Structure génétique des populations de saumon Atlantique en France

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Résumé

Cette thèse porte sur la structuration génétique des populations françaises de saumon Atlantique. Nous avons notamment étudié l'effet de facteurs environnementaux et des repeuplements sur la distribution spatiale de la diversité génétique. 1739 individus échantillonnés dans 34 rivières et provenant de cohortes anciennes (1965-1987) et récentes (1998-2006) ont été génotypés à 17 marqueurs microsatellites. Les analyses des échantillons récents montrent l'existence de cinq groupes de populations génétiquement et géographiquement distincts. La distance côtière entre les populations ainsi que la longueur des rivières sont deux facteurs fortement corrélés à la différentiation des populations. Le cas de la population de l'axe Loire-Allier suggère une adaptation locale à la difficulté de montaison liée à la grande distance entre les frayères et l'estuaire et illustrée par la grande taille des poissons, leur phénologie de migration particulière, et une forte différenciation de cette population. La comparaison d'échantillons anciens et récents montre d'une façon générale une relative stabilité temporelle de la structure génétique observée associée à une réduction de la différentiation entre les populations. Certaines des variations temporelles observées pourraient être les conséquences d'introgressions des populations natives par celles utilisées lors des repeuplements. Pour certaines rivières dépeuplées et non sujettes au repeuplement, nous avons observé des recolonisations spontanées par des poissons provenant de stocks voisins et distants. Afin de quantifier l'impact des repeuplements dans certaines populations pour lesquelles ces pratiques étaient bien documentées, nous avons développé une approche utilisant des simulations individus centrées temporellement explicites. Cette étude suggère une faible survie des poissons déversés. Enfin, parallèlement aux analyses génétiques, nous avons réalisé des analyses microchimiques sur les otolithes d'une centaine d'individus issus de populations repeuplées. Le couplage d'analyses microchimiques et génétiques a permis de déterminer si les poissons ayant des caractéristiques génétiques de pisciculture provenaient de repeuplement ou de reproduction *in natura* de poissons précédemment déversés.

Mots clefs : adaptation locale, introgression, marqueurs microsatellites, microchimie, otolithe, repeuplements, saumon Atlantique, simulations, structure génétique.

Abstract

This thesis investigates the genetic structure among Atlantic salmon populations from France. We focused on the influence of environmental factors and stocking on the spatial distribution of genetic diversity. We genotyped 1739 individuals from 34 rivers at 17 microsatellite markers. Samples were collected in old (1965-1987) and recent (1998-2006) cohorts. Clustering analyses revealed the existence of five genetically and geographically distinct groups. Distance among estuaries and river length were strong predictors of population structure. Local adaptation to upstream migration difficulty linked to the large distance from the sea to the spawning grounds is suggested in the Loire-Allier population given the large body size of fish, their particular run timing, and the high differentiation of this population. Comparing recent and old samples revealed a general reduction of differentiation among populations and high introgression by stocking strains in some populations most probably resulting from stocking. In some depopulated rivers were no stocking was performed we observed natural recolonization by individuals from neighbouring and distant stocks. We developed an approach using temporally explicit simulations to quantify the impact of stocking on some populations. This study suggested a lower fitness of stocked fish compared to wild individuals. In parallel to genetic analyses, we carried out microchemistry analyses of otoliths from individuals collected in stocked populations. Coupling genetic and microchemistry analyses on the same individuals allowed identifying river-born fish with hatchery pedigrees, discriminating them from hatchery-born fish with similar genetic characteristics.

Key words: Atlantic salmon, genetic structure, introgression, local adaptation, microchemistry, microsatellite markers, otolith, simulations, stocking.

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Chapitre I. Introduction

Au cours des derniers siècles, les populations de nombreuses espèces ont connus de profondes modifications dans leur répartition et leur abondance entraînant notamment une importante perte de biodiversité. Les principales causes de cette perte de biodiversité sont liées à l'impact des activités humaines se traduisant par la disparition et la fragmentation des habitats, la pollution, les invasions biologiques, la surexploitation, le transfert d'individus allochtones dans des populations sauvages et le réchauffement climatique. Ces effets peuvent toucher directement une espèce en provoquant son extinction qui par effet cascade peut entraîner la disparition d'une autre espèce dont l'existence n'est pas menacée directement par les activités humaines (coextinction). En 2010, l'IUCN estime que 34% des espèces animales survivantes et décrites sont menacées (http://www.iucnredlist.org). Par ailleurs, la FAO estime qu'environ 74% des espèces de poissons sont pleinement exploitées, surexploitées, ou éteintes (http://www.fao.org).

Il existe depuis de nombreuses années une prise de conscience importante des effets néfastes de la croissance humaine sur l'environnement. Cette prise de conscience a débouché sur la nécessité de développer des moyens pour essayer d'enrayer cette crise écologique qualifiée de sixième extinction. La quantification et le maintien de la diversité génétique est une composante importante de ces moyens qui se mettent en œuvre. En effet, il est nécessaire de comprendre comment les espèces peuvent réagir à ces bouleversements environnementaux en analysant et déterminant les composantes de leur capacité (résilience, modification de l'histoire de vie) à évoluer dans un environnement en pleine mutation.

L'étude de cette capacité à réagir passe par la génétique des populations qui étudie l'évolution des caractéristiques génétiques des groupes d'individus dans l'espace et le temps. L'évolution de la diversité génétique au sein et entre les populations d'une même espèce renseigne sur leur histoire de vie, leur démographie, les flux d'individus et de gènes, et leur potentiel évolutif. Ainsi, le maintien de la diversité génétique totale chez une espèce exige de prendre en compte différentes échelles spatiales, depuis l'aire de répartition de l'espèce jusqu'à la structure à l'intérieur d'une population, et temporelles, du court au long terme. L'analyse de la diversité génétique à ces différentes échelles nécessite l'étude des mécanismes sous-jacents à sa mise en place et à son maintien.

Les populations de poissons migrateurs, et en particulier de saumon Atlantique (*Salmo salar*), ont subi et subissent des réductions significatives de leurs effectifs en lien avec des effets négatifs cumulés des activités humaines (Schlindler, 2001). Les populations de

saumons localisées sur le territoire français n'ont pas fait exception à ce déclin général et semblent même plus affectées en raison de leur situation au sud de l'aire de répartition de l'espèce (Anonyme, 2003). Ainsi, le saumon atlantique est considéré comme une espèce vulnérable et est inscrite sur la liste rouge des espèces menacées en France comme en Europe (Porcher & Baglinière, 2001 et http://www.uicn.fr/Liste-rouge-poissons-d-eau-douce.html).

Cette thèse se propose d'étudier la structuration génétique des populations de saumon en France, en s'attachant à déterminer les niveaux de différenciation entre les populations et comment ils sont influencés par les facteurs environnementaux, les repeuplements, et les processus de recolonisation naturelle des rivières. Caractériser et comprendre la structure génétique de ces populations et les facteurs qui l'influencent sont des éléments essentiels pour analyser leur évolution et leur capacité d'adaptation aux forçages d'origine anthropique.

Distribution et cycle de vie du saumon atlantique

Aire de répartition

La répartition native du saumon Atlantique s'étend, à l'Ouest de l'Océan Atlantique, de la rivière Hudson, Etat de New York, US, jusqu'à la Baie d'Ungava, Québec, Canada (Fig. 1). En Europe, la répartition du saumon s'étend de la rivière Pechora, Russie, jusqu'à la rivière Minho, Portugal, Europe (Maccrimmon & Gots 1979; Klemetsen *et al.* 2003). Divers introductions de saumon Atlantique ont été réalisées hors de l'aire originelle de répartition de l'espèce, via l'implantation de fermes aquacoles ou afin d'implanter de nouvelles populations sauvages, notamment en Amérique du Sud, en Afrique du Sud, En Asie, et en Australie à des fins de pêche sportive. Cependant ces populations demeurent artificielles et dépendantes des opérations de repeuplements[1] (Maccrimmon & Gots 1979).

[1] Le repeuplement (stocking) peut être défini comme une introduction (ou injection) de poisson dans un écosystème dans lequel une population de cette espèce est naturellement présente ou bien dans lequel l'espècc n'était originellement pas présente mais a été préalablement introduite (Cowx, 1998).

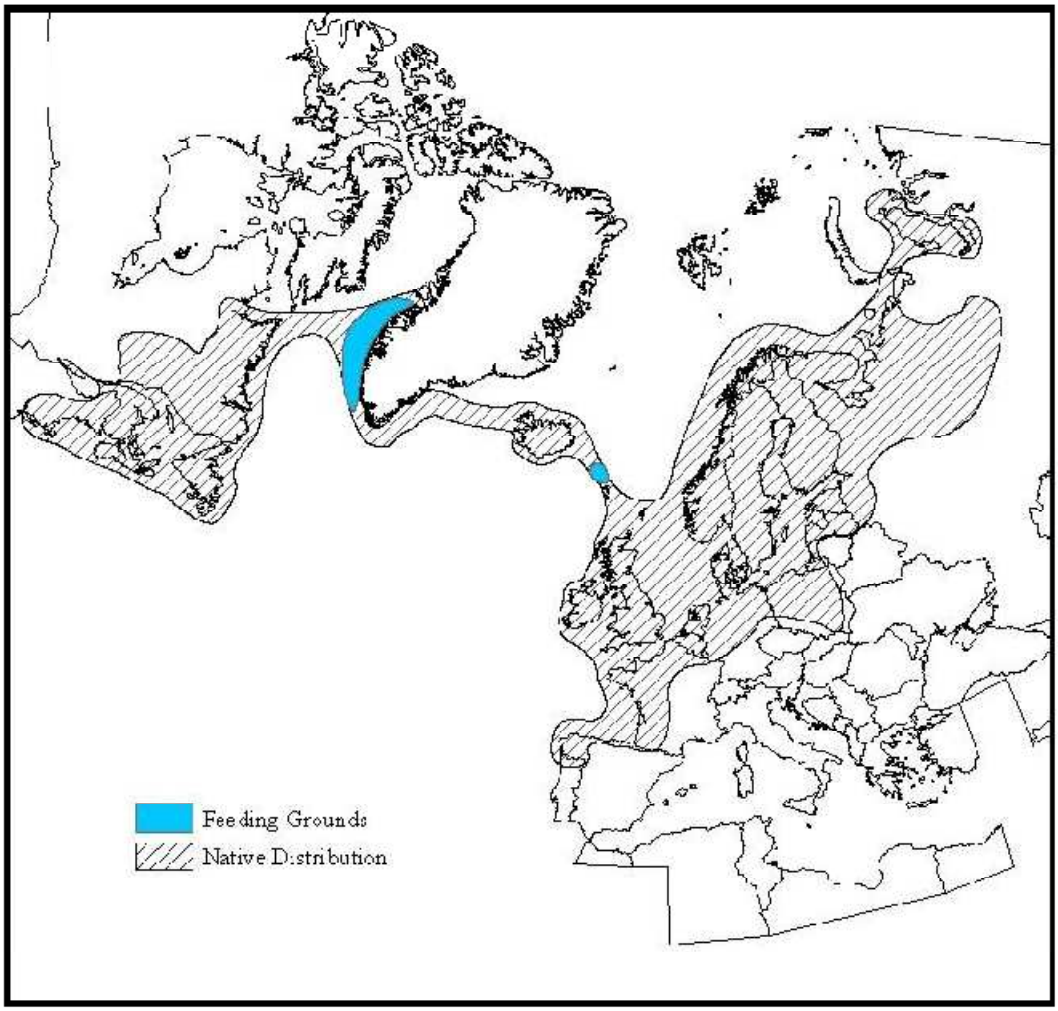

Figure 1 : Distribution des populations de saumon Atlantique. Modifié par (Finnengan 2009) d'après (Maccrimmon & Gots 1979) et (Klemetsen et al. 2003). Les zones d'engraissement figurées sont les plus importantes connues à ce jour. Elles ne concernent pas les populations de saumon de la Baltique et toutes celles de la Norvège.

Cycle de vie

Le saumon Atlantique est un poisson migrateur amphihalin, anadrome et phylopatrique. En d'autres termes, le saumon atlantique se reproduit en eau douce ; le juvénile grossit en rivière avant de migrer en mer puis revient au stade adulte se reproduire dans sa rivière natale (Fig. 2). Il existe des populations de saumon bouclant leur cycle uniquement en eau douce mais elles restent peu nombreuses et très localisées dans l'aire de répartition de l'espèce (Quelques lacs d'Amérique du Nord et du Nord de l'Europe). La forme anadrome est donc la forme majoritaire. Le saumon atlantique est itéropare, c'est-à-dire qu'il peut se reproduire plusieurs fois au cours de son cycle de vie. Ce taux d'itéroparité varie selon le stock et le sexe. Il est très faible pour l'ensemble des populations françaises puisque ces saumons de plusieurs remontées sont très peu représentés (en moyenne 1,1%) (Baglinière & Porcher, 1994).

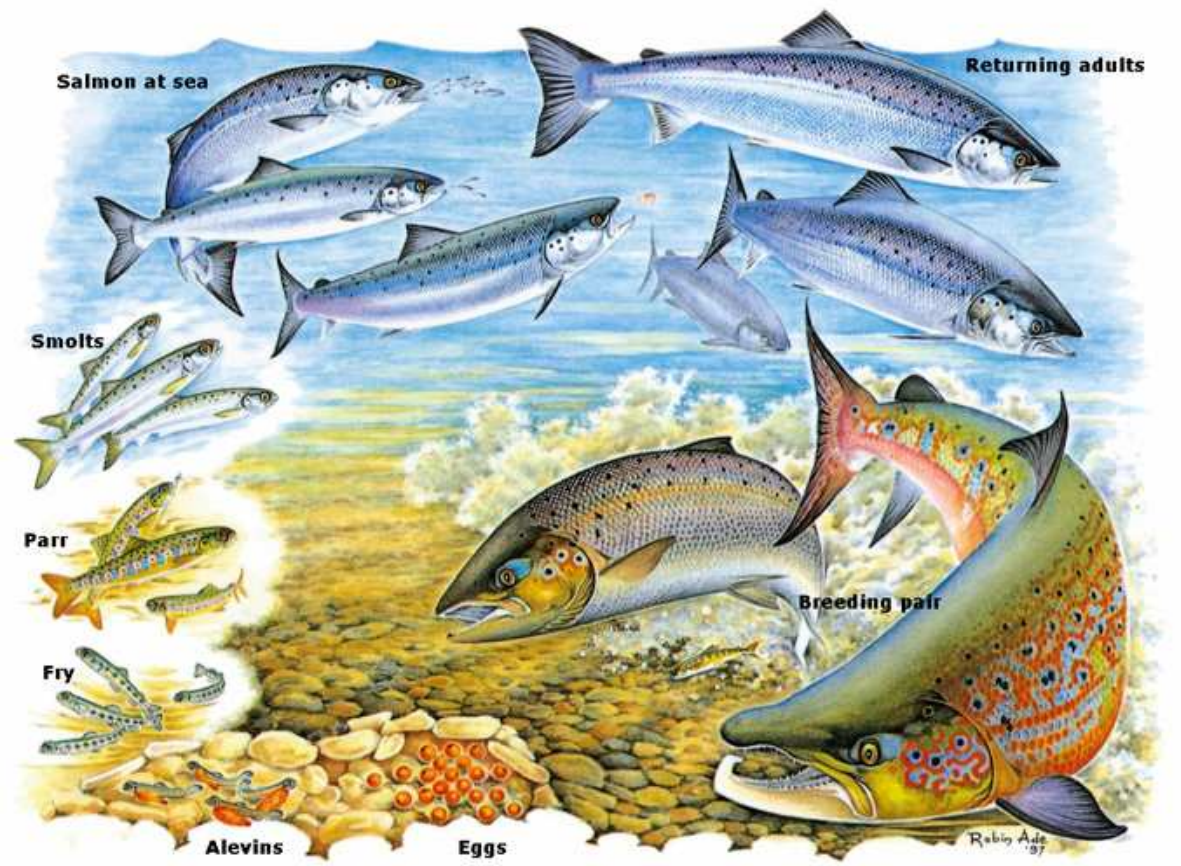

Figure 2 : Cycle de vie du saumon Atlantique (Source Atlantic Salmon Trust & Robin Ade).

Figure 3 : œufs de saumon. (Source G.Evanno)

La reproduction a lieu en rivière, sur des zones de graviers appelées frayères. L'incubation des œufs (Fig. 3) dans la frayère dure deux à trois mois suivant la température de l'eau. À l'éclosion, les alevins sont pourvus d'une vésicule vitelline qui assure leur survie jusqu'à ce qu'ils sortent des frayères pour se nourrir d'invertébrés aquatiques dans la rivière.

Le stade tacon dure de 1 à 8 ans (Heland & Dumas 1994). En France, la durée est généralement d'1 à 2 ans, avec une récente tendance au raccourcissement du temps de séjour en rivière (Rivot et al. 2009). Lorsqu'il atteint une taille et une condition suffisante, le tacon subit dès la fin de l'hiver un ensemble de modifications morphologiques, physiologiques et comportementales, appelé la smoltification, permettant la transformation du tacon en smolt

Figure 4 : Tacon de Saumon atlantique (Source INRA et ORE PFC).

Figure 5 : Smolt de Saumon atlantique (Source INRA et ORE PFC).

et le passage du milieu dulçaquicole au milieu marin (Fig. 4 & 5) (Saunders *et al.* 1985; Stefansson *et al.* 1991; McCormick *et al.* 1998). La descente vers la mer à lieu au printemps. Deux principales zones d'engraissement ont été identifiées : Groenland-Labrador et îles Féroé (Fig. 1). Il existe cependant deux autres zones de grossissement en mer : mer de Norvège et mer Baltique, concernant un nombre plus limités de stocks. La croissance des saumons en mer est élevée.

Après un temps de séjour en mer de 1 an (castillons, Fig. 6) à 2 ou 3 ans (saumon de printemps) (Baglinière & Porcher, 1994), les individus reviennent dans leur rivière natale respectivement en été ou au printemps. Cet instinct de retour est appelé « homing ». L'olfaction joue un rôle primordial dans l'orientation des individus qui font alors appel à leur mémoire olfactive des milieux traversés et des phéromones dégagés par leurs congénères lors de leur descente vers la mer en tant que smolts (Hasler *et al.* 1978; Stabell 1984; Nevitt *et al.* 1994; Dittman & Quinn 1996). La dispersion des adultes dans d'autres rivières que leur rivière natale est relativement faible, en moyenne de 5% (Quinn 1993; Jonsson *et al.* 2003; Pedersen *et al.* 2007). Le « homing » des poissons d'élevage semble beaucoup plus variable que celui des individus sauvages et dépendent des conditions, des stades et des sites d'élevage et de déversement. Leur dispersion est en moyenne 3 fois plus forte que celle des individus sauvages (soit 15%) (Quinn 1993; Jonsson *et al.* 2003; Pedersen *et al.* 2007). Néanmoins, des taux de homing jusqu'à 99,6 % ont put être observés chez des poissons déversés (Hansen & Jonsson, 1994).

Figure 6 : Saumon Atlantique mâle lors de la reproduction (Source G.Evanno).

Sous nos latitudes, la reproduction s'étale de fin novembre à début février avec une majorité d'œufs déposés en Décembre (Mills 1971). L'acte implique au moins deux adultes anadromes de sexes opposés. La femelle creuse une frayère dans des zones de graviers où l'oxygénation est importante (Mills 1971; Fleming 1998). Les œufs sont déposés par la femelle puis fécondés par les mâles. La femelle recouvre par la suite les œufs avec des graviers (Fleming, 1998).

Les saumons mâles peuvent aussi maturer sexuellement précocement au stade de tacon avant la migration marine (Fleming 1998; Klemetsen *et al.* 2003) et participer efficacement à

la reproduction (Klemetsen et al. 2003). Ces petits mâles peuvent en effet féconder jusqu'à 60% des œufs d'une frayère (Saunders *et al.* 1982; Bagliniere & Maisse 1985; Fleming 1998).

Modifications de l'aire de répartition et des caractéristiques des populations

Diminution de l'abondance des stocks et changements dans le cycle biologique

Au cours du siècle dernier, en Europe, aux Etats-Unis et au Canada, de nombreuses populations de saumon Atlantique ont vu leur abondance diminuer ou se sont éteintes. Ainsi l'aire de répartition de l'espèce et la taille des stocks s'est réduite (Maccrimmon & Gots 1979; WWF 2001; Friedland *et al.* 2003; ICES 2004; Jonsson & Jonsson 2004a; NASCO 2004). Selon le document publié par le WWF en 2001 et traitant du statut des populations de saumon Atlantique dans le monde, sur 2005 rivières classées comme rivière à saumon (sur 2615), 15% avaient des stocks complètement éteints, 20% des stocks en danger, 12% en état critique, 10% vulnérables, et 43% en bonne conditions. Selon ce même document du WWF, sur les 47 rivières française historiquement colonisées par le saumon Atlantique, 14 avaient des stocks éteints, 15 en état critique, 10 en danger, 3 vulnérables, et 5 inclassables (pas assez de données).

Tableau 1 : Status des rivières à saumon Atlantique dans 19 pays (WWF 2001).

Country	Total number of historically salmon bearing rivers	Unknown Status	Healthy	Vulnerable	Endangered	Critical	Extinct
Finland	25	0%	8%	0%	0%	0%	92%
Poland	8	0%	0%	0%	0%	12%	88%
Portugal	7	0%	0%	0%	0%	14%	86%
United States	50	0%	0%	0%	0%	16%	84%
Spain	43	0%	9%	2%	7%	14%	67%
Denmark	9	0%	0%	11%	0%	22%	67%
Sweden	28	0%	14%	11%	7%	18%	50%
Lithuania	2	0%	50%	0%	0%	0%	50%
France	47	11%	0%	6%	21%	32%	30%
Estonia	9	0%	0%	0%	0%	78%	22%
N. Ireland	44	18%	0%	36%	32%	0%	14%
Ireland	339	8%	38%	27%	7%	8%	12%
England and Wales	76	5%	33%	14%	25%	14%	9%
Latvia	11	55%	9%	9%	18%	0%	9%
Norway	667	10%	47%	3%	23%	8%	9%
Russia	224	42%	4%	19%	16%	11%	8%
Canada	550	72%	8%	4%	2%	11%	3%
Iceland	103	0%	99%	0%	0%	0%	1%
Sweden - West	23	0%	52%	9%	4%	35%	0%
Scotland	350	0%	63%	0%	37%	0%	0%

Ces modifications de l'aire de répartition et de l'abondance des stocks s'accompagnent également d'évolution des traits de vie des individus. En effet diverses études ont montré des raccourcissement des temps de séjours des juvéniles en eau douce (Friedland *et al.* 2000; Jonsson *et al.* 2005; Rivot *et al.* 2009) comme des adultes en milieu marin (Kuparinen *et al.* 2009) ainsi qu'un décalage dans les dates de remontée en rivière (Juanes *et al.* 2004; Quinn *et al.* 2006).

Causes de ces modifications

Le facteur primaire de la modification de l'aire de répartition et de l'abondance des populations a été la construction de nombreux barrages pour la production d'électricité, l'amélioration de la navigabilité, les besoins en eau potable ou la prévention des inondations (Bednarek, 2001). Cette édification a considérablement modifié les conditions de circulation des saumons que ce soit au stade adulte lors de la migration de reproduction ou au stade juvénile lors de la descente vers la mer (franchissement souvent difficile voire impossible). (Boet *et al.* 1999; Levin & Tolimieri 2001; Stefansson *et al.* 2003; Thorstad *et al.* 2008). Par ailleurs, de tels obstacles modifient non seulement les flux biologiques mais également les flux hydrosédimentaires et donc l'ensemble de la connectivité entre les compartiments dulçaquicoles et marins.

D'autres facteurs tels que les pollutions industrielles, urbaines et agricoles sont venus se surajouter pour entraîner de fortes modifications dans les populations de saumon avec notamment des fortes perturbations lors de la phase de croissance et sur la survie en eau douce des juvéniles (Stefansson et al. 2003).

Les pêches professionnelles et de loisir en rivière comme en mer en surimposant leur impacts sur des stocks déjà fragilisés sont susceptibles de réduire les effectifs de façon dramatique et d'entrainer des modifications dans la composition des populations *via* la sélectivité des pêches (Kendall & Quinn 2009).

Les changements climatiques pourraient avoir des conséquences importantes sur la croissance (Fig. 7) et la survie des saumons en rivière (Friedland *et al.* 2000; Friedland *et al.* 2003; Jonsson *et al.* 2005; Rivot *et al.* 2009) comme en mer (Jonsson & Jonsson 2004b; Juanes *et al.* 2004; Todd *et al.* 2008; Bacon *et al.* 2009).

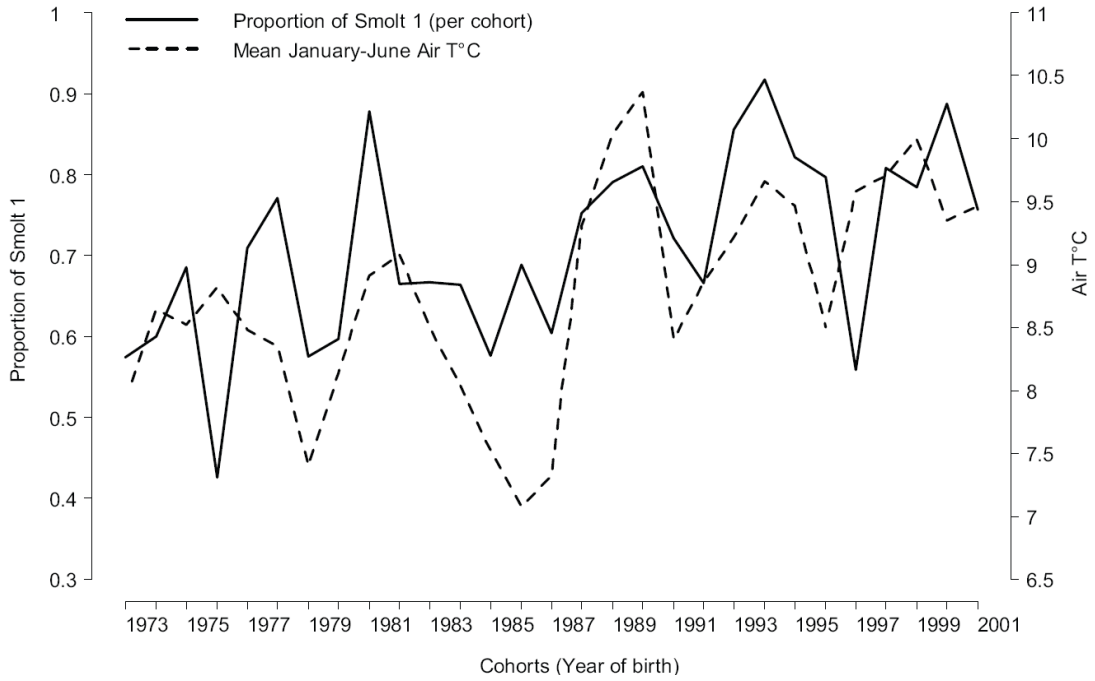

Figure 7 : Evolution de la proportion de smolts d'un an dans les populations de saumon Atlantique de Bretagne, France, de 1973 à 2001 (Rivot et al. 2009).

Enfin, il existe un impact potentiel des interactions avec des poissons d'élevage provenant de lignées domestique déversés pour soutenir, restaurer les stocks et/ou échappés des fermes marines. En effet, ces saumons ont une forte probabilité d'intéragir avec des individus sauvages entraînant des transmissions de pathogènes et parasites inféodés aux élevages (Heggberget et al. 1993) mais également une réduction de la diversité génétique de la population sauvage (Verspoor 1988a; Aho *et al.* 2006; Lage & Kornfield 2006; Horreo *et al.* 2008; Eldridge *et al.* 2009) et des risques d'interactions génétiques négatives lors de la reproduction (McGinnity *et al.* 2003; Fraser *et al.* 2008). On observe notamment une introgression[2] par les lignées utilisées (Campos *et al.* 2008; Finnegan & Stevens 2008; Grandjean *et al.* 2009), potentiellement éloignées et inadaptées aux conditions locales (Fraser *et al.* 2008; Darwish & Hutchings 2009; McGinnity *et al.* 2009), ou ayant connus de forts épisodes de sélection durant leur phase d'élevage (Blanchet *et al.* 2008; Fraser 2008).

Sauvegarde et restauration des populations

Selon la NASCO (North Atlantic Salmon Commission Organisation), la sauvegarde des populations de saumon Atlantique sauvage passe par les actions suivantes : mise en place de mesures réglementaires notamment concernant la pêche, restauration de la libre circulation, restauration de l'habitat, et repeuplement.

[2] Introgression : Introduction de nouveaux allèles ou gène(s) dans une population à partir d'individus d'une population ou d'une espèce différente.

Réduction de l'effort de pêche

La pêche intensive du saumon sur les aires d'engraissement du Groenland et des îles Féroé a commencé à la fin des années 1950. Après avoir atteint des pics de 2689 tonnes en 1971 et 1025 tonnes en 1981, respectivement au Groenland et dans les eaux féringiennes, des quotas de pêche sont mis en place (Fig. 8) (Davaine & Prouzet 1994). Les premiers quotas de pêche sont instaurés en de 1972 pour les pêcheries du Groenland et fixés à 1100 tonnes. D'une manière générale, suivant l'objectif global de protection de l'espèce (Mills 1987), la pêche sportive a été développée, et la pêche commerciale réduite jusqu'à l'arrêt de l'exploitation aux îles Féroé en 1991. En France, la majeure partie de la pêche professionnelle a été arrêtée au début des années 1990 tandis que la pêche sportive s'est vue imposée des quotas par bassin fluvial (Totaux autorisés de capture) de façon à maintenir le renouvellement des populations de saumon.

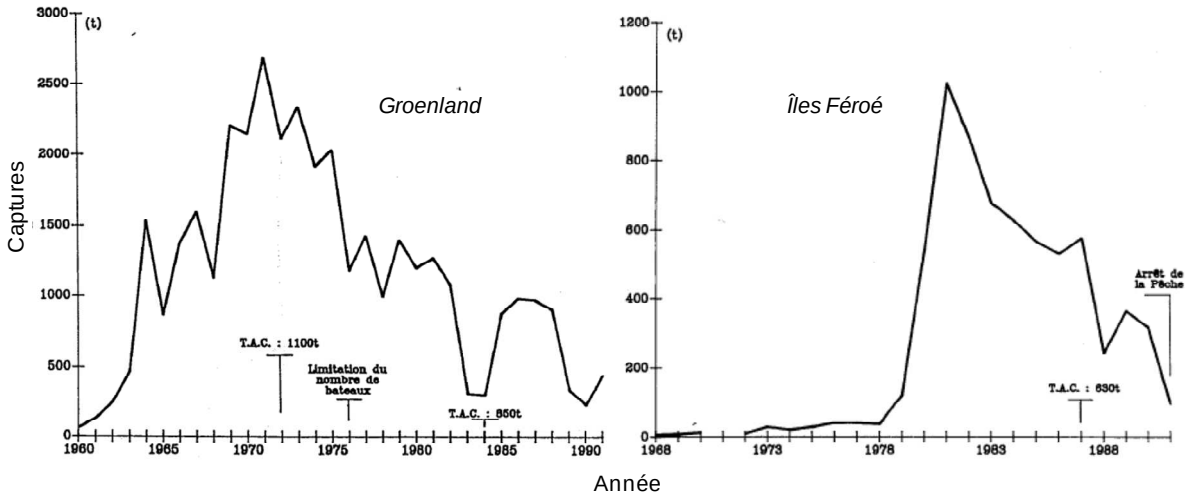

Figure 8 : Evolution des captures de saumon au Groenland et Îles Féroé (modifié d'après (Davaine & Prouzet 1994)).

Restauration de la libre circulation

L'amélioration du franchissement des barrages voir leur suppression reste un moyen permettant aux saumons de retrouver leurs zones de frayères historiques généralement situées dans les parties amont des bassins. De très gros progrès ont été réalisés sur le comportement de l'espèce face aux obstacles lors de sa migration de reproduction permettant de construire des passes à poisson plus efficaces car en adéquation avec les besoins du poisson (Larinier, 1992). Néanmoins, cette efficacité est difficile à évaluer, varie suivant la localisation de l'obstacle barrage sur le cours d'eau et de l'implantation de la passe sur le barrage. Enfin, il existe un fort effet cumulatif des obstacles sur la migration du poisson voire sur sa survie lors de sa migration de reproduction (Croze, 2008).

Utilisation de poissons de repeuplement

De nombreux repeuplements ont été ou sont encore effectués afin de compenser la baisse de la productivité naturelle de populations. Les suivis parfois effectués montrent en général un succès modeste des déversements à partir de stades juvéniles traduits par des taux de retours d'adultes faibles. Par exemple, la rivière Connecticut (Etats-Unis) a vu sa population de saumon s'éteindre à la fin du 19ème siècle et a bénéficié de déversement de 10 000 à 1 000 000 de juvéniles par an de 1967 à 1994 (Spidle et al. 2004). Les retours d'adultes ont été alors de l'ordre de quelques individus à 550 poissons correspondant à des taux de retour faibles par rapport à ceux des populations naturelles. Dans les années récentes, ces retours ont chuté (<100 individus) suite à une forte diminution de l'intensité des déversements (quelques milliers seulement). Cette situation démontre alors que le stock ne peut pas se maintenir naturellement et que la pratique du repeuplement n'est pas une solution si la qualité des habitats et leur connectivité ne sont pas restaurées.

Certaines populations françaises bénéficient ou ont bénéficié de programmes de repeuplements utilisant des poissons natifs ou non (Fig. 9) (Baglinière & Dumas 1988; Baglinière *et al.* 1990; Vauclin 2007). Des années 1950 à 1990, des repeuplements avec des juvéniles au stade smolt de souches étrangères ont été effectués. Les géniteurs utilisés provenaient essentiellement d'Ecosse mais également de Scandinavie et du Canada. De nombreuses rivières ont été repeuplées, notamment la Bresle, l'Arques, l'Orne, la Vire l'Aulne, l 'Elorn, l'Allier, la Garonne, la Dordogne, l'Adour et la Nivelle. A partir de la fin des années 1980, les repeuplements utilisant des juvéniles issus de géniteurs français ont été privilégiés. L'Aulne, l'Elorn, l'Allier, et l'Adour ont été repeuplées avec des juvéniles issus de géniteurs locaux. Certaines rivières de Normandie, principalement le Couesnon, la Sélune, la Sée, la Sienne, et l'Orne, ont été repeuplées avec des juvéniles issus de géniteurs provenant de l'Aulne et de l'Adour. La Garonne et la Dordogne ont notamment été repeuplées avec des juvéniles issus de géniteurs de l'Adour et de l'Allier. Actuellement, l'Aulne, l'Elorn, l'Allier, la Garonne, la Dordogne, et l'Adour bénéficient de repeuplements à partir de poissons natifs de ces rivières. Le Couesnon est la seule rivière bénéficiant encore de déversements de juvéniles issus de géniteurs non-natifs, issus de l'Aulne. En règle générale, le succès de ces opérations était limité au regard du faible taux de retour au stade adulte (Baglinière & Dumas 1988; Martinez *et al.* 2001).

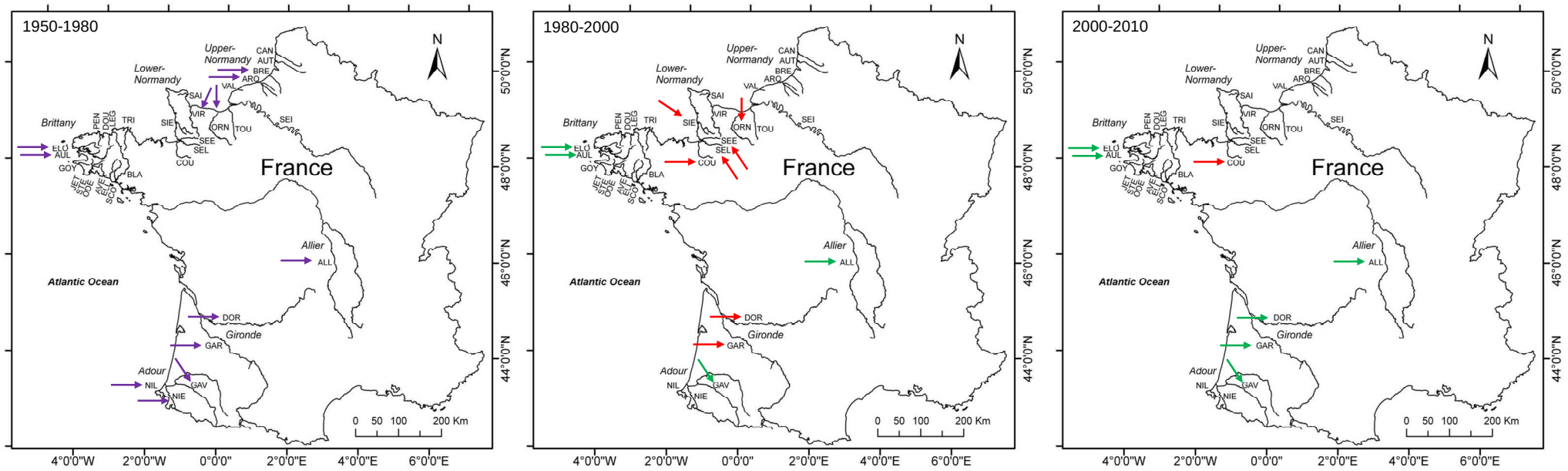

Figure 9 : Repeuplements effectués sur les rivières françaises de 1950 à 1980, de 1980 à 2000, et dans les années 2000. Les flèches indiquent les rivières repeuplées et les couleurs violettes, rouges, et vertes correspondent respectivement à des repeuplements utilisant des juvéniles issus de géniteurs « étrangers », français non-natif de la rivière, et français natif de la rivière.

Peu d'exemples de recolonisation de rivières où le saumon Atlantique avait totalement disparu existent (cas de la rivière Ouelle au Québec (Vion, 2005)). Pourtant l'espèce a une certaine capacité de dispersion autour de sa rivière d'origine et même sur de plus longues distances lors de sa migration de reproduction (Quinn 1993; Jonsson *et al.* 2003), ce qui rend possible la recolonisation de systèmes dépeuplés (Vasemagi *et al.* 2001; Perrier *et al.* 2010).

Génétique des populations de saumon atlantique

La variabilité génétique d'une espèce se répartit au sein des individus, d'une population et entre plusieurs populations. Cette variabilité est influencée par la mutation, la dérive, la migration et la sélection (en lien avec la fitness[3] des individus). Dès lors, l'analyse de la variabilité génétique au sein d'une espèce permet d'étudier le fonctionnement des populations de cette espèce et d'évaluer son potentiel évolutif.

Au fur et à mesure des avancées scientifiques et méthodologiques, la structuration génétique des populations de saumon Atlantique s'est révélée être organisée de façon hiérarchique depuis des patrons de différenciation importants à larges échelles (continents) jusqu'à des différences plus fines entre des sous-populations au sein d'une même rivière.

Méthodes de caractérisation de la structure génétique

De nombreux marqueurs génétiques[4] ont été développés relativement récemment pour l'étude de la diversité génétique neutre au sein et entre les populations.

Les allozymes ont été les premiers marqueurs à être largement utilisés pour établir la structure génétique inter ou intra populations. Les allèles enzymatiques différent par leur taille et leur charge électrique et sont révélés par électrophorèse (Verspoor et al. 2005).

Les RFLPS (Restriction Fragment Length Polymorphisms) sont des séquences d'ADN (Acide Désoxyribonucléique) générés par PCR[5] (Polymerase Chain Reaction) et fragmentées par l'action d'enzymes de restriction coupant l'ADN à des sites spécifiques. Les séquences

[3] Fitness : capacité qu'a l'individu à transmettre ses gènes.
[4] Marqueur génétique : séquence polymorphe (présentant des variations selon les individus) d'ADN aisément détectable.
[5] PCR : Amplification en Chaîne par Polymérase. Permet d'obtenir, à partir d'un échantillon complexe et peu abondant, d'importantes quantités (million de copies en quelques heures) d'un fragment d'ADN spécifique.

créées peuvent être de tailles différentes suite à des insertions, substitutions ou réarrangements de l'ADN. La puissance des RFLPs quand utilisés à une petite échelle géographique est relativement faible. Cependant, l'utilisation de RFLP d'ADN mitochondrial (mtDNA) est très utile pour les études de phylogéographie et permet d'étudier l'histoire des populations sur un temps relativement long et sur une large échelle géographique (Nielsen *et al.* 1996; Nilsson 1997; Finnengan 2009).

Les minisatellites et microsatellites consistent en des répétitions en tandem, respectivement de 10 à 100, et 2 à 6 paires de bases (pb). Ces séquences montrent un important polymorphisme et peuvent être utilisées pour étudier la diversité génétique au sein et entre des populations qui présenteraient des polymorphismes plus faibles en utilisant des allozymes ou des RFLP. Les microsatellites, beaucoup plus petits que les minisatellites, sont d'une amplification plus facile par PCR et ont des taux de mutation plus importants que les autres marqueurs utilisés, de 10^{-2} to 10^{-6} par locus et par génération (Ellegren 2000; Chistiakov *et al.* 2006). Le nombre important d'allèles présent par locus microsatellite permet de nombreuses applications en biologie et a fait que ce type de marqueur est devenu le plus utilisé. Chez le saumon Atlantique, ces marqueurs ont été notamment utilisés pour l'étude de la différentiation génétique entre des populations (King *et al.* 2001; Dionne *et al.* 2008; Grandjean *et al.* 2009; Tonteri *et al.* 2009), des assignations de migrants (Vasemagi *et al.* 2001; Glover *et al.* 2009; Perrier *et al.* 2010), et des analyses de parenté (Letcher & King 2001; Saura *et al.* 2008).

Structure génétique interspécifique

Le saumon Atlantique (*Salmo salar* L.) est un poisson téléostéen de la famille des *Salmonidae*. La famille des Salmonidae comprend trois sous-familles : les *Coregoninae*, les *Thymallinae* et les *Salmoninae* (Allendorf & G.H. 1984; Crespi & Fulton 2004). La sous-famille des Salmoninae comporte 5 genres et 30 espèces, dont *Salmo salar* (Fig. 10).

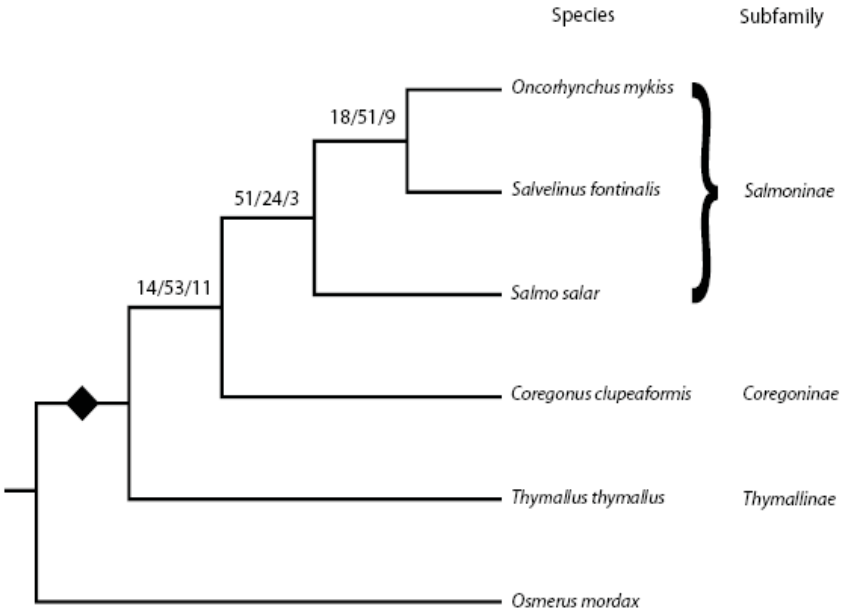

Figure 10 : Phylogénie des salmonidae majeurs (Koop et al. 2008)

Structure génétique intraspécifique

Les populations sauvages de saumon Atlantique sont fortement structurées (Verspoor 1997). D'importants taux de différenciation ont été observés entre différentes régions (King *et al.* 2001; Nilsson *et al.* 2001; Tonteri *et al.* 2009), rivières (Dionne et al. 2008), et même entre différentes localités au sein d'un bassin versant (Primmer *et al.* 2006; Vaha *et al.* 2006; Dillane *et al.* 2008).

Les populations de saumons sont structurés en quatre groupes génétiques principaux : Europe, Amérique du Nord, Atlantique Nord et Mer Baltique (Ståhl 1987; Guyomard 1994). Il existe d'importantes différences génétiques entre le Saumon atlantique d'Europe (est) et celui d'Amérique du Nord (Ouest) (Fig 11). Le caryotype des saumons de l'Atlantique Est présente 29 chromosomes avec 58 paires et 74 bras

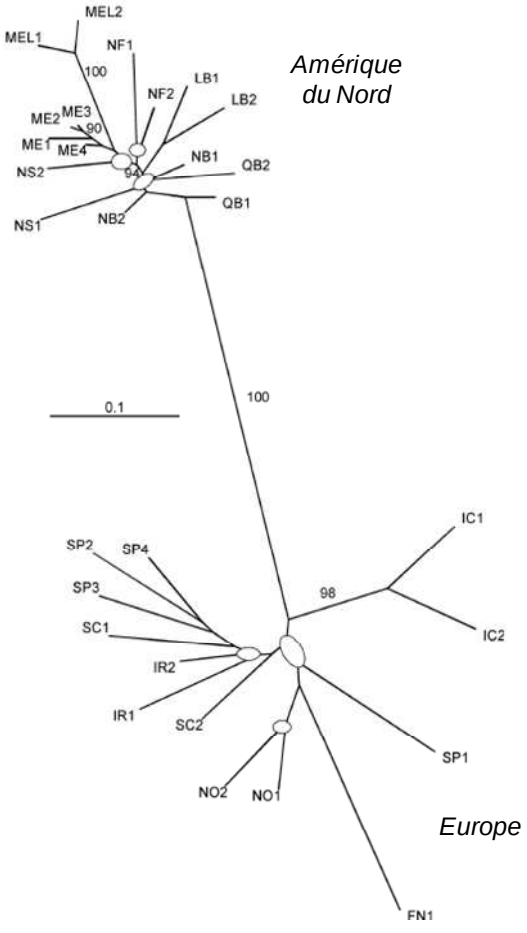

Figure 11 : Arbre phylogénétique les populations Européennes et d'Amérique du Nord. Neigbor joining sur distance de Nei (Da) basé sur 12 microsatellites, modifié d'après (King et al. 2001)).

chromosomiques alors que ceux de l'Atlantique Ouest montre 27 chromosomes avec 54 paires et 72 bras chromosomique (Hartley & Horne 1984; Hartley 1987; Phillips & Rab 2001) (Fig. 12). (Nilsson *et al.* 2001) ont estimé que ces deux principaux groupes ont divergé il y a plus d'un million d'années. La divergence entre les lignées européennes auraient eu lieu beaucoup plus récemment, il y a 22,000 ans (Verspoor et al. 1999).

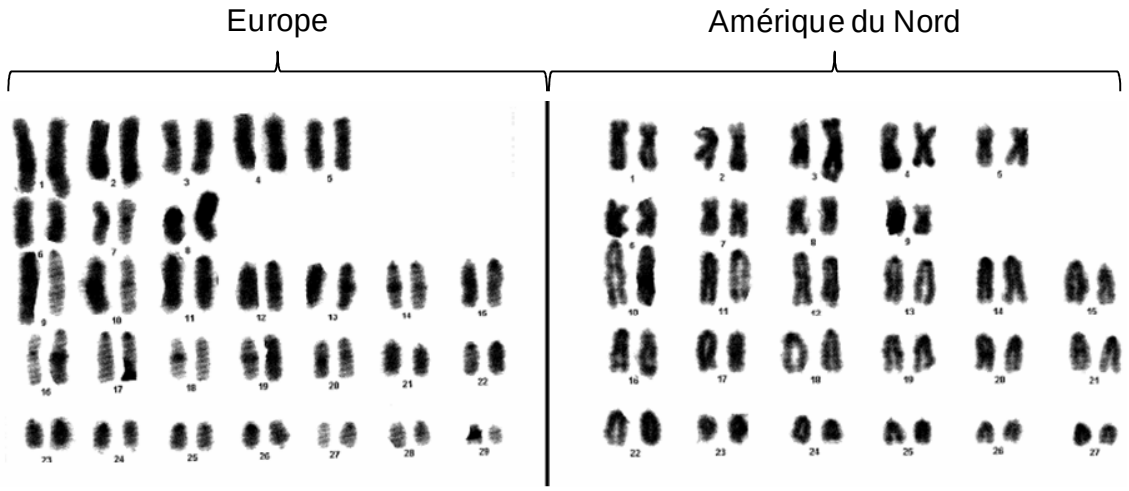

Figure 12 : Photos de caryotypes du saumon Atlantique Est et Ouest (modifié d'après (Hartley 1987)).

D'importants taux de différenciation ont pu être détectés entre les populations d'une même région géographique à l'aide des allozymes (Verspoor et al. 2005), de l'ADN mitochondrial (Verspoor *et al.* 1999; Campos *et al.* 2008; Finnengan 2009), ou encore des microsatellites (King *et al.* 2001; Dionne *et al.* 2008; Finnengan 2009; Tonteri *et al.* 2009). Ces variations ont été observées au sein des quatre principaux groupes : en Amérique du Nord (Verspoor *et al.* 2005; Dionne *et al.* 2008), en Atlantique Nord (Tonteri et al. 2009), en mer Baltique (Saisa et al. 2005), et en Europe (King *et al.* 2001; Verspoor *et al.* 2005; Finnengan 2009). Mais des différences génétiques peuvent exister également entre des populations vivant dans des rivières voisines à l'intérieur d'un groupe (Dionne *et al.* 2008; Grandjean *et al.* 2009; Tonteri *et al.* 2009). Elles sont attribuables à un homing strict (Quinn 1993; Jonsson *et al.* 2003) et à un fort potentiel d'adaptation locale[6] des individus, entraînant chez les dispersants un faible succès reproducteur (Garcia de Leaniz *et al.* 2007; Dionne *et al.* 2008).

