

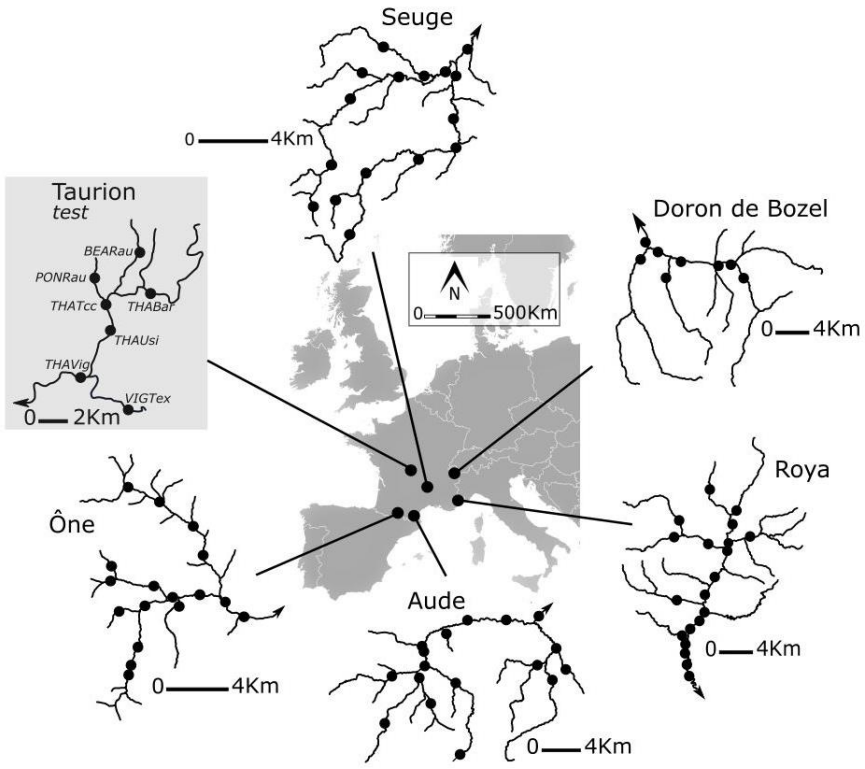


*Figure 1.1: Map of the five river basins and sampling sites (black dots) used to test for genotyping success. The Seuge and Ône Rivers are part of the Atlantic catchment, therefore naturally harboring AT trout, whereas the Aude, Roya and Doron de Bozel Rivers are part of the Mediterranean catchment, naturally populated with ME lineage. Maps were generated by authors on ArcGis and assembled using Inkscape.*

The sampling sessions were performed in 2016, using a single-pass electrofishing approach from a total of 79 sites (between 8 and 21 sites per river basin, Appendix S5), with an aim of sampling 30 individuals of brown trout per site. In total, we captured 1862 individuals (26 individuals per site in average; see Appendix S5) from which a fin clip was taken (after Eugenol anesthesia), and kept in 70% TE Ethanol for genotyping. All individuals were released alive to their site of capture. Fin samples were sent to the LGC Genomics company for DNA extraction and multilocus genotyping of the 192 SNPs markers using KASPAR® (Smith and Maughan, 2015). Genotyping success was measured at the individual level, by the proportion of SNPs which were not genotyped (either because not amplified or because the allele could not be read). Finally, these basins were tested for patterns of isolation-by-distance, by using a mantel test with 1000 permutations on pairwise Fst matrices (calculated with the pairwise.fst *adegenet* function) and riparian distances (measured in meters with STARS ArcGis package).

*Efficiency of the low-density SNPs array*.

The low-density SNP array was further used for classic population genetic questions in order to compare its efficiency with thirteen microsatellite markers previously used in brown trout population genetic studies (e.g. Estoup et al., 1998; Grimholt et al., 2002; Slettan et al., 1995). A total of 190 brown trout individuals were sampled in a small river basin (the Taurion River in the Massif Central Mountains; Figure 1, "test") in 2017 using electrofishing. Seven sites were sampled with 21 to 30 individuals per site (Appendix S6). For each individual, a pelvic fin clip was taken for genetic analyses. All individuals were released to their original sampling site. We additionally sampled 30 individuals of domestic Atlantic brown trout from a local hatchery used for stocking purposes (the Soueich trout hatchery), to quantify genetic admixture with wild populations from the Taurion basin. Fin samples were sent to the LGC Genomics Company for DNA extraction and for multilocus genotyping to 192 SNPs markers using the KASPAR® (Smith and Maughan, 2015). Note that 30 SNP markers from the initial 192 SNP panel did not amplify (see the Results), and were hence replaced by 30 other SNPs from the 245 SNPs filtered (see methods) to improve the SNP array (see Appendix S2 for details). Additionally, all individuals were genotyped at thirteen microsatellites assembled in PCR multiplexes (see Appendix S7, and Saint-Pé et al., 2018 for details). We tested whether the selected SNPs were likely influenced by selection using the Fst outlier detection method implemented in the *fsthet* R package (Flanagan and Jones, 2017), in which outlier values of $F_{ST}$ can be identified in a plot of $F_{ST}$ *vs*. heterozygosity (Beaumont and Nichols, 1996).

From both SNP and microsatellite datasets, we removed individuals with more than a third of missing data, and kept only individuals for which we had both SNPs and microsatellites genotypes. We first compared genetic admixture between wild and captive-bred strains using STRUCTURE 2.3.1 (Pritchard et al., 2000) with the admixture model and the correlated allele frequency model, without prior population information. Twenty runs assuming two clusters (K=2, in order to discriminate between wild and captive-bred individuals, see (Saint-Pé et al., 2018)) were performed with a burn-in period of 200,000 and 200,000 subsequent MCMC repetitions. The ten best runs (highest LnP(D) values) were compiled using CLUMPP (Jakobsson and Rosenberg, 2007) to obtain final averaged individual Q-values. Individuals were assigned to one of the two clusters with the greatest Q-value, provided that value exceeded 0.7 (as in (Saint-Pé et al., 2018)).

Individuals with intermediate Q-values were considered genetically admixed individuals between hatchery and wild strains. Individual assignment Q-values to the cluster containing all Soueich hatchery individuals (i.e. degree of assignment to the "captive-bred" cluster = individual level of "hatchery ancestry") were compared between SNPs and microsatellites using a Spearman correlation test, as admixed ancestry was not normally distributed.

We then compared population differentiation assessment between markers by calculating pairwise Fst between sites using the *adegenet* R package and *mantel* R function. Finally, we compared the informativeness of both sets of markers for population structure by calculating the informativeness for assignment ($I_{n;}$ (Rosenberg et al., 2003)). A higher index indicates a higher informativeness of the set of markers. It was calculated with R as follows: for $i$ = 1, 2,..., $K$ populations and $m$ = 1, 2, ..., $L$ loci, with $K \geq 2$ and $L \geq 1$. Locus $m$ has alleles $j$ = 1, 2, ..., $N_{(m)}$. The average frequency of allele $j$ at locus $m$ across the $K$ populations is defined as $P_j = \sum_{i=1}^{K} \frac{P_{ij\,(m)}}{K}$, where $P_{ij(m)}$ is the relative frequency for allele $j$ of locus $m$ in population $i$. The informativeness is defined as:

$$I_n = \sum_{i=1}^{N}(-P_j log P_j + \sum_{i=1}^{K} \frac{P_{ij}}{K} log P_{ij}).$$

## Results

*Development of the large SNPs resource and characteristics of the low-density SNP array*

After applying filters, we identified a RAD data set of 12,204 sequences each containing one or two SNPs that met our specifications and that are made readily available to the scientific community (see Appendix S1). The number of RADs per linkage groups (LGs) varies between 137 (LG 33) and 563 (LG 6). These RAD tags are spaced by 0.119 cM (+/- 0.039) in average. They were found in all LGs and they cover the linkage map relatively evenly, although there were large gaps on LGs 11 (top), 12 (top and bottom), 27 (bottom), 32 (top), 33 (bottom), 37 (bottom), and 39 (top) (Figure 2A). One estimate of the local recombination rate for each SNP (i.e. one index of the relative power of markers for LD-based mapping approaches) is provided in Appendices S1 and S2.

The 245 SNPs selected from this set of RADs were spread over all linkage group so as to cover the linkage map as homogeneously as possible (Figure 2B). The final low-density 182 SNP array, to which we added 10 SNPs previously developed (see the Methods) is presented in Appendix S2.

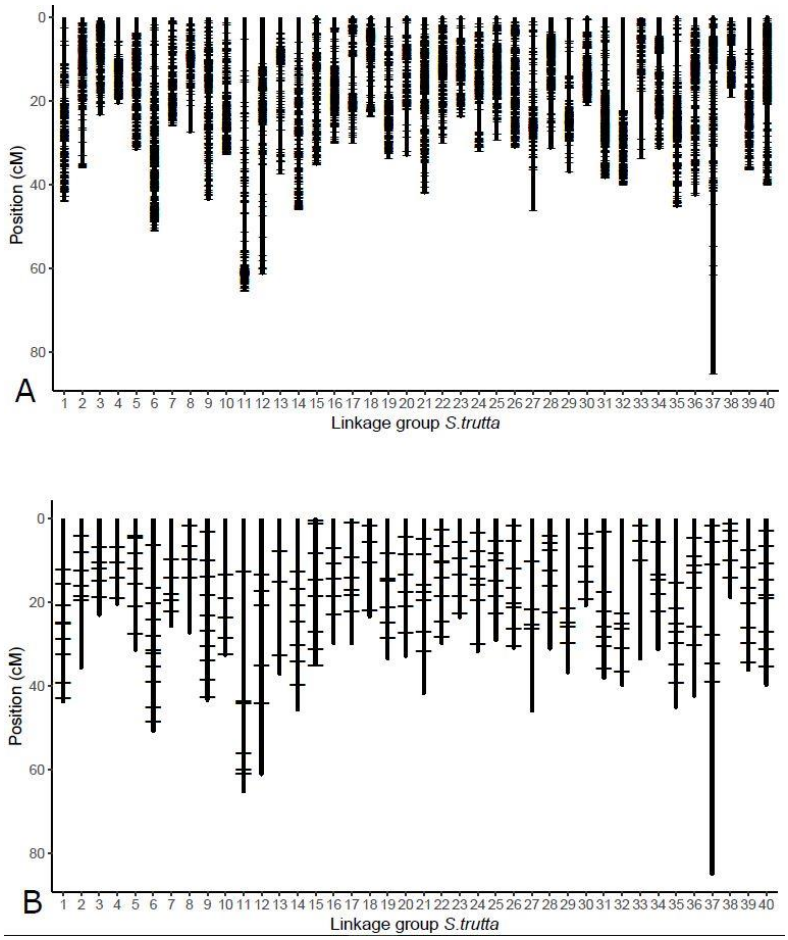


*Figure 1.2: Positions on the brown trout linkage map of the 12204 RADs (A; containing one or two SNPs with MAF ≥ 5% based on all individuals (AT + ME) and positions between the first or last 30 bp of the RAD with no undetermined nucleotides), and of the 245 SNPs (B; informative for at least the AT and ME lineages, positions between the first or last 50 bp of RADs (primer design zones), and spaced by at least 3.5 cM)*

*Genotyping success of the low-density SNPs array*

Thirty of the 192 SNPs (among which a mitochondrial marker) that were initially genotyped in the five watersheds did not amplify, suggesting primers failure to properly bind their target DNA site. For the loci that successfully amplified, the overall genotyping success was high, with less than 1% of missing data per individual, irrespective of the river basin and the lineage (Table 1). Nonetheless, the number of polymorphic SNPs greatly varied from one river to another, ranging from 91 (for the Seuge River) to 160 (for the Aude River) out of the 162 SNPs that were successfully genotyped in that portion of our work (Table 1). The isolation-by-distance patterns were significant (pairwise Fst significantly correlated to pairwise riparian distance) for the Seuge River ($r_{Mantel}$ = 0.55, p-value <0.001) and the Roya River ($r_{Mantel}$ = 0.65, p-value = 0.01). Fst and riparian distance were also positively although non-significantly correlated for the Aude River ($r_{Mantel}$ = 0.19, p-value = 0.15), the One River ($r_{Mantel}$ = 0.28, p-value = 0.07) and the Doron River ($r_{Mantel}$ = 0.02, p-value = 0.36) (see Appendix S3 for the plots).

| River basin (lineage) | Number of polymorphic loci | Average (±SD) number of non-genotyped loci per individual on polymorphic loci Min-max | He on polymorphic loci | Basin surface ($Km^2$) |
|---|---|---|---|---|
| **Aude (Mediteranean)** | 160/162 | 1.04 (±1.68) 0-24 | 0.16 | 240 |
| **Doron de Bozel (Mediteranean)** | 153/162 | 0.71 (±0.99) 0-8 | 0.30 | 180 |
| **One (Atlantic)** | 119/162 | 0.87 (±1.23) 0-13 | 0.14 | 155 |
| **Roya (Mediteranean)** | 158/162 | 1.36 (±4.51) 0-63 | 0.30 | 360 |
| **Seuge (Atlantic)** | 91/162 | 0.66 (±1.06) 0-15 | 0.09 | 90 |
| **Average** | 136 | 0.95 | 0.20 | 205 |

*Table 1.1: Number of polymorphic loci, number of non-genotyped loci per individual considering polymorphic loci (average, min and max), expected heterozygosity (He) on polymorphic loci in each of the five river basins, and surface drained by the river basin (upstream from the lowest sampling site).*

*Efficiency of the low-density SNPs array*

Among the 30 replaced SNPs for the empirical test in the Taurion River, 10 failed at the amplification step (see Appendix S2). This resulted in a set of 182 markers that successfully amplified, although only 92 of them were polymorphic in this river (among which only one mitochondrial marker of the 10 INRA and mitochondrial added markers). After removing individuals with more than a third of missing data, and keeping only individuals for which we had both SNPs and microsatellite data, our final dataset comprised 197 individuals (167 individuals from the Taurion basin and 30 from the Soueich trout farm). Expected heterozygosity ranged from 0.18 to 0.22 for SNPs and from 0.58 to 0.73 for microsatellites. Fst ranged from 0.022 to 0.070 for SNPs and from 0.019 to 0.040 for microsatellites (details for each site are shown in Table 2). We found one marker that was a significant Fst outlier, suggesting it may be influenced by selection (ID 295415; Fst>>Expected heterozygosity), but we decided to keep it in our analyses (see the Discussion).

| | | *SNPs* | | | | | *Microsatellites* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **N** | **Ho** | **He** | **Fis** | **Fst** | **Ar** | **Ho** | **He** | **Fis** | **Fst** | **Ar** |
| **BEA-Rau** | 27 | 0.18 ± 0.19 | 0.18 ± 0.18 | 0.011 | 0.070 | 1.58 | 0.44 ± 0.20 | 0.58 ± 0.25 | 0.226 | 0.04 | 4.42 |
| **PON-Rau** | 12 | 0.22 ± 0.23 | 0.21 ± 0.20 | -0.038 | 0.030 | 1.66 | 0.67 ± 0.19 | 0.70 ± 0.17 | 0.027 | 0.022 | 5.63 |
| **THA-Bar** | 26 | 0.22± 0.23 | 0.22 ± 0.20 | -0.025 | 0.022 | 1.65 | 0.65 ± 0.17 | 0.67 ± 0.18 | 0.015 | 0.017 | 5.85 |
| **THA-Tcc** | 30 | 0.19 ± 0.20 | 0.20 ± 0.18 | 0.028 | 0.027 | 1.69 | 0.61 ± 0.18 | 0.69 ± 0.17 | 0.107 | 0.019 | 6.18 |
| **THA-Usi** | 29 | 0.22 ± 0.22 | 0.21 ± 0.19 | -0.023 | 0.025 | 1.67 | 0.68 ± 0.12 | 0.73 ± 0.16 | 0.057 | 0.019 | 6.79 |
| **THA-Vig** | 21 | 0.21 ± 0.21 | 0.22 ± 0.20 | 0.056 | 0.028 | 1.68 | 0.64 ± 0.17 | 0.72 ± 0.12 | 0.125 | 0.024 | 6.40 |
| **VIG-Tex** | 22 | 0.21 ± 0.21 | 0.21 ± 0.19 | -0.004 | 0.031 | 1.66 | 0.69 ± 0.14 | 0.70 ± 0.15 | 0.033 | 0.02 | 5.91 |
| **PIS-Sou** | | 0.34 ± 0.18 | 0.33 ± 0.16 | -0.008 | 0.153 | 1.96 | 0.80 ± 0.11 | 0.84 ± 0.09 | 0.052 | 0.070 | 8.14 |

*Table 1.2: For each site, sample size ("N"), mean expected heterozygosity over all loci ("He") and standard deviation between loci, mean observed heterozygosity over all loci ("Ho") and standard deviation between loci, mean allelic richness computed using a rarefaction approach over all loci ("Ar"), mean Fis over all loci ("Fis"), mean Fst over all loci (i.e. uniqueness at the site level; Fst = 1-$He_{Site}/He_{Total}$).*

Individual inferences of hatchery ancestry measured with either SNPs or microsatellites are presented in Figure 3 in the form of individual barplots. The distribution of captive-bred ancestry was bimodal, meaning that most individuals were either purely wild or captive-bred, with relatively low numbers of admixed genotypes (Figure 3, Figure 4A). Levels of hatchery ancestry

were significantly and moderately correlated ($r_{Spearman}$ = 0.60, d.f. = 193, $p < 0.001$; Figure 4A), although for some individuals, there was a discrepancy between markers with one of the two marker types detecting introgressed fish while the other marker type assigned them as pure wild fish (Figure 3, Figure 4A). Pairwise Fst values are presented for both SNPs and microsatellites in Appendix S4. They ranged from 0.010 to 0.088 for SNPs (mean = 0.033 ± 0.025) and from 0.010 to 0.053 for microsatellites (mean = 0.025 ± 0.013). Pairwise Fst values between sites assessed with SNPs and microsatellites were strongly correlated ($r_{Mantel}$ = 0.92, p = 0.001 based on 1000 permutations), and SNPs had higher values (Figure 4B, the regression coefficient is significantly higher than the 1:1 line (dotted line on figure) since its 95% CI is 1.46-2.18).

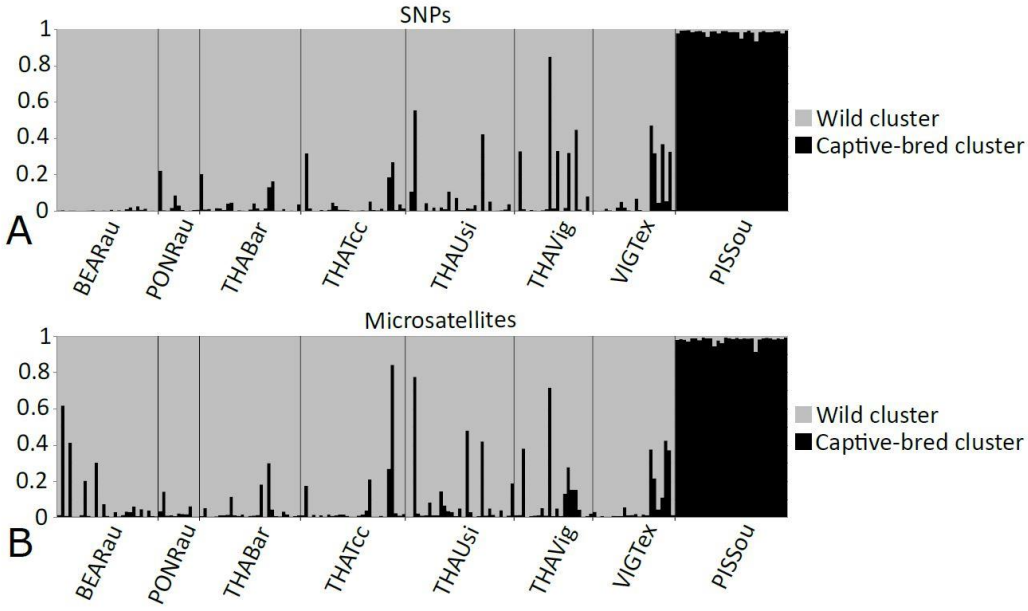


*Figure 1.3: Structure barplots of assignment to the wild (grey) and the captive-bred (black) clusters, using both SNPs (A) and microsatellites (B)*

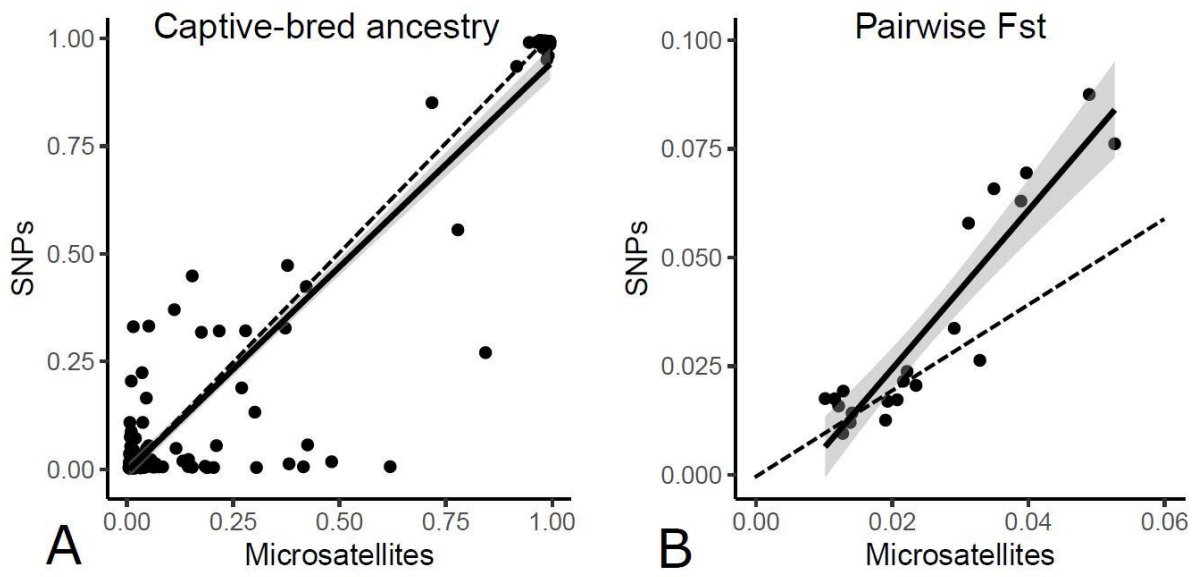


*Figure 1.4: Plot of individual hatchery ancestry proportion measured with microsatellites against that measured with SNPs (A) and pairwise Fst between sites measured with microsatellites against pairwise Fst between sites measured with SNPs (B). Black lines represent the linear regression with its confidence interval; spotted lines represent the 1:1 line.*

Finally, within the Taurion River, the 92 SNPs displayed a slightly lower informativeness (I) than the 13 microsatellites (2.08 vs. 2.48; Figure 5). We found that the informativeness of these 92 SNPs is actually equivalent to that of 10 of the microsatellites (Figure 5). Based on the equation linking the number of our SNPs and informativeness ($I = 0.023*Nsnps+0.0038$; $r^2 = 0.98$, p-value $< 0.001$), we extrapolated that 108 SNPs are required to be equivalent to the panel of 13 microsatellites in terms of informativeness for individual assignment.

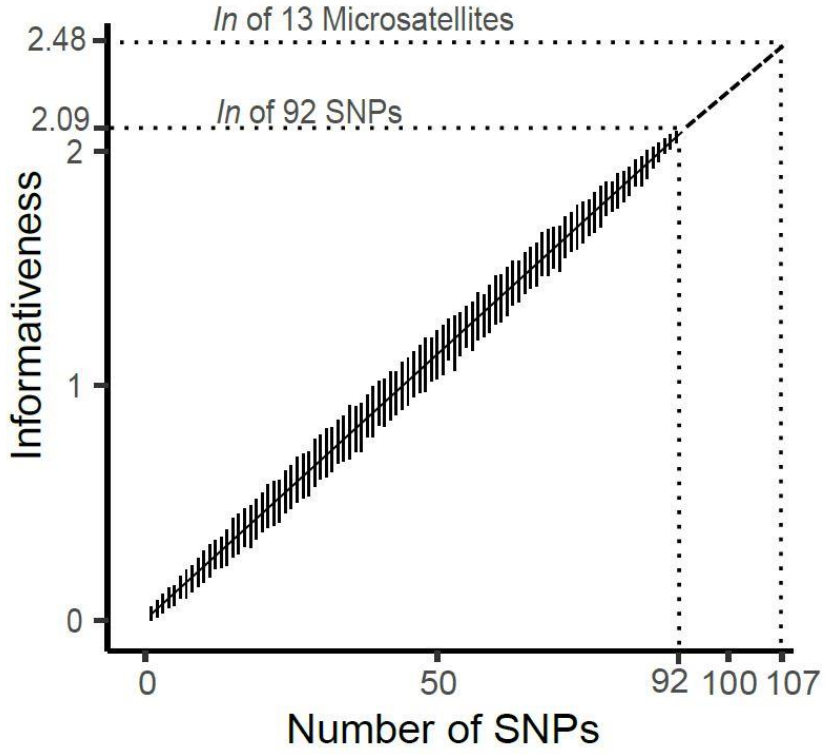


*Figure 1.5: Plot of informativeness against number of SNPs. 107 SNPs would be equivalent to the 13 microsatellites in terms of informativeness.*

## Discussion

The SNP panel developed here was shown to be efficient to study the population genetics of Atlantic and Mediterranean brown trout lineages from Western Europe, which gathered a huge number of studies in the past decades (Poteaux, 1999; Aurelle and Berrebi, 2001; Almodóvar et al., 2012). We provided a panel of 12,204 RADs which are relatively evenly spread across the whole genome, and, from a sample of this panel, we proved its efficiency in terms of genotyping success, and measuring patterns of isolation-by-distance. We also proved that these SNPs successfully detect population structure, which opens new insights for many applications with great potential compared to commonly used markers. These advantages are multiple, going from lower error rates and a simple mutation model with low homoplasy (Morin and Mccarthy, 2007) to usability on poor quality samples. In terms of costs, SNPs and microsatellites are roughly similar, however in terms of time efficiency, SNPs are highly advantageous: samples are directly sent to

the genotyping platform, and within a month, data is ready to be analyzed. Moreover, SNPs can be easily reused in other studies, and are more powerful in detecting hybridization (McFarlane and Pemberton, 2019).

*Development of a large variation map for population genomic studies in brown trout*

The 12,204 RAD-derived SNPs were relatively homogeneously distributed across the 40 linkage groups of *Salmo trutta* (with 137 to 563 RADs per LG), and showed an average spacing of 0.119 cM. There are some gaps in coverage, which does not necessarily mean that information is missing, they could be due to high recombination rates in these regions or to the positions of the centromeres. Although these gaps exist, genome coverage is satisfying, and with the marker density obtained, it should be sufficient for most genome-scale studies that need to tag a large fraction of genomic variation through linkage disequilibrium. However, this marker density may still be limited when a rapid decay of linkage disequilibrium leaves many genomic regions unattainable, as it might be the case for some applications like the search for loci underlying fitness in the wild (Kaeuffer et al., 2007). Therefore, an estimate of the local recombination rate for each SNP as an index of the relative power of markers for LD-based mapping approaches is provided in Appendices S1 and S2.

The proposed SNP resource only includes SNPs with a MAF higher than 0.05 (i.e. removing of rare variants), a criterion that has been set for two main reasons. First, SNPs with very low MAF can in many cases be genotyping errors (Ahn et al., 2009; Wang et al., 2008). Second, we chose a relatively high MAF because this panel of SNPs is primarily designed for studying populations for most of the species range. Therefore, SNPs that are discovered in the populations used to develop the panel are more likely to be polymorphic in other populations from which they were not developed if their MAF is high (Morin et al., 2009). Although filtering SNPs on their MAF could lead to ascertainment bias (Helyar et al., 2011; Nielsen et al., 2009; Rosenblum and Novembre, 2007), we suggest this is not an issue in our case study because we applied the MAF criteria regarding distinct glacial lineages, which limits marker choice bias. Moreover, for individually-centered investigations such as population structure, kinship and individual assignment, SNPs with higher MAF were shown to generally be the most powerful (Krawczak, 1999; Morin et al., 2004; Ryman et al., 2006).

In the test panel, and for the Taurion river (test basin), one locus might be potentially affected by selection. Outlier markers are usually removed before analyses for inferring neutral evolutionary processes, such as genetic drift and gene flow (Beaumont and Nichols, 1996). In our analyses, we decided not to remove it because in the case of detection of admixture between strains, it was shown that these ancestry informative markers can increase accuracy for detecting differentiation and assignment of individuals to populations (Funk et al., 2012; Gagnaire et al., 2015; Nielsen et al., 2009). Removing loci displaying selection is thus up to the users of the resource, depending on the aim of the study. For instance, if the aim is to determine if a population is at HWE, or to quantify gene flow/inbreeding, or calculate effective population sizes, loci affected by selection should be removed, whereas more individual-based questions do not necessarily require removing these loci.

*Genotyping success*

Genotyping success was very high in all basins, and except for two basins, the number of polymorphic SNPs among the 162 amplified (from the 192 set) was satisfactory (73 to 99 %), confirming the potential versatility of this tool. This set of 162 SNPs already benefits to the field of population genetics for the species as to our knowledge, most studies used less than 40 SNPs (Pustovrh et al., 2012; Drywa et al., 2013; Sušnik Bajec et al., 2015). Therefore, the 12,204 RAD panel is a promising tool for genome wide studies on brown trout. It would be of interest however to further test this panel on other evolutionary lineages, or on populations which have been shown to have diverged from the continental populations such as western Mediterranean populations found in Iberian Peninsula (Cortey et al., 2004), Italian populations (Splendiani et al., 2016), and Corsican populations (Berrebi et al., 2019), as well as on the other main lineages (e.g. Adriatic and Danube lineages; Bernatchez, 2001) or remote populations inhabiting at the edges of the species' range (Iran: Hashemzadeh Segherloo et al., 2012; Osinov, 2009, Morocco: Tougard et al., 2018).

As a first approach we found this panel to be efficient for detecting patterns of isolation-by-distance, although we had no other markers to compare with. Patterns of isolation-by-distance were found to be significant only in the Seuge and the Roya Rivers, although there was a tendency in the other three basins. Interestingly, it seems that even when the number of polymorphic SNPs was low (Seuge River: 92 polymorphic loci), detecting a pattern of isolation-by-distance was still possible. In other basins, we suggest that the strength of the relation between genetic and riparian

distance may also be affected by stocking events, or by characteristics of the watershed and demographic histories. However, we did not investigate these issues further in the present manuscript.

*Tests of the low-density SNP array*

Studies in which low-density SNP arrays equal or outperform a handful of microsatellites for population structure and differentiation are common, particularly when sample sizes are large and populations are strongly structured (Freamo et al., 2011; Gärke et al., 2012; Liu et al., 2005). Although we found no literature on this particular aspect in brown trout, it has been shown in Atlantic Salmon : genetic divergence, structuring and isolation-by-distance were assessed as successfully using only 7 SNPs and 14 microsatellites, although genetic diversity estimates were less concordant (Ryynanen et al., 2007). Twenty-six SNPs were also shown to be nearly as efficient as 16 microsatellites for parentage assignment in this species (Rengmark et al., 2006), which makes our set of SNPs appear very promising. The panel of 192 SNPs tested here performed well in terms of detection of admixture and population differentiation, although only 92 SNPs were polymorphic in the Taurion basin. The low level of polymorphism is probably due to the fact that the study scale is extremely low (less than 15 km between the two most extreme sampling sites; Figure 1) and/or that the biogeographic area in which this river basin is situated has historically low level of diversity (see below).

We found similar introgression level when measured with SNPs compared to microsatellites (Figure 4A), and higher pairwise Fst values between sites with SNPs, suggesting that SNPs have – at least - a similar discriminatory and assignment power. However, the low number of polymorphic SNPs in this panel (92 polymorphic SNPs) compared to other SNP-microsatellite comparison case studies (Gärke et al., 2012; Hauser et al., 2011; Kaiser et al., 2017) lowered its' informativeness : it was outperformed by the 13 microsatellites, and we found that it would actually require 107 SNPs to be equivalent to microsatellites in terms of informativeness for assignment. However, the advantage of SNPs may here not be accounted for when calculating informativeness. Indeed, they enable to better detect introgression and admixture compared to microsatellites, and show that individuals considered as « pure » with microsatellites may in fact be introgressed (Haasl and Payseur, 2011). This difference in individual admixture proportions

calculated with microsatellites and SNPs might results from the fact that our panel is characterized by an even repartition of SNPs along the genome of the brown trout, which is expected to improve the global assessment of genome-wide admixture proportions (Leitwein et al., 2018). Hence, the strong advantage of a SNP panel of this type is that it ensures a better representativeness of the entire genome of the brown trout. Moreover, although informativeness is lower, the 92 SNPs still give sufficient information on admixture and differentiation, highly correlated with that given by the microsatellites: the trade-off between cost and power must also be taken into account in regard of the questions asked and the means of the user.

For other empirical case studies, such as the five river basins on which genotyping success was measured, and in which individuals are variable on more loci (except for the Seuge river, other basins showed 119 to 160 polymorphic loci, Table 1), we can expect higher informativeness. We even expect SNPs to equal or outperform microsatellites in terms of informativeness for these basins (indeed, around 107 SNPs should be equivalent to the panel of 13 microsatellites, as pairwise Fst values were higher for SNPs than microsatellites), and with the advantages of SNPs mentioned previously. This is particularly true in Mediterranean rivers, probably because they contain both domestic Atlantic and natural Mediterranean ancestries. Indeed, these SNPs were discovered using a mix of Mediterranean and Atlantic individuals, and are therefore more likely to be polymorphic if both lineages are present. Additionally, the mitochondrial and INRA markers were also developed to have a fixed allele in each lineage, explaining why in the Taurion for instance, in which only the Atlantic lineage is present, they were not polymorphic (except for one).

We make this SNP panel freely available as a resource. As it contains many more untested SNPs, future users will be able to choose the number, density, and position of markers in the linkage map, and considering the local recombination rate around each SNP in order to adjust their own panel to their objectives. This should also be considered for the Massif Central Rivers (in our case Taurion and Seuge Rivers), in which the number of polymorphic loci is lower than in the other basins studied here, probably because of the past demographic histories (colonization, connectivity, stocking, bottlenecks, population sizes and habitat). However, as this subpanel was tested on only 7 populations (190 individuals), we first suggest that increasing the sample size should increase statistical power better than increasing the number of SNPs, especially when Fst is low (< 0.01; Kalinowski, 2005; Morin et al., 2009).

## Conclusion

The SNP panel presented here appears as a novel tool to study diverse aspects of population genetics in the brown trout. The possibility to genotype many loci in a fast and affordable way will open many perspectives. It opens new insights into the species life history, with many potential applications both for fundamental population genetics, conservation and management questions, but also for more biological questions such as mapping of quantitative trait loci, or investigating links between genetic and environmental divergence. This resource has the potential to offer high flexibility for many possible applications, outperforming previously used markers in many ways: genome coverage and ancestry detection for instance, but also in terms of cost and efficiency to obtain individual genotypes. We hope that it will be useful to the population geneticists' community working on brown trout and call for future studies across the species' range.

## Declarations

*Ethics approval and consent to participate*

The Direction Départmentale des Territoires et de la Mer of departments gave their ethical approval for the field sessions and trout capture.

*Availability of data and material*

SNPs sequences and information are available on Figshare.

DOI: 10.6084/m9.figshare.8174708

*Competing interests*

We have no competing interests and we declare no conflict of interests.

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EDF; Poulet Nicolas, AFB), and have contributed to the design of the study, the collection, analysis, and interpretation of data, and in writing the manuscript.

*Authors' contributions*

KSP, SB, NP and LT designed the experiment and coordinated the study; KSP, ML, BG, LT, NP, GM, JML, PB, and SB conducted sampling; ML, PAG, BG and PB generated the variation map used for developing the SNP panel; KSP, ML, GM and SB carried out the experimental lab work; KSP and ML ran the statistical analyses; KSP, ML, PAG, GB, LT, NP, and SB interpreted the data; KSP, ML PAG, and SB wrote the first draft of the manuscript; BG, LT, NP, GM, JML and PB read, commented and corrected the initial draft, and all authors gave final approval for publication.

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## Supplementary material for Chapter I

*Appendix S1.1 : RAD data set of 12204 sequences each containing one or two SNPs, with minimum allele frequency of 5% or higher, no SNPs in the first or last 30 bp of the RAD, no unsequenced nucleotides, and which can be positioned on the S. trutta linkage map*

This file contains for each RAD, identification (Locus_ID ), chromosome on Atlantic Salmon CHROMsalar), linkage group on the Brown trout linkage map (LG_Trutta),Position on the brown trout linkage group (Pos_cM_Trutta), positions on the Atlantic salmon chromosome (POSsalar_snp1, POSsalar_snp2), position of the SNP on the Rad tag (POStag_snp1, POStag_snp2), recombination rate ( recombination_rate_cM.Mb), sequence of the tag (TAG), sequencing direction (Orientation), and position of the rad (POS_RAD).

See file «12204RADsFinal.txt» in DOI: *10.6084/m9.figshare.8174708*

*Appendix S1.2: SNPs used for low density arrays (5 basins and Taurion test)*

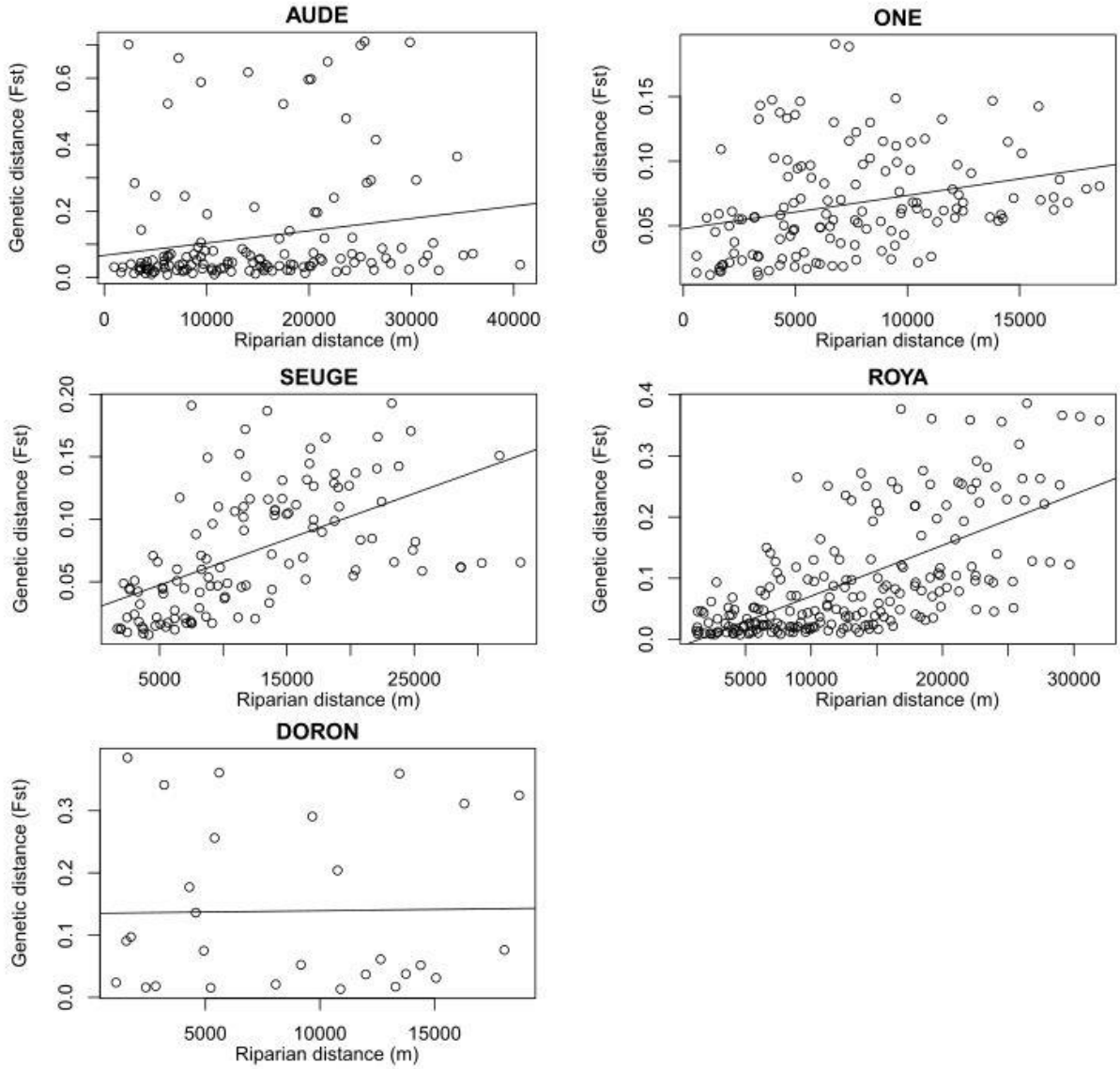
See file «SNPsLowDensityFinal245.txt» in DOI*: 10.6084/m9.figshare.8174708*

This file contains for each SNP, identification (SNP_ID), linkage group on the Brown trout linkage map (LG_Trutta), position of the SNP on the Rad tag (POStag_snp), recombination rate (recombination_rate_cM.Mb), sequence of the tag (TAG_sequence).

It also gives success at genotyping information:

- listSuccessTests162 : SNPs which have been genotyped (when TRUE) in the five river basins tested for genotyping success.
- listTaurion182: SNPs which have been genotyped (when TRUE) in the five river basins tested for genotyping success, and added SNPs which have been genotyped in the Taurion basin (after removing the 30 which did not work in the five basins, then the 10 which did not work in the Taurion).