Même si une population est souvent associée à une rivière, de récentes études ont également montré l'existence de structuration au sein de larges rivières (Beacham & Dempson 1998; Garant *et al.* 2000; Primmer *et al.* 2006; Vaha *et al.* 2007; Dillane *et al.* 2008; Dionne *et al.* 2009). Certaines de ces études suggèrent que cette structuration est due à

[6] Adaptation locale : changements dans la fréquence des gènes et des phénotypes qui en résultent, en réponse à des pressions sélectives associées à l'environnement local.

l'isolement par la distance au sein du cours d'eau et un homing à fine échelle géographique (Primmer et al. 2006). D'autres études montrent que la structure d'âge (Vaha et al. 2007) et l'hétérogénéité de l'habitat (Dillane et al. 2008) semblent expliquer davantage la structuration observée.

Structure génétique des populations de saumon en France

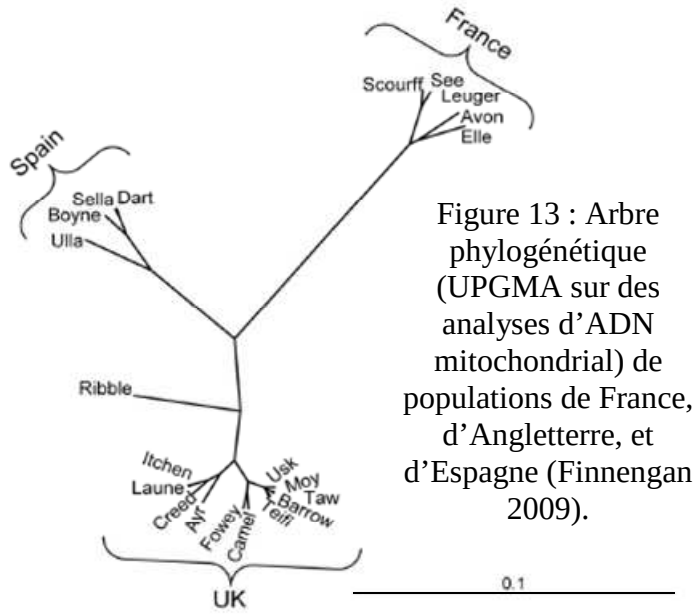

Figure 13 : Arbre phylogénétique (UPGMA sur des analyses d'ADN mitochondrial) de populations de France, d'Angletterre, et d'Espagne (Finnengan 2009).

Jusqu'à très récemment, la structuration génétique des populations de saumon en France a fait l'objet de peu d'études (Guyomard 1987; Martinez *et al.* 2001; Ayllon *et al.* 2006) et un nombre très restreint de populations a été étudié (Nivelle, Elorn, et Allier). Trois études récentes (2009) ont inclus des échantillons issus de rivières du Nord et de l'Ouest de la France. (Finnengan 2009) a montré des différences importantes entre des populations françaises (Sée, Leguer, Aven, Ellé, et Scorff), anglaises et espagnols en étudiant à la fois le polymorphisme d'ADN mitochondrial (Fig. 13) et d'ADN microsatellite (Finnengan 2009). De même, (Grandjean *et al.* 2009) ont montré une différenciation entre des populations à l'échelle régionale (Normandie : Depuis la Bresle jusqu'au Couesnon). Ces auteurs ont également suggéré des phénomènes de recolonisation et des impacts variables d'opérations de repeuplements. Enfin, Nikolic et al. (2009) ont étudié les tailles efficaces[7] de quatre populations de France (Sélune et Scorff) et d'Ecosse présentant des niveaux d'abondance très différents.

[7] Taille efficace (*Ne*) : concept englobant la capacité d'une population à transmettre sa variabilité génétique d'une génération à l'autre. Il est possible d'approcher la taille d'une population à partir de sa taille efficace.

Influence des facteurs environnementaux sur la structure génétique des populations de saumons

Une des questions principales en biologie évolutive est de comprendre comment l'environnement, notamment la géographie, influence la structure génétique des populations (Wright 1943). D'une part, la structure du paysage, notamment la connectivité des habitats, influence les processus de dispersion entre les populations inféodées à ces différents habitats et par conséquent la structure génétique neutre de ces populations. D'autre part, l'hétérogénéité des habitats, d'un point de vue abiotiques ou biotiques, peut générer différents régimes de sélection et influencer la structure génétique des populations.

L'influence de divers facteurs environnementaux sur la structure génétique neutre des populations de saumon a récemment été étudiée. La distance géographique entre les populations, généralement mesurées comme la longueur de la côte entre les estuaires des rivières, apparaît comme étant une variable importante pour expliquer la différenciation des populations (Fig. 14a) (Verspoor *et al.* 2005; Dionne *et al.* 2008; Grandjean *et al.* 2009; Tonteri *et al.* 2009). Certains auteurs ont également observé un phénomène d'isolement par la distance à l'intérieur d'une même rivière entre les différentes populations ou sous-populations étudiées, ainsi qu'une correlation entre la diversité génétique d'une sous-population et l'accessibilité de la zone à laquelle elle est inféodée (Fig. 14 b) (Primmer *et al.* 2006; Vaha *et al.* 2007; Gomez-Uchida *et al.* 2009).

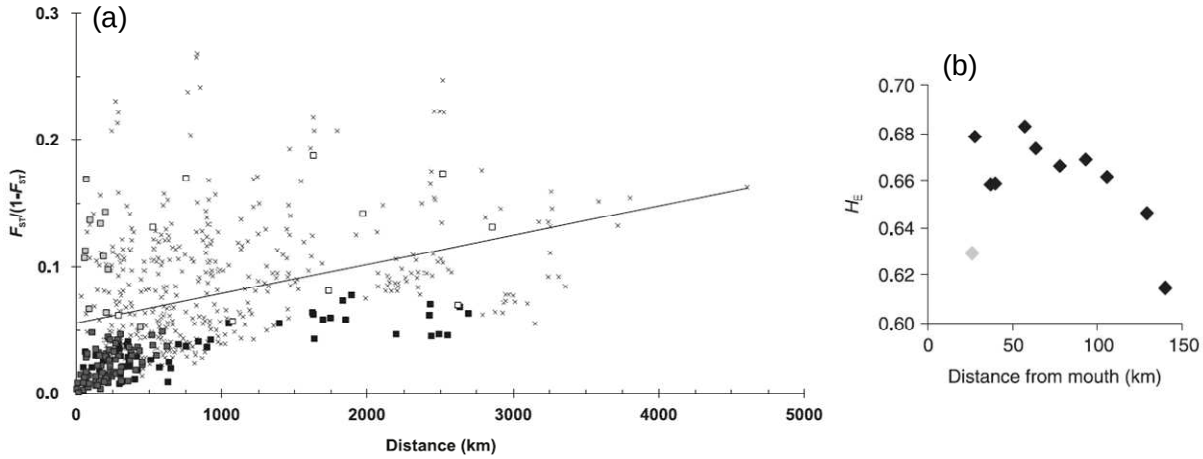

Figure 14 : (a) Relation entre distance genetique (Fst/(1-Fst)) et géographique pour des populations d'Europe du nord (Tonteri et al. 2009). (b) Relation entre diversité génétique (He) et distance à l'estuaire pour des populations de la rivière Varzuga (Primmer et al. 2006).

La différenciation des populations de saumon peut être corrélée à d'autres variables environnementales comme la température (Dionne et al. 2008) et l'oscillation Nord-Atlantique (Valiente *et al.* 2010).

Impacts des repeuplements sur la structure génétique des populations de saumons

Les repeuplements sont généralement pratiqués dans le but d'augmenter les effectifs et donc la taille d'une population. Cependant ces pratiques de gestion peuvent modifier les caractéristiques biologiques des populations, notamment leur structure génétique. Ces modifications du niveau de diversité génétique sont variables et vont dépendre notamment de l'origine des individus déversés : en particulier si ils sont issus de géniteurs locaux ou non, et en fonction du degré de domestication[8] des individus.

Figure 15 : Introgression chez Salmo trutta après repeuplement non-natif, modifié d'après (Hansen et al. 2009).

Les repeuplements utilisant des individus non-natifs, génétiquement différenciés, peuvent entrainer de fortes introgressions génétiques des populations voir même un remplacement du pool de gènes natifs (Hansen 2002; Campos *et al.* 2008; Finnegan & Stevens 2008; Sonstebo *et al.* 2008; Hansen *et al.* 2009) (Fig. 15). Ces pratiques peuvent également conduire à une homogénéisation entre les populations (Ayllon *et al.* 2006; Marie *et al.* 2010) ou sous-populations (Finnengan & Stevens 2008; Eldridge *et al.* 2009) (Fig. 16). Le phénomène inverse peut s'observer mais cette augmentation de la structure entre les populations reste artificielle (Campos *et al.* 2008). Etant donné l'adaptation locale probable des populations de saumon Atlantique (Garcia de Leaniz *et al.* 2007; Dionne *et al.* 2008; Fraser *et al.* 2008), toutes ces modifications de structure génétique peuvent s'accompagner d'une réduction de fitness et d'une perte de cette adaptation (Taylor 1991; Araki *et al.* 2007; Garcia de Leaniz *et al.* 2007; Fraser *et al.* 2008).

Les repeuplements utilisant des juvéniles issus de souches natives présentent l'avantage de minimiser les risques de modification de la structure génétique et de diminution d'adaptation locale de la population. Cependant, ces repeuplements peuvent également modifier la structure existante notamment en réduisant la structure au sein des populations repeuplées (Eldridge *et al.* 2009). De plus, l'adaptation locale peut diminuer suite aux pressions de sélection s'exerçant en milieu d'élevage et qui différent de celles survenant en milieu naturel (Fleming & Einum 1997; Blanchet *et al.* 2008; Ford *et al.* 2008; Neregard *et al.*

[8] Domestication : acquisition, perte ou développement par une espèce animale ou végétale, de caractères héréditaires morphologiques, physiologiques et/ou comportementaux résultant d'une interaction prolongée, d'un contrôle, et/ou d'une sélection délibérée par l'homme.

2008; Darwish & Hutchings 2009; Lawlor *et al.* 2009).

Les individus déversés pourraient également affecter la structure génétique des populations voisines en raison d'un taux de dispersion plus élevé (Quinn 1993; Jonsson *et al.* 2003).

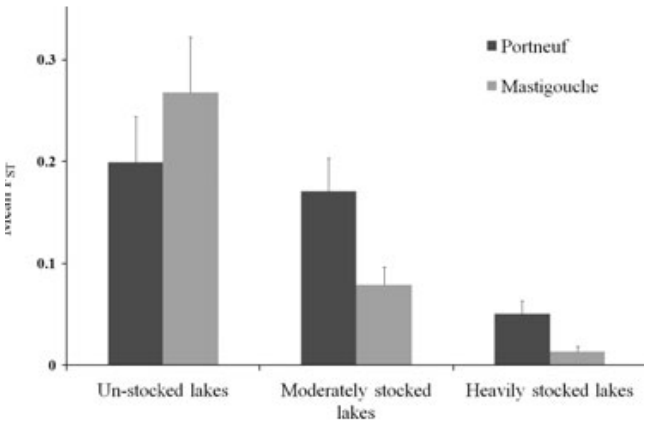

Figure 16 : Differenciation génétique entre des populations de Salvelinus fontinalis du Québec, pour trois intensités de repeuplement non-natif (Marie et al. 2010).

Certaines études ont porté sur les effets des repeuplements sur la structure génétique de certaines populations françaises. La Nivelle (Martinez *et al.* 2001; Moran *et al.* 2002), la Nive (Campos *et al.* 2008), le Couesnon, la Sélune, et l'Orne (Grandjean *et al.* 2009) ont notamment été étudiées. Ces travaux suggèrent de sensibles modifications génétiques dans les populations de la Nivelle et de la Nive bien que les taux de retour des individus deversés soient relativement faibles. Les analyses des populations du Couesnon, de la Sélune et de l'Orne suggéraient une introgression variable et relativement importante par les souches de l'Adour et de l'Aulne utilisées comme sources de déversements importants et réguliers.

Utilisation de la microchimie pour inférer l'histoire de vie des saumons

Une meilleure compréhension des processus de fonctionnement des populations de salmonidés nécessitent la connaissance des tactiques d'histoire de vie des individus, notamment les tactiques migratoires. Des méthodes de piégeage et de marquage individuel sont couramment réalisées pour estimer le nombre de juvéniles migrants, la phénologie des migrations, et la contribution de types migratoires en lacs, rivières ou océans (Kwain 1981) (Seelbach 1993). Cependant, ces méthodes traditionnelles restent fastidieuse et chères, notamment en raison de la forte mortalité naturelle des juvéniles engendrant la perte de nombreuses marques. Or les poissons produisent eux-mêmes des marques naturelles intégrées dans la structure des otolithes.

Analyses microchimique des otolithes

L'analyse de la microchimie des otolithes est en pleine expansion depuis quelques années, notamment grâce à l'émergence de techniques analytiques extrêmement sensibles permettant de travailler sur les éléments traces. Les otolithes sont des concrétions minérales localisées dans l'oreille interne des poissons téléostéens. Ils sont composés principalement de carbonate de calcium sous forme d'aragonite qui est déposée en couches journalières sur une matrice organique. De part leur croissance continue, ils fournissent un enregistrement de l'histoire du poisson et de son environnement. En effet, les éléments présents dans le milieu passent dans l'endolymphe et sont incorporés à la surface des otolithes au fur et à mesure de leur croissance (Campana 1999; Campana & Thorrold 2001). De plus, les couches successives ainsi constituées de l'otolithe conservent leurs caractéristiques tout au long de la vie du poisson (Campana & Thorrold 2001), excepté en cas de stress extrêmes (Mugiya & Uchimura 1989). Ainsi, les changements de milieu ou d'habitat du poisson et les variations de leur composition chimique sont visibles sur les otolithes *via* des modifications de leur composition chimique (Kalish 1991).

L'étude de la composition chimique des otolithes a plusieurs applications dans les sciences halieutiques. Elle permet en effet de valider l'âge de l'individu, de détecter des pollutions (Hanson & Zdanowicz 1999) (Saquet et al. 2002), de discriminer des stocks ou des structures de population (Thorrold *et al.* 1998; Campana *et al.* 2000; Gillanders & Kingsford 2000; Gillanders 2002). Mais cette étude de la composition chimique permet également d'identifier les divers milieux traversés par le poisson et de mettre en évidence d'éventuelles migrations ou changement d'habitat (Rieman *et al.* 1994; Secor *et al.* 2001; Elsdon *et al.* 2008). Ces différentes applications reposent sur l'hypothèse de stabilité temporelle des compositions chimiques des différentes eaux et sur l'existence de différences spatiales de ces compositions. On parle ainsi de signature chimique d'une eau ou d'un milieu. Cette signature est influencée par la composition des substrats géologiques traversés par l'eau (en rivière) (Radtke & Shafer 1992) et par d'éventuelles pollutions résultants d'activités humaines (Hanson & Zdanowicz 1999). Elle est établie à l'aide de l'analyse des rapports de concentrations en calcium et autres éléments substitutifs au calcium dont une trentaine ont été détectés dans les otolithes (Campana 1999).

Ces éléments sont présents en quantité très faible. Leur analyse nécessite donc de travailler à l'échelle de la partie par billion. De plus, pour profiter au maximum de l'information temporelle donnée par les stries de l'otolithe, il convient de réaliser ces analyses

sur des échantillons extraits de l'otolithe avec une précision de l'ordre de la dizaine de µm. Ce type de résolution peut être atteint grâce notamment grâce à la technique *laser ablation inductively coupled plasma-mass spectrometer* (LA-ICP-MS) (Gemperline *et al.* 2002; Brophy *et al.* 2003; Barnett-Johnson *et al.* 2005; Arai & Hirata 2006). Cette méthode offre le double avantage d'une grande précision de dosage et d'une analyse microstructurale de l'otolithe. La technique LA-ICP-MS peut ainsi permettre de lever de nombreux verrous de connaissances dans un cadre aussi complexe que celui lié à l'étude des migrations chez les poissons.

Etude de la dispersion des poissons grâce à l'utilisation de la microchimie

Certains travaux en LA-ICP-MS sur Zn et Sr ont permis d'établir l'existence d'une bonne corrélation entre la composition de l'eau et la composition de l'otolithe (Arai *et al.* 2007). Ainsi, s'il existe des différences de composition chimique entre des milieux, ces différences peuvent être retrouvées entre ou au sein des otolithes. La microchimie des otolithes peut alors permettre d'étudier les mouvements des individus entre des milieux aux caractéristiques chimiques différentes (Elsdon & Gillanders 2004; Halden & Friedrich 2008). La microchimie des otolithes a également été utilisée avec succès pour discriminer à fine échelle spatiale des stocks naturels de poissons inféodés à différents milieux de vie aux caractéristiques chimiques distinctes (Thorrold *et al.* 2001; Tomas *et al.* 2005; Veinott & Porter 2005). Cette technique est également applicable pour discriminer des stocks de pisciculture (Gibson-Reinemer *et al.* 2009).

Couplage microchimie - génétique

La microchimie des otolithes qui renseigne sur les milieux traversés par les individus a été couplée à la génétique qui renseigne sur la généalogie des individus. La combinaison de ces deux méthodes peut s'avérer intéressante pour étudier l'histoire de vie des individus. Récemment, des études couplant ces deux méthodes ont été réalisées pour analyser la dispersion de poissons diadromes, la microchimie renseignant alors sur la possible dispersion par le milieu marin et la génétique sur la population source (Bradbury *et al.* 2008; Ohara *et al.* 2009). Ce type de couplage offre des opportunités nouvelles car il permet d'étudier l'origine génétique des individus mais également géographique et/ou leur dispersion et migration à

travers différents milieux. Nous verrons plus loin comment ce type de couplage peut-être utilisé pour étudier des populations faisant l'objet de repeuplements.

Objectifs de la thèse

L'objectif général de cette thèse est d'étudier les caractéristiques génétiques des populations françaises de saumon Atlantique en répondant à différents questionnements :

1- Quel est le niveau de structuration génétique des populations inféodées aux rivières françaises et comment varie la diversité au sein de ces populations ?

2- Comment a évolué cette structure génétique au cours des dernières décennies ?

3- Quel sont les influences de certains facteurs environnementaux (distance géographique entre les rivières et longueur des rivièeres) et de certaines activités humaines (repeuplement et amélioration de l'habitat) sur cette structuration et son évolution temporelle ?

Après une description des méthodes utilisées (chapitre II), les résultats sont présentés en cinq chapitres, correspondant chacun à un article scientifique rédigé en anglais :

- Chapitre III. Dans ce chapitre la structuration génétique des populations françaises de saumon Atlantique est déterminée à l'aide de marqueurs microsatellites et d'un échantillonnage des stocks le plus exhaustif possible du territoire français. Puis, l'influence de facteurs environnementaux tels que la distance côtière entre les estuaires, la longueur des rivières et l'impact des repeuplements récents sur cette structure génétique est analysée. Cet article sera soumis à *Molecular Ecology*.

- Chapitre IV. Dans ce chapitre l'évolution récente de la diversité génétique entre et au sein de ces populations est analysée en comparant des échantillons récents (1998-2006) et anciens (1965-1985) à l'échelle nationale. L'impact des repeuplements anciens et récents sur cette évolution est évalué. Cet article sera soumis à *Conservation Genetics*.

- Chapitre V. Dans ce chapitre la survie et la dispersion des individus repeuplés et leur impact sur la structure génétique des populations de saumon sont estimés à l'aide d'une approche comparative entre données réelles anciennes et récentes, et données simulées. Cette étude est réalisée sur les populations de la baie du Mont-Saint-Michel où sont présentes quatre

populations de saumon présentant des niveaux d'abondance et des intensités de repeuplement différents. Cet article sera soumis à *Evolutionary Applications*.

- Chapitre VI. Dans ce chapitre la signature microchimique des otolithes (analyse LA-ICP-MS) est utilisée pour discriminer les deux origines possibles de saumons ayant les caractéristiques génétiques d'individus issus de repeuplement : né en élevage et déversé, ou issus de la reproduction *in natura* d'individus précédemment déversés. Cet article est accepté avec modifications dans *Canadian Journal of Fisheries and Aquatic Sciences*.

- Chapitre VII. Dans ce chapitre l'origine de saumons recolonisant naturellement le fleuve Seine est identifiée en utilisant les résultats issus de l'analyse génétique de la structure nationale des populations. Cet article est paru dans *Canadian Journal of Fisheries and Aquatic Sciences*.

Le chapitre VIII correspond à une discussion générale qui synthétise et discute l'ensemble des résultats obtenus.

Sont présentés en annexe deux articles. Le premier traite de la mise en évidence d'un marqueur microsatellite permettant de distinguer saumon Atlantique, truite fario, et leurs hybrides ; cet article est sous presse dans *Conservation Genetics Ressources*. Le second article est en préparation et traite de la structure génétique des populations de saumon du bassin de l'Adour. Cet article approfondit l'étude de la structure génétique des populations françaises en montrant qu'il existe une différenciation génétique à une plus fine échelle géographique, au sein de la rivière Adour.

Chapitre II. Méthodes

Populations étudiées

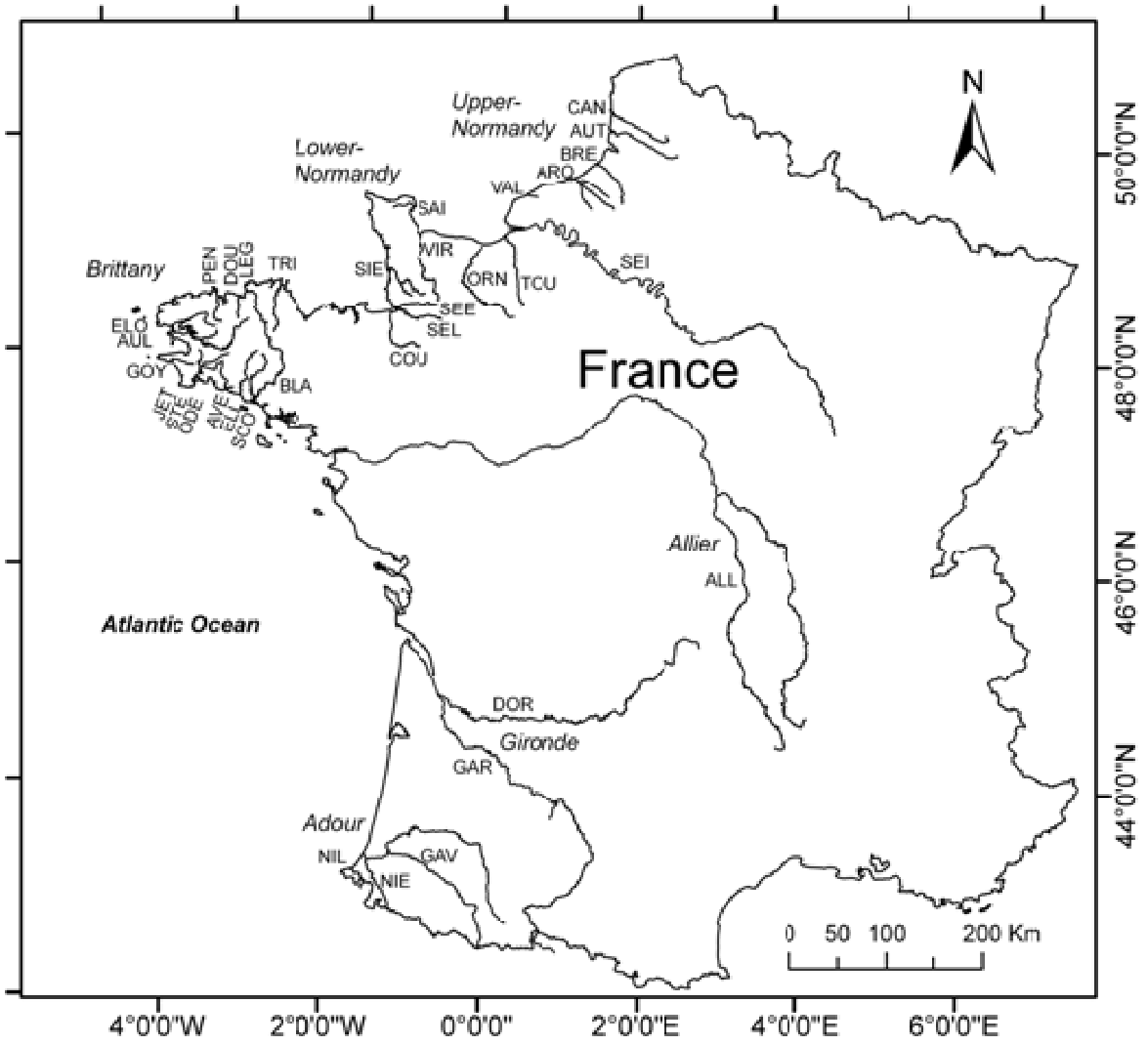

Figure 17 : Carte des populations de saumon Atlantique étudiées.

Nous avons étudiés 34 populations françaises de saumon Atlantique (Fig. 17, tableau 2) représentant les principaux stocks de cette espèce (Baglinière & Porcher 1994). Ces populations sont réparties sur 1,662 km de côtes. Les distances géographiques entre les estuaires des rivières ont étés mesurées en utilisant Google Earth (http://earth.google.com) et étaient comprises entre 0 (estuaire commun) to 1,662 km avec une valeur médiane de 741 km. Les longueurs des rivières, de la source à l'estuaire, ont été calculées en utilisant ESRI® ArcGIS 9.2 et étaient comprises entre 14 et 1,013 km avec une valeur médiane de 78 km. Les pourcentages de poissons de plusieurs hivers de mer étaient renseignés pour chaque région étudiée suite à la « lecture » des écailles de chaque individu (INRA, ONEMA and Fédération

Nationale pour la Pêche en France – FNPF - unpublished data). Pour certaines études nous avons également utilisé 23 échantillons provenant de diverses rivières écossaises.

Tableau 2 : Caractéristiques des rivières étudiées.

Rivière	Longitude	Latitude	Distance de la Canche (km)	Longueur de la rivière (km)	Pourcentage de poissons de plusieurs hivers de mer	Abréviation
Canche	1°36	50°32	0	88		CAN
Authie	1°34	50°22	19	103	18%	AUT
Bresle	1°22	50°03	57	72		BRE
Arques	1°05	49°56	82	67		ARQ
Valmont	0°22	49°45	138	14		VAL
Seine	0°07	49°26	185	777		SEI
Touques	0°04	49°21	193	108		TOU
Orne	0°14	49°17	218	170		ORN
Vire	1°07	49°21	284	128		VIR
Saire	1°14	49°36	315	31	13%	SAI
Sienne	1°34	49°00	465	93		SIE
Sée	1°29	48°39	505	78		SEE
Sélune	1°29	48°39	505	91		SEL
Couesnon	1°30	48°37	508	101		COU
Trieux	3°04	48°49	649	72		TRI
Leguer	3°33	48°43	716	60		LEG
Douron	3°38	48°40	726	27		DOU
Penzé	3°56	48°40	755	30		PEN
Elorn	4°21	48°24	881	57		ELO
Aulne	4°15	48°17	903	140		AUL
Goyen	4°32	48°00	966	36	17%	GOY
Steir	4°06	47°52	1018	62		STE
Jet	4°06	47°52	1018	62		JET
Odet	4°06	47°52	1018	62		ODE
Aven	3°44	47°48	1052	37		AVE
Ellé	3°32	47°46	1069	76		ELL
Scorff	3°22	47°42	1087	78		SCO
Blavet	3°22	47°42	1087	149		BLA
Allier	2°10	47°16	1197	1013	95%	ALL
Dordogne	1°06	45°34	1412	483	?	DOR
Garonne	1°06	45°34	1412	647		GAR
Gave	1°31	43°31	1642	309	19%	GAV
Nive	1°31	43°31	1642	80		NIE
Nivelle	1°40	43°23	1662	45		NIL

Analyses génétiques

Echantillons

Les échantillons utilisés étaient généralement des écailles prélevées sur des individus adultes pêchés à la ligne dans le cadre des activités de pêche amateur. Les échantillons provenant de l'Allier, la Dordogne et la Garonne proviennent de piégeages. Des juvéniles ont également étés utilisés dans le cadre de l'étude microchimique et ont étés prélevés par pêche électrique.

Les écailles étaient conservées dans de l'éthanol à 95% ou dans des enveloppe en papier. Nous avons focalisé notre échantillonnage sur les cohortes 1998-2006 pour les échantillons récents et sur les cohortes 1965 à 1988 pour les anciens (Tableau 3). Au total, 1739 individus adultes ont étés génotypés à 17 marqueurs microsatellites. 99 individus supplémentaires ont également été génotypés à 6 de ces 17 marqueurs microsatellites et utilisés dans le chapitre VI.

Tableau 3 : Détail de l'ensemble des échantillons analysés dans les chapitres I, II, et III.

Rivière	Echantillons anciens			Echantillons récents		
	Abréviation	Cohortes	N individus	Abréviation	Cohortes	N individus
Scotland	FOR80	1980	23	-	-	-
Canche	-	-	-	CAN03	1999-2006	23
Authie	-	-	-	AUT03	2003-2006	8
Bresle	BRE68	1968	19	BRE03	1998-2004	29
Arques	-	-	-	ARQ03	2003	31
Valmont	-	-	-	VAL03	2003-2005	5
Seine	-	-	-	SEI03	1998-2006	7
Touques	-	-	-	TOU03	19982006	12
Orne	-	-	-	ORN03	2001	31
Vire	-	-	-	VIR03	1998-2004	19
Saire	-	-	-	SAI03	2005-2006	9
Sienne	SIE86	1985-1987	40	SIE03	2002-2003	37
Sée	SEE77	1977-1986	97	SEE03	2002-2003	66
Sélune	SEL77	1977-1986	77	SEL03	2002-2003	80
Couesnon	COU82	1978-1986	11	COU03	2002-2003	34
Trieux	TRI77	1968-1981	17	TRI03	2002	16
Leguer	LEG77	1976-1977	22	LEG03	2002-2003	16
Douron	DOU82	1978-1984	29	DOU03	2002-2003	27
Penzé	PEN78	1969-1982	25	PEN03	2002-2003	23
Elorn	ELO75	1969-1970	18	ELO03	2003	30
Aulne	AUL69	1969-1984	30	AUL03	2003	34
Goyen	GOY81	1972-1984	33	GOY03	2003	24
Steir	STE72	1971-1972	21	STE03	2002	14
Jet	JET72	1971-1973	11	JET03	2000-2004	17
Odet	ODE72	1972-1973	19	ODE03	2003	14
Aven	AVE77	1973-1978	40	AVE03	2003	34
Ellé	ELL68	1968-1968	17	ELL03	2003	32
Scorff	SCO77	1966-1985	64	SCO03	2002-2003	64
Blavet	BLA77	1977-1978	65	BLA03	2002-2003	63
Allier	ALL67	1965-1967	49	ALL03	2001-2002	31
Dordogne	-	-	-	DOR03	2002	15
Garonne	-	-	-	GAR03	2002	30
Gave d'Oloron	GAV84	1984-1984	25	GAV03	2003	29
Nive	NIE84	1984-1984	26	NIE03	2001-2006	8
Nivelle	NIL80	1977-1987	26	NIL03	1998-2004	17

Analyses moléculaires

L'extraction d'ADN a été réalisée à partir d'une ou deux écailles par individus. Ces tissus ont été digérés dans une solution de protéinase K, de TE (Tris/EDTA) et de Chelex 5%, chauffée à 55°C pendant 2 heures puis à 100°C pendant 10min (Estoup *et al.* 1996). Après repos d'une nuit à 4°C, celui-ci a été centrifugé à 4000 tours/minutes pendant 5 minutes et le surnageant a été récupéré pour être transféré dans un tube contenant du Chelex 5% et conservée à -20°C.

L'ADN a été amplifié à 17 microsatellites: BHMS176, BHMS179A, BHMS184B, BHMS429, BHMS235, BHMS217, BHMS111, BHMS377, BHMS365 (Hoyheim 2000), SsA85, SsA197, SsA171 (Oreilly *et al.* 1996), SsA65, SsA9, SsA224 (O'Reilly *et al.* 1997), SsOSL85 (Slettan *et al.* 1995) et SsSP2216 (Paterson *et al.* 2004). La méthode M13 (Schuelke 2000) a été utilisée pour marquer l'ADN. Le volume final de 10µL était composé de tampon PCR 1X (GoTaq, Promega), 200µM de dNTP, 1.5mM MgCl2, 0.1µM d'amorce Up allongé (M13_U), 0.15µM d'amorce Down, 0.15µM d'amorce M13 marqué par Fluorescence, 25 à 50ng d'ADN et 0.5U d'ADN Polymérase (GoTaq Promega). La réaction d'amplification PCR a été réalisée dans un thermocycler GeneAmp PCR System 9700 (Applied Biosystems). Les conditions d'amplification étaient les suivantes : 5min de dénaturation initiale à 94°C puis de 42 à 45 cycles avec 30s de dénaturation à 94°C, 30s d'hybridation à la température optimale pour chaque couple d'amorces et 30 s d'élongation à 72°C, enfin 30min de synthèse finale à 72°C. Pour chacune de nos PCR, nous avons introduit des témoins négatifs afin de vérifier l'absence de contamination, et des échantillons de référence afin de valider la reproductibilité du génotypage.

Les fragments d'ADN ont été analysés avec le logiciel Genmapper 3.5 (Applied Biosystems).

Analyses microchimiques

Echantillons

84 otolithes de juvéniles 0+ de la Sienne, la Sée, la Sélune et du Couesnon ont été analysés. Des rempoissonnements à partir de juvéniles de la pisciculture du Favot (basée sur la rivière Aulne, Finistère, Bretagne) ont été effectués dans ces cours d'eau. Six otolithes de juvéniles de cette pisciculture ont donc également été analysés. Enfin, neuf otolithes d'adultes retrouvés morts sur la Sélune après la reproduction ont été analysés. Ces 99 individus ont également été

génotypés à 6 marqueurs microsatellites, SSA65 (O'Reilly et al. 1997); BHMS235; BHMS111; BHMS377 (Hoyheim 2000); SSA197; SSA171 (O'Reilly et al. 1996).

Analyses LAICPMS

L'extraction des otolithes est réalisée avec des outils chimiquement inertes, en polymères, décontaminés à l'acide et l'eau ultrapure. Les otolithes ont été inclus dans une résine Araldite (type 2020), poncés à l'aide de papier abrasif jusqu'à obtenir un plan passant par le nucléus, puis polis à l'aide de poudre de diamant.

Chaque otolithe a ensuite été analysé au laboratoire UT2A (Université de Pau) par LA-ICP-MS, analyse par Spectrométrie de Masse Couplée à un Plasma Inductif avec prélèvement par Ablation Laser. L'ablation laser a consisté en l'application d'impact laser à une cadence de 3kHz sur une couronne de 200µm de large autour d'un cercle de 100µm de diamètre centré sur le nucleus (zone considérée comme représentative du milieu continental). Les éléments suivants ont ensuite étés quantifies par spectrometrie de masse: ^{7}Li, ^{24,25}Mg, ^{27}Al, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66,68}Zn, ^{85}Rb, ^{111,113}Cd, ^{138}Ba, ^{208}Pb ^{23}Na ^{86}Sr and ^{43}Ca, ^{43}Ca.

Chapitre III. Structure génétique des populations françaises de saumon Atlantique

L'objectif de ce chapitre est de déterminer la structure génétique entre les populations françaises de saumon Atlantique à l'aide de marqueurs microsatellites et d'étudier l'influence sur cette structure génétique de facteurs environnementaux tels que la distance côtière entre les estuaires et la longueur des rivières, et l'impact des repeuplements récents.

La structure génétique des populations de saumons est soumise à l'influence de nombreux paramètres intrinsèques à l'espèce, de facteurs environnementaux, et d'activités humaines. Ces différents facteurs peuvent être difficile à distinguer, notamment lorsque les populations sont sujettes à des repeuplements avec des poissons allochtones.

Afin d'étudier l'influence de ces paramètres sur la structure génétique des populations françaises de saumon Atlantique, nous avons génotypé à 17 marqueurs microsatellites 975 poissons issus de 34 rivières et provenant des cohortes 1998 à 2006.

Une analyse bayesienne a permit de classer ces individus en cinq principaux groupes génétiquement et géographiquement distincts. La différenciation entre les populations au sein des groupes était relativement faible en comparaison de la structure entre ces groupes. Ces résultats suggèrent des flux important d'individus et de gènes au sein des régions identifiées et des flux beaucoup plus restreint entre ces régions. La distance côtière entre les estuaires s'est révélé être significativement corrélée à la différenciation génétique entre les populations. La longueur de la rivière s'est également révélée comme étant un bon proxy de la structure génétique observée. Cependant, ce dernier résultat est principalement dû à la population de l'Allier, étant situé sur la rivière avec les sites de frais les plus éloignés de la mer (> 700 km), et étant la plus différentiée génétiquement. Cette population était composée majoritairement de poissons adultes ayant séjourné plusieurs hivers en mer et donc de grande taille et remontant tôt dans l'estuaire. Ces résultats suggèrent une adaptation locale, morphologique et comportementale, à la difficulté à la montaison.

Parmi les populations étudiées, certaines étaient sujettes à des opérations de repeuplement utilisant des poissons autochtones ou allochtones. Alors que les repeuplements avec des poissons locaux ne semblaient pas modifier la diversité génétique au sein et entre les rivières, d'importants taux d'introgression de certaines populations attestent des effets des repeuplements effectués avec des poissons non natifs.

Ces résultats ont d'importantes implications pour la conservation et la gestion des populations de saumon en exposant les principales unités génétiques présentes sur le territoire français, et en montrant les effets des repeuplements sur la diversité génétique au sein et entre les populations. Cette étude suggère également l'adaptation locale des poissons à la longueur de la rivière. D'une façon générale, ces résultats suggèrent l'importance de la conservation à une fine échelle étant donné la potentielle adaptation locale des populations et l'introgression suivant la transplantation des individus.

Determinants of hierarchical genetic structure in Atlantic salmon populations: environmental factors *versus* anthropogenic influences

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Running title: Environmental and anthropogenic influences on Atlantic salmon genetics

Key words: Atlantic salmon, genetic structure, landscape genetics, introgression, local adaptation

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Abstract

The effects of ecological factors on the genetic structure of natural populations can be difficult to disentangle from those linked to human activities. For instance, stocking practices are known to obscure genetic makeup of populations. Here we investigated the influence of landscape features and stocking on the genetic structure of 34 French Atlantic salmon (*Salmo salar*) populations. 975 individuals were genotyped at 17 microsatellite loci. A Bayesian analysis revealed a pattern of hierarchical genetic structure into five geographically distinct groups. Coastal distance among estuaries was a strong predictor of population structure ($r = 0.71$) and the effect of river length was also significant ($r = 0.62$). However the latter was mainly due to one population having both the farthest spawning grounds off the river mouth (> 700 km) and the highest level of differentiation. This population also exhibited very particular morphological (large size) and behavioral traits (run timing), suggesting a role for local adaptation to river length. While stocking with local fish did not strongly impact the distribution of genetic diversity within and among rivers, some populations were highly admixed with populations used for stocking. Overall, these results have important implications for the conservation of salmon populations *via* the delineation of conservation units and the identification of coastal distance and river length as major environmental factors influencing gene flow and potentially local adaptation. This study also illustrates the introgression of natural populations by stocking strains at a large spatial scale.

Introduction

Delineating genetic structure among groups of individuals is a central issue in population genetics (Waples & Gaggiotti 2006). Patterns of population genetic structure are also increasingly considered for the conservation of endangered species (Palsboll *et al.* 2007) (Schwartz *et al.* 2007). The management of threatened species is often based on captive breeding programs that can have major impacts on the genetic structure (Hansen *et al.* 2009) and evolutionary potential of natural populations (Araki *et al.* 2007; Fraser 2008; Williams & Hoffman 2009). As a result, it could be difficult to disentangle the effects of supportive breeding from those of 'natural' environmental factors when studying the distribution of genetic diversity within and among populations. For instance gene flow is frequently reduced when distance increases between populations, but such a pattern of isolation by distance may be disrupted by artificial translocations (Finnegan & Stevens 2008; Marie *et al.* 2010).

A major question in population genetics is to determine how landscape-level geographical and environmental factors are involved in the spatial distribution of genetic variation (Manel *et al.* 2003; Storfer *et al.* 2007). The role of ecological variables on mediating gene flow across populations has been investigated in a wide range of plants (Sork *et al.* 1999; Albaladejo *et al.* 2009; Schmidt *et al.* 2009) and animals (Keyghobadi *et al.* 1999) (Sokal & Thomson 1998) (Castric *et al.* 2001) using a growing variety of dedicated statistical tools (Manel *et al.* 2003; Balkenhol *et al.* 2009; Guillot *et al.* 2009). Landscape genetics studies usually focus on the distribution of neutral genetic variation and attempt to identify landscape features that constrain or facilitate dispersal (McRae 2006). However, landscape heterogeneity can ultimately result in divergent selection among habitats and such local adaptation should be detected by analyzing adaptive genetic variation (Hendry 2004; Nosil *et al.* 2005; Bolnick & Nosil 2007). Indirect evidence for local adaptation can also be uncovered by genome scans based on the genotyping of a large number of putatively neutral molecular markers in populations thought to undergo divergent selection. Loci exhibiting a differentiation higher or lower than a simulated set of neutral values are thought to be under selection (Oleksyk *et al. 2010*; Storz *et al.* 2004). Alternatively, pattern of passive (movement of individuals) *versus* effective dispersal (gene flow) can also provide some evidence for local adaptation. Passive dispersal is expected to be higher than effective dispersal among locally adapted populations (Currat *et al.* 2008; Rasanen & Hendry 2008). Such a selection against immigrants has been suggested in chum salmon and Atlantic salmon (Tallman 1994; Dionne *et al.* 2008).

As mentioned above, major modifications in the spatial distribution of genetic diversity can result from translocations of individuals for management purposes. Consequences of such translocations have been well documented in fish species and are in most cases a decrease of genetic structure between supplemented and donors stocks *via* the genetic introgression of supplemented populations (Finnegan & Stevens 2008; Hansen *et al.* 2009; Marie *et al.* 2010). If populations experience different selection regimes (Dionne *et al.* 2007; Nielsen *et al.* 2009), alterations in adaptive genetic variation may occur in recipient populations. Captive breeding programs where individuals are bred in captivity for one or several generations before being released in their population of origin may also alter the genetic make-up of natural populations (Araki *et al.* 2007; Fraser *et al.* 2008; Darwish & Hutchings 2009).

Atlantic salmon (*Salmo salar*) is a long distance migratory (Hansen *et al.* 1993) and philopatric fish species that returns to spawn in its natal river after a feeding period at sea (Stabell 1984) (Quinn 1993). This homing behavior is not fully strict but often invoked to explain the moderate to high differentiation observed among salmon populations (King *et al.* 2001) (Verspoor *et al.* 2005). Accordingly, coastal distance between rivers is negatively linked to the amount of gene flow among populations, (Dionne *et al.* 2008; Tonteri *et al.* 2009), this pattern of isolation by distance being also observed within rivers (Primmer *et al.* 2006; Vaha *et al.* 2006; Dillane *et al.* 2008). River length, water flow and more generally upstream migration difficulty could also have a constraining effect on gene flow among populations. These environmental factors may also generate local adaptation (Taylor 1991; Garcia de Leaniz *et al.* 2007), potentially reflected by variations in size and age of returning adults (Schaffer & Elson 1975) (Power 1981) (Dionne *et al.* 2008).

In many declining Atlantic salmon populations, conservation programs based on supplementation with non-native fish have been implemented (Hindar *et al.* 1991; Aprahamian *et al.* 2003). As *S. salar* populations are highly structured throughout their native range (Verspoor *et al.* 2005; Lehtonen *et al.* 2009; Tonteri *et al.* 2009), fish stocked from genetically distant populations or farms are traceable and genetic admixture in wild populations can be estimated (Campos *et al.* 2008; Finnegan & Stevens 2008; Hansen *et al.* 2009). These fish translocations can decrease genetic structure (Ayllon *et al.* 2005; Lage & Kornfield 2006), alter local adaptation and reduce the fitness of recipient populations (Hindar *et al.* 1991; McGinnity *et al.* 2003; Araki *et al.* 2007; Ford & Myers 2008). Overall, supplementation operations may have antagonistic or synergistic effects with the processes of

natural dispersal or local adaptation but few studies have investigated such effects at a large spatial scale (Dionne *et al.* 2008).

Here we describe the genetic structure among 34 French Atlantic salmon populations using 975 samples genotyped at 17 microsatellite markers. A first aim of this study was to determine the spatial scale of genetic structure and the potential hierarchical clustering of populations. Second, we investigated the influence of coastal distance between rivers and of river length on gene flow among populations and on potential local adaptation. Third, we took advantage of a detailed knowledge of supplementation operations to assess the consequences of such a management on the spatial distribution of genetic diversity.

Materiel and methods

Study populations and sampling

We studied 34 Atlantic salmon populations (Fig 1, table 1) representing the mains stocks of this species in France (Baglinière & Porcher 1994) along 1,662 km of coastline. Only a few small populations inhabiting small coastal rivers were not sampled. Adult fish were collected by angling or trapping and scales were stored by the INRA laboratory in Rennes and by the *Office National de l'Eau et des Milieux aquatiques* (ONEMA). Scales were kept in 95% ethanol or in paper envelopes. The age of each individual was determined from its scales (Bagliniere *et al.* 1991). We focused on cohorts 2002-2003 and collected between eleven and 80 samples per population except in 5 small populations where only between five and nine samples were available (Table 1). Whenever possible, we added some samples from cohorts 1998 to 2001 and 2004-2006 to increase sample size.

Percentages of multi-sea-winter fish were available for each regional group (INRA, ONEMA and Fédération Nationale pour la Pêche en France – FNPF - unpublished data). Supplementation operations were performed in some rivers using non-native or native fish (INRA & ONEMA, unpublished data; (Baglinière & Dumas 1988; Baglinière *et al.* 1990)). The Orne River was stocked with fish from Gave d'Oloron in 1995. The Sélune was stocked with individuals from Gave d'Oloron in 1995 and from Aulne in 1996 and 1997. The Couesnon was stocked with Gave d'Oloron individuals in 1995 and has been annually stocked with fish from Aulne since 1996. Garonne and Dordogne were stocked with non-native fish from Scotland, Allier and Gave d'Oloron from 1980 and 1977 respectively to 1991

and have been stocked with native individuals since 1992. Elorn, Aulne, Allier, and Gave d'Oloron have been stocked with native fish since 1995. The populations Authie, Canche, Valmont, Touques and Saire were very low at the time of sampling and were considered as extinct or not viable. In the Seine River, although no stocking was performed, more than 162 adult salmon were observed in 2008 by video-counting and this recolonization event involved individuals from multiple origins (Perrier *et al.* 2010).

Geographic coastal distances between estuaries of the study rivers were calculated following coastline using Google Earth (http://earth.google.com) and ranged from 0 to 1,662 km with a median value of 741 km. River lengths were calculated as the distance between the source and the mouths of the river using ESRI® ArcGIS 9.2 and ranged from 14 to 1,013 km with a median value of 78 km.

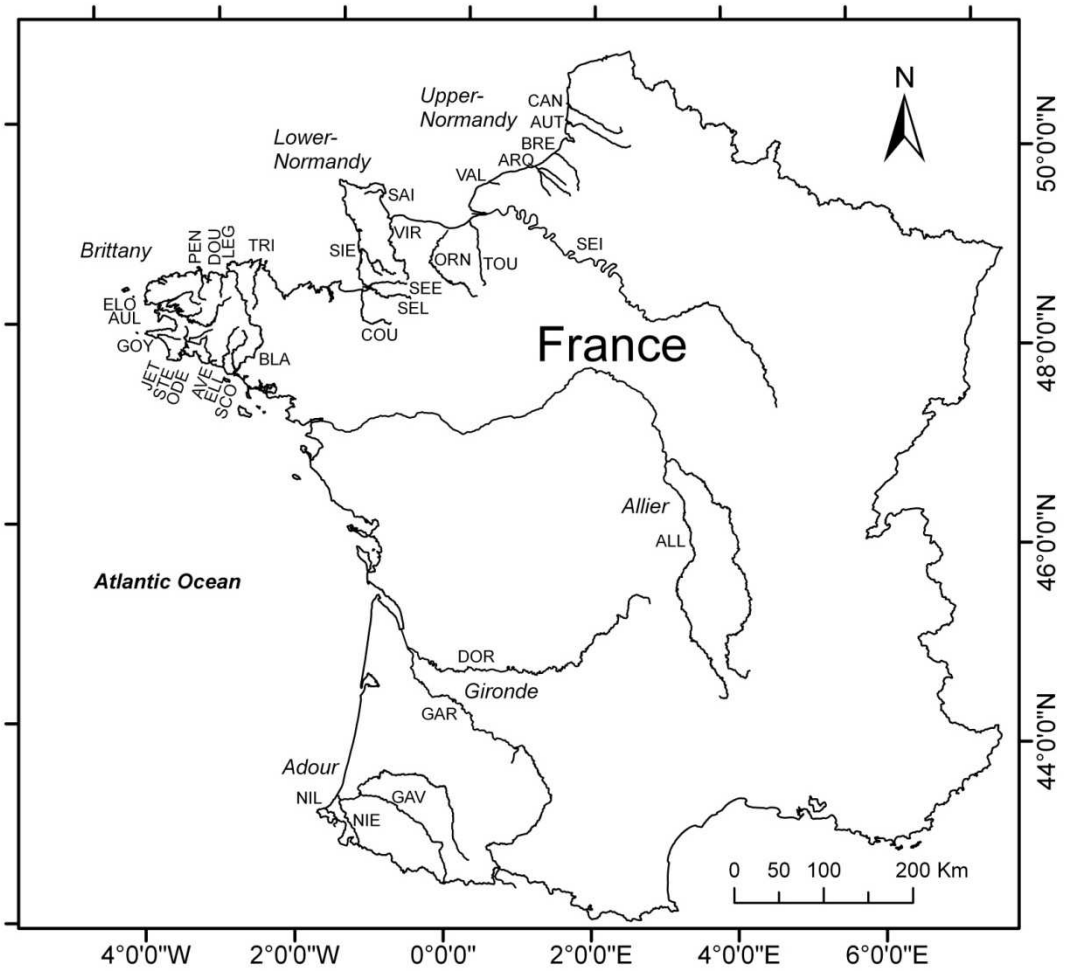

Figure 1: Map of the locations of the study populations (see also Table 1).

Table 1: Sampling and genetic diversity data for the 34 rivers studied. N is the number of alleles, AR is allelic richness (based on samples of 11 individuals), HE is the unbiased expected heterozygosity, HO is the observed heterozygosity, FIS is the inbreeding coefficient (significance is indicated by*).

Sampling sites						Samples				Genetic diversity indices					
River	Longitude	Latitude	Distance from Canche	River length (km)	Percentage of Multi-sea-winter fish	Abbreviation	Sample size	Cohorts	excluded from spatial analyses	N	A_R	H_E	H_O	F_{IS}	Private alleles (n ; max.
Canche	1°36	50°32	0	88		CAN	8	1999-2006	/	6.1	/	0.76	0.74	0.03	0
Authie	1°34	50°22	19	103	18%	AUT	11	2003-2006	/	7.6	6.7	0.76	0.68	*0.11**	4 ; 0.090
Bresle	1°22	50°03	57	72		BRE	30	1998-2004	/	8.7	5.5	0.72	0.72	0.00	7 ; 0.027
Arques	1°05	49°56	82	67		ARQ	31	2003	/	7.3	5.3	0.71	0.70	0.02	1 ; 0.016
Valmont	0°22	49°45	138	14		VAL	5	2003-2005	yes	5.4	/	0.82	0.72	*0.13**	3 ; 0.125
Seine	0°07	49°26	185	777		SEI	7	1998-2006	yes	6.9	/	0.79	0.75	0.06	1 ; 0.055
Touques	0°04	49°21	193	108		TOU	11	19982006	yes	8.4	7.5	0.83	0.75	*0.11**	2 ; 0.050
Orne	0°14	49°17	218	170		ORN	31	2001	yes	11.7	7.4	0.83	0.81	0.02	1 ; 0.016
Vire	1°07	49°21	284	128		VIR	19	1998-2004	yes	10.4	7.3	0.83	0.78	0.06	1 ; 0.026
Saire	1°14	49°36	315	31	13%	SAI	9	2005-2006	yes	7.1	/	0.80	0.80	0.00	1 ; 0.055
Sienne	1°34	49°00	465	93		SIE	37	2002-2003	/	11.6	6.8	0.80	0.80	0.00	1 ; 0.014
Sée	1°29	48°39	505	78		SEE	66	2002-2003	/	11.5	6.1	0.78	0.76	0.02	0
Sélune	1°29	48°39	505	91		SEL	80	2002-2003	/	13.4	6.8	0.79	0.79	0.01	2 ; 0.006
Couesnon	1°30	48°37	508	101		COU	34	2002-2003	yes	10.7	6.7	0.81	0.80	0.01	1 ; 0.014
Trieux	3°04	48°49	649	72		TRI	26	2002	/	9.8	6.9	0.82	0.81	0.02	2 ; 0.019
Leguer	3°33	48°43	716	60		LEG	27	2002-2003	/	10.4	7.3	0.82	0.79	0.04	0
Douron	3°38	48°40	726	27		DOU	27	2002-2003	/	9.8	6.8	0.80	0.80	0.01	0
Penzé	3°56	48°40	755	30		PEN	26	2002-2003	/	10.2	6.6	0.81	0.81	0.00	1 ; 0.019
Elorn	4°21	48°24	881	57		ELO	33	2003	/	10.4	6.4	0.79	0.78	0.01	0
Aulne	4°15	48°17	903	140		AUL	34	2003	/	11.5	6.9	0.81	0.80	0.02	4 ; 0.033
Goyen	4°32	48°00	966	36	17%	GOY	34	2003	/	10.1	6.5	0.80	0.77	0.04	0
Steir	4°06	47°52	1018	62		STE	20	2002	/	9.0	6.8	0.81	0.81	0.00	2 ; 0.041
Jet	4°06	47°52	1018	62		JET	20	2000-2004	/	8.6	6.3	0.78	0.78	0.00	1 ; 0.050
Odet	4°06	47°52	1018	62		ODE	19	2003	/	8.4	6.4	0.78	0.77	0.01	1 ; 0.026
Aven	3°44	47°48	1052	37		AVE	34	2003	/	10.2	6.4	0.79	0.79	0.01	1 ; 0.014
Ellé	3°32	47°46	1069	76		ELL	34	2003	/	10.2	6.4	0.78	0.79	-0.01	2 ; 0.016
Scorff	3°22	47°42	1087	78		SCO	64	2002-2003	/	11.6	6.3	0.79	0.78	0.01	3 ; 0.033
Blavet	3°22	47°42	1087	149		BLA	64	2002-2003	/	12.5	6.5	0.79	0.78	0.02	3 ; 0.008
Allier	2°10	47°16	1197	1013	95%	ALL	35	2001-2002	/	8.1	5.4	0.74	0.74	0.00	2 ; 0.016
Dordogne	1°06	45°34	1412	483	?	DOR	15	2002	yes	8.1	6.5	0.79	0.83	-0.05	0
Garonne	1°06	45°34	1412	647		GAR	30	2002	yes	10.2	6.6	0.80	0.82	-0.02	1 ; 0.017
Gave d'Oloron	1°31	43°31	1642	309	19%	GAV	29	2003	/	11.6	7.2	0.81	0.77	0.06	6 ; 0.051
Nive	1°31	43°31	1642	80		NIE	8	2001-2006	/	5.5	/	0.77	0.75	0.04	0
Nivelle	1°40	43°23	1662	45		NIL	17	1998-2004	/	7.9	6.4	0.80	0.80	-0.01	0

Molecular analyses

Genomic DNA was extracted from fin tissues and scales by heating samples in solution of proteinase K, TE (Tris/EDTA) buffer and chelex, at 55°C 2 hours and then at 100°C for 10min (Estoup *et al.* 1996). The M13 method (Schuelke 2000) was used to label DNA polymerase chain reaction (PCR) amplifications. DNA was amplified using 17 microsatellites: BHMS176, BHMS179A, BHMS184B, BHMS429, BHMS235, BHMS217, BHMS111, BHMS377, BHMS365 (Hoyheim 2000), SsA85, SsA197, SsA171 (Oreilly *et al.* 1996), SsA65, SsA9, SsA224 (O'Reilly *et al.* 1997), SsOSL85 (Slettan *et al.* 1995) and SsSP2216 (Paterson *et al.* 2004) (Table S1). PCR was carried out in a 10 ml reaction volume containing 1.5mM MgCl2, 200mM dNTPs, 0.1 mM forward primer, 0.15mM reverse primer, 0.15mM M13-Fluo, 25–50ng DNA and 0.5U Taq DNA polymerase. The amplification conditions were as follows: an initial denaturation for 5min at 94°C, then 42–45 cycles for 30s at 94°C, 30s at annealing temperature, 30 s at 72°C and a final synthesis for 30min at 72°C.

Within-population genetic diversity

We used TEXTPAD 4.7.3 (Helios Software Solutions), Convert 1.31 (Glaubitz 2004), GENEPOP 4.0.7 (Rousset 2008), and GENALEX 6 (Peakall & Smouse 2006) to format the data sets for different sofwares. We used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to assess the frequency of null alleles and scoring errors due to stuttering or large allelic dropout. Allele number and allelic richness were obtained using FSTAT 2.9.3.2 (Goudet 1995). Tests for linkage and Hardy-Weinberg disequilibria were conducted with FSTAT 2.9.3.2 based on 1,000 permutations. Expected heterozygosity, HE, (Nei 1978) and observed heterozygosity (HO), were calculated with GENETIX 4.05.2 (Belkhir *et al.* 1996). GENALEX 6 (Peakall & Smouse 2006) was used to calculate the number and frequency of private alleles in each population. FDIST 2 (Beaumont & Nichols 1996) was employed to validate the neutrality of the markers. BOTTLENECK 1.2.02 (Cornuet & Luikart 1996; Piry *et al.* 1999) was used to investigate the occurrence of recent bottleneck in the study populations. We assumed a two-phase model of mutation (TPM) with 95% single-step, 5% multi-step mutations, and variance of 12% authors' recommendations, using 1,000 iterations and a Wilcoxon sign-rank test.

Table S1: List and characteristics of the microsatellite markers used.

Microsatellite locus	Genbank	Name	Repeat	Forward primer	Reverse primer	Reference
BHMS176	AF256672	SsA0021NVH	2	GACTTGGAGACTCTTTGG	GAGAGGGAGATAGCATCG	Hoyheim, 2000
BHMS179A	AF257058	SsA0146NVH	2	AGTGCGTCTGTGGCTTTG	GGGCAGAGAGAAGAGATAG	Hoyheim, 2000
BHMS184B	AF257049	SsA0149NVH	2	GCAGCATAGAGAGTAATGG	TGGAAAAGTCCCTCACCC	Hoyheim, 2000
BHMS429	AF256719	SsA0071NVH	2	CCCCTGTCAAACGTCTTC	AGCACACTGGATTCAAGG	Hoyheim, 2000
SsA85	U43692	SsA85	2	ACTGCGGGACATTTGAGG	TCAGATTTCTTACACTCTCG	O'Reilly et al, 1996
SsA65	AF019184	SsA65	2	TGTTGTGGCTCGTGACAG	GAACACAGGGTAGAGTGG	O'Reilly et al, 1997
SsOSL85	Z48596	SsOSL85	2	AGACTAGGGTTTGACCAAG	ATTCAGTACCTTCACCACC	Slettan,A. 1995
SsA9	AF019197	SsA9	2	ACAATTACCAGAGCCAGG	TCTGACGACAAATTACCAC	O'Reilly et al, 1997
BHMS235	AF256846	SsA0217NVH	2	AGCGAGCTTTCTTTCCAG	AGCTGTCTATTCACGACTC	Hoyheim, 2000
BHMS217	AF256786	SsA0152NVH	2	GCTGTTCATTTCTGAGCAG	GACACACCGAATCAGTGC	Hoyheim, 2000
BHMS111	AF256661	SsA0008NVH	2	TCCTCTATCATACGGCTG	ATCAGATGACCCAGTGGC	Hoyheim, 2000
SsA197	U43694	SsA197	3	TGAGTAGGGAGGCTTGTG	TGACATAACTCTTCTATGGC	O'Reilly et al, 1996
SsA171	U43693	SsA171	3	TGATGGCAGGGTAAGAGG	GGTCAAAACCTCGCTGTG	O'Reilly et al, 1996
BHMS377	AF256707	SsA0057NVH	2	TGGCTACAACAGGGATAC	AGTCTCTTACATGGAGGC	Hoyheim, 2000
SsSP2216	AY081811	SsSP2216	4	GGCCCAGACAGATAAACAAACACGC	GCCAACAGCAGCATCTACACCCAG	Paterson et al, 2004
BHMS365	AF256703	SsA0053NVH	2	GGAATTGTGTGTAGATGGG	ACTTGGCAAGGAGCAGAC	Hoyheim, 2000
SsA224	AF019168	SsA224	2	ACAGACAGAACTGTGCATC	TCGATTTTGGTTGACTGCAT	O'Reilly et al, 1997

Individual Bayesian clustering

In order to estimate the number of genetic clusters in our data set without taking into account any predefined populations we used the Bayesian clustering method implemented in STRUCTURE 2.2 (Pritchard et al 2000). This analysis also allowed us to compute admixture proportions among the inferred clusters. STRUCTURE analyses were performed assuming an admixture model and a number of genetic clusters (*k*) from 1 to 14 (15 replicates for each k). Each run started with a burn-in period of 50,000 steps followed by 300,000 Markov Chain Monte Carlo (MCMC) replicates. We used DISTRUCT to plot STRUCTURE output data (Rosenberg 2004).

Inter-population genetic divergence

Pairwise F_{ST} were computed in FSTAT 2.9.3.2 and tested with 1,000 permutations. Pairwise Nei (Da) genetic distances (Nei *et al.* 1983) were estimated using POPULATIONS 1.2.30 (http://bioinformatics.org/~tryphon/populations/). Neighbor-Joining dendrograms were constructed from Nei distances using TreeView (Page 1996). Analyses of Molecular Variance (AMOVA) were performed using ARLEQUIN (Excoffier *et al.* 2005) with groups of populations defined using geographic criteria and results from Bayesian clustering analyses. We performed three AMOVAs: i) with the 34 populations distributed in six groups: Upper-Normandy, Lower-Normandy, Brittany, Allier, Gironde, and Adour (see Figure 2), ii) with a subset of 32 populations similarly clustered in five groups, the non-native Gironde group being excluded, and c) with a subset of 25 populations considered as "unstocked" with non-native fish and distributed in the same five groups (see Table 1). The latter analysis aimed at testing the effect of stocking on hierarchical genetic structure. In this analysis we also excluded populations recently recolonized or populations close to those stocked with non-native fish.

Spatial analyses

To test the effect of coastal distance (between river mouths) and river length on genetic differentiation, we used Mantel tests and partial Mantel tests implemented in PASSAGE (http://www.passagesoftware.net/index.php). Pairwise genetic differentiation was estimated by the F_{ST} /(1- F_{ST}) quantity for the subset of 25 populations using Fstat (Goudet 1995). The significance of correlation coefficients was estimated with 9,999 permutations in PASSAGE.

We also investigated the effect of coastal distance and river length on recent migration rates using the Bayesian model implemented in BIMr 1.0 (Faubet & Gaggiotti 2008). The best model was chosen from the run showing the lowest Bayesian assignment deviance out of 15 MCMC replicates. We used 20 short pilots of 1,000 iterations. Given the relatively low genetic structure among several populations and according to authors' recommendations, we used MCMC chains starting with 1,000,000 iterations for burn in to ensure convergence, followed by 20,000 iterations for sampling and thinning intervals of 50 iterations between consecutive samples.

A finer investigation of isolation by distance was carried out by autocorrelation analyses. The autocorrelation coefficient (r) was calculated in GENALEX 6 (Peakall & Smouse 2006) at the individual level (Smouse & Peakall 1999; Peakall *et al.* 2003) for the subset of 25 populations. Distance classes of 50 km were calculated from populations pairwise coastal distances. Significance of r-values was tested using 999 permutations.

Results

Summary statistics

Successive cohorts analyzed in Sienne, Sée, Sélune, Scorff, and Blavet were not significantly differentiated. We thus combined genotypes from successive cohorts of the same population in our analyses. We found evidence of null alleles or large allele drop-out for 18 out of 578 tests, but no association with any particular marker or population. Considering the full dataset, there was no evidence of departure from Hardy-Weinberg-Equilibrium associated with a particular marker (Table S2). The loci used showed no evidence of linkage disequilibrium or influence of natural selection. Significant heterozygote deficits were detected in Authie, Valmont and Touques populations (Tables 1 & S2). An evidence of recent bottleneck was only detected in Saire ($p < 0.05$). Indices of genetic diversity per population are presented in Table 1. Average gene diversity (He) over all populations was 0.79, ranging from 0.71 to 0.83. Allelic richness and He were the lowest in the Bresle, Arques, and Allier populations and the highest in the Touques, Orne, Vire and Leguer populations.

Table S2: Allelic richness (AR) estimated for 4 and 8 individuals, number of alleles (AN), observed (Ho) and expected heterozygoties (HE), inbreeding coefficient (FIS, significant values in bold) and presence of null alleles for every locus and population.

		SSA0021NVH	SSA0146NVH	SSA0149NVH	SSA0071NVH	SSA85	SSA65	SSOSL85	SSA9	SSA0217NVH	SSA0152NVH	SSA0008NVH	SSA197	SSA171	SSA0057NVH	SSSP2216	SSA0053NVH	SSA224	All loci
	AR, 4 ind	3.76	2.46	3.25	5.30	5.65	4.67	3.75	5.26	5.53	4.07	2.50	4.98	4.58	4.96	4.53	5.31	4.39	4.41
	AR, 8 ind	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AN	5.00	3.00	4.00	7.00	9.00	7.00	5.00	8.00	8.00	5.00	4.00	7.00	6.00	7.00	6.00	7.00	6.00	6.12
CAN	Ho	0.88	0.50	0.88	0.75	0.75	0.63	0.75	0.86	0.75	0.88	0.25	0.75	0.83	0.71	0.86	0.83	0.71	0.74
	He	0.74	0.49	0.68	0.88	0.88	0.79	0.73	0.82	0.89	0.77	0.35	0.85	0.76	0.81	0.81	0.86	0.75	0.76
	Fis	-0.20	-0.02	-0.32	0.16	0.16	0.22	-0.02	-0.04	0.17	-0.15	***0.30***	0.13	-0.11	0.13	-0.06	0.04	0.05	0.03
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	4.29	2.46	2.73	6.18	4.45	4.43	4.50	5.72	5.38	4.41	3.22	6.07	5.17	5.04	4.75	4.86	4.17	4.58
	AR, 8 ind	5.58	3.91	3.65	9.47	6.34	6.77	6.62	9.22	7.39	6.26	4.90	9.10	7.57	6.70	7.60	7.82	4.92	6.69
	AN	6.00	5.00	4.00	11.00	7.00	8.00	8.00	10.00	8.00	7.00	6.00	10.00	9.00	7.00	9.00	10.00	5.00	7.65
AUT	Ho	0.82	0.27	0.36	0.91	0.60	0.36	0.73	0.89	0.80	0.55	0.55	0.60	0.82	0.90	0.90	0.73	0.82	0.68
	He	0.79	0.34	0.46	0.93	0.79	0.74	0.79	0.89	0.88	0.77	0.53	0.92	0.86	0.86	0.80	0.81	0.80	0.76
	Fis	-0.03	0.20	0.22	0.02	0.25	0.52	0.08	0.00	0.10	0.30	-0.03	0.36	0.05	-0.05	-0.13	0.11	-0.02	***0.11***
	Null Allele	no	no	no	no	no	yes	no	no	no	no	no	yes	no	no	no	no	no	
	AR, 4 ind	4.01	2.07	2.41	4.79	4.48	3.78	4.35	4.25	5.10	3.99	2.89	4.29	5.02	5.42	4.10	4.14	3.68	4.05
	AR, 8 ind	4.83	2.87	2.84	6.75	6.16	5.53	5.53	5.69	7.48	5.19	3.76	6.39	7.00	8.04	5.38	6.02	4.54	5.53
	AN	6.00	5.00	3.00	11.00	9.00	11.00	8.00	8.00	13.00	8.00	5.00	10.00	11.00	14.00	9.00	11.00	6.00	8.71
BRE	Ho	0.87	0.27	0.38	0.83	0.90	0.73	0.83	0.89	0.86	0.76	0.50	0.69	0.87	0.66	0.76	0.72	0.70	0.72
	He	0.78	0.27	0.44	0.83	0.80	0.66	0.80	0.78	0.85	0.75	0.52	0.73	0.85	0.87	0.77	0.73	0.74	0.72
	Fis	-0.11	0.03	0.15	0.00	-0.12	-0.11	-0.04	-0.15	-0.02	-0.02	0.05	0.05	-0.02	0.25	0.02	0.00	0.06	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	
	AR, 4 ind	3.34	2.08	2.17	4.97	3.71	3.18	4.56	4.85	5.10	4.37	2.67	4.29	4.57	4.92	4.58	4.40	3.95	3.98
	AR, 8 ind	3.88	2.44	2.65	6.69	5.09	4.41	5.76	6.82	7.25	5.46	3.62	6.22	6.16	6.92	5.93	5.99	4.95	5.31
	AN	4.00	3.00	3.00	9.00	8.00	7.00	7.00	9.00	12.00	6.00	5.00	9.00	8.00	10.00	8.00	9.00	7.00	7.29
ARQ	Ho	0.68	0.39	0.35	0.81	0.57	0.58	0.84	0.87	0.84	0.84	0.53	0.68	0.73	0.71	0.80	0.79	0.84	0.70
	He	0.68	0.37	0.36	0.85	0.70	0.56	0.83	0.84	0.85	0.81	0.45	0.74	0.81	0.84	0.83	0.78	0.76	0.71
	Fis	0.00	-0.05	0.00	0.06	0.19	-0.04	-0.02	-0.04	0.02	-0.04	***-0.20***	0.08	0.10	0.16	0.03	-0.01	-0.10	0.02
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.60	3.60	2.78	5.00	5.00	6.76	6.76	5.00	7.00	4.58	3.60	5.51	7.38	6.00	3.00	5.98	2.98	4.97
	AR, 8 ind	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AN	4.00	4.00	3.00	5.00	5.00	8.00	8.00	5.00	7.00	5.00	4.00	6.00	9.00	6.00	3.00	7.00	3.00	5.41
VAL	Ho	0.80	0.40	0.60	0.75	0.75	0.80	1.00	1.00	1.00	0.60	0.80	1.00	0.80	0.75	0.25	0.80	0.20	0.72
	He	0.71	0.71	0.51	0.86	0.86	0.96	0.96	0.89	0.96	0.84	0.71	0.91	0.98	0.89	0.61	0.91	0.69	0.82
	Fis	-0.14	***0.47***	***-0.20***	0.14	0.14	0.18	***-0.05***	***-0.14***	***-0.04***	0.31	-0.14	***-0.11***	***0.20***	***0.18***	***0.63***	0.14	***0.73***	***0.13***
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.35	2.43	2.86	6.02	4.69	4.81	5.72	6.47	5.72	3.15	3.73	5.90	4.93	6.42	5.46	5.42	3.00	4.71
	AR, 8 ind	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AN	4.00	3.00	4.00	10.00	6.00	7.00	9.00	10.00	9.00	4.00	5.00	10.00	7.00	10.00	8.00	8.00	4.00	6.94
SEI	Ho	0.78	0.56	0.33	0.89	0.56	0.56	0.89	1.00	0.67	0.78	0.89	0.89	0.89	0.78	0.78	0.78	0.67	0.75
	He	0.71	0.54	0.48	0.91	0.84	0.84	0.90	0.94	0.90	0.67	0.75	0.90	0.84	0.94	0.89	0.88	0.54	0.79
	Fis	-0.10	-0.03	0.31	0.02	0.36	0.35	0.01	***-0.07***	0.27	-0.17	-0.21	0.02	-0.06	0.18	0.13	0.13	***-0.25***	0.06
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	4.10	3.08	3.03	4.96	4.87	6.27	5.62	5.42	6.13	4.72	3.62	5.41	6.31	6.20	5.92	6.79	4.18	5.10
	AR, 8 ind	4.94	4.18	3.77	7.30	6.38	9.62	8.02	8.00	10.31	5.86	5.12	7.82	10.21	10.20	8.75	11.88	5.91	7.54
	AN	5.00	5.00	4.00	8.00	7.00	11.00	9.00	8.00	12.00	6.00	6.00	9.00	11.00	12.00	10.00	13.00	7.00	8.41
TOU	Ho	0.60	0.45	0.70	0.70	0.82	0.73	0.73	0.88	0.80	0.64	0.73	0.73	1.00	0.70	0.91	0.67	0.91	0.75
	He	0.78	0.64	0.63	0.82	0.85	0.93	0.90	0.88	0.91	0.84	0.67	0.88	0.93	0.92	0.91	0.95	0.75	0.83
	Fis	0.24	0.30	-0.13	0.15	0.04	0.23	0.20	0.00	0.12	0.26	-0.10	0.18	***-0.08***	0.25	0.01	***0.31***	-0.22	***0.11***
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	
	AR, 4 ind	3.77	2.24	2.71	5.89	6.18	5.67	5.63	6.31	6.52	4.83	3.96	4.95	5.41	6.64	5.63	5.53	3.63	5.03
	AR, 8 ind	4.49	2.52	3.31	8.90	9.82	8.42	8.43	10.04	10.62	6.29	5.13	7.31	7.86	11.05	8.13	8.01	4.70	7.35
	AN	5.00	4.00	5.00	14.00	18.00	14.00	13.00	18.00	19.00	8.00	6.00	12.00	12.00	21.00	12.00	12.00	6.00	11.71
ORN	Ho	0.87	0.55	0.48	0.84	0.93	0.90	0.97	0.97	0.87	0.77	0.74	0.81	0.90	0.73	0.80	0.97	0.70	0.81
	He	0.75	0.52	0.60	0.91	0.92	0.89	0.89	0.93	0.94	0.85	0.75	0.83	0.88	0.94	0.89	0.89	0.68	0.83
	Fis	-0.16	-0.05	0.19	0.08	-0.02	-0.01	-0.09	-0.04	0.07	0.09	0.01	0.03	-0.03	***0.23***	0.11	-0.09	-0.03	0.02
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	
	AR, 4 ind	3.54	2.82	2.62	5.38	5.73	5.92	5.49	6.79	6.11	4.49	4.78	5.46	5.77	6.07	5.35	5.87	3.10	5.02
	AR, 8 ind	3.94	3.68	3.26	7.92	8.54	8.95	7.83	11.45	9.63	5.97	6.36	8.18	8.42	9.53	7.85	8.89	3.79	7.31
	AN	4.00	6.00	5.00	10.00	12.00	13.00	10.00	18.00	15.00	8.00	8.00	13.00	11.00	15.00	11.00	13.00	4.00	10.35
VIR	Ho	0.58	0.42	0.53	0.79	0.79	0.79	0.84	1.00	0.95	0.78	0.84	0.79	0.88	0.89	0.89	0.89	0.63	0.78
	He	0.74	0.59	0.59	0.86	0.90	0.91	0.88	0.95	0.92	0.81	0.84	0.88	0.90	0.92	0.87	0.91	0.59	0.83
	Fis	0.22	0.29	0.10	0.09	0.12	0.14	0.05	***-0.05***	-0.04	0.04	-0.01	0.10	0.02	0.03	-0.03	0.02	-0.07	0.06
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.49	2.42	1.93	5.42	5.72	5.18	5.29	5.54	6.90	4.76	4.44	4.81	5.66	6.61	5.83	5.83	2.64	4.85
	AR, 8 ind	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AN	4.00	3.00	2.00	8.00	9.00	8.00	8.00	7.00	12.00	6.00	6.00	7.00	8.00	11.00	9.00	9.00	3.00	7.06
SAI	Ho	0.89	0.44	0.22	0.89	0.67	0.89	0.67	1.00	1.00	0.89	0.78	0.89	0.88	1.00	0.89	0.89	0.67	0.80
	He	0.70	0.50	0.37	0.88	0.90	0.86	0.86	0.89	0.96	0.85	0.80	0.84	0.90	0.95	0.91	0.91	0.52	0.80
	Fis	-0.29	0.12	0.41	-0.01	0.27	-0.04	0.24	***-0.13***	***-0.04***	-0.05	0.03	-0.07	0.03	***-0.06***	0.02	0.02	***-0.30***	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.26	2.19	2.66	5.21	4.96	5.50	5.38	5.88	5.87	4.58	3.79	4.86	5.48	6.67	5.22	5.83	3.56	4.76
SIE	AR, 8 ind	3.92	2.39	3.17	7.37	7.13	8.08	7.79	9.09	8.99	5.92	5.17	6.93	8.05	11.10	7.54	8.75	4.81	6.84
	AN	5.00	3.00	5.00	12.00	12.00	14.00	13.00	18.00	17.00	8.00	7.00	13.00	15.00	22.00	12.00	14.00	7.00	11.59

	Ho	0.68	0.62	0.50	0.86	0.86	0.89	0.92	0.95	0.92	0.69	0.54	0.81	0.89	1.00	0.86	0.86	0.72	0.80
	He	0.68	0.51	0.58	0.86	0.83	0.88	0.87	0.90	0.90	0.82	0.68	0.83	0.88	0.95	0.86	0.90	0.64	0.80
	Fis	0.00	-0.22	0.15	0.00	-0.04	-0.01	-0.05	-0.05	-0.01	0.17	0.21	0.03	-0.01	***-0.06***	-0.01	0.05	-0.13	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	yes	yes	no	no	no	no	no	no	
	AR, 4 ind	2.91	2.22	2.29	4.81	5.28	5.50	5.39	5.62	5.78	4.19	3.86	4.14	4.37	5.85	4.70	5.11	3.19	4.42
	AR, 8 ind	3.41	2.45	2.57	6.71	7.75	8.00	7.63	8.50	8.91	5.28	5.12	5.28	5.65	9.07	6.86	7.25	4.05	6.15
	AN	4.00	4.00	5.00	12.00	17.00	14.00	13.00	16.00	19.00	8.00	8.00	8.00	11.00	19.00	13.00	19.00	5.00	11.47
SEE	Ho	0.57	0.38	0.53	0.73	0.79	0.84	0.85	0.92	0.89	0.70	0.79	0.88	0.80	0.88	0.84	0.88	0.63	0.76
	He	0.63	0.53	0.54	0.83	0.86	0.88	0.88	0.88	0.89	0.79	0.71	0.78	0.80	0.90	0.79	0.86	0.62	0.78
	Fis	0.10	0.29	0.02	0.12	0.08	0.05	0.03	-0.05	0.00	0.12	-0.11	-0.12	0.01	0.02	-0.06	-0.03	-0.03	0.02
	Null Allele	no	yes	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.03	2.22	2.32	5.72	5.29	5.77	5.62	5.97	6.20	4.22	3.82	4.65	4.88	6.24	5.06	5.54	3.56	4.71
	AR, 8 ind	3.56	2.46	2.67	8.53	7.79	8.57	8.33	9.36	9.84	5.37	5.01	6.48	6.85	9.98	7.37	8.32	4.75	6.78
	AN	4.00	4.00	6.00	15.00	17.00	15.00	17.00	19.00	20.00	8.00	9.00	15.00	14.00	22.00	13.00	22.00	8.00	13.41
SEL	Ho	0.69	0.49	0.54	0.84	0.85	0.87	0.89	0.86	0.90	0.70	0.76	0.79	0.79	0.95	0.87	0.85	0.72	0.79
	He	0.64	0.51	0.52	0.90	0.86	0.90	0.89	0.91	0.92	0.79	0.71	0.82	0.83	0.92	0.84	0.88	0.66	0.79
	Fis	-0.07	0.05	-0.04	0.07	0.01	0.03	-0.01	0.05	0.02	0.11	-0.07	0.04	0.05	-0.03	-0.04	0.04	-0.10	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.34	2.45	3.27	5.42	5.23	5.61	5.82	5.55	5.50	4.74	3.69	4.68	4.65	6.18	5.27	5.83	3.56	4.75
	AR, 8 ind	3.82	2.75	3.79	7.87	7.78	8.29	8.88	8.40	8.28	5.97	4.74	6.46	6.67	9.83	7.60	8.98	4.64	6.75
	AN	4.00	3.00	4.00	12.00	13.00	13.00	15.00	14.00	14.00	7.00	7.00	11.00	11.00	18.00	12.00	18.00	6.00	10.71
COU	Ho	0.71	0.65	0.56	0.82	0.88	0.85	0.87	0.82	0.88	0.84	0.74	0.76	0.77	0.91	0.88	0.82	0.82	0.80
	He	0.70	0.55	0.67	0.88	0.85	0.89	0.90	0.88	0.88	0.84	0.71	0.82	0.80	0.92	0.87	0.90	0.68	0.81
	Fis	-0.01	-0.18	0.17	0.07	-0.04	0.05	0.03	0.07	-0.01	0.00	-0.03	0.07	0.04	0.01	-0.02	0.09	-0.22	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.33	2.39	3.14	4.96	5.36	5.24	5.16	6.42	5.96	4.62	4.08	5.23	5.72	5.56	5.95	5.56	3.50	4.83
	AR, 8 ind	3.87	2.68	3.53	7.17	7.90	7.50	7.24	10.32	9.28	6.09	5.09	7.44	8.49	8.38	8.90	8.53	4.34	6.87
	AN	4.00	3.00	4.00	11.00	13.00	11.00	11.00	17.00	16.00	8.00	6.00	12.00	11.00	12.00	11.00	12.00	5.00	9.82
TRI	Ho	0.64	0.69	0.56	0.81	0.96	0.88	0.73	0.96	0.88	0.77	0.72	0.85	0.94	0.94	0.88	0.80	0.69	0.81
	He	0.68	0.55	0.68	0.83	0.87	0.86	0.86	0.94	0.91	0.83	0.78	0.87	0.90	0.89	0.91	0.88	0.70	0.82
	Fis	0.05	-0.27	0.19	0.03	-0.11	-0.03	0.16	-0.03	0.03	0.07	0.08	0.02	-0.05	-0.06	0.04	0.09	0.02	0.02
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.26	2.99	2.91	5.65	5.28	5.54	5.51	6.20	6.17	4.63	3.76	5.62	5.63	6.36	6.27	6.11	2.97	4.99
	AR, 8 ind	4.06	3.79	3.27	8.23	7.88	8.07	8.06	9.66	9.63	6.36	4.66	8.45	8.45	10.06	9.75	9.53	3.78	7.28
	AN	5.00	5.00	4.00	12.00	12.00	12.00	12.00	14.00	16.00	8.00	6.00	15.00	11.00	14.00	13.00	14.00	4.00	10.41
LEG	Ho	0.48	0.59	0.52	0.85	0.96	0.96	0.89	0.96	0.93	0.81	0.63	0.89	0.81	0.81	0.94	0.75	0.60	0.79
	He	0.66	0.58	0.65	0.89	0.86	0.88	0.88	0.92	0.92	0.81	0.73	0.89	0.89	0.93	0.93	0.92	0.54	0.82
	Fis	0.28	-0.03	0.20	0.05	-0.13	-0.09	-0.01	-0.04	0.00	0.00	0.14	0.00	0.09	0.13	-0.01	0.19	-0.13	0.04
	Null Allele	yes	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.29	2.53	2.52	6.26	5.52	5.24	5.56	5.10	6.25	4.17	3.53	5.24	5.14	5.54	5.59	6.04	3.39	4.76
	AR, 8 ind	3.80	2.96	2.89	9.75	8.11	7.37	8.15	7.26	9.77	5.39	4.43	7.68	7.06	8.17	8.25	9.57	4.27	6.76
	AN	5.00	4.00	3.00	15.00	12.00	11.00	13.00	11.00	15.00	7.00	6.00	13.00	10.00	14.00	13.00	16.00	5.00	10.18
DOU	Ho	0.78	0.59	0.33	0.89	0.88	0.85	0.96	0.88	0.85	0.81	0.67	0.85	0.96	0.81	0.78	0.93	0.70	0.80
	He	0.71	0.58	0.50	0.93	0.88	0.87	0.89	0.85	0.93	0.78	0.70	0.86	0.87	0.88	0.89	0.91	0.64	0.80
	Fis	-0.11	-0.03	0.34	0.04	0.00	0.02	-0.09	-0.03	0.08	-0.05	0.05	0.01	-0.12	0.08	0.13	-0.02	-0.10	0.01
	Null Allele	no	no	yes	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.43	2.39	3.16	5.30	4.99	4.28	4.91	6.44	5.22	4.70	3.53	5.28	5.85	6.00	5.69	5.55	3.34	4.71
	AR, 8 ind	3.91	2.68	3.52	7.26	6.98	5.88	6.76	10.31	7.93	6.36	4.34	7.85	8.75	9.30	8.22	8.67	4.25	6.65
	AN	4.00	3.00	4.00	10.00	9.00	9.00	11.00	16.00	13.00	9.00	6.00	12.00	13.00	15.00	11.00	16.00	5.00	9.76
PEN	Ho	0.64	0.46	0.73	0.81	0.79	0.83	0.88	0.91	0.85	0.85	0.77	0.92	0.91	0.83	0.91	0.87	0.74	0.81
	He	0.71	0.56	0.69	0.88	0.84	0.77	0.85	0.94	0.85	0.83	0.72	0.86	0.91	0.91	0.90	0.87	0.63	0.81
	Fis	0.10	0.18	-0.06	0.08	0.07	-0.09	-0.05	0.03	0.00	-0.02	-0.07	-0.07	-0.01	0.09	-0.02	0.01	-0.17	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.48	2.62	3.05	5.00	5.49	4.95	4.90	6.25	6.09	4.37	3.74	4.80	5.72	6.33	5.34	6.35	3.23	4.81
	AR, 8 ind	3.91	2.89	3.44	7.28	8.31	7.47	7.03	9.94	9.50	5.72	4.69	6.80	8.81	10.14	7.61	10.19	3.77	6.91
	AN	4.00	3.00	4.00	13.00	14.00	13.00	13.00	18.00	17.00	9.00	6.00	12.00	15.00	20.00	11.00	19.00	4.00	11.47
AUL	Ho	0.87	0.39	0.61	0.73	0.93	0.77	0.79	0.97	0.94	0.85	0.74	0.85	0.78	0.79	0.88	0.97	0.76	0.80
	He	0.73	0.60	0.66	0.84	0.88	0.81	0.83	0.92	0.92	0.80	0.74	0.82	0.89	0.93	0.87	0.93	0.66	0.81
	Fis	-0.20	0.36	0.07	0.13	-0.07	0.05	0.05	-0.05	-0.03	-0.07	0.00	-0.04	0.12	0.15	-0.01	-0.04	-0.15	0.02
	Null Allele	no	yes	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	
	AR, 4 ind	3.17	2.76	2.86	4.55	5.71	4.63	5.10	6.21	4.85	4.20	4.08	4.42	5.90	5.37	5.13	5.25	3.08	4.54
	AR, 8 ind	3.72	3.25	3.48	6.52	8.44	6.75	7.18	9.64	7.17	5.27	5.14	6.05	9.01	8.46	7.45	7.81	3.57	6.41
	AN	4.00	4.00	4.00	11.00	13.00	11.00	12.00	16.00	13.00	8.00	7.00	11.00	14.00	18.00	12.00	15.00	4.00	10.41
ELO	Ho	0.59	0.64	0.67	0.79	0.94	0.85	0.82	1.00	0.73	0.78	0.79	0.73	0.90	0.87	0.80	0.73	0.60	0.78
	He	0.67	0.60	0.55	0.80	0.90	0.79	0.85	0.93	0.82	0.79	0.77	0.80	0.91	0.85	0.85	0.86	0.67	0.79
	Fis	0.12	-0.07	-0.22	0.01	-0.05	-0.07	0.04	***-0.08***	0.12	0.02	-0.02	0.09	0.01	-0.02	0.06	0.15	0.10	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.06	2.83	3.23	5.05	4.80	4.97	5.21	6.31	5.57	4.43	3.30	5.26	5.37	5.94	5.03	5.29	3.15	4.64
	AR, 8 ind	3.61	3.18	3.85	7.20	6.60	7.02	7.30	9.98	8.52	5.76	4.00	7.54	7.64	9.08	7.20	8.17	3.74	6.49
	AN	4.00	4.00	5.00	12.00	11.00	10.00	11.00	18.00	16.00	8.00	5.00	12.00	11.00	14.00	12.00	14.00	4.00	10.06
GOY	Ho	0.53	0.65	0.65	0.85	0.85	0.88	0.90	0.97	0.76	0.74	0.62	0.94	0.83	0.83	0.75	0.71	0.67	0.77
	He	0.65	0.62	0.68	0.85	0.83	0.83	0.87	0.93	0.88	0.81	0.65	0.87	0.88	0.91	0.85	0.84	0.65	0.80
	Fis	0.19	-0.04	0.05	-0.01	-0.02	-0.06	-0.04	-0.04	0.13	0.09	0.06	-0.09	0.05	0.09	0.12	0.17	-0.03	0.04
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.24	2.70	3.09	5.59	4.83	4.88	5.09	6.23	5.44	4.63	3.43	5.23	5.28	6.36	5.50	6.36	4.11	4.82
	AR, 8 ind	3.98	3.28	3.40	7.97	6.52	7.55	7.05	9.78	7.85	5.91	3.92	7.71	7.67	10.14	7.65	10.63	5.39	6.85
	AN	5.00	4.00	4.00	10.00	9.00	12.00	10.00	13.00	11.00	7.00	4.00	12.00	9.00	13.00	9.00	15.00	6.00	9.00
STE	Ho	0.60	0.55	0.50	0.85	0.84	0.95	1.00	1.00	0.84	0.80	0.65	0.90	0.83	0.92	0.92	0.79	0.86	0.81
	He	0.68	0.53	0.69	0.89	0.84	0.80	0.86	0.93	0.88	0.83	0.71	0.86	0.87	0.93	0.89	0.92	0.75	0.81
	Fis	0.12	-0.04	0.28	0.05	0.00	-0.20	***-0.17***	***-0.08***	0.05	0.04	0.08	-0.05	0.04	0.01	-0.04	0.15	-0.16	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.29	2.35	2.60	4.89	4.88	4.68	4.93	6.33	5.87	4.38	2.96	4.47	4.58	5.75	5.22	5.73	2.97	4.46
	AR, 8 ind	3.85	2.65	2.88	6.93	7.14	6.51	6.51	9.99	9.05	5.84	3.38	6.41	6.59	9.18	7.13	9.11	3.59	6.28
JET	AN	4.00	3.00	3.00	9.00	9.00	9.00	9.00	14.00	14.00	8.00	4.00	10.00	9.00	14.00	9.00	14.00	4.00	8.59
	Ho	0.60	0.45	0.55	0.80	0.92	0.85	0.70	0.88	0.90	0.80	0.60	0.75	0.76	1.00	1.00	0.94	0.76	0.78
	He	0.67	0.54	0.60	0.83	0.83	0.82	0.86	0.93	0.90	0.80	0.63	0.78	0.80	0.89	0.87	0.88	0.63	0.78

	Fis	0.11	0.17	0.08	0.04	-0.12	-0.04	0.19	0.06	0.00	0.00	0.05	0.04	0.04	***-0.13***	***-0.15***	-0.07	-0.22	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.44	2.71	2.84	5.53	4.04	5.17	5.25	5.74	5.55	4.84	3.24	4.29	5.92	5.16	5.49	4.91	2.47	4.51
	AR, 8 ind	4.22	3.24	3.37	8.24	4.99	7.98	7.58	8.54	8.04	6.56	4.10	6.27	8.90	7.69	8.05	7.73	2.83	6.37
	AN	5.00	4.00	4.00	11.00	6.00	12.00	11.00	10.00	11.00	9.00	5.00	10.00	11.00	10.00	10.00	11.00	3.00	8.41
ODE	Ho	0.53	0.58	0.63	0.89	0.68	0.79	0.89	0.77	0.94	0.84	0.67	0.79	0.71	0.93	0.79	0.93	0.71	0.77
	He	0.70	0.60	0.57	0.88	0.78	0.84	0.86	0.90	0.89	0.84	0.62	0.77	0.91	0.85	0.88	0.80	0.54	0.78
	Fis	0.26	0.03	-0.10	-0.02	0.13	0.06	-0.04	0.15	-0.06	0.00	-0.08	-0.03	0.22	-0.10	0.11	-0.17	-0.33	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	no	
	AR, 4 ind	3.42	2.51	3.03	5.43	4.74	4.65	4.87	6.30	5.41	4.60	3.49	4.21	5.15	5.77	5.32	5.19	3.25	4.55
	AR, 8 ind	3.85	2.81	3.40	7.75	6.65	6.82	6.75	10.04	8.20	6.15	4.41	5.73	7.38	8.81	7.70	7.96	3.80	6.37
	AN	4.00	3.00	4.00	12.00	11.00	11.00	11.00	17.00	15.00	9.00	7.00	9.00	13.00	17.00	12.00	14.00	4.00	10.18
AVE	Ho	0.65	0.56	0.53	0.91	0.76	0.76	0.79	0.94	0.85	0.85	0.65	0.82	0.79	0.76	0.97	0.91	0.82	0.79
	He	0.72	0.55	0.66	0.88	0.82	0.78	0.84	0.93	0.87	0.82	0.69	0.77	0.86	0.90	0.87	0.84	0.67	0.79
	Fis	0.10	-0.02	0.20	-0.04	0.07	0.02	0.05	-0.01	0.02	-0.04	0.07	-0.08	0.08	0.15	-0.12	-0.09	-0.24	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	
	AR, 4 ind	3.41	2.56	2.60	5.46	5.01	4.50	5.28	5.67	5.29	4.57	3.68	4.65	5.26	5.90	5.11	5.50	3.16	4.57
	AR, 8 ind	4.05	2.85	3.10	8.19	7.11	6.65	7.59	8.50	7.98	6.20	5.02	6.69	7.56	8.96	6.89	8.41	3.70	6.44
	AN	5.00	3.00	4.00	12.00	12.00	11.00	13.00	16.00	15.00	8.00	7.00	11.00	13.00	15.00	10.00	14.00	4.00	10.18
ELL	Ho	0.84	0.68	0.55	0.78	0.77	0.78	0.85	0.88	0.91	0.88	0.56	0.79	0.88	0.88	0.88	0.91	0.68	0.79
	He	0.70	0.58	0.51	0.87	0.84	0.76	0.87	0.89	0.86	0.81	0.67	0.81	0.87	0.91	0.86	0.87	0.66	0.78
	Fis	-0.19	-0.17	-0.08	0.11	0.09	-0.03	0.02	0.01	-0.07	-0.10	0.16	0.02	-0.02	0.03	-0.02	-0.05	-0.02	-0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.08	2.63	2.95	5.52	4.85	4.76	5.18	5.63	4.99	4.10	3.45	5.03	5.18	5.83	5.16	5.49	3.22	4.53
	AR, 8 ind	3.60	2.99	3.40	8.00	6.64	6.89	7.41	8.34	7.21	5.09	4.23	7.23	7.51	9.04	7.35	8.38	3.83	6.30
	AN	4.00	4.00	5.00	14.00	12.00	13.00	15.00	15.00	15.00	8.00	6.00	16.00	13.00	19.00	14.00	20.00	5.00	11.65
SCO	Ho	0.62	0.47	0.66	0.88	0.80	0.83	0.88	0.89	0.76	0.77	0.70	0.86	0.87	0.86	0.83	0.91	0.67	0.78
	He	0.66	0.57	0.63	0.89	0.84	0.81	0.86	0.89	0.84	0.78	0.68	0.84	0.85	0.90	0.86	0.87	0.66	0.79
	Fis	0.06	0.17	-0.04	0.01	0.05	-0.02	-0.03	0.00	0.09	0.02	-0.03	-0.02	-0.02	0.04	0.03	-0.04	-0.02	0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.24	2.37	2.74	5.33	4.95	4.25	5.08	6.32	5.58	4.60	3.36	5.30	4.99	5.89	5.48	5.61	3.26	4.61
	AR, 8 ind	3.84	2.74	3.08	7.63	7.13	6.29	7.15	10.10	8.40	6.00	4.32	7.75	6.89	9.22	7.98	8.75	3.87	6.54
	AN	5.00	5.00	4.00	13.00	15.00	13.00	13.00	21.00	17.00	8.00	7.00	17.00	13.00	21.00	15.00	20.00	5.00	12.47
BLA	Ho	0.68	0.56	0.52	0.81	0.85	0.73	0.88	0.92	0.87	0.84	0.59	0.92	0.82	0.86	0.83	0.89	0.59	0.78
	He	0.68	0.52	0.58	0.87	0.84	0.73	0.85	0.93	0.88	0.82	0.65	0.86	0.85	0.90	0.88	0.88	0.66	0.79
	Fis	0.00	-0.08	0.09	0.07	-0.02	0.00	-0.03	0.01	0.01	-0.02	0.10	-0.07	0.04	0.05	0.06	-0.02	0.10	0.02
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.50	2.04	2.63	4.32	4.36	4.37	3.86	5.25	4.93	3.35	3.37	4.29	4.40	5.22	4.39	5.33	3.77	4.08
	AR, 8 ind	4.12	2.38	3.12	5.42	6.03	5.90	4.76	7.43	6.79	4.05	4.30	6.10	5.73	7.66	5.98	7.46	4.57	5.40
	AN	5.00	3.00	4.00	8.00	9.00	9.00	8.00	11.00	11.00	5.00	7.00	11.00	9.00	13.00	9.00	10.00	5.00	8.06
ALL	Ho	0.62	0.31	0.60	0.83	0.85	0.86	0.83	0.81	0.89	0.66	0.60	0.74	0.71	0.81	0.74	0.90	0.84	0.74
	He	0.71	0.35	0.51	0.81	0.78	0.79	0.76	0.87	0.85	0.67	0.68	0.76	0.81	0.85	0.78	0.88	0.74	0.74
	Fis	0.13	0.11	-0.19	-0.03	-0.10	-0.09	-0.09	0.06	-0.05	0.01	0.12	0.02	0.12	0.06	0.05	-0.03	-0.14	0.00
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.65	2.51	3.29	5.11	4.84	5.04	4.93	6.09	5.97	3.92	4.19	5.95	4.49	5.07	5.25	6.05	3.03	4.67
	AR, 8 ind	3.98	3.00	4.21	6.85	7.25	7.32	6.90	9.37	9.04	5.02	5.73	9.22	6.00	7.62	7.48	9.45	3.72	6.60
	AN	4.00	5.00	6.00	10.00	13.00	13.00	11.00	15.00	14.00	7.00	10.00	16.00	8.00	13.00	10.00	14.00	5.00	10.24
GAR	Ho	0.80	0.43	0.68	0.82	0.83	0.87	0.90	0.96	0.93	0.86	0.72	0.93	0.81	0.81	0.90	0.81	0.81	0.82
	He	0.76	0.57	0.64	0.87	0.81	0.85	0.84	0.92	0.91	0.75	0.77	0.91	0.81	0.83	0.86	0.91	0.59	0.80
	Fis	-0.06	0.25	-0.07	0.05	-0.03	-0.03	-0.07	-0.05	-0.02	-0.15	0.06	-0.03	0.00	0.03	-0.05	0.12	***-0.37***	-0.02
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.70	2.73	2.62	5.16	4.36	4.71	5.87	5.73	5.43	3.94	3.05	5.83	5.28	6.12	4.97	5.84	3.52	4.64
	AR, 8 ind	3.99	3.32	3.41	7.19	6.59	6.59	8.74	8.36	7.89	5.15	4.13	8.77	7.89	9.88	6.50	8.86	3.97	6.54
	AN	4.00	4.00	4.00	9.00	9.00	9.00	11.00	10.00	10.00	6.00	6.00	11.00	10.00	13.00	7.00	11.00	4.00	8.12
DOR	Ho	0.80	0.60	0.47	0.87	0.73	0.80	0.93	0.92	0.93	0.80	0.67	0.87	1.00	1.00	0.92	1.00	0.83	0.83
	He	0.77	0.60	0.45	0.86	0.73	0.83	0.91	0.90	0.88	0.75	0.63	0.90	0.87	0.92	0.86	0.91	0.73	0.79
	Fis	-0.04	0.01	-0.04	0.00	0.00	0.03	-0.03	-0.03	-0.07	-0.07	-0.06	0.04	***-0.16***	***-0.10***	-0.08	***-0.11***	-0.15	-0.05
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.23	2.88	3.54	5.31	5.60	5.05	4.83	6.09	6.15	3.47	3.99	5.80	6.26	6.08	5.94	6.36	3.16	4.93
	AR, 8 ind	3.72	3.66	4.59	7.45	8.25	7.32	6.72	9.80	9.68	4.83	4.83	8.74	9.77	10.02	9.06	10.36	4.09	7.23
	AN	4.00	6.00	6.00	10.00	13.00	12.00	10.00	18.00	16.00	8.00	6.00	15.00	13.00	21.00	15.00	18.00	6.00	11.59
GAV	Ho	0.83	0.52	0.62	0.90	0.86	0.86	0.72	0.86	0.83	0.62	0.59	0.93	0.93	0.79	0.93	0.62	0.62	0.77
	He	0.68	0.62	0.69	0.87	0.89	0.84	0.83	0.91	0.92	0.62	0.78	0.90	0.93	0.91	0.91	0.93	0.60	0.81
	Fis	-0.23	0.16	0.11	-0.03	0.03	-0.02	0.14	0.05	0.10	0.00	0.25	-0.03	-0.01	0.13	-0.02	***0.34***	-0.03	0.06
	Null Allele	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	no	yes	no	
	AR, 4 ind	3.36	2.00	4.07	3.99	4.72	3.76	5.37	5.88	5.66	3.65	4.51	5.32	4.29	3.70	5.06	5.22	2.67	4.31
	AR, 8 ind	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	AN	4.00	2.00	5.00	6.00	6.00	5.00	7.00	7.00	8.00	4.00	6.00	7.00	5.00	5.00	7.00	7.00	3.00	5.53
NIE	Ho	0.88	0.50	0.63	0.75	0.88	0.75	0.88	1.00	0.88	0.63	0.50	0.88	0.71	0.86	1.00	0.71	0.29	0.75
	He	0.69	0.50	0.77	0.74	0.85	0.74	0.89	0.92	0.90	0.76	0.82	0.88	0.81	0.70	0.85	0.86	0.48	0.77
	Fis	-0.29	0.00	0.20	-0.01	-0.03	-0.01	0.02	***-0.09***	0.03	0.19	0.40	0.00	0.13	-0.24	***-0.20***	0.18	0.43	0.04
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	
	AR, 4 ind	3.63	2.47	3.15	5.66	5.14	4.99	3.54	5.88	4.65	3.56	3.61	5.62	5.00	6.40	5.37	6.04	3.61	4.61
	AR, 8 ind	4.37	2.94	3.91	8.19	6.87	6.53	4.60	9.09	6.59	5.19	3.97	8.46	6.89	10.44	7.59	9.23	3.99	6.40
	AN	5.00	4.00	5.00	10.00	8.00	7.00	6.00	12.00	9.00	7.00	4.00	12.00	8.00	14.00	9.00	11.00	4.00	7.94
NIL	Ho	0.59	0.65	0.65	0.88	0.76	0.82	0.76	0.92	0.88	0.65	0.88	0.94	0.92	0.69	0.92	0.75	0.92	0.80
	He	0.73	0.57	0.63	0.90	0.87	0.86	0.71	0.90	0.81	0.61	0.75	0.89	0.85	0.93	0.88	0.92	0.74	0.80
	Fis	0.20	-0.14	-0.03	0.02	0.12	0.04	-0.08	-0.02	-0.08	-0.07	-0.18	-0.06	-0.09	0.27	-0.06	0.19	-0.26	-0.01
	Null Allele	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	no	no	no	
	AR, 4 ind	3.44	2.52	2.85	5.27	5.02	4.96	5.13	5.84	5.70	4.29	3.61	5.05	5.28	5.81	5.20	5.59	3.37	4.64
	AR, 8 ind	4.03	3.00	3.36	7.61	7.18	7.21	7.18	9.07	8.52	5.67	4.61	7.28	7.64	9.13	7.58	8.64	4.21	6.58
	AN	4.47	3.85	4.21	10.65	10.74	10.68	10.62	13.44	13.44	7.21	6.09	11.29	10.65	14.38	10.41	13.44	4.88	9.44
All pop	Ho	0.70	0.51	0.55	0.83	0.81	0.80	0.85	0.92	0.87	0.77	0.67	0.83	0.85	0.84	0.85	0.83	0.70	0.77
	He	0.71	0.54	0.58	0.86	0.84	0.82	0.85	0.90	0.89	0.79	0.69	0.84	0.86	0.89	0.86	0.88	0.66	0.79
	Fis	0.00	0.05	0.06	0.04	0.04	0.03	0.01	-0.02	0.03	0.02	0.03	0.01	0.02	0.06	0.01	0.05	-0.07	0.02

Bayesian individual clustering

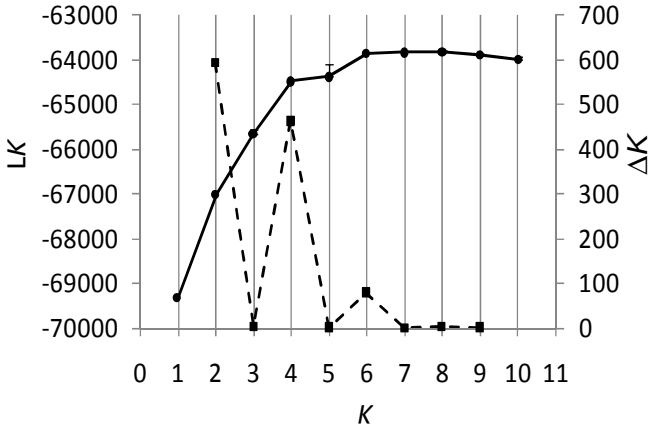

Figure S1: Mean L(K) (±SD) and ΔK over 15 runs for each K from 1 to 11 based on Structure analysis.