*Appendix S1.3: Isolation by distance patterns for each river basin, represented by plots of pairwise Fst values against pairwise riparian distance between sites*



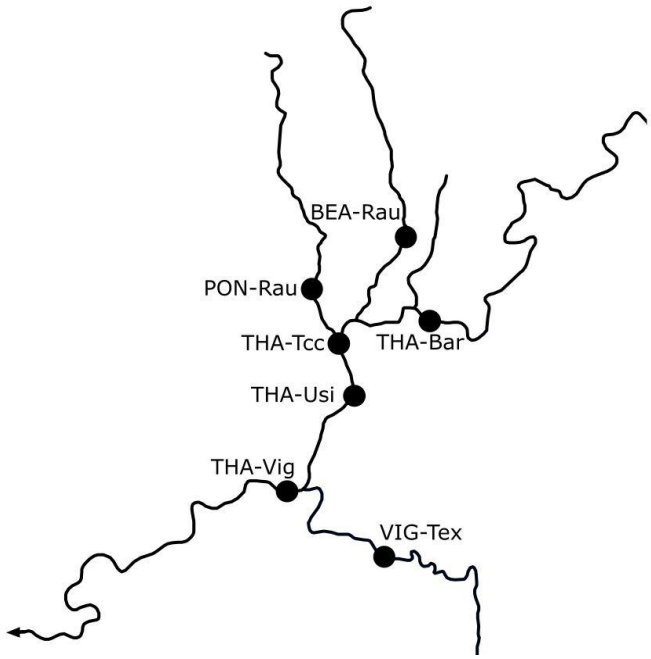
*Appendix S1.4: Pairwise Fst values (calculated with adegenet R package) between sites measured with SNPs and with microsatellites*

| | Pop | BEA-Rau | PON-Rau | THA-Bar | THA-Tcc | THA-Usi | THA-Vig | VIG-Tex |
|---|---|---|---|---|---|---|---|---|
| SNPs | **PON-Rau** | 0.063 | | | | | | |
| | **THA-Bar** | 0.0579 | 0.0173 | | | | | |
| | **THA-Tcc** | 0.0658 | 0.0175 | 0.0175 | | | | |
| | **THA-Usi** | 0.0695 | 0.0215 | 0.0095 | 0.0193 | | | |
| | **THA-Vig** | 0.0762 | 0.0263 | 0.0126 | 0.0206 | 0.0158 | | |
| | **VIG-Tex** | 0.0875 | 0.0337 | 0.0169 | 0.0238 | 0.012 | 0.0142 | |
| | **PIS-Sou** | 0.199907 | 0.153376 | 0.145969 | 0.153629 | 0.141601 | 0.138166 | 0.140239 |
| Microsatellites | **PON-Rau** | 0.0389 | | | | | | |
| | **THA-Bar** | 0.0312 | 0.0207 | | | | | |
| | **THA-Tcc** | 0.0349 | 0.0115 | 0.0102 | | | | |
| | **THA-Usi** | 0.0397 | 0.0216 | 0.0127 | 0.0128 | | | |
| | **THA-Vig** | 0.0527 | 0.0329 | 0.019 | 0.0235 | 0.0121 | | |
| | **VIG-Tex** | 0.0489 | 0.0291 | 0.0193 | 0.0222 | 0.0138 | 0.0141 | |
| | **PIS-Sou** | 0.0971 | 0.0626 | 0.0706 | 0.0677 | 0.0562 | 0.0664 | 0.0687 |

*Appendix S1.5: Sample sizes and mean (± standard error) body length in mm of fish sampled in each site and each basin for testing the 192 SNPs panel's genotyping success.*

| AUDE | | | DORON | | | ONE | | | ROYA | | | SEUGE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | N | Mean body length (±SE) | Site | N | Mean body length (±SE) | Site | N | Mean body length (±SE) | Site | N | Mean body length (±SE) | Site | N | Mean body length (±SE) |
| Agu-Sou | 27 | 157(±42) | All-All | 17 | 126(±55) | Cou-Lar | 30 | 111(±26) | Ben-Cas | 8 | 158(±33) | Ber-Ben | 11 | 114(±28) |
| Aig-Pou | 30 | 139(±33) | Boz-Car | 12 | 173(±56) | Lab-Cas | 17 | 112(±23) | Bie-Cas | 30 | 157(±47) | Bui-Pie | 6 | 96(±27) |
| Aig-Sou | 30 | 143(±38) | Boz-Gib | 15 | 127(±36) | Nga-Jur | 30 | 152(±40) | Bie-Mai | 30 | 172(±33) | Cla-Tou | 9 | 105(±55) |
| Art-Lau | 30 | 138(±38) | Boz-Vil | 18 | 165(±41) | Nga-Mar | 30 | 135(±18) | Bie-Min | 30 | 171(±36) | Lav-Riv | 15 | 138(±51) |
| Aud-Car | 30 | 166(±34) | Cha-Chi | 22 | 133(±40) | Nga-Vga | 30 | 127(±35) | Cai-Gaf | 30 | 170(±48) | Lav-Suc | 30 | 122(±31) |
| Aud-Far | 30 | 165(±33) | Chv-Ger | 23 | 177(±69) | Noo-Ast | 30 | 135(±26) | Lev-Bri | 30 | 140(±27) | Pon-Amo | 30 | 127(±26) |
| Aud-Fou | 30 | 135(±25) | Pra-Fra | 14 | 153(±62) | Noo-Cas | 30 | 144(±18) | Lev-Ten | 30 | 153(±30) | Pon-Bom | 30 | 133(±32) |
| Aud-Nen | 30 | 123(±54) | Ros-Mug | 3 | 137(±72) | Noo-Esp | 30 | 129(±38) | Mag-Cem | 30 | 176(±32) | Pon-Cha | 30 | 148(±29) |
| Aud-Puy | 30 | 121(±31) | | | | Noo-Lac | 30 | 109(±26) | Ref-Ric | 30 | 123(±23) | Pon-Tis | 30 | 129(±18) |
| Aud-Ser | 30 | 156(±22) | | | | Noo-Sav | 30 | 111(±63) | Roy-Chi | 24 | 196(±24) | Ser-Sau | 6 | 143(±28) |
| Bai-Pas | 14 | 153(±28) | | | | Noo-Tre | 30 | 157(±33) | Roy-Dal | 30 | 143(±27) | Seu-Cch | 30 | 110(±21) |
| Bru-Mij | 30 | 127(±26) | | | | Noo-Voo | 30 | 138(±30) | Roy-Ort | 30 | 131(±18) | Seu-Cou | 30 | 134(±27) |
| Bru-Uss | 30 | 151(±21) | | | | Nou-Boo | 28 | 173(±43) | Roy-Pie | 21 | 207(±31) | Seu-Cro | 30 | 165(±33) |
| Cam-Sau | 14 | 118(±46) | | | | Nou-Cir | 30 | 134(±42) | Roy-Sca | 30 | 172(±47) | Seu-Fag | 30 | 126(±32) |
| Que-Mas | 29 | 131(±36) | | | | Nou-May | 30 | 155(±34) | Roy-Ten | 30 | 141(±27) | Seu-Rod | 30 | 157(±38) |
| Que-Ria | 30 | 158(±37) | | | | Nou-Spo | 30 | 140(±38) | ROY-Fon | 30 | 199(±65) | Seu-Sau | 30 | 138(±19) |
| Roq-Sau | 9 | 154(±25) | | | | One-Bag | 30 | 147(±28) | ROY-Evc | 30 | 196(±59) | | | |
| | | | | | | | | | Roy-Bre | 30 | 193(±63) | | | |
| | | | | | | | | | Roy-Amb | 30 | 171(±52) | | | |
| | | | | | | | | | Roy-Gia | 30 | 162(±47) | | | |
| | | | | | | | | | ROY-Vei | 30 | 182(±40) | | | |
| Total | 453 | 143(±37) | | 124 | 150(±56) | | 495 | 136(±38) | | 413 | 167(±23) | | 377 | 134(±34) |

*Appendix S1.6: Map of the Taurion River showing sampling points (black dots). Sample sizes by site, and mean (±SE) body length in mm) from the Taurion River in table. Map was generated by authors on ArcGis and assembled using Inkscape.*




| Site | N | Mean (±SE) body length (mm) |
|---|---|---|
| BEA-Rau | 30 | 111(±38) |
| PON-Rau | 21 | 111(±37) |
| THA-Bar | 28 | 221(±108) |
| THA-Tcc | 30 | 144(±46) |
| THA-Usi | 30 | 193(±40) |
| THA-Vig | 26 | 167(±51) |
| VIG-Tex | 25 | 151(±44) |

The Taurion River is a Snow/rain fed stream from the Massif central (France). We sampled between 21 and 30 individuals per site. 4 sites were located on the mainstream (THA sites), the 3 others on tributaries. These sampling sites are represented by black dots on the map.

*Appendix S1.7: Genotyping microsatellites*

Individual multilocus genotypes were obtained at a total of 13 microsatellite markers (BS131, One9, SSosL311, SsoSL438, T3-13, Sfo1, Ssa064, Ssa103, Ssa417, Ssa-60NVH, Ssa85DU, Ssa-TAP2a and SsoSL417; see Saint-Pé et al. [1] for details on these markers).

The 13 markers were assembled in 3 multiplexes allowing co-amplification of several markers in a single Polymerase Chain Reaction (PCR). Genomic DNA was extracted from the fin clips using a salt-extraction protocol [2]. The loci were amplified using the QIAGEN Multiplex PCR Kit (Qiagen, Valencia, CA, USA). PCR reactions were carried out in a 10 µL final volume containing 5–20 ng of genomic DNA, 5 µL of 2xQIAGEN Multiplex PCR Master Mix, and locus-specific optimized combination of primers. PCR amplifications were performed in a Mastercycler PCR apparatus (Eppendorf, Hauppauge, NY, USA) under the following conditions: 15 min at 95°C followed by 30 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C and finally followed by a 60 min elongation step at 72°C. Amplified fragments were then separated on an ABI PRISM 3730 automated capillary sequencer. Allelic sizes were finally scored using GENEMAPPER v.4.0 (Applied Biosystems, Foster City, CA, USA). We investigated for anomalies owed to genotyping (e.g. large allele drop; null alleles) using MicrocheckerV 2.2 [3]. As in Saint-Pé et al. [1], we tested for linkage disequilibrium and selection, which none of these 13 loci displayed of.

*References Appendix S7*

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# Chapter II. Combining genetics, stable isotopes and morphometrics improves assignments for movement detection: a case study in Brown trout (*Salmo trutta*) populations

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**Key words:** Dispersal, brown trout, SNPs, isotopes, morphometrics, individual assignment.

## Summary

Assessing landscape connectivity is of crucial importance for biodiversity dynamics, and assignment methods are valuable approaches for this purpose. Assignment methods are generally performed using a single data type such as genetic or isotopic markers. However, relying on a single marker type for detecting movements can be sub optimal, for instance at small spatial scales when populations are poorly differentiated. Here, we tested whether combining genetic, (stable) isotopic and morphometric markers improves power and accuracy of assignments, and we assessed the contribution of each data type to assignment rate. To do so, we developed an approach based on a machine learning algorithm (random Forest) and a permutation procedure that we applied to two brown trout (*Salmo trutta*) populations. We genotyped 830 brown trout using SNP markers, quantified their stable isotope signatures (δ15N and δ13C values), and inferred they morphometry using a geometrical approach. We found a significant increase in assignment power when combining some data sets, in particular genetics and isotopes, as indicated by the detection of nearly three times more residents as well as a several-fold reduced number of unassigned individuals compared to these data types used alone. Then, we found that isotopes were the most powerful data type to discriminate and assign individuals to their population of origin, while morphometrics were the least powerful.

Our findings demonstrate the usefulness of combining marker types for inferring movements at small spatial extents (within river basin), and that stable isotopes appeared particularly relevant for identifying local scale movements. Finally, we provide an operational statistical framework to generalize the use of multiple marker types to assess movements in natural populations.

## Introduction

Landscape connectivity, the permeability of the landscape matrix to individual movements, has widely been shown to be crucial for individuals to complete their life cycle (Dunning et al., 1992), ensuring within- and between- population dynamics, and thus viability (Hanski, 1998; Yen, 1999; Hanski and Gilpin, 1991; Wiens, 2001; Van Dyck and Baguette, 2005; Clobert, 2012; Wiens et al., 1997). Understanding how habitat fragmentation, now considered as one of the first causes of biodiversity loss (Wilson et al., 2016), affects population functioning through movements is therefore a growing concern worldwide (Young et al. 1996; Fischer, and Lindenmayer 2007; Cote et al. 2017; Fahrig and Merriam, 1994). Various methods and data types have been used to measure individual movements (Cayuela et al., 2018, Kindlmann and Burel 2008). Directly measuring movement has been done successfully using capture-recapture protocols, pit-tagging and telemetry for instance (Cooke et al., 2013). However, it is a difficult, expensive and time consuming task that rarely allows for large sample sizes (Koenig et al., 1996; Birnie-Gauvin et al., 2018).

More recently developed, genetic-based methods allow indirect estimations of movements, under the hypothesis that population genetic structure reflects the impact of natural and anthropogenic features on patterns of landscape functional connectivity (Davey et al., 2011; Forester et al., 2018; Manel et al., 2003; Holderegger et al., 2006; Broquet and Petit, 2009; Baguette et al., 2013). Genetic data have traditionally been used to assess patterns of effective dispersal, that is, inter-population movements followed by reproduction events, using methods such as edge detection methods, clustering algorithms, parentage analyses, Fst based estimates, and many others (see Cauyela et al. 2018 for a recent review). However, these methods reflect rather long-term dispersal events, with a deferred response regarding ‘real-time’ changes in movement patterns (Landguth et al. 2010, Whitlock and McCauley, 1999).

Individual-based genetic assignment methods, based on the use of genotypic data to discriminate among resident and immigrant individuals in local populations and determine the population of origin of immigrants, appear as an interesting alternative for rapid estimation of “real-time” movements. Indeed, they do not rely on any theoretical assumptions as other genetic tools and allow detecting different kinds of movements, such as non-effective dispersal events, but also migration events and/or foraging movement (Paetkau et al., 2004; Raeymaekers et al., 2008; Saint-

Pé et al., 2018). Genetic assignment methods are highly accurate for studying movement particularly in cases where dispersal is unlikely, thus between distant and/or well differentiated populations (Berry et al., 2004; Manel et al., 2002; Paetkau et al., 1995). However, they were also shown to be poorly accurate between less differentiated populations, for instance at fine spatial scale (Anderson et al., 2008; Berry et al., 2004; Manel et al., 2005).

Although assignment procedures have been initially developed for genetic markers, these approaches can use other data types, and some studies have recently take advantage of this (Begg and Waldman, 1999). Data types that have been successfully used for individual assignments are various, and include morphological traits (Bagherian and Rahmani, 2007; Caccamise et al., 2000), parasites (Timi et al., 2005), fatty acid composition (Czesny et al., 2000), and elemental or isotopic composition contains in solid structures (fish otoliths: Martin et al., 2013; bird feathers: Hobson, 1999; invertebrate shells: Becker et al., 2007). They generally provide complementary and alternative information to genetic markers, mainly because they vary at different spatial and temporal scales than genetic markers (Cook et al., 2007). Moreover, combining different maker types may provide better discrimination among individuals and populations than genetics alone, and thus higher assignment accuracy and power (Clegg et al., 2003; Gómez-Díaz and González-Solís, 2007; Kelly et al., 2005; Smith and Campana, 2010). An interesting example of how coupling methods can better capture movement events concern Atlantic salmon (*Salmo salar*) in which descendants of hatchery-born Salmon were shown to be born in the wild using genetics and geochemistry of otoliths, whereas they would have been identified as hatchery-raised juveniles if considering only genetic data (Perrier et al. 2011).

Recently, new methods of supervised machine learning (Kotsiantis, 2007) were developed, and appeared very efficient for robustly discriminating among populations and assigning individuals to their populations or origin with the use of different types of data. Supervised learning aims at predicting the value of either a categorical or a continuous variable through the use of a training set of labeled data whose true outcome response is known, to ultimately train a predictive algorithm. Among these methods, Random forests in particular are highly interesting for this purpose (Chen et al., 2018; Cutler et al., 2007). They consist in “bagged decision tree models”, i.e. predicting a value or a class by averaging the value obtained by many different uncorrelated decision trees (Breiman, 2001). Moreover, these methods present high versatility and low bias, do

not suffer from the numerous requirements and assumptions of classical (genetic) assignment methods, and have already been used successfully for assignment purposes (Guinand, 2002; Schrider and Kern, 2018; Sheehan and Song, 2016).

Rivers are particularly concerned with fragmentation, and conservation of these ecosystems is of growing concern (Dudgeon et al., 2006; Birnie-Gauvin et al., 2017; Reid et al., 2018). There are more than 45 000 large dams in the world (Duflo and Pande 2007), and in France only for instance, the estimated number of riverine obstacles is above 80 000 (ROE: référentiel des obstacles à l'écoulement). Their effects on riverine fish notably are of high concern (Calò et al., 2013; Heggenes et al., 2006; Junker et al., 2012), and have thus been widely studied using assignment methods (Barnett-Johnson et al., 2010; Morrissey and Ferguson, 2011; Saint-Pé et al., 2018). However, studies combining data types are scarce, and in most cases, occur at very large scales and/or concern highly differentiated populations within contrasted environments. For instance, isotopes are often used as a tool to measure movement at very large scales, between freshwater and saltwater movements (Acolas et al., 2008; McCarthy and Waldron, 2000), or movements between anthropized and natural environments, the former being characterized with enhanced $\delta^{15}N$ (Hansson et al., 1997). To our knowledge, no studies have combined different data types to assess fish movements at a within-basin fine-scale resolution.

Here, we used a headwater stream fish, the brown trout (*Salmo trutta*), in two independent river catchments (i) to assess the benefits of combining genetics, carbon and nitrogen stable isotopes, and morphometrics in assigning individuals to their population of origin and (ii) to identify which of these markers is the most powerful for assignment. We used a random forest classification algorithm that we combined to a robust permutation procedure to determine individual probabilities of originating from each sampled site. We apply this approach to every possible combination of data (i.e. genetics only, isotopes only, morphometrics only, genetics + isotopes, genetics + morphometrics, isotopes + morphometrics, and genetics + isotopes + morphometrics) to assess which data type(s) can be of use at this spatial extent, and how combining datasets can improve assignment power. At this relatively small spatial scale, we expected between-population discrimination to be low, regardless the data type used. However, different data types are supposed to be uniquely distributed within landscapes for different reasons: genetic drift and selection for genetic markers (Hartl and Clark, 2007), geology and trophic interactions for isotopes (Peterson

and Fry, 1987), phenotypic responses to environmental constraints for morphometrics, either through selection or plasticity (Cunico and Agostinho, 2006). We thus expected that by combining data types, populations should be better discriminated than with only one data type used. Higher discrimination between populations should provide better assignment accuracy, that is, higher assignment probabilities for each individual, which in turn translates into a reduced number of unassigned individuals, and into the detection of more "residents" (Manel et al., 2002; Paetkau et al., 1995). Combining data types should also imply higher statistical power, with a reduced type I and type II error rates (i.e. erroneously classifying a resident as migrant or a migrant as resident, respectively; Maxwell et al., 2008). Following these ideas, we expected that combining all datasets (genetics + isotopes + morphometrics) should provide the highest discrimination between brown trout populations, and therefore the highest assignment accuracy and statistical power. Finally, we discuss the idea that data types might convey unique biological information about the spatio-temporal dynamics of different kinds of movements (foraging, migration, natal dispersal), further highlighting the potential benefits of considering different data types in supervised assignment methods.

## Methods

*Field sampling*

We sampled two independent French river basins (Figure 1) in which the fish community is dominated by brown trout (*Salmo trutta*): the Aude River (Mediterranean outlet) and the Seuge River (Atlantic outlet). Brown trout were sampled using a single-pass electric-fishing protocol from 33 sites in total, with an aim of 30 individuals per site. We focused on small individuals in order to only sample one age class (1 year-old), based on unpublished length-frequency distributions (Tissot et al., 2016-2018) as we did not use scale analysis. In total, we captured 830 individuals (453 from the Aude River, 377 from the Seuge River), with sample sizes ranging from 6 to 30 per site. Each individual was measured (total length in mm; Table 1) and a pelvic fin clip was taken for genetic and isotopic analyses. All individuals were released alive to their original sampling site.

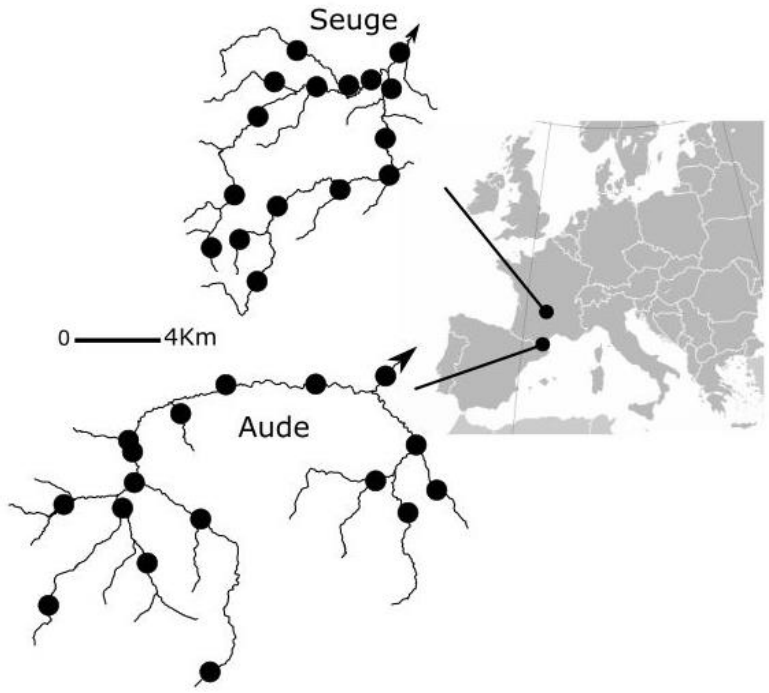


*Figure 2.1: Location of the two studied systems in France and locations of sampled sites within each basin. The Seuge River is part of the Atlantic lineage, whereas the Aude River is part of the Mediterranean lineage. Thirty three sampling sites have been selected within each river basin (black dots on river networks).*

| AUDE | | | SEUGE | | |
|---|---|---|---|---|---|
| Site | N | Mean Body length (±SE) | Site | N | Mean Body length (±SE) |
| Agu-Sou | 27 | 157(±42) | Ber-Ben | 11 | 114(±28) |
| Aig-Pou | 30 | 139(±33) | Bui-Pie | 6 | 96(±27) |
| Aig-Sou | 30 | 143(±38) | Cla-Tou | 9 | 105(±55) |
| Art-Lau | 30 | 138(±38) | Lav-Riv | 15 | 138(±51) |
| Aud-Car | 30 | 166(±34) | Lav-Suc | 30 | 122(±31) |
| Aud-Far | 30 | 165(±33) | Pon-Amo | 30 | 127(±26) |
| Aud-Fou | 30 | 135(±25) | Pon-Bom | 30 | 133(±32) |
| Aud-Nen | 30 | 123(±54) | Pon-Cha | 30 | 148(±29) |
| Aud-Puy | 30 | 121(±31) | Pon-Tis | 30 | 129(±18) |
| Aud-Ser | 30 | 156(±22) | Ser-Sau | 6 | 143(±28) |
| Bai-Pas | 14 | 153(±28) | Seu-Cch | 30 | 110(±21) |
| Bru-Mij | 30 | 127(±26) | Seu-Cou | 30 | 134(±27) |
| Bru-Uss | 30 | 151(±21) | Seu-Cro | 30 | 165(±33) |
| Cam-Sau | 14 | 118(±46) | Seu-Fag | 30 | 126(±32) |
| Que-Mas | 29 | 131(±36) | Seu-Rod | 30 | 157(±38) |
| Que-Ria | 30 | 158(±37) | Seu-Sau | 30 | 138(±19) |
| Roq-Sau | 9 | 154(±25) | | | |
| Total | 453 | 143(±37) | | 377 | 134(±34) |

*Table 2.1 Number of individuals sampled and their average (±Standard error) body length for each site (in mm).*

*Genotyping*

Individual multilocus genotypes were obtained for 162 SNPs markers (Saint-Pé et al., 2018), using KASPAR technology performed by LGC Genomics (Smith and Maughan, 2015). All polymorphic markers were kept regardless of selection and linkage disequilibrium, as these processes may increase assignment power and may thus be exploited for investigating population structure (Morin et al., 2009; Nielsen et al., 2009; Waples and Gaggiotti, 2006). For further analyses, we discarded individuals for which more than one third of SNPs were not genotyped, leaving us with 824 individuals (449 in the Aude River, 375 in the Seuge River).

As stocking is a common practice in brown trout and has been shown to affect dispersal and spatial patterns of genetic diversity in the river basins studied here (Saint-Pé et al., 2018), we decided to filter out all individuals showing more than 30% of captive-bred ancestry in order to only keep supposedly native individuals. Based on genetics, individual level of hatchery ancestry was determined using STRUCTURE (Pritchard et al., 2000) as in Saint-Pé et al. (2018, 2019).

This only concerned the Aude basin (48 individuals), as the Seuge basin showed only very slight traces of hatchery ancestry (all individuals assigned at less than 0.3 to the captive-bred cluster). The native genetic dataset comprised 776 individuals (401 for the Aude River, 375 for the Seuge River), and only these individuals were kept for subsequent analyses.

*Carbon and Nitrogen stable isotopes*

For all 776 native individuals, fin samples were dried at 60 °C for 72 hours, and then loaded into 1.5mL Eppendorf tubes, labeled and parafilmed. These fins were analyzed at Cornell University (COIL, Ithaca NY), using an elemental analyzer plumbed into a Finnigan MAT Delta Plus isotope ratio mass spectrometer to measure samples for $\delta^{13C}$ and $\delta^{15}N$. Isotope analysis failed for three of the individuals, which were therefore discarded from all analyses. The final isotope dataset comprised 773 individuals (399 for the Aude River, 374 for the Seuge River).

*Morphometrics*

While on the sampling sites, each individual was placed (after having been anaesthetized) on a polystyrene board with a ruler and a color sampler, and was photographed with a digital camera, always at the same magnification (X 2.8). Using the TPS suite (tpsDig 2.10 and tpsUtil 1.38; Rohlf, 2015), 12 morphological landmarks (Details in Appendix S1) were digitized from each fish. We then used the GeoMorph R package (Adams and Otárola-Castillo, 2013) to calculate the position of each individual along relative warps, which consist in major axes of body shape variation after Principal Components Analyses (Park et al., 2013). We kept the first six relative warps (75.7% of explained variance) in order to explain most of the morphometric variation. However, as the first relative warp represented a deformation that likely result from a bias in fish positioning on the board (29.8% of explained variance), we discarded it from the morphometrics dataset.

The final dataset therefore comprised 773 individuals with all completed data types.

*Assignment method*

First, and for each combination of datasets (i.e. G = genetics only, I = isotopes only, M = morphometrics only, G+I = genetics + isotopes, G+M = genetics + morphometrics, I+M =

isotopes + morphometrics, and G+I+M = genetics + isotopes + morphometrics), we calculated individual assignment probability to each site using the random forest classification method (R-package randomForest; Liaw and Wiener, 2002) with the following formula: "site ~ data combination". The number of trees as well as the number of splits of the variables ("ntree" and "mtry" parameters, respectively) were chosen to provide the lowest and more stable out-of-bag error rate (OOB; classification to sites different from capture site).

When markers are poorly discriminant (e.g. between populations are poorly differentiated), Type I (erroneously classifying a resident individual as a migrant) and Type II (erroneously classifying a migrant individual as a resident) errors can be high, and can be difficult to identify. In order to set a conservative threshold in assignment probabilities and limit these errors, we developed a permutation-based procedure. The aim of this procedure was to calculate the theoretical maximum probability of assignment to a site under the assumption that sites were totally undifferentiated, so as to increase the overall statistical power of the assignment procedure and limit type I and II errors (at the cost of more unassigned individuals that cannot confidently be assigned to a population). To do so, we performed 1000 random permutations of individuals across capture sites and ran RF classification on all the 1000 permuted datasets, with the same number of trees and dataset splits as in the first step, to compute the $95^{th}$ quantile of maximum possible assignment values over the 1000 runs.

Following these classification and permutation procedures, individuals were considered as "resident" if the observed probability of assignment was the highest for their capture site (which is conservative and the most parcimonious). Individuals were considered as "migrant" *if* the observed probability of assignment was highest for another site than its site of capture, and only *if* this probability was higher than the theoretical maximum assignment probability expected if populations are undifferentiated and as computed from permutations. Individuals assigned to a site different from their capture site, but with a probability lower than the theoretical assignment probability were considered as "unknown" (as for these individuals type I errors are expected to be high). This approach was performed for each basin separately and for each combination of variables (G, I, M, G+I, G+M, I+M and G+I+M).

*Contribution of combining data types*

First, *we used the* mean decrease in Gini coefficient (Breimann et al. 2001) to calculate the contribution of each variable type (genetics, isotopes and morphometrics) to the randomForest model using the G+I+M combination. The mean decrease in Gini coefficient represents the mean decrease in accuracy of the model due to the exclusion (or permutation) of a variable, which is determined during the OOB error calculation: the more the accuracy decreases due to the exclusion of a variable (or group of variables in our case), the more important that variable or group of variables is for classification of the data and individual assignments.

*Then,* we assessed the assignment accuracy of each data combination across river basins. If combining different data types yields higher inter-population discrimination, the assumption that we considered as the most parsimonious is that the inferred number of residents should be higher. We thus considered individual assignment probability to the capture site as a proxy for assignment accuracy. Differences in accuracy between combinations of variables were assessed using a linear mixed-effect model (*lme* function from the *nlme* R-package), with the individual assignment probability to the capture site as the dependent variable, the data combination as a fixed effect, and sites nested within river basins as random effects. As we expected that combining all datasets (G+I+M) should provide the highest assignment accuracy, we used the *relevel* R function to set this combination as the reference factor in our model. Therefore, the model provides the gain/loss in accuracy of all possible data combinations compared to the full combination of genetic, isotopic and morphometric data.

To assess the differences in assignment accuracy between basins, we investigated the conditional modes of the basin random effects using the *ranef* function from the *nlme* R-package. Conditional modes measure the difference between the average predicted response in each basin and the response predicted for a particular individual.

*Individual decisions: migrant, resident, unknown*

Finally, for each basin, we counted individuals considered as migrants, residents and unknown after the permutation threshold was applied for each data type and combination (G, I, M, G+I, G+M, I+M and G+I+M). The first objective was here to estimate whether combining datasets

would indeed increase between site discrimination, and thus accuracy (under the hypothesis that increasing accuracy results in maximizing the number of residents as discussed above). The second objective was to assess the differences in statistical power between data types and combinations, and therefore to determine which combinations enabled to minimize the number of unassigned ("unknown") individuals.

## Results

*Data types and combinations comparison*

Relative contributions of each data type to the model combining them all (G+I+M) are shown in Figure 2. The contribution index was much higher for isotopes than for the two other markers, especially in the Aude River. Surprisingly, genetics contributed little to this model, less than morphometrics, although assignment accuracy was slightly higher for genetics, as indicated below.


*Figure 2.2: Mean decrease in Gini, i.e. the contribution of each variable type to the randomForest model when all variables are used in combination to explain individual assignment probability to each sampling site.*

Assignment accuracy results for each marker combination and for each river basin are shown in Figure 3. Overall, the combination of genetics and isotopes (G+I) showed the highest assignment accuracy (although Isotopes alone were slightly more accurate in the Aude River). The combination of genetics, isotopes and morphometrics (G+I+M) was not significantly less accurate

(p-value = 0.91, Table 2A), although including morphometric markers did not increase accuracy. Conversely, all other combinations (and marker types considered alone) were significantly less accurate (p-value < 0.001, Table 2A), in particular morphometrics (M, -0.52 in accuracy compared to the reference combination G+I+M), genetics (G, -0.41 in accuracy compared to the reference combination) and their combination (G+M, -0.43 in accuracy compared to the reference combination).

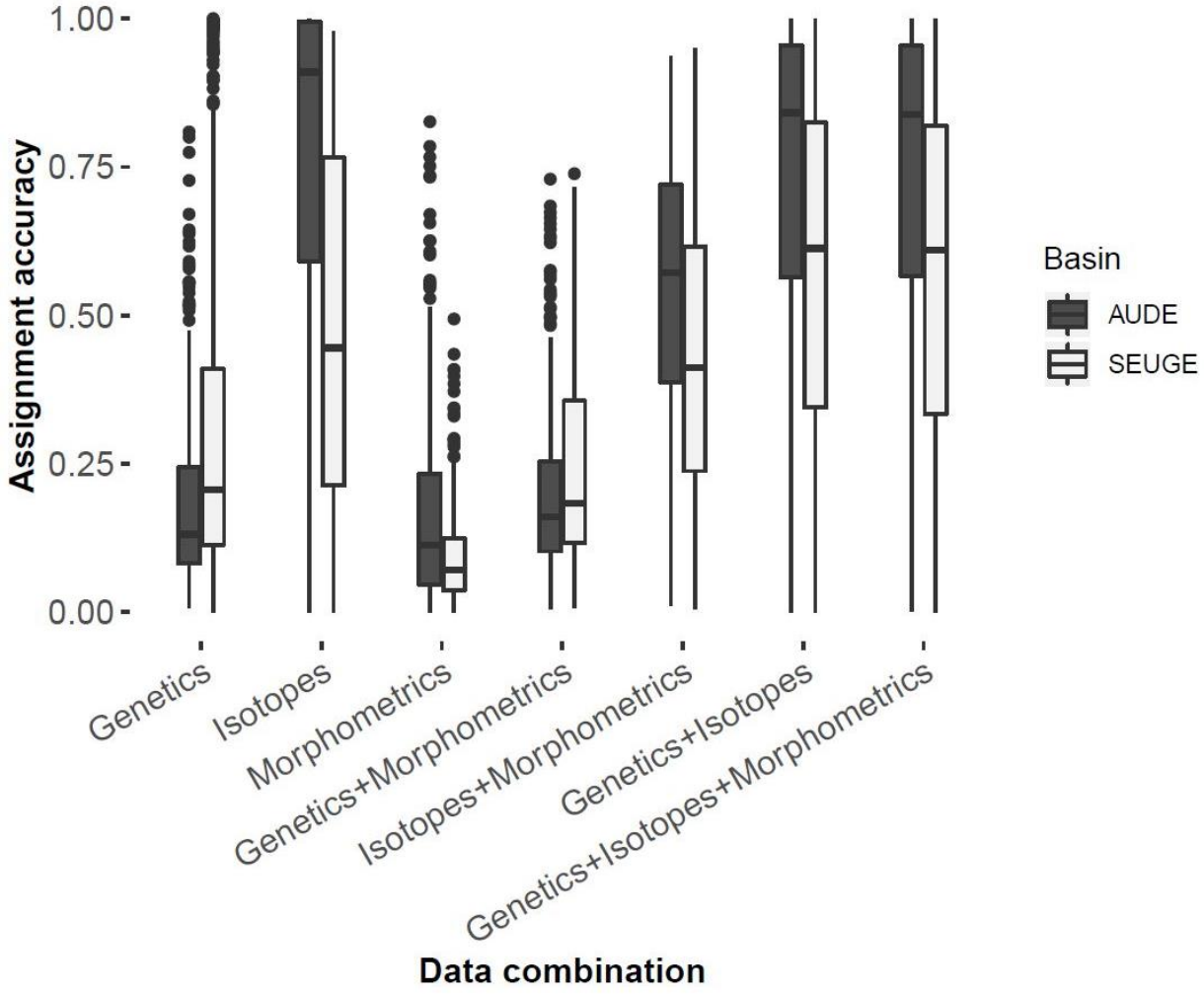


*Figure 2.3: Assignment accuracy boxplots for all data types combinations and each river basin.*

| **A** | Value | Std.Error | DF | t-value | p-value |
|---|---|---|---|---|---|
| Intercept (G+I+M) | 0.645 | 0.050 | 5399.000 | 12.849 | 0.000 |
| G | -0.408 | 0.012 | 5399.000 | -35.394 | 0.000 |
| G+I | 0.001 | 0.012 | 5399.000 | 0.118 | 0.906 |
| G+M | -0.433 | 0.012 | 5399.000 | -37.568 | 0.000 |
| I | -0.045 | 0.012 | 5399.000 | -3.896 | 0.000 |
| I+M | -0.160 | 0.012 | 5399.000 | -13.899 | 0.000 |
| M | -0.523 | 0.012 | 5399.000 | -45.351 | 0.000 |

| Random effects | (Intercept) | Residual |
|---|---|---|
| ~1\|BV StdDev | 0.065 | |
| ~1\|Site/BV StdDev | 0.105 | 0.227 |

| **B** | Ranef, level=BV |
|---|---|
| Aude | 0.042 |
| Seuge | - 0.042 |

*Table 2.2: Summary of the linear mixed-effect model explaining assignment accuracy with data combination used as a fixed effect, and sites nested within river basins used as random effects. (A) Gain/loss in assignment accuracy for all combinations compared to the reference combination (G+I+M); (B) Difference in assignment accuracy between basins across all data combinations.*

When comparing averaged assignment accuracy between basins, we found that assignment accuracy was lower in the Seuge River. Indeed, assignment accuracy was overall 0.084 higher in the Aude River than in the Seuge River (Table 2B, Figure 3), however, the uncertainty (standard deviation = 0.065) on the basin effect was higher than the deviation to the intercept due to random effect of the basin.

*Counts of individuals detected as migrants, residents or unassigned*

The percentage of migrants, residents and unknown individuals for each combination of markers used are shown in figure 4. As expected, data combinations with low assignment accuracy led to the highest numbers of both migrants and unknown individuals (Figure 4: G, M and G+M for instance). Interestingly, we found that the two combinations of data types that maximized assignment accuracy (i.e., that maximized the number of individual actually assigned to their capture site; G+I and G+I+M) did not classify any individual as unknown except in the Seuge for the combination G+I+M, despite a dedicated permutation procedure designed to minimize Type 1

error rate by setting conservative theoretical thresholds. These two combinations thus showed both high assignment accuracy and statistical power.

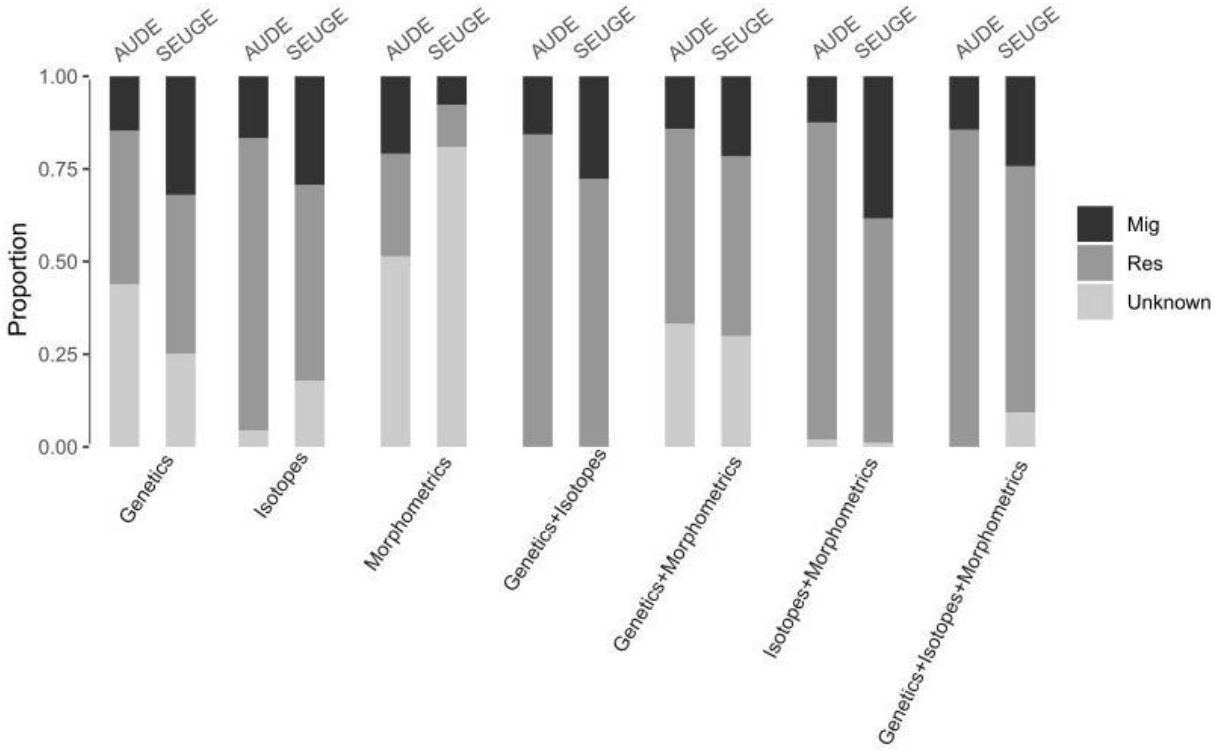


*Figure 2.4: Number of migrants, residents and unclassified individuals for all data types combinations and each river basin.*

## Discussion

*Increasing statistical power in assignment procedures*

We used three marker types that could potentially be of interest to detect movements at the spatial extents we considered, i.e. headwater river basins of a few tens of kilometers across. First, genetics, which have already been used very widely for this purpose, rely on inter-population differentiation to enable sufficient discrimination among populations for detecting movements. As the brown trout exhibits strong population structure at relatively small spatial extents (Carlsson et al., 1999; Jensen et al., 2006), we expected that these markers would be particularly powerful in our case studies. Second, isotopes are also widely used to study movements, however, as they are mainly used in contrasted environments, we expected that their discriminant ability and thus their

power would be lower than genetics in the river basins studied. Finally, we also expected that morphometrics should be poorly discriminant mainly because differential selection pressures should be low at such reduced spatial scales.

Methodologically, the framework we used is similar to that developed in Chen et al. (2018), where assignment accuracy is compared using different data types alone and in combination. However, conversely to Chen et al. (2018), we stuck to random forest classifications to set conservative thresholds in assignment probabilities using a permutation approach we developed, instead of using existing resampling methods. Similarly to these methods, this enabled to diminish Type I and II assignment error rates, particularly in the case of lowly discriminant data. Individuals that do not meet theoretical thresholds are discarded (classified as unknown), which implies a gain in assignment power yet with an increase in the number of unassigned individuals as a counterpart.

Morphometrics exhibited the lowest accuracy and power for assignment. This result is surprising, as most morphological traits have a polygenic basis and are heavily influenced by environmental factors through plasticity (Allendorf and Leary, 1988; Keeley et al., 2007). Diet and vegetation cover (Svanbäck and Eklöv, 2006) for instance were shown to strongly act on morphology in fish even at small spatial scales, and in particular for salmonids, water velocity (Pakkasmaa and Piironen, 2000). At the spatial scales considered, selection on morphological features was expected to be relatively high (Stelkens et al., 2012). We thus suggest that differences in morphology at these scales may be very context dependent, thus in our study systems, the environmental variables acting on morphometrics probably vary insufficiently to differentially select morphotypes. Moreover, even if factors such as competition for resource can select for different morphotypes (Ward et al., 2006), these morphotypes may also be living in sympatry (Moore and Bronte, 2001) in our study systems, thus being unusable for discrimination between populations.

Genetics were not as accurate and powerful as expected for assignment, especially for the Aude River, in which almost half of individuals were not assigned, although less SNPs were available for the Seuge River. This result is in accordance with the expectation that genetic assignment is often more efficient when populations are more genetically differentiated (Berry et al., 2004), as suggested by higher levels of genetic uniqueness measured in the Seuge River than in the Aude

River (average 0.074 in the Seuge, 0.042 in the Aude; Appendix S2). Each river basin being unique, discrimination between populations, and thus of assignment accuracy and power will strongly depend on the history of the river considered in terms of colonization history, demographic history (bottlenecks for instance), selection patterns and many other factors such as the panel of selected markers for instance (Banks et al., 2003). We thus suggest that in cases in which genetic differentiation is low at the "sampling site" level, assignment and migrant detection should be more accurate at the "cluster" level (Berry et al., 2004). However, in terms of management and straightforwardness for instance, working at this abstracted level could be problematic for both sampling designs and interpretation. Therefore in these low genetic differentiation cases, other markers can here be highly valuable, and in our results, isotopes appeared an excellent alternative, particularly in the Aude basin.

Isotopes exhibited the highest assignment accuracy and power. This result was surprising, as we were expecting low inter-population variation given the reduced spatial extent of the studied areas, which is expected to translate into low inter-population differences in isotopic signatures of resources (Ben-David and Flaherty, 2012; Gray et al., 2004; Kennedy et al., 2005). Moreover, resource competition could also have been expected to increase intra-population divergence as a result of specialization at the individual level (Ward et al., 2006). However this was not observed in our results, suggesting that contrary to the variables which act on morphometrics, those acting on isotopic signatures (i.e. geochemical processes) are very contrasted between sites at this spatial extent. This hypothesis was also supported by a similar pattern in periphyton and in invertebrate prey, whose isotopic signatures also exhibited high inter-population and low intra-population variation (Appendix S3). Isotopes therefore appeared as highly discriminant, thus accurate and powerful at the extent of our study system. This is also of great interest because being highly accurate and powerful at the "sampling site" scale, (whereas genetics could bore useful at the "cluster" scale), it can have direct applications, in particular for managers (e.g. for targeting a specific location and identifying specific landscape features which could be constraining movements).

Although in some cases, one data type can be more discriminating and powerful than another, one of our most valuable results is that combining datasets was the best way to gain in assignment efficiency. For instance, if genetics alone were more powerful in the Seuge than in the Aude River,

it was the opposite in the case of isotopes, but by combining genetics and isotopes, accuracy and power were gained in both rivers, although they were still higher in the Aude River, mostly because isotopes were extremely discriminating in this basin. Moreover, we found that the combination of genetics and isotopes was the most powerful, as it was the only combination for which there were no unassigned individuals. We thus argue that combining data types in assignment protocols can highly improve the detection of movements occurring between populations, as suggested in Chen et al. (2018), thus providing valuable information in terms of functional connectivity (Holderegger et al., 2006; Broquet and Petit, 2009; Baguette et al., 2013). However, some cases have shown that this is not always true, for instance, Smith and Campana, (2010) found that combining genetics and otoliths isotopes decreased assignment success. As suggested in Chen et al. (2018), our study also highlights that methods for integrating different data types are still to be improved for bettering assignment.

*Considerations when combining different data types*

The advantage of combining different data types appeared as a very valuable method to increase assignment statistical performance. However, different data types may be associated with different spatio-temporal resolutions and may thus capture different biological processes at different scales (Hall and Beissinger, 2017). For instance, and contrary to the genetic signature of an individual, the isotopic signature of an individual may change over time depending on diet and isotopic turnover rate (Hobson and Wassenaar 2008): assignment procedures based on isotopes will thus only allow detecting short-term movements, depending on the capture vs. movement timing. Therefore, and this has rarely been discussed in assignment methods studies, combining data types such as genetics and isotopes could blur the biological interpretation of inferred movement patterns. We thus suggest that comparing data types in addition to combining them might provide unprecedented biological insights as to the studied movement patterns, although assignment procedures would then lose in accuracy and statistical power. For instance, an individual captured soon after emigrating and before isotopic turnover would be correctly detected as “migrant” with both genetics and isotopes, whereas an individual captured long after dispersal would be assigned to its population of origin and thus detected as “migrant” using genetics, but to the population where it was recently foraging and thus detected as “resident” when using isotopes. On the opposite, for instance in the case of seasonal migration events, an individual captured right after it

returned to its site of origin would be detected as “migrant” using isotopes, but detected as “resident” using genetics.

In our study for instance, the higher proportion of individuals detected as residents when using isotopes than when using genetics (suggesting higher assignment accuracy) could hide the fact that a high proportion of inferred residents are actually longer-term migrants, with a short-term “resident” isotopic signatures but a long-term ”migrant” genetic signature. These hypotheses might of course require further investigation, but similar considerations have been discussed for morphometrics of developing larvae (therefore with a short “turnover time”), where variation among populations differed through time, demonstrating the importance of evaluating divergence over multiple time periods and at a time relevant to the processes being studied (Zellmer, 2018). Nevertheless, we believe that combining different data types may be the most valuable tool when the aim of the study is only to assess landscape functional connectivity regardless of the type of movement and underlying biological mechanisms, as we demonstrated that this procedure provides greater discrimination, and thus accuracy and statistical power.