Using Structure, the highest likelihood was observed for k=6, while the Δ*k* statistic showed multiple peaks for k = 2, 4 and 6 (Fig S1). The six clusters inferred corresponded to five geographic regions: Upper-Normandy, Lower-Normandy, Brittany, Allier, and Adour (Fig 2). However individuals from Brittany were distributed into two clusters not clearly associated with the spatial locations of populations. A similar analysis with BAPS software (Corander *et al.* 2003) confirmed the existence of five clusters without any sub-structure in Brittany (data not shown). We also separately analyzed samples from Brittany with STRUCTURE without detecting any sub-structure in this region. Therefore, the two clusters from Brittany were pooled into a single one in subsequent analyses.

Three clusters (Brittany, Allier, and Adour) were weakly admixed (Fig 2). In contrast, we detected high contributions from Adour and Brittany clusters into some populations from Lower-Normandy. For instance the contribution of the Brittany clusters to the Couesnon population was 0.49 and contributions of Adour cluster to the

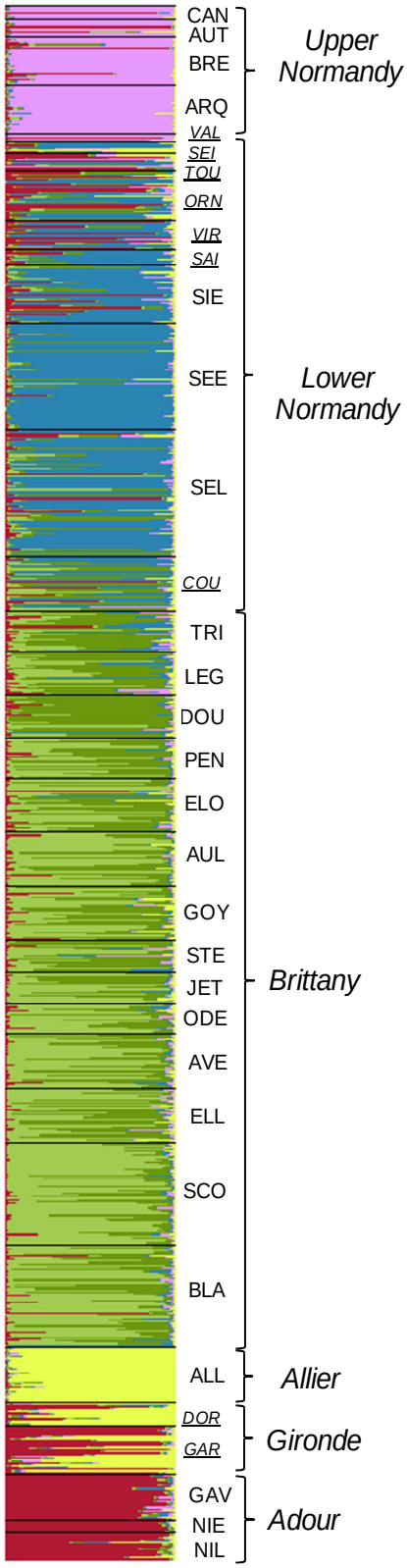

Figure 2: Bayesian individual clustering results with Structure for k=6. Colored bars represent proportions of membership of each individual to each cluster. Populations extensively stocked with non-native fish or recently recolonized are given in italic and underlined.

Touques, Orne, and Vire populations were 0.15, 0.18, and 0.24 respectively (Table S3). In populations from Upper-Normandy, we found variable admixture from the Adour cluster (from 0.01 to 0.54). Garonne and Dordogne populations were almost made of the admixture by the Allier and Adour clusters (0.63 and 0.27 in Garonne and 0.42 and 0.49 in Dordogne, respectively).

Three clusters (Brittany, Allier, and Adour) were weakly admixed (Fig 2). In contrast, we detected high contributions from Adour and Brittany clusters into some populations from Lower-Normandy. For instance the contribution of the Brittany clusters to the Couesnon population was 0.49 and contributions of Adour cluster to the Touques, Orne, and Vire populations were 0.15, 0.18, and 0.24 respectively (Table S3). In populations from Upper-Normandy, we found variable admixture from the Adour cluster (from 0.01 to 0.54). Garonne and Dordogne populations were almost made of the admixture by the Allier and Adour clusters (0.63 and 0.27 in Garonne and 0.42 and 0.49 in Dordogne, respectively).

Table S3: Population admixture estimated with Structure for k=6 clusters.

		Upper-Normandy	Lower-Normandy	Brittany (1)	Brittany (2)	Allier	Adour
Upper-Normandy	CAN	0.837	0.012	0.012	0.009	0.012	0.117
	AUT	0.702	0.007	0.015	0.025	0.023	0.227
	BRE	0.875	0.01	0.022	0.009	0.007	0.078
	ARQ	0.952	0.012	0.007	0.008	0.011	0.009
	VAL	0.429	0.008	0.007	0.006	0.009	0.541
Lower-Normandy	SEI	0.057	0.488	0.065	0.017	0.212	0.162
	TOU	0.302	0.159	0.073	0.025	0.055	0.386
	ORN	0.043	0.392	0.184	0.033	0.057	0.29
	VIR	0.035	0.51	0.057	0.042	0.025	0.331
	SAI	0.013	0.721	0.103	0.042	0.025	0.096
	SIE	0.025	0.679	0.091	0.06	0.022	0.123
	SEE	0.014	0.876	0.057	0.024	0.014	0.016
	SEL	0.016	0.722	0.121	0.06	0.019	0.063
	COU	0.028	0.372	0.376	0.117	0.022	0.085
Brittany	TRI	0.043	0.141	0.52	0.211	0.027	0.057
	LEG	0.035	0.072	0.592	0.239	0.02	0.042
	DOU	0.017	0.053	0.778	0.099	0.016	0.036
	PEN	0.015	0.026	0.476	0.44	0.015	0.028
	ELO	0.02	0.058	0.445	0.388	0.035	0.054
	AUL	0.011	0.011	0.28	0.65	0.016	0.032
	GOY	0.015	0.013	0.432	0.457	0.036	0.047
	STE	0.049	0.043	0.532	0.333	0.013	0.029
	JET	0.012	0.023	0.37	0.559	0.02	0.016
	ODE	0.018	0.03	0.259	0.643	0.017	0.032
	AVE	0.015	0.019	0.296	0.633	0.014	0.023
	ELL	0.03	0.011	0.423	0.493	0.026	0.016
	SCO	0.012	0.014	0.193	0.753	0.011	0.017
	BLA	0.014	0.037	0.313	0.577	0.011	0.047
Allier	ALL	0.008	0.008	0.012	0.01	0.951	0.011
Gironde	DOR	0.029	0.022	0.024	0.019	0.419	0.489
	GAR	0.02	0.034	0.034	0.017	0.629	0.266
Adour	GAV	0.028	0.022	0.013	0.044	0.014	0.879
	NIE	0.009	0.011	0.013	0.02	0.011	0.936
	NIL	0.009	0.024	0.032	0.023	0.059	0.852

Divergence among populations

F_{ST} among the 34 populations was 0.043 (CI: 0.038-0.049) and pairwise F_{ST} ranged from -0.006 to 0.162 (Table S4). F_{ST} was low and often not significant among populations from the same cluster (105 values out of 166 were not significant). Pairwise F_{ST}s including the Allier population were particularly high, ranging from 0.09 to 0.16. The AMOVAs revealed a significant and higher proportion of the total genetic variance among clusters than among populations within clusters (4.6% and 1.0% respectively, Table 2). When removing Garonne and Dordogne, and when considering the subset of 25 'unstocked' populations, the proportions of variance among groups (4.8% and 5.7% respectively) were higher and the proportions of variance within groups (0.8% and 0.5% respectively) were lower than those

Table S4: Pairwise Fst (above diagonal, non significant values in bold) and coastal distances among populations.

		CAN	AUT	BRE	ARQ	VAL	SEI	TOU	ORN	VIR	SAI	SIE	SEE	SEL	COU	TRI	LEG	DOU	PEN	ELO	AUL	GOY	STE	JET	ODE	AVE	ELL	SCO	BLA	ALL	DOR	GAR	GAV	NIE	NIL
Upper-Normandy	CAN		***0.01***	***0.01***	***0.02***	***0.01***	***0.08***	***0.02***	0.06	0.06	***0.09***	0.07	0.08	0.06	0.06	0.06	0.07	0.08	0.08	0.08	0.06	0.08	***0.06***	***0.08***	***0.08***	0.08	0.07	0.08	0.08	0.14	0.09	0.09	0.09	***0.12***	***0.11***
	AUT	19		***0.01***	***0.01***	***0.02***	***0.06***	***0.02***	0.06	***0.06***	***0.06***	0.07	0.08	0.06	0.07	0.06	***0.05***	0.06	0.08	0.08	0.06	0.07	***0.05***	***0.08***	***0.07***	0.07	0.06	0.07	0.06	0.15	0.10	***0.09***	***0.09***	***0.10***	***0.10***
	BRE	57	38		***<0,01***	***0.03***	0.09	***0.04***	0.07	0.08	0.09	0.08	0.10	0.08	0.08	0.07	0.08	0.09	0.09	0.09	0.07	0.09	0.08	0.09	0.08	0.08	0.07	0.08	0.07	0.16	0.12	0.10	0.10	0.12	0.12
	ARQ	82	63	25		***0.03***	0.09	***0.04***	0.08	0.09	0.10	0.09	0.10	0.08	0.09	0.08	0.08	0.09	0.10	0.10	0.08	0.10	0.08	0.10	0.09	0.09	0.08	0.09	0.08	0.16	0.12	0.11	0.11	***0.13***	***0.13***
	VAL	138	119	81	56		***0.04***	***<0,01***	0.03	***0.03***	***0.05***	***0.05***	0.06	0.04	0.06	***0.05***	***0.05***	0.05	***0.06***	0.07	0.05	***0.06***	***0.06***	***0.07***	***0.06***	0.07	0.07	0.06	0.06	0.12	***0.07***	***0.06***	***0.05***	***0.08***	***0.07***
Lower-Normandy	SEI	185	166	128	103	47		***0.01***	***0.02***	***0.01***	***<0,01***	0.02	0.03	0.02	0.04	0.03	0.03	0.04	0.04	0.05	0.03	0.05	***0.03***	***0.04***	0.05	0.05	0.05	0.04	0.04	0.10	0.06	0.04	0.06	***0.06***	0.07
	TOU	193	174	136	111	55	8		***0.01***	***0.01***	***0.02***	0.02	0.03	0.02	0.02	***0.03***	***0.02***	0.03	0.03	0.04	***0.02***	0.03	***0.02***	***0.03***	0.03	0.03	0.04	0.03	0.04	0.09	***0.04***	0.03	***0.03***	***0.05***	***0.04***
	ORN	218	199	161	136	80	33	25		***<0,01***	***0.01***	<0,01	0.02	0.01	0.01	0.02	0.02	0.02	0.03	0.03	0.02	0.02	***0.02***	0.03	0.03	0.03	0.03	0.03	0.03	0.08	0.04	0.03	0.03	0.05	0.04
	VIR	284	265	227	202	146	99	91	66		***<0,01***	***<0,01***	0.01	***0.01***	0.02	***0.01***	0.01	0.03	0.03	0.04	0.02	0.03	0.02	0.03	0.04	0.03	0.04	0.03	0.04	0.09	0.03	0.02	***0.02***	***0.04***	***0.03***
	SAI	315	296	258	233	177	130	122	97	31		0.02	***0.01***	***0.01***	***0.02***	***0.02***	***0.02***	***0.02***	0.03	0.04	***0.03***	0.03	***0.02***	***0.03***	***0.03***	0.03	0.03	0.04	0.04	0.12	***0.07***	0.05	***0.05***	***0.06***	0.06
	SIE	465	446	408	383	327	280	272	247	181	150		***<0,01***	***<0,01***	0.01	0.02	0.02	0.03	0.04	0.05	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.09	0.05	0.04	0.04	0.06	0.05
	SEE	505	486	448	423	367	320	312	287	221	190	40		***<0,01***	0.02	0.03	0.03	0.04	0.05	0.06	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.11	0.07	0.06	0.07	0.08	0.08
	SEL	505	486	448	423	367	320	312	287	221	190	40	0		0.01	0.02	0.02	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.03	0.10	0.06	0.05	0.05	0.07	0.06
	COU	508	489	451	426	370	323	315	290	224	193	43	3	3		0.01	0.01	0.03	0.02	0.03	0.01	0.01	***0.01***	0.01	0.03	0.02	0.03	0.02	0.02	0.08	0.05	0.04	0.05	0.07	0.05
Brittany	TRI	649	630	592	567	511	464	456	431	365	334	184	144	144	141		***<0,01***	***0.01***	***<0,01***	0.02	***<0,01***	0.01	***<0,01***	***0.01***	***0.01***	0.01	0.02	0.01	0.01	0.09	0.05	0.04	***0.05***	***0.06***	0.05
	LEG	716	697	659	634	578	531	523	498	432	401	251	211	211	208	67		***0.01***	***0.01***	0.02	***0.01***	***0.01***	***<0,01***	***0.02***	***0.01***	0.01	0.01	0.01	0.01	0.10	0.06	0.04	0.05	0.06	0.05
	DOU	726	707	669	644	588	541	533	508	442	411	261	221	221	218	77	10		0.02	0.02	***0.01***	0.01	***0.01***	***0.02***	***0.01***	0.02	0.01	0.02	0.01	0.10	0.07	0.05	0.06	0.07	0.06
	PEN	755	736	698	673	617	570	562	537	471	440	290	250	250	247	106	39	29		***0.01***	***<0,01***	***<0,01***	***<0,01***	***0.01***	***<0,01***	***<0,01***	***0.01***	0.01	***0.01***	0.10	0.06	0.05	0.05	***0.06***	0.05
	ELO	881	862	824	799	743	696	688	663	597	566	416	376	376	373	232	165	155	126		***0.01***	***0.01***	***0.01***	***0.01***	***<0,01***	***0.01***	***0.01***	0.01	***0.01***	0.11	0.08	0.07	0.07	0.08	0.07
	AUL	903	884	846	821	765	718	710	685	619	588	438	398	398	395	254	187	177	148	22		***<0,01***	***<0,01***	***<0,01***	***<0,01***	***<0,01***	***0.01***	0.01	***0.01***	0.09	0.05	0.04	0.05	***0.06***	0.05
	GOY	966	947	909	884	828	781	773	748	682	651	501	461	461	458	317	250	240	211	85	63		***-0.01***	***<0,01***	***<0,01***	***<0,01***	***<0,01***	<0,01	***<0,01***	0.09	0.05	0.05	0.05	0.06	0.05
	STE	1018	999	961	936	880	833	825	800	734	703	553	513	513	510	369	302	292	263	137	74	52		***<0,01***	***<0,01***	***<0,01***	***0.01***	***<0,01***	***<0,01***	0.11	0.06	0.05	0.05	***0.07***	0.05
	JET	1018	999	961	936	880	833	825	800	734	703	553	513	513	510	369	302	292	263	137	74	52	0		***<0,01***	***<0,01***	***0.01***	***0.01***	***<0,01***	0.10	***0.06***	0.06	0.06	***0.08***	0.07
	ODE	1018	999	961	936	880	833	825	800	734	703	553	513	513	510	369	302	292	263	137	74	52	0	0		***<0,01***	***<0,01***	***<0,01***	***<0,01***	0.11	0.07	0.06	0.06	***0.07***	0.07
	AVE	1052	1033	995	970	914	867	859	834	768	737	587	547	547	544	403	336	326	297	171	108	86	34	34	34		***<0,01***	<0,01	***<0,01***	0.11	0.07	0.06	0.06	0.07	0.06
	ELL	1069	1050	1012	987	931	884	876	851	785	754	604	564	564	561	420	353	343	314	188	125	103	51	51	51	17		***<0,01***	***<0,01***	0.11	0.08	0.07	0.07	0.08	0.08
	SCO	1087	1068	1030	1005	949	902	894	869	803	772	622	582	582	579	438	371	361	332	206	143	121	69	69	69	35	18		***<0,01***	0.11	0.07	0.06	0.06	0.07	0.07
	BLA	1087	1068	1030	1005	949	902	894	869	803	772	622	582	582	579	438	371	361	332	206	143	121	69	69	69	35	18	0		0.11	0.07	0.06	0.06	0.07	0.07
Allier	ALL	1197	1178	1140	1115	1059	1012	1004	979	913	882	732	692	692	689	548	481	471	442	316	253	231	179	179	179	145	128	110	110		0.03	0.05	0.10	0.12	0.09
Gironde	DOR	1412	1393	1355	1330	1274	1227	1219	1194	1128	1097	947	907	907	904	763	696	686	657	531	468	446	394	394	394	360	343	325	325	215		***0.01***	0.05	***0.08***	0.05
	GAR	1412	1393	1355	1330	1274	1227	1219	1194	1128	1097	947	907	907	904	763	696	686	657	531	468	446	394	394	394	360	343	325	325	215	0		0.03	***0.03***	0.03
Adour	GAV	1642	1623	1585	1560	1504	1457	1449	1424	1358	1327	1177	1137	1137	1134	993	926	916	887	761	698	676	624	624	624	590	573	555	555	445	230	230		***0.03***	0.02
	NIE	1642	1623	1585	1560	1504	1457	1449	1424	1358	1327	1177	1137	1137	1134	993	926	916	887	761	698	676	624	624	624	590	573	555	555	445	230	230	0		***0.04***
	NIL	1662	1643	1605	1580	1524	1477	1469	1444	1378	1347	1197	1157	1157	1154	1013	946	936	907	781	718	696	644	644	644	610	593	575	575	465	250	250	20	20	

Table 2: Results of AMOVAs for a) the 34 populations grouped in six clusters by geographic location (those from figure 2), b) 32 populations grouped in five clusters excluding Garonne and Dordogne populations, and c) 25 populations considered as lowly introgressed by stocking.

	% of variation explained		
	a) n=34	b) n=32	c) n=25
Among groups	4.6	4.8	5.7
Among populations within groups	1.0	0.8	0.5
Within populations	94.4	94.3	93.8

obtained with the entire dataset. The Neighbourg-Joining phylogram confirmed the structuration into five clusters. However, in this analysis Touques and Couesnon populations were not found in the Lower-Normandy cluster but respectively closer to the Upper-Normandy and Brittany clusters (Fig 3). Garonne and Dordogne populations were closer to the Allier than to the Adour cluster.

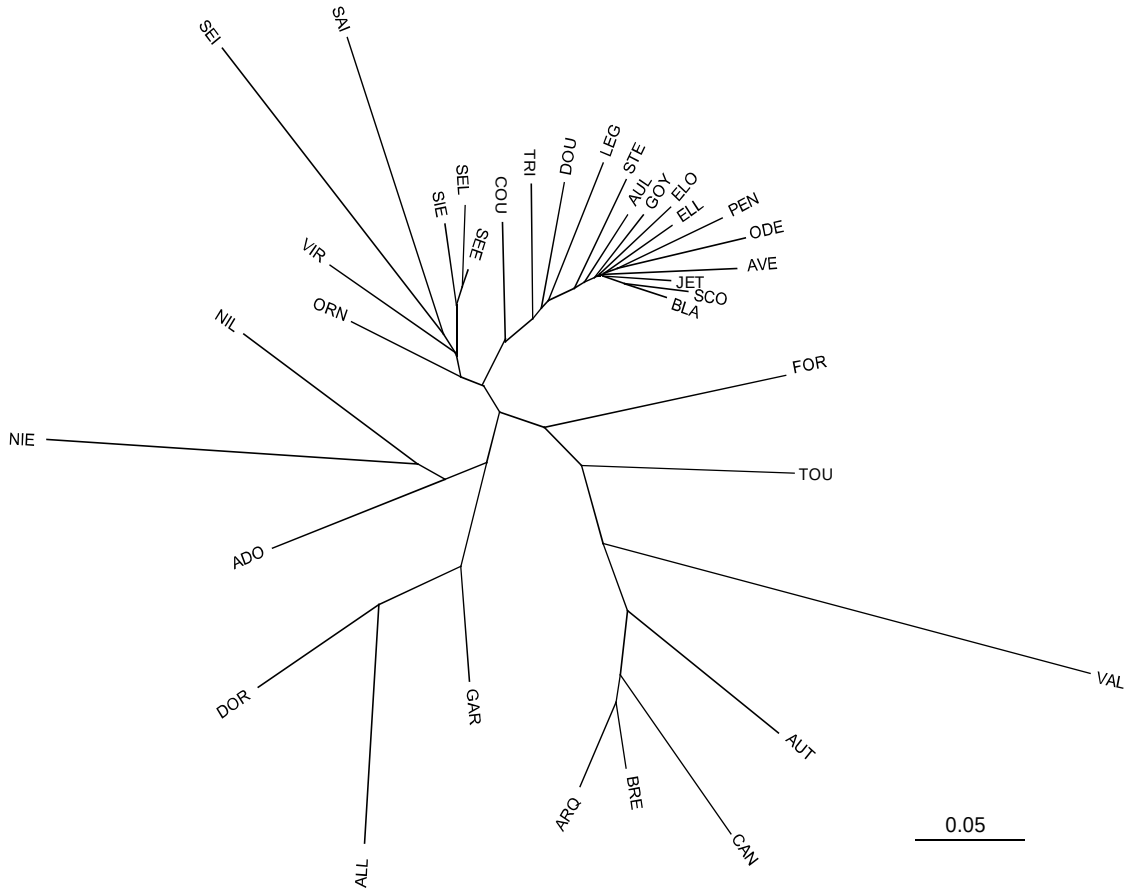

Figure 3: Neighbor-Joining tree based on Nei genetic distances among the 34 populations.

Spatial analyses

Using the subset of 25 populations not introgressed by non-native stocks, we found a significant pattern of isolation by distance with both coastal distance and river length being highly correlated with genetic distance (Table 3, fig 4). The best model included coastal

distance with river length kept constant (r = 0.80). When we excluded the Allier population, the effect of river length was not significant anymore while coastal distance still had a significant effect (Table 3). We also detected only a significant effect of coastal distance when considering populations from one single cluster, Brittany (Table 3, fig 4). Pairwise genetic distances including the Allier population were higher than most other pairwise comparisons and we also found a significant effect of coastal distance for this particular set of comparisons (Fig 4). Using BIMr we also found a significant impact of coastal distance and river length on recent migration rates (data not shown) with the highest posterior probability (0.78) obtained for the model containing both coastal distance and river length.

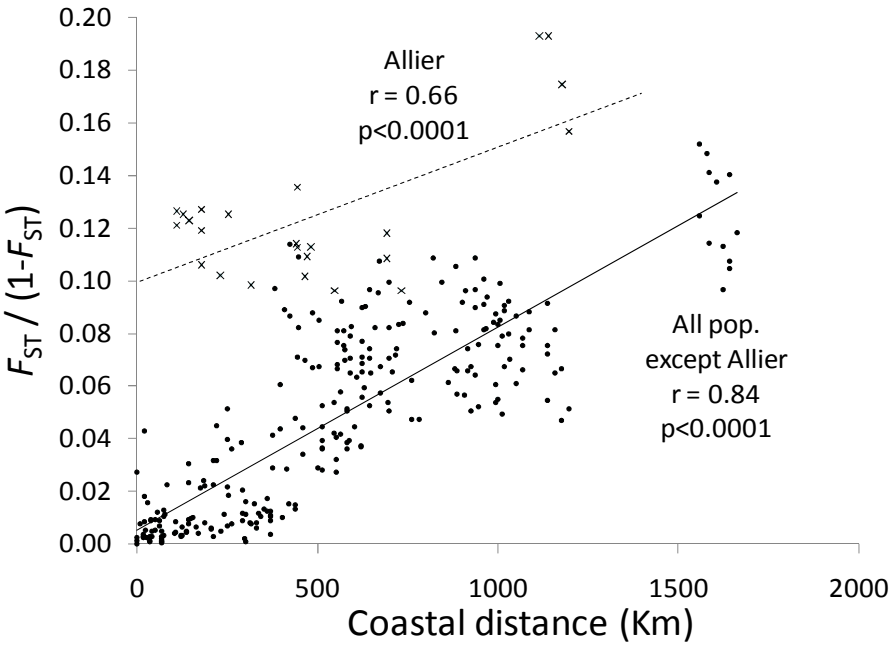

Figure 4: Pairwise genetic distances (FST/(1- FST)) as a function of coastal distance for a subset of 25 populations (see methods). Pairs only including the Allier population (crosses) or excluding this population (black circles) are presented.

At the individual level, we found positive and significant autocorrelation coefficients for the 50 km and 100 km distance classes, intercepting the *x*-axis at 145 km (Figure S2). Consistently negative coefficients were observed from the 400-km size class and above.

Discussion

A hierarchical genetic structure into five clusters was observed among the 34 Atlantic salmon populations considered in this study. Some populations were genetically admixed with populations from other clusters. This pattern is concordant with the origin of individuals used for supplementation, indicating an introgression linked to stocking operations. The five clusters were highly differentiated and corresponded to different geographic regions. A low

differentiation was found within clusters even between relatively distant populations, suggesting high gene flow. Spatial analyses revealed significant influences of coastal distance and river length on genetic structure and recent migration rates. The Allier population was highly differentiated from all other populations, constituted a single cluster and the significant effect of river length was mainly due to this population.

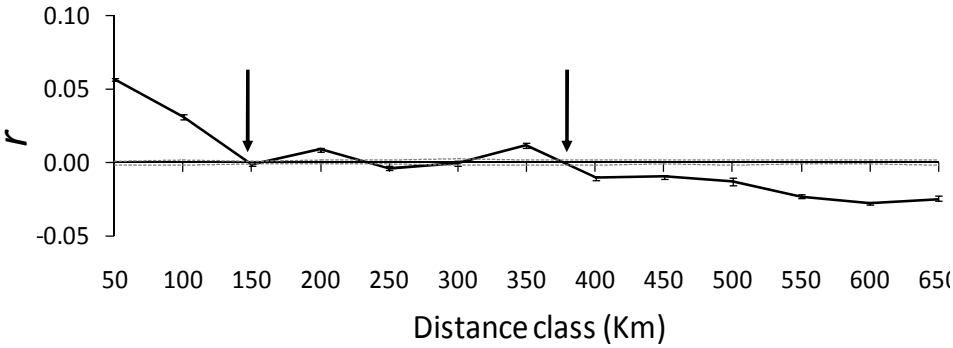

Figure S2: Individual autocorrelation analysis of the genetic correlation (r) as a function of various distance classes. Dotted lines indicates 95% CI around the null hypothesis of no genetic structure, and error bars indicate 95% CI determined by permutations on r. Results are given for a subset of 25 populations (see methods).

Spatial genetic structure

The level of genetic differentiation among our study populations was moderate to high, similar to those previously observed in East Atlantic populations (King *et al.* 2001; Saisa *et al.* 2005; Verspoor *et al.* 2005; Grandjean *et al.* 2009; Tonteri *et al.* 2009). The proportion of genetic variance among the five clusters was much higher than among populations within clusters suggesting high gene flow within clusters. Interestingly, geographic distances between populations from the same cluster could be much higher than between populations from different clusters. Such a hierarchical genetic structure has been described among Atlantic salmon populations from Baltic sea (Saisa *et al.* 2005), Russia (Tonteri *et al.* 2009) and Quebec (Dionne *et al.* 2008). However, the level of variation among clusters we observed was higher than those reported in these studies.

The level of structure found in Upper-Normandy and Lower-Normandy clusters was low suggesting a metapopulation structure and (or) recent recolonizations of some rivers by neighboring populations (Grandjean *et al.* 2009; Perrier *et al.* 2010). The genetic structure among Brittany populations was also low despite the large spatial distribution of this cluster (up to 438 km among populations). We suppose that the significant IBD combined with the high relative number of individuals found in this cluster, may explain why STRUCTURE built two clusters in Brittany, which were not clearly geographically differentiated. Indeed, recent

studies demonstrated that Bayesian clustering methods generally overestimate genetic structure in cases of isolation by distance (Frantz *et al.* 2009) (Schwartz & McKelvey 2009). Such a pattern of low genetic structure had already been reported but only among neighboring salmon rivers (Dionne *et al.* 2008) (Tonteri *et al.* 2009) or within river systems with large populations (Dionne *et al.* 2009). The weak differentiation and the relatively small sizes of Brittany populations suggests high gene flow and a metapopulation structure (Rieman & Dunham 2000). These results may also indicate that the homing behaviour may be relaxed and dispersal favored among rivers having similar geological substrates, due to the difficulty of discriminating the natal river in such situations. The recognition of specific river odors partly resulting from geological nature of the stream bed, could indeed be an important mechanism allowing salmon to return in their natal river (reviewed in (Stabell 1984) (Dittman *et al.* 1996). However, homing is known to be lower in stocked fish compared to wild individuals (Quinn 1993; Jonsson *et al.* 2003), so we cannot exclude that local fish stocked in Aulne and Elorn may have strayed among other Brittany rivers leading to a decrease of genetic differentiation.

Natural recolonization and management impact

Natural recolonizations of Authie, Valmont, Seine, Touques and Saire were suggested by high levels of heterozygosity, bottleneck evidence on Saire, and admixture of some of these populations by several clusters. Owing to the straying of some salmon out of their natal river (Stabell 1984) (Jonsson *et al.* 2003), recolonization of depopulated but restored river systems can occur from nearby populations (Vasemagi *et al.* 2001) (Grandjean *et al.* 2009) but also from distant stocks (Perrier *et al.* 2010).

Hatchery-reared fish used for stocking have generally lower genetic diversity (Verspoor 1988a; Aho *et al.* 2006; Machado-Schiaffino *et al.* 2007; Karlsson *et al.* 2010) and higher straying rates (Quinn 1993; Jonsson *et al.* 2003) than wild fish. However, native stocking in Elorn, Aulne and Gave d'Oloron did not appear to have lowered genetic diversity of these populations. In contrast, the allelic richness and heterozygosity of Allier were slighlty lower than in other populations and could suggest a stocking impact. However, analyses of 30 years old samples from this river did not provide different estimates of genetic diversity (Perrier et al., unpublished data).

Introgression of some populations following stocking with non-native fish was supported by moderate to high levels of admixture with donor populations. The Orne, Sélune,

and Couesnon populations were stocked with fish originating from genitors caught in Aulne and Gave d'Oloron and were strongly admixed with them. Such examples of introgression by stocked non-native fish have been described in Atlantic salmon (Finnegan & Stevens 2008) (Campos *et al.* 2008). Populations close to stocked rivers also showed relatively high introgression rates (*e.g.* Vire or Sienne), suggesting straying of stocked fish (Quinn 1993; Jonsson *et al.* 2003; Pedersen *et al.* 2007) or hybridization between local and stocked fish into these rivers, a similar pattern was described in. Dordogne and Garonne populations had gone through extinction before being stocked using genitors from Allier and Gave d'Oloron. Accordingly they appeared as an admixture between the two donor clusters.

Isolation by distance, coastal distance and river length

Our analyses identified coastal distance as the main factor influencing genetic differentiation in Atlantic salmon populations. This finding is consistent with previous observations on Atlantic salmon populations from across the species range (King *et al.* 2001), Eastern Atlantic (Verspoor *et al.* 1999),Western Atlantic (McConnell *et al.* 1997), Northern Atlantic (Tonteri *et al.* 2009), Baltic sea (Saisa *et al.* 2005) and at smaller geographic scale, among Irish (Dillane *et al.* 2007) or some French populations (Grandjean *et al.* 2009). IBD was significant for the subset of 25 populations, and among the 14 populations from Brittany. However, while populations from Brittany were distributed along a large area, their genetic differentiation was relatively low. In contrast, the differentiation of Allier population was higher than predicted by IBD. Other studies also reported such cases of populations uncommonly highly differentiated from neighbouring ones (Primmer *et al.* 2006; Dillane *et al.* 2007; Tonteri *et al.* 2009).

Dillane *et al.* (2007) showed that IBD among several salmon populations from Ireland was obscured by some samples probably affected by cultured salmon. While stocking operations using non-local fish were performed in Allier from the 50s to the 80s, an analysis of old samples (from 1965 to 1967) from this population suggested little genetic modifications and thus low impact of such practices (Perrier et al., unpublished data). Primmer *et al.* (2006) found a sample of the Varzuga River unusually more differentiated than predicted by IBD, and suggested a higher genetic drift resulting from low Ne and potential bottleneck events. In the case of the Allier River, we did not found evidence of bottleneck or particularly low population size (≈500 individuals, Com. Pers. LOGRAMI). Primmer *et al.* (2006) also showed a negative association between genetic diversity and distance from river mouth, suggesting low upstream gene flow. We found a significant influence of river length

on the differentiation and recent gene flow among populations. The effect of river length was no more significant when Allier was excluded from the analyses. Spawning grounds of the Allier River are located far away from the sea, up to 700 km, leading to high upstream migration difficulty. Atlantic salmon usually begin their homing run in the Allier River several months earlier than in other rivers (Bachelier 1963). Moreover, the Allier population is mainly composed of multi-sea-winter fish, which are biggest and run earlier, contrary to other French populations (respectively 95% and from 13% to 19 % on average). Some studies suggested high heritability of morphological traits (Riddell *et al.* 1981) or sea age at maturity (Naevdal *et al.* 1978). Furthermore, several studies showed a positive correlation between the proportion of multi-sea-winter fish and river length and the water discharge or difficulty to upstream migration (Schaffer & Elson 1975) (Power 1981) (Dionne *et al.* 2008). Overall these results suggest a phenotypic and behavioral adaptation of Atlantic salmon in the Allier River in response to the high difficulty to upstream migration in this river.

To conclude, our results show a hierarchical genetic structure among Atlantic salmon populations with a strong isolation by distance. We also found important impacts of stocking using non-local fish on the distribution of genetic diversity within and among populations. Finally, the unique characteristics of the Allier River and of its salmon population suggest a local adaptation to long upstream migration. Overall, this study provides important information for the conservation management of salmon populations, in particular the importance of preserving local genetic diversity. Future studies focusing on adaptive genetic variation in the Allier population could help understanding the mechanisms of local adaptation and provide some clues to preserve such distinctive populations.

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Chapitre IV. Evolution récente de la structure génétique des populations : effets des repeuplements

L'objectif général de ce chapitre est d'analyser l'évolution récente de la diversité génétique des populations françaises de saumon via l'analyse comparée d'échantillons récents (1998-2006) et relativement anciens (1965-1985). Il est notamment question d'évaluer l'impact des repeuplements anciens et récents sur la structure génétique entre et au sein des populations.

La structure génétique des populations de saumon est relativement stable sur le court-terme. Cependant les pratiques de repeuplement, courantes chez les salmonidés, sont à même de modifier la structure génétique des populations. Les résultats exposés dans le chapitre IV montrent des taux d'introgression variables de populations repeuplées avec des poissons allochtones. Cependant, afin de statuer précisément sur le rôle des repeuplements quant à l'état actuel des populations, il est nécessaire de réaliser des analyses sur des échantillons anciens, si possible avant repeuplement. Similairement, afin d'étudier l'effet de repeuplement ayant été opéré par la passé, il peut s'avérer utile d'analyser des échantillons récent et des échantillons anciens, contemporains de ces repeuplements.

Afin d'étudier l'influence des repeuplements sur la structure génétique des populations françaises de saumon Atlantique, nous avons génotypé à 16 marqueurs microsatellites 1627 poissons issus de 34 rivières et provenant des cohortes anciennes et récentes (1965-1987 et 1998-2006). Nous avons également analysé des individus issus de plusieurs rivières écossaises.

Nos analyses classent les individus dans six groupes génétiquement et géographiquement distincts. Un groupe correspond aux poissons écossais, les cinq autres correspondent aux cinq groupes français précédemment exposés dans le chapitre IV. Des analyses de variance moléculaire ont révélé une plus grande différenciation entre les échantillons anciens qu'entre les échantillons récents, à la fois au sein et entre les groupes, suggérant une homogénéisation génétique des populations suite aux repeuplements. Cependant, la diversité génétique ne semble pas avoir évolué de la même façon dans toutes les populations. La diversité génétique des populations non repeuplées ou repeuplées avec des poissons autochtones ne semble pas, en général, avoir beaucoup évolué. Au contraire, les populations repeuplées avec des individus non-natifs montrent des changements variables de diversité. Celles repeuplées récemment montrent des taux d'admixture importants et une réduction des taux de différenciation avec les populations d'où provenaient les individus déversés. Celles repeuplées « historiquement » montrent des taux d'admixture « historiques »

variables et des taux récent beaucoup plus faibles, ce qui pourrait suggérer une fitness des individus relâchés, et un succès des repeuplements, faibles.

Ces résultats ont d'importantes implications pour la gestion des populations, notamment en termes de repeuplement. Ils montrent la stabilité de la structure génétique des populations de saumon non soumises aux repeuplements ou à des repeuplements utilisant des poissons autochtones. Au contraire, ils montrent que les repeuplements utilisant des poissons allochtones sont à même de modifier la diversité génétique au sein des populations repeuplées et entre ces dernières et celles utilisées comme sources de repeuplement. Ces résultats suggèrent néanmoins une résilience relativement rapide des caractéristiques génétique originelle, suggérant des phénomènes de sélection contre les individus non-natifs et non adaptés localement.

Temporal variation in genetic structure among Atlantic salmon populations: Impacts of old and contemporary stocking revealed by old DNA microsatellite analyses

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Abstract

Supplementation of wild populations with captive-bred individuals is a common practice that can have major consequences on the genetic makeup of recipient populations. Here we investigated the influence of stocking on the evolution of genetic structure among 34 French Atlantic salmon (*Salmo salar*) populations. We genotyped 1627 individuals from old and recent cohorts (1965-1987 and 1998-2006) at 16 microsatellite loci. Bayesian analyses clustered the individuals into five genetically and geographically distinct groups in a similar way for old and recent samples. An analysis of molecular variance revealed a higher differentiation among and within groups for old compared to recent samples (6.85% versus 4.99% among groups and 1.53% versus 0.68% within groups, respectively). We observed few changes in genetic diversity within populations non-stocked or stocked with native fish. In contrast, recent samples from populations stocked with non-native individuals were strongly admixed (up to 62%) with the clusters of origin of the donor populations. Accordingly the genetic differentiation decreased between donor and recipient populations compared to old samples. In contrast we detected few effects of old stocking operations on the genetic structure of old samples suggesting short term effects of these practices and / or short term detection abilities of microsatellite markers. Overall, these results demonstrate that stocking can influence the distribution of genetic diversity within and among salmon populations at multiple spatial and temporal scales.

Key words: *Salmo salar*, population genetics, temporal stability, stocking, conservation

Introduction

Supplementation of declining wild populations with individuals from a distant location or individuals reared in captivity is a common management practice of vertebrate populations (Maccrimmon & Gots 1979; Hindar *et al.* 1991; Levin *et al.* 2001; Aprahamian *et al.* 2003). Fish populations are frequently supplemented with captive-bred individuals to restore declining populations and / or enhance the number of catches for sportive angling. However, stocking operations can produce important changes in the genetic diversity of recipient populations. First, supportive breeding using native stock could result in a loss of genetic diversity and reductions in effective population size (Verspoor 1988a; Ryman & Laikre 1991; Gaffney *et al.* 1996; Aho *et al.* 2006; Horreo *et al.* 2008; Small *et al.* 2009). Second, stocking a population with genetically differentiated fish can lead to partial introgression or even complete replacement of native populations (Hansen 2002; Campos *et al.* 2008; Finnegan & Stevens 2008; Sonstebo *et al.* 2008; Hansen *et al.* 2009). Genetic introgression can induce major changes in genetic structure among populations (Ayllon *et al.* 2006; Marie *et al.* 2010) or subpopulations within a large population (Finnengan & Stevens 2008; Eldridge *et al.* 2009). Finally, stocking could greatly impact non-target populations due to the frequent straying of hatchery-reared fish (Quinn 1993; Jonsson *et al.* 2003). All these modifications of genetic diversity could provoke a fitness reduction in the local population and a loss of local adaptation resulting from the transfer of misadapted fish (Taylor 1991; Araki *et al.* 2007; Garcia de Leaniz *et al.* 2007) selected in hatchery for behavioral and physiological traits that are disadvantageous in nature (Fleming & Einum 1997; Ford *et al.* 2008; Neregard *et al.* 2008; Darwish & Hutchings 2009; Lawlor *et al.* 2009).

In the case of Atlantic salmon (*Salmo salar*) populations that are highly structured throughout their native range (Verspoor *et al.* 2005; Lehtonen *et al.* 2009; Tonteri *et al.* 2009), fish stocked from genetically distant stocks are traceable and their impact on wild populations genetic structure can be estimated (Campos *et al.* 2008; Finnegan & Stevens 2008; Hansen *et al.* 2009). Furthermore, large fish scales collections sometimes exist and are of great interest to finely track temporal evolution of genetic structure of populations subject to stocking (Nielsen *et al.* 1999; Tessier & Bernatchez 1999; Nielsen & Hansen 2008; Hansen *et al.* 2009). While major modifications of genetic structure in stocked populations were frequently detected, some studies suggested relatively low and brief impacts of stocking. For instance, low impacts of intensive stocking were reported on the genetic structure of Brown Trout and some Atlantic salmon populations (Hansen 2002; Santos *et al.* 2006; Caudron *et al.* 2009; Hansen *et al.* 2009) ; (Tessier & Bernatchez 1999; Ciborowski *et al.* 2007; Finnengan

& Stevens 2008). Despite these various results, there is a large body of data demonstrating that hatchery-reared fish do not perform well in nature, or not as well as their wild congeners (Fleming & Einum 1997; Araki *et al.* 2007; Ford *et al.* 2008; Fraser 2008; Neregard *et al.* 2008; Darwish & Hutchings 2009). However, most studies were carried out at a local scale in few populations and during a short time period. In addition, the fate of one stocking strain was generally investigated while in many situations several stocks are used to supplement wild populations.

Atlantic salmon populations from France represent an ideal case study to investigate the admixture between wild and stocked fish at multiple spatial and temporal scales. Several supplementation programs using a variety of non-local individuals have been performed from the 1950s to the 1980s ((Baglinière & Dumas 1988; Baglinière *et al.* 1990; Vauclin 2007; Grandjean *et al.* 2009); Perrier et al., in prep). In the 1990s, stocking programs were mainly using local fish except in a group of population from Lower-Normandy in which non-local individuals from Brittany have been stocked. Various admixture rates were documented in French populations suggesting variable impacts of stocking ((Grandjean *et al.* 2009); Perrier et al., in prep). However, these studies were solely based on genetic analyses of recent samples collected a long time after the beginning of stocking operations. The consequences of ancient stocking operations (from the 1950s to the 1980s) remain unknown and require the analysis of old samples contemporary to these management actions to be investigated. Thanks to scientific and management programs initiated in the 1960s by INRA (French National Institute for Agronomic Research) and ONEMA (French National Agency for Water and Aquatic Environments) a large amount of salmon scales have been collected for most of the French rivers, allowing comparative analyses of past and recent samples.

In this study, we genotyped old (up to 40 years) and contemporary samples of adult Atlantic salmon at 16 microsatellite loci to investigate the impacts of both ancient and recent stocking practices. We aimed at i) infer the admixture rates between wild and stocked fish using pre- and post-stocking samples in populations supplemented with different stocks, ii) compare the differentiation among populations before and after stocking, iii) test whether a decrease of genetic diversity is observed in populations stocked with local individuals, iv) investigate whether non-target populations are also introgressed by stocked fish from other rivers and v) infer whether stocking has short-term or long-lasting effects on the genetic diversity of wild populations.

Table 1: Sampling and genetic diversity details for the 34 French sampling sites chosen.

Location	Old cohorts				Recent cohorts			
	Abbreviation	Cohorts	Sample size	Stocking from 1950 to 1988	Abbreviation	Cohorts	Sample size	Stocking from 1989 to 2003
Scotland	FOR80	1980	23	-	-	-	-	-
Canche	-	-	-	-	CAN03	1999-2006	23	-
Authie	-	-	-	-	AUT03	2003-2006	8	-
Bresle	BRE68	1968	19	Scotland	BRE03	1998-2004	29	-
Arques	-	-	-	Scotland	ARQ03	2003	31	-
Valmont	-	-	-	-	VAL03	2003-2005	5	-
Seine	-	-	-	-	SEI03	1998-2006	7	-
Touques	-	-	-	-	TOU03	19982006	12	-
Orne	-	-	-	Scotland, Sélune	ORN03	2001	31	Gave d'Oloron
Vire	-	-	-	Scotland	VIR03	1998-2004	19	-
Saire	-	-	-	-	SAI03	2005-2006	9	-
Sienne	SIE86	1985-1987	35	-	SIE03	2002-2003	36	Aulne
Sée	SEE77	1977-1978	61	-	SEE03	2002-2003	66	Aulne
Sélune	SEL77	1977-1978	39	-	SEL03	2002-2003	80	Aulne and Gave d'Oloron
Couesnon	COU82	1978-1986	11	Sélune	COU03	2002-2003	34	Aulne and Gave d'Oloron
Trieux	TRI77	1968-1981	17	-	TRI03	2002	16	-
Leguer	LEG77	1976-1977	22	-	LEG03	2002-2003	16	-
Douron	DOU82	1978-1984	29	-	DOU03	2002-2003	27	-
Penzé	PEN78	1969-1982	25	-	PEN03	2002-2003	23	-
Elorn	ELO75	1969-1970	18	Scotland, local	ELO03	2003	30	local
Aulne	AUL69	1969-1984	30	Scotland, local	AUL03	2003	31	local
Goyen	GOY81	1972-1984	33	-	GOY03	2003	24	-
Steir	STE72	1971-1972	21	-	STE03	2002	14	-
Jet	JET72	1971-1973	11	-	JET03	2000-2004	17	-
Odet	ODE72	1972-1973	19	-	ODE03	2003	14	-
Aven	AVE77	1973-1978	40	local	AVE03	2003	34	-
Ellé	ELL68	1968-1968	17	local	ELL03	2003	32	-
Scorff	SCO77	1966-1985	64	-	SCO03	2002-2003	64	-
Blavet	BLA77	1977-1978	65	-	BLA03	2002-2003	63	-
Allier	ALL67	1965-1967	49	Scotland, Canada, local	ALL03	2001-2002	31	local
Dordogne	-	-	-	Scotland, Allier, local	DOR03	2002	15	local
Garonne	-	-	-	Scotland, Allier, Gave d'Oloron, local	GAR03	2002	30	local
Gave	GAV84	1984-1984	25	Scotland, local	GAV03	2003	29	local
Nive	NIE84	1984-1984	26	Scotland, local	NIE03	2001-2006	8	-
Nivelle	NIL80	1977-1987	26	Scotland, local	NIL03	1998-2004	17	-

Material and methods

Study populations and sampling

We studied 34 Atlantic salmon populations from France and also analysed 23 samples from several Scottish rivers to study the effects of stocking with Scottish individuals (Fig. 1, table 1). Adult fish were collected by angling or trapping and scales were sampled and stored by INRA and ONEMA in 95% ethanol or in paper envelopes. The age of each individual was determined by scalimetry. We collected samples from cohorts 1998-2006 and cohorts 1965 to 1988.

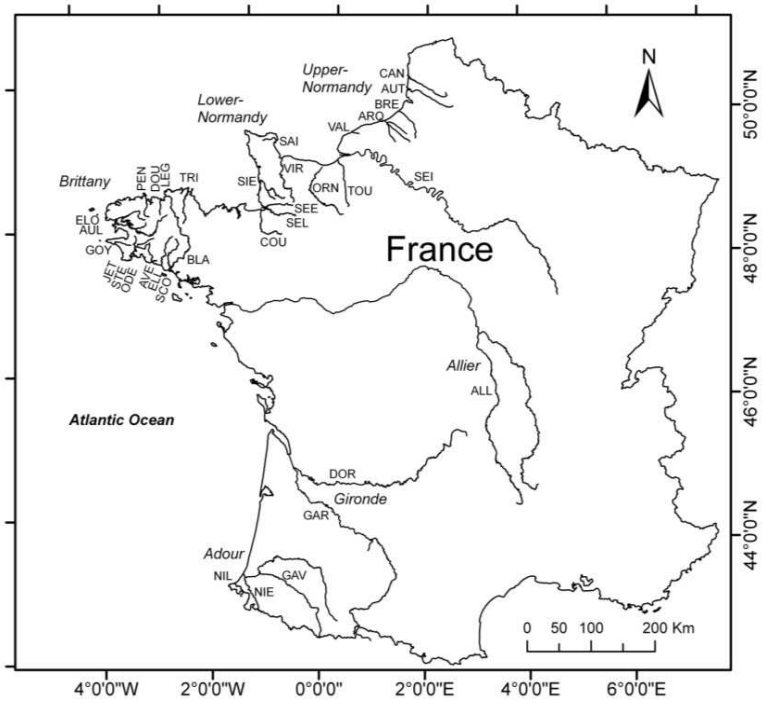

Figure 1: Locations of the populations studied.

Supplementation operations have occurred in many rivers and at different times. From 1950 to 1988, stocking programs using non-local fish mainly originating from Scotland but also from different French rivers were performed in Bresle, Arques, Orne, Vire, Elorn, Aulne, Allier, Garonne, Dordogne, Gave d'Oloron, Nive, and Nivelle rivers (Baglinière & Dumas 1988; Baglinière *et al.* 1990); Table 1). Since 1989, stocking with native individuals has become the rule (e.g. in Aulne, Elorn, Allier, and Gave d'Oloron rivers) but in Orne, Couesnon, Sélune, Sée, and Sienne rivers, stocking with non-native individuals has occurred using progeny from genitors caught in Aulne or / and Gave d'Oloron Rivers).

Molecular analyses

Genomic DNA was extracted from *S. salar* scales by heating samples in solution of proteinase K, TE (Tris/EDTA) buffer and chelex, at 55°C 2 hours and then at 100°C for 10min (Estoup *et al.* 1996). The M13 method (Schuelke 2000), was used to label DNA polymerase chain reaction (PCR) amplifications. We used 16 microsatellites: BHMS176; BHMS179A; BHMS184B; BHMS429; SSA85; SSA65; SSOSL85; BHMS235; BHMS217; BHMS111; SSA197; SSA171; BHMS377; SSSP2216; SSA224 and BHMS365 (references in (Nikolic *et al.* 2009)). PCR conditions are detailed in Perrier et al. (2010).

Data analyses

Textpad 4.7.3 (Helios Software Solutions), Convert 1.31 (Glaubitz 2004), Genepop 4.0.7 (Rousset 2008), and Genalex 6 (Peakall & Smouse 2006) were used to format the data sets used in the subsequent programs. We used Micro-Checker 2.2.3 (Van Oosterhout *et al.* 2004) to assess the frequency of null alleles and scoring errors due to stuttering or large allelic dropout. Allele number and allelic richness were obtained using Fstat 2.9.3.2 (Goudet 1995). Tests for linkage and Hardy-Weinberg disequilibria were conducted with Fstat 2.9.3.2. Expected heterozygosity, He, (Nei 1978) and observed heterozygosity, Ho, were calculated with Genetix 4.05.2 (Belkhir *et al.* 1996). Fdist 2 (Beaumont & Nichols 1996) was used to verify the neutrality of the markers used.

Populations pairwise F_{ST} and tests of differentiation were computed in Fstat 2.9.3.2. Analyses of Molecular Variance (AMOVA) were performed using Arlequin (Excoffier *et al.* 2005) on a subset of 23 populations for which samples were available for the two time periods. The hierarchical grouping of populations was defined with Bayesian clustering analysis using STRUCTURE (Pritchard et al. 2000). Temporal pairwise F_{ST} were computed in each river with Fstat 2.9.3.2. As all rivers were not sampled at the same time, temporal differentiation was not directly comparable among populations so we also divided the temporal F_{ST} by the number of years between the two cohorts sampled.

We used the Bayesian individual clustering implemented in STRUCTURE to determine i) the hierarchical genetic structure of the study populations and ii) the admixture rates between clusters. Analyses were performed assuming an admixture model (i.e. allowing the genetic composition of individuals to be a mixture from different populations). We tested from 1 to 15 genetic clusters (k) (15 replicates for each k). Each run started with a burn-in period of 50,000 steps followed by 300,000 Markov Chain Monte Carlo (MCMC) replicates. We selected the k with the highest likelihood (Pritchard *et al.* 2000) and according to the Δk method (Evanno *et al.* 2005).

Potential isolation by distance among populations was investigated through the relation between pairwise F_{ST} /(1- F_{ST}) and the coastal distance between river mouths. This analysis was restricted to a subset of 23 French populations for which both old and recent samples were available. This analysis was also performed separately for samples from Brittany. The significance of correlation coefficients was estimated with Mantel tests implemented in Passage (http://www.passagesoftware.net/index.php), using 9 999 permutations. Geographic coastal distances between rivers' estuaries were calculated

following coastline using Google Earth (http://earth.google.com) and ranged from 0 to 1,662 km with a median value of 741 km.

Results

Multi-locus genotype information was obtained for 1627 individuals (Table 1). Amplification success was generally high, ranging from 91.6% to 99.9% depending on the sample, and from 87.9% to 99.9% depending on the locus. It was of 94.0% for ancient samples and of 98.0% for recent ones with an overall rate of 96.2%. Average expected heterozygosity was 0.78 and ranged from 0.27 to 0.98 (Table S1). A total of 45 out of 928 HWE tests were significant. Micro-checker suggested only 7 out of these 45 cases may result from the presence of null alleles, and no evidence of large allelic dropout or stuttering was found.

Considering The Bayesian clustering analysis delineated nine genetic groups (Table 2) corresponding to six distinct geographic regions: Scotland, Upper-Normandy, Lower-Normandy, Brittany, Allier, and Adour. Individuals from Brittany were grouped into four clusters not consistently associated with the geographic locations of the populations. This apportionment suggested instead a pattern of isolation by distance. As a result, these four clusters were pooled for further admixture analyses. Considering the whole data set, most populations never stocked with non-native fish were mainly composed of the local cluster, e.g. South Brittany populations (from Goyen to Blavet). In contrast, we found some non-stocked populations moderately to highly admixed with non-native clusters. For instance, we detected admixtures rates from 0.12 to 0.57 by the Scottish cluster into the CAN03, AUT03, VAL03, SEI03, TOU03, and SAI03 populations.

Populations stocked with non-native fish were usually strongly admixed with the cluster of origin of the population they were stocked with. As examples, admixture rates of the Scottish cluster into BRE68 and ORN03 populations were 0.45 and 0.27 respectively and admixtures of the Brittany cluster into SIE03, SEL03, and COU03 populations were 0.17, 0.20, and 0.50, respectively. Accordingly, admixture rates of the Scotland, Allier, and Adour clusters into DOR03 and GAR03 samples ranged from 0.10 to 0.57. In contrast, some populations appeared weakly admixed with the cluster of origin of the population they were stocked with: e.g. the ALL67, ALL03, BRE03, ARQ03, NIE84, and NIE03 populations (less than 6% of admixture with the non-native cluster).

Table S1: Allelic richness (AR) estimated for 3 or 4 individuals, number of alleles (AN), observed (Ho) and expected heterozygoties (HE). Significant inbreeding coefficient (FIS) are given in bold, and in italic if possibly associated with null alleles.

		SSA0021NVH	SSA0146NVH	SSA0149NVH	SSA0071NVH	SSA85	SSA65	SSOSL85	SSA0217NVH	SSA0152NVH	SSA0008NVH	SSA197	SSA171	SSA0057NVH	SSSP2216	SSA0053NVH	SSA224	All loci
	AR, 4 ind	3.76	2.46	3.25	5.30	5.65	4.67	3.75	5.53	4.07	2.50	4.98	4.58	4.96	4.53	5.31	4.39	4.36
	AN	5.00	3.00	4.00	7.00	9.00	7.00	5.00	8.00	5.00	4.00	7.00	6.00	7.00	6.00	7.00	6.00	6.00
CAN03	Ho	0.88	0.50	0.88	0.75	0.75	0.63	0.75	0.75	0.88	0.25	0.75	0.83	0.71	0.86	0.83	0.71	0.73
	He	0.74	0.49	0.68	0.88	0.88	0.79	0.73	0.89	0.77	0.35	0.85	0.76	0.81	0.81	0.86	0.75	0.75
	Fis	-0.20	-0.02	-0.32	0.16	0.16	0.22	-0.02	0.17	-0.15	**0.30**	0.13	-0.11	0.13	-0.06	0.04	0.05	0.03
	AR, 4 ind	4.29	2.46	2.73	6.18	4.45	4.43	4.50	5.38	4.41	3.22	6.07	5.17	5.04	4.75	4.86	4.17	4.51
	AN	6.00	5.00	4.00	11.00	7.00	8.00	8.00	8.00	7.00	6.00	10.00	9.00	7.00	9.00	10.00	5.00	7.50
AUT03	Ho	0.82	0.27	0.36	0.91	0.60	0.36	0.73	0.80	0.55	0.55	0.60	0.82	0.90	0.90	0.73	0.82	0.67
	He	0.79	0.34	0.46	0.93	0.79	0.74	0.79	0.88	0.77	0.53	0.92	0.86	0.86	0.80	0.81	0.80	0.75
	Fis	-0.03	0.20	0.22	0.02	0.25	*0.52*	0.08	0.10	0.30	-0.03	*0.36*	0.05	-0.05	-0.13	0.11	-0.02	0.12
	AR, 3 ind	3.60	2.18	2.41	4.22	4.78	4.38	4.23	4.98	4.28	3.50	5.04	4.24	5.04	4.47	4.12	3.24	4.04
	AN	5.00	4.00	3.00	10.00	11.00	11.00	11.00	14.00	9.00	8.00	15.00	8.00	16.00	11.00	10.00	6.00	9.50
BRE68	Ho	0.68	0.58	0.47	0.79	0.83	0.50	0.63	0.95	0.84	0.63	0.63	0.75	0.95	0.74	0.69	0.56	0.70
	He	0.79	0.47	0.56	0.84	0.91	0.87	0.83	0.92	0.85	0.74	0.93	0.85	0.93	0.87	0.82	0.70	0.81
	Fis	0.13	-0.23	0.17	0.07	0.09	***0.43***	*0.25*	-0.03	0.01	0.15	***0.33***	0.12	-0.02	0.16	0.16	0.21	**0.13**
	AR, 4 ind	4.01	2.07	2.41	4.79	4.48	3.78	4.35	5.10	3.99	2.89	4.29	5.02	5.42	4.10	4.14	3.68	4.03
	AN	6.00	5.00	3.00	11.00	9.00	11.00	8.00	13.00	8.00	5.00	10.00	11.00	14.00	9.00	11.00	6.00	8.75
BRE03	Ho	0.87	0.27	0.38	0.83	0.90	0.73	0.83	0.86	0.76	0.50	0.69	0.87	0.66	0.76	0.72	0.70	0.71
	He	0.78	0.27	0.44	0.83	0.80	0.66	0.80	0.85	0.75	0.52	0.73	0.85	0.87	0.77	0.73	0.74	0.71
	Fis	-0.11	0.03	0.15	0.00	-0.12	-0.11	-0.04	-0.02	-0.02	0.05	0.05	-0.02	*0.25*	0.02	0.00	0.06	0.01
	AR, 4 ind	3.34	2.08	2.17	4.97	3.71	3.18	4.56	5.10	4.37	2.67	4.29	4.57	4.92	4.58	4.40	3.95	3.93
	AN	4.00	3.00	3.00	9.00	8.00	7.00	7.00	12.00	6.00	5.00	9.00	8.00	10.00	8.00	9.00	7.00	7.19
ARQ03	Ho	0.68	0.39	0.35	0.81	0.57	0.58	0.84	0.84	0.84	0.53	0.68	0.73	0.71	0.80	0.79	0.84	0.69
	He	0.68	0.37	0.36	0.85	0.70	0.56	0.83	0.85	0.81	0.45	0.74	0.81	0.84	0.83	0.78	0.76	0.70
	Fis	0.00	-0.05	0.00	0.06	0.19	-0.04	-0.02	0.02	-0.04	**-0.20**	0.08	0.10	0.16	0.03	-0.01	-0.10	0.01
	AR, 4 ind	3.60	3.60	2.78	5.00	5.00	6.76	6.76	7.00	4.58	3.60	5.51	7.38	6.00	3.00	5.98	2.98	4.97
	AN	4.00	4.00	3.00	5.00	5.00	8.00	8.00	7.00	5.00	4.00	6.00	9.00	6.00	3.00	7.00	3.00	5.44
VAL03	Ho	0.80	0.40	0.60	0.75	0.75	0.80	1.00	1.00	0.60	0.80	1.00	0.80	0.75	0.25	0.80	0.20	0.71
	He	0.71	0.71	0.51	0.86	0.86	0.96	0.96	0.96	0.84	0.71	0.91	0.98	0.89	0.61	0.91	0.69	0.82
	Fis	-0.14	**0.47**	**-0.20**	0.14	0.14	0.18	**-0.05**	**-0.04**	0.31	-0.14	**-0.11**	**0.20**	**0.18**	**0.63**	0.14	**0.73**	0.15
	AR, 4 ind	3.35	2.43	2.86	6.02	4.69	4.81	5.72	5.72	3.15	3.73	5.90	4.93	6.42	5.46	5.42	3.00	4.60
	AN	4.00	3.00	4.00	10.00	6.00	7.00	9.00	9.00	4.00	5.00	10.00	7.00	10.00	8.00	8.00	4.00	6.75
SEI03	Ho	0.78	0.56	0.33	0.89	0.56	0.56	0.89	0.67	0.78	0.89	0.89	0.89	0.78	0.78	0.78	0.67	0.73
	He	0.71	0.54	0.48	0.91	0.84	0.84	0.90	0.90	0.67	0.75	0.90	0.84	0.94	0.89	0.88	0.54	0.78
	Fis	-0.10	-0.03	0.31	0.02	0.36	0.35	0.01	0.27	-0.17	-0.21	0.02	-0.06	0.18	0.13	0.13	**-0.25**	0.06
	AR, 4 ind	4.10	3.08	3.03	4.96	4.87	6.27	5.62	6.13	4.72	3.62	5.41	6.31	6.20	5.92	6.79	4.18	5.08
	AN	5.00	5.00	4.00	8.00	7.00	11.00	9.00	12.00	6.00	6.00	9.00	11.00	12.00	10.00	13.00	7.00	8.44
TOU03	Ho	0.60	0.45	0.70	0.70	0.82	0.73	0.73	0.80	0.64	0.73	0.73	1.00	0.70	0.91	0.67	0.91	0.74
	He	0.78	0.64	0.63	0.82	0.85	0.93	0.90	0.91	0.84	0.67	0.88	0.93	0.92	0.91	0.95	0.75	0.83
	Fis	0.24	0.30	-0.13	0.15	0.04	0.23	0.20	0.12	0.26	-0.10	0.18	**-0.08**	0.25	0.01	***0.31***	-0.22	0.11
	AR, 4 ind	3.77	2.24	2.71	5.89	6.18	5.67	5.63	6.52	4.83	3.96	4.95	5.41	6.64	5.63	5.53	3.63	4.95
	AN	5.00	4.00	5.00	14.00	18.00	14.00	13.00	19.00	8.00	6.00	12.00	12.00	21.00	12.00	12.00	6.00	11.31
ORN03	Ho	0.87	0.55	0.48	0.84	0.93	0.90	0.97	0.87	0.77	0.74	0.81	0.90	0.73	0.80	0.97	0.70	0.80
	He	0.75	0.52	0.60	0.91	0.92	0.89	0.89	0.94	0.85	0.75	0.83	0.88	0.94	0.89	0.89	0.68	0.82
	Fis	-0.16	-0.05	0.19	0.08	-0.02	-0.01	-0.09	0.07	0.09	0.01	0.03	-0.03	***0.23***	0.11	-0.09	-0.03	0.02
	AR, 4 ind	3.54	2.82	2.62	5.38	5.73	5.92	5.49	6.11	4.49	4.78	5.46	5.77	6.07	5.35	5.87	3.10	4.91
	AN	4.00	6.00	5.00	10.00	12.00	13.00	10.00	15.00	8.00	8.00	13.00	11.00	15.00	11.00	13.00	4.00	9.88
VIR03	Ho	0.58	0.42	0.53	0.79	0.79	0.79	0.84	0.95	0.78	0.84	0.79	0.88	0.89	0.89	0.89	0.63	0.77
	He	0.74	0.59	0.59	0.86	0.90	0.91	0.88	0.92	0.81	0.84	0.88	0.90	0.92	0.87	0.91	0.59	0.82
	Fis	0.22	0.29	0.10	0.09	0.12	0.14	0.05	-0.04	0.04	-0.01	0.10	0.02	0.03	-0.03	0.02	-0.07	0.07
	AR, 4 ind	3.49	2.42	1.93	5.42	5.72	5.18	5.29	6.90	4.76	4.44	4.81	5.66	6.61	5.83	5.83	2.64	4.81
	AN	4.00	3.00	2.00	8.00	9.00	8.00	8.00	12.00	6.00	6.00	7.00	8.00	11.00	9.00	9.00	3.00	7.06
SAI03	Ho	0.89	0.44	0.22	0.89	0.67	0.89	0.67	1.00	0.89	0.78	0.89	0.88	1.00	0.89	0.89	0.67	0.78
	He	0.70	0.50	0.37	0.88	0.90	0.86	0.86	0.96	0.85	0.80	0.84	0.90	0.95	0.91	0.91	0.52	0.79
	Fis	-0.29	0.12	0.41	-0.01	0.27	-0.04	0.24	**-0.04**	-0.05	0.03	-0.07	0.03	**-0.06**	0.02	0.02	**-0.30**	0.02
	AR, 3 ind	2.00	1.99	2.27	4.04	4.31	4.57	4.55	4.62	3.33	3.74	3.64	3.91	4.46	3.90	4.16	2.59	3.63
	AN	3.00	3.00	4.00	11.00	11.00	14.00	14.00	16.00	7.00	9.00	7.00	9.00	15.00	11.00	12.00	5.00	9.44
SIE86	Ho	0.57	0.60	0.57	0.85	0.77	0.79	0.90	0.97	0.79	0.80	0.80	0.76	0.80	0.88	0.80	0.47	0.76
	He	0.47	0.46	0.55	0.83	0.86	0.88	0.88	0.89	0.72	0.77	0.79	0.81	0.87	0.79	0.84	0.52	0.74
	Fis	-0.23	-0.32	-0.05	-0.03	0.10	0.11	-0.03	-0.10	-0.10	-0.03	-0.02	0.06	0.08	-0.12	0.05	0.10	-0.02
	AR, 4 ind	3.26	2.19	2.66	5.21	4.96	5.50	5.38	5.87	4.58	3.79	4.86	5.48	6.67	5.22	5.83	3.56	4.69
	AN	5.00	3.00	5.00	12.00	12.00	14.00	13.00	17.00	8.00	7.00	13.00	15.00	22.00	12.00	14.00	7.00	11.19
SIE03	Ho	0.68	0.62	0.50	0.86	0.86	0.89	0.92	0.92	0.69	0.54	0.81	0.89	1.00	0.86	0.86	0.72	0.79
	He	0.68	0.51	0.58	0.86	0.83	0.88	0.87	0.90	0.82	0.68	0.83	0.88	0.95	0.86	0.90	0.64	0.79
	Fis	0.00	-0.22	0.15	0.00	-0.04	-0.01	-0.05	-0.01	*0.17*	*0.21*	0.03	-0.01	**-0.06**	-0.01	0.05	-0.13	0.00
	AR, 3 ind	2.26	1.97	1.97	3.95	4.31	4.48	4.42	4.76	3.65	3.59	3.38	3.94	4.90	4.39	4.33	2.80	3.69
	AN	5.00	2.00	2.00	10.00	12.00	14.00	16.00	17.00	7.00	8.00	9.00	12.00	20.00	16.00	15.00	7.00	10.75
SEE77	Ho	0.44	0.48	0.38	0.84	0.78	0.89	0.73	0.93	0.68	0.75	0.80	0.67	0.97	0.72	0.79	0.65	0.72
	He	0.49	0.50	0.50	0.82	0.86	0.88	0.86	0.90	0.78	0.76	0.75	0.82	0.92	0.86	0.86	0.59	0.76
	Fis	0.10	0.04	0.24	-0.02	0.08	-0.01	*0.16*	-0.04	0.14	0.01	-0.08	*0.19*	-0.06	*0.16*	0.08	-0.10	**0.05**
	AR, 4 ind	2.91	2.22	2.29	4.81	5.28	5.50	5.39	5.78	4.19	3.86	4.14	4.37	5.85	4.70	5.11	3.19	4.35
	AN	4.00	4.00	5.00	12.00	17.00	14.00	13.00	19.00	8.00	8.00	8.00	11.00	19.00	13.00	19.00	5.00	11.19
SEE03	Ho	0.57	0.38	0.53	0.73	0.79	0.84	0.85	0.89	0.70	0.79	0.88	0.80	0.88	0.84	0.88	0.63	0.75
	He	0.63	0.53	0.54	0.83	0.86	0.88	0.88	0.89	0.79	0.71	0.78	0.80	0.90	0.79	0.86	0.62	0.77
	Fis	0.10	*0.29*	0.02	*0.12*	0.08	0.05	0.03	0.00	0.12	-0.11	-0.12	0.01	0.02	-0.06	-0.03	-0.03	0.03
	AR, 3 ind	2.61	1.96	2.18	4.27	4.46	4.63	4.42	4.96	3.38	3.23	3.76	3.71	4.88	4.17	4.24	2.52	3.71
	AN	5.00	2.00	3.00	11.00	12.00	13.00	12.00	19.00	7.00	8.00	8.00	6.00	18.00	15.00	13.00	5.00	9.81
SEL77	Ho	0.51	0.44	0.45	0.69	0.82	0.67	0.79	0.92	0.75	0.69	0.77	0.74	0.82	0.68	0.66	0.49	0.68
	He	0.60	0.49	0.53	0.86	0.87	0.89	0.87	0.92	0.72	0.69	0.80	0.79	0.91	0.82	0.85	0.50	0.76
	Fis	0.15	0.12	0.16	*0.20*	0.07	***0.26***	0.10	0.00	-0.04	0.00	0.03	0.06	0.10	*0.17*	*0.23*	0.02	**0.10**
	AR, 4 ind	3.03	2.22	2.32	5.72	5.29	5.77	5.62	6.20	4.22	3.82	4.65	4.88	6.24	5.06	5.54	3.56	4.63
	AN	4.00	4.00	6.00	15.00	17.00	15.00	17.00	20.00	8.00	9.00	15.00	14.00	22.00	13.00	22.00	8.00	13.06
SEL03	Ho	0.69	0.49	0.54	0.84	0.85	0.87	0.89	0.90	0.70	0.76	0.79	0.79	0.95	0.87	0.85	0.72	0.78
	He	0.64	0.51	0.52	0.90	0.86	0.90	0.89	0.92	0.79	0.71	0.82	0.83	0.92	0.84	0.88	0.66	0.79
	Fis	-0.07	0.05	-0.04	0.07	0.01	0.03	-0.01	0.02	0.11	-0.07	0.04	0.05	-0.03	-0.04	0.04	-0.10	0.00
	AR, 3 ind	2.57	2.78	1.92	4.46	4.57	4.58	4.75	5.20	3.33	3.05	3.76	4.36	4.75	4.34	4.42	3.28	3.88
	AN	3.00	5.00	2.00	7.00	8.00	10.00	10.00	12.00	6.00	6.00	6.00	8.00	10.00	12.00	9.00	6.00	7.50
COU82	Ho	0.64	0.64	0.40	0.88	0.75	0.70	0.82	0.91	0.73	0.45	0.73	0.91	1.00	0.73	0.91	0.73	0.74
	He	0.61	0.65	0.44	0.88	0.88	0.88	0.90	0.94	0.71	0.63	0.80	0.87	0.90	0.83	0.87	0.71	0.78
	Fis	-0.05	0.02	0.10	0.01	0.16	0.22	0.10	0.04	-0.03	0.29	0.10	-0.05	**-0.11**	0.13	-0.05	-0.03	0.05

	AR, 4 ind	3.34	2.45	3.27	5.42	5.23	5.61	5.82	5.50	4.74	3.69	4.68	4.65	6.18	5.27	5.83	3.56	4.70
	AN	4.00	3.00	4.00	12.00	13.00	13.00	15.00	14.00	7.00	7.00	11.00	11.00	18.00	12.00	18.00	6.00	10.50
COU03	Ho	0.71	0.65	0.56	0.82	0.88	0.85	0.87	0.88	0.84	0.74	0.76	0.77	0.91	0.88	0.82	0.82	0.80
	He	0.70	0.55	0.67	0.88	0.85	0.89	0.90	0.88	0.84	0.71	0.82	0.80	0.92	0.87	0.90	0.68	0.80
	Fis	-0.01	-0.18	0.17	0.07	-0.04	0.05	0.03	-0.01	0.00	-0.03	0.07	0.04	0.01	-0.02	0.09	-0.22	0.00
	AR, 3 ind	2.90	1.90	2.69	3.76	4.31	4.22	4.16	4.42	3.28	3.28	4.12	4.24	4.68	4.44	5.40	3.32	3.82
	AN	5.00	2.00	3.00	8.00	12.00	8.00	8.00	12.00	5.00	6.00	10.00	9.00	13.00	9.00	14.00	6.00	8.13
TRI77	Ho	0.47	0.47	0.41	0.90	0.82	0.67	0.76	0.94	0.71	0.76	0.82	0.73	0.82	0.81	0.90	0.64	0.73
	He	0.67	0.43	0.65	0.75	0.86	0.86	0.84	0.87	0.74	0.73	0.84	0.84	0.89	0.88	0.96	0.71	0.78
	Fis	0.30	-0.10	0.37	-0.22	0.04	0.23	0.10	-0.09	0.05	-0.04	0.02	0.14	0.07	0.08	0.06	0.09	0.07
	AR, 4 ind	3.33	2.39	3.14	4.96	5.36	5.24	5.16	5.96	4.62	4.08	5.23	5.72	5.56	5.95	5.56	3.50	4.73
	AN	4.00	3.00	4.00	11.00	13.00	11.00	11.00	16.00	8.00	6.00	12.00	11.00	12.00	11.00	12.00	5.00	9.38
TRI03	Ho	0.64	0.69	0.56	0.81	0.96	0.88	0.73	0.88	0.77	0.72	0.85	0.94	0.94	0.88	0.80	0.69	0.80
	He	0.68	0.55	0.68	0.83	0.87	0.86	0.86	0.91	0.83	0.78	0.87	0.90	0.89	0.91	0.88	0.70	0.81
	Fis	0.05	-0.27	0.19	0.03	-0.11	-0.03	0.16	0.03	0.07	0.08	0.02	-0.05	-0.06	0.04	0.09	0.02	0.02
	AR, 3 ind	2.62	2.62	2.90	4.63	3.94	4.20	3.84	4.76	3.75	2.90	4.47	4.16	5.21	4.32	4.83	3.26	3.90
	AN	4.00	4.00	4.00	10.00	11.00	11.00	7.00	15.00	7.00	4.00	11.00	8.00	17.00	9.00	11.00	6.00	8.69
LEG77	Ho	0.45	0.45	0.85	0.79	0.55	0.64	0.77	0.82	0.59	0.41	0.86	0.81	0.82	1.00	0.79	0.91	0.72
	He	0.62	0.60	0.68	0.89	0.81	0.84	0.81	0.90	0.80	0.66	0.87	0.85	0.94	0.86	0.91	0.73	0.80
	Fis	*0.28*	0.25	-0.27	0.13	***0.33***	*0.25*	0.05	0.10	*0.26*	*0.39*	0.01	0.04	*0.14*	**-0.17**	0.14	-0.26	**0.10**
	AR, 4 ind	3.26	2.99	2.91	5.65	5.28	5.54	5.51	6.17	4.63	3.76	5.62	5.63	6.36	6.27	6.11	2.97	4.92
	AN	5.00	5.00	4.00	12.00	12.00	12.00	12.00	16.00	8.00	6.00	15.00	11.00	14.00	13.00	14.00	4.00	10.19
LEG03	Ho	0.48	0.59	0.52	0.85	0.96	0.96	0.89	0.93	0.81	0.63	0.89	0.81	0.81	0.94	0.75	0.60	0.78
	He	0.66	0.58	0.65	0.89	0.86	0.88	0.88	0.92	0.81	0.73	0.89	0.89	0.93	0.93	0.92	0.54	0.81
	Fis	0.28	-0.03	0.20	0.05	-0.13	-0.09	-0.01	0.00	0.00	0.14	0.00	0.09	0.13	-0.01	0.19	-0.13	0.04
	AR, 3 ind	3.00	2.37	2.70	4.28	3.90	4.22	4.51	4.78	3.84	3.18	4.07	4.01	4.97	4.42	4.97	2.88	3.88
	AN	5.00	4.00	3.00	8.00	11.00	10.00	10.00	13.00	7.00	6.00	10.00	10.00	18.00	11.00	20.00	6.00	9.50
DOU82	Ho	0.45	0.48	0.74	0.78	0.68	0.71	0.86	0.89	0.69	0.79	0.86	0.74	0.79	0.86	0.93	0.64	0.74
	He	0.70	0.54	0.65	0.86	0.80	0.85	0.88	0.91	0.81	0.70	0.83	0.82	0.92	0.87	0.92	0.61	0.79
	Fis	*0.36*	0.11	-0.14	0.10	0.15	0.16	0.02	0.01	0.15	-0.13	-0.04	0.10	*0.14*	0.01	-0.01	-0.05	0.06
	AR, 4 ind	3.29	2.53	2.52	6.26	5.52	5.24	5.56	6.25	4.17	3.53	5.24	5.14	5.54	5.59	6.04	3.39	4.74
	AN	5.00	4.00	3.00	15.00	12.00	11.00	13.00	15.00	7.00	6.00	13.00	10.00	14.00	13.00	16.00	5.00	10.13
DOU03	Ho	0.78	0.59	0.33	0.89	0.88	0.85	0.96	0.85	0.81	0.67	0.85	0.96	0.81	0.78	0.93	0.70	0.79
	He	0.71	0.58	0.50	0.93	0.88	0.87	0.89	0.93	0.78	0.70	0.86	0.87	0.88	0.89	0.91	0.64	0.80
	Fis	-0.11	-0.03	*0.34*	0.04	0.00	0.02	-0.09	0.08	-0.05	0.05	0.01	-0.12	0.08	0.13	-0.02	-0.10	0.02
	AR, 3 ind	2.86	2.43	2.50	4.18	4.38	4.42	4.05	4.53	3.74	2.99	4.13	4.33	5.11	4.19	5.03	2.94	3.86
	AN	4.00	3.00	3.00	8.00	14.00	12.00	12.00	15.00	6.00	5.00	10.00	8.00	18.00	9.00	14.00	5.00	9.13
PEN78	Ho	0.60	0.40	0.65	0.67	0.76	0.71	0.68	0.80	0.52	0.76	0.80	0.88	0.88	0.56	0.86	0.52	0.69
	He	0.68	0.59	0.59	0.83	0.85	0.87	0.83	0.87	0.80	0.67	0.83	0.87	0.93	0.84	0.93	0.61	0.79
	Fis	0.11	0.32	-0.10	0.21	0.11	*0.19*	0.18	0.09	*0.35*	-0.14	0.04	-0.01	0.06	***0.34***	0.08	0.15	**0.13**
	AR, 4 ind	3.43	2.39	3.16	5.30	4.99	4.28	4.91	5.22	4.70	3.53	5.28	5.85	6.00	5.69	5.55	3.34	4.60
	AN	4.00	3.00	4.00	10.00	9.00	9.00	11.00	13.00	9.00	6.00	12.00	13.00	15.00	11.00	16.00	5.00	9.38
PEN03	Ho	0.64	0.46	0.73	0.81	0.79	0.83	0.88	0.85	0.85	0.77	0.92	0.91	0.83	0.91	0.87	0.74	0.80
	He	0.71	0.56	0.69	0.88	0.84	0.77	0.85	0.85	0.83	0.72	0.86	0.91	0.91	0.90	0.87	0.63	0.80
	Fis	0.10	0.18	-0.06	0.08	0.07	-0.09	-0.05	0.00	-0.02	-0.07	-0.07	-0.01	0.09	-0.02	0.01	-0.17	0.00
	AR, 3 ind	2.79	2.63	2.75	3.13	3.88	3.51	3.39	3.82	3.39	3.17	4.23	4.15	4.35	4.17	3.32	2.85	3.47
	AN	4.00	4.00	3.00	4.00	9.00	9.00	10.00	9.00	6.00	6.00	12.00	7.00	13.00	10.00	6.00	6.00	7.38
AUL69	Ho	0.70	0.63	0.40	0.56	0.72	0.85	0.60	0.90	0.60	0.70	0.83	0.73	0.83	0.67	0.62	0.80	0.70
	He	0.66	0.63	0.67	0.70	0.81	0.74	0.69	0.80	0.76	0.69	0.84	0.85	0.86	0.84	0.74	0.63	0.74
	Fis	-0.06	0.00	*0.41*	0.22	0.11	-0.15	0.13	-0.13	0.21	-0.01	0.00	0.14	0.03	*0.21*	0.17	-0.28	0.07
	AR, 4 ind	3.48	2.62	3.05	5.00	5.49	4.95	4.90	6.09	4.37	3.74	4.80	5.72	6.33	5.34	6.35	3.23	4.72
	AN	4.00	3.00	4.00	13.00	14.00	13.00	13.00	17.00	9.00	6.00	12.00	15.00	20.00	11.00	19.00	4.00	11.06
AUL03	Ho	0.87	0.39	0.61	0.73	0.93	0.77	0.79	0.94	0.85	0.74	0.85	0.78	0.79	0.88	0.97	0.76	0.79
	He	0.73	0.60	0.66	0.84	0.88	0.81	0.83	0.92	0.80	0.74	0.82	0.89	0.93	0.87	0.93	0.66	0.81
	Fis	-0.20	*0.36*	0.07	0.13	-0.07	0.05	0.05	-0.03	-0.07	0.00	-0.04	0.12	*0.15*	-0.01	-0.04	-0.15	0.02
	AR, 3 ind	3.20	2.22	3.05	3.67	3.89	4.35	3.89	4.94	3.84	3.29	3.94	4.49	4.92	4.20	4.52	2.82	3.83
	AN	5.00	3.00	5.00	5.00	9.00	10.00	7.00	15.00	7.00	6.00	10.00	9.00	14.00	10.00	8.00	4.00	7.94
ELO75	Ho	0.83	0.67	0.50	0.83	0.72	0.76	0.78	0.94	0.67	0.78	0.67	0.73	0.83	0.78	1.00	0.67	0.76
	He	0.71	0.51	0.68	0.79	0.80	0.87	0.81	0.92	0.81	0.74	0.81	0.88	0.92	0.84	0.88	0.61	0.79
	Fis	-0.18	-0.32	0.27	-0.06	0.10	0.12	0.04	-0.03	0.18	-0.06	0.19	0.18	0.10	0.08	**-0.14**	-0.09	0.04
	AR, 4 ind	3.17	2.76	2.86	4.55	5.71	4.63	5.10	4.85	4.20	4.08	4.42	5.90	5.37	5.13	5.25	3.08	4.44
	AN	4.00	4.00	4.00	11.00	13.00	11.00	12.00	13.00	8.00	7.00	11.00	14.00	18.00	12.00	15.00	4.00	10.06
ELO03	Ho	0.59	0.64	0.67	0.79	0.94	0.85	0.82	0.73	0.78	0.79	0.73	0.90	0.87	0.80	0.73	0.60	0.76
	He	0.67	0.60	0.55	0.80	0.90	0.79	0.85	0.82	0.79	0.77	0.80	0.91	0.85	0.85	0.86	0.67	0.78
	Fis	0.12	-0.07	-0.22	0.01	-0.05	-0.07	0.04	0.12	0.02	-0.02	0.09	0.01	-0.02	0.06	0.15	0.10	0.02
	AR, 3 ind	3.32	2.66	2.96	4.40	4.38	3.72	4.23	4.77	3.86	3.19	4.00	4.28	4.73	4.39	4.30	3.04	3.89
	AN	5.00	3.00	4.00	12.00	10.00	12.00	11.00	16.00	9.00	7.00	13.00	9.00	21.00	14.00	19.00	4.00	10.56
GOY81	Ho	0.67	0.79	0.66	0.84	0.76	0.76	0.88	0.85	0.76	0.76	0.88	0.90	0.81	0.85	0.84	0.85	0.80
	He	0.75	0.63	0.67	0.87	0.87	0.76	0.85	0.90	0.81	0.70	0.81	0.86	0.89	0.86	0.83	0.69	0.80
	Fis	0.11	-0.25	0.02	0.04	0.13	0.00	-0.03	0.06	0.07	-0.09	-0.08	-0.05	0.09	0.01	-0.02	-0.23	-0.01
	AR, 4 ind	3.06	2.83	3.23	5.05	4.80	4.97	5.21	5.57	4.43	3.30	5.26	5.37	5.94	5.03	5.29	3.15	4.53
	AN	4.00	4.00	5.00	12.00	11.00	10.00	11.00	16.00	8.00	5.00	12.00	11.00	14.00	12.00	14.00	4.00	9.56
GOY03	Ho	0.53	0.65	0.65	0.85	0.85	0.88	0.90	0.76	0.74	0.62	0.94	0.83	0.83	0.75	0.71	0.67	0.76
	He	0.65	0.62	0.68	0.85	0.83	0.83	0.87	0.88	0.81	0.65	0.87	0.88	0.91	0.85	0.84	0.65	0.79
	Fis	0.19	-0.04	0.05	-0.01	-0.02	-0.06	-0.04	0.13	0.09	0.06	-0.09	0.05	0.09	0.12	0.17	-0.03	0.04
	AR, 3 ind	2.77	2.71	2.66	3.62	3.54	2.85	4.59	4.60	3.60	2.78	4.14	3.18	4.69	4.53	4.15	2.62	3.56
	AN	5.00	3.00	4.00	7.00	7.00	7.00	12.00	12.00	8.00	5.00	10.00	4.00	14.00	10.00	6.00	6.00	7.50
STE72	Ho	0.62	0.71	0.62	0.60	0.71	0.43	0.81	0.95	0.58	0.48	0.86	0.83	0.90	0.67	0.33	0.67	0.67
	He	0.62	0.66	0.61	0.78	0.76	0.63	0.89	0.89	0.74	0.58	0.84	0.71	0.89	0.88	0.85	0.60	0.75
	Fis	0.00	-0.09	-0.02	0.23	0.06	*0.32*	0.09	-0.08	0.23	0.19	-0.03	-0.19	-0.01	*0.25*	***0.63***	-0.12	0.10
	AR, 4 ind	3.24	2.70	3.09	5.59	4.83	4.88	5.09	5.44	4.63	3.43	5.23	5.28	6.36	5.50	6.36	4.11	4.74
	AN	5.00	4.00	4.00	10.00	9.00	12.00	10.00	11.00	7.00	4.00	12.00	9.00	13.00	9.00	15.00	6.00	8.75
STE03	Ho	0.60	0.55	0.50	0.85	0.84	0.95	1.00	0.84	0.80	0.65	0.90	0.83	0.92	0.92	0.79	0.86	0.80
	He	0.68	0.53	0.69	0.89	0.84	0.80	0.86	0.88	0.83	0.71	0.86	0.87	0.93	0.89	0.92	0.75	0.81
	Fis	0.12	-0.04	0.28	0.05	0.00	-0.20	**-0.17**	0.05	0.04	0.08	-0.05	0.04	0.01	-0.04	0.15	-0.16	0.01
	AR, 3 ind	3.18	2.23	2.45	3.70	3.44	3.97	4.60	4.34	3.71	3.37	4.18	4.00	3.86	4.43	3.00	2.66	3.57
	AN	4.00	3.00	3.00	6.00	5.00	7.00	9.00	9.00	5.00	5.00	8.00	4.00	8.00	8.00	3.00	4.00	5.69
JET72	Ho	0.91	0.55	0.45	0.78	0.91	0.91	0.67	0.82	0.70	0.73	0.91	0.67	1.00	0.56	0.33	0.45	0.71
	He	0.73	0.54	0.60	0.80	0.77	0.82	0.89	0.85	0.81	0.74	0.84	0.80	0.79	0.88	0.73	0.57	0.76
	Fis	-0.26	-0.01	0.25	0.03	-0.20	-0.12	0.26	0.04	0.14	0.01	-0.08	**0.20**	**-0.28**	0.38	**0.60**	0.21	0.08
	AR, 4 ind	3.29	2.35	2.60	4.89	4.88	4.68	4.93	5.87	4.38	2.96	4.47	4.58	5.75	5.22	5.73	2.97	4.35
	AN	4.00	3.00	3.00	9.00	9.00	9.00	9.00	14.00	8.00	4.00	10.00	9.00	14.00	9.00	14.00	4.00	8.25
JET03	Ho	0.60	0.45	0.55	0.80	0.92	0.85	0.70	0.90	0.80	0.60	0.75	0.76	1.00	1.00	0.94	0.76	0.77
	He	0.67	0.54	0.60	0.83	0.83	0.82	0.86	0.90	0.80	0.63	0.78	0.80	0.89	0.87	0.88	0.63	0.77
	Fis	0.11	0.17	0.08	0.04	-0.12	-0.04	0.19	0.00	0.00	0.05	0.04	0.04	**-0.13**	**-0.15**	-0.07	-0.22	0.00
	AR, 3 ind	2.86	2.42	2.08	2.73	3.59	4.02	4.51	4.16	3.57	3.21	4.16	3.46	4.86	4.13	3.99	3.01	3.55
	AN	4.00	5.00	3.00	3.00	7.00	9.00	9.00	10.00	7.00	6.00	12.00	6.00	16.00	12.00	7.00	6.00	7.63
ODE72	Ho	0.79	0.53	0.32	0.80	0.74	0.61	0.74	0.89	0.78	0.84	0.74	0.73	1.00	0.58	0.50	0.74	0.71
	He	0.67	0.57	0.42	0.62	0.78	0.82	0.88	0.84	0.77	0.72	0.82	0.76	0.91	0.83	0.80	0.67	0.74
	Fis	-0.19	0.08	0.26	**-0.33**	0.05	*0.26*	0.17	-0.07	0.00	-0.18	0.10	0.05	**-0.10**	*0.31*	*0.39*	-0.11	0.05
	AR, 4 ind	3.44	2.71	2.84	5.53	4.04	5.17	5.25	5.55	4.84	3.24	4.29	5.92	5.16	5.49	4.91	2.47	4.43
ODE03	AN	5.00	4.00	4.00	11.00	6.00	12.00	11.00	11.00	9.00	5.00	10.00	11.00	10.00	10.00	11.00	3.00	8.31
	Ho	0.53	0.58	0.63	0.89	0.68	0.79	0.89	0.94	0.84	0.67	0.79	0.71	0.93	0.79	0.93	0.71	0.77