## Conclusions

Our results showed that different data types can be used separately and combined to increase accuracy in individual assignment probabilities. In particular, stable isotopes of Carbon and Nitrogen were very accurate tools at a short spatial extent, whereas genetic data did not show such a discriminatory power among populations. Cheaper and less time consuming than the use of genetics and morphometrics, our study suggests that isotopes thus have great potential to study movement in stream fish at a small spatial scale. Combining different data types may yield very high assignment accuracy and statistical power, providing valuable insight in terms of landscape functional connectivity. Concomitantly, comparing assignment probabilities based on marker types with different spatial and temporal resolutions may offer unprecedented opportunities to characterize movement patterns, although future studies should now be conducted to further assess the possible benefits of such comparisons.

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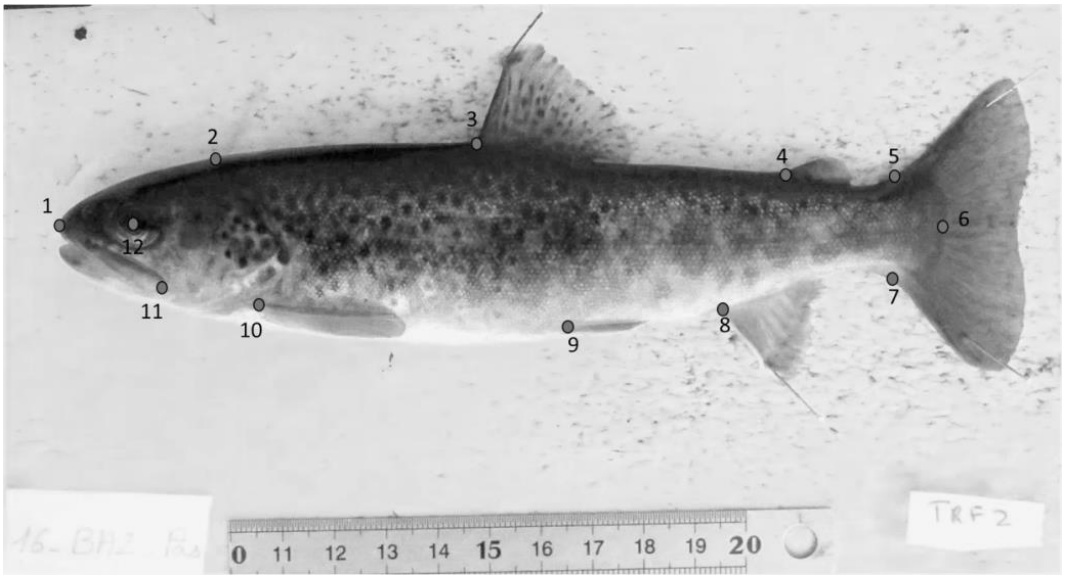
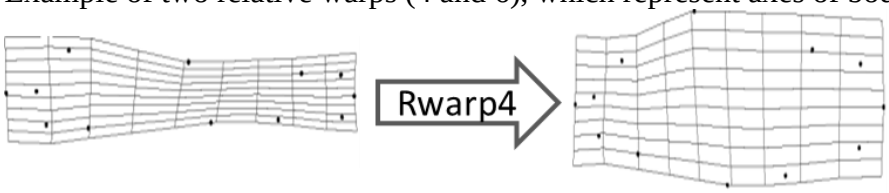
## Supplementary material for Chapter II

*Appendix S2.1: Morphological landmarks used to calculate relative warps*




Example of two relative warps (4 and 6), which represent axes of body shape deformation.




*Appendix S2.2: pairwise genetic differentiation between sample sites for each river basin*

We computed site uniqueness as the average of pairwise FST values observed between a deme and all other demes (Coleman, Weeks, & Hoffmann, 2013; Hedrick, 2005; Prunier et al. 2018) using the adegenet R-package (Jombart et al. 2010), to assess whether higher genetic differentiation would indeed enable better discrimination between populations, and therefore increase assignment accuracy and power.

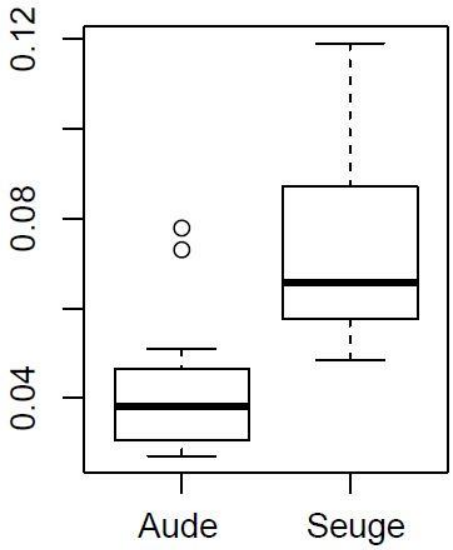
A : Aude pairwise Fst

| | AGUSou | AIGPou | AIGSou | ARTLau | AUDCar | AUDFar | AUDFou | AUDNen | AUDPuy | AUDSer | BAIPas | BRUMij | BRUUss | CAMSau | QUEMas | QUERia | ROQSau |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AGUSou | 0.000 | 0.053 | 0.022 | 0.075 | 0.052 | 0.024 | 0.032 | 0.040 | 0.038 | 0.025 | 0.522 | 0.063 | 0.039 | 0.036 | 0.021 | 0.020 | 0.140 |
| AIGPou | 0.053 | 0.000 | 0.047 | 0.059 | 0.017 | 0.023 | 0.029 | 0.019 | 0.104 | 0.022 | 0.702 | 0.057 | 0.032 | 0.070 | 0.071 | 0.051 | 0.284 |
| AIGSou | 0.022 | 0.047 | 0.000 | 0.072 | 0.047 | 0.023 | 0.034 | 0.037 | 0.038 | 0.029 | 0.524 | 0.067 | 0.042 | 0.044 | 0.022 | 0.024 | 0.143 |
| ARTLau | 0.075 | 0.059 | 0.072 | 0.000 | 0.048 | 0.041 | 0.038 | 0.045 | 0.118 | 0.045 | 0.708 | 0.080 | 0.062 | 0.080 | 0.091 | 0.067 | 0.293 |
| AUDCar | 0.052 | 0.017 | 0.047 | 0.048 | 0.000 | 0.017 | 0.020 | 0.013 | 0.106 | 0.021 | 0.699 | 0.041 | 0.027 | 0.063 | 0.070 | 0.050 | 0.285 |
| AUDFar | 0.024 | 0.023 | 0.023 | 0.041 | 0.017 | 0.000 | 0.009 | 0.011 | 0.065 | 0.010 | 0.597 | 0.032 | 0.015 | 0.032 | 0.036 | 0.022 | 0.196 |
| AUDFou | 0.032 | 0.029 | 0.034 | 0.038 | 0.020 | 0.009 | 0.000 | 0.013 | 0.074 | 0.010 | 0.618 | 0.028 | 0.020 | 0.029 | 0.046 | 0.027 | 0.212 |
| AUDNen | 0.040 | 0.019 | 0.037 | 0.045 | 0.013 | 0.011 | 0.013 | 0.000 | 0.088 | 0.013 | 0.661 | 0.035 | 0.020 | 0.046 | 0.057 | 0.040 | 0.245 |
| AUDPuy | 0.038 | 0.104 | 0.038 | 0.118 | 0.106 | 0.065 | 0.074 | 0.088 | 0.000 | 0.062 | 0.364 | 0.117 | 0.086 | 0.056 | 0.022 | 0.034 | 0.067 |
| AUDSer | 0.025 | 0.022 | 0.029 | 0.045 | 0.021 | 0.010 | 0.010 | 0.013 | 0.062 | 0.000 | 0.588 | 0.031 | 0.018 | 0.027 | 0.035 | 0.020 | 0.191 |
| BAIPas | 0.522 | 0.702 | 0.524 | 0.708 | 0.699 | 0.597 | 0.618 | 0.661 | 0.364 | 0.588 | 0.000 | 0.710 | 0.650 | 0.596 | 0.415 | 0.479 | 0.246 |
| BRUMij | 0.063 | 0.057 | 0.067 | 0.080 | 0.041 | 0.032 | 0.028 | 0.035 | 0.117 | 0.031 | 0.710 | 0.000 | 0.025 | 0.061 | 0.071 | 0.044 | 0.293 |
| BRUUss | 0.039 | 0.032 | 0.042 | 0.062 | 0.027 | 0.015 | 0.020 | 0.020 | 0.086 | 0.018 | 0.650 | 0.025 | 0.000 | 0.041 | 0.052 | 0.031 | 0.240 |
| CAMSau | 0.036 | 0.070 | 0.044 | 0.080 | 0.063 | 0.032 | 0.029 | 0.046 | 0.056 | 0.027 | 0.596 | 0.061 | 0.041 | 0.000 | 0.036 | 0.025 | 0.197 |
| QUEMas | 0.021 | 0.071 | 0.022 | 0.091 | 0.070 | 0.036 | 0.046 | 0.057 | 0.022 | 0.035 | 0.415 | 0.071 | 0.052 | 0.036 | 0.000 | 0.012 | 0.088 |
| QUERia | 0.020 | 0.051 | 0.024 | 0.067 | 0.050 | 0.022 | 0.027 | 0.040 | 0.034 | 0.020 | 0.479 | 0.044 | 0.031 | 0.025 | 0.012 | 0.000 | 0.120 |
| ROQSau | 0.140 | 0.284 | 0.143 | 0.293 | 0.285 | 0.196 | 0.212 | 0.245 | 0.067 | 0.191 | 0.246 | 0.293 | 0.240 | 0.197 | 0.088 | 0.120 | 0.000 |

B : Seuge pairwise Fst

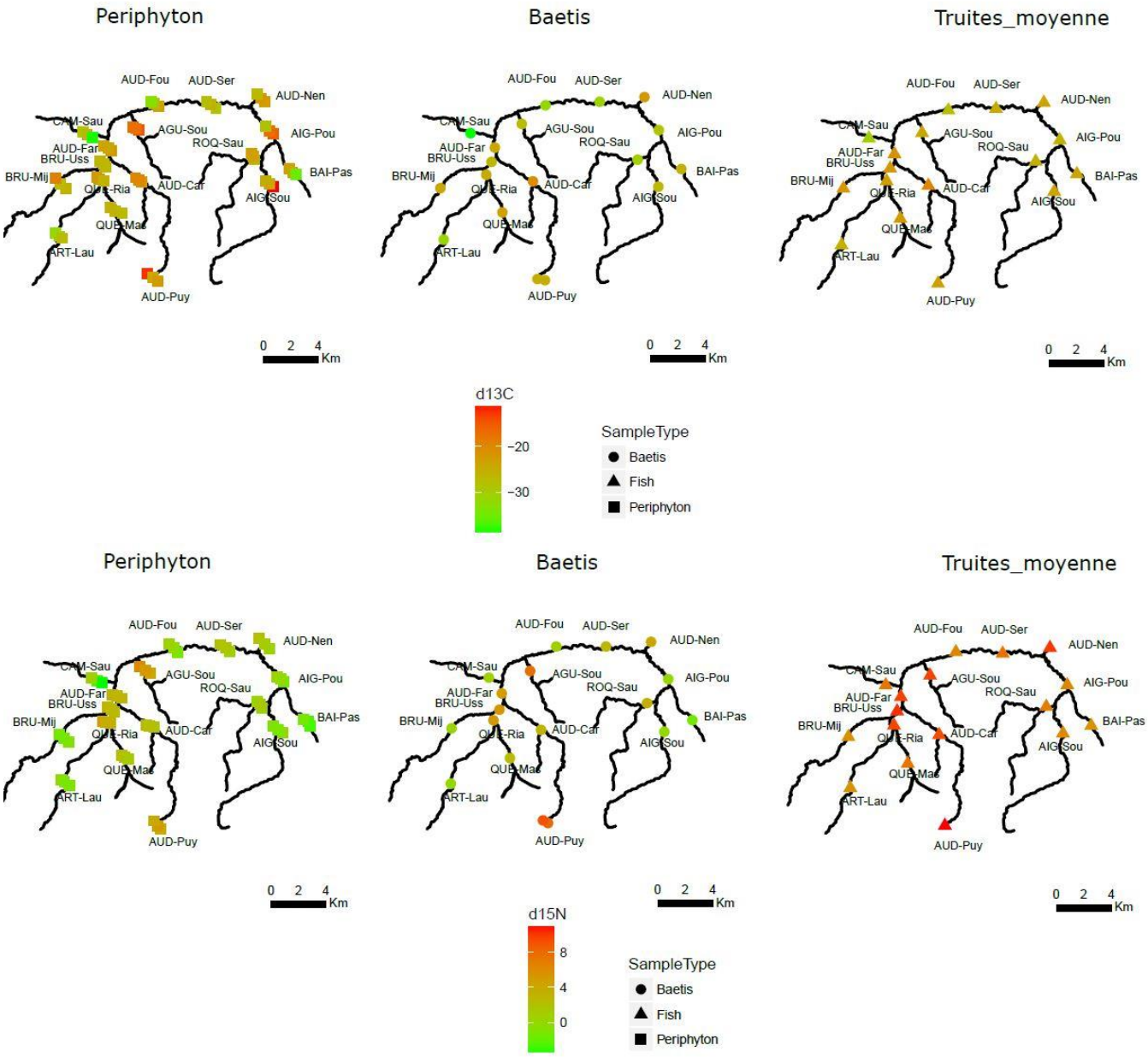
| | BUIPie | CLATou | LAVRiv | LAVSuc | PONAmo | PONBom | PONCha | PONTet | PONTis | SERSau | SEUCch | SEUCou | SEUCro | SEUFag | SEURod | SEUSau |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BUIPie | 0.000 | 0.191 | 0.166 | 0.099 | 0.066 | 0.062 | 0.094 | 0.151 | 0.083 | 0.193 | 0.022 | 0.110 | 0.134 | 0.024 | 0.100 | 0.105 |
| CLATou | 0.191 | 0.000 | 0.064 | 0.047 | 0.052 | 0.085 | 0.037 | 0.171 | 0.044 | 0.069 | 0.096 | 0.044 | 0.066 | 0.071 | 0.038 | 0.042 |
| LAVRiv | 0.166 | 0.064 | 0.000 | 0.018 | 0.061 | 0.104 | 0.016 | 0.165 | 0.017 | 0.047 | 0.142 | 0.020 | 0.049 | 0.110 | 0.017 | 0.021 |
| LAVSuc | 0.099 | 0.047 | 0.018 | 0.000 | 0.060 | 0.110 | 0.012 | 0.131 | 0.012 | 0.027 | 0.137 | 0.017 | 0.045 | 0.112 | 0.008 | 0.014 |
| PONAmo | 0.066 | 0.052 | 0.061 | 0.060 | 0.000 | 0.045 | 0.051 | 0.071 | 0.045 | 0.032 | 0.082 | 0.072 | 0.091 | 0.059 | 0.054 | 0.060 |
| PONBom | 0.062 | 0.085 | 0.104 | 0.110 | 0.045 | 0.000 | 0.102 | 0.051 | 0.088 | 0.068 | 0.065 | 0.125 | 0.157 | 0.059 | 0.107 | 0.116 |
| PONCha | 0.094 | 0.037 | 0.016 | 0.012 | 0.051 | 0.102 | 0.000 | 0.116 | 0.009 | 0.021 | 0.129 | 0.019 | 0.044 | 0.103 | 0.009 | 0.012 |
| PONTet | 0.151 | 0.171 | 0.165 | 0.131 | 0.071 | 0.051 | 0.116 | 0.000 | 0.106 | 0.172 | 0.066 | 0.141 | 0.127 | 0.061 | 0.126 | 0.132 |
| PONTis | 0.083 | 0.044 | 0.017 | 0.012 | 0.045 | 0.088 | 0.009 | 0.106 | 0.000 | 0.021 | 0.114 | 0.021 | 0.047 | 0.090 | 0.012 | 0.017 |
| SERSau | 0.193 | 0.069 | 0.047 | 0.027 | 0.032 | 0.068 | 0.021 | 0.172 | 0.021 | 0.000 | 0.075 | 0.033 | 0.046 | 0.055 | 0.022 | 0.029 |
| SEUCch | 0.022 | 0.096 | 0.142 | 0.137 | 0.082 | 0.065 | 0.129 | 0.066 | 0.114 | 0.075 | 0.000 | 0.152 | 0.187 | 0.015 | 0.136 | 0.145 |
| SEUCou | 0.110 | 0.044 | 0.020 | 0.017 | 0.072 | 0.125 | 0.019 | 0.141 | 0.021 | 0.033 | 0.152 | 0.000 | 0.049 | 0.117 | 0.017 | 0.014 |
| SEUCro | 0.134 | 0.066 | 0.049 | 0.045 | 0.091 | 0.157 | 0.044 | 0.127 | 0.047 | 0.046 | 0.187 | 0.049 | 0.000 | 0.149 | 0.040 | 0.042 |
| SEUFag | 0.024 | 0.071 | 0.110 | 0.112 | 0.059 | 0.059 | 0.103 | 0.061 | 0.090 | 0.055 | 0.015 | 0.117 | 0.149 | 0.000 | 0.107 | 0.116 |
| SEURod | 0.100 | 0.038 | 0.017 | 0.008 | 0.054 | 0.107 | 0.009 | 0.126 | 0.012 | 0.022 | 0.136 | 0.017 | 0.040 | 0.107 | 0.000 | 0.012 |
| SEUSau | 0.105 | 0.042 | 0.021 | 0.014 | 0.060 | 0.116 | 0.012 | 0.132 | 0.017 | 0.029 | 0.145 | 0.014 | 0.042 | 0.116 | 0.012 | 0.000 |

C: Boxplots of uniqueness (mean pairwise Fst values) for the Aude and the Seuge Rivers. Black lines represent the median (higher in the Seuge whereas the mean is lower).

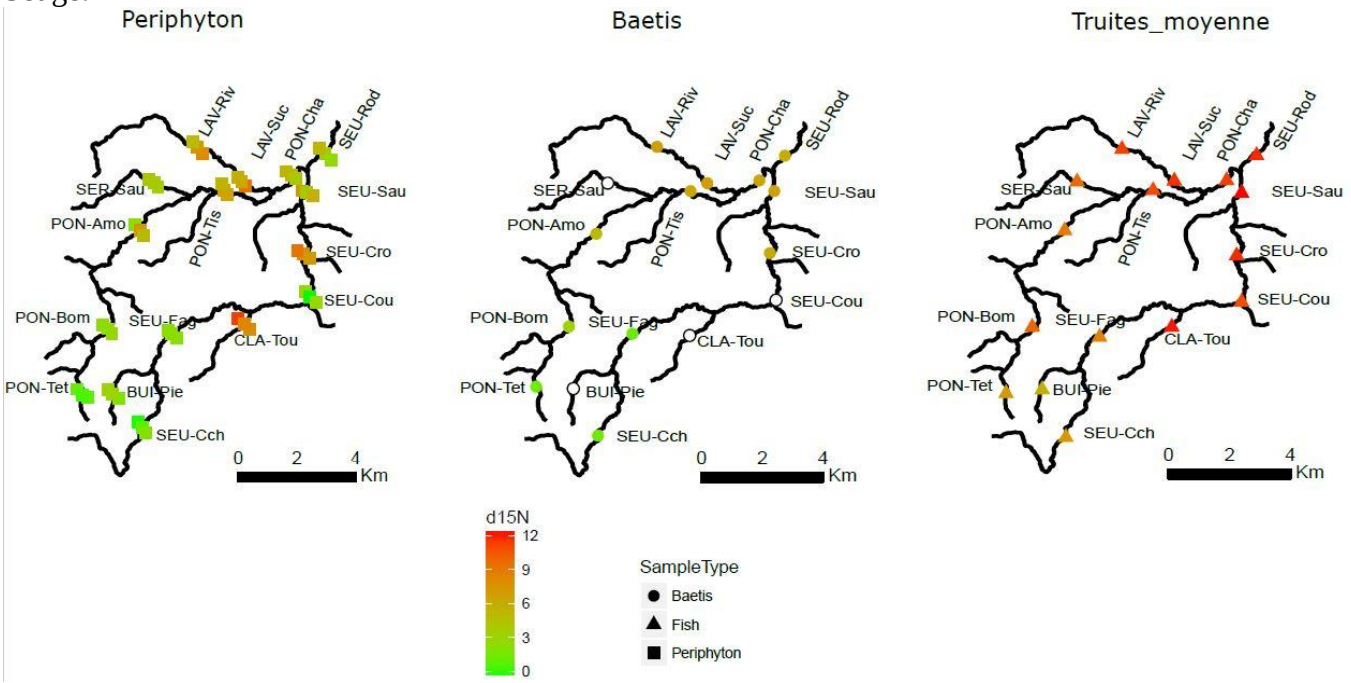


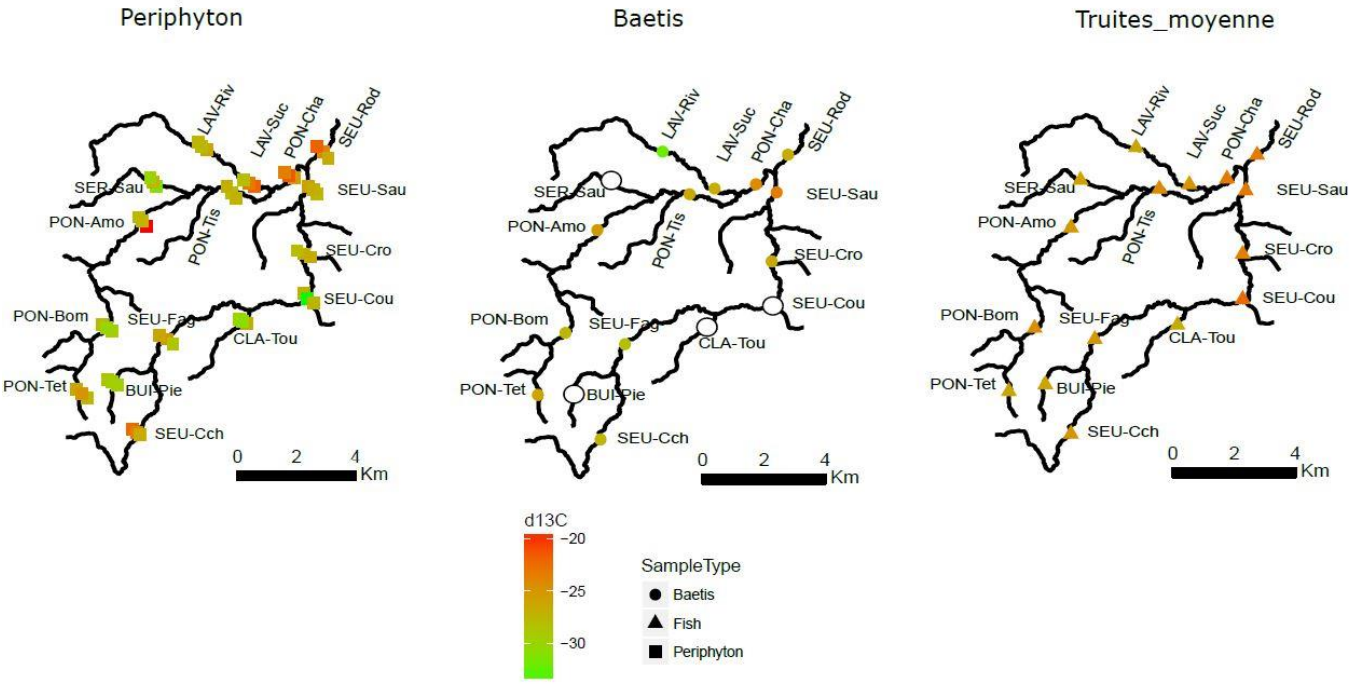
*Appendix S2.3: Isotoscapes for periphyton, baetis and trout*

A: Aude:



Seuge:


Periphyton
Baetis
Truites_moyenne
LAV-Riv
LAV-Suc
PON-Cha
SEU-Rod
SER-Sau
SEU-Sau
PON-Amo
PON-Tis
SEU-Cro
SEU-Cou
PON-Bom
SEU-Fag
CLA-Tou
PON-Tet
BUI-Pie
SEU-Cch
0 2 4
Km
d15N
12
9
6
3
0
SampleType
Baetis
Fish
Periphyton


Periphyton
Baetis
Truites_moyenne
LAV-Riv
LAV-Suc
PON-Cha
SEU-Rod
SER-Sau
SEU-Sau
PON-Amo
PON-Tis
SEU-Cro
SEU-Cou
PON-Bom
SEU-Fag
CLA-Tou
PON-Tet
BUI-Pie
SEU-Cch
0 2 4
Km
d13C
−20
−25
−30
SampleType
Baetis
Fish
Periphyton

# Chapter III. Captive-bred ancestry affects spatial patterns of genetic diversity and differentiation in Brown trout (*Salmo trutta*) populations

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**Key words:** Genetic admixture, SNPs, diversity and differentiation patterns, brown trout, individual assignment, captive breeding, stocking, hatchery.

## Summary

Intraspecific patterns of diversity and differentiation are key features for populations. Understanding how human induced perturbations affect these patterns is therefore a crucial question. Among the numerous pressures which directly or indirectly affect patterns of diversity and differentiation, stocking natural populations with captive-bred strains is a common practice worldwide.

Using 1738 individuals genotyped at 192 SNP loci, we quantified and described the spatial distribution of captive-bred ancestry in brown trout (*Salmo trutta*) populations from four watershed having been stocked using Bayesian clustering. We then tested the effect of captive-bred ancestry on the spatial distribution of genetic diversity and differentiation. Individuals with a strong assignment to the captive strain occurred almost exclusively in upper reaches of the basins.

We found that captive-bred genotypes increased diversity and differentiation, and that patterns such as downstream increase in genetic diversity and higher differentiation upstream were reversed in the case of higher captive-bred genotypes occurring at the level of populations.

The heterogeneous distribution of captive-bred genotypes, even stocked many generations ago, as well as their effect on diversity and differentiation patterns, is a crucial facet of eco evolutionary dynamics which is far overlooked in most studies on spatial processes in stocked species. Indeed, as natural patterns of diversity and differentiation reflect important processes driving the dynamics of populations, understanding how human induced changes can affect them has great implications.

## Introduction

Describing and understanding spatial patterns of biodiversity is a central question in ecology, evolution and conservation sciences (Chave 2013). Large spatial scale patterns of interspecific diversity have been widely studied, such as distribution of species' richness across latitudinal ranges (Gaston 2000). It has further been shown that intraspecific diversity follows the same global patterns as interspecific diversity, with higher diversity in the tropics and a decrease toward the poles (Miraldo et al. 2016). At more local scales (i.e. within landscapes) many studies have assessed patterns of diversity, such as functional diversity (Raffard et al. 2017) and genetic diversity (Charlesworth et al. 1997; Manel et al. 2003; Paz-Vinas et al. 2015). Describing and understanding small scale patterns of intraspecific diversity is of utmost importance for designing proper conservation strategies (Paz-Vinas et al. 2018) given that intraspecific diversity is the first biodiversity facet being affected by human pressures (Mimura et al. 2017), and that it constitutes the evolutionary potential of species (Franklin and Frankham 1998).

In river ecosystems, intraspecific patterns of genetic diversity and differentiation have been widely studied (Labonne et al. 2008; Paz-Vinas et al. 2015). River ecosystems are characterized by their tree-like geometric branching pattern (dendritic network; Benda et al. 2004; Campbell Grant et al. 2007), and strongly structured by elevation, making water flow unidirectional. These characteristics were shown to modulate patterns of genetic diversity and differentiation. Indeed, these landscapes strongly constrain movements of individuals and hence dispersal, with for instance smaller and less connected patches upstream (therefore experiencing more drift; Raeymaekers et al. 2008; Carrara et al. 2014) and asymmetrical gene flow due to the downstream direction of the water flow (Morrissey and de Kerckhove 2009). Moreover, colonization history (which usually occurred from downstream towards upstream reaches; Cyr and Angers 2011) was also shown to be crucial in shaping these patterns in river ecosystems.

Altogether, these mechanisms have been shown to lead to recurrent spatial patterns consisting in a downstream increase in genetic diversity (hereafter DIGD, Kikuchi et al. 2011; Alp et al. 2012; Torterotot et al. 2014; Paz-Vinas et al. 2015) and an upstream increase in differentiation. Although numerous and thorough, studies on these patterns have to our knowledge rarely taken into account the fact that anthropologic pressures can alter patterns of intraspecific diversity and differentiation

(but see Prunier et al. 2018) by modifying their underlying mechanisms (Mimura et al. 2017). River fragmentation by weirs or dams for instance, generally reduces gene flow (Keller and Largiadèr 2003; Raeymaekers et al. 2008; Blanchet et al. 2010) which may reinforce DIGD and upstream genetic differentiation (Blanchet et al. 2010; Faulks et al. 2011). Conversely, a human-induced reduction in effective population size in lower reaches (for instance due to pollution or habitat destruction; Almodóvar et al. 2012; Comte et al. 2013), should reduce genetic diversity of these downstream demes (Ellstrand and Elam 1993), and hence cancel or even invert DIGD and increase downstream genetic differentiation.

Moreover, some pressures are more insidious, and may not affect spatial patterns through population size and connectivity directly. Among these pressures, stocking of captive-bred individuals is a widespread practice used to sustain and/or enhance populations to improve recreational fishing (Borsuk et al. 2006; Hansen et al. 2009; Burnside et al. 2016). Because they are from different origin (lineage, mainly from north Europe AT), and because of unnatural selection pressures and small population sizes, captive-bred individuals strongly differ genetically from wild ones (Frankham et al. 1986; Blanchet et al. 2008; Christie et al. 2016). The genetic effects of stocking have thus been a concern for both scientists and river managers, in particular admixture between wild and captive-bred strains (Cagigas et al. 1999; Heggenes et al. 2002; Perrier et al. 2013a). The differences between strains and the potential genetic effects of stocking on populations suggest that this practice could modify natural patterns of intraspecific diversity. First, direct impacts of stocking on genetic diversity and differentiation (Moran et al. 2005) should, depending on the location of stocking events, modify the natural patterns. Moreover, as for instance captive-bred were shown to disperse differently than wild individuals, and occupy higher reaches (Saint-Pé et al. 2018), we can expect that their location in a river system will strongly influence natural patterns, by homogenizing structure and increasing diversity upstream.

The Brown trout (*Salmo trutta*), as most salmonids, naturally exhibits strong patterns of genetic diversity and differentiation at small spatial scales (Aurelle and Berrebi 2001, Swatdipong et al. 2010; Vøllestad et al. 2012), which have been studied widely because of high interest for river managers and researchers (Berrebi 2015). As it is highly associated to human interests (Mills 1989; Butler et al. 2009), the Brown trout has been domesticated since the 19$^{th}$ century (Laikre et al. 1999), and hatchery strains have been largely used to sustain wild populations worldwide

(Elliott 1994; Berrebi et al. 2000; Bohling 2016; Lobón-Cervía and Sanz 2017). However, the effects of this practice were rapidly shown to be highly concerning, because of its negative effects on natural populations (Hansen 2002; Araki and Schmid 2010; Christie et al. 2012). Therefore, assessing the effect of passed stocking activities at a multi-river shed level, with the aim of describing a general effect of this practice on expected spatial patterns of genetic diversity and differentiation is of crucial importance for both scientists and managers.

Here, we tested the effect of stocking and admixture between wild and captive-bred Brown trout (*Salmo trutta*) on spatial patterns of genetic diversity and differentiation in replicated river systems. By combining extensive river scale sampling design and a series of tools derived from population genetics approaches, we specifically aimed at (i) quantifying captive-bred ancestry of supposedly native populations (ii) testing how captive-bred ancestry was spatially distributed within a river catchment and (iii) testing how captive-bred ancestry affects the relations between genetic diversity/differentiation and distance to the river mouth in four independent river basins. We expect that, captive-bred individuals and their descendants are not distributed homogeneously throughout river basins, and are mostly present in upstream reaches, where the density of native counterparts is generally lower resulting in lower intraspecific competition and prior effects (Weber and Fausch 2003; Saint-Pé et al. 2018). Partly due to this effect, we therefore expect that spatial patterns of differentiation and diversity will be different according to the degree of "nativeness" of individuals/populations. We expect that DIGD for instance will be inexistent (or even inverted) when considering captive-bred individuals and their descendants. Finally, as these strains tend to live upstream, in unconnected reaches although they are from a "homogeneous cluster", we thus expect that differentiation in these upper reaches in which it is naturally expected to be very high, will be lower because of the common origin and "sink effect" of hatchery genotypes.

## Methods

*Study area*

We focused on four independent French river basins: the Aude, the Ône, the Seuge and the Roya Rivers (Figure 1), in which the fish community is dominated by brown trout. These basins vary considerably in terms of geography and environmental conditions, and in stocking activity (Table

1). These four river basins, located in southern France, flow from three different mountain ranges: the Ône and the Aude Rivers flow from the Pyrenees Mountains, the Seuge River from the Massif Central, and the Roya River flows from the Alps. They naturally harbor two different lineages of brown trout, due to their oceanic outlet (Bernatchez 2001): Atlantic (AT) lineage in the Ône and the Seuge Rivers, Mediterranean (ME) lineage for the Aude and the Roya Rivers. The study areas concern the upper reaches of the catchments: above the lowest sampling sites, the basins drain in average 205 $km^2$ (240 $km^2$ for the Aude catchment, 155 $km^2$ for the Ône, 360 $km^2$ for the Roya, and 90 $km^2$ for the Seuge basin).

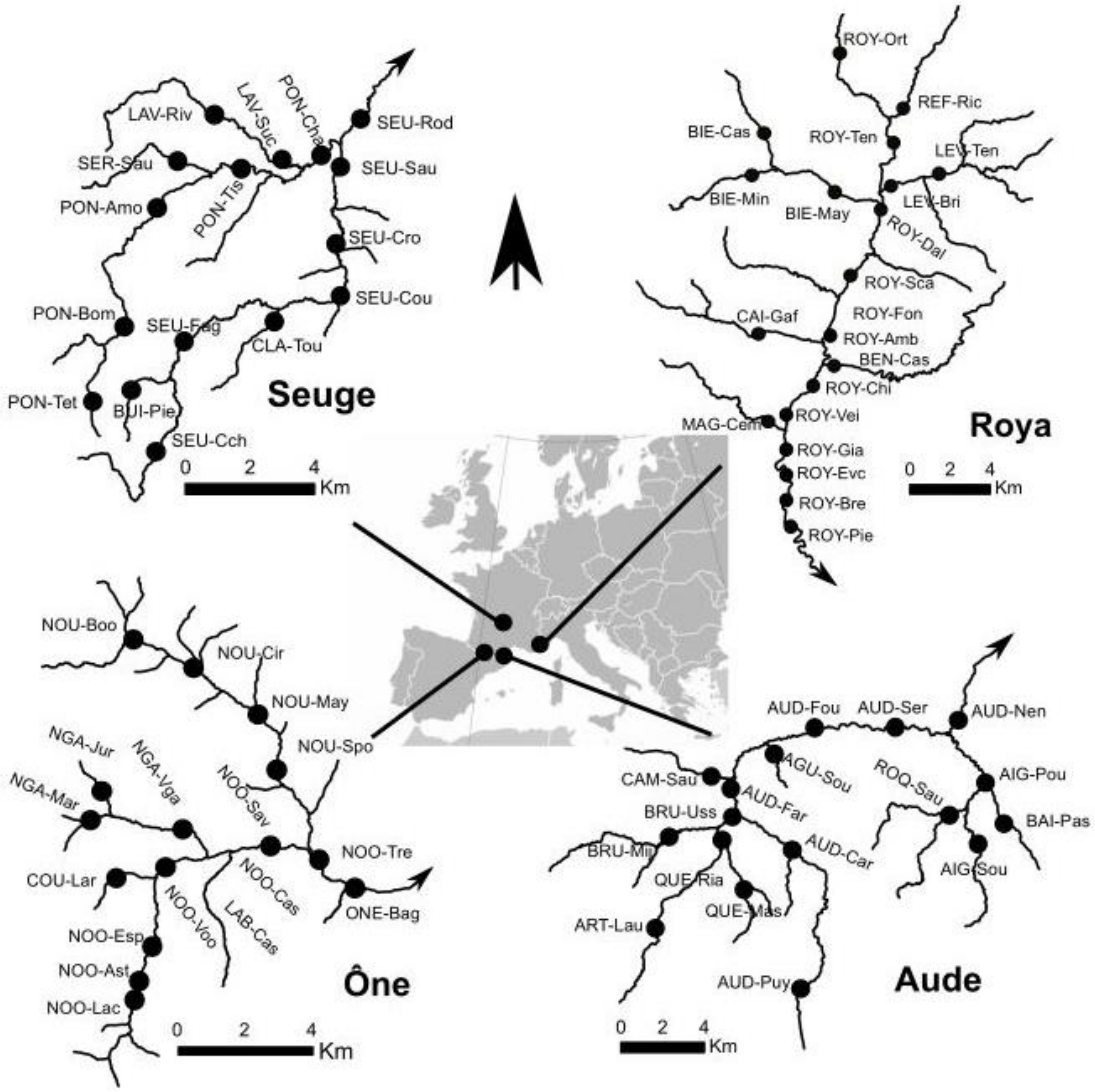


*Figure 3.1: Map of the four river basins studied. The Seuge and Ône Rivers are part of the Atlantic lineage, whereas the Aude and the Roya Rivers are part of the Mediterranean lineage. Sampling sites = black dots*

| | Ône | Seuge | Aude | Roya |
|---|---|---|---|---|
| Outlet | Atlantic | Atlantic | Mediterranean | Mediterranean |
| Mountain range | Pyrenees | Massif Central | Pyrenees | Alps |
| Elevation range (m) | 630-1400 | 960-1250 | 380-1420 | 300-1550 |
| Surface drained by the basin above lowest sampling point ($km^2$) | 240 | 180 | 155 | 360 |
| Flow source | Snow-rain | Snow-rain | Snow-rain | Snow-glacier-rain |
| Hatcheries used in the basins | Soueich | Vourzac, Federation of Lozere | Gesse, Fagolle | Roquebillère, Italian Atlantic hatcheries |
| Hatcheries successfully sampled | Soueich | Vourzac | Fagolle | Roquebillère + Soueich |
| Stocking range | 1972-1999 | 1970's-1995 | 1970's-2000 | 1970's-present |
| Stocking intensity | ++ | - | + | +++ |

*Table 3.1: Features for each basin, presenting the outlet (Atlantic or Mediterranean), the mountain range, elevation range, basin surface, flow source, the hatcheries used (of which we know), hatcheries sampled, time range during which stocking occurred, and intensity of stocking.*

*General stocking practices in France*

For more than a century (and especially in the last 50 years), stocking practices have led to massive introductions of hatchery-reared AT (majority of Danish origin) trout (Laikre et al. 1999), even in rivers naturally inhabited by ME trout (Krieg and Guyomard 1985). However, because this had tremendous consequences on native populations (Largiader and Scholl 1996; Berrebi et al. 2000; Caudron et al. 2006), French managers changed their stocking practices since the early 2000s: stocking was either stopped or shifted toward the use of more local strains (e.g. through the use of ME strains for Mediterranean rivers; Caudron et al. 2006) in many stocking reaches. Nonetheless, in France, data on stocking is generally scarce, and only qualitative data about the source of stoking is properly informed by local managers. Regarding the four target rivers, we

provide in Table 1 the information that we were able to collect from formal discussions with angling departments and local managers about stocking history, intensity and strategy (see also hereafter).

*Sampling hatcheries according to stocking practices*

Quantifying the impacts of stocking and the potential for admixture between wild and stocked fish requires to genetically characterizing the stocked fish. Here, we focused on the hatchery strains having been used in the last 10-30 years in the basins since information anterior to this period is inexistent. For three rivers (the Ône, Seuge and Aude Rivers), stocking practices were officially stopped in the early 2000's (unpublished data, Table 1). In the Ône River, stocking was done from an Atlantic strain local trout hatchery (Soueich, origin of fish is a mix between local rivers and Danish strains) that is administrated by the departmental angling association (Fédération Départementale pour la Pêche et la Protection des Milieux Aquatiques de Haute Garonne). The Aude River was stocked with two different Atlantic strains from local hatcheries: Gesse and Fagolle. We did not manage to get samples of the Gesse hatchery as it has ceased its activity some years ago. These hatcheries were used until the 1980ies, and stocking has officially been stopped since then. The Seuge River was stocked with fish from the hatchery of the departmental angling association of Lozère (Fédération Départementale pour la Pêche et la Protection des Milieux Aquatiques de Lozère) until 1989, with Atlantic strain individuals (the exact origin being unknown). After contacting many actors involved, we were unable to obtain samples from this hatchery that does no longer exist. Nonetheless, we collected samples from a small local hatchery derived from it, which was also used to stock this basin until 1989 (Pisciculture des eaux du Vourzac). Finally, in the Roya River, the story is more complex as it is managed according to two practices. In the downstream sections of the river (below Fontan, "ROY-Fon", see Figure 1) stocking practices have been stopped recently (2007), whereas in the upstream sections stocking practices are still ongoing. This downstream part of the river has been stocked by fish from multiple hatcheries: first by the Fontan hatchery (Atlantic strain, unknown origin, stopped in 2006), then by the Roquebilière hatchery (Mediterranean strain, origin unknown) administrated by the departmental angling association of Alpes-Maritimes (Fédération Départementale pour la Pêche et la Protection des Milieux Aquatiques des Alpes-Maritimes). Stocking was officially done until 2007 with this hatchery. Contrastingly, the upper reaches are still stocked with Atlantic

strains (unknown origin, most likely from Italian hatcheries), but their tracking is extremely complex, as it exists little exchange between local managers and legal authorities. Roquebilière hatchery has been successfully sampled, but Fontan samples were not available. We therefore used Soueich as an Atlantic hatchery to represent stocking in this basin (note that using Soueich or one of the other Atlantic hatcheries did not change the results qualitatively and quantitatively). As a result of information gathered (Table 1), we considered the Roya River as having been (and being) the most potentially impacted by stocking practices, particularly in its upper reach. According to interviews with local managers, we considered that the second most potentially impacted (number and duration of stocking events) is the One River, then the Aude River and finally the Seuge River that have been only weakly impacted (Table 1).

For each basin, we therefore sampled 30 fish from each of the trout hatcheries used, which were available, in order to genetically characterize the captive-bred strain, and hence quantify genetic admixture with captive-bred trout. In Table 1, we give the hatcheries used (which we know of), and the ones we were able to obtain samples for.

*Field sampling*

Brown trout were sampled using a single-pass electric-fishing approach from 71 sites in total, with an aim of catching 30 individuals per site. The number of sites per river basin varied from 16 (Seuge River) to 21 (Roya River) so as to cover the whole river network (all main tributaries and the upstream-downstream gradient of the main stem) of each river (Figure 1, Appendix S1). We focused on small-bodied individuals during sampling in order to focus mainly on individuals from one age class (1 year-old). In total, we captured 1738 individuals, with sample sizes ranging from 6 to 30 (Appendix S1 for details). Each individual was measured (total length in mm, see Appendix S1 for more details on the size distribution), and a pelvic fin clip was taken for the genetic analysis. All individuals were released alive to their original sampling site.

*Genotyping*

Individual multilocus genotypes were obtained for 162 polymorphic SNPs markers (Saint-Pé et al., 2019), using the KASP technology performed by LGC Genomics® (Smith and Maughan 2015). These markers were developed from a high-density linkage map (Leitwein et al. 2017), and

filtered to be highly polymorphic, evenly spread and spaced on the linkage map, and present in both AT and ME brown trout lineages (see Saint-Pé et al., 2019 for details). Five ancestry informative SNPs used in previous studies to distinguish individuals (and infer admixture) from the AT and ME lineages (OMM1164, OMM1105, OMM1154, Str541INRA, Str591INRA; Estoup et al. 2000; Caudron et al. 2006, 2012), were included in the array.

*Quantification of captive-bred ancestry and genetic diversity and differentiation*

For each river independently, we performed genetic clustering using STRUCTURE 2.3.1 (Pritchard et al. 2000) with the admixture model and the correlated allele frequency model, without prior population information. Runs were performed with a burn-in period of 200.000 and 200.000 subsequent MCMC repetitions. The number K of clusters ranged from 1 to 10 and 5 runs were performed for each value. We checked MCMC convergence making sure the alpha plots showed no substantial fluctuation before the end of the burn-in. Log-likelihood plots and ΔK statistics were obtained via the Evanno's method (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and vonHoldt 2012), and were used to infer the optimal K-value. Twenty runs were then performed with this optimal K-value and the ten best runs (the ones with highest LnP(D) values) were compiled using the Greedy algorithm from CLUMPP (Jakobsson and Rosenberg 2007) to get final averaged individual Q-values. Individuals were assigned to the cluster for which their Q-value was highest, and provided this value was higher than 0.7. Graphical displays of STRUCTURE plots were generated using DISTRUCT software (Rosenberg 2003) with the membership of each individual representing the mean membership over the replicate runs. We directly used the probability of assignment (individual Q-value) to the cluster containing all individuals from the trout hatcheries used to stock each basin (see the Results section) as an estimate of individual captive-bred ancestry (Hansen et al. 2001; Vähä and Primmer 2005; Valiquette et al. 2014). This probability thus varies from 0 to 1 and the higher the score, the more the individual carries captive-bred ancestry (individuals with a score close to 1 are likely captive-bred fish released or pure descendants of captive-bred fish). In the case of the Roya basin, as there were two distinct hatchery strains used to stock (ME and AT), we used two steps to identify ancestry origin of captive-bred ancestry: first, we assessed the percentage of AT ancestry, then we assessed, for individuals considered as "Mediterranean", the probability of assignment to the ME hatchery of Roquebillière.