	He	0.70	0.60	0.57	0.88	0.78	0.84	0.86	0.89	0.84	0.62	0.77	0.91	0.85	0.88	0.80	0.54	0.77
	Fis	0.26	0.03	-0.10	-0.02	0.13	0.06	-0.04	-0.06	0.00	-0.08	-0.03	*0.22*	-0.10	0.11	-0.17	-0.33	-0.01
	AR, 3 ind	2.70	2.85	2.64	4.21	4.11	4.20	3.62	4.15	3.82	3.14	3.90	4.15	4.94	4.23	4.30	3.24	3.76
	AN	4.00	4.00	4.00	8.00	11.00	9.00	10.00	14.00	6.00	6.00	11.00	11.00	16.00	10.00	18.00	4.00	9.13
AVE77	Ho	0.70	0.64	0.49	0.85	0.78	0.76	0.73	0.83	0.67	0.70	0.75	0.86	0.88	0.80	0.83	0.75	0.75
	He	0.63	0.68	0.61	0.86	0.83	0.85	0.78	0.83	0.81	0.68	0.81	0.84	0.92	0.85	0.84	0.74	0.78
	Fis	-0.11	0.06	0.20	0.01	0.06	0.10	0.07	0.01	*0.18*	-0.03	0.07	-0.03	0.05	0.06	0.01	-0.02	0.04
	AR, 4 ind	3.42	2.51	3.03	5.43	4.74	4.65	4.87	5.41	4.60	3.49	4.21	5.15	5.77	5.32	5.19	3.25	4.44
	AN	4.00	3.00	4.00	12.00	11.00	11.00	11.00	15.00	9.00	7.00	9.00	13.00	17.00	12.00	14.00	4.00	9.75
AVE03	Ho	0.65	0.56	0.53	0.91	0.76	0.76	0.79	0.85	0.85	0.65	0.82	0.79	0.76	0.97	0.91	0.82	0.78
	He	0.72	0.55	0.66	0.88	0.82	0.78	0.84	0.87	0.82	0.69	0.77	0.86	0.90	0.87	0.84	0.67	0.78
	Fis	0.10	-0.02	0.20	-0.04	0.07	0.02	0.05	0.02	-0.04	0.07	-0.08	0.08	*0.15*	-0.12	-0.09	-0.24	0.01
	AR, 3 ind	3.17	2.84	2.42	4.46	3.88	3.92	4.23	4.51	3.50	3.28	4.04	3.31	5.01	4.06	3.50	3.76	3.74
	AN	5.00	4.00	3.00	8.00	8.00	10.00	9.00	11.00	5.00	6.00	10.00	4.00	16.00	8.00	4.00	6.00	7.31
ELL68	Ho	0.65	0.53	0.41	0.56	0.76	0.40	0.85	0.82	0.67	0.59	0.88	0.50	1.00	0.53	1.00	0.63	0.67
	He	0.72	0.64	0.59	0.87	0.81	0.80	0.85	0.88	0.78	0.70	0.82	0.76	0.92	0.83	0.79	0.80	0.78
	Fis	0.10	0.18	0.31	*0.38*	0.05	***0.51***	0.01	0.07	0.15	0.17	-0.08	0.36	**-0.09**	*0.36*	**-0.33**	0.22	0.15
	AR, 4 ind	3.41	2.56	2.60	5.46	5.01	4.50	5.28	5.29	4.57	3.68	4.65	5.26	5.90	5.11	5.50	3.16	4.50
	AN	5.00	3.00	4.00	12.00	12.00	11.00	13.00	15.00	8.00	7.00	11.00	13.00	15.00	10.00	14.00	4.00	9.81
ELL03	Ho	0.84	0.68	0.55	0.78	0.77	0.78	0.85	0.91	0.88	0.56	0.79	0.88	0.88	0.88	0.91	0.68	0.79
	He	0.70	0.58	0.51	0.87	0.84	0.76	0.87	0.86	0.81	0.67	0.81	0.87	0.91	0.86	0.87	0.66	0.78
	Fis	-0.19	-0.17	-0.08	0.11	0.09	-0.03	0.02	-0.07	-0.10	0.16	0.02	-0.02	0.03	-0.02	-0.05	-0.02	-0.02
	AR, 3 ind	3.18	2.65	2.75	4.44	4.15	4.01	3.85	3.65	3.59	3.06	3.95	3.85	4.96	4.46	4.60	2.90	3.75
	AN	5.00	3.00	5.00	12.00	13.00	15.00	11.00	15.00	7.00	6.00	15.00	12.00	19.00	16.00	19.00	4.00	11.06
SCO77	Ho	0.70	0.63	0.53	0.80	0.90	0.78	0.71	0.70	0.59	0.63	0.77	0.63	0.86	0.84	0.73	0.64	0.72
	He	0.71	0.63	0.62	0.88	0.84	0.82	0.80	0.76	0.78	0.65	0.80	0.79	0.92	0.87	0.88	0.66	0.78
	Fis	0.02	0.01	0.14	0.08	-0.08	0.05	0.12	0.07	*0.25*	0.04	0.05	*0.20*	0.07	0.03	*0.17*	0.04	**0.08**
	AR, 4 ind	3.08	2.63	2.95	5.52	4.85	4.76	5.18	4.99	4.10	3.45	5.03	5.18	5.83	5.16	5.49	3.22	4.46
	AN	4.00	4.00	5.00	14.00	12.00	13.00	15.00	15.00	8.00	6.00	16.00	13.00	19.00	14.00	20.00	5.00	11.44
SCO03	Ho	0.62	0.47	0.66	0.88	0.80	0.83	0.88	0.76	0.77	0.70	0.86	0.87	0.86	0.83	0.91	0.67	0.77
	He	0.66	0.57	0.63	0.89	0.84	0.81	0.86	0.84	0.78	0.68	0.84	0.85	0.90	0.86	0.87	0.66	0.78
	Fis	0.06	0.17	-0.04	0.01	0.05	-0.02	-0.03	0.09	0.02	-0.03	-0.02	-0.02	0.04	0.03	-0.04	-0.02	0.02
	AR, 3 ind	3.03	2.56	2.97	4.18	3.92	3.97	4.14	4.48	3.76	2.99	4.03	3.97	4.94	4.34	4.31	2.74	3.77
	AN	4.00	3.00	6.00	11.00	9.00	11.00	11.00	16.00	9.00	7.00	13.00	13.00	20.00	13.00	20.00	5.00	10.69
BLA77	Ho	0.69	0.55	0.65	0.83	0.80	0.82	0.70	0.80	0.67	0.60	0.88	0.70	0.92	0.89	0.75	0.60	0.74
	He	0.70	0.62	0.68	0.84	0.82	0.81	0.84	0.87	0.80	0.64	0.83	0.81	0.92	0.86	0.85	0.63	0.78
	Fis	0.01	0.10	0.05	0.02	0.02	0.00	*0.17*	0.08	*0.17*	0.06	-0.06	*0.14*	-0.01	-0.04	*0.11*	0.04	0.05
	AR, 4 ind	3.24	2.37	2.74	5.33	4.95	4.25	5.08	5.58	4.60	3.36	5.30	4.99	5.89	5.48	5.61	3.26	4.50
	AN	5.00	5.00	4.00	13.00	15.00	13.00	13.00	17.00	8.00	7.00	17.00	13.00	21.00	15.00	20.00	5.00	11.94
BLA03	Ho	0.68	0.56	0.52	0.81	0.85	0.73	0.88	0.87	0.84	0.59	0.92	0.82	0.86	0.83	0.89	0.59	0.77
	He	0.68	0.52	0.58	0.87	0.84	0.73	0.85	0.88	0.82	0.65	0.86	0.85	0.90	0.88	0.88	0.66	0.78
	Fis	0.00	-0.08	0.09	0.07	-0.02	0.00	-0.03	0.01	-0.02	0.10	-0.07	0.04	0.05	0.06	-0.02	0.10	0.02
	AR, 3 ind	3.00	1.99	2.28	3.50	2.88	3.29	3.68	4.53	3.14	3.37	3.77	3.62	4.65	3.59	4.50	2.52	3.39
	AN	5.00	3.00	4.00	8.00	6.00	8.00	9.00	13.00	8.00	5.00	10.00	8.00	13.00	7.00	13.00	5.00	7.81
ALL67	Ho	0.84	0.39	0.53	0.70	0.71	0.84	0.86	0.82	0.64	0.71	0.84	0.62	0.90	0.61	0.77	0.63	0.71
	He	0.68	0.36	0.48	0.76	0.60	0.72	0.79	0.88	0.66	0.76	0.79	0.76	0.89	0.75	0.88	0.57	0.71
	Fis	-0.24	-0.07	-0.10	0.07	-0.19	-0.16	-0.08	0.08	0.04	0.06	-0.06	*0.19*	0.00	*0.19*	0.12	-0.10	-0.01
	AR, 4 ind	3.50	2.04	2.63	4.32	4.36	4.37	3.86	4.93	3.35	3.37	4.29	4.40	5.22	4.39	5.33	3.77	4.01
	AN	5.00	3.00	4.00	8.00	9.00	9.00	8.00	11.00	5.00	7.00	11.00	9.00	13.00	9.00	10.00	5.00	7.88
ALL03	Ho	0.62	0.31	0.60	0.83	0.85	0.86	0.83	0.89	0.66	0.60	0.74	0.71	0.81	0.74	0.90	0.84	0.74
	He	0.71	0.35	0.51	0.81	0.78	0.79	0.76	0.85	0.67	0.68	0.76	0.81	0.85	0.78	0.88	0.74	0.73
	Fis	0.13	0.11	-0.19	-0.03	-0.10	-0.09	-0.09	-0.05	0.01	0.12	0.02	0.12	0.06	0.05	-0.03	-0.14	-0.01
	AR, 4 ind	3.65	2.51	3.29	5.11	4.84	5.04	4.93	5.97	3.92	4.19	5.95	4.49	5.07	5.25	6.05	3.03	4.58
	AN	4.00	5.00	6.00	10.00	13.00	13.00	11.00	14.00	7.00	10.00	16.00	8.00	13.00	10.00	14.00	5.00	9.94
GAR03	Ho	0.80	0.43	0.68	0.82	0.83	0.87	0.90	0.93	0.86	0.72	0.93	0.81	0.81	0.90	0.81	0.81	0.81
	He	0.76	0.57	0.64	0.87	0.81	0.85	0.84	0.91	0.75	0.77	0.91	0.81	0.83	0.86	0.91	0.59	0.79
	Fis	-0.06	0.25	-0.07	0.05	-0.03	-0.03	-0.07	-0.02	-0.15	0.06	-0.03	0.00	0.03	-0.05	0.12	**-0.37**	-0.02
	AR, 4 ind	3.70	2.73	2.62	5.16	4.36	4.71	5.87	5.43	3.94	3.05	5.83	5.28	6.12	4.97	5.84	3.52	4.57
	AN	4.00	4.00	4.00	9.00	9.00	9.00	11.00	10.00	6.00	6.00	11.00	10.00	13.00	7.00	11.00	4.00	8.00
DOR03	Ho	0.80	0.60	0.47	0.87	0.73	0.80	0.93	0.93	0.80	0.67	0.87	1.00	1.00	0.92	1.00	0.83	0.83
	He	0.77	0.60	0.45	0.86	0.73	0.83	0.91	0.88	0.75	0.63	0.90	0.87	0.92	0.86	0.91	0.73	0.79
	Fis	-0.04	0.01	-0.04	0.00	0.00	0.03	-0.03	-0.07	-0.07	-0.06	0.04	**-0.16**	**-0.10**	-0.08	**-0.11**	-0.15	-0.05
	AR, 3 ind	2.94	2.83	3.42	4.05	4.47	4.12	3.78	4.50	2.66	3.67	4.46	3.98	4.50	4.54	4.91	3.31	3.88
	AN	4.00	5.00	6.00	9.00	9.00	9.00	7.00	13.00	6.00	6.00	10.00	10.00	14.00	12.00	13.00	6.00	8.69
GAV84	Ho	0.72	0.60	0.80	0.84	0.92	0.92	0.68	0.68	0.52	0.76	0.88	0.75	0.76	0.92	0.95	0.80	0.78
	He	0.68	0.63	0.73	0.81	0.88	0.84	0.79	0.87	0.54	0.79	0.88	0.80	0.88	0.88	0.92	0.72	0.79
	Fis	-0.06	0.05	-0.10	-0.03	-0.05	-0.10	0.14	*0.23*	0.04	0.04	0.00	0.07	0.13	-0.04	-0.03	-0.12	0.01
	AR, 4 ind	3.23	2.88	3.54	5.31	5.60	5.05	4.83	6.15	3.47	3.99	5.80	6.26	6.08	5.94	6.36	3.16	4.85
	AN	4.00	6.00	6.00	10.00	13.00	12.00	10.00	16.00	8.00	6.00	15.00	13.00	21.00	15.00	18.00	6.00	11.19
GAV03	Ho	0.83	0.52	0.62	0.90	0.86	0.86	0.72	0.83	0.62	0.59	0.93	0.93	0.79	0.93	0.62	0.62	0.76
	He	0.68	0.62	0.69	0.87	0.89	0.84	0.83	0.92	0.62	0.78	0.90	0.93	0.91	0.91	0.93	0.60	0.81
	Fis	-0.23	0.16	0.11	-0.03	0.03	-0.02	0.14	0.10	0.00	*0.25*	-0.03	-0.01	0.13	-0.02	***0.34***	-0.03	0.06
	AR, 3 ind	2.72	1.90	3.44	3.32	4.28	2.93	3.92	4.16	3.27	3.24	4.02	3.07	3.41	4.00	4.07	2.17	3.37
	AN	4.00	2.00	5.00	7.00	8.00	6.00	7.00	10.00	6.00	6.00	9.00	8.00	7.00	9.00	14.00	4.00	7.00
NIE84	Ho	0.69	0.36	0.92	0.83	0.88	0.69	0.81	0.85	0.85	0.73	0.92	0.63	0.79	0.80	0.73	0.42	0.74
	He	0.64	0.43	0.76	0.73	0.86	0.61	0.81	0.84	0.72	0.72	0.83	0.66	0.75	0.83	0.80	0.47	0.71
	Fis	-0.09	0.16	-0.22	-0.15	-0.03	-0.13	0.01	-0.01	-0.18	-0.01	-0.12	0.05	-0.06	0.03	0.09	0.09	-0.04
	AR, 4 ind	3.36	2.00	4.07	3.99	4.72	3.76	5.37	5.66	3.65	4.51	5.32	4.29	3.70	5.06	5.22	2.67	4.21
	AN	4.00	2.00	5.00	6.00	6.00	5.00	7.00	8.00	4.00	6.00	7.00	5.00	5.00	7.00	7.00	3.00	5.44
NIE03	Ho	0.88	0.50	0.63	0.75	0.88	0.75	0.88	0.88	0.63	0.50	0.88	0.71	0.86	1.00	0.71	0.29	0.73
	He	0.69	0.50	0.77	0.74	0.85	0.74	0.89	0.90	0.76	0.82	0.88	0.81	0.70	0.85	0.86	0.48	0.76
	Fis	-0.29	0.00	0.20	-0.01	-0.03	-0.01	0.02	0.03	0.19	0.40	0.00	0.13	-0.24	**-0.20**	0.18	0.43	0.05
	AR, 3 ind	3.00	2.23	3.05	4.24	4.22	4.42	4.36	4.37	3.44	3.70	4.40	4.06	4.79	4.80	4.34	2.87	3.89
	AN	4.00	3.00	5.00	8.00	12.00	16.00	14.00	16.00	7.00	7.00	11.00	11.00	18.00	13.00	9.00	6.00	10.00
NIL80	Ho	0.65	0.42	0.77	0.95	0.77	0.72	0.76	0.88	0.65	0.81	0.73	0.72	0.81	0.76	0.71	0.56	0.73
	He	0.68	0.52	0.70	0.86	0.85	0.87	0.86	0.85	0.72	0.79	0.87	0.81	0.90	0.90	0.86	0.60	0.79
	Fis	0.04	0.19	-0.10	-0.12	0.09	0.17	0.12	-0.04	0.09	-0.02	0.16	0.11	0.10	*0.16*	0.17	0.07	0.08
	AR, 4 ind	3.63	2.47	3.15	5.66	5.14	4.99	3.54	4.65	3.56	3.61	5.62	5.00	6.40	5.37	6.04	3.61	4.53
	AN	5.00	4.00	5.00	10.00	8.00	7.00	6.00	9.00	7.00	4.00	12.00	8.00	14.00	9.00	11.00	4.00	7.69
NIL03	Ho	0.59	0.65	0.65	0.88	0.76	0.82	0.76	0.88	0.65	0.88	0.94	0.92	0.69	0.92	0.75	0.92	0.79
	He	0.73	0.57	0.63	0.90	0.87	0.86	0.71	0.81	0.61	0.75	0.89	0.85	0.93	0.88	0.92	0.74	0.79
	Fis	0.20	-0.14	-0.03	0.02	0.12	0.04	-0.08	-0.08	-0.07	-0.18	-0.06	-0.09	*0.27*	-0.06	0.19	-0.26	-0.01
	AN	9.00	17.00	6.00	28.00	23.00	26.00	27.00	30.00	13.00	12.00	31.00	31.00	36.00	26.00	44.00	13.00	
All pop	Ho	0.68	0.52	0.55	0.81	0.80	0.77	0.81	0.87	0.73	0.68	0.82	0.80	0.86	0.81	0.80	0.68	
	He	0.69	0.54	0.59	0.84	0.83	0.82	0.85	0.88	0.77	0.70	0.84	0.84	0.89	0.85	0.87	0.65	
	Fis	0.01	0.03	0.07	0.04	0.05	0.06	0.04	0.02	0.06	0.02	0.02	0.05	0.04	0.06	0.08	-0.04	

Table S2: Matrix of pairwise F_{ST}.

	FOR80	CAN03	AUT03	BRE68	BRE03	ARQ03	VAL03	SEI03	TOU03	ORN03	VIR03	SAI03	SIE86	SIE03	SEE77	SEE03	SEL77	SEL03	COU82	COU03	TRI77	TRI03	LEG77	LEG03	DOU82	DOU03	PEN78	PEN03
CAN03	0.05																											
AUT03	0.05	0.01																										
BRE68	0.00	0.04	0.03																									
BRE03	0.06	0.02	0.01	0.05																								
ARQ03	0.07	0.03	0.01	0.06	0.00																							
VAL03	0.02	0.01	0.02	0.01	0.04	0.03																						
SEI03	0.00	0.06	0.05	0.02	0.08	0.09	0.02																					
TOU03	0.00	0.01	0.02	-0.01	0.04	0.04	-0.01	0.00																				
ORN03	0.01	0.05	0.06	0.01	0.07	0.08	0.03	0.01	0.00																			
VIR03	0.01	0.06	0.06	0.02	0.08	0.09	0.03	0.00	0.01	0.00																		
SAI03	0.02	0.08	0.06	0.03	0.09	0.10	0.05	-0.01	0.02	0.01	0.00																	
SIE86	0.05	0.09	0.09	0.06	0.11	0.12	0.08	0.04	0.04	0.03	0.02	0.03																
SIE03	0.03	0.06	0.07	0.03	0.08	0.09	0.04	0.02	0.02	0.00	0.00	0.01	0.01															
SEE77	0.05	0.09	0.09	0.06	0.11	0.12	0.07	0.04	0.04	0.02	0.02	0.02	0.00	0.01														
SEE03	0.04	0.08	0.08	0.05	0.10	0.10	0.06	0.02	0.03	0.02	0.01	0.01	0.00	0.00	0.00													
SEL77	0.05	0.08	0.08	0.05	0.10	0.11	0.06	0.04	0.04	0.02	0.01	0.02	0.00	0.01	0.00	0.00												
SEL03	0.03	0.06	0.07	0.03	0.08	0.08	0.04	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.01											
COU82	0.04	0.07	0.07	0.04	0.10	0.10	0.05	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00										
COU03	0.03	0.06	0.07	0.04	0.08	0.09	0.05	0.03	0.02	0.01	0.02	0.02	0.03	0.01	0.03	0.02	0.03	0.02	0.03									
TRI77	0.03	0.08	0.08	0.04	0.10	0.10	0.06	0.04	0.03	0.03	0.04	0.04	0.06	0.04	0.07	0.06	0.06	0.04	0.05	0.02								
TRI03	0.02	0.07	0.06	0.04	0.08	0.09	0.05	0.02	0.03	0.02	0.02	0.02	0.05	0.03	0.04	0.03	0.04	0.02	0.03	0.01	0.03							
LEG77	0.03	0.06	0.06	0.04	0.08	0.09	0.05	0.02	0.03	0.04	0.04	0.03	0.06	0.04	0.06	0.04	0.06	0.04	0.04	0.02	0.02	0.01						
LEG03	0.02	0.07	0.06	0.03	0.09	0.09	0.05	0.02	0.02	0.02	0.01	0.02	0.04	0.02	0.04	0.03	0.04	0.03	0.03	0.01	0.02	0.00	0.01					
DOU82	0.03	0.07	0.06	0.04	0.08	0.09	0.06	0.03	0.03	0.02	0.03	0.03	0.05	0.03	0.05	0.04	0.05	0.03	0.04	0.02	0.02	0.01	0.02	0.01				
DOU03	0.03	0.07	0.06	0.04	0.09	0.09	0.05	0.03	0.03	0.02	0.03	0.02	0.06	0.03	0.05	0.04	0.05	0.03	0.03	0.02	0.02	0.01	0.02	0.01	0.01			
PEN78	0.03	0.08	0.06	0.04	0.09	0.09	0.06	0.02	0.02	0.02	0.02	0.02	0.05	0.02	0.05	0.03	0.05	0.02	0.03	0.01	0.01	0.01	0.02	0.00	0.01	0.00		
PEN03	0.03	0.08	0.08	0.04	0.09	0.09	0.06	0.03	0.03	0.03	0.03	0.03	0.06	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.00	0.02	0.01	0.01	0.01	0.01	
ELO75	0.06	0.11	0.10	0.06	0.13	0.14	0.10	0.07	0.06	0.05	0.05	0.06	0.07	0.05	0.07	0.06	0.07	0.06	0.06	0.04	0.04	0.03	0.04	0.03	0.04	0.05	0.04	0.03
ELO03	0.03	0.06	0.06	0.04	0.07	0.08	0.05	0.02	0.02	0.02	0.02	0.03	0.05	0.03	0.05	0.04	0.05	0.03	0.04	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.00	0.00
AUL69	0.03	0.08	0.07	0.04	0.09	0.09	0.06	0.01	0.03	0.03	0.02	0.02	0.06	0.03	0.06	0.04	0.06	0.03	0.05	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.01	0.01
AUL03	0.04	0.08	0.08	0.06	0.10	0.10	0.07	0.04	0.04	0.04	0.04	0.04	0.07	0.05	0.07	0.06	0.07	0.04	0.05	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.01
GOY81	0.03	0.07	0.06	0.04	0.08	0.08	0.06	0.03	0.03	0.03	0.04	0.04	0.07	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
GOY03	0.03	0.07	0.07	0.04	0.09	0.10	0.06	0.03	0.03	0.02	0.03	0.03	0.06	0.03	0.05	0.04	0.05	0.03	0.04	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.00	0.00
STE72	0.06	0.08	0.07	0.07	0.09	0.10	0.08	0.07	0.05	0.05	0.07	0.06	0.09	0.06	0.08	0.08	0.09	0.06	0.07	0.04	0.04	0.04	0.03	0.03	0.04	0.02	0.03	0.02
STE03	0.03	0.07	0.06	0.04	0.09	0.09	0.06	0.03	0.03	0.02	0.03	0.03	0.05	0.03	0.06	0.05	0.05	0.04	0.05	0.01	0.02	0.01	0.01	0.00	0.01	0.01	0.01	0.00
JET72	0.04	0.09	0.08	0.04	0.09	0.10	0.06	0.03	0.04	0.03	0.03	0.03	0.07	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.00
JET03	0.04	0.08	0.08	0.05	0.09	0.10	0.07	0.03	0.03	0.03	0.03	0.03	0.05	0.03	0.05	0.04	0.05	0.03	0.04	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01
ODE72	0.04	0.10	0.09	0.06	0.10	0.10	0.07	0.04	0.04	0.04	0.05	0.04	0.08	0.05	0.07	0.06	0.08	0.05	0.06	0.04	0.03	0.02	0.03	0.02	0.04	0.02	0.02	0.01
ODE03	0.04	0.08	0.07	0.05	0.09	0.09	0.06	0.03	0.04	0.04	0.04	0.03	0.07	0.04	0.07	0.05	0.06	0.04	0.04	0.02	0.03	0.01	0.01	0.02	0.03	0.01	0.01	0.00
AVE77	0.04	0.08	0.08	0.05	0.10	0.10	0.07	0.04	0.03	0.03	0.04	0.04	0.06	0.04	0.06	0.05	0.06	0.04	0.04	0.03	0.02	0.02	0.02	0.01	0.03	0.02	0.01	0.01
AVE03	0.04	0.08	0.07	0.05	0.08	0.09	0.07	0.04	0.03	0.03	0.04	0.03	0.07	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.01	0.00
ELL68	0.04	0.07	0.06	0.05	0.08	0.08	0.06	0.03	0.03	0.03	0.04	0.04	0.07	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02
ELL03	0.04	0.07	0.05	0.05	0.07	0.07	0.07	0.04	0.04	0.03	0.04	0.03	0.07	0.04	0.06	0.05	0.07	0.04	0.05	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01
SCO77	0.04	0.08	0.07	0.05	0.09	0.09	0.06	0.04	0.03	0.04	0.04	0.04	0.07	0.04	0.07	0.06	0.07	0.04	0.05	0.02	0.03	0.02	0.02	0.02	0.03	0.02	0.01	0.01
SCO03	0.04	0.08	0.07	0.05	0.08	0.09	0.06	0.04	0.03	0.03	0.04	0.04	0.06	0.03	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.01
BLA77	0.04	0.08	0.08	0.05	0.09	0.09	0.06	0.04	0.04	0.03	0.04	0.04	0.07	0.04	0.06	0.05	0.06	0.04	0.05	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.01
BLA03	0.04	0.07	0.06	0.04	0.07	0.08	0.06	0.04	0.03	0.03	0.04	0.03	0.06	0.04	0.06	0.05	0.06	0.03	0.05	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.00
ALL67	0.11	0.17	0.18	0.12	0.19	0.18	0.14	0.10	0.11	0.10	0.10	0.14	0.15	0.11	0.13	0.12	0.13	0.12	0.13	0.10	0.13	0.11	0.12	0.12	0.11	0.12	0.12	0.12
ALL03	0.09	0.14	0.16	0.10	0.17	0.17	0.12	0.08	0.09	0.08	0.09	0.12	0.13	0.09	0.12	0.11	0.12	0.10	0.12	0.09	0.11	0.09	0.11	0.10	0.09	0.10	0.11	0.10
DOR03	0.03	0.09	0.09	0.03	0.11	0.11	0.06	0.03	0.03	0.03	0.02	0.05	0.07	0.04	0.06	0.06	0.06	0.05	0.07	0.04	0.06	0.04	0.06	0.04	0.04	0.05	0.05	0.05
GAR03	0.04	0.10	0.11	0.05	0.13	0.13	0.07	0.04	0.04	0.04	0.04	0.08	0.09	0.05	0.07	0.07	0.07	0.06	0.07	0.05	0.08	0.05	0.07	0.06	0.05	0.07	0.06	0.06
ADO84	0.05	0.11	0.11	0.05	0.13	0.14	0.08	0.07	0.05	0.05	0.04	0.08	0.10	0.07	0.09	0.09	0.08	0.07	0.09	0.07	0.07	0.07	0.09	0.07	0.07	0.08	0.08	0.08
ADO03	0.04	0.10	0.09	0.04	0.11	0.11	0.05	0.05	0.04	0.03	0.02	0.05	0.08	0.05	0.07	0.07	0.06	0.05	0.07	0.05	0.07	0.05	0.07	0.05	0.06	0.06	0.06	0.05
NIE84	0.09	0.15	0.15	0.08	0.17	0.18	0.14	0.10	0.08	0.08	0.07	0.10	0.12	0.09	0.11	0.11	0.11	0.10	0.11	0.10	0.11	0.10	0.12	0.10	0.10	0.10	0.10	0.11
NIE03	0.05	0.12	0.10	0.05	0.13	0.14	0.08	0.05	0.05	0.05	0.04	0.06	0.10	0.07	0.09	0.08	0.08	0.07	0.08	0.07	0.08	0.06	0.08	0.07	0.07	0.07	0.06	0.06
NIL80	0.04	0.11	0.10	0.05	0.12	0.13	0.07	0.05	0.04	0.04	0.03	0.06	0.09	0.06	0.08	0.08	0.08	0.07	0.08	0.06	0.07	0.06	0.07	0.06	0.06	0.06	0.06	0.06
NIL03	0.04	0.11	0.10	0.04	0.13	0.14	0.07	0.05	0.04	0.04	0.03	0.06	0.09	0.05	0.08	0.08	0.07	0.06	0.08	0.05	0.07	0.05	0.07	0.05	0.05	0.06	0.05	0.05

Table S2. Continued.

	ELO75	ELO03	AUL69	AUL03	GOY81	GOY03	STE72	STE03	JET72	JET03	ODE72	ODE03	AVE77	AVE03	ELL68	ELL03	SCO77	SCO03	BLA77	BLA03	ALL67	ALL03	DOR03	GAR03	ADO84	ADO03	NIE84	NIE03	NIL80
ELO03	0.03																												
AUL69	0.05	0.01																											
AUL03	0.04	0.01	0.01																										
GOY81	0.03	0.00	0.01	0.01																									
GOY03	0.02	0.00	0.00	0.01	0.00																								
STE72	0.05	0.01	0.03	0.02	0.02	0.01																							
STE03	0.03	0.00	0.01	0.02	0.01	0.00	0.02																						
JET72	0.03	0.00	0.01	0.01	0.01	0.01	0.02	0.02																					
JET03	0.04	0.00	0.01	0.01	0.01	0.00	0.02	0.00	0.00																				
ODE72	0.06	0.01	0.01	0.01	0.02	0.00	0.01	0.02	0.01	0.01																			
ODE03	0.04	0.00	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	0.00																		
AVE77	0.04	0.01	0.01	0.01	0.01	0.01	0.03	0.02	0.03	0.02	0.02	0.01																	
AVE03	0.04	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01																
ELL68	0.04	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.02	0.01															
ELL03	0.04	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.01														
SCO77	0.04	0.01	0.01	0.02	0.01	0.01	0.03	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.01													
SCO03	0.04	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01												
BLA77	0.03	0.01	0.01	0.01	0.00	0.00	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00											
BLA03	0.04	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.00										
ALL67	0.15	0.11	0.12	0.13	0.12	0.12	0.16	0.13	0.13	0.11	0.14	0.13	0.13	0.13	0.12	0.13	0.13	0.13	0.12	0.13									
ALL03	0.13	0.09	0.11	0.11	0.11	0.10	0.14	0.12	0.11	0.10	0.13	0.12	0.11	0.11	0.10	0.11	0.12	0.11	0.11	0.12	0.01								
DOR03	0.07	0.04	0.05	0.07	0.06	0.05	0.09	0.05	0.06	0.06	0.07	0.07	0.06	0.06	0.06	0.07	0.07	0.06	0.06	0.06	0.06	0.06							
GAR03	0.08	0.06	0.06	0.08	0.06	0.06	0.11	0.07	0.07	0.06	0.09	0.08	0.07	0.07	0.06	0.08	0.07	0.07	0.07	0.08	0.03	0.03	0.01						
ADO84	0.08	0.07	0.08	0.09	0.08	0.07	0.10	0.07	0.09	0.09	0.10	0.09	0.08	0.08	0.08	0.09	0.09	0.08	0.08	0.08	0.12	0.11	0.04	0.06					
ADO03	0.07	0.05	0.06	0.07	0.06	0.06	0.09	0.06	0.06	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.06	0.12	0.10	0.03	0.05	0.01				
NIE84	0.10	0.10	0.10	0.12	0.11	0.10	0.14	0.11	0.12	0.12	0.12	0.13	0.11	0.11	0.12	0.12	0.12	0.11	0.11	0.12	0.14	0.14	0.05	0.10	0.05	0.06			
NIE03	0.08	0.06	0.06	0.08	0.07	0.06	0.09	0.07	0.07	0.08	0.08	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.07	0.07	0.14	0.12	0.03	0.08	0.03	0.03	0.01		
NIL80	0.07	0.05	0.07	0.08	0.07	0.06	0.10	0.07	0.07	0.08	0.09	0.08	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.11	0.09	0.03	0.06	0.02	0.01	0.04	0.03	
NIL03	0.07	0.05	0.06	0.07	0.06	0.05	0.10	0.05	0.06	0.07	0.08	0.08	0.07	0.06	0.07	0.08	0.07	0.07	0.07	0.07	0.12	0.10	0.03	0.05	0.03	0.02	0.06	0.04	0.01

Table 2: Results from admixture analysis carried out using Structure for k=9. Values corresponding to the native clusters were highlighted in grey and values corresponding to the clusters used for stocking before sampling were underlined.

		Scotland	Upper-Normandy	Lower-Normandy	Σ_{1-4} Brittany	Allier	Adour
Scotland	FOR80	0.84	0.02	0.02	0.08	0.02	0.02
Upper-Normandy	CAN03	0.12	0.82	0.01	0.04	0.01	0.01
	AUT03	0.12	0.70	0.01	0.07	0.01	0.10
	BRE68	0.45	0.29	0.02	0.05	0.04	0.15
	BRE03	0.06	0.87	0.01	0.03	0.01	0.03
	ARQ03	0.01	0.94	0.01	0.03	0.01	0.01
	VAL03	0.57	0.39	0.01	0.02	0.01	0.01
Lower-Normandy	SEI03	0.40	0.01	0.32	0.08	0.15	0.04
	TOU03	0.41	0.27	0.11	0.08	0.02	0.11
	ORN03	0.27	0.04	0.34	0.18	0.04	0.13
	VIR03	0.20	0.02	0.46	0.11	0.01	0.20
	SAI03	0.19	0.01	0.57	0.19	0.01	0.02
	SIE86	0.02	0.02	0.88	0.07	0.01	0.02
	SIE03	0.11	0.02	0.62	0.17	0.02	0.06
	SEE77	0.04	0.01	0.88	0.05	0.01	0.02
	SEE03	0.01	0.01	0.84	0.11	0.01	0.02
	SEL77	0.03	0.01	0.87	0.06	0.01	0.01
	SEL03	0.07	0.01	0.68	0.20	0.01	0.03
	COU82	0.05	0.01	0.77	0.15	0.01	0.01
	COU03	0.05	0.04	0.34	0.50	0.02	0.05
Brittany	TRI77	0.12	0.02	0.06	0.72	0.02	0.06
	TRI03	0.08	0.03	0.11	0.73	0.02	0.02
	LEG77	0.06	0.02	0.02	0.86	0.02	0.03
	LEG03	0.10	0.03	0.05	0.79	0.01	0.02
	DOU82	0.04	0.01	0.03	0.88	0.03	0.02
	DOU03	0.06	0.02	0.05	0.85	0.01	0.01
	PEN78	0.11	0.02	0.03	0.81	0.01	0.03
	PEN03	0.04	0.01	0.02	0.91	0.01	0.01
	AUL69	0.11	0.01	0.02	0.81	0.02	0.04
	AUL03	0.04	0.01	0.01	0.91	0.01	0.02
	ELO75	0.07	0.01	0.02	0.84	0.01	0.06
	ELO03	0.08	0.02	0.04	0.81	0.03	0.02
	GOY81	0.09	0.01	0.02	0.86	0.01	0.01
	GOY03	0.05	0.02	0.01	0.86	0.03	0.03
	STE72	0.03	0.02	0.01	0.91	0.01	0.03
	STE03	0.07	0.04	0.03	0.83	0.01	0.02
	JET72	0.02	0.01	0.01	0.92	0.02	0.01
	JET03	0.03	0.01	0.02	0.91	0.02	0.01
	ODE72	0.03	0.02	0.01	0.92	0.01	0.02
	ODE03	0.04	0.01	0.03	0.89	0.01	0.02
	AVE77	0.02	0.02	0.02	0.91	0.01	0.02
	AVE03	0.03	0.02	0.01	0.92	0.01	0.02
	ELL68	0.07	0.04	0.02	0.83	0.03	0.01
	ELL03	0.03	0.03	0.01	0.89	0.03	0.01
	SCO77	0.03	0.01	0.02	0.91	0.01	0.01
	SCO03	0.04	0.01	0.01	0.93	0.01	0.01
	BLA77	0.04	0.01	0.01	0.92	0.01	0.01
	BLA03	0.06	0.01	0.04	0.86	0.01	0.02
Allier	ALL67	0.01	0.01	0.01	0.03	0.95	0.01
	ALL03	0.01	0.01	0.01	0.04	0.93	0.01
Gironde	DOR03	0.10	0.03	0.02	0.08	0.36	0.42
	GAR03	0.18	0.02	0.03	0.09	0.57	0.12
Adour	GAV84	0.07	0.02	0.01	0.04	0.01	0.85
	GAV03	0.15	0.04	0.04	0.09	0.01	0.69
	NIE84	0.01	0.01	0.01	0.03	0.01	0.94
	NIE03	0.01	0.01	0.01	0.06	0.01	0.91
	NIL80	0.16	0.05	0.03	0.07	0.03	0.67
	NIL03	0.10	0.01	0.03	0.12	0.05	0.70

Considering the 23 populations sampled for the two periods, we found a lower genetic differentiation among recent samples (F_{ST} = 0.034, pairwise F_{ST} from 0.00 to 0.16) than among old ones (F_{ST} = 0.058, pairwise F_{ST} from -0.01 to 0.17) (Fig 2, table S2). Among the 14 populations from Brittany, F_{ST} was 0.023 (from -0.003 to 0.057) and 0.007 (from -0.002 to 0.023) for old and recent samples, respectively.

Analyses of Molecular Variance revealed a higher proportion of genetic variance among and within groups in old samples (6.85% and 1.53%, respectively) than in recent ones (4.99% and 0.68%). In most cases we observed a reduction in population differentiation with time, except for the Bresle population whose differentiation with other locations increased (Fig. 2). In particular, Bresle was more differentiated from the Scottish sample recently (F_{ST} = 0.058) than in the past (F_{ST} = 0.005). Lower-Normandy populations, especially Sienne and Couesnon, were especially less differentiated from Brittany samples recently than historically.

Average within river temporal F_{ST} was 0.13 and ranged from 0.00 to 0.46 (Fig. 3). When corrected by the time between the two sampling periods (26 years on average, range: 17 to 34), average temporal F_{ST} was 4.9E-04 and

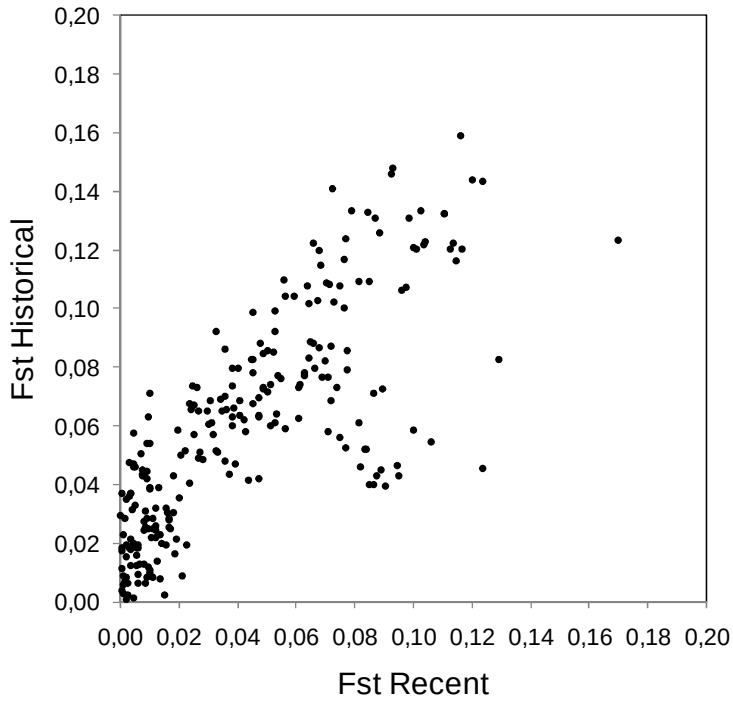

Figure 2: Relationship between historical and recent pairwise genetic differentiation among 23 Atlantic salmon populations. Empty symbols indicate pairs including the Bresle population.

ranged from 1.5E-06 to 1.4E-03.

Temporal F_{ST} were variable among rivers. High temporal differentiations were detected in Bresle, Couesnon, and Trieux rivers. Notable changes were also observed in the Sienne, Douron, Aulne, Steir, and Nivelle populations. In contrast, we did not observe consistent changes in other populations.

We found a significant pattern of isolation by distance (IBD) among populations for both old and recent samples (Figure 4). IBD was stronger in recent samples than in old ones (correlation coefficients of 0.62 and 0.53 respectively, $p<0.0001$). When excluding old Bresle samples we found a stronger IBD: $r = 0.61$, $p<0.0001$ (instead of 0.53). We also detected a significant IBD among Brittany samples, which was also lower in old compared to recent samples (Figure 4).

Discussion

Our main objective was to compare the genetic structure among old and recent samples from the main Atlantic salmon populations from France. We focused on the effects of stocking practices on this evolution. Our results show that the general pattern of genetic structure has been relatively stable over the period studied. However, we found a lower differentiation among recent samples than among old ones, and variable admixture rates between hatchery and wild populations in both ancient and recent samples. Non-target populations also appeared to be affected by stocking practices *via* the dispersal of stocked fish. While stocking with non-local individuals had significant impacts on the genetic structure of wild populations, our results suggest that such impacts are not long-lasting.

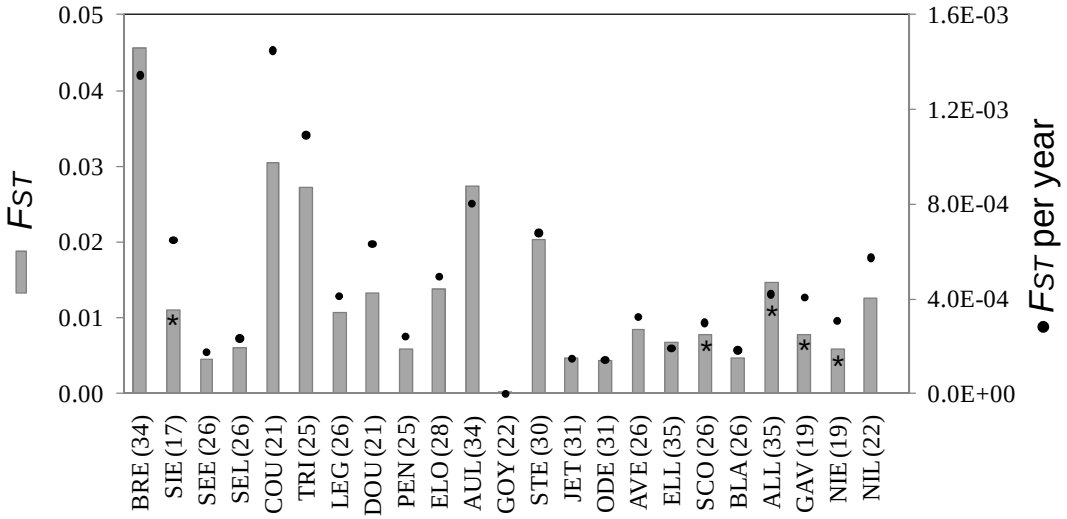

Figure 3: Temporal differentiation in each population. F_{ST} between the two cohorts sampled is presented as well as F_{ST} divided by the number of years between two cohorts. Asterisks indicate significant values and numbers in brackets indicate the number of years between the cohorts sampled.

According to Perrier et al (unpublished data) and (Grandjean *et al.* 2009), French *S. salar* populations cluster into five geographically distinct groups. We observed a higher proportion of genetic variance among these five groups than among populations within these groups, supporting the existence of a hierarchical genetic structure for both old and recent periods (Dionne *et al.* 2008; Tonteri *et al.* 2009). We also detected a significant isolation by distance among Atlantic salmon populations from old and recent periods. This finding is consistent with previous observations across the species range (McConnell *et al.* 1997; Verspoor *et al.* 1999; King *et al.* 2001; Grandjean *et al.* 2009). While the general pattern of genetic structure was stable over time, we show a lower differentiation among recent samples than among old ones, both among and within groups. In contrast, we found a stronger isolation by distance among recent samples than among old ones. These changes in genetic structure seem to result primarily from stocking practices.

Native hatchery-reared fish generally have a lower genetic diversity than their wild conspecifics, hence a potential decline of diversity in stocked populations (Verspoor 1988a; Aho *et al.* 2006; Horreo *et al.* 2008). However, we did not observe such a decline in recent samples from Elorn, Allier, and Gave d'Oloron populations that have been stocked using such native hatchery-reared fish. We found relatively low allelic richness and heterozygosity in recent and old samples of the Allier population, hence before and after/during native stocking. This result may be due to the geographic isolation of this population linked to probable local

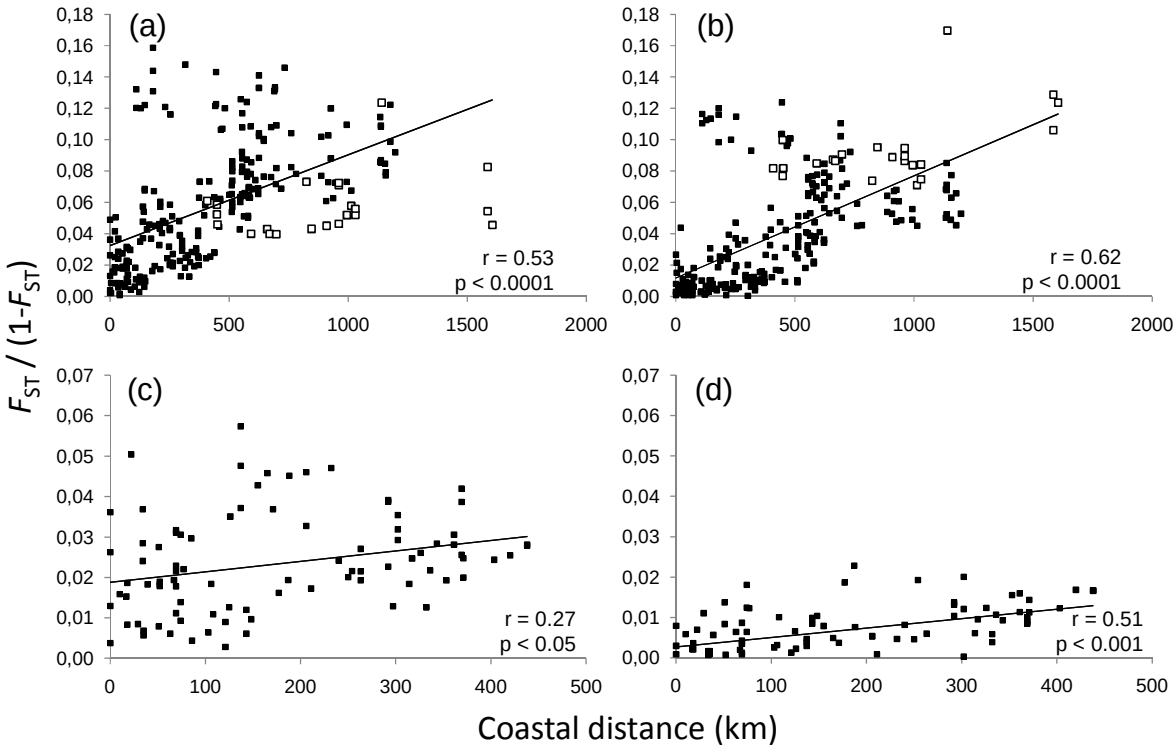

Figure 4: Isolation by distance among study populations at different time periods: old samples (a), recent samples (b), old samples from Brittany (c) and recent samples from Brittany (d). Correlation coefficients and results from Mantel tests are given for each dataset and empty symbols indicate pairs with the Bresle population.

adaptation, an hypothesis mainly supported by the high differentiation, low admixture by other clusters and extremely large proportion of multi-sea-winter fish in the Allier population (Perrier et al., in prep).

As suggested in many studies, stocking with non-local fish can be associated to variable but relatively high admixtures and modifications of genetic differentiation among stocks (Campos *et al.* 2008; Finnegan & Stevens 2008); Perrier et al, unpublished data). Sélune and Couesnon populations were highly stocked with fish originating from genitors caught in Aulne and Gave d'Oloron and were thus strongly admixed by Brittany and Adour clusters. Similarly, in old samples from Bresle, Aulne and Nivelle and in recent samples from Orne and Vire we found high admixtures with clusters of Scotland or/and Adour used in stocking programs. Dordogne and Garonne populations had gone through extinction before being stocked using genitors from Scotland, Allier, and Gave d'Oloron. Accordingly, we found high admixture rates from these sources in recent samples from Dordogne and Garonne, while no specific cluster to these two populations was detected.

Non-target populations may also be impacted by stocking operations in neighbouring populations. While native stocking may not have major effects on the genetic structure of

stocked populations, it could influence the genetic structure among populations of a larger area *via* the dispersal of hatchery fish that is usually greater than dispersal of wild fish (Quinn 1993; Jonsson *et al.* 2003; Pedersen *et al.* 2007). We found a decrease of genetic differentiation among populations from Brittany where native stocking have been practiced during the last decades. One could formulate the hypothesis that local fish recently stocked in Aulne and Elorn have dispersed among Brittany rivers and eroded the genetic structure among populations. Moreover, adipose fin clipped fish are often caught in many rivers from Brittany (Baglinière, pers. com.), suggesting the dispersal of released hatchery fish. Alternatively, the reduced genetic structure among Brittany populations could be due to old non-native stocking, an hypothesis discussed later in this study. Dispersal of non-native fish stocked in Bresle, Arques, Orne, and Vire could explain the admixture rates with Scottish samples of Canche, Authie, Valmont, Seine, Touques, and Saire populations. However, natural dispersal and/or recolonization from other stocked rivers could also explain the observed admixtures (Vasemagi *et al.* 2001; Grandjean *et al.* 2009; Perrier *et al.* 2010).

Our results demonstrate that stocking using non-local fish induces important modifications in the genetic makeup of wild populations. However, some of our results also suggest cases of short term and especially low impacts of such practices. The Allier River was stocked with a variety of non-local fish (mainly from Scotland but also Canada) from the 60s to the 80s and hence we expected relatively high admixture rates of both old and recent samples with the Scotland cluster. However, we did not detect any significant admixture in the Allier populations, neither in old nor in recent samples. Several non exclusive hypotheses could explain this particularly low impact of stocking programs. We suggested in a precedent study that the Allier population could be locally adapted to the extremely large length of the river compared to other European salmon populations. Stocked fish may face difficulties during both downstream and upstream migrations resulting in poor survival and explaining the lack of introgression in this population. Maladaptation and low fitness of stocked fish (McGinnity *et al.* 1997; Verspoor & de Leaniz 1997; Fleming *et al.* 2000; Aprahamian *et al.* 2003; Finnegan & Stevens 2008) could also explain the short term influence of stocking in Brittany and in Bresle populations. Indeed, stocking performed in Brittany from the 50s to the 80s could have artificially increased genetic differentiation and decreased isolation by distance among populations during this period. The end of stocking with non-native individuals would have then allowed natural short-distance gene flow to progressively reduce global genetic differentiation but incresase isolation by distance. However the potential increased dispersal of native fish stocked in Aulne and Elorn could have also played a role in this process. Scottish fish were used to stock the Bresle River from the 60s to the 80s and we

found a strong admixture (0.45) of this population with the Scottish cluster in samples from the 1968 cohort. Accordingly, we observed no differentiation between these two stocks (Fst = 0.004). In contrast, the recent admixture of this population with the Scottish cluster was much lower (0.06) and differentiation between both samples was much higher (Fst = 0.058). The isolation by distance among French populations was stronger among recent samples than among old ones, and removing the Bresle population from old samples produced a correlation similar to the one found in recent samples. These results strongly suggest short term genetic influences of stocking practices using non-native fish. Maladaptation and lower fitness of stocked fish may not be the only reasons for such weak influences of stocking on the genetic diversity of wild populations. Firstly, gene flow among populations may dilute the introgression signal, more or less rapidly depending on the sizes of wild populations, dispersal rates, and on the number of stocked fish. Secondly, highly variable microsatellite loci may not be best suited to estimate introgression after a long period of hybridization among local and stocked fish. The less variable mtDNA could help studying introgression among samples collected a long time after stocking operations (Campos *et al.* 2008).