Genetic diversity within each of the sampling site was estimated over all loci by computing unbiased expected heterozygosity (*He*), while genetic differentiation was assessed by computing the global *Fst* for each site using the *adegenet* R package (Jombart 2008). This later measure of differentiation indicates to which extent a site is genetically unique/differentiated compared to all other sites form the river.

*Statistical analyses*

*Spatial patterns of captive-bred ancestry.* In order to describe the spatial structuration of strains in river basins, we tested the relationship between site riverine distance to the river mouth (calculated with STARS ArcGis package, Peterson and Hoef 2014) and proportion of captive-bred ancestry (averaged at the site level over all sampled individuals), using a beta regression model (from the *betareg* R package, as the proportion of captive-bred ancestry is comprised between 0 and 1) that also included a two-way interaction term between the identity of the river basin and the distance to the mouth to test if the relationship varied between basins. Using the *dredge* R function, we selected the best models based on AIC, and determined the proportion of models displaying a ΔAIC lower than 4 in which each variable appeared.

*Spatial patterns of genetic diversity and differentiation, and the effect of introgression.* We tested the effect of the proportion of captive-bred ancestry on genetic diversity across basins, running a beta regression model in which the mean expected heterozygosity (as a measure of genetic diversity, comprised between 0 and 1) at the site level was the response variable, while the proportion of captive-bred ancestry, the distance to the mouth, the river basin and all resulting two-term interactions were the predictors. In the same way, we tested the effect of captive-bred ancestry on the spatial distribution of genetic differentiation using a linear model (*lm* from the glm R package). In that case, the mean *Fst* (as a measure of population differentiation) at the site level was the response variable, while the proportion of captive-bred ancestry, the distance to the mouth, the river basin and all resulting two-term interactions were the predictors. The best models were also selected based on AIC using dredge, and we determined the proportion of models displaying a ΔAIC lower than 4 in which each variable appeared. A significant interaction term between the proportion of captive-bred ancestry and site river distance to the river mouth would indicate that

spatial patterns of intraspecific diversity (either the DIGD or the increase in differentiation upstream) are affected by introgression by stocked individuals.

## Results

*Spatial patterns of captive-bred ancestry*

Captive-bred ancestry presence strongly varied across rivers (Figure 2A). At the individual level, it ranged from 0 to 0.99, with an average of 0.28 ± 0.31 (mean ± SD) (see Figure 2A and Appendix S2 for details). Moreover, we found that the spatial distribution of captive-bred ancestry was heterogeneous, mostly found in the upper parts of the basins. This was statistically confirmed as we found that a single model was best supported by the data for captive-bred ancestry (Appendix S3A): this model comprised "river basin" and "riparian distance to the river mouth" as simple terms (Table 2a), indicating that captive-bred ancestry was different in average among basins (as shown in figure 2A), and positively related to riparian distance to the river mouth (Figure 2B).

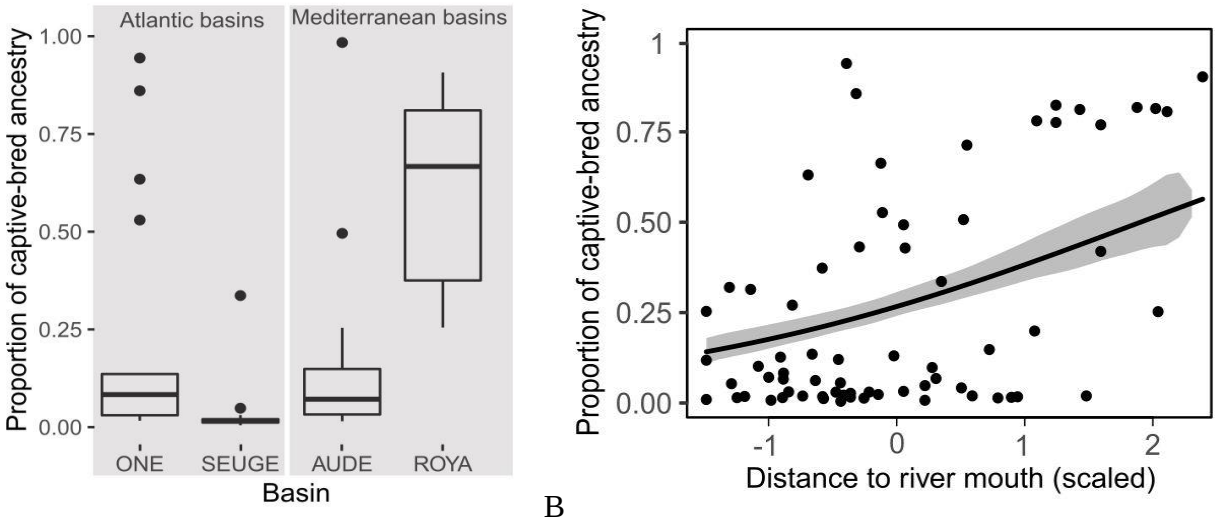


*Figure 3.2: (A) Boxplots of captive-bred ancestry for each basin, averaged at the population level. (B) Plot of captive-bred ancestry against distance to the river mouth (scaled). Line and 95% confidence interval correspond to the fitted values of the beta regression.*

| | Basin | Dist-mouth | Dom_anc | Basin:Dist_mouth | Basin: Dom_anc | Dist_mouth: Dom_anc |
|---|---|---|---|---|---|---|
| (A) Dom_anc (Betareg) | 1 | 1 | / | 0 | / | / |
| (B) He (Betareg) | 1 | 0.75 | 1 | 0.25 | 1 | 0.25 |
| (C) Fst (lm) | 1 | 0.5 | 1 | 0.83 | 0.33 | 0.67 |

*Table 3.2: Proportion of models (with DeltaAIC<4 after using dredge) containing each explanatory variable tested (River basin ("Basin"), riparian distance to the river mouth ("Dist-mouth"), proportion of captive-bred ancestry ("Dom_anc"), and two way interactions (":") for explaining the proportion of captive-bred ancestry ("Dom_anc"), diversity ("He") and differentiation ("Fst"). The type of model used is detailed in the first column: beta regression ("betareg") or linear model ("lm").*

More precisely, in the Ône River, fish with a high proportion of captive-bred ancestry (q > 0.7) were present in the most upstream site of the Neste d'Oueil ("NOU-Boo", which has not been stocked since 1998, although illegal non-controled stocking cannot be ruled out). The Neste de Garin tributary ("NGA sites") was almost exclusively populated with fish with a high proportion of captive-bred ancestry. Other sites were mostly populated with native fish, which were separated in two main genetic clusters (Figure 3A). In the Seuge River, all individuals were assigned by less than 0.3 to the captive-bred cluster, which indicated that this basin was populated with only native fish, separated in two clusters (Wild 1, in the upper part, and Wild 2 in the downstream part, and some sites being populated with hybrids; Figure 3B). In Mediterranean Rivers (Aude and Roya Rivers), captive-bred ancestry was also heterogeneous spatially. The Aude basin displayed relatively low captive-bred ancestry (Figure 3C), except in two tributaries: "BAI-Pas", in which all individuals were highly assigned to the captive-bred cluster, and "ROQ-Sau" populated with mostly hybrids between native and captive-bred strains. On the other hand, the Roya basin displayed a very high proportion of fish with a high proportion of captive-bred ancestry. Upstream from Fontan ("ROY-Fon", Figure 3D), there were virtually no fish assigned as pure native fish. Atlantic fish are mostly present in the upper reaches, and a relatively high proportion of these fish are hybrids between Atlantic and Mediterranean strains. These hybrids are most likely captive-bred Mediterranean in these reaches, as no native ancestry was found. Captive-bred ancestry from the Mediterranean hatchery was also ubiquitous in these upper reaches, and hybrids between this strain and native individuals were also present in high proportions in the downstream reaches (Figure 3D).

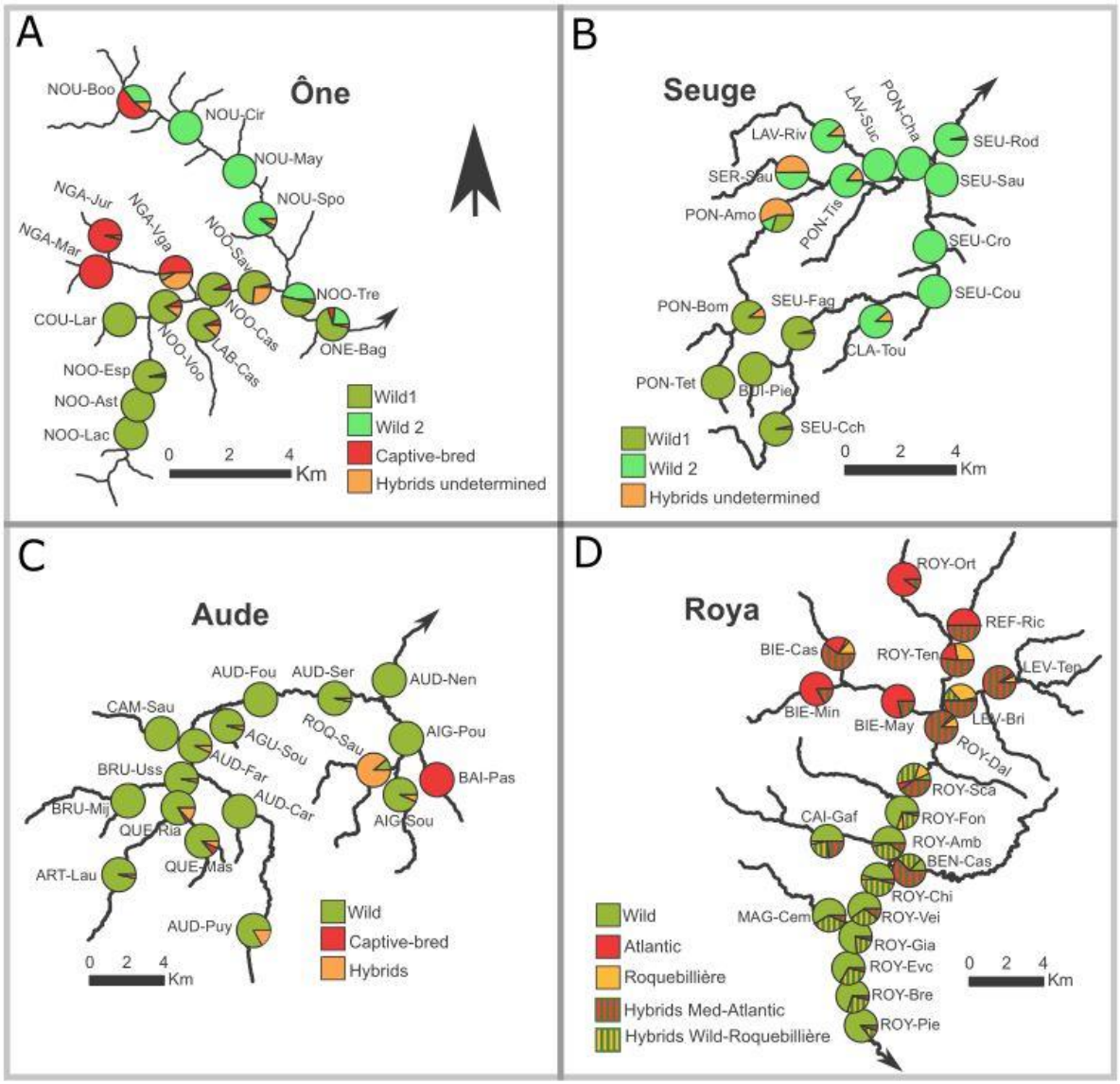


*Figure 3.3: Maps of each river basin, with proportion of individuals assigned to each cluster (native, domestic and hybrids clusters) at each site*

*Effects of captive-bred ancestry on spatial patterns of genetic diversity and differentiation*

Expected heterozygosity over all loci (*He*) and all populations was comprised between 0.041 and 0.40 (mean = 0.18 ± 0.10), and *Fis* within each site ranged from -0.27 to 0.26 (0.01 ± 0.07). Genetic differentiation (*Fst*) estimated across loci at the site level ("uniqueness") was comprised between 0.04 and 0.53 (mean = 0.09 ± 0.06). Trout from the Roya River were –in average- the

most diverse genetically, whereas the highest levels of differentiation were observed in the trout from the Aude River (Table 3 and Appendix S1 for details).

| | *He* | | | | *Fis* | | | | *Fst* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | max | mean | sd | min | max | mean | sd | min | max | mean | sd |
| Aude River | 0.066 | 0.388 | 0.162 | 0.088 | -0.129 | 0.262 | 0.032 | 0.086 | 0.063 | 0.534 | 0.114 | 0.112 |
| Ône River | 0.091 | 0.182 | 0.139 | 0.022 | -0.053 | 0.070 | 0.003 | 0.034 | 0.042 | 0.135 | 0.063 | 0.024 |
| Roya River | 0.220 | 0.398 | 0.305 | 0.064 | -0.029 | 0.072 | 0.023 | 0.031 | 0.048 | 0.229 | 0.091 | 0.043 |
| Seuge River | 0.041 | 0.110 | 0.090 | 0.019 | -0.269 | 0.151 | -0.035 | 0.112 | 0.045 | 0.112 | 0.069 | 0.020 |

*Table 3.3: Per basin summary statistics of expected heterozygosity (He), Fis and genetic differentiation (Fst) estimated across loci for each site*

Regarding *He*, four models displayed ΔAIC<4 (Appendix S3B), and basin identity, captive-bred ancestry and the interaction term between these two variables were selected in all these four models. This indicated that *He* was positively linked to the proportion of captive-bred ancestry for all rivers, although the slope of this relation significantly varied across basins (Figure 4). In particular, the positive relationship between *He* and captive-bred ancestry was stronger for the Aude and Roya Rivers, than for the Ône and Seuge Rivers where the relationship was extremely weak (Figure 4). Moreover, three out of the four models with a ΔAIC<4 models retained the term "riparian distance to the river mouth", and one model retained the interaction term between riparian distance to the river mouth and basin identity (Appendix S3B, Table 2b). This indicated that the relationship between He and distance from the mouth varied between river basins, overall this relationship was negative for the Seuge River (as expected according to DIGD), but positive for other basins (the highest slope was for the Roya River; Appendix S4). Finally, the interaction term between captive-bred ancestry (at the site level) and distance to the river mouth was retained in one out of the four model with ΔAIC<4 (Table 2b; Appendix S3B) in explaining *He*, indicating that the relationship between He and distance from the mouth tends to vary according to the probability of assignment to the captive-bred cluster. In particular, a visual inspection of these links reveals that there was –as expected according to the DIGD- a general downstream increase in expected heterozygosity for populations composed of individuals strongly assigned to the native cluster, whereas populations composed of individuals strongly assigned to the captive-bred cluster showed a striking increase in expected heterozygosity in upstream reaches (Figure 5a). As captive-bred ancestry proportion was different between basins, for instance inexistent in the Seuge and

very high in the Roya, these latter results also thus explain why the relation between He and riparian distances vary between basins: they interact with the level of captive-bred ancestry.

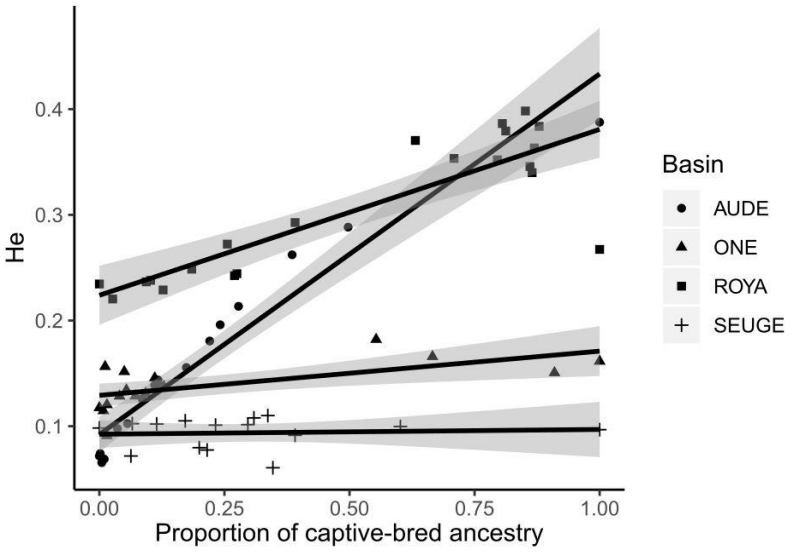


*Figure 3.4: Plots of genetic diversity (expected heterozygosity, He) against proportion of captive-bred ancestry depending on the river basin as an interaction term*

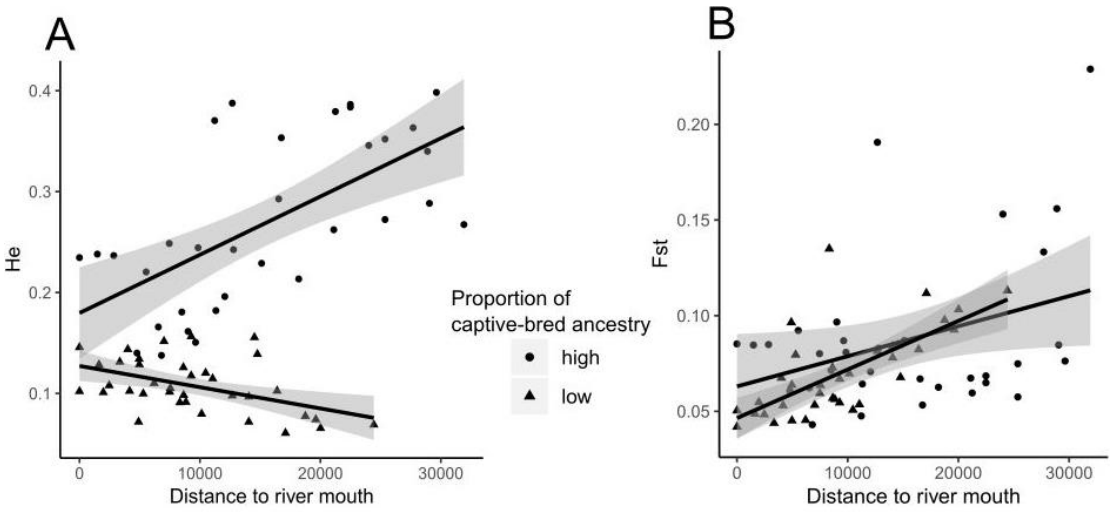


*Figure 3.5: Plots of genetic diversity (He) (A) and differentiation (Fst) (B) against riparian distance to the river mouth (in m) depending on proportion of captive-bred ancestry as an interaction term*

Regarding genetic differentiation, 6 models displayed ΔAIC<4 (Appendix S3B), and basin identity and captive-bred ancestry were selected in all these 6 models indicating that these two variables were extremely important to explain patterns of Fst (Table 2c). To a lesser extent distance from the mouth was selected in half of the models displaying a ΔAIC<4 (Table 2c). Nonetheless, three interaction terms involving these three terms were also selected in some of the most likely models (Table 2c), indicating that these variables likely have interactive effects on Fst. Overall, genetic differentiation tended to increase upstream (as expected), but this increase (i) tended to vary among rivers, with the lowest slope for the Aude River, the highest for the Seuge (interaction "basin:dist_mouth", Appendix S5), (ii) tended to be lower in slope for sites with a higher proportion of captive-bred ancestry than for sites mainly containing native assigned fish (interaction "dist_mouth:dom_anc", Figure 5b). Moreover, higher *Fst* values were observed for sites with a higher proportion of captive-bred ancestry.

Overall, we found that captive-bred ancestry is a strong driver of both genetic diversity and differentiation. Indeed, in our models, this variable appears very frequently (Appendices S3A and S3B), and is more present than distance to the river mouth, a variable which would be most expected to drive these patterns.

## Discussion

*Captive-bred ancestry extent*

Although stocking was stopped years ago for most basins (i.e. 6-8 trout generations ago), captive-bred ancestry is still present, from very low percentages, 4% in the Seuge River (26 years without stocking), to 59% in the Roya River (still heavily stocked in its upstream part nowadays). This high variability is congruent with other studies which show that admixture rates between captive-bred and wild trout populations are highly variable, ranging from undetectable contributions to total replacement of native gene pools (Poteaux 1999; Cagigas et al. 1999; Perrier et al. 2013b; Kazyak et al. 2018). Captive-bred ancestry and introgression outcomes depending on stocking strategies and durations have been widely studied (Barbat-Leterrier et al. 1989; Largiadèr et al. 1996), and shown to be highly variable depending on the context. For instance, Almodovar et al.

(2006) found alarmingly high rates in some Mediterranean rivers, whereas North Atlantic populations showed little or no introgression, which is congruent with our results.

Although not quantified statistically, we observed a tendency for captive-bred ancestry presence and introgression to be positively associated to stocking intensity and to how recent the last stocking events are (Martinez et al. 1993; Marie et al. 2010; Gossieaux et al. 2019) as the Seuge River (where stocking was stopped long ago) is the least impacted whereas the Roya (still partially heavily stocked) is the most impacted. Populations could thus recover from stocking (Post 2013; Valiquette et al. 2014; Létourneau et al. 2018), thanks to several mechanisms: lower fitness and survival of captive-bred individuals (Aarestrup et al. 2005; Pedersen et al. 2008), genetic drift purging exogenous alleles (Frankham et al. 2010), competitive exclusion (Saint-Pé et al., 2018), or difference in spawning time (Hansen et al. 2006). Fishing pressure after stocking can also be determinant for the survival of stocked fish, as it was shown that stocked fish were more likely to be caught (Mezzera and Largiadèr, 2005; García-Marín et al. 1998). However, although the cessation of stocking has in some cases enabled to return to the "natural state" (Hansen et al. 1995; Almodovar et al. 2001), we believe that in an extreme case such as the Roya, post-stocking recovery rates would be low even at long-term if stocking was ceased, as it was observed in populations of France and Spain (Poteaux 1999; Araguas et al. 2004).

A major limitation in studies on the genetic impact of stocking, at least in southern Europe, is the lack of reliable information in terms of activity, density, and time range (de Sostoa and Lobon-Cervia 1989). Moreover, from our experience, we found that historical archives are scarce, and because of numerous illegal and hidden stocking events (unconsented by Angling departments), conclusions on the persistence of captive-bred strains are often impossible to make. Other salmonids (e.g. brook trout; Marie et al. 2010; Gossieaux et al. 2019) have been much better surveyed in terms of stocking, and therefore, provided the impacts of stocking are consistent across salmonids, they potentially represent more reliable models.

*Captive-bred ancestry distribution*

Interestingly, for all basins, the spatial distribution of captive-bred ancestry in the watershed was not homogeneous (consistent with Saint-Pé et al., 2018), whereas stocking activities are generally

done homogeneously over river basins. In all basins, captive-breeding ancestry was majorly present in upstream reaches, with some upper sites exclusively populated with fish strongly assigned to the captive-bred cluster, whereas in lower reaches, fish strongly assigned to the captive-bred cluster were rare. For instance in the Ône basin, captive-bred ancestry was present in the upper reaches of the Neste d'Oueil ("NOU-Bou" site), and the Neste de Garin ("NGA" sites, Figure 3). In the Roya basin, this pattern was also observed (Figure 3), as all the upper reaches were exclusively populated with captive-bred strains, although in this basin, stocking practices are different between upstream and downstream reaches, and are thus propbably the main cause for this pattern. These visual patterns were confirmed statistically as we found a significant relationship between distance to the river mouth of each site and the proportion of captive-bred ancestry. Conditions in these upper reaches may differ significantly from those downstream in terms of habitat availability and stability (Vannote et al. 1980; Grant et al. 2012), therefore, fish assigned to captive-bred clusters may be favored by harsher conditions. Indeed, higher introgression has been linked to low pH, high water temperature (Marie et al. 2012; Harbicht et al. 2014; Létourneau et al. 2018), environmental instability and smaller habitat size (Splendiani et al. 2013; White et al. 2018). There is however a counter-example of this pattern (with higher introgression in fertile waters and stable discharge; Almodovar et al. 2006), but in all cases, there is a spatial segregation between strains. Various mechanisms could underlie this pattern. First, movement patterns between wild and captive-bred strains may differ (Vasemägi et al. 2005; Finnegan and Stevens 2008; Saint-Pé et al. 2018). Second, competition with the native strain may probably be low or inexistent in these harsher stretches, thus facilitating the settlement of non-native strains and improving their reproductive success (Saint-Pé et al., 2018).

*Effect of stocking on spatial patterns of diversity and differentiation*

The distribution of allochtonous alleles introduced with stocking has modified spatial patterns of genetic diversity and differentiation. This is consistent with Prunier et al. (2018), who found that among various anthropogenic stressors, stocking had a strong and consistent influence on patterns of genetic diversity and genetic differentiation. Love Stowell et al. (2015) also found that the actual distribution of lineages was predicted by stocking pressure and source, but not by which lineage was historically native in a Cutthroat population (*Oncorhynchus clarkii*). Stocking

therefore has a tremendous effect on wild populations, which foreshadows concerning implications.

Captive-bred fish generally harbor lower genetic diversity than wild ones (Hansen et al. 2006; Hutchings and Fraser 2008; Blanchet et al. 2008). Therefore, stocking with captive-bred individuals should be expected to decrease diversity in natural populations (Eldridge et al. 2009; Gossieaux et al. 2019), which has been a conservation and management conundrum for decades (Hansen 2002; Hansen et al. 2009). Our results clearly contrast with this prediction, as we found a positive relation between genetic diversity and captive-bred ancestry. Marie et al. (2010, 2012) and Almodóvar et al. (2006) show that this is due to differences in origins and genetic drift processes between captive-bred and wild populations, thus introducing new alleles into wild populations. This result must thus be taken very cautiously because even if it increases genetic diversity locally, stocking could overall decrease the species' genetic diversity and effective population sizes (Almodóvar et al. 2006; Gossieaux et al. 2019).

When considering populations composed of individuals mostly assigned to the native cluster (i.e. the supposedly native individuals), we found an overall downstream increase in genetic diversity (DIGD), meeting the theoretical expectation (Ritland 1989; Morrissey and de Kerckhove 2009; Paz-Vinas and Blanchet 2015; Paz-Vinas et al. 2015). Conversely, in populations composed of individuals mostly assigned to the captive-bred cluster, genetic diversity significantly increased upstream. Therefore, as captive-breeding ancestry is mostly present upstream, and harbors higher levels of diversity, its observed effect on spatial patterns of diversity could simply be the result of the distribution of strains. Moreover, the theoretical differentiation pattern (upstream increase in differentiation) was weaker when considering individuals of high captive-bred ancestry. We therefore suggest that stocking has homogenized genotypes among locations in which captive-bred ancestry is present, and that genetic differentiation between captive-bred and introgressed populations is lower than between wild populations (Hansen et al. 2006; Eldridge and Naish 2007; Halbisen and Wilson 2009; Marie et al. 2010). Captive-bred strains are thus now maintained in upper reaches in which naturally, populations would have diverged (Labonne et al. 2008; Paz-Vinas and Blanchet 2015). Moreover, in Saint-Pé et al, (2018), we found that captive-bred individuals tended to exhibit higher propensity for movement, moved on longer distances, and moved preferentially from and towards tributaries (in which they are mostly present). This could

thus explain why these upstream populations of captive-bred fish and/or descendants are less differentiated than the theoretical expectation (when comparing to the "*Fst*-distance to source" slope of wild individuals).

## Conclusions and implications

This study, along with others before it, confirms that the effects of stocking on spatial population patterns can be substantial, and could highly affect conservation and management decisions. In most of Europe, stocking has been highly underestimated both in terms of timescale and quantities (Horreo and Garcia-Vazquez 2011; Miró and Ventura 2013). Its effects are thus still to be assessed, as it is a major threat to species' genetic integrity, but also potentially to ecosystems. Indeed, despite its potential direct effects on fitness (Hansen et al. 2009; Lamaze et al. 2012; Morissette et al. 2018), its effects on spatial patterns of genetic diversity and differentiation also have strong potential implications, and exist even in cases in which it was stopped many generations ago (Hansen et al. 2009; Marie et al. 2010). Modifying spatial or temporal structure of populations can for instance profoundly affect local food webs, with important subsequent ecological consequences through community structure of invertebrate prey and primary productivity for instance (Harmon et al. 2009; Raffard et al. 2017), but also through non-trophic effects (Whitham et al. 2003; Matthews et al. 2011). Finally, the potential disappearance of conservation units of high interest for both representation and persistence of diversity is another conundrum posed by stocking (Antunes et al. 2001; Moritz 2002; Ferguson 2004). Indeed, as these patterns of genetic diversity and differentiation can be "context-dependent", we suggest that they form very unique populations, which with stocking, are potentially threatened through changes in these patterns.

## Authors' contributions

KSP, SB, NP and LT designed the experiment and coordinated the study; KSP, LT, NP, and SB conducted sampling; KSP and SB carried out the experimental lab work; KSP ran the statistical analyses; KSP, LT, NP, and SB interpreted the data; KSP and SB wrote the first draft of the manuscript; LT and NP, read, commented and corrected the initial draft, and all authors gave final approval for publication.

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## Conflict of Interest Statement

We have no competing interests and we declare no conflict of interests.

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## Supplementary material for Chapter III.

*Appendix S3.1: Fish sampled; numbers and sizes*

| Basin | N | Mean body length (mm) | Mean Fst | Mean He | Mean Fis |
|---|---|---|---|---|---|
| Aude | 453 | 143(±37) | 0.114(±0.112) | 0.162(±0.089) | 0.032(±0.086) |
| One | 495 | 136(±38) | 0.063(±0.024) | 0.139(±0.022) | 0.003(±0.034) |
| Roya | 413 | 167(±23) | 0.091(±0.043) | 0.305(±0.064) | 0.024(±0.031) |
| Seuge | 377 | 134(±34) | 0.069(±0.021) | 0.091(±0.019) | -0.036(±0.112) |

| Basin | Site | N | Mean body length (mm)(±SE) | Uniqueness (mean pairwise Fst) | He | Fis | X | Y |
|---|---|---|---|---|---|---|---|---|
| AUDE | AGUSou | 27 | 157(±42) | 0.071 | 0.196 | 0.058 | 627088.1 | 6185199.75 |
| | AIGPou | 30 | 139(±33) | 0.096 | 0.072 | 0.01 | 637243.95 | 6183735 |
| | AIGSou | 30 | 143(±38) | 0.071 | 0.181 | 0.018 | 636876.2 | 6180792.25 |
| | ARTLau | 30 | 138(±38) | 0.113 | 0.069 | 0.018 | 621320.8 | 6176817 |
| | AUDCar | 30 | 166(±34) | 0.093 | 0.074 | 0.027 | 627921.65 | 6180545.5 |
| | AUDFar | 30 | 165(±33) | 0.068 | 0.139 | -0.028 | 625004.1 | 6183550.5 |
| | AUDFou | 30 | 135(±25) | 0.073 | 0.126 | 0.059 | 629048.35 | 6186449 |
| | AUDNen | 30 | 123(±54) | 0.081 | 0.098 | 0.001 | 635955.25 | 6186755.75 |
| | AUDPuy | 30 | 121(±31) | 0.085 | 0.288 | -0.092 | 628263.15 | 6173847.75 |
| | AUDSer | 30 | 156(±22) | 0.067 | 0.144 | 0.004 | 632930.0927 | 6186461.501 |
| | BAIPas | 14 | 153(±28) | 0.534 | 0.177 | 0.090 | 638128.2 | 6181735.75 |
| | BRUMij | 30 | 127(±26) | 0.103 | 0.066 | 0.059 | 621999.45 | 6181223.75 |
| | BRUUss | 30 | 151(±21) | 0.082 | 0.103 | 0.047 | 625055 | 6182171.25 |
| | CAMSau | 14 | 118(±46) | 0.085 | 0.156 | -0.129 | 624813.2 | 6184020.75 |
| | QUEMas | 29 | 131(±36) | 0.067 | 0.262 | 0.262 | 625591 | 6178665.75 |
| | QUERia | 30 | 158(±37) | 0.063 | 0.213 | 0.137 | 624547.7 | 6181059.25 |
| | ROQSau | 9 | 154(±25) | 0.191 | 0.388 | -0.004 | 635481.2 | 6182155.75 |
| ONE | COULar | 30 | 111(±26) | 0.135 | 0.091 | 0.028 | 494582.72 | 6191615.94 |
| | LABCas | 17 | 112(±23) | 0.061 | 0.14 | 0.042 | 497950.61 | 6192263.67 |
| | NGAJur | 30 | 152(±40) | 0.087 | 0.151 | -0.004 | 494142.91 | 6194213.37 |
| | NGAMar | 30 | 135(±18) | 0.097 | 0.161 | -0.016 | 494232.47 | 6193507.03 |
| | NGAVga | 30 | 127(±35) | 0.062 | 0.166 | -0.053 | 496537.4 | 6193069.2 |
| | NOOAst | 30 | 135(±26) | 0.051 | 0.12 | -0.03 | 495260.7 | 6188546.68 |
| | NOOCas | 30 | 144(±18) | 0.045 | 0.129 | 0.065 | 497578.93 | 6192335.96 |
| | NOOEsp | 30 | 129(±38) | 0.055 | 0.118 | -0.023 | 495647.88 | 6189592.87 |
| | NOOLac | 30 | 109(±26) | 0.054 | 0.115 | -0.011 | 495122.63 | 6187988.33 |
| | NOOSav | 30 | 111(±63) | 0.044 | 0.131 | -0.002 | 499144.09 | 6192586.61 |
| | NOOTre | 32 | 157(±33) | 0.049 | 0.128 | -0.014 | 500610.5 | 6192174.74 |
| | NOOVoo | 28 | 138(±30) | 0.043 | 0.138 | 0.043 | 496043.43 | 6191942.32 |
| | NOUBoo | 28 | 173(±43) | 0.064 | 0.182 | 0.07 | 495090.59 | 6198734.52 |
| | NOUCir | 30 | 134(±42) | 0.067 | 0.156 | 0 | 496859.39 | 6197875.96 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NOUMay | 30 | 155(±34) | 0.053 | 0.152 | -0.001 | 498770.59 | 6196493.34 |
| | NOUSpo | 30 | 140(±38) | 0.064 | 0.134 | -0.02 | 499342.58 | 6194843.9 |
| | ONEBag | 30 | 147(±28) | 0.042 | 0.146 | -0.015 | 501655.8 | 6191325.5 |
| SEUGE | BuiPie | 6 | 96(±27) | 0.1 | 0.041 | 0.044 | 736154.95 | 6421760.5 |
| | CLATou | 9 | 105(±55) | 0.07 | 0.08 | -0.006 | 740628.2 | 6424059.5 |
| | LAVRiv | 15 | 138(±51) | 0.064 | 0.105 | -0.018 | 738719.1508 | 6430339.667 |
| | LAVSuc | 30 | 122(±31) | 0.053 | 0.102 | -0.097 | 740929.25 | 6428709.75 |
| | PONAmo | 30 | 127(±26) | 0.056 | 0.091 | -0.01 | 736948.8429 | 6427435.268 |
| | PONBom | 30 | 133(±32) | 0.084 | 0.072 | -0.08 | 735932.9 | 6423741.5 |
| | PONCha | 30 | 148(±29) | 0.048 | 0.108 | -0.014 | 742060.2 | 6428955.5 |
| | PONTet | 11 | 114(±28) | 0.112 | 0.061 | -0.269 | 734965.3332 | 6421406.405 |
| | PONTis | 30 | 129(±18) | 0.045 | 0.11 | -0.006 | 739554.4898 | 6428659.981 |
| | SERSau | 6 | 143(±28) | 0.057 | 0.098 | 0.125 | 737591.5467 | 6428895.11 |
| | SEUCch | 30 | 110(±21) | 0.098 | 0.078 | -0.162 | 736912.1 | 6419850.75 |
| | SEUCou | 30 | 134(±27) | 0.059 | 0.102 | -0.081 | 742646.6581 | 6424696.732 |
| | SEUCro | 30 | 165(±33) | 0.08 | 0.1 | 0.151 | 742507.05 | 6426326.75 |
| | SEUFag | 30 | 126(±32) | 0.078 | 0.097 | -0.21 | 737803.35 | 6423289.5 |
| | SEURod | 30 | 157(±38) | 0.05 | 0.102 | 0.059 | 743269.65 | 6430201.25 |
| | SEUSau | 30 | 138(±19) | 0.055 | 0.101 | 0.006 | 742641.15 | 6428719.25 |
| ROYA | BENCas | 8 | 158(±33) | 0.048 | 0.37 | 0.043 | 1064681.159 | 6330776.294 |
| | BIECas | 30 | 157(±47) | 0.076 | 0.398 | 0.062 | 1061599.65 | 6342372.25 |
| | BIEMai | 30 | 172(±33) | 0.153 | 0.346 | -0.029 | 1065152.9 | 6339425 |
| | BIEMin | 30 | 171(±36) | 0.156 | 0.34 | 0.024 | 1060994.8 | 6340253.25 |
| | CAIGaf | 30 | 170(±48) | 0.067 | 0.293 | 0.01 | 1061323.924 | 6332296.13 |
| | LEVBri | 30 | 140(±27) | 0.057 | 0.352 | -0.028 | 1070230.357 | 6340355.202 |
| | LEVTen | 30 | 153(±30) | 0.069 | 0.386 | 0.004 | 1067621.35 | 6339593.25 |
| | MAGCem | 30 | 176(±32) | 0.075 | 0.272 | 0.041 | 1061971.15 | 6327821.25 |
| | REFRic | 30 | 123(±23) | 0.133 | 0.363 | -0.012 | 1068559.35 | 6343613.75 |
| | ROYAmb | 30 | 171(±52) | 0.083 | 0.243 | 0.041 | 1018145.09 | 1901873.82 |
| | ROYBre | 30 | 193(±63) | 0.085 | 0.238 | 0.012 | 1016478.36 | 1894038.21 |
| | ROYChi | 24 | 196(±24) | 0.081 | 0.244 | -0.005 | 1064068.351 | 6329711.933 |
| | ROYDal | 30 | 143(±27) | 0.06 | 0.379 | -0.002 | 1067460.706 | 6338535.476 |
| | ROYEvc | 30 | 196(±59) | 0.085 | 0.237 | 0.031 | 1016219.44 | 1895386.21 |
| | ROYFon | 30 | 199(±65) | 0.087 | 0.229 | 0.054 | 1018390.04 | 1902415.5 |
| | ROYGia | 30 | 162(±47) | 0.092 | 0.22 | 0.072 | 1016240.41 | 1897091.32 |
| | ROYOrt | 30 | 131(±18) | 0.229 | 0.267 | -0.017 | 1065392.1 | 6346381.5 |
| | ROYPie | 21 | 207(±31) | 0.085 | 0.235 | 0.029 | 1062946.375 | 6322646.07 |
| | ROYSca | 30 | 172(±47) | 0.053 | 0.353 | 0.048 | 1065916.912 | 6335228.32 |
| | ROYTen | 30 | 141(±27) | 0.065 | 0.384 | 0.07 | 1068075.713 | 6341877.007 |
| | ROYVei | 30 | 182(±40) | 0.08 | 0.249 | 0.046 | 1016385.91 | 1897993.54 |

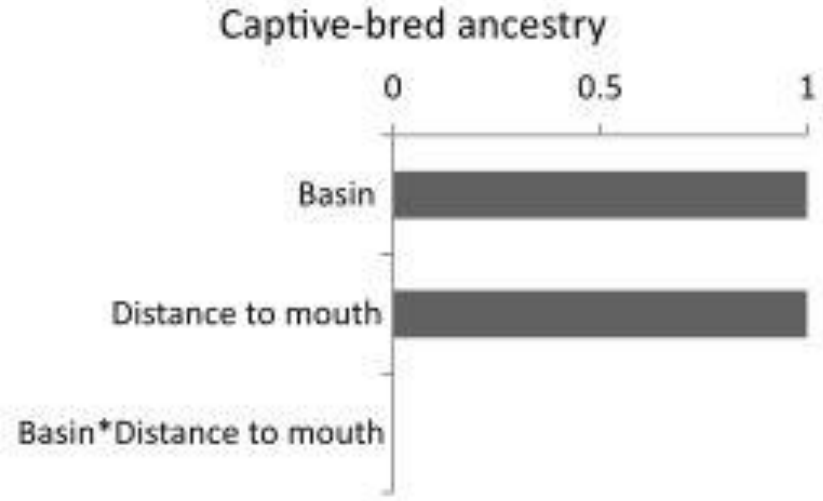
*Appendix S3.2: Summary of individual proportion of captive-bred ancestry for each basin (in %)*

| | Min | Max | Median | Mean | Sd |
|---|---|---|---|---|---|
| AUDE | 1.5 | 98.4 | 7.1 | 16.3 | 24.3 |
| ONE | 1.6 | 94.4 | 8.3 | 22.6 | 30.9 |
| SEUGE | 0.5 | 33.6 | 1.6 | 3.7 | 8.0 |
| ROYA | 25.5 | 90.7 | 66.7 | 59.1 | 22.9 |

*Appendix S3.3: Model selection for explaining spatial patterns of admixture (A), and spatial patterns of genetic diversity (B) and differentiation (C)*

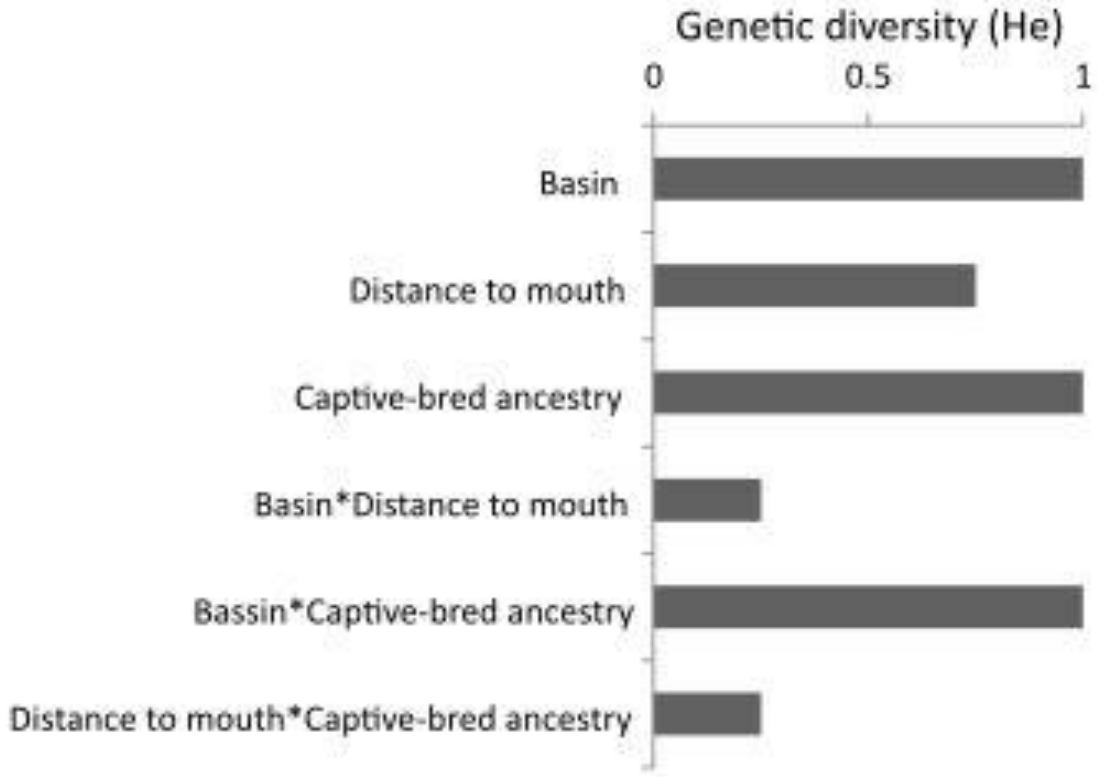
(A)

| Introg | (Int) | BV | dist_drn | BV:dist_drn | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|
| 4 | -1.98 | + | 0.42 | | 6.00 | 63.05 | -112.70 | 0.00 | 0.87 |
| 8 | -1.84 | + | 0.07 | + | 9.00 | 64.84 | -108.60 | 4.10 | 0.11 |
| 2 | -1.70 | + | | | 5.00 | 57.90 | -104.80 | 7.89 | 0.02 |
| 3 | -1.00 | | 0.53 | | 3.00 | 39.09 | -71.80 | 40.92 | 0.00 |
| 1 | -0.94 | | | | 2.00 | 32.56 | -60.90 | 51.81 | 0.00 |