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Chapitre V. Estimation des effets des repeuplements par simulation

Le but de ce chapitre est d'estimer, par une approche de simulations, les taux de survie et de dispersion des individus repeuplés et leur impact sur la structure génétique de 4 populations de saumon de la baie du Mont-St-Michel.

Le rempoissonnement est une pratique courante qui consiste à déverser des poissons dans un cours d'eau afin d'augmenter la population locale et / ou augmenter le nombre de captures par la pêche. Cette pratique implique l'utilisation de poissons issus de stocks locaux ou bien allochtones. Le transfert d'individus dans divers cours d'eau peut affecter la structure génétique des populations et provoquer une introgression génétique par les souches importées. Les résultats exposés dans les chapitres IV et V montrent des taux d'admixture variables de populations repeuplées avec des poissons allochtones. Le but de cette étude était d'investiguer la survie des individus issus de repeuplement et leur contribution aux populations repeuplées. Afin de quantifier l'impact relatif des repeuplements il est nécessaire de prendre en compte le nombre d'individus repeuplés et la taille des populations.

Les populations de la Baie du Mont Saint Michel sont idéales pour cette étude car elles sont étudiées depuis plus d'une quinzaine d'années par l'INRA et parce que les repeuplements non-natifs effectués sont bien documentés. Ainsi, 545 saumons provenant de quatre rivières de la Baie et 2 populations utilisées pour les repeuplements ont été génotypés à 17 marqueurs microsatellites afin de quantifier l'impact génétique de ces pratiques de gestion. Parallèlement, afin de quantifier la survie des individus non-natifs relâchés, relativement à celle des individus sauvages, nous avons utilisé des simulations individus centrées intégrant les tailles des populations et les effectifs déversés chaque année. Il nous est ainsi possible de comparer l'admixture observée dans les populations avec celles simulées selon divers scénarios implémentant plusieurs valeurs de survie et de dispersion.

Les analyses génétiques montrent que les déversements de poissons allochtones ont conduit à des taux d'admixture relativement importants et à une réduction de la variabilité entre les populations repeuplées et celles utilisées pour les repeuplements. Ces pratiques semblent en outre avoir des conséquences sur la diversité génétique des populations voisines via la dispersion des individus déversés. Les simulations suggèrent que les saumons déversés ont une survie beaucoup moins importante que leurs congénères sauvages. De plus, il semble que la dispersion des individus déversés, comme celle des sauvages, soit relativement importante entre les rivières de la Baie.

Ces résultats, en quantifiant l'introgression et la survie relative des individus repeuplés, ont d'importantes implications en termes de gestion. De plus, les simulations utilisées pourraient être appliquées pour prévoir l'impact génétique de repeuplements. Ce type de simulation pourrait également trouver d'importantes implications dans les études d'adaptation locale et plus particulièrement d'interactions entre les poissons domestiques et sauvages.

Estimating admixture in Atlantic salmon (*Salmo salar*) populations supplemented with non-native individuals: a case study combining molecular analyses and temporally explicit simulations

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Key words: *Salmo salar*, introgression, stocking, conservation, simulation

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Abstract

Supplementation of wild populations with non-native individuals is a common practice to manage endangered or exploited species. However, these operations can alter the genetic make up as well as the long-term viability of supplemented populations. Here we aim at evaluating the admixture rates in four Atlantic salmon populations from the Bay of Mont-Saint-Michel (France) supplemented with non-native fish. We combine microsatellite analyses of old (1969-1987) and recent samples with individual-based simulations to infer the effects of differential survival and dispersal between stocked and wild fish on observed admixture levels. We detected a decrease of differentiation between donor and supplemented populations following stocking. We also observed a high but variable admixture at the population level (from 12 % to 60 %). Simulation data suggested that observed levels of admixture are best explained by a much lower survival of hatchery fish relative to wild individuals in most populations. In contrast, these data indicated that a higher dispersal of hatchery fish would only have a marginal influence on observed admixture levels. Finally, the low number of hybrids observed between wild and hatchery populations further suggested a reduced fitness of hatchery fish. Overall, our results demonstrate that combining analyses of both real and simulated genetic data strongly improves our understanding of mechanisms underlying the admixture between wild and non-native populations.

Introduction

Temporal variations in population genetic structure can occur depending on changes in gene flow among populations. Genetic drift can also influence these variations when local effective population sizes vary over time. In endangered or exploited species, temporal changes in the genetic make up of populations can be linked to anthropogenic pressures like habitat fragmentation, destruction or introduction of non-native individuals for conservation or exploitation purposes. Such changes in population genetic parameters over time can be detected by analyzing successive temporal samples (Schwartz *et al*. 2007).

The analyses of archived old biological samples have been increasingly used in population genetic studies of a wide range of species, including mammals (Pertoldi *et al.* 2001), birds (Bouzat *et al*. 1998) and fishes (Larsson *et al.* 2010; Watts *et al.* 2010). Times series analyses of fish scales collections are of great interest to finely track temporal evolution of population genetic structure (Nielsen *et al.* 1999; Nielsen & Hansen 2008). Such studies either showed a temporal stability of population genetic structure over several decades (Hansen et al. 2002; Vaha et al. 2008) or strong variations often linked to translocations among populations (Hansen 2002; Ruzzante *et al.* 2004; Santos *et al.* 2006; Finnegan & Stevens 2008; Sonstebo *et al.* 2008).

Stocking of declining or extinct populations with non-native individuals collected in the wild or reared in captivity is a common management practice of fish populations (MacCrimmon & Gots 1979; Hindar, Ryman & Utter 1991; Aprahamian, Smith, McGinnity, McKelvey & Taylor 2003; Araki, Cooper & Blouin 2007). Salmonids populations are highly structured throughout their native range (Verspoor *et al.* 2005; Lehtonen *et al.* 2009; Tonteri *et al.* 2009) meaning that individuals released into genetically distant populations are traceable and admixture between stocks can be estimated (Campos *et al.* 2008; Finnegan & Stevens 2008; Hansen *et al.* 2009). Fish translocations can lead to a decrease in genetic structure (Ayllon *et al.* 2005) and they may ultimately result in a loss of local adaptation and a fitness reduction in recipient populations (Hindar *et al.* 1991; McGinnity *et al.* 2003; Araki *et al.* 2007; Ford & Myers 2008). Overall hatchery fish usually suffer from increased mortality relative to their wild conspecifics and it has been shown that their dispersal rate can be higher than wild individuals (Quinn 1993; Jonsson *et al.* 2003). However, such effects are difficult to detect since molecular analyses of pre- and post-stocking samples can reveal the genetic admixture between stocks but provide no information on the fitness of hatchery fish relative to wild conspecifics. Alternatively, comparative analyses of observed admixture levels with

simulated admixture data based on realistic demographic scenarios may give some insights into the mechanisms of introgression in wild populations.

Several individual-based simulations methods are available to study the temporal evolution of population genetic structure (e.g. the softwares EASYPOP (Balloux 2001), SPLATCH (Currat *et al.* 2004), NEMO (Guillaume & Rougemont 2006) and HYBRILAB (Nielsen *et al.* 2006)). Some of them were successfully used to simulate genetic introgression following demographic expansions or bioinvasions (Currat et al. 2008). In the case of human mediated inter-populations transfers, local population sizes and the number of non-native individuals transferred are generally known. As a result it can be possible to compare observed admixture levels with expected levels simulated according to the quantitative data available on transfers of individuals. For instance, (Hansen 2002) compared the introgression computed with simulations to the one estimated from microsatellite analyses in a brown trout population. Simulations were based on the assumption of equal survival and reproductive performances of hatchery and wild fish. Results revealed a much higher expected than observed genetic contribution of hatchery fish to the wild population suggesting poor performances of hatchery fish. Recent methods allow modeling more complex scenarios where not only survival but also dispersal can be modified between groups of individuals in a metapopulation framework. For example, the version 2.1.0 (2009) of the Nemo program allows the implementation of temporal parameters to vary dispersal rates or population sizes during the course of a simulation.

Here we assessed the admixture between wild and hatchery fish in four Atlantic salmon (*Salmo salar*) populations from the bay of Mont-Saint-Michel (France) using microsatellite DNA analyses of pre- and post-stocking samples as well as individual-based simulations. From 1988 to 2003, about 1 052 000 juvenile 0+ salmon have been transplanted in the Couesnon, Sélune, Sée, and Sienne rivers. Individuals released were produced by genitors caught in distant French populations, mainly the Aulne River (Brittany), and to a lesser extent the Gave d'Oloron River (Aquitania). Scales samples of fish caught by anglers have been collected since the late 1960s in the populations studied. In addition, estimates of population sizes are available and the numbers and origins of fishes stocked in each river are known. Therefore, this study aimed at i) compare the genetic structure and admixture levels of the study populations before and after stocking, ii) simulate expected admixture levels in each population according to stocking and demographic data and iii) investigate with simulation data whether increased dispersal and / or lower survival of hatchery fish relative to wild individuals may explain the observed levels of admixture.

Material and methods

Study populations and sampling

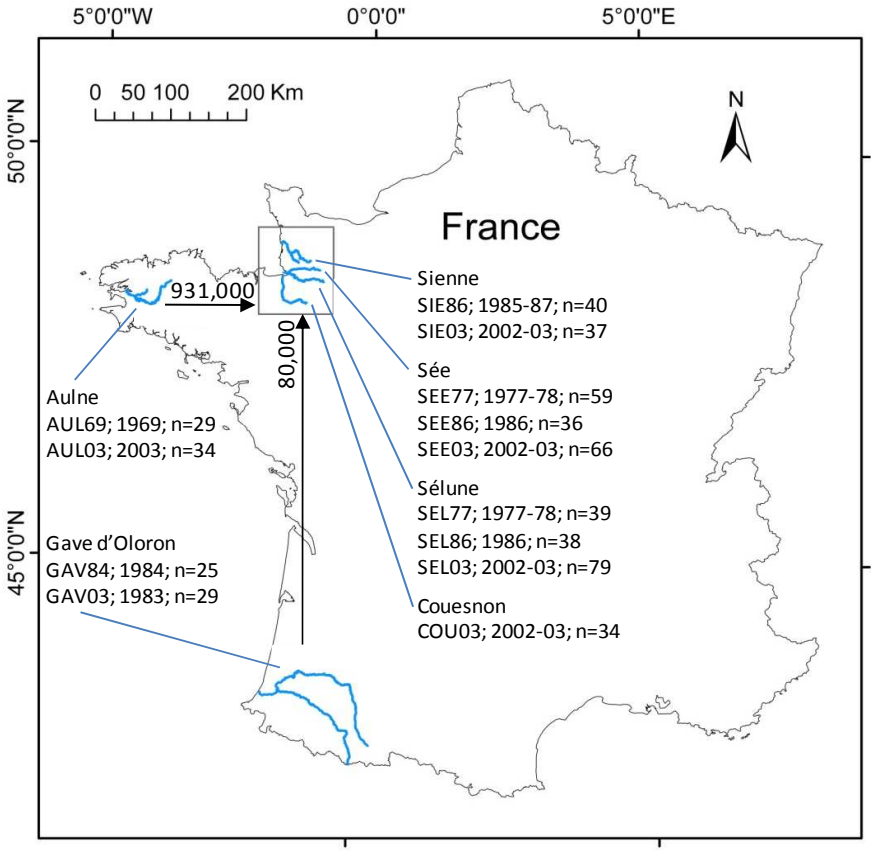

Figure 1: Map of sampling locations. Aulne and Adour rivers have been used to stock Couesnon, Sélune, Sée, and Sienne rivers.

The location of the fours studied populations (Couesnon (COU), Sélune (SEL), Sée (SEE), and Sienne (SIE)), is presented in Figure 1. These populations declined in the 1950s probably due to important habitat degradation. COU population was considered extinct (no natural reproduction) in the 1970s (A. Richard, ONEMA, and J.-L. Baglinière, INRA, Com pers.). As a result, supplementation operations in this river started in 1989 using young of the year progeny produced in the Favot hatchery, Brittany. Adult individuals used to produce the fish stocked have been caught in the Aulne River (AUL) and in the Gave d'Oloron River (GAV, see Figure 1). The four rivers were not stocked with the same number of individuals, these numbers are given in Table S1. From 1989 to 1994 and from1996 to 2003, approximately 931,000 fish produced by AUL genitors were stocked (Table S1). In 1995, about 80,000 juveniles originating from GAV genitors were released. COU has been stocked annually from 1989 to 2003 with a total of 623 518 fish. SEL has been stocked annually from 1989 to 1996 with a total of 363 500 fish. SEE and SIE were stocked once in 1990 with respectively 14 000 and 10 000 individuals.

Table S1: Population and stocking details for the Couesnon, Sélune, Sée, and Sienne rivers from 1989 to 2009. Stocking operations used genitors from Gave d'Oloron (given in bold italic) or from Aulne. We also give average populations sizes and average 0+ autumn par productions according to angler's catches and electrofishing (Anonymous 2008) (Baglinière Pers Com).

	Average pop size	Average 0+ parr production	Stocking																			
			1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
COU	200	4,000	22,000	53,500	25,000	64,500	25,000	16,600	***50,267***	43,197	89,020	59,665	48,200	30,782	35,049	33,172	27,566	29,381	23,585	22,988	25,519	20,090	27,094
SEL	300	6,000	4,000	36,000	25,900	30,000	101,000	66,000	***29,800***	61,000	9,800	-	-	-	-	-	-	-	-	-	-	-	-
SEE	800	16,000	-	14,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SIE	400	8,000	-	10,000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table S2: Sample sizes, locations, cohorts and abbreviations of the different temporal samples collected in each population. Populations used to produce hatchery fish are noticed in italics.

Population	Coordinates	Cohort	Abbreviation	Sample size
Sienne	49.02 : -1.51	1985-87	SIE86	40
Sienne		2002-03	SIE03	37
Sée	48.68 : -1.38	1977-78	SEE77	59
Sée		1986	SEE86	36
Sée		2002-03	SEE03	66
Sélune	48.65 : -1.37	1977-78	SEL77	39
Sélune		1986	SEL86	38
Sélune		2002-03	SEL03	79
Couesnon	48.62 : -1.51	2002-03	COU03	34
Aulne	48.28 : -4.27	1969	AUL69	29
Aulne		2003	AUL03	34
Gave d'Oloron	43.53 : -1.52	1984	GAV84	25
Gave d'Oloron		2003	GAV03	29

All samples were scales collected from anadromous adults caught by anglers and stored in dryly paper envelopes by INRA (UMR ESE and U3E) and ONEMA. Age of each individual was determined from its scales. For contemporary samples, we focused on cohorts 2002-2003 (Fig 1, Table S2). For old ones, we chose cohorts 1977-1978 and 1985 to 1987, which were not yet impacted by local stocking. Concerning the two populations where genitors were collected for the hatchery, we focused on cohorts 1969 and 2003 for AUL, and 1984 and 2003 for GAV.

Molecular analyses

Genomic DNA was extracted from *S. salar* fin tissues and scales by heating samples in solution of proteinase K, TE (Tris/EDTA) buffer and chelex, at 55°C 2 hours and then at 100°C for 10min (Estoup *et al.* 1996). Individuals were genotyped at 17 microsatellites (BHMS176; BHMS179A; BHMS184B; BHMS429; SSA85; SSA65; SSOSL85; SSA9; BHMS235; BHMS217; BHMS111; SSA197; SSA171; BHMS377; SSSP2216; SSA224; BHMS365, see Nikolic et al. 2009 for references) following the procedure described in Perrier et al. (2010).

Molecular data analyses

TEXTPAD 4.7.3 (Helios Software Solutions), Convert 1.31 (Glaubitz 2004), GENEPOP 4.0.7 (Rousset 2008), and GENALEX 6 (Peakall & Smouse 2006) were used to format the data sets used in the subsequent programs. We used MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) to assess the frequency of null alleles and scoring errors due to stuttering or large allelic dropout. Allele number and allelic richness were obtained using FSTAT 2.9.3.2 (Goudet 1995). Tests for linkage and Hardy-Weinberg disequilibrium (*via* F_{IS}) were also conducted with FSTAT. Expected heterozygosity, HE, (Nei 1978) and observed heterozygotie, HO, were calculated with GENETIX 4.05.2 (Belkhir *et al.* 1996). Fdist 2 (Beaumont & Nichols 1996) was used to verify the neutrality of the markers used. Pairwise F_{ST} and tests of differentiation were conducted in FSTAT 2.9.3.2. Pairwise Nei (Da) genetic distances (Nei *et al.* 1983) were estimated at 17 loci using populations 1.2.30 (http://bioinformatics.org/~tryphon/populations/). Factorial Analysis of Correspondences was made using GENETIX 4.05.2 to display the genetic structure among old and recent samples.

Admixture between wild and hatchery stocks was estimated at the individual level using the Bayesian method implemented in the STRUCTURE software (Pritchard 2000). Accordingly, STRUCTURE analyses were performed assuming an admixture model (i.e.

allowing the genetic composition of individuals to be a mixture from different clusters). We ran STRUCTURE from 1 to 6 genetic clusters (*k*) (15 replicates for each *k*). Each run started with a burn-in period of 50,000 steps followed by 300,000 Markov Chain Monte Carlo (MCMC) replicates. We selected the *k* with the highest likelihood (Pritchard *et al.* 2000) and according to the Δ*k* method (Evanno *et al.* 2005). We then calculated the average population admixtures based on 3 runs at the best clustering solution.

Simulation study

We used NEMO 2.1.0, a stochastic individual-based genetically-explicit framework (Guillaume & Rougemont 2006) to simulate the evolution of virtual populations made of wild and hatchery individuals. An individual based approach was used to produce data as close as possible from the real situation. Simulation data were analyzed analogously to molecular data. Model organisms were diploid with separate genders and lived in a structured metapopulation of 6 demes with the following local carrying capacities: 2000, 7000, 200, 300, 800, and 400 adult, corresponding to the GAV, AUL, COU, SEL, SEE, and SIE populations, respectively (see also Table S1 and fig. S1). These Carrying capacities were assumed to correspond to the population sizes for COU, SEL, SEE, and SIE estimated from census data (Anonymous 2008). For GAV and AUL, these values were chosen to allow high dispersal rates simulating stocking operations. We implemented the following semelparous life cycle: (1) breed; (2) dispersal; (3) random regulation of local populations, which reduced the pool of competing individuals to the local carrying capacity (with equal sex ratios); (4) reproduction during which females were assigned a fecundity value drawn from a Poisson distribution with a mean value of 40 offspring and were mated with one randomly chosen male (monogamy). We

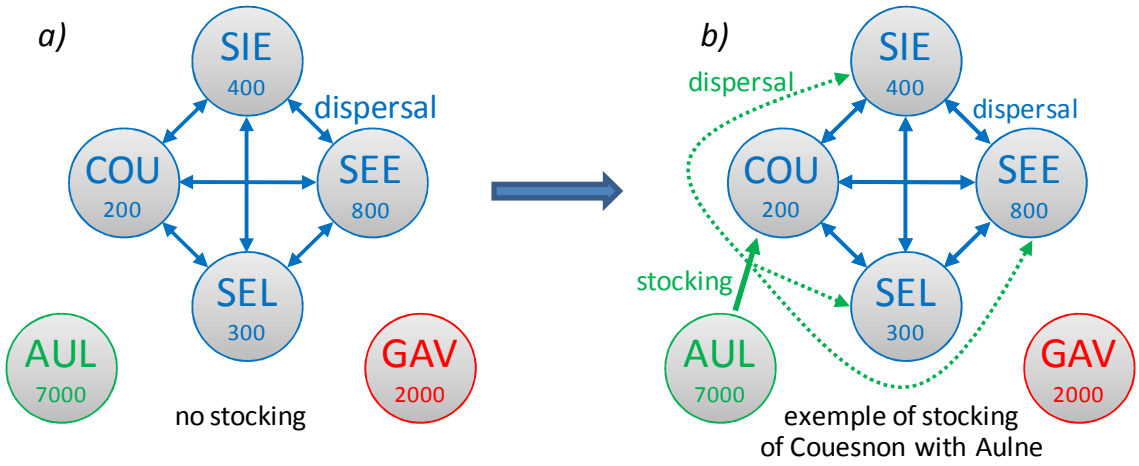

Figure S1: Schematic representation of the metapopulation implemented in Nemo a) without stocking and b) with an example of stocking in Couesnon.

also investigated the potential effect of multiple mating by testing 20% and 50% of polyandry for the best scenario, i.e. the scenario leading to the admixture proportions closest to real data. Adults died after reproduction and the cycle started again. We simulated 17 neutral loci with 15 alleles per locus, a mutation rate u=0.0001, and a recombination rate U=0.5. Alleles at neutral loci were inherited randomly (i.e., no linkage or epistasis). Sex was set randomly (with equal sex ratio). *F*-statistics and multilocus genotypes were recorded every generation. There was no dispersal between AUL, GAV, and the four other Normandy populations before stocking events. According to values of Atlantic salmon dispersal reported in the literature (Jonsson et al. 2003; Pedersen et al. 2007), we implemented two dispersal rates for wild fish among Normandy populations (0.06 and 0.15) and three rates for stocked individuals (0.06, 0.15, and 0.24) giving combinations (see Table 1). These values corresponded to total dispersal from a population and individual flow was equally divided among the three connected populations. We investigated the effects of potential lower survival of hatchery compared to wild fish by testing four ratios of hatchery / wild individuals' survival rates (1; 0.1; 0.05 and 0.01). Each configuration was run 3 times for 290 generations to obtain a genetic structure similar to molecular data. Then we implemented stocking events from the generation 290 on according to stocking data. We ran the program until generation 302.

Table 1: Parameters used for the different scenarios implemented in Nemo. Survival of hatchery fish is given relative to wild fish. Dispersal rates of wild and hatchery fish represent the proportion of individuals leaving a given population and then dispersing with an equal probability into the wild populations.

Scenario	Mating system	Survival	Dispersal Wild	Dispersal Hatchery
A1	monogamy	1	0.06	0.06
A2		1	0.06	0.15
A3		1	0.15	0.15
A4		1	0.15	0.24
B1		0.1	0.06	0.06
B2		0.1	0.06	0.15
B3		0.1	0.15	0.15
B4		0.1	0.15	0.24
C1		0.05	0.06	0.06
C2		0.05	0.06	0.15
C3		0.05	0.15	0.15
C4		0.05	0.15	0.24
D1		0.01	0.06	0.06
D2		0.01	0.06	0.15
D3		0.01	0.15	0.15
D4		0.01	0.15	0.24
E1	polyandry 20%	0.05	0.15	0.15
E2	polyandry 50%	0.05	0.15	0.15

For each run of NEMO, we ran STRUCTURE 3 times assuming an admixture model and using a burn-in periods of 50,000 steps followed by 300,000 MCMC replicates, for the best clustering partition. We then calculated the average population admixture for each simulation conditions. To illustrate the patterns of introgression, 150 genotypes per population were randomly sampled.

Results

Genetic variation within populations

All 17 microsatellite loci were successfully analyzed, with an average successful amplification and scoring per locus of 97.5% for contemporary samples and 92.3% for historical ones with no difference between loci or populations. No significant differences in genetic differentiation (F_{ST}) were found between successive cohorts in SIE, SEE, and SEL indicating temporal stability of allele frequencies. We thus combined genotypes from successive cohorts for each population in our analyses. We found evidence of null alleles or large allele drop-out for 6 out of 238 tests. Considering the overall dataset, there was no evidence of departure from HWE (Table S3) and from LD associated with a particular marker thus ruling out locus-specific factors. Average gene diversity (He) over all populations was 0.77, ranging from 0.74 to 0.80.

Table S3: Sample size (n), number of alleles per locus (N), allelic richness (AR), F_{IS}, expected (H_E) and observed heterozygosity (H_O) of the study populations. Significant F_{IS} values are given in bold.

		SIE86	SIE03	SEE77	SEE86	SEE03	SEL77	SEL86	SEL03	COU03	AUL70	AUL03	GAV80	GAV03	All
	n	40	37	59	36	65	38	38	79	34	29	30	25	29	564
BHMS176	N	3	5	5	5	4	5	5	4	4	4	4	4	4	5
	A_R	2.5	4.0	3.1	3.5	3.5	3.2	3.5	3.6	3.9	3.3	3.9	3.9	3.8	3.7
	F_{IS}	-0.17	0.00	0.06	0.08	0.10	-0.07	0.17	-0.08	-0.01	-0.10	-0.20	-0.11	-0.23	
	H_E	0.49	0.67	0.52	0.59	0.63	0.56	0.53	0.64	0.69	0.65	0.72	0.67	0.67	
	Ho	0.58	0.68	0.49	0.56	0.57	0.61	0.45	0.70	0.71	0.72	0.87	0.76	0.83	
BHMS179A	N	3	3	2	3	4	2	2	4	3	4	3	5	6	7
	A_R	2.2	2.4	2.0	2.3	2.5	2.0	2.0	2.5	2.8	3.2	2.9	4.2	3.8	2.7
	F_{IS}	-0.36	-0.22	-0.02	-0.08	0.29	-0.07	0.11	0.04	-0.18	0.01	0.36	0.01	0.16	
	H_E	0.46	0.50	0.49	0.51	0.53	0.50	0.47	0.51	0.54	0.61	0.59	0.64	0.61	
	Ho	0.63	0.62	0.51	0.56	0.38	0.54	0.42	0.49	0.65	0.62	0.39	0.64	0.52	
BHMS184B	N	5	5	2	4	5	3	3	6	4	3	4	6	6	6
	A_R	3.0	3.3	2.0	2.5	2.6	2.6	2.2	2.8	3.8	3.0	3.5	5.2	4.8	3.4
	F_{IS}	-0.01	0.15	0.28	-0.07	0.02	-0.02	0.04	-0.05	0.17	0.38	0.07	-0.10	0.11	
	H_E	0.55	0.58	0.49	0.51	0.54	0.54	0.51	0.52	0.66	0.65	0.65	0.71	0.68	
	Ho	0.56	0.50	0.36	0.56	0.53	0.55	0.50	0.55	0.56	0.42	0.61	0.80	0.62	
BHMS429	N	11	12	10	7	12	12	11	15	12	4	13	9	10	18
	A_R	7.0	7.7	6.6	5.7	7.0	7.8	7.5	9.1	8.3	4.0	7.7	7.1	7.8	8.4
	F_{IS}	-0.08	0.00	-0.05	0.18	0.12	0.12	-0.03	0.06	0.07	0.22	0.13	0.02	-0.03	
	H_E	0.81	0.85	0.82	0.73	0.82	0.84	0.86	0.89	0.86	0.66	0.83	0.80	0.86	
	Ho	0.89	0.86	0.87	0.61	0.73	0.76	0.89	0.85	0.82	0.56	0.73	0.80	0.90	
SSA85	N	11	12	12	12	17	12	11	17	13	9	14	9	13	19
	A_R	7.8	7.5	8.1	7.3	8.2	8.0	6.7	8.3	8.2	6.2	8.8	7.6	8.7	8.7
	F_{IS}	0.12	-0.04	0.09	-0.02	0.08	0.02	0.05	0.00	-0.04	0.11	-0.07	-0.05	0.03	
	H_E	0.85	0.82	0.86	0.81	0.85	0.84	0.79	0.86	0.84	0.79	0.86	0.86	0.87	

	Ho	0.76	0.86	0.79	0.83	0.79	0.84	0.76	0.86	0.88	0.71	0.93	0.92	0.86	
SSA65	N	14	14	14	12	14	12	10	15	13	9	13	9	12	19
	A_R	8.8	8.5	8.4	8.0	8.4	8.2	8.0	9.1	8.8	6.0	8.0	6.8	7.7	9.0
	F_{IS}	0.13	-0.01	0.00	0.09	0.05	0.14	-0.07	0.03	0.05	-0.14	0.05	-0.11	-0.02	
	H_E	0.87	0.87	0.88	0.87	0.87	0.87	0.87	0.90	0.88	0.73	0.80	0.81	0.83	
	Ho	0.76	0.89	0.88	0.81	0.84	0.76	0.95	0.87	0.85	0.84	0.77	0.92	0.86	
SSOSL85	N	14	13	16	11	13	11	12	17	15	10	13	7	10	23
	A_R	8.6	8.2	8.5	8.1	8.0	7.8	8.1	8.8	9.4	6.4	7.4	6.3	7.1	8.5
	F_{IS}	-0.01	-0.05	0.15	0.04	0.03	0.10	0.01	-0.01	0.03	0.12	0.05	0.11	0.14	
	H_E	0.86	0.86	0.86	0.87	0.87	0.85	0.86	0.88	0.89	0.69	0.82	0.79	0.82	
	Ho	0.88	0.92	0.74	0.85	0.85	0.78	0.87	0.89	0.87	0.62	0.79	0.72	0.72	
SSA9	N	19	18	14	14	16	15	14	19	14	19	18	11	18	26
	A_R	10.1	9.7	8.7	9.6	9.0	9.7	10.2	10.0	8.9	10.9	10.6	8.7	10.5	10.7
	F_{IS}	-0.02	-0.05	-0.02	-0.14	-0.05	0.02	0.08	0.04	0.07	-0.04	-0.05	0.07	0.05	
	H_E	0.89	0.89	0.88	0.86	0.88	0.89	0.90	0.90	0.87	0.91	0.91	0.68	0.89	
	Ho	0.92	0.95	0.90	1.00	0.92	0.89	0.85	0.87	0.82	0.97	0.97	0.67	0.86	
BHMS235	N	15	17	17	15	19	17	14	20	14	9	17	13	16	27
	A_R	9.0	9.6	9.5	9.1	9.5	9.5	9.0	10.6	8.8	6.3	10.1	8.8	10.3	10.3
	F_{IS}	-0.10	-0.01	-0.03	0.02	0.00	-0.08	0.00	0.03	-0.01	-0.13	-0.03	0.19	0.10	
	H_E	0.88	0.89	0.90	0.87	0.89	0.89	0.88	0.92	0.86	0.78	0.90	0.87	0.90	
	Ho	0.97	0.92	0.93	0.86	0.89	0.97	0.89	0.90	0.88	0.90	0.94	0.72	0.83	
BHMS217	N	7	8	7	6	8	7	7	8	7	6	9	6	8	9
	A_R	5.1	6.1	5.2	5.1	5.4	5.2	5.6	5.5	6.1	4.6	5.9	4.4	5.1	5.6
	F_{IS}	-0.13	0.17	0.07	0.05	0.12	-0.07	-0.11	0.12	0.00	0.19	-0.07	0.00	0.00	
	H_E	0.71	0.81	0.76	0.77	0.78	0.73	0.77	0.78	0.83	0.75	0.79	0.55	0.61	
	Ho	0.82	0.69	0.72	0.74	0.70	0.80	0.86	0.70	0.84	0.62	0.85	0.56	0.62	
BHMS111	N	9	7	8	8	8	8	8	9	7	6	6	6	6	10
	A_R	6.2	5.4	5.8	5.9	5.3	5.2	5.9	5.1	4.9	4.7	4.8	5.3	4.9	5.6
	F_{IS}	-0.11	0.21	-0.04	-0.06	-0.11	-0.08	-0.02	-0.07	-0.03	-0.03	0.00	0.00	0.25	
	H_E	0.75	0.67	0.75	0.74	0.71	0.68	0.76	0.71	0.70	0.69	0.73	0.79	0.76	
	Ho	0.85	0.54	0.78	0.80	0.79	0.74	0.79	0.76	0.74	0.72	0.74	0.80	0.59	
SSA197	N	8	13	9	9	8	8	10	15	11	12	12	9	15	25
	A_R	5.7	7.3	5.1	6.3	5.5	5.9	6.3	6.8	6.8	8.0	7.2	7.6	9.3	7.1
	F_{IS}	-0.04	0.03	-0.11	0.09	-0.12	0.04	0.14	0.04	0.07	0.01	-0.04	0.00	-0.03	
	H_E	0.78	0.82	0.75	0.80	0.78	0.78	0.81	0.81	0.81	0.82	0.81	0.86	0.89	
	Ho	0.82	0.81	0.83	0.74	0.88	0.76	0.71	0.78	0.76	0.83	0.85	0.88	0.93	
SSA171	N	11	15	12	7	11	6	8	14	11	7	15	9	13	25
	A_R	6.7	8.5	6.6	5.3	5.9	5.2	6.0	7.2	7.0	6.5	9.7	6.8	10.4	7.8
	F_{IS}	0.03	-0.01	0.16	-0.08	0.01	-0.04	-0.06	0.05	0.04	0.17	0.06	0.11	-0.01	
	H_E	0.82	0.87	0.82	0.78	0.80	0.76	0.81	0.83	0.79	0.82	0.88	0.75	0.90	
	Ho	0.81	0.89	0.70	0.86	0.80	0.80	0.86	0.79	0.77	0.71	0.85	0.68	0.93	
BHMS377	N	15	22	20	15	19	17	15	22	18	13	15	13	21	33
	A_R	8.7	12.0	10.2	9.4	9.7	9.5	9.5	10.7	10.5	8.2	9.7	8.4	10.8	11.2
	F_{IS}	0.06	-0.06	-0.09	0.01	0.02	-0.02	0.04	-0.03	0.01	-0.01	0.16	0.06	0.13	
	H_E	0.85	0.93	0.91	0.89	0.89	0.89	0.89	0.92	0.91	0.84	0.90	0.84	0.89	
	Ho	0.81	1.00	1.00	0.89	0.88	0.92	0.86	0.95	0.91	0.86	0.77	0.81	0.79	
SSSP2216	N	11	12	17	10	13	13	11	13	12	10	11	12	15	22
	A_R	7.0	8.0	8.8	7.3	7.3	7.6	7.1	7.8	8.0	7.3	8.1	8.5	9.6	8.3
	F_{IS}	-0.08	-0.01	0.12	0.01	-0.06	0.16	0.13	-0.05	-0.02	0.22	0.02	-0.07	-0.02	
	H_E	0.78	0.85	0.86	0.83	0.79	0.78	0.78	0.84	0.85	0.83	0.86	0.87	0.89	
	Ho	0.86	0.86	0.76	0.83	0.84	0.67	0.69	0.88	0.88	0.66	0.86	0.95	0.93	
BHMS365	N	11	14	15	12	19	12	12	22	18	6	17	13	18	33
	A_R	6.6	9.3	7.8	7.8	7.6	7.6	7.6	8.9	9.6	5.1	11.2	10.2	11.1	9.2
	F_{IS}	0.01	0.05	0.07	0.07	-0.03	0.05	0.07	0.03	0.09	0.17	-0.07	-0.08	0.34	
	H_E	0.82	0.89	0.86	0.84	0.85	0.84	0.84	0.88	0.89	0.71	0.92	0.90	0.91	
	Ho	0.83	0.86	0.81	0.79	0.88	0.81	0.79	0.86	0.82	0.62	1.00	1.00	0.62	
SSA224	N	6	7	7	6	5	6	5	8	6	6	4	6	6	9
	A_R	4.5	5.0	4.5	4.4	4.2	4.0	4.0	4.9	4.8	4.4	3.8	5.0	4.3	4.7
	F_{IS}	0.07	-0.13	-0.12	-0.11	-0.03	-0.06	-0.08	-0.11	-0.22	-0.30	-0.18	-0.08	-0.03	
	H_E	0.56	0.63	0.61	0.54	0.61	0.48	0.64	0.65	0.67	0.63	0.66	0.68	0.59	
	Ho	0.53	0.72	0.69	0.60	0.63	0.51	0.70	0.72	0.82	0.83	0.79	0.75	0.62	
All loci	F_{IS}	-0.03	0.00	0.03	0.01	0.02	0.02	0.02	0.01	0.01	0.05	0.01	0.00	0.06	
	H_E	0.75	0.79	0.76	0.75	0.77	0.75	0.76	0.79	0.80	0.74	0.80	0.77	0.80	
	Ho	0.78	0.80	0.75	0.76	0.76	0.75	0.76	0.79	0.80	0.72	0.81	0.79	0.77	
	m(A_R)	6.45	7.21	6.52	6.33	6.45	6.41	6.44	7.16	7.10	5.77	7.27	6.76	7.65	

Genetic variation among populations

The pairwise tests of genetic differentiation yielded significant outcomes among GAV, AUL, and Normandy populations for both historical and contemporary samples (Table S4).

However, the genetic differentiation between these populations was in average higher among historical samples (F_{ST} = 0.08) than among contemporary samples (0.04). COU03 was less differentiated from GAV03 and AUL03 than SEL, SEE, and SIE. There was no significant differentiation among both historical and contemporary from SEL, SEE, and SIE (F_{ST} <0.01), However, F_{ST} between COU03 and SEL03, SEE03, and SIE03 was significant. The Factorial Analysis of Correspondences highlighted the existence of 3 genetic groups and lower genetic distances between contemporary samples than old ones (Fig 2, A). In particular, COU03 was much close to AUL03 than to Normandy populations.

Table S4: Pairwise F_{ST} with non-significant values given in bold italic.

	SIE03	SEE77	SEE86	SEE03	SEL77	SEL86	SEL03	COU03	AUL69	AUL03	GAV84	GAV03
SIE86	0.011	***0.003***	***0.003***	***0.003***	***0.003***	***0.004***	0.008	0.028	0.068	0.052	0.096	0.075
SIE03		0.008	0.009	0.005	***0.007***	0.008	***0.003***	0.010	0.053	0.028	0.068	0.045
SEE77			***0.009***	***0.005***	***0.004***	***0.003***	0.008	0.026	0.067	0.048	0.087	0.065
SEE86				***0.002***	***0.007***	***0.008***	0.008	0.021	0.075	0.045	0.095	0.071
SEE03					***0.003***	0.006	***0.003***	0.021	0.064	0.041	0.088	0.067
SEL77						***0.006***	***0.004***	0.025	0.070	0.049	0.094	0.067
SEL86							0.005	0.030	0.067	0.046	0.087	0.063
SEL03								0.015	0.057	0.028	0.073	0.051
COU03									0.039	0.013	0.074	0.052
AUL69										0.025	0.079	0.073
AUL03											0.065	0.046
GAV84												***0.011***

Admixture between populations

The STRUCTURE program consistently identified 3 genetic clusters (*k*) corresponding to AUL, GAV, and samples from Normandy. Admixture proportions from AUL and GAV clusters in historical samples of Normandy were small: from 0.02 to 0.05. Admixture proportions of AUL and GAV were of same magnitude into SEL77 and SEL86 and in SEE77 and SEE86. In contrast, we found a high genetic contribution of AUL cluster in COU03, SEL03, SEE03, and SIE03: 0.53, 0.20, 0.09 and 0.16, respectively (Table 2). GAV cluster contribution in COU03, SEL03, SEE03 and SIE03 was also higher than in old samples but remained relatively small: 0.07, 0.08, 0.03 and 0.14, respectively. Considering these four Normandy populations and their relative sizes, the overall admixture by AUL and GAV was 0.25. A close inspection of individual admixture in COU03 revealed many individuals with high proportions of the AUL cluster (Fig 3). We also found some individuals with high proportions of the AUL cluster in SEL03, SEE03 and SIE03. Individuals with intermediate admixtures among the different clusters were observed in each river but in low abundance compared to the “pure” individuals from each clusters.

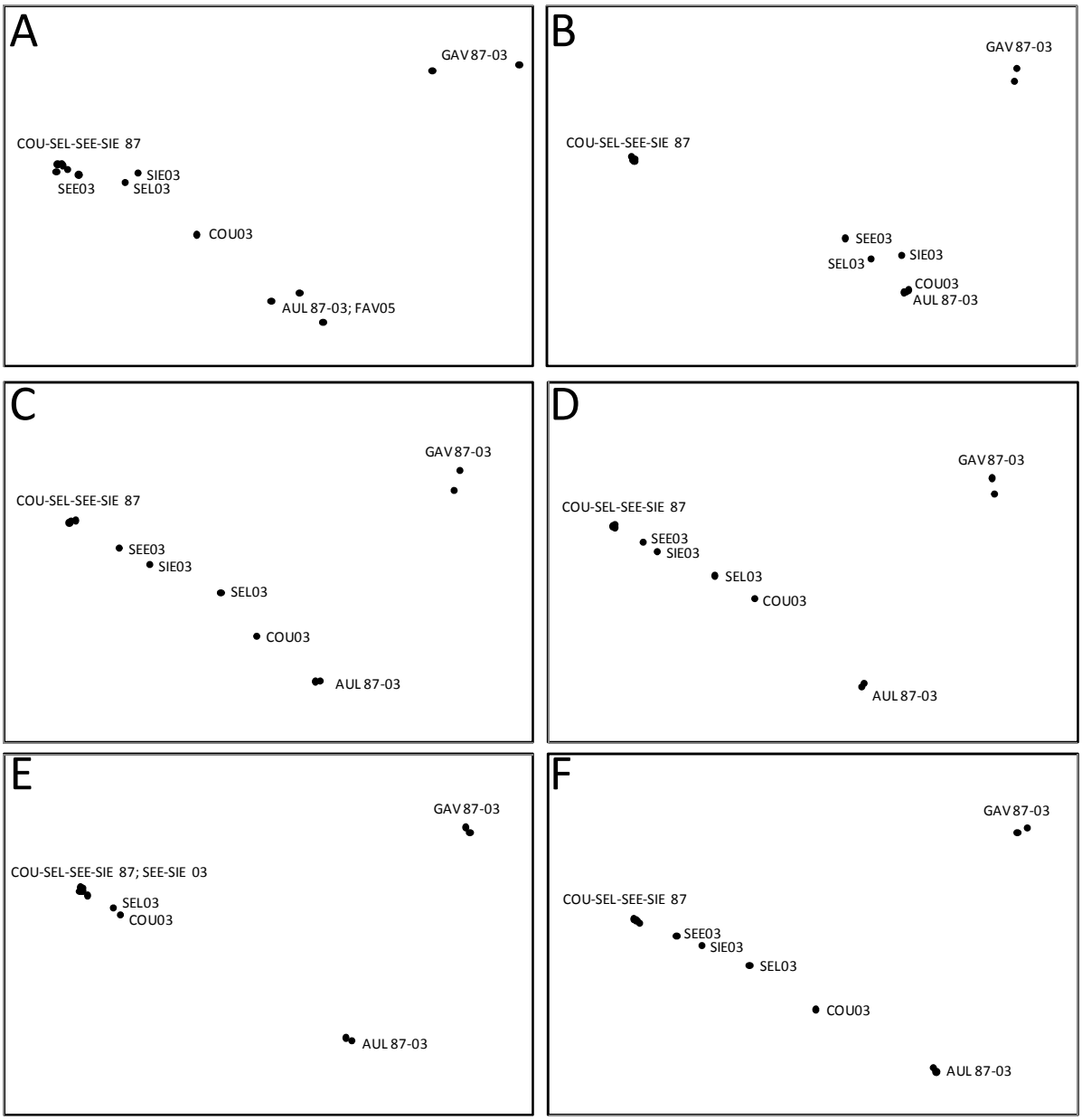

Figure 2: Multidimensional scaling analysis of pairwise Nei's (1978) genetic distances between samples for the real data set (A) and for simulated data from the following scenarios: A3 (B), B3 (C), C3 (D), D3 (E) and E2 (F).

Admixture between simulated populations

The STRUCTURE program also identified the 3 genetic clusters (*k*) corresponding to AUL, GAV, and samples from Normandy. Variability between Nemo and Structure runs were relatively low for a same scenario (average deviations between populations' admixture: 0.03 and 0.01, respectively). Populations Admixture proportions from AUL and GAV clusters into samples of Normandy before stocking events were small, 0.01 on average. The admixture in COU03, SEL03, SEE03, and SIE03 by GAV was in most cases lower than estimated. In contrast, we found a wide range of admixtures in COU03, SEL03, SEE03, and SIE03 by AUL: from 0.01 to 0.96 depending on the scenario, the origin of the genitors, and the river

considered (Table 2, Fig 3). Populations' admixtures were very different depending on survival rates of stocked fish. Scenarios A and B led to a strong admixture of AUL into Normandy rivers and 29 out of 32 values were higher than for real data (Table 2). AUL admixture proportions for A and B scenarios were from 0.68 to 0.96 in COU03, from 0.39 to 0.88 in SEL03, from 0.05 to 0.79 in SEE03, and from 0.10 to 0.87 in SIE03. Scenarios C gave the AUL admixture values the more compatible with real data, especially scenarios C3 and C4 which gave AUL admixture proportions of 0.55 and 0.47 in COU03, of 0.30 and 0.31 in SEL03, of 0.07 in SEE03, and of 0.14 in SIE03. Finally, scenarios D led to low admixture proportions of AUL: from 0.12 to 0.24 in COU03, from 0.06 to 0.11 in SEL03, from 0.01 to 0.02 in SEE03 and from 0.01 to 0.04 in SIE03. Populations' admixtures were also different depending on dispersal of both wild and stocked individuals. Dispersal of stocked fish was globally negatively linked to admixture for COU03 but positively linked for SEE03 and SIE03. For SEL03 general pattern was more difficult to sort out. In the same way, dispersal of wild fish was globally negatively linked to admixture for COU03 but positively linked for SEL03, SEE03, and SIE03. Implementing a 20% and 50% polyandry mating with system for the scenario C3, we found only very small admixture differences. Individual admixture proportions for Scenario C were relatively consistent with those observed for real data, especially the scenario C3 that led to a similar pattern of introgression (Fig 3). However, we globally found more individuals with "intermediate" genetic admixture signatures and fewer individuals with "pure" hatchery profiles in simulated than molecular data.

Table 2: Observed and simulated admixture proportions of hatchery stocks (Gave d'Oloron and Aulne) with Couesnon, Sélune, Sée, and Sienne in cohorts 2002-2003.

	Admixture proportion of GAV				Admixture proportion of AUL				Average total admixture within Bay of Mont-Saint-Michel
	COU03	SEL03	SEE03	SIE03	COU03	SEL03	SEE03	SIE03	
Observed	0.07	0.08	0.03	0.14	0.53	0.2	0.09	0.16	0.33
A1	0.04	0.19	0.06	0.07	0.87	0.58	0.19	0.34	0.59
A2	0.02	0.13	0.07	0.08	0.96	0.84	0.69	0.82	0.90
A3	0.02	0.09	0.07	0.06	0.96	0.87	0.79	0.85	0.93
A4	0.02	0.08	0.05	0.08	0.96	0.88	0.62	0.87	0.89
B1	0.05	0.1	0.02	0.03	0.82	0.62	0.05	0.1	0.45
B2	0.05	0.1	0.02	0.04	0.8	0.59	0.08	0.19	0.47
B3	0.05	0.07	0.03	0.04	0.7	0.45	0.14	0.2	0.42
B4	0.05	0.08	0.03	0.03	0.68	0.39	0.11	0.23	0.40
C1	0.05	0.07	0.01	0.02	0.7	0.47	0.03	0.06	0.35
C2	0.07	0.06	0.02	0.03	0.67	0.41	0.05	0.1	0.35
C3	0.05	0.06	0.03	0.03	0.55	0.3	0.07	0.14	0.31
C4	0.06	0.05	0.02	0.03	0.47	0.31	0.07	0.14	0.29
D1	0.04	0.04	0.01	0.01	0.24	0.11	0.01	0.01	0.12
D2	0.03	0.04	0.01	0.01	0.23	0.11	0.02	0.02	0.12
D3	0.03	0.03	0.01	0.01	0.16	0.07	0.02	0.02	0.09
D4	0.02	0.02	0.01	0.01	0.12	0.06	0.02	0.04	0.08
E1	0.06	0.04	0.02	0.02	0.47	0.31	0.08	0.08	0.27
E2	0.04	0.06	0.03	0.04	0.53	0.3	0.08	0.09	0.29

Genetic variation among simulated populations

The Factorial Analysis of Correspondences highlighted the existence of 3 genetic groups among old generations of simulated populations for the scenarios A3, B3, C3, D3, and E2 (Fig 2, B, C, D, E, & F). Considering the scenario A3 with the highest survival rate of stocked fish, the analysis reveals that all contemporary populations have genetic characteristics close to AUL (Fig 2, B). The scenarios B3 and C3 showed a clear gradient of genetic modifications from the original signature of SEE, SIE, SEL and COU (Fig 2, C & D). Implementing the scenario D3 with the lowest survival rate of stocked fish, the genetic characteristics of the studied populations remain mostly unchanged (Fig 2, E). Overall, the modifications of genetic distances observed with the scenario C3 (Fig 2, D) were closest to those observed in real data. Finally, considering 50% polyandry for the scenario C3, we only detected a slight modification of the original pattern (Fig 2, F).

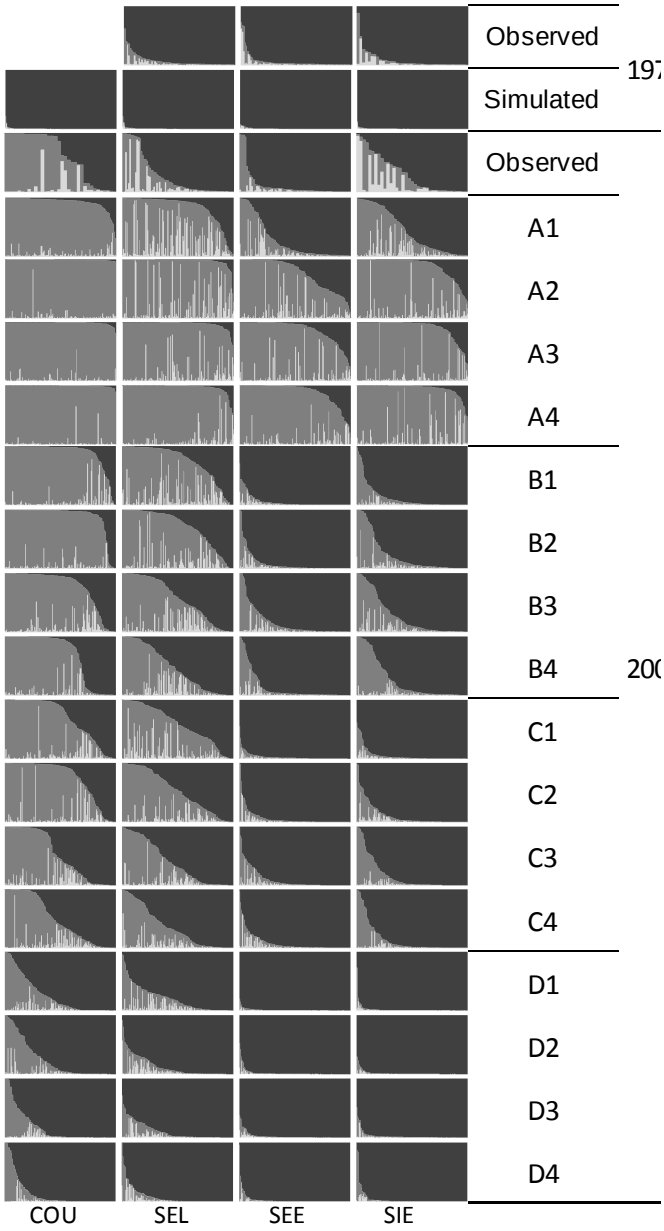

Figure 3: Observed and simulated individual admixture proportions in COU, SEL, SEE and SIE of three genetic clusters inferred with the Structure software: Gave (▫), Aulne (▪), and Normandy (■). Vertical bars show individual membership to each cluster. Admixture analyses of old and recent cohorts are presented for real and simulated data according to the scenarios described in table 1.