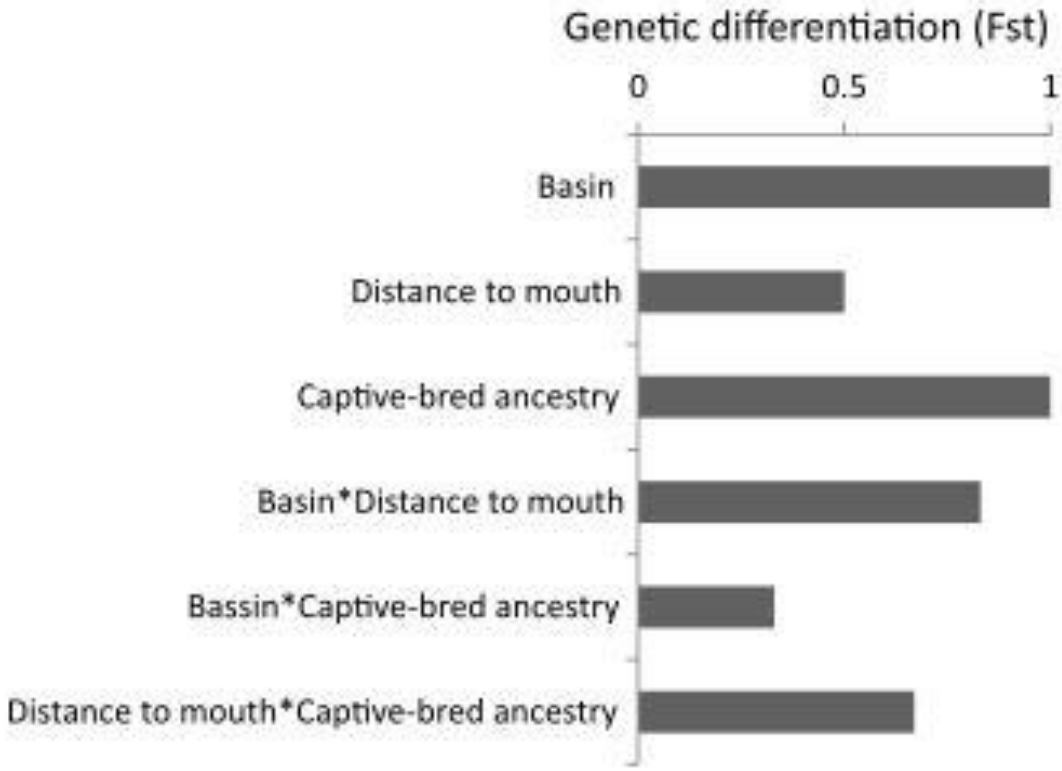
(B)

| He | (Int) | BV | dist_drn | Introg | BV:dist_drn | BV:Introg | dist_drn:Introg | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22.0 | -2.2 | + | | 4.2 | | + | | 9.0 | 155.9 | -290.6 | 0.0 | 0.4 |
| 24.0 | -2.1 | + | -6.82E-06 | 4.2 | | + | | 10.0 | 157.0 | -290.3 | 0.4 | 0.3 |
| 56.0 | -2.2 | + | -2.80E-06 | 4.4 | | + | -1.47E-05 | 11.0 | 157.6 | -288.6 | 2.1 | 0.1 |
| 32.0 | -2.3 | + | 4.32E-06 | 4.2 | + | + | | 13.0 | 159.9 | -287.2 | 3.5 | 0.1 |
| 64.0 | -2.3 | + | 7.78E-06 | 4.5 | + | + | -2.56E-05 | 14.0 | 161.0 | -286.2 | 4.5 | 0.0 |
| 6.0 | -1.8 | + | | 1.0 | | | | 6.0 | 123.7 | -234.0 | 56.6 | 0.0 |
| 8.0 | -1.8 | + | -1.79E-06 | 1.0 | | | | 7.0 | 123.7 | -231.6 | 59.0 | 0.0 |
| 40.0 | -1.7 | + | -1.02E-05 | 0.8 | | | 1.81E-05 | 8.0 | 124.5 | -230.6 | 60.0 | 0.0 |
| 16.0 | -1.9 | + | 7.40E-06 | 1.1 | + | | | 10.0 | 125.8 | -227.8 | 62.9 | 0.0 |
| 48.0 | -1.9 | + | 4.49E-06 | 0.7 | + | | 2.89E-05 | 11.0 | 126.5 | -226.3 | 64.3 | 0.0 |
| 4.0 | -2.0 | + | 1.41E-05 | | | | | 6.0 | 112.0 | -210.7 | 79.9 | 0.0 |
| 12.0 | -1.9 | + | 8.67E-06 | | + | | | 9.0 | 114.4 | -207.8 | 82.8 | 0.0 |
| 2.0 | -1.7 | + | | | | | | 5.0 | 108.8 | -206.7 | 83.9 | 0.0 |
| 5.0 | -2.0 | | | 1.6 | | | | 3.0 | 103.8 | -201.2 | 89.5 | 0.0 |
| 7.0 | -2.0 | | 8.54E-06 | 1.4 | | | | 4.0 | 104.7 | -200.7 | 90.0 | 0.0 |
| 39.0 | -1.9 | | -1.89E-06 | 1.1 | | | 2.39E-05 | 5.0 | 105.6 | -200.3 | 90.3 | 0.0 |
| 3.0 | -1.9 | | 3.47E-05 | | | | | 3.0 | 77.5 | -148.6 | 142.1 | 0.0 |
| 1.0 | -1.5 | | | | | | | 2.0 | 69.6 | -135.0 | 155.6 | 0.0 |

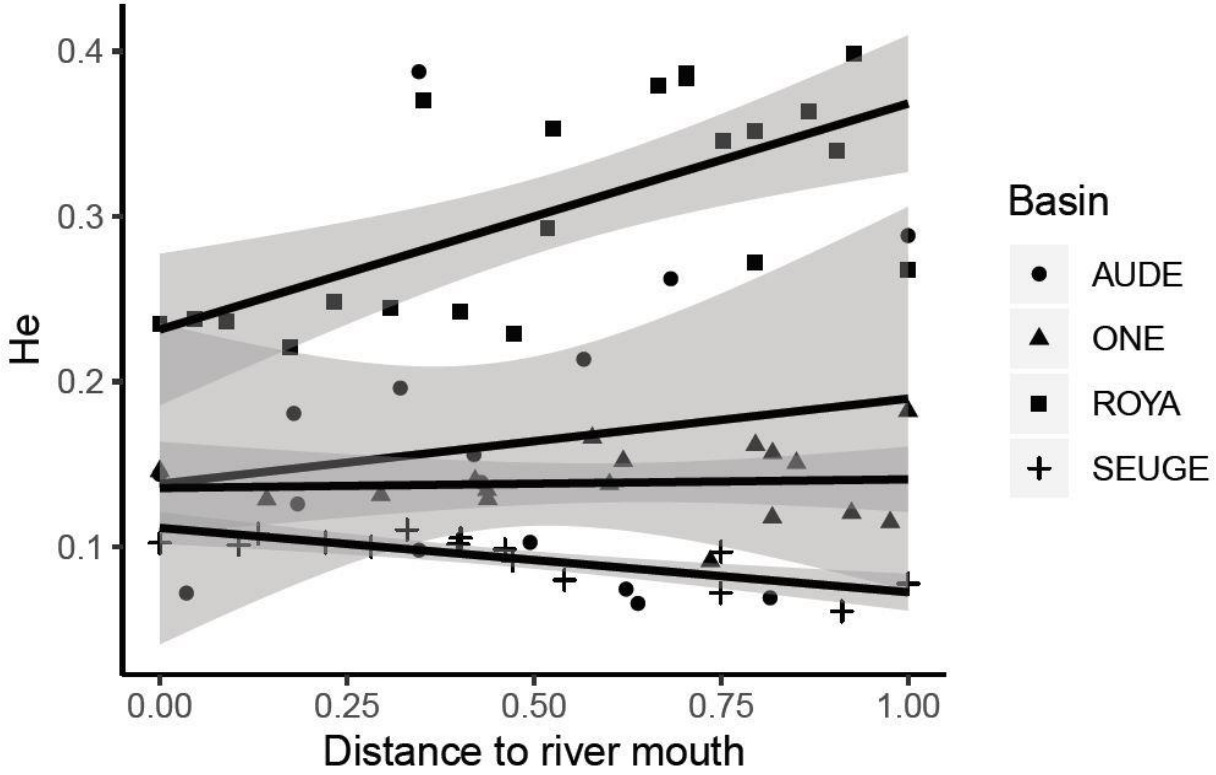


(C)

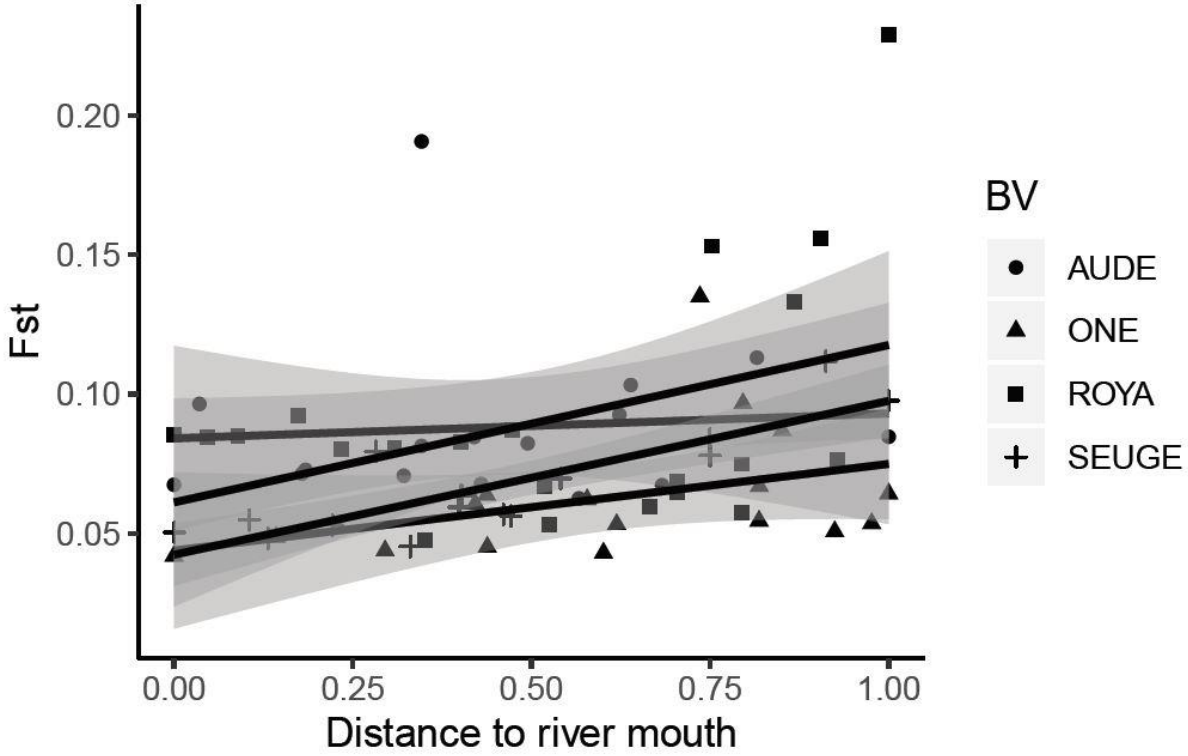
| Fst | (Int) | BV | dist_drn | Introg | BV:dist_drn | BV:Introg | dist_drn:Introg | df | logLik | AICc | delta | weight |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3.00 | 0.05 | | 2.02E-06 | | | | | 3.00 | 146.53 | -286.70 | 0.00 | 0.35 |
| 7.00 | 0.05 | | 1.68E-06 | 0.02 | | | | 4.00 | 147.37 | -286.10 | 0.59 | 0.26 |
| 39.00 | 0.06 | | 1.29E-06 | 0.00 | | | 1.13E-06 | 5.00 | 147.67 | -284.40 | 2.31 | 0.11 |
| 64.00 | 0.09 | + | -1.34E-06 | -0.02 | + | + | 1.13E-05 | 14.00 | 159.76 | -283.70 | 2.95 | 0.08 |
| 48.00 | 0.09 | + | -7.76E-07 | -0.06 | + | | 8.30E-06 | 11.00 | 154.80 | -283.00 | 3.73 | 0.06 |
| 56.00 | 0.07 | + | 1.66E-07 | 0.05 | | + | 6.37E-06 | 11.00 | 154.71 | -282.80 | 3.90 | 0.05 |
| 8.00 | 0.07 | + | 1.25E-06 | 0.03 | | | | 7.00 | 148.68 | -281.50 | 5.18 | 0.03 |
| 4.00 | 0.06 | + | 1.67E-06 | | | | | 6.00 | 147.39 | -281.40 | 5.27 | 0.03 |
| 40.00 | 0.08 | + | 5.14E-07 | 0.00 | | | 1.91E-06 | 8.00 | 149.47 | -280.50 | 6.16 | 0.02 |
| 6.00 | 0.08 | + | | 0.05 | | | | 6.00 | 146.26 | -279.20 | 7.54 | 0.01 |
| 24.00 | 0.05 | + | 1.47E-06 | 0.14 | | + | | 10.00 | 150.76 | -277.70 | 8.96 | 0.00 |
| 5.00 | 0.07 | | | 0.04 | | | | 3.00 | 142.02 | -277.70 | 9.02 | 0.00 |
| 12.00 | 0.08 | + | 3.56E-07 | | + | | | 9.00 | 148.73 | -276.40 | 10.29 | 0.00 |
| 16.00 | 0.08 | + | 2.78E-07 | 0.03 | + | | | 10.00 | 149.96 | -276.10 | 10.57 | 0.00 |
| 22.00 | 0.07 | + | | 0.15 | | + | | 9.00 | 148.24 | -275.40 | 11.28 | 0.00 |
| 2.00 | 0.09 | + | | | | | | 5.00 | 142.21 | -273.50 | 13.23 | 0.00 |
| 32.00 | 0.07 | + | -8.82E-09 | 0.15 | + | + | | 13.00 | 152.60 | -272.60 | 14.12 | 0.00 |
| 1.00 | 0.08 | | | | | | | 2.00 | 136.89 | -269.60 | 17.10 | 0.00 |



*Appendix S4: Plot of expected heterozygosity (He) against riparian distance to the river mouth, depending on the river basin as an interaction term*



*Appendix S3.5: Plot of genetic differentiation (Fst) against riparian distance to the river mouth, depending on the river basin as an interaction term*



# Chapter IV. Genetic admixture between captive-bred and wild individuals affects patterns of dispersal in a brown trout (*Salmo trutta*) population

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**Key words:** Genetic admixture, dispersal, brown trout, microsatellites, individual assignment, captive breeding, stocking, hatchery.

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## Summary

1. Genetic admixture between captive-bred and wild individuals has been demonstrated to affect many individual traits, although little is known about its potential influence on dispersal, an important trait governing the eco-evolutionary dynamics of populations.
2. Here, we quantified and described the spatial distribution of genetic admixture in a brown trout (Salmo trutta) population from a small watershed that was stocked until 1999, and then tested whether or not individual dispersal parameters were related to admixture between wild and captive-bred fish.
3. We genotyped 715 fish at 17 microsatellite loci sampled from both the mainstream and all populated tributaries, as well as 48 fish from the hatchery used to stock the study area. First, we used Bayesian clustering to infer local genetic structure and to quantify genetic admixture. We inferred first generation migrants to identify dispersal events and test which features (genetic admixture, sex and body length) affected dispersal parameters (i.e. probability to disperse, distance of dispersal and direction of the dispersal event).
4. We identified two genetic clusters in the river basin, corresponding to wild fish on the one hand and to fish derived from the captive strain on the other hand, allowing us to define an individual gradient of admixture. Individuals with a strong assignment to the captive strain occurred almost exclusively in some tributaries, and were more likely to disperse towards a tributary than towards a site of the mainstream. Furthermore, dispersal probability increased as the probability of assignment to the captive strain increased, and individuals with an intermediate level of admixture exhibited the lowest dispersal distances.
5. These findings show that various dispersal parameters may be biased by admixture with captive-bred genotypes, and that management policies should take into account the differential spread of captive-bred individuals in wild populations.

## Introduction

Captive breeding programs are a management practice that is commonly used to sustain endangered species and populations (Seddon et al. 2007; Frankham 2008), and/or to enhance populations in order to improve recreational activities such as fishing and hunting (Hansen et al. 2009; Burnside et al. 2016). However, captive-bred individuals can differ in their genetic and phenotypic characteristics from wild ones, mainly because captivity and the selective pressures associated with it strongly differ from natural environments (Frankham et al. 1986; Blanchet et al. 2008; Christie et al. 2016). Therefore, genetic admixture between wild populations and their captive-bred conspecifics is a major conservation and wildlife management conundrum (Randi 2008; Araki and Schmid 2010).

Genetic admixture between captive-bred and wild individuals can strongly influence individual fitness, notably through its consequences on behavioral, morphological and physiological traits (Geiser and Ferguson 2001; Stoinski et al. 2003). Among these traits, dispersal (which is defined here as all movements of individuals or propagules, potentially responsible for gene flow across space and time; Ronce 2007) is a determinant mechanism for population dynamics (Clobert 2012). Dispersal indeed plays a key role in the persistence of local populations and evolution of species' spatial distribution, in particular because dispersal enables gene flow among populations, underlies colonization and reduces extinction risk (Hanski 1998; Campbell Grant et al. 2010). Dispersal also directly benefits individuals' fitness in response to environmental changes, kin competition, and risk of inbreeding (Cressman and Křivan 2006). Moreover, differential dispersal (in terms of probability to disperse, distance travelled and characteristics of destination sites) among individuals varying in their genetic background (i.e. wild fish, admixed fish or descendants of captive-bred fish) could condition the spatial distribution of admixture.

The individual determinants of dispersal have been widely studied (Clobert 2012; Lowe and McPeek 2014) which permits generating predictions regarding how patterns of dispersal may differ between captive-bred, wild and resulting admixed individuals. For instance, Dingemanse et al. (2003) showed that individuals exhibiting higher exploratory behavior are more likely to disperse, and do so over longer distances. Similarly, more aggressive and bold individuals should disperse less, because they can successfully hold on to resources and to avoid costs of dispersal

(Cote et al. 2010; Hudina et al. 2015). Given that captive-bred individuals tend to show a lower propensity for exploration (Robert et al. 1987; Johnsson and Abrahams 1991) and higher aggression and dominance levels (Kelley et al. 2006; Frumkin et al. 2016), we may expect captive-bred individuals to show a less pronounced dispersal behavior than wild ones. Alternatively, larger or heavier individuals with higher growth rate may show higher dispersal propensity and move over longer distances (Debeffe et al. 2014; Radinger and Wolter 2014; Dahirel et al. 2015). Because captive-bred individuals generally exhibit higher growth rates and higher body mass (Tymchuk and Devlin 2005), we may thus conversely expect captive-bred individuals to show a more pronounced dispersal behavior than wild ones. To sum up, captive-bred and wild individuals vary in many traits related to dispersal: clear predictions regarding differences between strains and admixed individuals (i.e. those sharing the genome of both captive-bred and wild individuals), in terms of dispersal propensity and distance, strongly depend on the relative importance of these traits on dispersal behavior.

Studies comparing dispersal differences between wild and captive-bred strains focus generally on immediate post-release movements. For instance, captive-bred fish released into a natural system are less likely to disperse (Symons 1969; Jorgensen and Berg 1991), but those that disperse do so over longer distances than wild ones (Bettinger and Bettoli 2002; Ebner and Thiem 2009). In birds, captive-bred individuals released in the wild tend to disperse less, and over shorter distances than wild ones, probably because of both shorter life span and lower migration speed in captive-bred individuals (Amar et al. 2008; Söderquist et al. 2013). To our knowledge however, no study has yet focused on the direct link between genetic admixture and dispersal in a population in which captive-bred strains have been implanted for a relatively long time, and in which admixture is thus a part of the population dynamics.

Here, we used a headwater stream fish, the brown trout (*Salmo trutta*), to test whether or not long-term admixture between wild individuals and captive-bred fish affects patterns of dispersal. We focused on a population residing in a small mountain watershed that was heavily stocked with captive-bred fish from 1972 to 1999, and in which we thoroughly sampled the whole watershed. By combining this fine scale sampling design and a series of tools derived from population genetics, we specifically aimed to (i) quantify genetic admixture of the supposedly wild trout with captive-bred trout at the individual level, (ii) test how admixture was spatially

distributed within the river basin, and (iii) assess the effect of genetic admixture on individual dispersal parameters (dispersal probability, dispersal direction and dispersal distances), while simultaneously taking into account the effects of sex and body size on dispersal parameters (two characteristics having been identified as major determinants of dispersal; Pusey 1987; Gutiérrez and Menéndez 2003). We expected admixed fish and descendants of captive-bred fish to display different dispersal behavior than wild fish, and dispersal traits divergence between groups to increase with the increase in the proportion of allochtonous ancestry in the genome.

## Material and Methods

*Study area*

The Neste d'Oueil is a snow/rain-fed river from the French Pyrenees (Figure 1). Its source is at 1850m altitude, it confluences with the Neste d'Oô River at 765m, after a 9.2 km course, and it is situated ~500 km from the Atlantic Ocean mouth of the Garonne River (from which it is a tributary). With a 1.8 % mean slope, its water flow varies from 0.4 $m^3.s^{-1}$ to 1 $m^3.s^{-1}$. Its basin drains 30.7 $km^2$ and is composed of 14 tributaries, all shorter than 3 km in riparian distance. The river basin is fragmented by ten obstacles, among which eight weirs (four of them are lower than 0.5 m and the other four are between 0.5 and ~1.5 m high), a culvert and a natural waterfall (1.5 m high) (Figure 1). According to local managers and a recent telemetric survey (unpublished data), all these obstacles are passable downstream, and upstream passage depends upon water flow conditions. Highest obstacles are passable (upstream) on rare high flow conditions, whereas smallest obstacles are passable during fall and spring normal high flows (Ovidio et al., 2015), which –overall– suggests that gene flow can occur across the whole river catchment. The fish community is dominated by sedentary brown trout (the population does not sustain anadromous individuals as the river is situated far too upstream to allow for saltwater migrations), with some rare bullhead individuals (*Cottus gobio*). The last brown trout stockings in the Neste d'Oueil occurred in 1999. Since 1972, these stockings were all done from a local trout hatchery (Soueich) that is administrated by the regional angling association (Fédération Départementale pour la Pêche et la Protection des Milieux Aquatiques de Haute Garonne) and used to stock most rivers in the area. This hatchery was created in 1971, and fish from this hatchery originated from crosses between Danish strains classically used in European hatcheries, and individuals from a

neighboring river basin (the Ger River). Unpublished studies showed that these hatchery fish form a population being genetically distinct from wild populations from local rivers, even from the Ger River itself. Until 1999, yearly stocking in the Neste d'Oueil river basin mainly consisted in releasing juvenile fish (young-of-the-year) and/or in placing incubated eggs across the entire river basin, including the mainstream and tributaries. The local anglers associations in charge of the stocking aimed at releasing fish evenly in the river basin, and did not target any particular locations (personal communication). In some occasions (i.e. ~ once every two years), the downstream part of the mainstream was additionally stocked with adults for recreational activity (see Appendix S1).

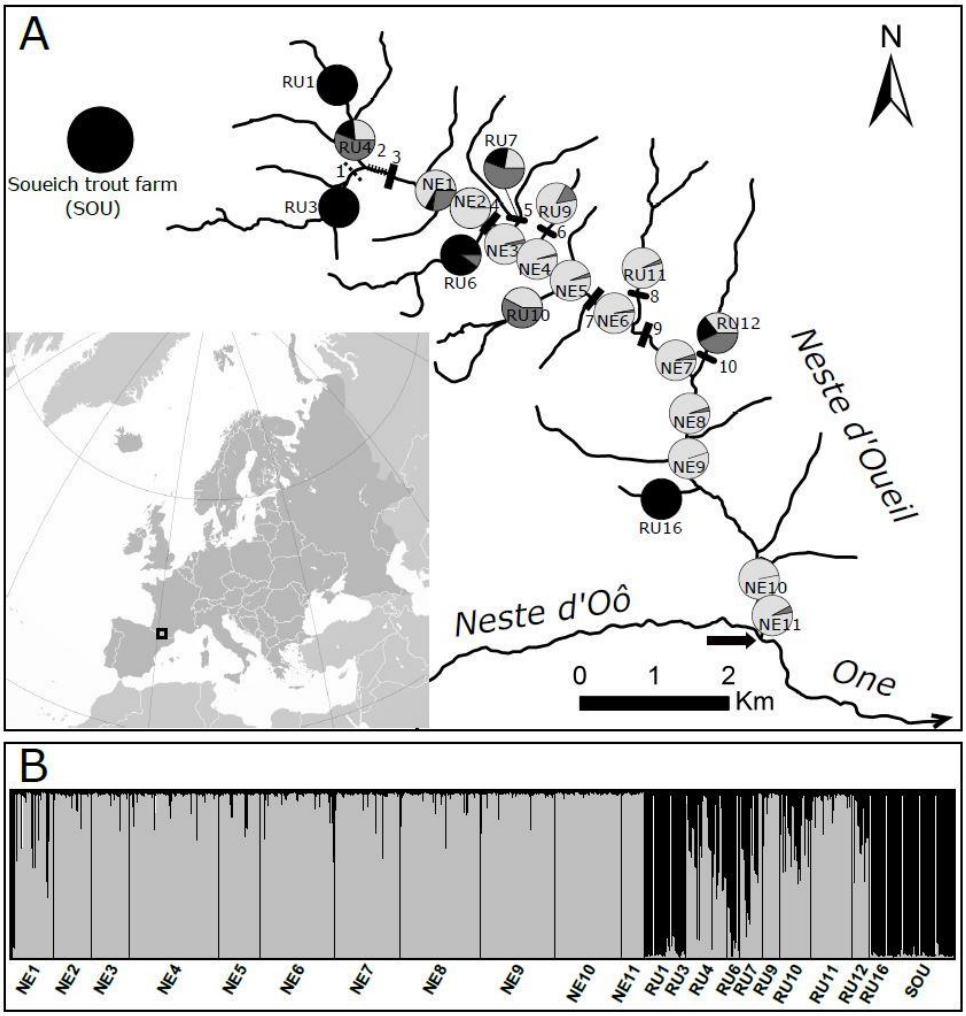


*Figure 4.1. (A) Maps representing the geographic situation of the Neste d'Oueil, as well as sampling sites, and obstacles (natural: square dashed line, culverts: rectangle dashed line, artificial weirs lower than 0.5m: black rounded rectangles, artificial weirs higher than 0.5m: black rectangles) in the river basin. Pie*

*charts represent the proportions of individuals from each site that can be assigned to clusters "wild" (light grey), "captive-bred" (black) and "mixed" (dark grey) as inferred from Q-values. The black arrow indicates the location of the river mouth. (B) Classical bar plot for STRUCTURE results ("wild" cluster in light grey and "captive-bred" cluster in black).*

*Field sampling*

Brown trout were sampled in July 2014, using electric-fishing from 21 sites in total, 11 on the mainstream ("NE" sites, Figure 1), and 10 on tributaries ("RU" sites, Figure 1). We failed to find brown trout in four out of the 14 tributaries sampled despite intensive sampling efforts, which indicated that they were probably fishless. We mainly sampled small individuals from the 1 year-old class age. In total, we captured 715 individuals, with sample sizes ranging from 5 (RU3) to 72 (NE4), and with an average of 35 ± 19 individuals per site (Appendix S2 and S3). We additionally sampled 48 fish from the Soueich trout hatchery to genetically characterize the captive-bred strain used in this river, and hence to quantify genetic admixture with captive-bred trout. Each individual was measured (total length in mm; see Appendix S2 for details), and a pelvic fin clip was taken for genetic analyses. All individuals were released alive to their original sampling site.

*Genotyping*

Individual multilocus genotypes were obtained at a total of 17 markers. Among these 17 markers, we used 16 microsatellite markers (BS31, One9, SsoSL311, SsoSL438, T3-13, Sfo1, Ssa064, Ssa417, Ssa103, Ots515NWFSC, Ssa-60NVH, Ssa-TAP2a, Ssa-UBA, Ssa14, Ssa85DU and SsoSL417; see Appendix S4 for details), and one sex-linked marker, Salmo-Sdy, which enables determination of the sex of each individual (Quéméré et al. 2014). The 17 markers were assembled in three PCR multiplexes.

Genomic DNA was extracted from the fin clips using a salt-extraction protocol (Aljanabi 1997). The loci were amplified using the QIAGEN Multiplex PCR Kit (Qiagen, Valencia, CA, USA). PCRs were carried out in a 10 µL final volume containing 5–20 ng of genomic DNA, 5 µL of 2X QIAGEN Multiplex PCR Master Mix, and locus-specific optimized combination of primers under the following conditions: 15 min at 95°C followed by 30 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C and finally followed by a 60 min elongation step at 72°C. Amplified fragments

were then separated on an ABI PRISM 3730 automated capillary sequencer. Allelic sizes were called using GENEMAPPER v.4.0 (Applied Biosystems, Foster City, CA, USA).

*Genetic diversity and differentiation*

We performed preliminary analyses on all markers except for the one linked to sex. We first investigated large allele drop-out and null alleles using Microchecker V2.2 (Van Oosterhout et al. 2004). We then tested for linkage disequilibrium between loci using FSTAT (Goudet 1995), and we used LOSITAN (Antao et al. 2008) to determine whether or not some of these loci displayed signs of natural selection, as suggested by previous studies (Blanchet et al. 2009; Keller et al. 2011). We did not detect significant linkage disequilibrium among loci but one of the 16 markers (Ssa-UBA) displayed a strong deficit in heterozygosity, most likely because of the presence of null alleles (Appendix S3). Two loci appeared to be under potential selection (Ots515 NWFSC and Ssa14). Ssa-UBA, Ots515 NWFSC, and Ssa14 were therefore discarded from the database for subsequent analyses. Overall, our final genetic dataset thus comprises 763 individuals genotyped at 13 microsatellite markers (plus the sex marker Salmo-Sdy), with in total 1.48% of missing data.

Genetic diversity within each of the sampling sites was estimated over all loci by computing unbiased expected heterozygosity (He) using GENETIX (Belkhir et al. 2004), standardized allelic richness (i.e. the mean number of alleles corrected for the smaller sample size, Ar) after exclusion of small sample locations (n<14; RU3, RU6, RU9 and RU12) using FSTAT, and the Fis index using GENEPOP (Rousset 2008). Departure from Hardy-Weinberg equilibrium within each sampling site and each locus was calculated using GENEPOP. Genetic differentiation was assessed by computing global Fst over all sites and pairwise Fst between sites using the adegenet R package (Jombart 2008).

*Population structure and admixture extent*

We assessed how *S. trutta* populations are genetically and spatially structured along the Neste d'Oueil, and whether there were differences in terms of spatial distribution between wild, captive-bred descendants and admixed individuals. We performed genetic clustering using STRUCTURE 2.3.1 (Pritchard et al. 2000) with the admixture model and the correlated allele frequency model, without prior population information. Runs were performed with a burn-in period of 200.000 and

200.000 subsequent MCMC repetitions. The number K of clusters ranged from 1 to 10 (5 runs each). Log-likelihood plots and ΔK statistics were obtained via the Evanno's method (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and vonHoldt 2012), and they were used to infer the optimal K-value. Twenty runs were then performed with this optimal K-value and the ten best runs (the ones with highest LnP(D) values) were compiled using CLUMPP (Jakobsson and Rosenberg 2007) to get final averaged individual Q-values. Individuals were assigned to the cluster with the greatest Q-value, provided that value exceeded 0.7, as this has been done for other salmonid species (Hansen et al. 2001; Vähä and Primmer 2005; Valiquette et al. 2014). Individuals with intermediate Q-values (i.e between 0.3 and 0.7) were considered genetically admixed. Graphical displays of STRUCTURE plots were generated using DISTRUCT software (Rosenberg 2003). We directly used the probability of assignment (individual Q-value) to the cluster containing all individuals from the Soueich trout hatchery as an estimate of individual genetic admixture with the captive-bred strain (Hansen et al. 2001).

In order to further describe the spatial structure of strains in the river basin, we tested the relationship between site distance to the river mouth (confluence between the Neste D'Oô and the One Rivers; Figure 1) and admixture level (averaged at the population level and log-transformed). We also tested whether or not admixture level and distance to the mouth (and the resulting interaction term) were significantly related to mean allelic richness and population differentiation (mean Fst) respectively. Relationships were tested using linear models, and all variables were standardized. Finally, we tested to what extent geographic isolation and fragmentation by weirs affected the spatial distribution of strains by testing the relationships between difference in mean admixture between pairs of sites, pairwise riparian distance and number of obstacles between pairs of sites, using multiple regressions on distance matrices (MRDM; Smouse et al. 1986) coupled with regression commonality analyses (CA; Prunier et al. 2015).

*Inferring dispersal from first generation migrants*

We then aimed at identifying individual dispersal events by inferring "first generation migrants" (i.e. F0 immigrants) using GENECLASS 2 (Piry et al. 2004). We used Paetkau's method (Paetkau et al. 1995) to assign "first generation migrants" to their population of origin which involves calculating the expected frequency of each individual's genotype in each population (product of

expected genotype frequency at each locus, based on the observed distributions of alleles) and subsequent assignment of each individual to the population where its expected genotype frequency is highest. We tested the null hypothesis that an individual was born in the population in which it was sampled using a Monte Carlo resampling method (Rannala and Mountain 1997; Cornuet et al. 1999). Rejection of the null hypothesis indicated an individual having dispersed from one site to another. The probability threshold for inferring first generation migrants was set to 0.01.

We tested the effect of level of admixture, sex and body length of each individual on four dispersal parameters (response variables): (i) the *individual dispersal probability* (obtained by transformation of the binomial variable "dispersal vs. non-dispersal" using the *predict()* R function), (ii) the *individual dispersal distance* (i.e. geographic river distance between the site of origin and the site of destination), (iii) the *individual dispersal direction* (i.e. upstream or downstream-directed regarding the water flow), and (iv) the *probability of stream type of the destination site* (i.e. transformed binomial variable "dispersal to a site of the mainstream vs. to a tributary" using the R *predict()* function). We used Generalized Linear Models (GLM; Gaussian or Binomial error terms depending on the response variable) and we standardized all continuous variables. We integrated the quadratic term for the level of admixture to test for potential non-linear relationships. For each of the four dependent variables, we then used a model selection procedure based on the Akaike Information Criteria (AIC) to identify the most parsimonious set of predictors.

## Results

*Genetic diversity and differentiation*

Expected heterozygosity over all loci (He) ranged from 0.42 to 0.74 (mean = 0.65 ± 0.09), standardized mean allelic richness (Ar) varied from 4.49 to 9.39 (6.24 ± 1.21), and Fis within each site ranged from -0.06 to 0.15 (0.053 ± 0.047) (Appendix S4). Eight out of 21 populations significantly deviated from Hardy-Weinberg equilibrium (Appendix S4). The mean genetic differentiation (Fst) estimated across sites and loci was 0.12 (± 0.07), and pairwise Fst values between sites ranged from 0 between NE8 and NE10 (geographically close sites) to 0.21 between RU1 and RU16 (geographically distant sites) with an average of 0.070 (± 0.023). Finally, the sex

ratio in each site did not significantly differ from an equal ratio within sites (i.e. 50:50; $\chi^2$=0.55, d.f. = 1, p = 0.46).

*Population structure and admixture extent*

Individuals were assigned to two main genetic clusters. The first cluster (Cluster A, in light gray in Figure 1) mainly regrouped individuals from the mainstream and from some of the tributaries (RU4, RU7, RU9, RU10, RU11 and RU12). All individuals from RU1, RU3, RU6, RU16 and from the Soueich trout farm were assigned to the second cluster (Cluster B, in black in Figure 1), as well as a few individuals from NE1, RU4, RU7 and RU10. We therefore considered the first cluster as the "natural" population of the basin (hereafter "wild cluster"), and the second cluster as derived from past stocking activities (hereafter "captive-bred cluster"). We found that 79 % of the fish were most likely assigned to the "wild cluster" (566 individuals with Q-value < 0.3), 11 % were most likely assigned to the "captive-bred cluster" (77individuals with Q-value > 0.7), while 10% were equally assigned to both the wild and captive-bred clusters. Moreover, within the captive-bred cluster, subclustering showed two different clusters (Appendix S5), with all individuals from the trout farm on the one hand and individuals caught within the river basin on the other hand, hence suggesting genetic drift and/or confirming a partial admixture with the wild population. We found a positive correlation between distance to the river mouth and level of genetic admixture with captive-bred trout (r = 0.55, d.f. = 19, P-value < 0.01), indicating that individuals strongly assigned to the captive-bred cluster were mostly found on a few upstream tributaries (Figure 1).

*Patterns of genetic diversity and admixture*

Regarding patterns of allelic richness, we found a significant interaction term between admixture level (at the site level) and distance to the river mouth (Appendix S6, *allelic richness*). This indicated that, for an intermediate level of admixture, allelic richness was higher in upstream than in downstream sites (Appendix S6; see also Appendix S7 for the interpretation of model parameters in presence of a first-order interaction). This spatial trend held true at a low level of admixture (wild individuals) but not at a high level of admixture (Figure 2): downstream sites

associated with high admixture rates (notably RU16; Figures 1 and 2) were hence responsible for an inversion in the "natural" downstream increase in genetic diversity.

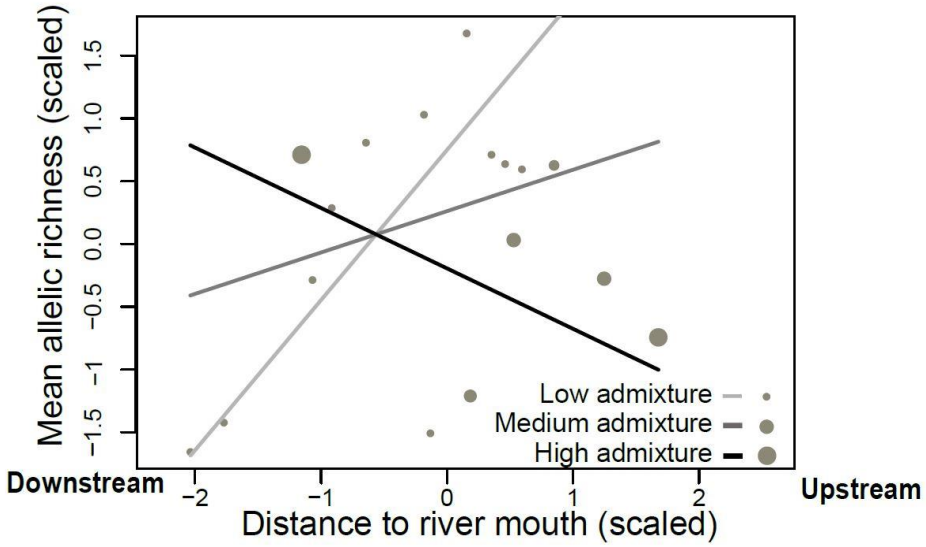


*Figure 4.2. Relationship between mean allelic richness and distance to the river mouth, depending on mean level of admixture at each site. In light grey, line for low admixture; in grey, line for average admixture; in black, line for high admixture.*

We further found a significant relationship between mean Fst measured at the site level and level of admixture: populations with a high proportion of fish assigned to the captive-bred cluster exhibited on average higher genetic differentiation (Appendix S6, *Fst*). Finally, we found that the number of obstacles significantly explained differences in admixture between sites (beta = 0.472, p-value = 0.004; Unique contribution = 0.109, Common contribution = 0.036), whereas riparian distance alone did not (beta = -0.127, p-value = 0.314; Unique contribution = 0.008, Common contribution = 0.036).

*Inferring dispersal from first generation migrants*

GENECLASS inferred 43 "first generation migrants" (24 females and 19 males) with a probability higher than 0.99, meaning that 5.6 % of individuals dispersed. Twenty individuals dispersed upstream, 23 dispersed downstream. Thirty-one of these migrants dispersed towards a site of the mainstream whereas 12 dispersed towards a tributary. Individual dispersal distances ranged from 322.8 to 8062.1 m with a median of 1921.3 m (± 2124.4).

The best model retained to explain individual dispersal probability included both genetic admixture with captive-bred trout (simple term only) and trout body length (Table 1, A). As shown in Appendix S8_A, dispersal probability was positively correlated with the probability of assignment to the captive-bred cluster (individuals with a high proportion of allochtonous ancestry were more likely to undergo a dispersal event). We additionally found a slight tendency for smaller individuals to be more likely to disperse. The model retained to explain individual dispersal distance also comprised body length as well as the simple and quadratic terms of genetic admixture (Table 1, B). Individuals strongly assigned either to the wild or to the captive-bred cluster tended to disperse over longer distances than individuals with mixed assignments (Appendix S8_B). Moreover, smaller individuals tended to disperse over longer distances. The best model explaining destination stream type comprised genetic admixture only (Table 1, D). The probability to disperse towards a site of the mainstream rather than towards a tributary was negatively correlated with the probability of being assigned to the captive-bred cluster. Finally, the null (intercept only) model was the best at explaining individual dispersal direction (Table 1, C), indicating that factors other than sex, body length and genetic admixture could explain individual variations in dispersal direction. Interestingly, sex was never retained in final simplified models, indicating no differences between males and females in dispersal traits.

| | A | | | B | | | C | | | D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dispersal probability | | | Dispersal Distance | | | Dispersal direction | | | Destination stream level | | |
| | β | SE | P | β | SE | P | β | SE | P | β | SE | P |
| Intercept | -2.800 | 0.166 | < 0.001** | < $10e^{-6}$ | 0.135 | 1.000 | 0.191 | 0.310 | 0.538 | 1.062 | 0.392 | 0.007** |
| Sex | | / | | | / | | | / | | | / | |
| Body length | -0.131 | 0.152 | 0.389 | -0.338 | 0.151 | 0.031* | | / | | | / | |
| Admixture | 0.288 | 0.131 | 0.028* | -0.957 | 0.640 | 0.143 | | / | | -0.966 | 0.365 | 0.008** |
| Admixture $^2$ | | / | | 1.274 | 0.628 | 0.049* | | / | | | / | |

*Table 4.1. Results from the final models retained for testing the role of sex, body length and admixture (along with the intercept) on the individual dispersal probability (A), the individual dispersal distance (B), the individual dispersal direction (C) and the type (i.e. mainstream or tributary) of the destination site of first generation migrants (D). Variables that were not retained in the final model are indicated by a slash bar. *: p-value < 0.05; **: p-value < 0.01.*

## Discussion

*Patterns of genetic admixture and genetic diversity at the basin scale*

Although stocking was stopped more than 16 years ago (i.e. 6-8 trout generations ago), we found that 21 % of individuals caught in the watershed were at least partially assigned to the captive-bred cluster. In particular, half of these individuals had a high assignment probability to the captive-bred cluster (> 0.7), suggesting either pure descendance from the captive-bred strain or back-crosses between admixed individuals and descendants from captive-bred fish. These results were difficult to compare to other systems because admixture rates between captive-bred and wild trout populations (and hence outcomes after stocking is stopped) are highly variable (Cagigas et al. 1999; Perrier et al. 2013).

Interestingly, the distribution of captive-bred genotypes in the watershed was not homogeneous. More precisely, some small tributaries, notably those situated in upstream parts of the basin, were exclusively populated with fish strongly assigned to the captive-bred cluster, whereas in the main river, fish strongly assigned to the captive-bred cluster were rare despite the homogeneous stocking effort in the watershed. This suggests that past stocking events significantly influenced the spatial distribution of alleles in this river basin. The persistence of fish strongly assigned to the captive-bred cluster in some tributaries could be due to the fact that these stretches were fishless or at very low density before stocking occurred: competition with the native strain was probably low, thus facilitating their settlement and reproductive success, enabling them to co-occur in parapatry with the original wild population. We also showed that obstacles partly explained differences in admixture between sites, and thus also probably contribute to favoring spatial segregation of the two strains in the basin. Indeed, some of these obstacles are difficult to cross upstreamwards at normal flow conditions, which may limit hybridization between wild and captive-bred individuals upstream of these obstacles. Nevertheless, additional information as to the historical spatial distribution of trout in this river system would be required to shed light on this spatial pattern.

Our results also suggested that past stocking activities and admixture with captive-bred trout affected spatial patterns of genetic diversity and genetic differentiation. We notably found an overall downstream decrease in allelic richness when levels of admixture were null to moderate

(Fig. 2 and Appendix S6), contrary to the traditional expectation of a downstream increase in genetic diversity (Morrissey and de Kerckhove 2009; Paz-Vinas et al. 2015). This pattern has already been observed though (Cyr and Angers 2011; Conti et al. 2015) and, it could be explained by upstream-biased gene flow, higher effective population sizes in upstream stretches, and/or a historical colonization of the river that began upstream and ended downstream (Paz-Vinas et al. 2015). Conversely, allelic richness slightly increased downstream for high levels of admixture, indicating that the distribution of allochtonous genotypes introduced with stocking events differed from the distribution of wild ones. Overall, our findings demonstrated that stocking, even when it occurred several generations ago, can strongly affect spatial patterns of allelic richness while increasing genetic differentiation of populations carrying a high proportion of allochtonous genotypes (Marie et al. 2010; Valiquette et al. 2014), highlighting the necessity for stocking events to be taken into account in riverscape genetics studies (Prunier et al. 2018).

*Captive breeding and genetic admixture affect patterns of dispersal*

Although we expected a male-biased dispersal or at least higher male mobility (McGinnity et al. 2003) due to the polygamous and/or polyandrous mating system of trout and to the strong competition for mates, we here found no evidence for an effect of sex on dispersal. Possible explanations could be a lack of statistical power, the lack of sex-biased dispersal for immature (1+)-trout as mainly sampled, or the actual absence of sex bias in dispersal at such a small spatial scale. We did not find any significant correlation between fish size and dispersal probability either, although younger, and therefore smaller individuals, are generally more likely to disperse because their territory is not yet established (Andreu and Barba 2006; Gachot-Neveu et al. 2009). Nevertheless, the "first generation migrant" assignment approach indicated that smaller fish disperse further than larger ones, a pattern already observed in riverine fish (Skalski and Gilliam 2000). Since shorter dispersal can enhance survival by reducing mortality risk (Johnsson et al. 1999), larger and more dominant fish may chose not to disperse over long distances, forcing the smaller ones to disperse further (Vøllestad et al. 2012). It is noteworthy that previous studies on brown trout populations regularly mentioned environmental variables (Cucherousset et al. 2005), fish densities (Olsson et al. 2006) or physiological status (Rustadbakken et al. 2004) as major determinants of dispersal, which were not considered in our study.

On the contrary, a constant driver of dispersal probability and dispersal distance in our study was the probability of assignment to the captive-bred cluster. Indeed, we found that: (i) dispersal probability was affected by the probability of assignment to the captive-bred cluster, and (ii) dispersal distances covered by dispersing individuals were lower in individuals with a mixed assignment than in individuals assigned either to the wild or captive-bred cluster. It is noteworthy that this later relationship was not due to the fact that captive-bred individuals were confined to tributaries (that we might expect to be farther away from a neighboring population than a population from the mainstream) since tributary-neighboring populations were actually not significantly further apart than mainstream-neighboring populations (mean distance to neighboring site in the mainstream: 733m *vs.* mean distance to neighboring site in tributaries: 826m; t-test, $t = -0.63$, $df = 17.25$, $p = 0.53$). Our results further revealed that individuals with genotypes closer to those of the trout farm were more likely to disperse towards a tributary whereas individuals strongly assigned to the wild cluster were more likely to disperse towards a site of the mainstream, which may contribute to maintaining the spatial segregation of strains observed in the watershed. Importantly, these findings were confirmed using another independent analysis based on dispersal measured by reconstructing full sibling families (see Appendix S9 for further details). To our knowledge, our study is one of the first to reveal such a pattern of "admixture-biased dispersal", which has implications for understanding population dynamics in an environment being -or having been- subject to stocking by fish of non-native origin.

This "admixture-biased dispersal" can have diverse explanations. For instance, admixed individuals may show differences in behavioral dominance and/or body size (Edelaar and Bolnick 2012). However, we did not identify any link between admixture and size, a main trait of dominance (Miller and Frey 1972). Alternatively –and non-exclusively–, the observed spatial distribution of admixture levels could result from differences in fitness between fish with a mixed assignment to the wild and captive-bred clusters (admixed fish *per se*) and pure (wild or captive-bred descendants) individuals (Johnson et al. 2010). Indeed, the average hybrid phenotypes have in many cases been shown to lie outside the phenotypic range of the parental ones, providing hybrid vigor or conversely hybrid depression (Facon et al. 2005; Rasmussen et al. 2012). These phenomena have been documented in salmonids (Wollebæk et al. 2012), although they are still ambiguous (McClelland and Naish 2007). Thus, predictions on the long-term genetic

consequences of stocking is still a challenge, and the link between our results and these differential fitness outcomes remains unclear (Harrison et al. 2005), thus adding a hypothesis for explaining our results. However, this pattern has many counterexamples in which admixed individuals show strong fitness increases enabling them to be better colonizers (Drake 2006; Keller and Taylor 2010).

Local selection pressures (Edelaar and Bolnick 2012) and/or differential habitat matching choice between the two strains (Edelaar et al. 2008) could also help explain both dispersal differences between strains and the observed heterogeneous spatial distribution of strains in the watershed. In habitat matching choice, individuals with a given phenotype aim to settle in the environment in which their fitness is optimized (Edelaar et al. 2008). Conversely to what is usually expected as a consequence of high gene flow (i.e. lower genetic differentiation among demes), matching habitat choice through dispersal could increase and/or maintain among-habitat divergence between the two different strains (Bolnick and Nosil 2007). In that case, this may contribute to maintain – together with fragmentation by weirs or natural obstacles– the spatial segregation between the "captive-bred" and the "wild" populations. Evaluating the relative relevance of such a hypothesis (compared to fragmentation) would require further investigations on the habitat characteristics (tributaries vs. mainstream) used by captive-bred and wild individuals and on the relative fitness of "captive-bred" and "wild" individuals in their respective habitats.

*Conclusions*

We demonstrated that parameters of dispersal can be affected by the proportion of allochtonous alleles brought by stocking activity, and that these effects of admixture can be observed long after stocking activities ceased. Although the mechanisms sustaining differences in dispersal along this gradient of admixture are still unknown, this finding has important implications for understanding and predicting the spread, distribution and maintenance of allochtonous alleles in wild populations. For instance, we here revealed a strong spatial segregation between "wild" and "captive-bred" strains at the watershed level. This heterogeneous spatial distribution could be maintained over time because of the differences in dispersal direction observed between wild and descendants of captive-bred fish, which in the long term could limit the spread of allochtonous alleles. However, this distribution could also be the result of competitive exclusion, where the

upstream populations act as sources of allochtonous alleles, which have trouble colonizing the downstream sites in which the wild strain is already present. We call for future studies completing these important findings and testing for underlying mechanisms. Indeed, it would be of interest to test whether differential dispersal parameters can affect the spatial dynamics of admixture in a watershed.

## Data accessibility

Raw data are deposited on figshare. DOI: 10.6084/m9.figshare.6886670.

## Authors' contributions

SB, NP and LT designed the experiment and coordinated the study; KSP, SB, NP, LT, OP, GL and CV conducted sampling; KSP, GL and CV carried out the experimental lab work; KSP and JGP ran the statistical analyses; KSP, JGP, SB, NP, LT, OP, GL and CV interpreted the data. KSP, SB, and JGP wrote the first draft of the manuscript. NP, LT, OP, GL and CV read, commented and corrected the initial draft, and all authors gave final approval for publication.

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## Conflict of Interest Statement

We have no competing interests and we declare no conflict of interests.

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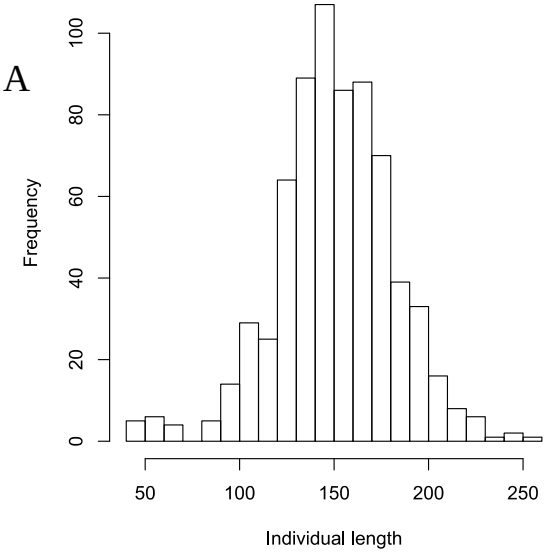
## Supplementary material for Chapter IV.

*Appendix S4.1: Pieces of information about stocking events in the Neste d'Oueil River gathered from local angling associations, in addition to informal interviews.*

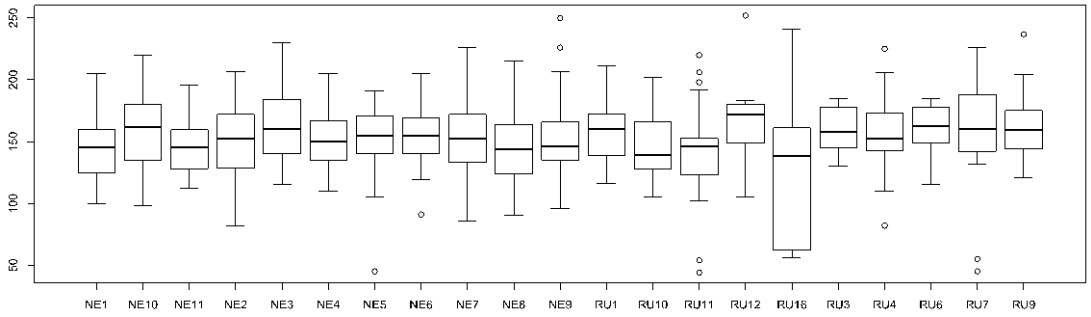
| *Year* | *Vibert Boxes (several thousand eggs each)* | *Larvae with yolk-sac* | *Juveniles 0+ (7/10 cm)* | *Adults (size in cm)* |
|---|---|---|---|---|
| *1987* | - | - | *4000* | *350 (24/30)* |
| *1988* | - | - | *10000* | *400 (24/30 and 40/50)* |
| *1989* | *40* | *20000* | - | *1200 (22/25)* |
| *1990* | *24* | - | - | - |
| *1991* | *20* | *8000* | - | *400 (20/25)* |
| *1992* | *46* | - | - | - |
| *1994* | *15* | - | - | - |
| *1995* | - | - | *50000* | - |
| *1996* | *16* | - | - | - |
| *1997* | - | - | - | - |
| *1998* | *20* | - | - | *300 (22/25)* |
| *1999* | - | - | *8000* | - |

*Appendix S4.2: Histogram of individual sizes (A, in mm), and distribution of individual sizes among sites (B) and between the mainstream and tributaries (C).*

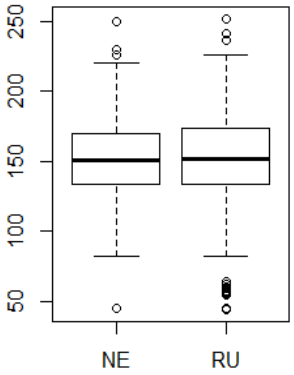
A




B




C




Average size varies among sites (Df = 20, F-value = 2.343, p-value = 0.000831). However, sizes do not differ significantly between the mainstream and the tributaries, (Df = 1, F-value = 0.28, p-value = 0.597).

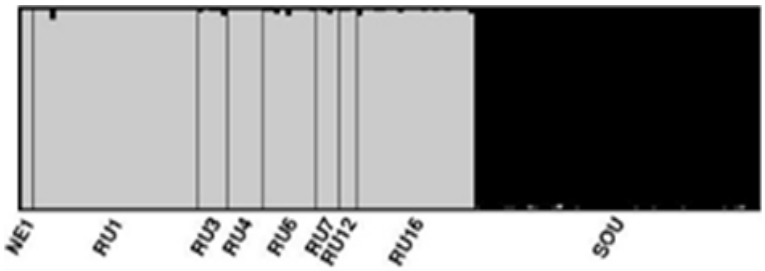
*Appendix S4.3: Table presenting for each site, sample size ("N"), geographical coordinates ("X" and "Y", extended Lambert II), mean expected heterozygosity over all loci ("He"), mean allelic richness computed using a rarefaction approach after exclusion of small populations (n<14) over all loci ("Ar"), mean Fis over all loci ("Fis"), mean Fst over all loci ("Fst"), p-value for Hardy-Weinberg equilibrium ("HW"; a significant p-value indicates departure from equilibrium), mean body length in mm and its standard deviation ("Length (+SD)"), and distance to the river mouth (DM) in meters.*

| Site | N | X | Y | He | Ar | Fis | Fst | HW | Length | (+SD) | DM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NE1 | 33 | 450086 | 1763239 | 0.70 | 6.64 | 0.08 | 0.07 | 0.000 | 146.0 | 27.7 | 8733 |
| NE2 | 31 | 450693 | 1762834 | 0.69 | 6.61 | 0.04 | 0.06 | 0.267 | 151.4 | 29.9 | 7972 |
| NE3 | 31 | 451002 | 1762645 | 0.69 | 6.65 | 0.12 | 0.06 | 0.004 | 163.8 | 29.7 | 7574 |
| NE4 | 72 | 451266 | 1762411 | 0.67 | 6.72 | 0.07 | 0.04 | 0.001 | 154.1 | 22.1 | 7251 |
| NE5 | 34 | 451654 | 1752079 | 0.72 | 7.63 | 0.08 | 0.04 | 0.000 | 148.6 | 33.6 | 6663 |
| NE6 | 60 | 452526 | 1761557 | 0.66 | 7.02 | 0.07 | 0.04 | 0.000 | 156.1 | 21.8 | 5652 |
| NE7 | 53 | 453493 | 1760779 | 0.64 | 6.81 | 0.02 | 0.04 | 0.184 | 153.4 | 31.6 | 4278 |
| NE8 | 65 | 453482 | 1760043 | 0.60 | 6.32 | 0.05 | 0.05 | 0.004 | 141.6 | 28.9 | 3467 |
| NE9 | 61 | 453438 | 1759580 | 0.56 | 5.78 | 0.06 | 0.06 | 0.032 | 149.7 | 31.8 | 3012 |
| NE10 | 54 | 454200 | 1758023 | 0.50 | 4.71 | 0.00 | 0.08 | 0.352 | 158.4 | 30.8 | 914 |
| NE11 | 19 | 454463 | 1757327 | 0.47 | 4.49 | 0.02 | 0.09 | 0.653 | 148.1 | 24.4 | 120 |
| RU1 | 28 | 448609 | 1764789 | 0.65 | 5.35 | 0.15 | 0.13 | 0.023 | 157.6 | 21.9 | 11203 |
| RU3 | 5 | 448965 | 1763495 | 0.66 | - | 0.13 | 0.08 | 0.024 | 159.2 | 22.8 | 10288 |
| RU4 | 34 | 449017 | 1763627 | 0.72 | 5.79 | 0.06 | 0.07 | 0.074 | 156.9 | 28.3 | 9919 |
| RU6 | 10 | 450687 | 1762692 | 0.74 | - | 0.03 | 0.08 | 0.654 | 157.4 | 24.9 | 8088 |
| RU7 | 18 | 451132 | 1762779 | 0.63 | 6.08 | 0.08 | 0.08 | 0.284 | 150.9 | 60.1 | 7777 |
| RU9 | 14 | 451576 | 1762593 | 0.66 | - | 0.01 | 0.06 | 0.535 | 163.1 | 31.3 | 7525 |
| RU10 | 26 | 451638 | 1761843 | 0.61 | 4.91 | 0.06 | 0.07 | 0.256 | 146.4 | 25.8 | 6750 |
| RU11 | 33 | 452762 | 1761782 | 0.55 | 4.63 | 0.02 | 0.08 | 0.883 | 141.2 | 37.4 | 5804 |
| RU12 | 14 | 453659 | 1760889 | 0.74 | - | -0.06 | 0.06 | 1.000 | 166.4 | 33.0 | 4426 |
| RU16 | 20 | 453432 | 1759173 | 0.72 | 6.72 | 0.04 | 0.12 | 0.360 | 127.2 | 59.0 | 2752 |
| SOU | 48 | - | - | 0.84 | 9.39 | 0.04 | 0.10 | 0.12 | - | - | - |
| AVERAGE | | - | - | 0.65 | 6.23 | 0.05 | 0.07 | 0.266 | 152.3 | 31.3 | |

*Appendix S4.4: List of microsatellites used, with their basic statistics (Na: number of alleles; Ar: mean allelic richness; He: expected heterozygosity; Ho: observed heterozygosity; Fis: fixation index; Hw: deviation from Hardy-Weinberg equilibrium (p-value); Fst: overall Fst), and references.*

| Locus | Na | Ar | He | Ho | Fis | HW | Fst | Reference |
|---|---|---|---|---|---|---|---|---|
| BS131 | 20 | 5.27 | 0.70 | 0.67 | 0.05 | 0.00 | 0.14 | (Estoup et al. 1998) |
| One9 | 9 | 4.03 | 0.62 | 0.65 | 0.00 | 0.15 | 0.13 | (Scribner et al. 1996) |
| Sfo1 | 17 | 5.28 | 0.71 | 0.74 | -0.01 | 0.09 | 0.12 | (Angers and Bernatchez 1996) |
| Ssa064 | 26 | 6.09 | 0.75 | 0.72 | 0.06 | 0.00 | 0.09 | Estoup et al. 1998 |
| Ssa103 | 20 | 4.92 | 0.65 | 0.65 | 0.04 | 0.04 | 0.15 | (Skaala et al. 2004) |
| Ssa417 | 16 | 4.31 | 0.66 | 0.65 | 0.04 | 0.09 | 0.08 | (Cairney et al. 2000) |
| SsosL311 | 25 | 5.16 | 0.69 | 0.68 | 0.05 | 0.00 | 0.10 | (Slettan et al. 1995) |
| SsoSL438 | 10 | 2.78 | 0.42 | 0.43 | 0.02 | 0.09 | 0.16 | Slettan et al. 1995 |
| T3-13 | 19 | 5.16 | 0.69 | 0.72 | -0.02 | 0.44 | 0.12 | Estoup et al. 1998 |
| Ots515NWFSC | 3 | 1.57 | 0.14 | 0.15 | -0.02 | NA | 0.02 | (Naish and Park 2002) |
| Ssa-60NVH | 26 | 5.65 | 0.74 | 0.74 | 0.02 | 0.00 | 0.10 | (Grimholt et al. 2002) |
| Ssa-TAP2a | 4 | 3.45 | 0.60 | 0.63 | -0.03 | 0.59 | 0.10 | Grimholt et al. 2002 |
| Ssa-UBA | 13 | 4.01 | 0.63 | 0.36 | 0.46 | NA | 0.08 | Grimholt et al. 2002 |
| Ssa14 | 5 | 1.78 | 0.19 | 0.17 | 0.07 | NA | 0.27 | (McConnell et al. 1995) |
| Ssa85DU | 9 | 3.72 | 0.52 | 0.50 | 0.07 | 0.23 | 0.15 | (O'Reilly et al. 1996) |
| SsoSL417 | 17 | 4.40 | 0.66 | 0.63 | 0.06 | 0.01 | 0.08 | Slettan et al. 1995 |

*Appendix S4.5: STRUCTURE barplot representing the assignment probability of individuals that were confidently assigned to the captive-bred cluster at the first level of the hierarchy (Q-value > 0.7).*




All individuals from the Soueich trout farm (SOU) were exclusively assigned to one subcluster (in black), whereas the other individuals from the river were assigned to a second subcluster (in gray).

*Appendix S4.6. Results from models testing the relationship between distance to the river mouth, level of admixture with captive-bred trout and the interaction between these two variables (along with the intercept) with allelic richness averaged at the site level and Fst calculated at each site (see Figure 2 in main text). The table provides beta weights (β) along with standard errors (SE) and p-values (P). *: p-value < 0.05; **: p-value < 0.01.*

| | Allelic richness | | | Fst | | |
|---|---|---|---|---|---|---|
| | β | SE | P | β | SE | P |
| (Intercept) | 0.262 | 0.218 | 0.251 | -0.157 | 0.182 | 0.401 |
| Distance to the river mouth | 0.330 | 0.232 | 0.179 | -0.355 | 0.200 | 0.094 |
| Level of admixture (log) | -0.313 | 0.253 | 0.238 | 0.792 | 0.201 | 0.001** |
| Distance to the river mouth * Level of admixture (log) | -0.557 | 0.184 | 0.010** | 0.298 | 0.154 | 0.070 |

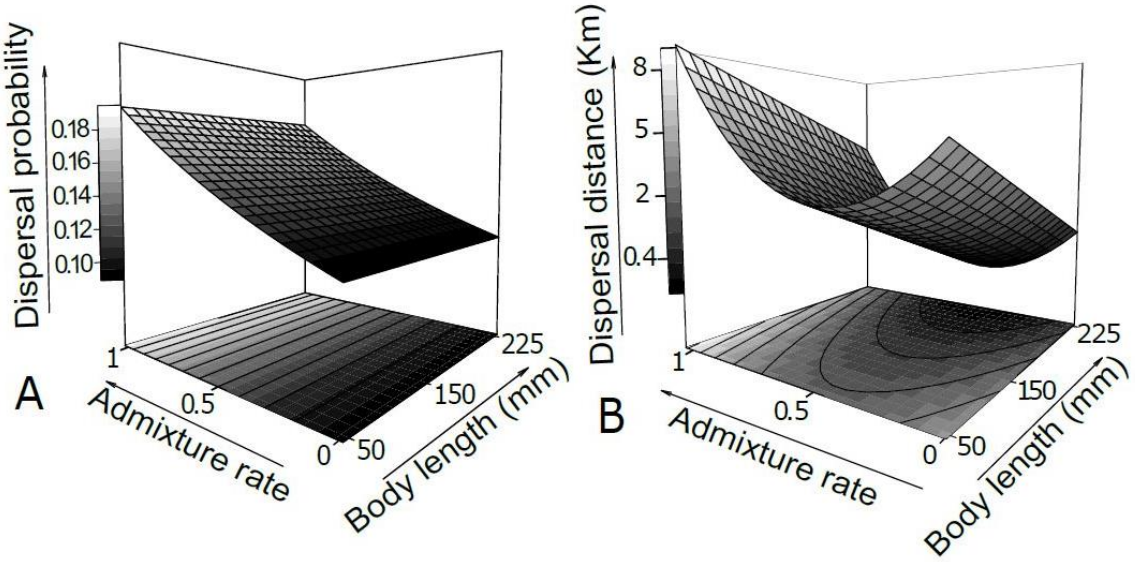
*Appendix S4.7: Interpretation of first-order interactions and of additive terms when predictors are involved in a first-order interaction.*

In the equation $Z \sim b_0 + b_1X + b_2Y + b_3XY$, the regression coefficient b3 (interaction) represents the amount of change in the regression of Z on X for a one unit change in Y. As the equation can be reformulated as $Z \sim b_0 + (b_1 + b_3Y)X + b_2Y$ (respectively $Z \sim b_0 + (b_2 + b_3X)Y + b_1X$ ), the regression coefficient $b_1$ (respectively $b_2$) represents the effect of the additive term X (respectively Y) when the other predictor is set to 0.

For instance, in the model presented in Appendix S6 (AR ~ DM * ADM, with AR standing for the standardized allelic richness, DM for the distance to the mouth and ADM for log-transformed admixture level), the beta coefficient associated with the additive term DM ($\beta = 0.330$) corresponds to the influence of DM on AR (upstream increase in allelic richness, that is, downstream decrease in genetic diversity) for an intermediate level of admixture ADM (that is, ADM = 0, as all variables were standardized). This specific additive effect of DM is also illustrated in Figure 2 (main text) in the form of the line corresponding to "medium admixture". In this fan-representation of the interaction, lines corresponding to "low" and "high admixture" stand for the specific effects of DM at null and maximum levels of admixture, respectively: Figure 2 shows an upstream increase in allelic richness for null to moderate levels of admixture, but an opposite trend for high levels of admixture.

Note that an additive regression coefficient may also be considered as the average effect of one predictor across all values of another variable (Aiken et al. 1991).

*Appendix S4.8. Three-dimensional plots of (A) individual dispersal propensity and (B) individual dispersal distance against level of admixture with captive-bred trout and body length in mm (see Table 1 in main text).*



*Appendix S4.9: Inferring dispersal from full-sibs families*

On top of inferring dispersal with GENECLASS2, we additionally inferred dispersal events using family reconstruction (Berry et al. 2004; Kanno et al. 2011). More specifically, we reconstructed full-sib families in order to determine whether admixture measured at the family level affected dispersal traits.

Method

Full-sib families of brown trout were reconstructed using the full-likelihood approach implemented in COLONY2 (Jones and Wang 2010). COLONY2 implements full-pedigree likelihood methods, i.e. with likelihood considered over the entire pedigree, to infer sibship among individuals. We assumed that both sexes are polygamous and we allowed for possible inbreeding. All individuals were considered as offspring and we defined no *a priori* candidate parental genotypes (neither males nor females). Allele frequencies were directly determined from the genetic dataset using COLONY2. Only the full-sib families with associated inclusion probability higher than 95 % were retained for further analyses. To ensure the strength of family reconstruction, we performed two COLONY runs with the same parameters as mentioned above, and only retained congruent families between both runs.

We then estimated the *family dispersal probability*, by isolating families clustered on a single site (suggesting no dispersal) and those spread on several sites (suggesting dispersal). Family dispersal probability values were obtained by transforming the binomial response variable (i.e. dispersal vs. non dispersal) with the R function *predict()*. For families spread over several sites, we measured the *family dispersal distance*, taking the river distance between the farthest sites containing members of a full-sib family.

We then tested the effects of admixture with captive-bred trout (i.e. mean level of admixture at the family level), sex (i.e. mean percentage of males in each full sib family), and body length (i.e. mean body length at the family level) on both family dispersal probability and family dispersal distance. We used Generalized Linear Models (GLM, with Gaussian or Binomial error terms depending on the response variable) with all continuous variables standardized. We included the quadratic term for admixture level to test for potential non-linear relationships. As the number of dispersed families was low (n = 39), we did not test for interaction terms. For each dependent variable (family dispersal probability and family dispersal distance), we then used a model

selection procedure based on the Akaike Information Criteria (AIC) to identify the most parsimonious set of predictors.

Results

Overall, the 715 fish were assigned to 519 full-sib families with probability higher than 95 %. On average, reconstructed full-sib families were composed of 1.38 individuals (ranging from 1 to 9). Among them, 404 individuals were found to be the only members of their families (i.e. singleton). The other 311 individuals were distributed over 115 families of at least two full sibs, with an average family size of 2.73 individuals (Figure S9-1A).

Among the 115 families of at least two individuals, 39 were found to be dispersed over at least two sites (and up to three sites). The distances covered by these families ranged from 367.7 to 7131.8 m, with a median of 1482 m (Figure S9-1B). The other 76 families had all their members on one unique site. The size of the families was not correlated to the distance covered by the family ($r = -0.020$, d.f. = 113, $p = 0.82$).

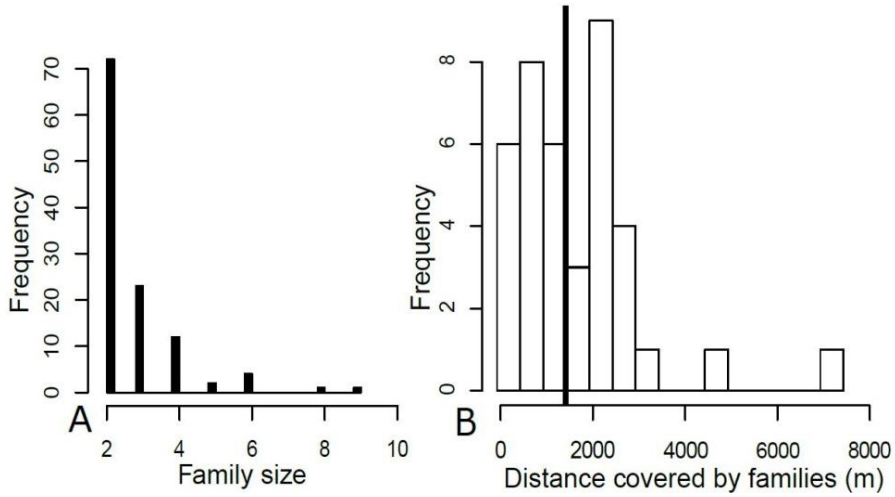



*Figure S9-1. (A) Histogram of full sibling family sizes, and (B) histogram of distances covered by full sibling families. Black vertical lines correspond to the median*

The best model retained to explain family dispersal probability only comprised simple and quadratic terms of genetic admixture with captive-bred trout (Table S9-1A). As shown in Figure S9-2A, families composed either of individuals strongly assigned to the wild cluster or to the captive-bred cluster are more likely to be spread over more than one site than families composed of individuals with mixed assignments. Similarly, the model retained to explain family dispersal distance only comprised simple and quadratic terms of genetic admixture (Table S9-1B); families with either a low or high assignment probability to the captive-bred cluster are more likely to be

spread over longer distances than families composed of individuals with mixed assignments (Figure S9-2B).

| | A | | | B | | |
|---|---|---|---|---|---|---|
| | Family dispersal probability | | | Family dispersal distance | | |
| | β | SE | P | β | SE | P |
| Intercept | 0.154 | 0.075 | 0.043 | -0.272 | 0.163 | 0.098 |
| Sex | | / | | | / | |
| Body length | | / | | | / | |
| Admixture | -0.385 | 0.095 | < 0.001*** | -0.610 | 0.206 | 0.004 ** |
| Admixture $^2$ | 0.187 | 0.063 | 0.004** | 0.275 | 0.137 | 0.048 * |

*Table S9-1. Results from the final models retained for testing the role of sex, body length and admixture (along with the intercept) on the family dispersal probability (A) and the family dispersal distance (B). Variables that were not retained in the final model are indicated by a slash bar. The table provides beta weights (β) along with standard errors (SE) and p values (P). *: p value < 0.05; **: p value < 0.01.*

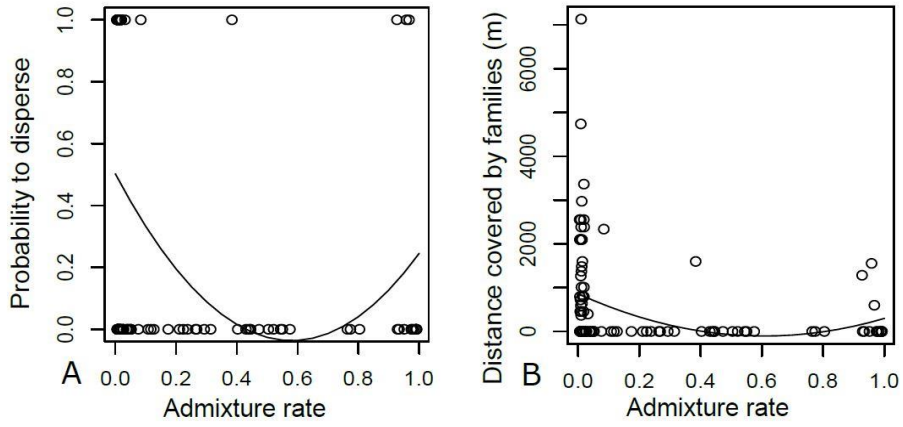



*Figure S9-2. (A) Observed (dots) and predicted (line) full sibling family dispersal probability against the mean level of admixture with captive-bred trout for each family; (B) Observed (dots) and predicted (line) distances covered by full sibling families against the mean level of admixture with captive-bred trout for each family.*

*Removing full sibling families of size two*

In salmonids, even in cases of large numbers of microsatellites, Colony has been shown to infer sibships of size two with low accuracy (Garza et al. 2014); we thus decided to perform the same analyses but only keeping the families composed of at least 3 siblings. Among the 43 families of at least three individuals, 17 were found to be dispersed over at least two sites (and up to three sites). We used the same statistical framework as described above.

Although statistical power was much lower due to the lower sample size, we found very similar patterns using this restricted dataset (Table S9-2, Figure S9-3). There was still a tendency for a quadratic relationship between dispersal probability and level of admixture (Figure S9-3A) and a negative relationship between dispersal distance and admixture (Figure S9-3B). Dispersal distance was also negatively to body size (Table S9-2), as found when using the first migrant approach.

| | Family dispersal probability | | | Family dispersal distance | | |
|---|---|---|---|---|---|---|
| | B | SE | P | B | SE | P |
| Intercept | 0.178 | 0.140 | 0.212 | <0.001 | 0.142 | 1.000 |
| Sex | / | / | / | / | / | / |
| Body length | / | / | / | -0.257 | 0.144 | 0.082 |
| Admixture | -0.359 | 0.129 | 0.008** | -0.332 | 0.144 | 0.027* |
| Admixture2 | 0.223 | 0.124 | 0.081 | / | / | / |

*Table S9-2. Results from the models retained for testing the role of admixture (along with the intercept) on the family dispersal probability (A) and the family dispersal distance (B), with only families of at least 3 individuals. The table provides beta weights (β) along with standard errors (SE) and p values (P).*

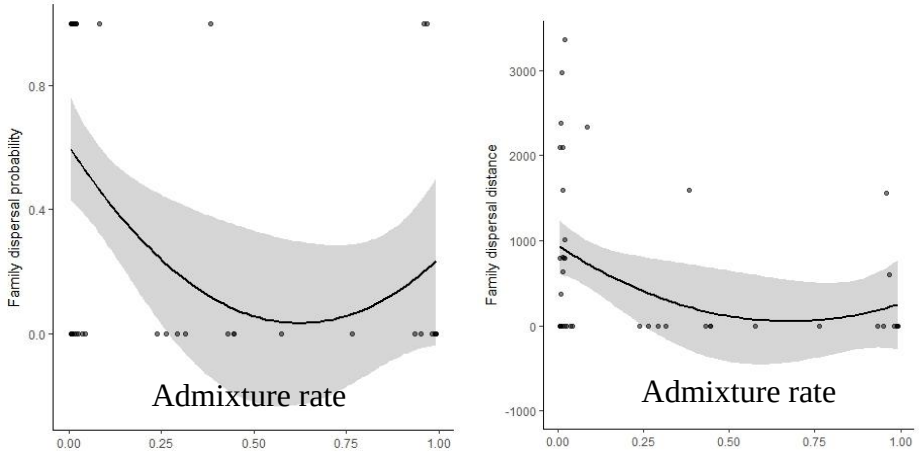



*Figure S9-3. (A) Observed (dots) and predicted (line) dispersal probability of full sibling families of at least 3 individuals against the mean level of admixture with captive-bred trout; (B) Observed (dots) and predicted (line) distances covered by full sibling families of at least 3 individuals against the mean level of admixture with captive-bred trout.*

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# Chapter V. Patterns of brown trout (*Salmo trutta*) movements and underlying drivers in replicated riverscapes: an approach combining isotopic and genetic markers

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**Key words:** Movement, brown trout, SNPs, isotopes, machine learning, individual assignment.

## Summary

In wild populations, determinants of movements lie in individual, environmental and populational characteristics. Assessing how these three components interact in shaping dispersal patterns is thus of high interest for both fundamental and applied issues. Using an original assignment method combining isotopic and genetic markers, we investigated the effects of individual phenotypes, environmental and populational indicators, and of connectivity features in shaping movement patterns in brown trout (*Salmo trutta*) populations living in three replicated riverscapes. To do so, we genotyped 1140 brown trout and collected fin tissue d15N and d13C values to identify residents and dispersers through individual assignment tests from random forest algorithms. We tested whether individual characteristics (morphology, heterozygosity…) are linked to dispersal propensity and movement characteristics. Then, we investigated –within each riverscape- which populations are more likely to receive immigrants or contrary to be emigrated from, and what are the population characteristics (biotic and abiotic) sustaining these dynamics. Finally, we tested whether individual exchanges between pairs of sites are linked to connectivity, and to environmental and populational similarity between sites. We found that individual size was a main driver of movement patterns. At the population level, we found that the position of the site within riverscapes, and habitat availability influenced the number of immigrants a site is likely to receive, and these relations varied between river basins. Finally, we found that sites that are directly connected by the water flow, and those that are similar in terms of elevation and stream order exchanged more migrants. These findings have implications for conservation, as identifying individual traits and environmental characteristics which may favor movements can be of use in defining populations or habitats of high conservation interest, and thus prioritizing conservation efforts.

## Introduction

Movement is a fundamental process for population dynamics and evolution, gene flow, and species distribution (Clobert, 2012). It is now common knowledge that important population processes depend on individual movement, almost as much as on fluctuations in population density (Patterson et al., 2008). This has widely been shown for various types of ecosystems, with examples on metapopulation ecology (Hanski, 1998), spread of diseases and invasive species (Kot et al., 1996), or home-range shifts (Simmons and Thomas, 2004), and thus has great implications in terms of management. Because movement is a determinant mechanism allowing species to respond to changing environmental conditions (Brooker et al., 2007; Haddad et al., 2015; Ims, 1995), understanding movement patterns and their causes appears crucial to optimize conservation efforts and predict consequences of environmental changes (Caplat et al., 2016). However, interactions between life history, physiology, behavior and habitat make individual movement an exceptionally complex phenomenon (Patterson et al., 2008). Movements are characterized by three distinct steps (Clobert, 2001; Clobert et al., 2009): emigration (i.e. leaving a location), transience (i.e. the movement per se, during which the displacement of the individual takes place), and immigration (i.e. the settlement in the destination location). Although an important component of movements is random (Cain et al., 2000; Hawkes, 2009), it is now acknowledged that many animals do not (only) move randomly as it is very commonly assumed in numerous models of spatial population dynamics and species distribution. Indeed, various studies showed that individuals move due to particular determinants which can act on all stages of movement: decision to leave, transience, and choice of destination location (Clobert, 2012; Cote et al., 2010). These factors driving movements have thus been a field of interest for many studies (Bowler and Benton, 2005; Ronce, 2007).

Movement implies costs and risks for individuals, at all stages of the process (Bonte et al. 2012). At a local scale, both biotic and abiotic features may indeed trigger emigration and/or motivate immigration, depending on individuals' expected fitness (e.g., Holt and Barfield, 2015). Dispersal patterns were for instance found to be linked to spatio-temporal changes in population density (McPeek and Holt, 1992), intraspecific competition and inbreeding risk (Branch, 1975; Szulkin and Sheldon, 2008; Johnson and Gaines, 1990), predation risk avoidance (Matthysen, 2012) or local habitat quality (Lin and Batzli, 2001; Lurz et al., 1997). Examples of movement patterns

shaped by these mechanisms are numerous, for instance increased numbers of post-natal dispersers (Monard and Duncan, 1996) and sex-biased dispersal (Cockburn et al., 1985; Pusey, 1987). At a regional scale, transient individuals may also struggle to access or find suitable habitats because of limited dispersal capacities (isolation-by-distance, IBD) or an increase in mortality risk, as they may for instance become exposed to predators or to physiologically harsh conditions (isolation-by-resistance, IBR; Edelaar et al., 2008; Bonte et al., 2012). For instance, a classical example is that of river dams, which impede movement, and increase mortality during passage of these obstacles (Gouraud et al., 2008). Paz-Vinas et al., (2015) also showed that individuals were more likely to move between two locations which are flow connected, i.e. between which water flows from the upstream deme to the downstream deme (Peterson et al., 2013).

These costs can be reduced through a plastic response of individuals or through the selection of specialized traits in terms of morphology, physiology or behavior (Clobert et al., 2009, Cote et al. 2010) . These specializations have been investigated for several decades now, and it is shown that there are both quantitative and qualitative differences between dispersing and resident individuals (Myers and Krebs, 1971), which are shaped by specializations needed to increase movement success (Clobert et al., 2009). For instance, some studies showed that dispersers are larger, fatter and have higher locomotor and feeding activities (O'Riain et al., 1996). Qualitative (behavioral) traits were also shown to play a key role in movements having important ecological consequences, with interesting examples concerning animal personality. Examples are numerous, where dispersers and residents have shown differences in activity, boldness, aggressiveness, social behavior, and mating decisions (Cote and Clobert, 2007; Dingemanse et al., 2003; O'Riain et al., 1996). Finally, in a study on mountain goats (Shafer et al., 2011), individuals exhibiting lower heterozygosity were found to disperse more than those with higher heterozygosity, and the authors suggest that higher fitness can be associated with more residency as heterozygosity is associated with fitness. Beyond phenotypic specialization, dispersing individuals may also increase their expected fitness by actively looking after environmental conditions that best match their phenotype, a hypothesis named habitat matching (Edelaar et al., 2008; Jacob et al., 2015) and resulting in patterns of isolation-by-environment (IBE, Cayuela et al., 2018; Wang and Bradburd, 2014), as individuals tend to settle in habitats similar to the one they come from (and are adapted to).

In riverine systems in particular, the shape of the network, as well as hydrological features (slope, flow, speed of flow, obstacles etc…) were shown to be determinant in shaping patterns of movements (Carrara et al., 2014; Hitt and Angermeier, 2008; Labonne et al., 2008; Paz-Vinas et al., 2015). For instance, upstream reaches, which are more isolated and provide smaller habitat availability were shown both theoretically and empirically to have much less immigrants than larger ones (Tonkin et al., 2018**).** The position of a deme within the riverscape is also crucial in shaping these patterns, as more central ones (i.e those which are physically easier to access to from surrounding ones, Paz-Vinas and Blanchet, 2015) were also shown to receive more immigrants than more isolated ones (Altermatt and Fronhofer, 2018). Moreover, anthropogenic pressures on these systems were also shown to modify dispersal patterns, either through the environment alteration (fragmentation, Brooks et al., 2018; Sondermann et al., 2015; pollution and flow reduction, Souza et al., 2008), or through the introduction of exotic phenotypes (for instance by stocking individuals whose genetics and phenotypic background is different; Jonsson et al., 1991; Saint-Pé et al., 2018). Altogether, this suggests that complex links between environment and individual characteristics are expected in river systems in shaping dispersal patterns. Describing and understanding patterns of connectivity and movements within river systems, and to which extent patterns and processes are similar and generalizable across river systems, are keys to investigate ecological processes in rivers, and to improve river management and conservation efforts (Pringle, 2001).

Here, we aimed at describing riverscape-scale movements in a widely distributed headwater stream fish (the brown trout, *Salmo trutta*) and at unraveling the links between environment and individual characteristics in replicated river systems. To that aim, we used an original assignment method combining isotopic and genetic markers in a machine learning algorithm to identify migrant/resident individuals and to determine the population of origin of immigrants (Berry et al., 2004; Manel et al., 2005). These markers have been used successfully for assignment (Cornuet et al., 1999; Paetkau et al., 1995; Hobson, 1999; Martin et al., 2013), and using them in combination, notably isotopic and genetic ones, was recently shown to be highly accurate (Clegg et al., 2003 ; Saint-Pé et al. in prep). More specifically, we aimed at identifying (i) individual specializations of individuals correlated with movement propensity and distance travelled (ii) environmental determinants influencing individual movement success including both transience and immigration

(IBD, IBR and IBE), and (iii) environmental/positional determinants of attractivity of a site, estimated by the proportion of immigrants detected at each site. First, we expected moving individuals to show higher body condition/length as they are more likely to afford the costs of movement (O'Riain et al., 1996), and that individuals exhibiting higher heterozygosity should be less mobile, as this was shown in Shafer et al., (2011). Further, we expected that IBD, IBR and IBE to shape patterns of movements between populations. More precisely, we expected that populations which are farther in terms of riparian distance, which are not flow connected, which are separated by more obstacles, and which are more dissimilar in terms of environmental conditions (temperature for instance) should exchange less migrants than well connected, close and environmentally similar populations. Finally, we expected that populations which are more central, and offering larger habitat availability, which are generally in lower reaches of the river catchment would be the most attractive within the network, therefore receiving more immigrants, as should locations in which environmental conditions are more favorable (lower temperatures for instance). We performed that work in three independent river systems in order to test whether patterns and processes underlying movement patterns in brown trout are similar across different ecological contexts, or on the contrary context-dependent. This last objective has strong implications for conservation strategies as it will help identifying whether conservation programs related to landscape connectivity should be context-dependent or not.

## Methods

*Field sampling*

We sampled three independent French river basins (Figure 1) in which the fish community is dominated by brown trout (*Salmo trutta*), the Aude River (Mediterranean outlet), the One River (Atlantic outlet), and the Seuge River (Atlantic outlet). Brown trout were sampled using a single-pass electric-fishing approach from 33 sites in total, with an aim of 30 individuals per site. We focused on small individuals in order to focus mainly on one age class (1 year-old). In total, we captured 1325 individuals (453 for the Aude River, 495 for the One river and 377 for the Seuge River), with sample sizes ranging from 6 to 30. Each individual was measured (total length in mm; Table 1) and a pelvic fin clip was taken for genetic and isotopic analyses. All individuals were released alive to their original sampling site.

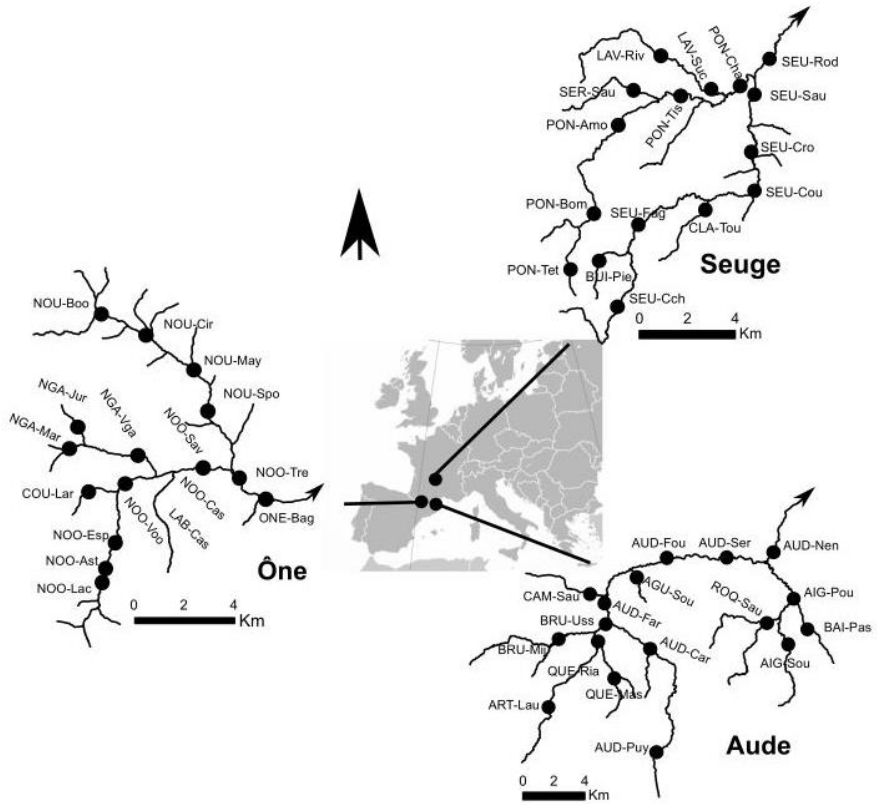


*Figure 5.1. Maps of the study sites, representing the three river basins sampled (the Aude, the One and the Seuge Rivers). Black dots represent sampling locations, arrows at the end of the river lines represent direction of the flow.*

| AUDE | | | ONE | | | SEUGE | | |
|---|---|---|---|---|---|---|---|---|
| Site | N | Body length (±SE) in mm | Site | N | Body length (±SE) in mm | Site | N | Body length (±SE) in mm |
| Agu-Sou | 27 | 157(±42) | Cou-Lar | 30 | 111(±26) | Ber-Ben | 11 | 114(±28) |
| Aig-Pou | 30 | 139(±33) | Lab-Cas | 17 | 112(±23) | Bui-Pie | 6 | 96(±27) |
| Aig-Sou | 30 | 143(±38) | Nga-Jur | 30 | 152(±40) | Cla-Tou | 9 | 105(±55) |
| Art-Lau | 30 | 138(±38) | Nga-Mar | 30 | 135(±18) | Lav-Riv | 15 | 138(±51) |
| Aud-Car | 30 | 166(±34) | Nga-Vga | 30 | 127(±35) | Lav-Suc | 30 | 122(±31) |
| Aud-Far | 30 | 165(±33) | Noo-Ast | 30 | 135(±26) | Pon-Amo | 30 | 127(±26) |
| Aud-Fou | 30 | 135(±25) | Noo-Cas | 30 | 144(±18) | Pon-Bom | 30 | 133(±32) |
| Aud-Nen | 30 | 123(±54) | Noo-Esp | 30 | 129(±38) | Pon-Cha | 30 | 148(±29) |
| Aud-Puy | 30 | 121(±31) | Noo-Lac | 30 | 109(±26) | Pon-Tis | 30 | 129(±18) |
| Aud-Ser | 30 | 156(±22) | Noo-Sav | 30 | 111(±63) | Ser-Sau | 6 | 143(±28) |
| Bai-Pas | 14 | 153(±28) | Noo-Tre | 30 | 157(±33) | Seu-Cch | 30 | 110(±21) |
| Bru-Mij | 30 | 127(±26) | Noo-Voo | 30 | 138(±30) | Seu-Cou | 30 | 134(±27) |
| Bru-Uss | 30 | 151(±21) | Nou-Boo | 28 | 173(±43) | Seu-Cro | 30 | 165(±33) |
| Cam-Sau | 14 | 118(±46) | Nou-Cir | 30 | 134(±42) | Seu-Fag | 30 | 126(±32) |
| Que-Mas | 29 | 131(±36) | Nou-May | 30 | 155(±34) | Seu-Rod | 30 | 157(±38) |
| Que-Ria | 30 | 158(±37) | Nou-Spo | 30 | 140(±38) | Seu-Sau | 30 | 138(±19) |
| Roq-Sau | 9 | 154(±25) | One-Bag | 30 | 147(±28) | | | |
| Total | 453 | 143(±37) | | 495 | 136(±38) | | 377 | 134(±34) |

*Table 5.1: For each river, site code, number N of individuals sampled in each site, and mean body length in mm (± standard error).*

*Genotyping*

Individual multilocus genotypes were obtained for 162 SNPs markers (Saint-Pé et al., 2019) on the 1325 individuals (453 for the Aude River, 495 for the One river and 377 for the Seuge River), using KASPAR technology performed by LGC Genomics (Smith and Maughan, 2015). All polymorphic markers were kept regardless of selection and linkage disequilibrium, as these processes can be exploited for investigating population structure and increasing assignment power (Morin et al., 2009; Nielsen et al., 2009; Waples and Gaggiotti, 2006). For further analyses, we discarded individuals for which more than one third of SNPs were not genotyped, leaving us with 1314 individuals (449 in the Aude River, 375 in the Seuge River, 490 in the One River).

As stocking with captive-bred individuals may heavily impact inferred movement patterns, notably in brown trout (Saint-Pé et al., 2018), we chose to focus on wild individuals only. We thus removed individuals which were assigned to captive-bred populations with a probability higher than 0.3, using STRUCTURE (Pritchard et al., 2000) and following the same procedure as in Saint-Pé et al. (2018). This concerned 48 individuals in the Aude River and 126 in the One River, the Seuge basin showed only very slight traces of hatchery ancestry (all individuals assigned at less than 0.3 to the captive-bred cluster). The "wild" genetic dataset comprised 1140 individuals (401 individuals in the Aude basin, 364 in the One, and 375 in the Seuge), and only these individuals were kept for subsequent analyses.

*Carbon and Nitrogen stable isotopes*

For all 1140 wild individuals and successfully genotyped, fin samples were dried at 60 °C for 72 hours, and then loaded into 1.5mL Eppendorf tubes, labeled and parafilmed. These samples were analyzed at Cornell University (COIL, Ithaca NY), using an elemental analyzer plumbed into a Finnigan MAT Delta Plus isotope ratio mass spectrometer to measure $\delta^{13}C$ and $\delta^{15}N$.

*Inference of movements*

Assignment was performed on all 1140 remaining individuals (i.e. wild and successfully genotyped). For each river basin, using the combination of genetic and stable isotopes of Carbon and Nitrogen, we calculated individual assignment probability to each site using a classification

method based on the Random Forest RF algorithm, in which the specified model aims at predicting the "site" from both genetic and isotopic data (Saint-Pé et al., in prep). The number of trees for the classification tested ranged from 1 to 6000, and the model retained made sure the number of trees was sufficient for the out of bag OOB error rate (classification to sites different than capture site) to be stable and lowest. The number of splits of the variables ("mtry" parameter) was also chosen to have the lowest OOB error rate (default value, then default*2, then number of variables). We then used a dedicated permutation procedure to filter out dubious individuals and avoid type 1 errors, as described in Saint-Pé et al. (in prep). In short, we permuted the site (capture site) 1000 times, and performed RF classification on all the 1000 permuted datasets, with the same number of trees and dataset splits retained in the first step. From this, we calculated for each individual the 95th quantile of maximum assignment over the 1000 runs. Individuals were considered as "resident" if the probability of assignment was highest for their capture site, and considered as "migrant" if the probability of assignment was highest for another site and this probability was higher than the $95^{th}$ quantile of maximum assignment over the 1000 permutations. Origins of individuals assigned to a site different than their capture site, but not with a probability higher than the quantile of the permutated analyses were considered as "unknown" (thus avoiding type 1 errors) and not considered further into analyses.

*Determinants of movement at the individual scale*

For individuals considered as migrants, we calculated the distances travelled from inferred site of origin to site of capture using the STARS ArcGis package (Peterson and Hoef, 2014). Migration propensity (binomial response: migrant or resident) and distances travelled by migrants were then used as response variables in subsequent analyses in order to identify individual determinants of movements.

We then measured/calculated individual characteristics that served as explanatory variables for migration propensity and migration distance. First, body length of each individual was measured in mm. Then we calculated Fulton's K body condition index (body weight/body length$^3$, Froese, 2006). Further, for morphometrics measures, each individual was placed on a polystyrene board with a ruler and a color sample, and was photographed with a digital camera, at the same magnification. First, using ImageJ, we measured a total of 12 traits having been related to

movement ability functions in fish (Albouy et al., 2011; Bracciali et al., 2016, Appendix S1). A Principal Component Analysis (PCA) was performed on these traits, and we used the first two axes of this PCA as explanatory responses because they explained more than 75% of variance (Appendix S2). The first axis represents the degree to which an individual is longiform, while the second axis (towards smaller values) represents the height and width of the back half of the body in comparison to the front. Finally, we measured individual heterozygosity as the number of loci on which an individual is heterozygote/number of loci.

We then tested movement propensity (migrant vs. resident) against the previously detailed explanatory variables using a binomial generalized linear model (*glm* R function), and with the river basin as an interaction term with all variables. We then performed a Type II ANOVA using the *Anova* function from the *car* R package to determine the terms in the model used (including interaction) which significantly participated in partitioning variance of the response variable. Similarly, we tested migration distance (after log-transformation as it was not normally distributed, data not shown) against the explanatory variables described above, using a Gaussian generalized linear model (*glm* R function), also with an interaction with the river basin with all simple terms. Again, we performed a Type II AVONA using the *Anova* function from the *car* R package.

*Environmental and populational determinants of emigration and immigration*

For each site, we calculated the proportion of immigrants as the number of migrants divided by the total number of individuals at the site level, and used that proportion as the response variable in subsequent analyses. We chose not to consider the possible number of emigrants inferred from assignment, as even if it is possible to know how many individuals were migrants and came from a specific site in our sample, we suggest that the majority of emigrants most likely moved to a location which we did not sample, therefore, this estimation should be highly erroneous because our sampling design was not exhaustive.

Then we retrieved for each site the environmental variables that were used as explanatory variables. First, the hierarchical classification of stream (Stralher index, Shreve, 1966) to which the sites belong; higher values indicating lower downstream stretches with lower branching

complexity and thus closer to the river mouth. Second, we calculated the centrality index with the STARS ArcGis package, that quantifies the positional importance of a node within a network (to which extent a site is connected to all others, Freeman, 1977). Third, we measured the physico-chemistry features of each site, including altitude, riverine distance to river mouth (calculated with the STARS ArcGis package), and water quality as measured from a multiparameter probe (water temperature, dissolved oxygen, and conductivity). For each site, measures of water quality were taken punctually, twice (once in the morning and once in the afternoon). We performed a PCA on these variables and we used the first two axes of this PCA as integrative explanatory responses as they explained more than 75% of variance (Appendix S3A). The first axis represents a temperature gradient, with higher temperature, and thus lower oxygen, towards high values, while the second is an altitudinal axis (higher values towards higher altitude and therefore farther from the river mouth). Finally, we characterized habitat availability by measuring mean depth and width of the river at each sampling site. Depth and width were averaged across two transects at each site during which water depth was measured every meter along transects. We performed a PCA to synthetize these two variables onto a single axis (Appendix S3B). The higher the value on that axis, the higher the depth and width, and thus the higher the habitat availability.

The proportions of immigrants at each site being typically beta-distributed (Ferrari and Cribari-Neto, 2004), they were then regressed against these environmental and populational characteristics using beta regressions (*betareg* function from the *betareg* R package). To control for zero inflation, proportions were transformed using the following equation: $(y * (n - 1) + 0.5)/n$ where n is the sample size (Smithson and Verkuilen 2006). The effect of river basin was considered in interaction with each other predictors. As previously, we finally performed a type II ANOVA (*Anova* function from the *car* R package).

*Environmental determinants of interdeme movement success*

Finally, we assessed if the number of movements between all pairs of sites (response variable) was related to (i) Flow connectivity (Paz-Vinas et al., 2015: two demes were considered flow-connected when water can flow from the upstream deme to the downstream deme; flow-unconnected demes are two demes that share a common confluence downstream but do not share flow as in Peterson et al., 2013) (ii) Riverine distance (calculated using the the STARS ArcGis

package, as mentioned previously) (iii) Number of riverine obstacles (obtained from the French water agency database "Référentiel des obstacles à l'écoulement", ROE), and (iv) isolation by environment parameters (calculated first by computing the distance (pairwise differences) along the previously described PCA axes, and then by computing pairwise difference in Strahler index between sites).

To control for the independence of pairwise data, we here considered a mixed-effect model with Maximum-likelihood-population-effect (MLPE) error structure. MLPE error structures are designed to account for the non-independence of pairwise data (Clarke et al., 2002; Van Strien et al., 2012).. MLPE models have been successfully used to identify landscape features that impact movements (Blair et al., 2013; Peterman et al., 2014; Row et al., 2017). The fixed part of our model included pairwise landscape matrices (described above), with the river basin as an interaction term. As we aimed at explaining numbers of movements between pairs of sites, we considered a MLPE mixed-effect model with a Poisson error distribution. The intercepts and slopes for the fixed effects were estimated with restricted maximum likelihood (REML, lme4 package in R; Clarke et al., 2002; Van Strien et al., 2012). Finally, we performed a Type II ANOVA (*Anova* function from the *car* R package) on this model.

## Results

*Description of movements detected*

The proportions of individuals classified as residents were 62% in the One River, 72% in the Seuge River and 84% in the River, whereas the proportions of immigrants (i.e. individuals not originating from their capture sites) were 12% in the One River, 24% in the Seuge River, and 15% in the Aude River (Figure 2A). Other individuals were classified as "unknown" and were not further considered in the analyses. Individuals which were significantly detected as migrants showed different movement patterns across rivers (Figure 2D; see also appendix S4 for a cartographic visualization). The distances travelled by these individuals ranged from 585 to 31584 meters, with a median of 7609 meters. The directions of these movements also varied among rivers (Figure 2B). For instance in the Aude River, there were roughly the same proportions of movement directions (28% downstream, 33% upstream, and 38% implying first downstream then upstream movement, Figure 2B), whereas in the One River, most movements occurred upstream

(64% vs 34% downstream and only 2% implying both down and upstream movement). Finally, most movements occurred between two streams of equal Strahler order (44%, 37% and 5% of movements of between sites belonging to 1, 2 and 3 Strahler orders respectively, whereas all other movements, between different types of streams were less frequent (>5%) (Figure 2C).

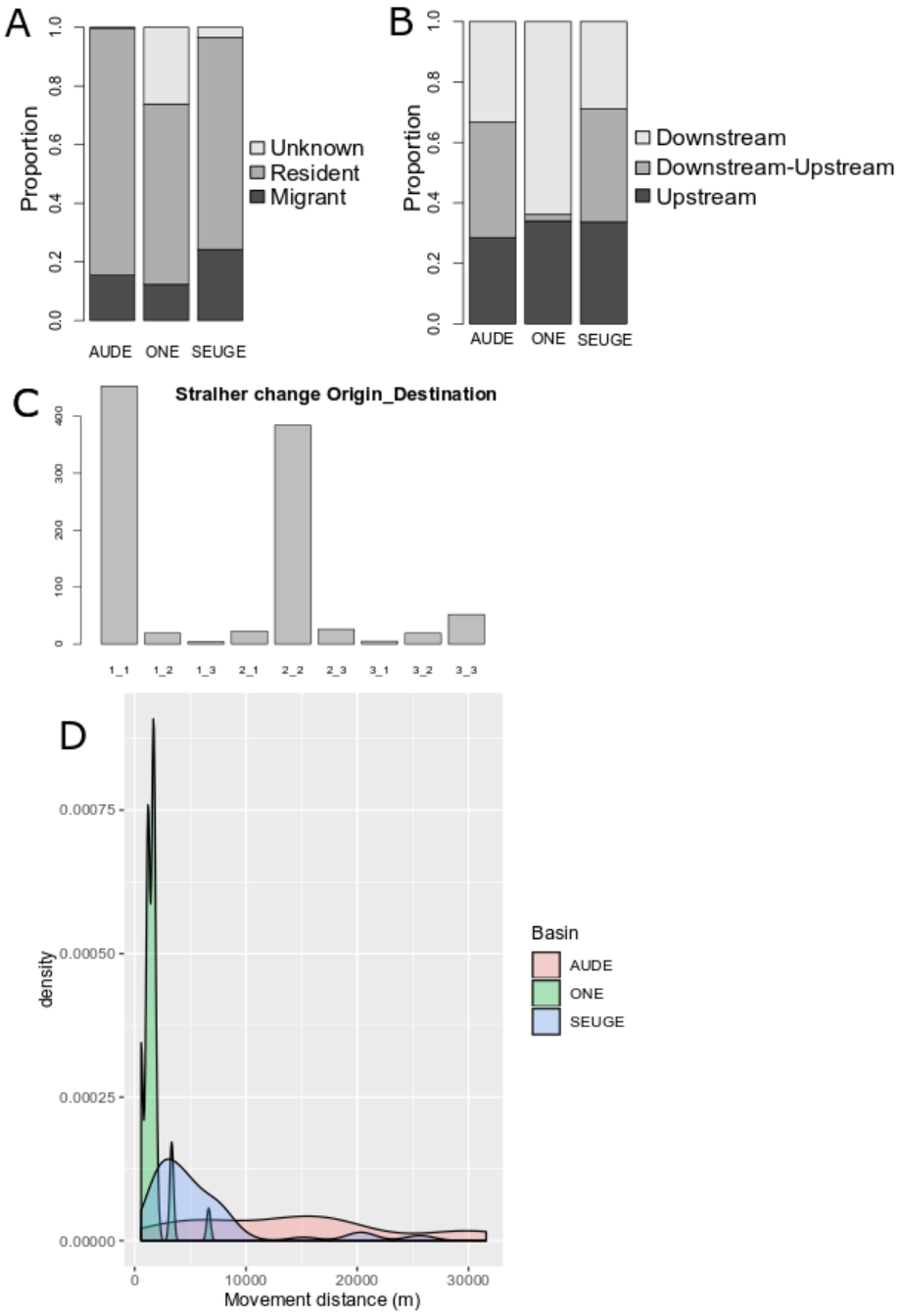


*Figure 5.2. Numbers of residents, migrants and non-assigned individuals (A), and for migrants, direction (B), Strahler of origin and destination sites (C), and density (Y axis) of distances travelled (D).*

*Individual specialization in movement*

The Fulton body condition index K in interaction with the river basin, and body length alone were found as significant predictors of movement propensity (Table 2, "movement propensity"). More precisely, we found that in the One River, individuals with a higher body condition tended to move less, whereas in the two other rivers (Aude and Seuge), individuals with higher body condition were more likely to move (Figure 3A; summary of complete model presented in Appendix S5). Additionally, longer individuals were more likely to move, a finding that holds true for all river basins (Figure 3B, Appendix S5).

| | Movement propensity | | | | Movement distance | | | |
|---|---|---|---|---|---|---|---|---|
| | Chisq | Df | Pr(>Chisq) | | Chisq | Df | Pr>Chisq) | |
| Body length | 10.958 | 1 | 0.001 | *** | 2.965 | 1 | 0.085 | . |
| BV | 13.799 | 2 | 0.001 | ** | 178.946 | 2 | <2e-16 | *** |
| MorphAx1 | 1.443 | 1 | 0.23 | | 1.866 | 1 | 0.172 | |
| MorphAx2 | 2.261 | 1 | 0.133 | | 1.376 | 1 | 0.241 | |
| FultonK | 0.347 | 1 | 0.556 | | 0.006 | 1 | 0.940 | |
| Heterozygosity | 2.811 | 1 | 0.094 | . | 0.855 | 1 | 0.355 | |
| Body length:BV | 3.722 | 2 | 0.156 | | 7.582 | 2 | 0.023 | * |
| BV:MoprphAx1 | 0.235 | 2 | 0.889 | | 0.979 | 2 | 0.613 | |
| BV:MorphAx2 | 3.249 | 2 | 0.197 | | 3.095 | 2 | 0.213 | |
| BV:FultonK | 9.42 | 2 | 0.009 | ** | 2.699 | 2 | 0.259 | |
| BV:Heterozygosity | 2.097 | 2 | 0.35 | | 1.169 | 2 | 0.557 | |

*Table 5.2: Individual determinants of movements. (A) Correlations between explanatory variables used, (B) ANOVA of both movement propensity and movement distance models, comprising simple terms and interactions with the river basin*

We further found that body length in interaction with the river basin was the only significant predictor of distance travelled (Table 2B, "movement distance"). Longer individuals travelled over shorter distances in the Aude and in the Seuge Rivers, whereas in the One River, longer individuals tended to move on longer distances (Figure 3C; summary of complete model presented in Appendix S6).

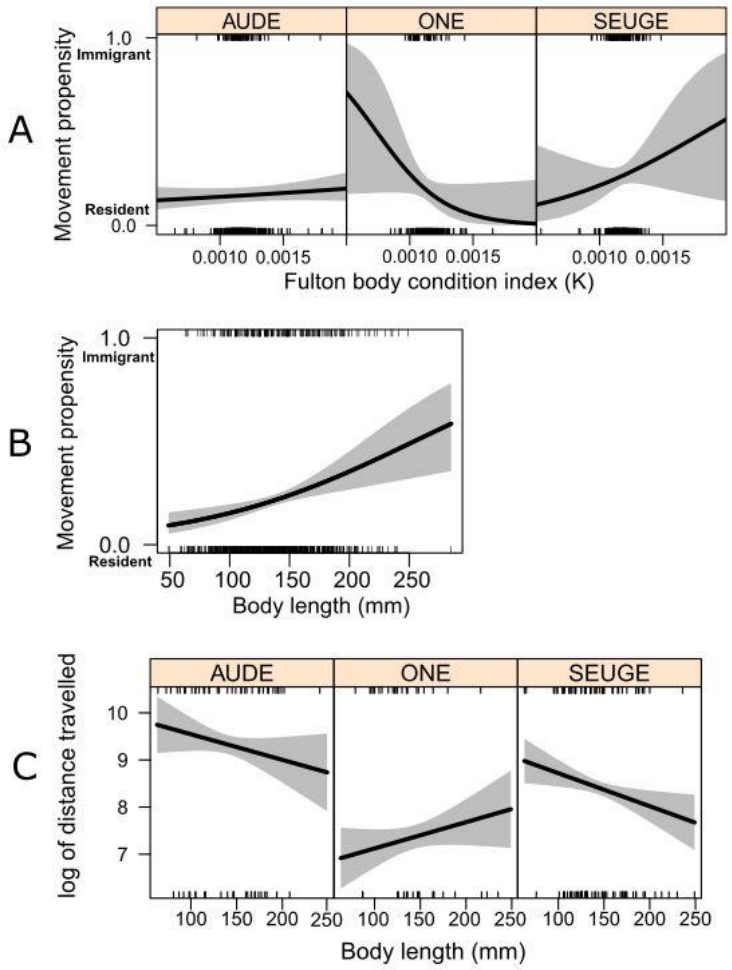


*Figure 5.3. Predictions for migration propensity according to (A) the interaction between body condition and river basin, and (B) body length. (C) Predictions for log of distance according to the interaction between body length and river basin. Confidence intervals (95% in grey).*

*Environmental determinants of individual movement success*

Using MLPE mixed-effect models to explain the number of movements among pairwise populations, we found that flow connectivity between sites, distance along the altitudinal axis, and difference in Strahler between sites significantly explained the pairwise number of movements between sites (Table 4B, summary of complete model presented in Appendix S7). More precisely, flow connectivity was positively correlated to pairwise movement between pairs of sites (Figure 5A), and negatively correlated with distance along the altitudinal axis and difference in Strahler index (Figure 5 B and C). This means that two sites which are flow connected, similar in terms of altitudinal conditions, and on the same type of stream are more likely to exchange individuals.

Interestingly, we found no relation between pairwise number of individual movements between sites and riparian distance (p-value = 0.94), which was one of the most expected relation, nor with obstacles separating sites (p-value = 0.79).

| | Chisq | Df | Pr(>Chisq) | |
|---|---|---|---|---|
| BV | 2.080 | 2 | 0.354 | |
| PairwiseObstacles | 0.073 | 1 | 0.787 | |
| PairwiseFlowConnect | 10.235 | 1 | 0.001 | ** |
| PairwiseDist | 0.007 | 1 | 0.935 | |
| dTempAx1 | 0.582 | 1 | 0.446 | |
| dAltAx2 | 7.392 | 1 | 0.007 | ** |
| dStrahler | 4.604 | 1 | 0.032 | * |
| BV:PairwiseObstacles | 0.588 | 2 | 0.745 | |
| BV:PairwiseFlowConnect | 5.238 | 2 | 0.073 | . |
| BV:PairwiseDist | 0.558 | 2 | 0.756 | |
| BV:dTempAx1 | 0.909 | 2 | 0.635 | |
| BV:dAltAx2 | 3.799 | 2 | 0.150 | |
| BV:dStrahler | 2.984 | 2 | 0.225 | |

*Table 5.4: Tables corresponding to the pairwise approach models. (A) Correlations between explanatory matrices used, (B) ANOVA of the model explaining the number of pairwise exchanges between sites, comprising simple terms and interactions with the river basin*

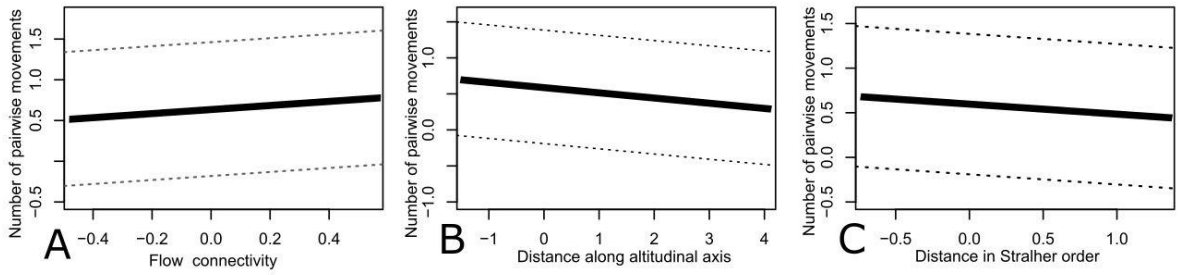


*Figure 5.5. Predictors for the number of pairwise movements between sites according to flow connectivity, difference in the altitudinal axis, and difference in Stralher index. Dotted lines represent confidence intervals (95%)*

*Environmental determinants of population attractivity*

At the local/population scale, the proportion of immigrants was significantly explained by three terms in interaction with the river basin: habitat availability, Strahler index and Centrality (Table 3B, "proportion of immigrants"). More precisely, the proportion of immigrants was positively

related to habitat availability in the One and the Seuge Rivers, meaning that larger habitats tend to receive more immigrants, whereas the opposite tendency was observed in the Aude River (Figure 4A). The proportion of immigrants was positively related to Strahler index in the Aude and the One Rivers, whereas this relation was negative in the Seuge River (Figure 4B). Proportion of immigrants was negatively correlated to centrality in the Aude and in the Seuge Rivers, but positively correlated to this variable in the One River, highlighting the importance of site position within the dendritic network (Figure 4C). On the other hand, variables participating to the temperature axis (i.e. oxygen, temperature, conductivity) and to the altitudinal axis (altitude, distance to the river mouth) did not explain the proportion of immigrants. Complete model summaries are presented in appendix S8.

| | Proportion of immigrants | | | |
|---|---|---|---|---|
| | Chisq | DF | Pr(>Chisq) | |
| TempAx1 | 0.125 | 1 | 0.723 | |
| BV | 3.226 | 2 | 0.199 | |
| AltAx2 | 0.599 | 1 | 0.439 | |
| HabAvailAx1 | 3.208 | 1 | 0.073 | . |
| Strahler | 0.700 | 1 | 0.403 | |
| Centrality | 0.720 | 1 | 0.396 | |
| TempAx1:BV | 4.357 | 2 | 0.113 | |
| BV:AltAx2 | 3.722 | 2 | 0.155 | |
| BV:HabAvailAx1 | 6.675 | 2 | 0.036 | * |
| BV:Strahler | 7.256 | 2 | 0.027 | * |
| BV:Centrality | 6.534 | 2 | 0.038 | * |

*Table 5.3: Environmental determinants of population attractivity ANOVA of both proportion of immigrants and proportion of emigrants at each site, comprising simple terms and interactions with the river basin*

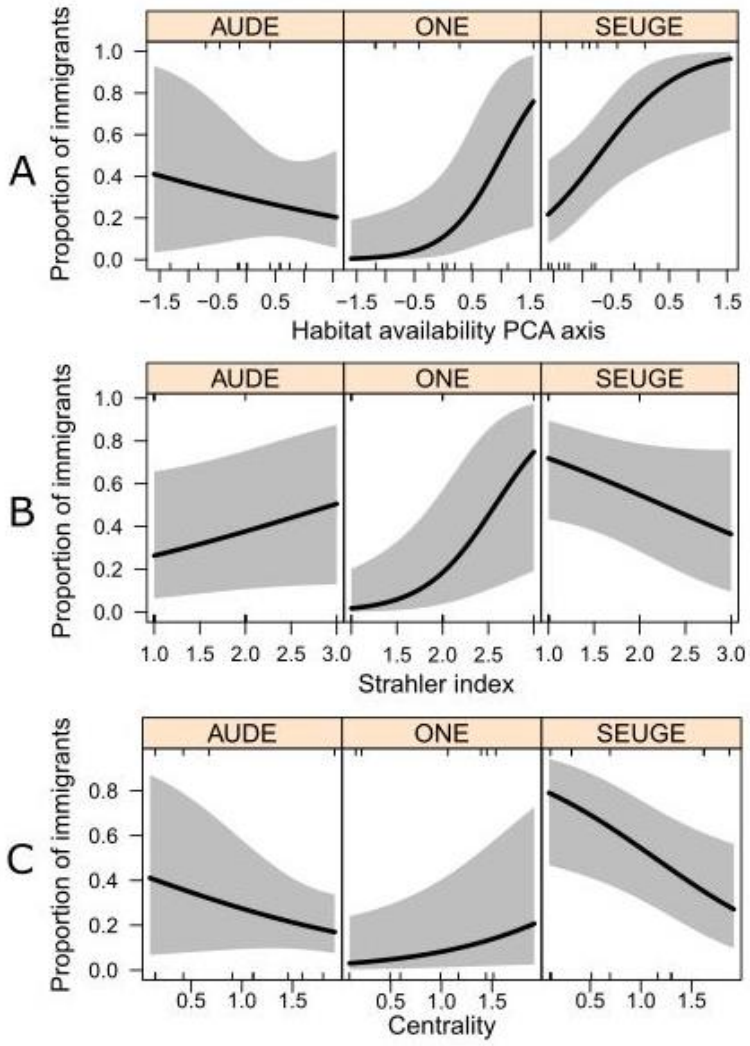


*Figure 5.4. Predictors for the proportion of immigrants at the site level (A) habitat availability, (B) Strahler index, and (C) centrality*

## Discussion

In this study, we investigated patterns and determinants of movements in three replicated river basins at different levels (from individual features to site and populational features). We found that individuals tend to move according to body size mainly, both in terms of propensity and movement distance. We also found that sites which are most likely to exchange migrants are those which are connected by water flow, and similar in terms of elevation and stream type. Finally, we found that according to river basins considered, some types of locations can be more or less likely to receive immigrants, in particular depending on both habitat quality (habitat size) and accessibility, notably due to their position in the river network (centrality and Strahler index). Overall, determinants were in the most cases river-dependent, which shows that patterns and processes of fish movements are hardly generalizable across riverscapes.

*Individual specialization in movement*

As suggested by our results, individual size is a major determinant of movements. More particularly in the case of the Brown trout, it was shown that these dynamics are often the result of hierarchical interactions between individuals, and exist at various life stages (Bremset and Berg, 1997; Cuinat and Heland, 1979). We found that regardless of the basin, longer individuals were more likely to move. However, in the Aude and the Seuge Rivers, these larger individuals moved on shorter distances, whereas in the One River longer individuals moved on longer distances. For the One River, we can therefore suggest that smaller individuals often disperse further because more dominant and often larger individuals attain habitats of first choice which they subsequently defend (Heland, 1980) for instance. Moreover, larger and more dominant fish tend to have a more fixed, but wider home range than smaller and subdominant individuals (Elliott, 1994; Höjesjö et al., 2007), although they have better movement capacities. Thus, movement patterns may be very context dependent, and determined by local trade-offs between remaining resident and moving which is modulated by body condition and size.

Body condition and metabolism in general have been shown to be determinant in individuals' decisions to move (Baines et al., 2015; Niitepõld et al., 2009), as a good condition may generally enable an individual to afford the cost of movement (Bonte et al., 2012). Accordingly, individuals

from the Aude and the Seuge Rivers exhibited a higher probability to have moved when their body condition index was higher. However, in the One River, the opposite pattern was observed. This could imply that in this particular basin, movement decisions may be forced instead of chosen: if their environment or biotic interactions do not enable them to sustain a suitable condition, individuals may decide to move or be forced to move out by individuals in better body condition (Cote et al., 2010; Hoset et al., 2011).

Morphometric ratios related to swimming ability were not retained as significant in the models explaining individual movement. This is relatively surprising, as even at these small spatial extents, variations in selection pressures would have been expected to be sufficient to induce morphological differentiation between individuals (Pakkasmaa and Piironen, 2001), however we suggest that different morphotypes may not be contrasted enough to enable to link them to differential movement behavior.

Individual heterozygosity was unexpectedly not linked to individual movement either. According to our predictions, we were expecting that due to the links between this variable and fitness (Shafer et al., 2011), individuals exhibiting lower heterozygosity should disperse more than those with higher heterozygosity.

*Pairwise determinants of exchange between sites*

In particular in the context of habitat fragmentation, movements between patches have been a vast field of research (Travis et al., 2013). Pairwise approaches are numerous, and generally, they all show that between sites movements are negatively related to inter patch resistance, i.e. the difficulty for an individual to move between patches. In rivers, this has also been investigated, for instance showing that gene flow between patches is negatively related to distance (Paz-Vinas et al., 2015), and obstacles to physical connectivity (Gouraud et al., 2008). Surprisingly, riparian distance did not affect the number of movements between two sites, however, other variables linked to physical and landscape resistance did, i.e. flow connectivity between sites, but also difference along the altitudinal axis and difference in stream type. This could suggest that movements of trout may have stronger biological causes than just spatial probability associated with proximity. On the other hand, movements between sites were not linked to variables related

to habitat choice: we found no effect of temperature, however, other variables which were not tested could be more likely to explain habitat suitability choice in movement decisions between sites. Finally, the fact that anthropic obstacles did not appear to reduce movements between locations although this was expected could suggest that in brown trout studies, these obstacles are often overestimated: depending on water flow in particular, assessing more thoroughly how they impede movement is a lacking field at these small scales.

*Environmental determinants of population attractivity*

Environmental factors which influence how a certain deme will contribute to the regional emigration-immigration dynamics within a river system were shown to be linked to the position of that deme within the dendritic network (Carrara et al., 2014; Paz-Vinas and Blanchet, 2015), but also to its size: upstream, smaller and less connected demes were shown to generally produce emigrants, which immigrate to downstream, more connected and larger demes. This is also very congruent with the life cycle of the Brown trout: migrations to mate upstream, with subsequent downstream dispersal (Maisse and Bagliniere, 1990; Ovidio et al., 1998; Roussel and Bardonnet, 2002). In our study, we found that these "positional" features at the site level influenced the proportion of immigrants: habitat availability, stream type and centrality. Except in the Aude River, sites with higher habitat availability for instance tended to receive more immigrants, however, there were many exceptions going against these theoretical expectations. For example in the Seuge river, higher Strahler index streams (that is, downstream river stretches) tended to receive less immigrants, and in the Aude and the Seuge Rivers, centrality was also negatively related to the proportion of immigrants. These counter intuitive exceptions thus highlight the need for very context-specific studies. Indeed, as different basins vary considerably in terms of environmental features, habitat use and spatial scales considered for different facets of ecology may be very different, and thus conclusions must be drawn in regards of these different possibilities.

We expected other variables, in particular temperature and oxygen but also altitude and distance to the river mouth to influence these movement choices because we expected that some sites may be better suited for immigrants. This was surprisingly not observed, as in explaining pairwise determinants of movement, suggesting that individuals may not decide to immigrate to a location

in which environmental conditions are more favorable (i.e. higher oxygen, lower temperature, etc…), or at least, these choices are negligible compared to those based on the size and position of a destination site.

*Technical considerations and conclusion*

Generally, rivers inhabited by Brown trout are all affected to a certain extent by human alteration. A major perturbation, which in this study we decided to not consider is stocking, however, its effects are of utmost importance: first, movement inference may strongly be biased by this practice, human induced displacements may be detected as movements when using assignment tests. Moreover, in terms of ecology, even if it may be possible to discard captive-bred individuals from these analyses, they were shown to exhibit different movement behavior (Saint-Pé et al., 2018), and as they are part of the population and its dynamics, their impact on movement behavior of wild populations with which they seem to be living with in parapatry mostly remains to be assessed.

A final however extremely important consideration is the use of data types in combination. By combining genetics and isotopes to detect movements, and in particular because the aim of this study is ecological, we suggest that we may be missing important biological insight, as the types of movements detected by isotopes may differ from that of genetics (Cook et al., 2007). We therefore call for future studies disentangling these time scale considerations, and in the best case, confirming that these markers can be used for very different purposes with direct tracking protocols such as telemetry.

Nevertheless, using a robust assignment method applied to wild individuals, we were able to identify key drivers of natural movement patterns in different river contexts. We showed that brown trout movements are driven by complex interactions between individual traits and environment. Moreover, these drivers are very context dependent, and seem to depend on the configuration of the riverscape. This has great implications in terms of conservation because it implies that each river must be studied and managed in its own way. This study opens new insight into multi-riverscape studies, because the approaches we developed make the study of movements possible at the spatial extent of a river basin. Thus we suggest that following research focuses on

more river basins to try and assess universal drivers of movement, and thus potentially identify key features for conservation.

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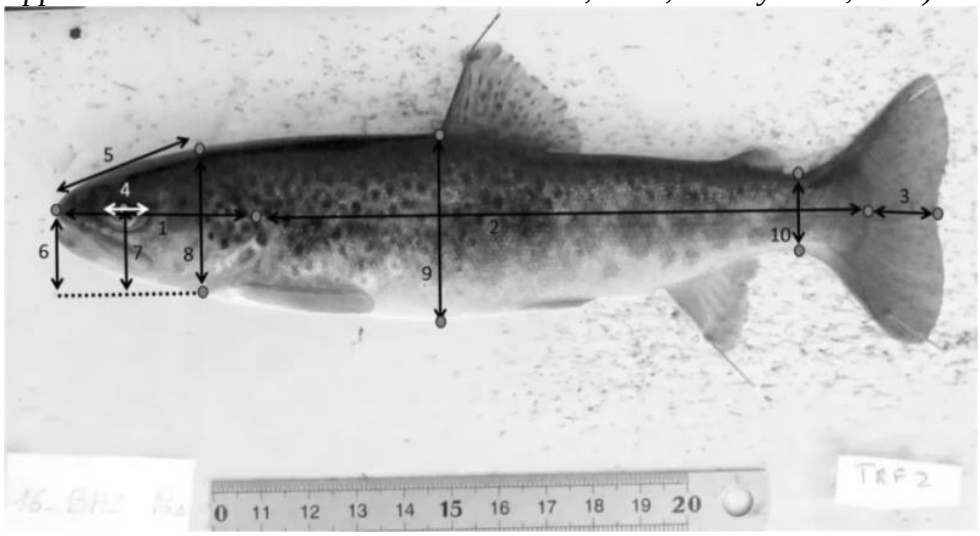
## Supplementary material for Chapter V.

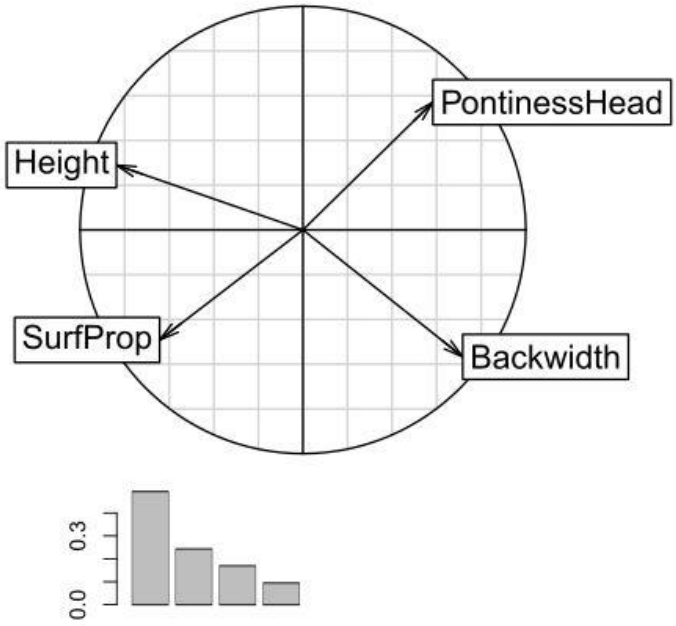
*Appendix S5.1Ratios based on Bracciali et al, 2005; Albouy et al., 2011)*



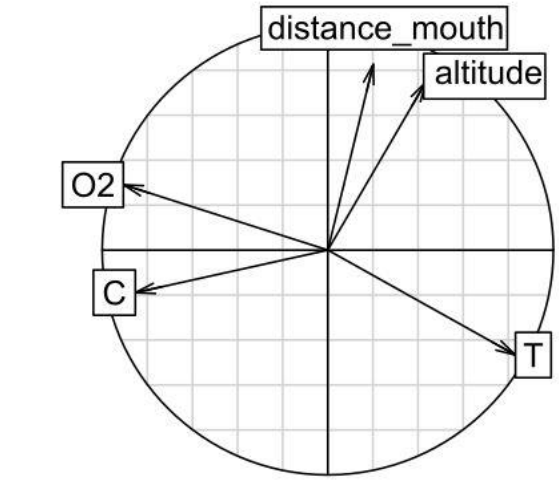

| Trait number | Measure |
|---|---|
| 1 | Head length |
| 2 | Body length |
| 3 | Caudal fin length |
| 4 | Eye diameter |
| 5 | Distance between snout and head-body junction |
| 6 | Nose tip height |
| 7 | Eye position |
| 8 | Head depth |
| 9 | Body height |
| 10 | Peduncle height |

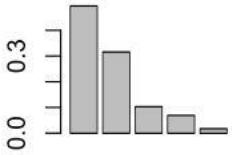
| Ratios name | Calculation |
|---|---|
| Pointiness of head | 1/8 |
| Body height | 9/(1+2) |
| Back width | 10/9 |
| SurfProp | 10*3/(1+2+3) |

*Appendix S5.2 PCA on morphometric ratios*

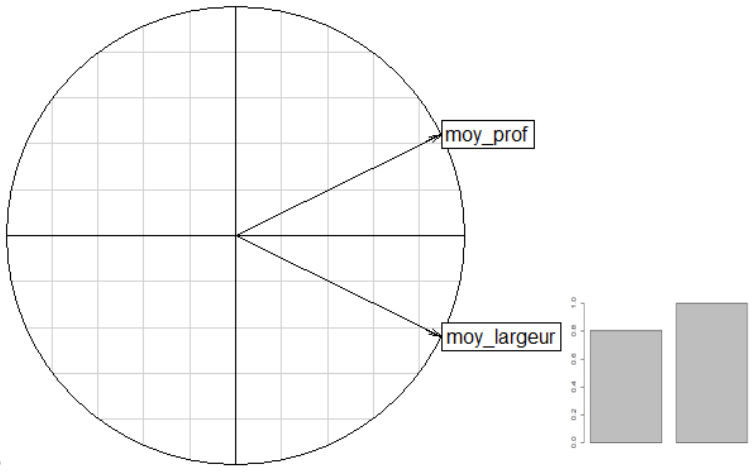


*Appendix S5.3. PCAs performed on (A) environmental predictors and on (B) habitat availability predictors*



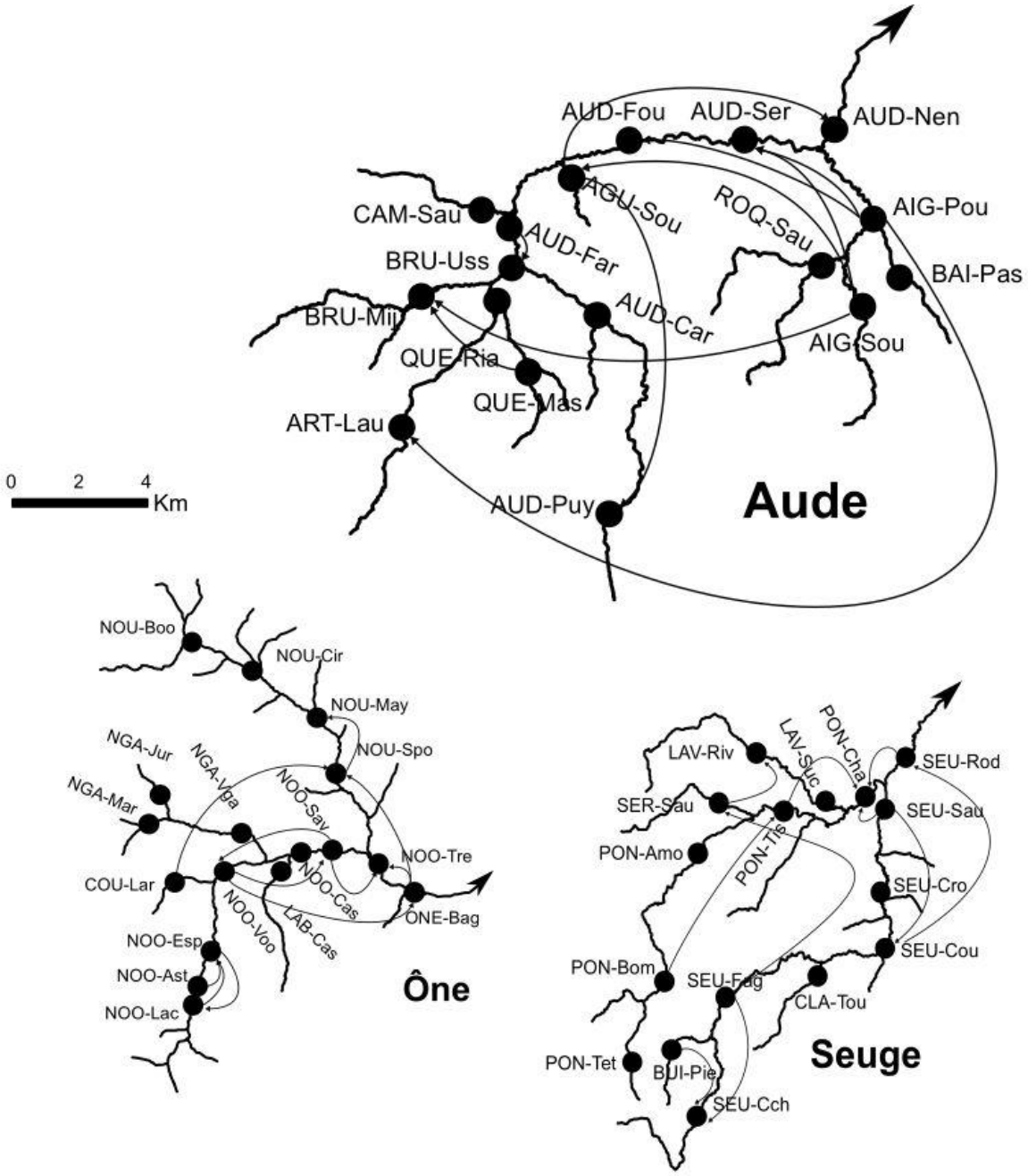




A




B

*Appendix S5.4: maps of a random selection of 10 movements detected for each river basin*




Cartographic illustration of the possible spatial extent of these movements in each river basin based on the random selection of 10 movements. Although this is far from representing the actual number of movements detected, it enables to represent possible events, showing the possible spatial extent of these movements.

*Appendix S5.5: summary of the complete model explaining individual movement propensity (A) and correlation matrix of response variables tested in this model (B)*

| **A** Movement propensity | Estimate | Std.Error | z-value | Pr(>\|z\|) | |
|---|---|---|---|---|---|
| (Intercept) | -1.65313 | 0.14257 | -11.595 | <2.00E-16 | *** |
| BVONE | -0.19713 | 0.34052 | -0.579 | 0.562659 | |
| BVSEUGE | 0.70176 | 0.20943 | 3.351 | 0.000806 | *** |
| scale(Taille) | 0.08202 | 0.20969 | 0.391 | 0.695698 | |
| scale(MoprphAx1) | 0.09561 | 0.19074 | 0.501 | 0.6162 | |
| scale(MoprphAx2) | -0.02341 | 0.17115 | -0.137 | 0.891225 | |
| scale(FultonK) | 0.28357 | 0.25715 | 1.103 | 0.270139 | |
| scale(Heterozygosity) | 0.09338 | 0.10628 | 0.879 | 0.379644 | |
| BVONE:scale(Taille) | 0.53737 | 0.34363 | 1.564 | 0.117864 | |
| BVSEUGE:scale(Taille) | 0.48108 | 0.28383 | 1.695 | 0.090081 | . |
| BVONE:scale(MoprphAx1) | 0.02449 | 0.34044 | 0.072 | 0.942654 | |
| BVSEUGE:scale(MoprphAx1) | 0.13095 | 0.27849 | 0.47 | 0.638202 | |
| BVONE:scale(MoprphAx2) | 0.54855 | 0.30765 | 1.783 | 0.074582 | . |
| BVSEUGE:scale(MoprphAx2) | 0.16483 | 0.21858 | 0.754 | 0.450795 | |
| BVONE:scale(FultonK) | -3.74559 | 1.91545 | -1.955 | 0.050528 | . |
| BVSEUGE:scale(FultonK) | 1.17945 | 1.27733 | 0.923 | 0.355817 | |
| BVONE:scale(Heterozygosity) | 0.57336 | 0.41332 | 1.387 | 0.165383 | |
| BVSEUGE:scale(Heterozygosity) | 0.11251 | 0.21045 | 0.535 | 0.592897 | |

| **B** | Body length | MoprphAx1 | MoprphAx2 | FultonK | Heterozygosity |
|---|---|---|---|---|---|
| Body length | 1 | -0.63 | -0.35 | -0.12 | -0.01 |
| MoprphAx1 | -0.63 | 1 | -0.01 | -0.04 | -0.02 |
| MoprphAx2 | -0.35 | -0.01 | 1 | 0.04 | 0.15 |
| FultonK | -0.12 | -0.04 | 0.04 | 1 | 0 |
| Heterozygosity | -0.01 | -0.02 | 0.15 | 0 | 1 |

*Appendix S5.6: summary of the complete model explaining individual movement distance (A) and correlation matrix of response variables tested in this model (B)*

| **A** Movement distance | Estimate | Std. Error | t value | Pr(>\|t\|) | |
|---|---|---|---|---|---|
| (Intercept) | 9.301329 | 0.09854 | 94.395 | < 2e-16 | *** |
| scale(Taille) | -0.20453 | 0.14133 | -1.447 | 0.1495 | |
| BVONE | -1.79321 | 0.20075 | -8.932 | 3.70E-16 | *** |
| BVSEUGE | -0.88851 | 0.12332 | -7.205 | 1.34E-11 | *** |
| scale(MoprphAx1) | -0.18276 | 0.11349 | -1.61 | 0.109 | |
| scale(MoprphAx2) | 0.08745 | 0.11064 | 0.79 | 0.4303 | |
| scale(FultonK) | 0.000995 | 0.06669 | 0.015 | 0.9881 | |
| scale(Heterozygosity) | 0.067265 | 0.06833 | 0.984 | 0.3262 | |
| scale(Taille):BVONE | 0.414534 | 0.20071 | 2.065 | 0.0403 | * |
| scale(Taille):BVSEUGE | -0.05933 | 0.17624 | -0.337 | 0.7368 | |
| BVONE:scale(MoprphAx1) | 0.141016 | 0.20501 | 0.688 | 0.4924 | |
| BVSEUGE:scale(MoprphAx1) | 0.139922 | 0.14973 | 0.934 | 0.3512 | |
| BVONE:scale(MoprphAx2) | -0.17694 | 0.1616 | -1.095 | 0.2749 | |
| BVSEUGE:scale(MoprphAx2) | -0.24829 | 0.14197 | -1.749 | 0.0819 | . |
| BVONE:scale(FultonK) | 0.986305 | 0.6201 | 1.591 | 0.1134 | |
| BVSEUGE:scale(FultonK) | -0.03389 | 0.11054 | -0.307 | 0.7595 | |
| BVONE:scale(Heterozygosity) | -0.31747 | 0.29357 | -1.081 | 0.2809 | |
| BVSEUGE:scale(Heterozygosity) | -0.01722 | 0.1157 | -0.149 | 0.8818 | |

| **B** | Body length | MoprphAx1 | MoprphAx2 | FultonK | Heterozygosity |
|---|---|---|---|---|---|
| Body length | 1 | -0.63 | -0.35 | -0.12 | -0.01 |
| MoprphAx1 | -0.63 | 1 | -0.01 | -0.04 | -0.02 |
| MoprphAx2 | -0.35 | -0.01 | 1 | 0.04 | 0.15 |
| FultonK | -0.12 | -0.04 | 0.04 | 1 | 0 |
| Heterozygosity | -0.01 | -0.02 | 0.15 | 0 | 1 |

*Appendix S5.7: summary of the complete model explaining pairwise movements between sites (A) and correlation matrix of response variables tested in this model (B)*

| **A** Pairwise movements between sites | Estimate | Std. Error | t value |
|---|---|---|---|
| (Intercept) | 0.62268 | 0.2606 | 2.389 |
| BVONE | -0.62383 | 0.85032 | -0.734 |
| BVSEUGE | 0.45996 | 0.36586 | 1.257 |
| scale(PairwiseObstacles) | 0.13235 | 0.36061 | 0.367 |
| scale(PairwiseFlowConnect) | 0.24562 | 0.26781 | 0.917 |
| scale(PairwiseDist) | 0.0848 | 0.25054 | 0.338 |
| scale(dTempAx1) | -0.11097 | 0.21026 | -0.528 |
| scale(dAltAx2) | -0.07212 | 0.249 | -0.29 |
| scale(dStrahler) | -0.11376 | 0.2392 | -0.476 |
| BVONE:scale(PairwiseObstacles) | -0.19079 | 0.90387 | -0.211 |
| BVSEUGE:scale(PairwiseObstacles) | -0.38614 | 0.50362 | -0.767 |
| BVONE:scale(PairwiseFlowConnect) | 1.03918 | 0.46154 | 2.252 |
| BVSEUGE:scale(PairwiseFlowConnect) | 0.20898 | 0.37656 | 0.555 |
| BVONE:scale(PairwiseDist) | -0.40669 | 0.58698 | -0.693 |
| BVSEUGE:scale(PairwiseDist) | -0.22123 | 0.5364 | -0.412 |
| BVONE:scale(dTempAx1) | -0.43107 | 0.53606 | -0.804 |
| BVSEUGE:scale(dTempAx1) | 0.13055 | 0.39322 | 0.332 |
| BVONE:scale(dAltAx2) | -0.74786 | 0.43178 | -1.732 |
| BVSEUGE:scale(dAltAx2) | -0.54182 | 0.35789 | -1.514 |
| BVONE:scale(dStrahler) | -0.66751 | 0.39548 | -1.688 |
| BVSEUGE:scale(dStrahler) | -0.13522 | 0.32943 | -0.41 |

| **B** | Obstacles | FlowConnect | Dist | dTempAx1 | dAltAx2 | dStrahler |
|---|---|---|---|---|---|---|
| Obstacles | 1 | -0.21 | 0.72 | 0.41 | 0.31 | 0.03 |
| FlowConnect | -0.21 | 1 | -0.37 | -0.14 | 0.23 | 0.4 |
| Dist | 0.72 | -0.37 | 1 | 0.37 | 0.34 | -0.01 |
| dTempAx1 | 0.41 | -0.14 | 0.37 | 1 | 0 | -0.05 |
| dAltAx2 | 0.31 | 0.23 | 0.34 | 0 | 1 | 0.2 |
| dStrahler | 0.03 | 0.4 | -0.01 | -0.05 | 0.2 | 1 |

*Appendix S5.8: summary of the complete model explaining proportion of immigrants (A) and correlation matrix of response variables tested in this model (B)*

| **A** Proportion of immigrants | Estimate | Std. Error | z value | Pr(>\|z\|) | |
|---|---|---|---|---|---|
| (Intercept) | -0.8468 | 1.2852 | -0.659 | 0.51 | |
| TempAx1 | 0.4938 | 0.5963 | 0.828 | 0.4077 | |
| BVONE | -5.3202 | 2.1937 | -2.425 | 0.0153 | * |
| BVSEUGE | 3.2601 | 1.7403 | 1.873 | 0.061 | . |
| AltAx2 | -0.6013 | 0.6972 | -0.862 | 0.3884 | |
| HabAvailAx1 | -0.5564 | 0.5903 | -0.943 | 0.3459 | |
| Strahler | 0.3492 | 0.3684 | 0.948 | 0.3432 | |
| Centrality | -0.6394 | 0.6463 | -0.989 | 0.3225 | |
| TempAx1:BVONE | 0.5943 | 0.9638 | 0.617 | 0.5375 | |
| TempAx1:BVSEUGE | -0.8801 | 0.6624 | -1.329 | 0.184 | |
| BVONE:AltAx2 | 2.0907 | 1.1604 | 1.802 | 0.0716 | . |
| BVSEUGE:AltAx2 | 0.3404 | 0.7432 | 0.458 | 0.6469 | |
| BVONE:HabAvailAx1 | 2.0268 | 0.9629 | 2.105 | 0.0353 | * |
| BVSEUGE:HabAvailAx1 | 1.8996 | 0.8278 | 2.295 | 0.0217 | * |
| BVONE:Strahler | 1.7005 | 0.912 | 1.865 | 0.0622 | . |
| BVSEUGE:Strahler | -0.8406 | 0.5864 | -1.434 | 0.1517 | |
| BVONE:Centrality | 1.5232 | 0.8693 | 1.752 | 0.0797 | . |
| BVSEUGE:Centrality | -0.4647 | 0.8476 | -0.548 | 0.5835 | |

| **B** | TempAx1 | AltAx2 | HabAvailAx1 | Strahler | Centrality |
|---|---|---|---|---|---|
| TempAx1 | 1 | 0 | -0.46 | -0.19 | -0.07 |
| AltAx2 | 0 | 1 | -0.29 | -0.52 | -0.29 |
| HabAvailAx1 | -0.46 | -0.29 | 1 | 0.47 | 0.29 |
| Strahler | -0.19 | -0.52 | 0.47 | 1 | 0.31 |
| Centrality | -0.07 | -0.29 | 0.29 | 0.31 | 1 |

# General discussion

In this thesis, I developed new methodological approaches to study movement at the scale of the river basin, and brought new insights in movement patterns of Brown trout, a key species in headwater ecosystems. First, I developed a genetic tool useful for population genetic studies in the brown trout, as well as hybridization with captive-bred strains. This Single Nucleotide Polymorphism array mapped on the brown trout linkage map is cost effective, and especially, should be a useful and universal tool for many population genetics studies on this species. This tool was used in all (but one) subsequent chapters of my thesis. This tool exhibited high genotyping success and enabled to successfully identify patterns of isolation-by-distance, yielding similar patterns for population differentiation and admixture assessments than those obtained with a panel of microsatellites but with better ability to detect introgression and differentiation. Then, based on recent methods, I developed a procedure combining different markers to study movement with improved accuracy and power. Using new methods of machine learning to combine them is a very promising approach, and at the scales of the rivers studied in this PhD, these novel statistical methods appear as an optimal way of investigating movements with high accuracy and power. In particular, isotopes appeared as very efficient markers to discriminate and assign individuals to their population of origin, and these non-lethal markers thus appear highly promising, as this is one of their first successful applications for detecting movements at these spatial extents. The second aim of my PhD was to identify biotic and abiotic factors underlying movement. However, as stocking was shown to modify spatial patterns of genetic diversity and differentiation, which are the base of movement detection, and thus interfere with movement inference, I first described the effects of captive breeding on spatial patterns of genetic diversity, and found that captive-bred genotypes increased diversity and differentiation. Moreover, I found that expected natural patterns could be reversed in the case of higher captive-bred genotypes occurring at the level of populations, thus showing that human induced changes can affect natural patterns therefore with great implications. Further, I assessed how different genetic background (i.e. wild vs. carrying captive-bred ancestry) created differential dispersal patterns, and found that this variable influence movement propensity, movement distances, and the direction of movements, with the two strains being highly parapatric. Finally, I aimed at better understanding how individual, environmental and landscape related factors influence movements in natural populations, thus taking into

consideration only individuals that were supposedly wild. We found that body size was a main and universal driver of movement patterns, and that the position of the site within riverscapes, and habitat availability influenced the number of immigrants, although this depended on the river considered. Finally, landscape features also influenced movement; we found that sites that are directly connected by the water flow, and those that are similar in terms of elevation and stream order exchanged more migrants.

This work was in large part funded to gather biological information which is useful in terms of management. In this aspect, we focused on two main conundrums posed by human threats: stocking and habitat fragmentation. Therefore, the main aspects which will be discussed here concern the conundrum posed by stocking in both studying movements and assessing its effect on this crucial facet of life history, and on the use of individual based methods for inferring movements along with the methodological considerations arising from this discipline when using different types of markers.

## FACTORS INFLUENCING MOVEMENT IN THE BROWN TROUT, AND MANAGEMENT/CONSERVATION IMPLICATIONS

### *Factors influencing movement*

In Chapters 4 and 5, we found that there were drivers of movements which seem to be generalizable, and others which are more context-dependent. Understanding how these relations function thus has strong implications in understanding dynamics of brown trout populations.

First for individual drivers, it appeared that body length was a main determinant of both movement propensity and movement distance. Indeed, in Chapter 5, we found that longer individuals tended to be more prone to movements, and this was observed for all river basins. This was rather unexpected, as generally, smaller individuals, which are younger, were widely shown to undergo dispersal because their territory is not yet established (Andreu and Barba, 2006; Gachot-Neveu et al., 2009). Moreover, in Chapter 4, although it was non-significant, there was an observed tendency for this more expected pattern. Therefore, as the life cycle of the Brown trout is highly complex and movements are closely linked to age and developmental stage, we suggest that the change in spatial scale (Chapter 4 concerned very high headwaters only whereas Chapter 5

concentrated on a wider scale, although also including the higher reaches) could translate a differential use of habitat depending on these time-scales.

These different spatio-temporal scales could also explain why in terms of movement distances, the One River in its globality revealed that larger fish disperse further than smaller ones, whereas when considering only the Neste d'Oueil (its tributary, Chapter 4), the inverse relation was observed: in this latter river, but also in the Aude and the Seuge Rivers, smaller fish exhibited longer movements than larger ones. This observation is more consistent with what was observed in riverine fish (Skalski and Gilliam, 2000): being resident can increase survival because it implies not spending the costs for movement (Johnsson et al., 1999), thus larger and more dominant fish may choose to hold on to their origin location, forcing smaller ones to move more (Vøllestad et al., 2012). However, this scenario could concern only a small window of life history. Indeed, other studies have shown that conversely, individuals which are larger, and in better body condition, tend to be more mobile, in part because they can afford the cost of it. These two diverging scenarios thus appear to be very dependent on the moment considered and when it arises within the life cycle and within the habitat used at that moment: when considering post natal dispersal for instance, smaller individuals should disperse more from higher reaches of the river, whereas when considering foraging movements, or migrations, we suggest that larger individuals should exhibit a more pronounced movement behavior.

At the population level, it appeared that several variables drove the number of immigrants which a location is likely to receive. This is of high interest because it gives an insight into the underlying mechanisms of source-sink dynamics in these systems. Also closely linked to the time and spatial scales considered, we suggest that several mechanisms among which some were identified in our study can play a crucial role in determining why a location should receive more immigrants than others. First, the positional features (as discussed in chapter 5) such as Strahler index, centrality, but also habitat availability which is closely linked to the distance to the river mouth were shown to strongly influence movement patterns. These findings are congruent with the fact that larger, more downstream and connected demes have been shown to generally act as sink populations (Paz-Vinas et al., 2015). However, in our study, it appeared that while these positional characteristics strongly drove movement patterns, features concerning environmental conditions, in particular those in relation with a temperature gradient (i.e. temperature, dissolved oxygen, and

conductivity) were not linked to immigration. This is relatively surprising, as we could expect that some habitats are more favorable for immigrants due to these physic-chemical features (Schlosser, 1998). However, this can be explained by what has been discussed previously: the dynamics of the population i.e dispersal of fry, seasonal migration etc… could be way more important in shaping movement patterns than local environmental conditions and the subsequent individual preferences in terms of local suitability. Similarly, when investigating the number of movements between sites, we also found that these positional variables were the only ones which influenced movements (i.e. similar sites in terms of altitudinal axis and Strahler index exchanged more individuals).

A main perspective in this direction would be to have a robust estimate of emigration. Indeed, to investigate source-sink dynamics, or simply whether some locations can be unfavorable thus tending to be emigrated from, having an accurate estimate of emigration would have been of high interest. Our sampling resolution did not correspond to measuring this parameter accurately, as discussed in chapter 5, therefore we suggest that direct methods could be the only way to track emigrating individuals. This would be highly interesting, as we believe that the factors which drive an individual to leave a location are as important as those driving immigration in understanding metapopulation dynamics, particularly in a species which exhibits such a complex life cycle. In particular, it would enable to assess the effect of inbreeding on the decision to emigrate, and also the effect of population density as these parameters were shown to be important triggers of movement, in particular, during the fry and parr stages. Indeed, density dependent emigration (as individuals flee over populated locations in which competition for space and resource high) has been observed (Bagliniere et al., 1989; Cuinat and Heland, 1979; Einum et al., 2006), and kin interactions and inbreeding were also shown to modulate movements (Carlsson et al., 2004, Sanz et al. 2011), either by concentrating related individuals, or conversely by separating them (kin avoidance, Kasuya, 2000; Perrin and Mazalov, 1999).

*Conservation*

In terms of conservation and management in general, these finding have, as discussed in the last chapter, strong implications, in particular because they may help to identify units of interest, at different scales, and particularly because they can be very dependent on the context, or on the

contrary generalizable. First at the individual level, size was shown to strongly drive movements, and depending on the context and on the spatial scale considered. In terms of management, the Brown trout is a species which, especially in France, is very widely fished for recreation, and deciding on the size at which an individual can be harvested is the topic of many arguments between managers. Our results could suggest that taking movements dynamics into account could perhaps add pertinence to these measures, for instance, protecting individuals which move most could be of interest in preserving gene flow. In this case this would potentially imply that contrary to what is usually done, protecting the large individuals from the downstream reaches could be more important than protecting the smaller ones in those areas, whereas protection the small individuals could be far more important in the upper reaches.

Second, at the landscape scale, the fact that positional features appear as the main drivers of movement within a riverscape, and that it is possible to identify the underlying mechanisms of what makes a population a source or a sink can also be useful in management. For instance, when putting effort on dam passages, these findings could help to better integrate movement dynamics in the prioritization of areas to protect/restore. For instance, we suggest that main sink populations are of high conservation interest because they concentrate diversity, which is crucial at the scale of a species (Groom et al., 2006). Therefore, in the rivers studied, the context-dependence of the relation between positional features and source-sink dynamics should be carefully taken into account for these matters. For instance in the One River, headwaters seem to receive a higher proportion of migrants than lower reaches, therefore, protecting sink populations in this basin would consist in protecting upper reaches, whereas in the Seuge and in the Aude, the sink populations were located on lower reaches. In this aspect, studying source populations and as discussed previously zones from which individuals emigrate would also have been of high interest because these locations were shown to be crucial in shaping sink demes: in river systems, these lowly diverse and highly differentiated locations generate diversity in the downstream sink populations (Finn et al., 2011) and are thus also highly important in the perspective of conservation, thus assessing this in our study systems remains to be done.

## STUDYING MOVEMENT IN A MANAGED AND STOCKED SPECIES

The brown trout is an emblematic species that has been stocked for decades, mainly for sustaining recreational fisheries. Assessing the short and long term consequences of this practice is a prerequisite for any study aiming at investigating "natural" processes in riverscapes, because stocking has profoundly affected this species. For instance in chapter 5, we first discarded individuals of captive-bred ancestry to investigate natural patterns of movement, however, even if in chapter 3, we found that there tended to be a strong spatial segregation between wild and captive bred strains, with relatively low admixture rates, it was widely shown that they can interact (Blanchet et al., 2008). Therefore, even by discarding individuals exhibiting captive-bred ancestry, natural patterns in wild populations can still strongly be affected by the presence of these non-local strains, in the same way that another species would. For instance, Blanchet et al. (2008) showed that captive-bred individuals revealed higher aggressiveness and directly compete with wild individuals. Moreover, as these strains were, in our case studies, mainly located in the upper reaches of the basins, we could also suggest that, given the impact of Brown trout on food resource, they could be affecting the lower reaches, in particular because of excretion and consummation of resource which may have drifted downstream without their presence (Morita et al., 2004; Moyle, 1986; Townsend, 1996). Among the effects that these exogenous strains may potentially have on wild ones, a main one we observed in these works was the modulation of dispersal patterns through their underlying mechanisms (Chapter 4). Therefore, studying movement in the context of stocking showed that the underlying mechanisms of movement are strongly affected by stocking.

On top of these biological effects on movements for wild populations, stocking is also a methodological conundrum in the detection of movements using genetics. As discussed in chapter 3, we suggest that all studies using genetics are likely to be biased by the presence of these captive-bred genotypes because massive displacements and introductions have most likely strongly affected population structure, and as shown in chapter 3, patterns of genetic diversity and differentiation. This therefore implies that for instance, studying movements based on genetics only may be somewhat erroneous, although it appears that captive-bred and wild strains seem to remain relatively isolated. This is also why using other types of data, and in particular isotopes could be the powerful way to go around these biases. However, behavioral differences between

strains could also lead to different feeding strategies, which would reflect on isotopic signatures in tissues (except in those which take the signature of water such as otoliths). The brown trout is a very interesting model, but universally affected by humans, and thus drawing conclusions on natural processes in this species is therefore very difficult. Studies must now integrate the fact that populations have been widely modified, and developing tools which could overcome or at least take into account these considerations is crucial.

In chapter one, the tool developed is suggested to function rather universally, and I demonstrated that it was actually the case in two lineages of Brown trout. The study systems in this PhD consist in different and independent river basins, thus it was crucial that all basins were studied using the same approach and tools to draw both methodological and biological conclusions. Indeed, replicability is often a standard for all scientific investigations, and we suggest that this tool will be used to compare findings from other rivers to the ones we studied. Moreover, the straightforwardness of these types of tools is a requisite for their use in conservation, particularly because managers need tools that are not time consuming and which they can extract information from simply. Indeed, this tool is highly advantageous in terms of efficiency to put in place: they require only sampling tissue, encapsulating the sample in ethanol and sent samples to the genotyping platform. Within a few weeks, and without having to perform DNA extraction because the sampling platforms generally offer this step at a very reasonable cost, the data is sent back ready to be analyzed. Additionally, each research group generally uses its own panel of microsatellites for these types of studies, which makes comparisons among studies relatively difficult. For instance, a global study gathering all generated datasets on the Brown trout would be impossible for this reason. Therefore, the SNP panel developed here appears as a straightforward tool which has the potential to now be used commonly for all next population genetics on this species, which is why we call for its application all across the species' range.

# INSIGHTS OF DETECTING MOVEMENTS WITH INDIVIDUAL BASED METHODS

*Assignment methods and combinations*

In this thesis, the tools used to discriminate between populations i.e. genetics and isotopes, revealed unexpected features at the spatial extent which we considered. First, genetics were not as discriminant and thus accurate as we expected for this species which generally exhibits strong genetic structure even at small spatial extents (Swatdipong et al., 2010; Vøllestad et al., 2012; Berrebi, 2015). However, the basins considered were only studied in their headwaters, thus the scale may be too small to detect this structure. Similarly, Cook et al., (2007) found similar lack of genetic structure in pygmy perch at relatively similar scales, resulting in relatively low assignment of individuals to their capture site, however, they found that stable isotopes of Carbon and Nitrogen from fin tissue on the other hand were much more discriminant. This is particularly congruent with our results, notably in the Aude River. Interestingly, we found very few other studies which go in this direction as generally, isotopes are used for assignment purposes at much wider and contrasted scales. However, there are some studies showing that isotopic signatures of local dietary origin, as in our case nitrogen and carbon, can be informative over relatively small spatial and temporal scales for some species (Cunjak et al., 2005; Maruyama et al., 2001) because they can vary over small spatial scales (Peterson and Fry, 1987). But in terms of sufficient power to detect movements, we expected that these markers would only provide high resolution at very broad geographical extents, and even when combined (accordingly to Clegg et al., 2003; Gómez-Díaz and González-Solis, 2007; Hall and Beissinger, 2017). This was not the case, as we found that fine-scale population resolution was satisfactory enough to make precise inferences about movements, even though using our permutation process to make sure that assignment power is sufficient, we lost some individuals. Another alternative to C and N could be trace elements as they were shown to be even more sensitive to micro geographical differences and were already shown to give even better assignment results at local scales, at least for birds, such as the example in Gómez-Díaz and González-Solis, 2007; Hall and Beissinger (2017), where the authors assigned Cory's Shearwaters caught in western Mediterranean long-liners to breeding colonies in Menorca, Ibiza and Crete.

*Comparison and significance of movement detection using different markers*

As these markers displayed different discriminatory power, we suggest that they may be very complementary tracers of animal movement especially because they concern different time scales, and therefore have the potential to detect different types of movements (Clegg et al., 2003; Rubenstein and Hobson, 2004). Additionally, the accuracy of detection of each type of movement will also depend on the discriminatory power of a given marker for a given context (as seen in figure 1). For instance, Cook 2007 found that classification to capture site was stronger for stable isotope data than genetics in a case study river, suggesting that short-term, small-scale population isolation is not maintained over longer timescales (Skalski and Gilliam, 2000).

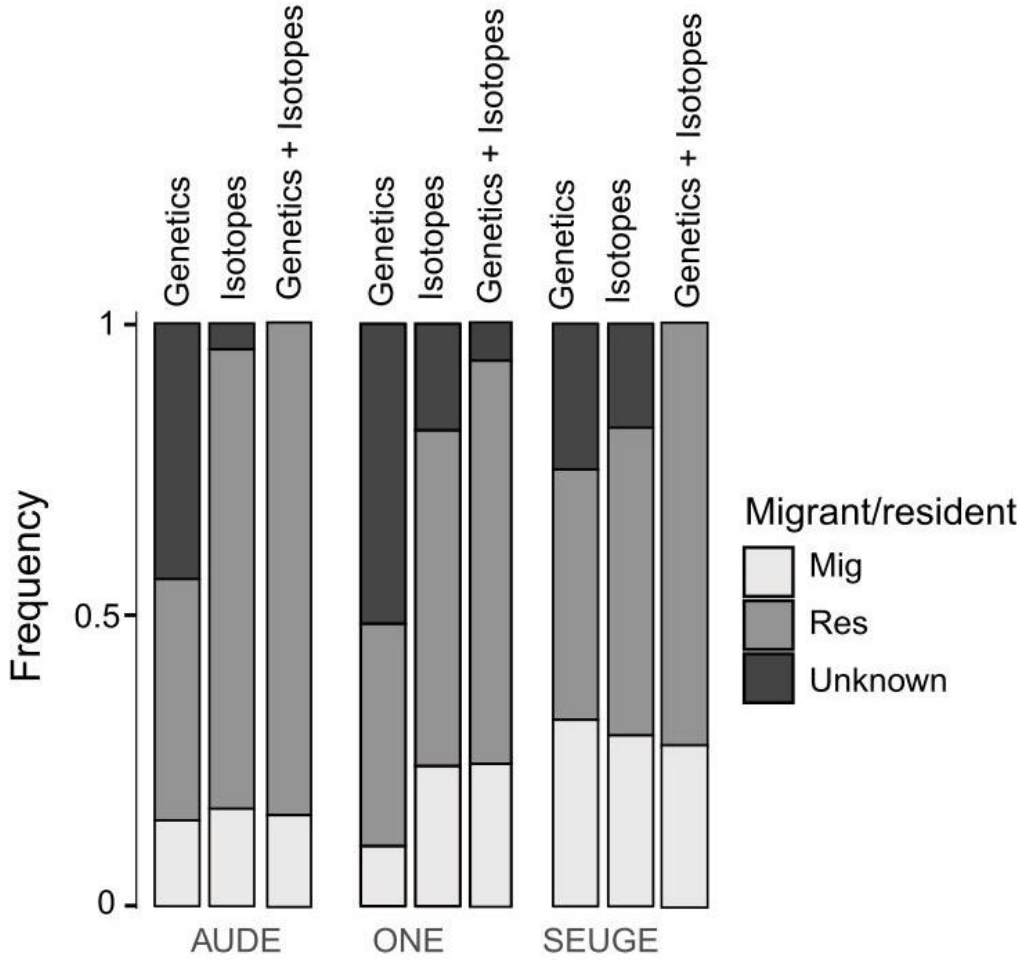



*Figure D1: Proportions of migrants, residents and unknown detected with genetics alone, isotopes alone, and the combination of genetics and isotopes*

Thus, the combined use of molecular markers and natural abundance isotopic signatures of nitrogen and carbon has the potential to be a powerful approach for studying movements and connectivity, and could enable to capture evens which would be missed by only considering one data type. Indeed, some authors suggest that information contained in genetic and isotope markers is largely additive and that therefore combining them is highly beneficial to increasing assignment (Clegg et al., 2003; Webster et al., 2002). Combining genetics with isotopes is therefore used more and more frequently. However, the fact that different types of movement can be detected depending on the markers used, in our eyes, also poses a conundrum for their use in combination, and very surprisingly, this consideration has to our knowledge never been discussed in these studies: in two of the chapters (2 and 4), I used these combinations of different data types do detect movements, and accordingly, I show that statistically, combining markers enabled a gain in discrimination among populations, and therefore in accuracy and power for assignment. However, as it is discussed in chapter 2, combining datasets may have some drawbacks, and we believe there is a counterpart for this increase in power: the potential loss of biologically information of interest. Indeed, the fact that each data sets enable to capture specific timescales (Hall and Beissinger, 2017) is, in the case of combination, not accounted for. Indeed, as discussed in chapter 2, the genetic signature of an individual is permanent but isotopic signature can change over time depending on diet and turnover rate (Hobson and Wassenaar, 2008). Therefore, assignment based on isotopes will depend on the time elapsed between actual movement and time of capture, and thus there are several possibilities of detecting individuals as migrants or residents according to the marker used, with two possible mismatches due to these differences (Figure 2).

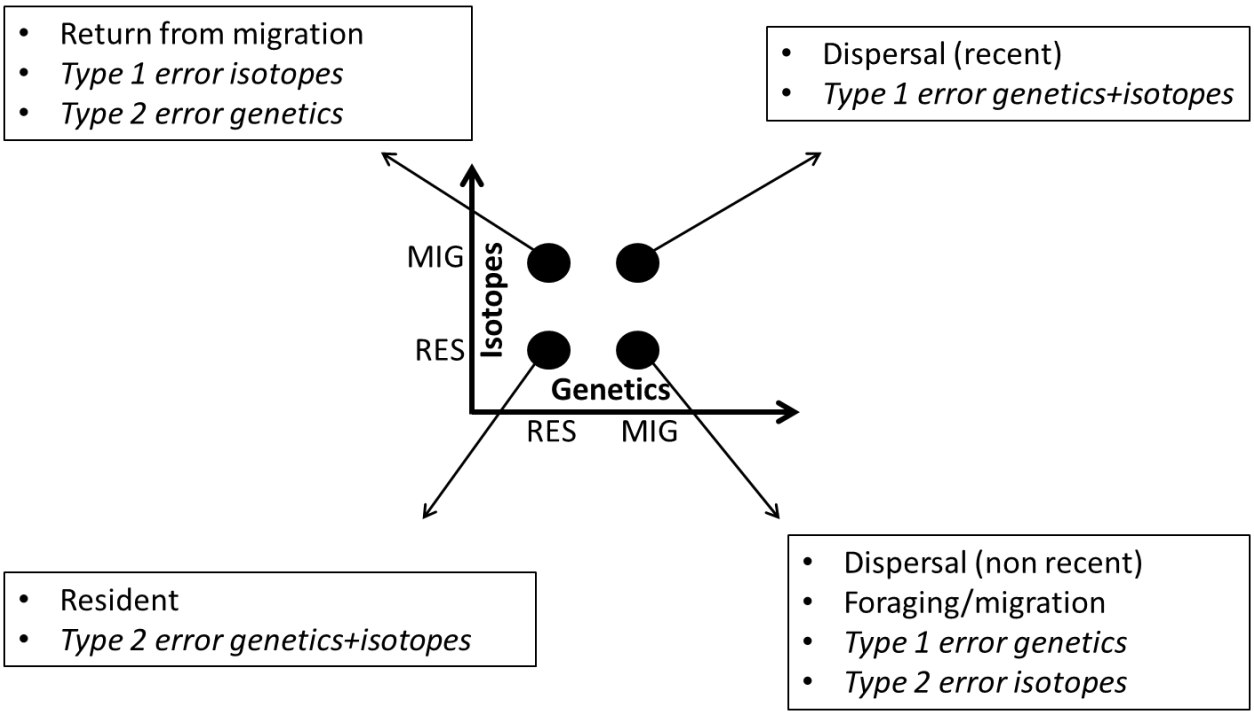



*Figure D2: Biological and statistical significance of detecting individuals as residents with both genetics and isotopes (bottom left), as residents according to genetics but migrants according to isotopes (top left), as migrants according to genetics but residents according to isotopes (bottom right), and as migrants using both markers (top right). There are two possible types of errors: Type I errors consist in assigning a resident individual as a migrant; Type II errors consist in assigning a migrant as a resident.*

Because these markers detect different movements, we therefore found that different individuals may be assigned differently according to the marker considered (Figure 3). And this has very strong implications on the legitimity of combining these datasets: in theory, an individual will be assigned according to the marker which is most discriminant. Thus, in the Aude for instance, if an individual is considered migrant according to isotopes, which for this basin are the most powerful, but resident considering genetics, it is most likely that when combining the markers, the individual will be considered as migrant because this decision is mainly driven by isotopes. And both of these assignments may be highly interesting, but the combination does not allow investigating these considerations: indeed, this could mean that the individual in question has returned to his capture (and origin) site after having emigrated to a location where its isotopic signature changed. Implications can thus vary considerably, because such an individual would be miss assigned in consideration of its genetic origin.

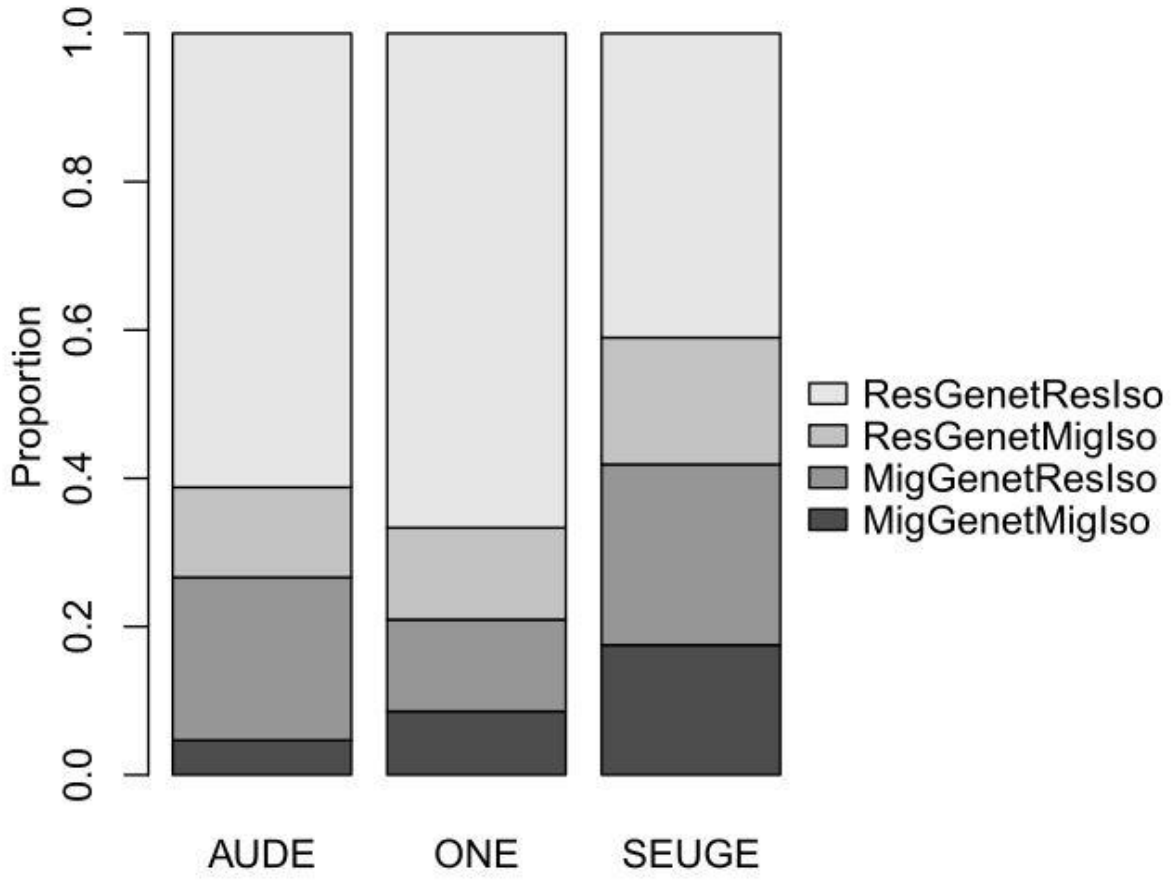



*Figure D3: Proportions of individuals detected as migrants with both genetics and isotpes (dark grey), of individuals detected as residents with both genetics and isotopes (white), of individuals detected as residents according to genetics but migrants according to isotopes (light gray), and of individuals detected as migrants according to genetics but residents according to isotopes (intermediate gray)*

In addition, other different scenarios emerge from these considerations, for instance, if an individual moves from a location to another, during this transience, it will get closer to its destination locations, thus isotopic signatures get closer while genetics do not change. This would mean that when an individual arrived recently, the distance moved according to genetics would be "true" whereas distance measured with isotopes should be shorter, because the individual has the isotopic signature of an intermediate site (between departure and destination site). Therefore, if an individual is detected as migrant according to genetics and also to isotopes, the site to which it is assigned can differ, although both markers consider the individual as a migrant. In our results, we

found quite a few cases following this scenario, but not all cases. This thus suggests that a wide variety of scenarios and mechanisms are probably taking place, and probably simultaneously.

Although they appear very intricate and question a large number of assumptions, among which some developed in this thesis, these considerations open new insights as to studying different types of movements, using different types of markers. Indeed, as detailed previously, the life cycle of the Brown trout is extremely complex, and presents a continuum of different possibilities regarding life-history strategies. Therefore, using markers such as genetics and isotopes, but also if possible coupled to direct tracking of individuals could be the direction to take when studying such complex species. Moreover, as we found that movement patterns are in some aspects very context dependent, using various methods in a large number of study systems should be the most accurate way to disentangle these different strategies, at different time and spatial scales.

## CONCLUSION AND PERSPECTIVES

In this thesis, I answered both a methodological goal, by developing a universal and efficient genetic tool, and assignment approaches to study movement in a machine learning framework. Then, I used these tools to first assess the impact of stocking on patterns of genetic diversity and differentiation, and, after assessing the effects of stocking on dispersal, I investigated the individual, environmental and landscape linked drivers of movements in different rivers. These works have also opened the door for many other questions about movement in the context of anthropic perturbations on natural habitats, and due to financial and temporal limitations, many aspects remain to be investigated. In particular, at these relatively large scales, it would be highly interesting to almost exhaustively monitor all individuals from different independent catchments, both in terms of biological findings, but also to enable a comparison of what it actually happening and what we found using indirect methods. In this optic, the genetic data of Chapter IV in the Neste d'Oueil have been used in combination with PIT tagging (Alp et al. in prep). Similarly, methods based on family reconstruction can also give very high definition information in particular in the study of dispersal of juveniles. This has been done in chapter IV, but in other basins, we suggest that the sampling design was too scarce to accurately use this approach, thus sampling smaller scale basins more thoroughly could have also been an interesting approach.

An interesting perspective from the markers used is that besides studying movements, they could enable to investigate many other questions. First, genetics are a tool which is accurate to investigate the genetic "health" of populations (without considering the effects of stocking), through calculation of effective size, inbreeding (although it appeared very low in the populations sampled). Furthermore, the genetic tool developed could also, by using different filters than those aiming at relative neutrality of markers, be used to investigate patterns of selection for instance using genome wide association studies. Second, with the data gathered, isotopes also still offer many unexplored perspectives fur future studies. For instance, investigating different trophic interactions within the river catchments of our study systems has not been done mainly because of temporal limitations. In particular, we showed that stocking affected natural populations in many ways (through genetic spatial patterns, and movement), thus investigating differences in diet, and in trophic position between wild and captive-bred strains, and competition for food resource between them could be another interesting way of assessing the effects of stocking on these populations. Finally, morphometrics were globally found to be relatively disappointing in our study systems, both for discriminating among populations and for explaining movement patterns at the individual level. Therefore, we suggested that this tool was most likely lacking spatial resolution. However, other studies have found it highly useful, thus perhaps using them differently by calculating other ratios than those used, or measuring variables which we ignored, in particular colorimetry or punctuation of the fish could have made these markers more exploitable.

Despite these considerations, our findings have brought insight of interest into the dynamics of the species studied. Indeed, for subsequent studies on movement, the new tools developed have shown to be efficient at the spatial scales of interest for management and conservation decisions, which was lacking compared to other studies. Moreover, the biological findings in this thesis are also of interest first because they open insights into the study of movement in a managed and stocked species, thus more globally in the context of human induced perturbations on natural systems. Finally, the identification of individual features, but also environments and landscape characteristics which shape movement patterns has participated to knowledge in this already widely studied species, which are also useful for management, and in particular for the definition of interesting units for conservation prioritization.

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