Discussion

Our first objective was to compare the genetic structure and admixture levels of the study populations before and after stocking. Our genetic analyses of old and recent samples strongly suggest that stocking greatly modified the genetic structure within the studied populations.

The following aims were to simulate expected admixture levels in each population according to stocking and demographic data and thus to investigate whether increased dispersal and / or lower survival of hatchery fish relative to wild individuals may explain the observed levels of admixture. The results of our simulation study fit well with observed data and suggest low survival and high dispersal of stocked fish.

According to previous studies on French populations (Perrier et al, in prep), the genetic differences were small and not significant between samples from the rivers Sélune, Sée, and Sienne but statistically significant differentiation were observed among these populations and Aulne and Gave d'Oloron. The observed level of differentiation among populations from the three geographic groups was similar to those found in other studies (Vaha *et al.* 2006; Dionne *et al.* 2008; Tonteri *et al.* 2009). Moreover, genetic signature of Aulne, Gave d'Oloron, and to a lesser extent Sée, appeared to be stable over the time, showing that these Atlantic salmon populations are likely to have a stable genetic composition, as suggested by several recent studies (Tessier & Bernatchez 1999; Palstra *et al.* 2006). The existence of a stable genetic differentiation between sources and sink is an important parameter allowing the detection of admixture following individuals transfer over temporal samples (Choisy *et al.* 2004; Vaha & Primmer 2006).

The Atlantic salmon populations from the Bay-du-Mont-Saint-Michel have been some of the most intensively stocked ones in France during the two last decade, and remain the unique place were stocking is still performed using non-local fish. Therefore, significant admixture levels with Aulne and Gave were found in recent samples from the Baie-du-Mont-Saint-Michel (from 12 to 60%) while old samples showed little admixture (from 2% to 5%). Furthermore, our results showed a decrease of genetic differentiation among donor and stocked populations. Also, admixture within old samples could result from migration while high degrees of admixture within recent samples were consistent with the quantities of fish stocked and corroborates precedent results on the same populations (Perrier et al., in prep; (Grandjean et al. 2009)). Indeed, Couesnon has been highly stocked and recent sample cumulated an admixture from Aulne and Gave of 60% while Sée have been stocked once and the recent admixture remains of 12%. These results were also consistent with studies showing a correlation between stocking intensities and admixture of wild populations of brown trout (Hansen & Mensberg 2009) and brook charr (Marie et al. 2010).

We found relatively high admixture rates within populations from the Bay-du-Mont-Saint-Michel but they correspond to relatively low relative survival of stocked fish in our simulation study. Indeed, our results suggest that each population may be almost totally

replaced by fish used for stocking for a survival rate of stocked fish equal to the one of native fish. For example, only 2% to 9% of the native gene pool may remain in Couesnon and 23% to 4% in Sélune. Also, the average observed admixture, 0.33, corresponded well, to scenario C implementing a survival rate of stocked fish 20 times inferior to the one of native fish. The relatively low admixture by stocked fish could result from a low survival in the wild or /and from low reproductive success. First, reduced survival of fish stocked appeared to be verified according to the low number of pure migrants found in each population and could reflect acclimation troubles consecutive to bad conditions during stocking and leading to highly variable survival rates (Mazur & Iwama 1993; Salminen *et al.* 2007).

Considering the abundance of pure individuals with Aulne or native genetic characteristics within Couesnon, we found fewer individuals with hybrids signature than we could expect. This could suggest low reproductive succes of hatchery reared fish in the wild (McGinnity *et al.* 1997; Fleming *et al.* 2000; Araki *et al.* 2007). This could also suggests that individuals breed with other individuals with similar genetic signature or that it exist a difference in timing of reproduction of these two strains (Hansen & Mensberg 2009). However, given the admixture expected in our simulations and according to the presence of relatively high hybrid abundance in other rivers from the Bay of Mont-Saint-Michel, the more probable reason for little abundance of hybrids in Couesnon is the low survival of offspring due to bad spawning grounds quality.

While observed admixture corresponded well to scenarios C, it corresponded better to scenario B in the case of Sienne and Sée, suggesting a higher admixture of these two populations. It could suggest better adaptation of these fishe in these environments of simply better environments. This can also suggest higher straying of stocked fish than the values retained in our scenarios. Indeed, we implemented straying rates of stocked fish of 6% or 15%, and equal to the native fish or 2,5 times higher according to (Jonsson *et al.* 2003), but higher values of straying were suggested in other studies (Pedersen *et al.* 2007). A last hypothesis could be that, given the high inter-annual variability of juvenile survival and populations sizes (Dumas & Prouzet 2003), some stocking events could have led to particularly high admixture because of particularly high survival of stocked fish and /or because of the concomitance of particularly low population size and abundant stocking.

The values chosen for simulation parameters were taken from literature and from in situ observations realized by INRA and fisheries organizations. However, other sets of parameters could be tested. For instance, various values of straying rates could be implemented (Jonsson *et al.* 2003; Pedersen *et al.* 2007). Life cycle parameters also kept

constant population sizes, while we know that they can vary a lot (Dumas & Prouzet 2003). Future studies could use more complex life cycle taking into account population size variability. Connectivity with populations from Brittany, Southern England, and other populations from Normandy may also influence admixture of the four populations from the Bay du Mont-Saint-Michel but probably in very low magnitude. Finally, we postulated that the increased admixture was almost exclusively due to stocking but we can not totally exclude a recent increase of straying of wild individuals. Indeed, recent studies proposed that dispersal rates could be variable over long time periods (Palstra *et al.* 2007) and could be influenced by global change (Valiente *et al.* 2010). However, the little admixture of Sée population and more generally of most of the French populations (Perrier *et al., in prep*) suggest that even if gene flow increased recently, it little contributed to observed admixture among populations from the Bay du Mont-Saint-Michel.

To our knowledge, this study represents the first analysis estimating the relative survival of stocked Atlantic salmon in wild populations. Atlantic salmon is an important species for recreational fishing in France and is considered as a great indicator of river quality and connectivity but is one of the most threatened fish species. Stocking French Atlantic populations is currently realized with native fish except for rivers from the bay du Mont-Saint-Michel. Also this study has several implications for the conservation of this species and the management of stocking practices. Firstly, this study shows how stocking practices analyzed here greatly modified the genetic structure within managed populations but also among these populations and donor's ones. Secondly, this study shows that these practices may be little efficient given the low survival rates of stocked fish. Finally, this study, and many other studies (Garcia de Leaniz *et al.* 2007; Fraser 2008), proposed strong selection against stocked fishes and potential loss of local adaptation. These considerations imply that these four rivers from the bay du Mont-Saint-Michel should be managed independently from other genetic groups in order to maintain local specific genetic diversity and local adaptation. Besides, this study demonstrates the usefulness of sample collection in conservation biology. Furthermore, the use of simulation tool and both contemporary and old sample can lead to a clearest analysis and interpretation of populations states. For example, in contrast with a precedent study on the same populations which only considered admixture rates without taking into account population sizes and number of fishe stocked integrated in our simulations (Grandjean *et al.* 2009) and suggesting a higher survival of fish from Gave, this study suggested the opposite result. We propose that this kind of approach could be generalized to other context where wild populations are subject to supplementation operations with genetically differentiated individuals.

Acknowledgments

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Chapitre VI. Utilisation couplée de la microchimie et de la génétique pour déterminer l'origine des poissons aux signatures génétiques de repeuplements : sauvages ou déversé.

Les objectifs de cette étude étaient d'utiliser des analyses LA-ICPMS des otolithes pour discriminer les deux origines possibles des saumons aux caractéristiques génétique des poissons repeuplés : né en élevage et déversé ou issus de la reproduction *in natura* d'individus précédemment déversés.

Les poissons déversés dans le cadre des programmes de repeuplement peuvent être détectés par des outils génétiques dès lors qu'ils sont issus de stocks génétiquement différenciés, comme exposé aux chapitres précédents. Cependant ces poissons sont à même de se reproduire dans le milieu dans le quel ils sont déversés et même de se croiser avec des poissons autochtones sauvages. Ainsi, la reproduction dans le milieu naturel de ces poissons issus de repeuplement peut conduire à la production « d'hybrides » aux signatures génétiques intermédiaires et de poissons sauvages aux caractéristiques génétiques des poissons repeuplés. Le chapitre VI montre la présence d'hybrides dans les quatre populations de la Baie du Mont-Saint-Michel. En revanche, les poissons ayant une signature caractéristique des stocks de repeuplements peuvent avoir été déversés ou être des hybrides résultants du croisement entre les poissons natifs et les individus repeuplés. Il peut alors être intéressant d'avoir recours à la microchimie des otolithes pour connaitre l'origine véritable de ces individus. En effet, discriminer les poissons aux caractéristiques de repeuplements issus de reproduction sauvage ou déversés est un point important pour mesurer les effets des pratiques de gestion et renseigner sur les éventuels processus de sélection locale.

Dans cette étude, nous avons couplé des analyses génétiques et des analyses microchimiques d'otolithes de juvéniles et adultes de populations de la Baie du Mont-Saint-Michel et de stocks utilisés pour le repeuplement. Nous avons échantillonné 90 juvéniles de quatre rivières de la baie et d'une pisciculture bretonne utilisée pour le repeuplement. Les individus ont été analysés à 6 marqueurs microsatellite et 14 éléments chimiques par ablation laser couplée à un spectromètre de masse à plasma inductif.

Les populations de la Baie étaient significativement différentes du stock breton utilisé pour les repeuplements. Au sein de la baie, peu de différences génétiques étaient observées entre les populations. En utilisant les analyses microchimiques, des différences significatives ont été observées parmi les otolithes des juvéniles des quatre rivières de la baie et de la pisciculture, permettant leur assignation avec une fiabilité de 83 à 100%. Les analyse

génétique ont révélé la présence i) de poissons sauvages avec des caractéristiques génétiques locales ii) d'hybrides entre des individus sauvages et repeuplée et iii) de juvéniles sauvages ayant des caractéristique de repeuplement et donc issus de la reproduction *in natura* de poissons déversés. Etant donné les différences microchimiques entre les poissons sauvages et ceux de pisciculture, l'approche couplée de la génétique et de la microchimie permet ensuite distinguer les adultes issus de repeuplements, de ceux issus de la reproduction naturelle de poissons déversés. Ainsi on peut classer les adultes en quatre groupes : i) sauvages aux caractéristiques génétiques locales, ii) sauvages aux caractéristiques de repeuplement, iii) sauvages aux caractéristiques hybrides, et iiii) de repeuplement.

Ces résultats ont d'importantes implications pour la gestion comme d'un point de vue strictement fondamental car mettent évidence la reproduction *in natura* des individus déversés dans le cadre des repeuplements. Par ailleurs, cette étude soulève le problème de l'interprétation de l'origine des individus généralement assignés aux stocks de repeuplements.

Coupling genetic and otolith trace element analyses to identify river-born fish with hatchery pedigrees in stocked Atlantic salmon populations

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Abstract

This study combines otolith trace element and genetic analyses to explore the origin of individuals when hatchery-reared fishes are released into wild populations. We sampled 90 juvenile Atlantic salmon in four rivers of Normandy (France) and in the hatchery stock. Individuals were analyzed at 6 microsatellite markers and their otolith elemental concentrations (14 elements) were measured using femto-second LA-ICPMS. Wild populations were genetically differentiated from the hatchery strain ($F_{ST} \approx 0.06$). Significant differences in elemental concentrations were found among otoliths of juveniles from the four rivers and the hatchery, allowing the identification of their geographic origin (83 to 100% correct assignment). Coupling genetic and trace element analyses on the same individuals provided formal evidence that hatchery-born juveniles released into the wild can migrate to the sea and return as adults to breed on natural spawning grounds. Their progeny have pure hatchery pedigrees but otoliths typical of river-born juveniles meaning that they can be mistaken for hatchery-raised juveniles if only genetic data are considered. The presence of hybrids also confirmed that individuals with hatchery pedigrees can breed with wild conspecifics.

Key words: Assignment, otolith microchemistry, genetic differentiation, hatchery, stocking

Introduction

The supplementation of wild populations with hatchery-reared individuals is a common practice in salmonid fisheries management (Aprahamian et al. 2003; Fraser 2008). The mortality of stocked fish is often supposed to be elevated given the relatively low percentages of released individuals returning to target rivers (Ruzzante et al. 2004). It has also been shown that stocked fish are more likely to stray than wild conspecifics when coming back to freshwater spawning grounds (Jonsson et al. 2003; Pedersen et al. 2007). Wild and stocked fish can breed together but detrimental effects of stocking on native stocks have been reported, notably a fitness decline of wild populations (Jonsson and Jonsson 2006; Youngson and Verspoor 1998; Araki et al. 2007).

The use of natural marks detected by genetic and chemical analyses allows the quick assignment of fish to their natal population with a relatively high accuracy. Assignment methods based on multilocus genotypes and Bayesian algorithms (Pritchard et al. 2000; Corander et al. 2003) have a large potential for identifying the geographic origin of individuals (Manel et al. 2005; Hauser et al. 2006). For instance, Griffiths et al. (2010) gathered a large amount of genetic data in Western Europe salmon populations, which can be used to identify individuals of unknown origin. Multilocus genotypes were also successfully used to identify hatchery-reared individuals and to study the hybridization between wild and hatchery fishes in stocked rivers (Vasemagi et al. 2001; Aurelle et al. 2002; Hansen 2002). However, non-native stocked individuals can breed together *in natura* and produce river-born juveniles with hatchery pedigrees (Hansen 2002; Araki et al. 2007; Hansen et al. 2009). Such individuals with hatchery pedigrees can thus either originate from the hatchery or from the breeding among hatchery-born adults that reproduced in the river.

In contrast with genetic characteristics, geochemical signatures from fish otoliths and scales are to a large extent not inherited from parents, but influenced by the local environment, especially elements in the water (Thorrold et al. 1997; Campana, 1999). Therefore, variations in environmental chemistry represent natural tracers for fish origin and movements when otoliths' elemental composition can be examined (Elsdon and Gillanders 2004; Gillanders, 2005; Friedrich and Halden 2008). Otolith trace element and stable isotope analyses have been successfully used to discriminate fish stocks even at small spatial scales (Thorrold et al. 2001; Tomas et al. 2005; Veinott and Porter 2005) and to distinguish wild from hatchery-reared fishes (Gao et al. 2004, Gao and Bean 2008, Gibson-Reinemer et al. 2009). The alternative use of scales is tricky since the inner portion of scales corresponding to juvenile growth includes collagen formed later in life (Hutchinson and Trueman 2006). As a

result, the continuous growth and concentric aggregation of material in the otolith make it more appropriate to study the early life history of fishes (but see Adey et al., 2009).

Several recent studies demonstrated how joint genetic and otolith analyses can be useful for stock identification (Miller et al., 2005; Higgins et al. 2010) and to explore patterns of dispersal in fishes (Bradbury et al., 2008; Ohara et al. 2009). In the present work, we studied four Atlantic salmon populations of the Bay of Mont-Saint-Michel (France), which have been moderately to heavily stocked over the past decades. Joint otolith trace element (femtosecond laser ablation inductively coupled mass spectrometry) and molecular (microsatellite markers) analyses were carried out on juveniles and adults collected in the wild and in the hatchery. Our objectives were to i) investigate whether natal rivers of wild individuals can be identified, ii) discriminate wild and hatchery-born individuals as well as their hybrids and iii) distinguish hatchery-born and river-born individuals among the fishes with hatchery pedigrees.

Materiel and methods

Study populations and restocking program

Four rivers of the Bay of Mont Saint-Michel (Normandy, France) were studied: Sienne, Sée, Sélune, and Couesnon Rivers (Fig. 1). These rivers have been stocked for years with 10-months-old juveniles produced at the Favot Hatchery, Brittany (Fig. 1). Genitors used in this hatchery are yearly caught in the nearby Aulne River, Brittany (Fig. 1). Earlier investigations on the genetic structure of French Atlantic salmon populations using 17 microsatellites revealed a significant differentiation between rivers from

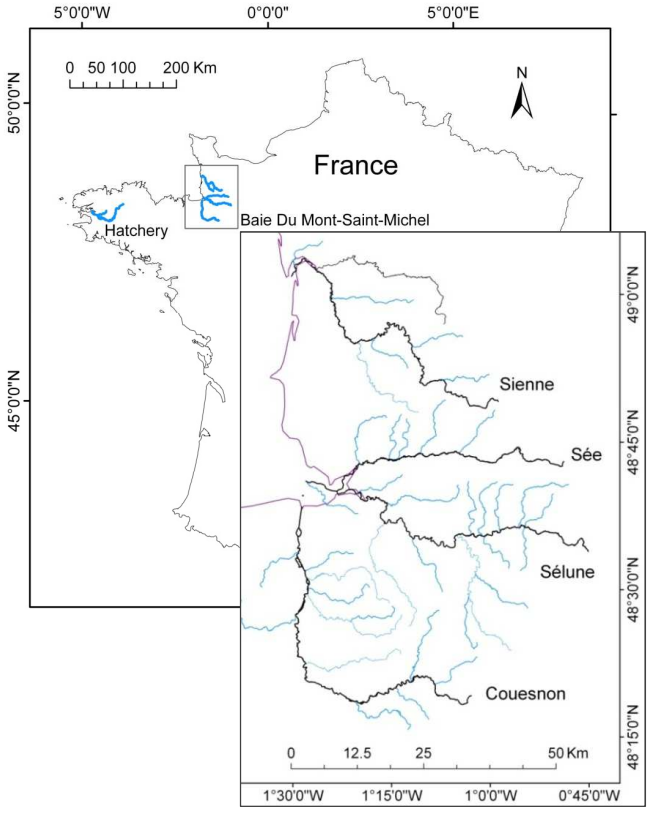


Figure 1. Location of Couesnon, Sélune, Sée and Sienne Rivers in the Bay of Mont-Saint Michel (Normandy, France). These rivers have been stocked with hatchery-reared fish originating from the Aulne River (Brittany, France).

Brittany and Normandy (Perrier et al. 2010, Perrier et al in preparation). Therefore, we expected individuals originating from Brittany to be readily identified among fishes collected in Normandy Rivers based on microsatellite data analyses. Hatchery data indicate that a total of 333 700 juveniles have been released into the Sélune River from 1989 to 1997. In the Couesnon River, 674 724 individuals have been stocked from 1989 to 2007. Sée and Sienne Rivers were stocked in 1990 with only with 14 000 and 10 000 individuals, respectively.

Sampling

In October 2007, 84 young-of–year Atlantic salmon ranging 55-100 mm (Total Length) were collected by electrofishing in four Rivers of the Bay. This survey was done before the annual restocking operation in the Couesnon River. Individuals were caught in several sites on each river catchment (3 to 7) depending on the area colonized by Atlantic salmon. Six young-of–year individuals (70–90 mm TL) were also collected in 2007 at the Favot Hatchery. All individuals were over-anaesthetized and preserved on ice at field, and then frozen at -20°C the same day. In addition, carcasses of 9 adult salmon were collected after spawning in the vicinity of the spawning grounds in the Sélune River, January 2008. Post-spawning fish were about to die or recently died (1 or 2 days) when collected. Finally, scales from 25 individuals caught in the Aulne river in 2006 were also collected for genetic analyses.

Genetic analyses

DNA analyses

Genomic DNA was extracted from scales (adults) or pelvic fins (juveniles) by heating samples in 230ml solution [proteinase K, TE (Tris/EDTA) buffer and chelex] at 55°C 2 hours and then at 100°C for 10min (Estoup et al. 1996). The M13 method (Schuelke 2000) was used to label DNA polymerase chain reaction (PCR) amplifications. DNA was amplified using 6 microsatellite markers (*BHMS235*; *BHMS111*; *BHMS377* (Hoyheim 2000); *SSA197*; *SSA171; SSA65* (O'Reilly et al. 1996)). PCR was carried out in a 10 ml reaction volume containing 1.5 mM $MgCl_2$, 200 mM dNTPs, 0.1 mM forward primer, 0.15 mM reverse primer, 0.15 mM M13-Fluo, 25–50 ng DNA and 0.5 U *Taq* DNA polymerase. The amplification conditions were as follows: an initial denaturation for 5min at 94°C, then 42–45 cycles for 30s at 94°C, 30s at annealing temperature, 30 s at 72°C and a final synthesis for 30min at 72°C.

Statistical analyses

Tests for linkage and Hardy-Weinberg disequilibrium were conducted with FSTAT 2.9.3.2 (Goudet 1995). The allelic richness (number of alleles corrected for sample size) was calculated for all loci and populations using FSTAT 2.9.3.2. The expected heterozygosity (*HE*, Nei 1978) and observed heterozygosity (*Ho*) were calculated with GENETIX 4.05.2 (Belkhir et al. 1996). GENETIX 4.05.2 was then used to compute a two dimensional Correspondence Factorial Analysis (CFA). We investigated the potential genetic structure among clusters of individuals without *a priori* knowledge on their geographic origin using the Structure software (Pritchard et al. 2000). The number of genetic groups (*k*) tested was from 1 to 5, each run consisting in a burn-in period of 50,000 steps followed by 300,000 MCMC replicates. Geneclass (Piry et al. 2004) was also used to assign individuals to the 5 potential origins using a leave one out procedure, excluding self assignment (the Baudouin and Lebrun (2001) approach was used).

Elemental analyses

Otolith preparation

Otoliths were extracted with plastic and glass tools cleaned with ultrapure Milli-Q water (18 Ω) and 10% nitric acid (HNO_3) to prevent contamination. Otoliths were then cleaned in ultrapure water to remove remaining tissues. They were finally embedded in epoxy resin and polished with diamond paste in the sagittal plane to expose the core.

Laser ablation ICPMS

A femtosecond laser ablation system (Alfamet, Novalase SA – Amplitude Systemes, France) fitted with a diode-pumped KGW-Yb laser source (360 fs, 1030 nm) was employed (Pecheyran et al. 2005). The laser was used at high repetition rate (3 kHz) with a laser beam diameter of 9 µm. A galvanometric scanning beam device fitted between the laser source and the objective permitted to rapidly move (up to 280 $mm \bullet s^{-1}$) the laser beam with a high repositioning precision (< 1 µm). When operated at high repetition rates, this system allowed ablating a sample very rapidly according to two dimensional trajectories as reported elsewhere (Fernandez et al. 2008; Claverie et al. 2009). The laser ablation system was coupled to an Elan DRC II ICPMS (Perkin Elmer). The optimization of gas flows (helium, Argon), plasma conditions, and lens voltage was performed ablating a NIST 612 (NIST, USA) glass series, with respect to oxides level less than 3% (ThO/Th<3%) and U/Th ratio of 1.00±0.05 to ensure optimal particle atomization and maximum signal sensitivity.

Laser ablation strategy

The ablated area corresponded to a 200-600 µm wide annulus centered on the primordium (Fig. 2). The ablation was realized using the 2D scanner according to a succession of 100 concentric circles ranging from 200 µm to 600 µm diameters, the laser beam moving rapidly at 8 mm•s^{-1}. This resulted in the ablation of a large amount of material (0.25 mm^2 area) in about 16 seconds, providing a global aerosol subsequently introduced into the ICPMS. The smallest diameter of the annulus (200 µm) was defined to fit with the largest size of otolith at the beginning of the juvenile period (exogenous feeding) according to preliminary tests and values in Metcalfe et al (1992). The largest diameter (600 µm) corresponded to the smallest otolith found in our juvenile samples. This original laser ablation strategy (Fig. 2) was essential to i) avoid nucleus material corresponding to maternal signature (Donohoe et al. 2008), ii) integrate the elemental signal corresponding to the entire growing period of juvenile in freshwater and to average fluctuations possibly encountered, *iii)* increase the quantity of material analyzed and improve detection limits, and *iv)* be able to reproduce such a design on otoliths of adults to obtain trace elemental signatures corresponding to the juvenile period only. Prior to record the signal, the otolith area to be ablated was cleaned by pre ablation with a low energy laser beam (2 µJ) to soften the surface contamination likely to occur.

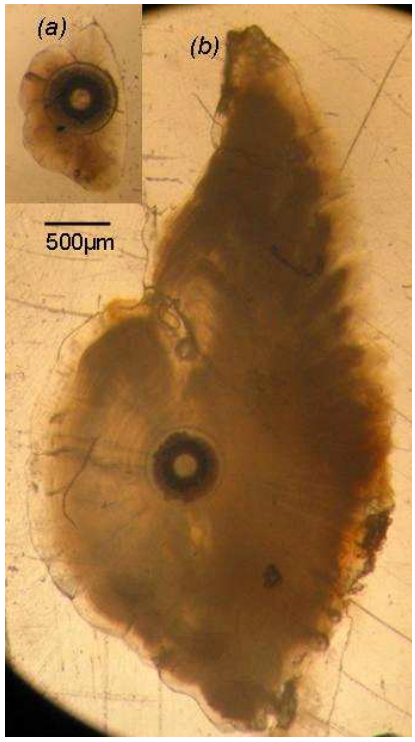


Figure 2. Pictures of (a) juvenile and b) adults Atlantic salmon otoliths, showing the area ablated by the laser prior to ICPMS analysis. The annulus ablated is 200-600 µm, centered on the primordium, and corresponds to the juvenile period of growth only.

Trace element quantification

14 elements (17 isotopes) of interest were recorded: ^{7}Li, ^{23}Na, ^{24,25}Mg, ^{27}Al, ^{43}Ca, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{66,68}Zn, ^{85}Rb, ^{86}Sr, ^{111,113}Cd, ^{138}Ba, and ^{208}Pb with ^{43}Ca being used as an internal standard to correct for mass removal variations due to uneven samples' surfaces as well as ICPMS drift. Signal processing was performed using Elan Instrumental Software (Perkin Elmer). A series of two calcium carbonate standards containing the co-precipitated trace elements of interest at expected concentrations ranging from 5 to 20 µg•g^{-1} were prepared

(Barats et al. 2007). For each calcium carbonate standards, 20 mg of the co-precipitated powder were used and pressed in a three mm die. The fish otolith certified reference material n°22 (National Institute for Environmental Studies, Japan) (Yoshinaga et al. 2000) was used to assess the reliability of the $CaCO_3$ pellets and to match with the high Sr concentrations (> 2000 $\mu g \cdot g^{-1}$) eventually found in marine fish otoliths (Daverat et al. 2005) and with ultra low concentrations (Zn, Cu) as well. Trace elements in otoliths were quantified by external calibration using the enriched $CaCO_3$ pellets. Calibration was carried out at the beginning and at the end of each analytical series to compensate for ICPMS drift. Each analytical series took about 3 hours. As mentioned above, a large amount of material was ablated in a short period of time, which resulted in the introduction of a large amount of thin particles into the ICPMS per time unit. This in turn generated a high ICPMS signal intensity and very low limits of detection at the $ng \cdot g^{-1}$ level. Limits of detection (in $ng \cdot g^{-1}$) obtained on calcium carbonate under our operating conditions and expressed as three times the standard deviation of the carrier gas background (He) were as follow: ^{7}Li: 2.8; ^{24}Mg:12.1; ^{55}Mn: 17.4; ^{63}Cu: 4.4 ; ^{66}Zn: 61.9; ^{68}Zn: 103.6; ^{138}Ba: 1.1; ^{208}Pb: 0.8; ^{23}Na: 458.0; ^{86}Sr: 182.1; ^{111,113}Cd: 0.09. Note that in order to prevent the detector's overloading ^{86}Sr and ^{23}Na signals were strongly attenuated, which explains the poor limits of detections for these elements.

Statistical analyses

Discriminant Function Analyses (DFA) including 12 elements (Li, Na, Mg, Al, Mn, Co, Cu, Zn, Rb, Sr, Ba, and Pb,) were used to differentiate samples from the four rivers and the hatchery. The significance of each element variation was tested with an F-statistic and the redundancy of variables was tested by a tolerance measurement using XLStat-Pro software (http://www.xlstat.com/). A jack-knifed classification matrix was used to estimate the correct assignment of individuals to their natal river.

Combination of genetic and trace element analyses

For each individual, genetic assignment scores were coupled with trace element scores. Genetic scores were the assignment proportions to the two genetic clusters detected (wild and hatchery) with the Structure software. A score close to 0 indicated a hatchery pedigree while a score close to 1 indicated wild pedigrees. Scores from 0.3 to 0.7 likely corresponded to hybrid pedigrees between hatchery-born and wild fishes. Trace element scores were calculated from canonical coordinates on the three first axes of the DFA (hereafter X, Y and Z, explaining 92.5% of variance). First, the five centroïds for juveniles originating from the hatchery (h) and from each river (r) were calculated as the mean value on each axis (X_h, Y_h and Z_h for hatchery

juveniles; X_r, Y_r and Z_r for wild juveniles of each river). Then, the distance from each individual *i* to the centroïd h was calculated following the equation:

$$D_{ih} = \sqrt[2]{(X_i - X_h)^2 + (Y_i - Y_h)^2 + (Z_i - Z_h)^2}$$

where X_i, Y_i and Z_i are coordinates of *i* on X, Y and Z axes, respectively. Similarly, the distance from each individual *i* to the centroïd r of its natal river was calculated as:

$$D_{ir} = \sqrt[2]{(X_i - X_r)^2 + (Y_i - Y_r)^2 + (Z_i - Z_r)^2}$$

Then, the trace element score of each individual S_i was calculated as:

$$S_i = \frac{D_{ih}}{D_{ih} + D_{ir}}$$

Elemental trace scores close to 0 or close to 1 indicated a hatchery or wild origin, respectively. The threshold score between hatchery and wild elemental trace signatures was set to 0.3, which corresponds to the maximum value observed among samples from the Favot Hatchery. For adult individuals S_i was calculated as for juveniles, choosing the statistically closest river to calculate D_{ir}. Genetic and elemental trace scores were then plotted together allowing the classification of individuals into six groups: i) river-born with wild pedigree, ii) river-born with hybrid pedigree, iii) river-born with hatchery pedigree, iv) hatchery-reared with hatchery pedigree, v) hatchery-reared with hybrid pedigree and vi) hatchery-reared with wild pedigree. The two last groups should theoretically contain no individual since no fish from Normandy was used at the Favot Hatchery.

Results

Molecular analyses

Only three out of 42 *FIS* were significant but they were not associated with a particular locus or population. In all populations *FIS* were not significant (Table 1). Average *Ho* and *HE* were 0.80 and 0.81, respectively. Allelic richness ranged from 5.3 to 5.9. Pairwise *FST* were extremely low and not significant among the four Normandy populations, ranging from 0.0002 to 0.012. Conversely, pairwise *FST* between hatchery and Normandy samples were

Table 1: Within population estimates of genetic diversity. Sample size (n), inbreeding coefficient (FIS), expected (HE) and observed (HO) heterozygosities, and average allelic richness (RA) per locus for 6 individuals are given.

Location	Life stage	n	Fis	He	Ho	Ra
Couesnon	Young of the year	12	-0.03	0.78	0.81	5.8
Sélune		25	-0.06	0.80	0.85	5.7
Sée		32	-0.06	0.77	0.80	5.3
Sienne		15	-0.01	0.79	0.80	5.4
Hatchery		6	0.07	0.77	0.72	5.3
Sélune	Adult	9	0.04	0.82	0.80	5.9
Hatchery		25	-0.01	0.81	0.82	5.9

significant, ranging from 0.03 to 0.08 (average = 0.06). The Genetics Correspondence Factorial Analysis (CFA) confirmed the genetic proximity among the four Normandy rivers since genotypes from these populations largely overlapped (Fig. 3a). This analysis also revealed clear differences between hatchery and wild individuals.

The Geneclass software correctly classified 25% to 47% of juveniles collected in rivers and 100% of hatchery-reared juveniles (Table 2). Among the 9 adults caught in the River Sélune, 7 were assigned to Normandy populations, and two were assigned to the hatchery. Similarly, the Bayesian clustering method implemented in Structure software did not reveal any structure among populations from Normandy but delineated two clusters corresponding to fish from the hatchery and wild populations. In juvenile hatchery samples the admixture with the wild cluster was low (0.09). In contrast, wild populations were significantly admixed with the hatchery cluster with values of 0.45, 0.32, 0.18, and 0.22 in the Couesnon, Sélune, Sée and Sienne Rivers, respectively. Eleven juveniles (12%) caught in Normandy Rivers clustered with hatchery samples and were considered as offspring of individuals originating from the hatchery. Thirteen juveniles (14%) showed intermediate scores (from 0.31 to 0.69) and were considered as hybrid progenies of hatchery and wild adults. The remaining 69 juveniles (74%) collected in Normandy Rivers clustered with native populations. The results obtained with Geneclass and Structure were mostly concordant: among the 15 juveniles assigned to the hatchery by Geneclass, Structure detected 11 individuals originating from the hatchery and four hybrids. The 9 remaining hybrids found by Structure were assigned to wild populations by Geneclass. Finally, two adults clustered with hatchery samples, three were hybrids and four clustered with wild populations.

Elemental analyses

Concentrations of Cd in otolith samples were extremely low and usually below the detection limit. Therefore, this element was not considered in subsequent data analyses. We detected significant differences among sites in otolith elemental concentrations of Li, Mg, Mn, Ba, Sr and Rb, but not for Al, Cu, Zn, Pb, Na, Co (Table 3). Mg concentrations were particularly higher in the hatchery otoliths. Discriminant Function Analyses revealed five groups of individuals corresponding to the four rivers and the hatchery (Fig. 3b). Most juveniles (83%-100%) were correctly assigned to their natal origin: river or hatchery (Table 2). Five out of nine adults were assigned to the Sélune River where they were caught; three were assigned to the Sienne River and one to the hatchery.

Table 2: Individual assignment to the population of origin using genetic and elemental data. In contrast with juveniles collected in their natal rivers and at the hatchery, the true natal origin of adults was unknown so the estimation of the proportion of correct assignment was not possible for these individuals.

Location	Life stage	Sample size	Assignment using genetic data						Assignment using elemental data					
			Couesnon	Sélune	Sée	Sienne	Hatchery	Correct	Couesnon	Sélune	Sée	Sienne	Hatchery	Correct
Couesnon	Young of the year	12	3	1	2	1	5	25%	10	1	0	1	0	83%
Sélune		25	2	8	6	3	6	32%	1	21	3	0	0	84%
Sée		32	3	8	15	5	1	47%	0	0	32	0	0	100%
Sienne		15	1	2	4	5	3	33%	2	0	0	13	0	87%
Hatchery		6	0	0	0	0	6	100%	0	0	0	0	6	100%
Sélune	Adult	9	1	3	2	1	2	-	0	5	0	3	1	-
Hatchery§		25	0	0	1	0	24	-	-	-	-	-	-	-

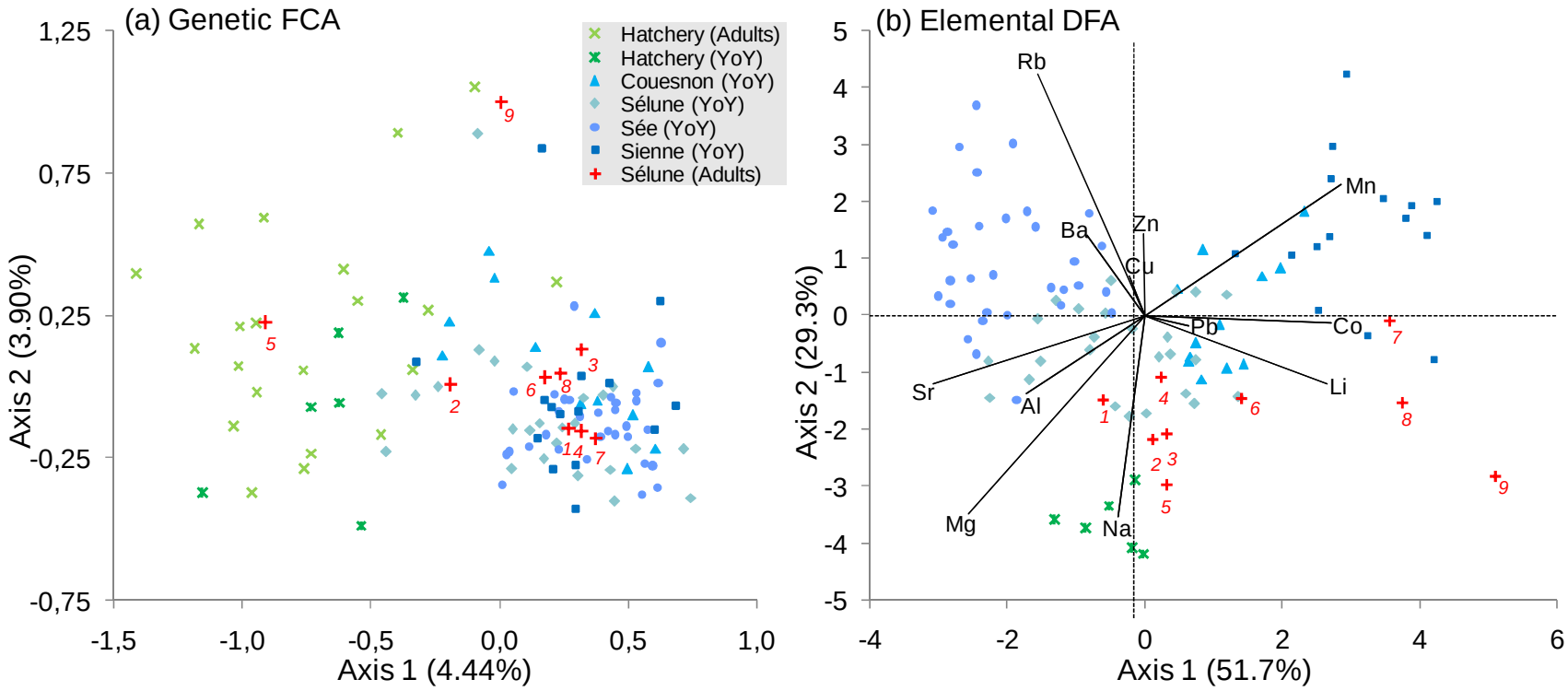
§ Adults used at hatchery are yearly collected in the Aulne River (Brittany). Because of their high conservation value, they were released just after artificial fecundation, and only scale tissue was sampled on each individual. No trace element analysis on otolith available.

Table 3: Spatial variability of 12 elements measured in juvenile Atlantic salmons collected in four Normandy Rivers and at the Favot Hatchery. Mean values (±SD) in micrograms per gram ($\mu g \bullet g^{-1}$) are given for each element in each site. Results from ANOVAs testing potential differences among sites are presented (***: p-value < 0.0001; NS: non significant).

Element	Concentrations for each study site					Test
	Couesnon	Selune	See	Sienne	Hatchery	
Li	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	***
Na	3815.16 ± 86.76	3813.88 ± 108.88	3764.62 ± 147.52	3669.31 ± 110.26	3496.46 ± 253.58	NS
Mg	15.28 ± 2.06	12.96 ± 1.52	18.27 ± 7.33	13.20 ± 1.77	45.39 ± 22.07	***
Al	0.25 ± 0.13	0.43 ± 0.26	0.41 ± 0.21	0.57 ± 0.30	0.34 ± 0.23	NS
Mn	7.33 ± 1.51	5.23 ± 1.23	4.49 ± 0.88	12.56 ± 1.62	2.81 ± 0.49	***
Co§	0.97 ± 0.02	0.95 ± 0.02	0.95 ± 0.02	0.97 ± 0.02	0.93 ± 0.02	NS
Cu	0.11 ± 0.02	0.10 ± 0.02	0.18 ± 0.17	0.13 ± 0.05	0.08 ± 0.01	NS
Zn	54.53 ± 2.71	52.58 ± 4.03	52.02 ± 6.18	49.44 ± 3.78	54.13 ± 10.03	NS
Rb§	11.65 ± 2.28	12.15 ± 2.15	20.78 ± 3.95	17.23 ± 4.34	6.29 ± 1.37	***
Sr	632.38 ± 34.11	766.18 ± 47.22	837.07 ± 41.96	604.74 ± 33.36	759.44 ± 75.78	***
Ba	11.27 ± 0.66	12.60 ± 2.39	16.45 ± 1.69	10.16 ± 1.09	7.81 ± 1.01	***
Pb	0.03 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0.25 ± 0.36	0.16 ± 0.19	NS

§: Values normalized against CRM NIES 22 $R = \frac{I_{M_{sample}} / I_{Ca_{sample}}}{I_{M_{Nies22}} / I_{Ca_{Nies22}}}$ where M is Rb or Co

Figure 3. (a) Correspondance Factorial Analysis of all genotypes analysed and (b) Discriminant Function Analysis of otolith elemental concentrations for individuals collected in Couesnon, Sélune, Sée and Sienne Rivers and at the hatchery. Adults collected on the Sélune River after spawning are labeled from 1 to 9. Note that the three first axes of the DFA were used for individual assignment to the source populations (Table 2), but only two axes are displayed for a convenient reading.



Coupling genetic and microchemistry

Juvenile otolith elemental concentrations and microsatellite analyses confirmed that hatchery-reared juveniles displayed specific genetic and trace element signatures from the hatchery (Fig. 4). All juveniles collected in Normandy Rivers were born in these rivers but molecular analyses revealed multiple genetic origins: wild, hatchery or hybrids. For adult salmon carcasses collected on the Sélune River after spawning, elemental and molecular signatures suggested multiple geographic and genetic origins. Adults #5 and #2 had hatchery pedigrees; elemental analysis revealed that they were born in the hatchery and the Sélune River, respectively. Adults #3 and #6 were hybrids born in the Sélune River, whereas Adult #9 was an hybrid born in the Sienne River. Finally, the four adults with wild pedigrees were born in the Sélune (Adult #1 and #4) and Sienne Rivers (#7 and #8).

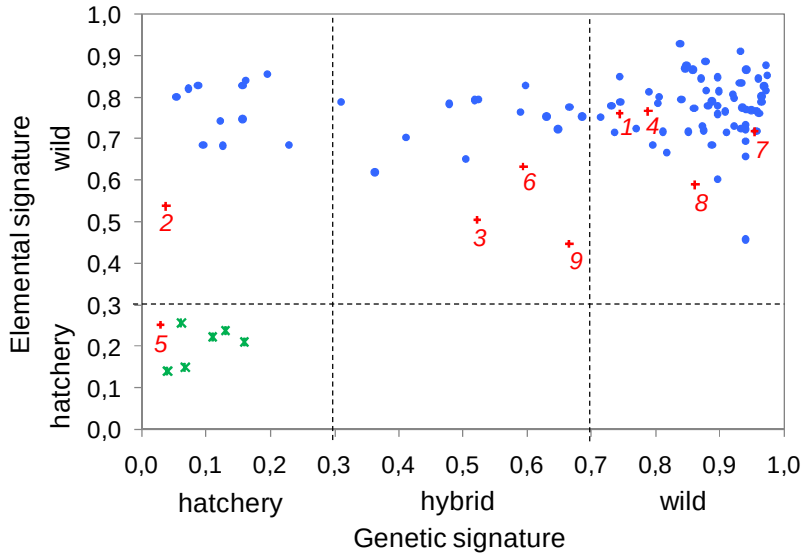


Figure 4. Dual plot of trace element and genetic scores for each fish analyzed in this study. Trace element scores were computed from the Discriminant Function Analysis and genetic scores with the Structure software (see text for detail). Juveniles were collected in Normandy Rivers (●) and at the hatchery (×); adults were collected on the Sélune River (+) after spawning and are labeled from 1 to 9.

Discussion

In this study, otolith trace element and genetic analyses were combined to explore the origin of Atlantic salmon in four adjacent rivers of the Mont Saint-Michel Bay (France). Microsatellite analyses confirmed the low level of genetic differentiation among these rivers observed by Grandjean et al (2009). The Assignment of wild individuals to their natal river based on molecular data was impossible, certainly because of the low differentiation among

populations (Faubet et al. 2007). Other cases of weak differentiation among Atlantic salmon populations living in adjacent river catchments have been previously reported and attributed to high gene flow between nearby stocks (Dionne et al. 2009).

The genetic differentiation between hatchery-reared and wild indviduals was significant enough to track hatchery pedigrees in wild populations. The Couesnon and Sélune Rivers have been intensively stocked over past decades with salmon originating from Brittany. Accordingly, a large proportion of juveniles collected in Normandy Rivers have complete or partial hatchery pedigrees. This result confirms the successful reproduction of some hatchery-reared Atlantic salmon after their release in Normandy Rivers. Other studies already reported the presence of hybrids between wild and hatchery fish in stocked populations (Finnegan and Stevens 2008, Hansen 2002; Hansen et al. 2009). We found that 14% of the juveniles collected in Normandy Rivers were such hybrids. However, juveniles with “pure” wild pedigrees were also collected, indicating indigenous salmon persistence in such introgressed populations, as observed in brown trout *Salmo trutta* by Hansen and Mensberg (2009). Conversely, young-of-year juveniles with “pure” hatchery pedigrees were detected in all rivers and before annual restocking operation in the Couesnon River, strongly suggesting the breeding of hatchery-reared individuals together on natural redds.

Unlike DNA, the geochemical signature of otoliths is mostly not inherited from parents (except a maternal signature in otolith primordium) but depends on the environment the organism lives in. Since otoliths integrate ambient water elements during fish growth, it was possible to discriminate river-born and hatchery-born juveniles thanks to trace elements incorporated in the portion of otoliths corresponding to juvenile growth. Recent studies based on isotopic and elemental analyses reported similar findings (Gao et al. 2004; Sohn et al. 2005; Gao and Bean 2008; Gibson-Reinemer et al. 2009). Elemental concentrations of Ba, Li, Mn, Rb, and Sr in juvenile otholiths significantly discriminated the four natal rivers. These elements are not strongly involved in physiological processes, which suggests that their concentrations in otoliths are mostly linked to the composition of the ambient water. These elements have already been pointed out as appropriate tracers in Allis shad (*Alosa alosa*, Tomas et al. 2005), gray snapper *(Lutjanus griseus*, Lara et al. 2008) and Atlantic croaker (*Micropogonias undulatus*, Thorrold et al. 1997). We also detected significant differences in elemental concentrations between individuals originating from the hatchery and Normandy rivers; such differences may be explained by variations in water chemistry, but also by variations in water temperature during growth (Mitsuguchi et al., 1996).

By identifying fish pedigrees and the environment where they grew as juveniles, joint genetic and trace element analyses provide new cues to study the life histories and origins of Atlantic salmon living in Normandy Rivers. Several studies used individual admixture analyses based on multilocus data to discriminate wild, stocked, and hybrid fishes (Hansen 2002; Hansen and Mensberg 2009). However, using molecular data only, it is impossible to certify the origin of individuals with hatchery genetic characteristics. Such individuals may indeed originate from the hatchery or be the progeny of hatchery-reared fishes having bred together *in natura*.

Atlantic salmon has a high conservation value in the Bay of Mont Saint-Michel making the lethal sampling of otoliths on adults returning to freshwater spawning grounds not feasible. We only found nine carcasses of adult salmons in the vicinity of spawning grounds in the Sélune River, despite substantial efforts in winter 2007 and winter 2008. However, these nine adult samples were very informative since it was possible to infer their natal origin thanks to the laser ablation of their otoliths. The region ablated on an adult's otolith covers the juvenile period of growth. The resulting elemental signatures were then compared with reference signatures obtained by similar laser ablations on otoliths of juveniles of known origins. Four of these nine adults were clearly identified as offspring of wild fish since they had both genetic and elemental signatures of wild juveniles. They were most probably born in the Sélune and Sienne Rivers and reproduced in the Sélune River where carcasses were found. Conversely, one adult was born in the hatchery since it had a hatchery pedigree and an elemental signature of the hatchery in its otolith. Scale reading revealed that this fish was three-year old. After being stocked in autumn 2005 in the Couesnon River, it migrated to the see and returned to the Sélune River to spawn in winter 2007-2008. Three adults likely resulted from the interbreeding *in natura* between wild fish and individuals with hatchery pedigrees. Elemental analyses suggested that these individuals were born in the Sélune and Sienne Rivers and then reproduced and died in the Sélune River. Finally, one adult with a pure hatchery pedigree likely resulted from the natural reproduction of two adults with a similar pedigree in the Sélune River since its otolith revealed an elemental signature of this River during the juvenile period. These results overall reveal an important straying of both hatchery-reared and wild adult salmons as previously suggested in several studies (Stabell 1984; Quinn 1993; Jonsson et al. 2003).

In conclusion, we detected significant differences in otolith trace element concentrations of juveniles Atlantic salmon from Normandy Rivers while no genetic differentiation was observed among these populations. Using elemental traces in otoliths,

more than 80% of juveniles collected in the wild were accurately assigned to their natal river. We also found trace element and genetic differences between wild and hatchery stocks allowing the assignment of individuals to these two groups and demonstrating the hybridization between fishes having hatchery pedigrees and wild individuals. This joint approach of elemental and genetic analyses also revealed that individuals with hatchery pedigrees can breed together in the wild. Their offspring consist in river-born juveniles with pure hatchery pedigrees that can be mistaken for hatchery-raised and released juveniles. This study overall suggests that combining otolith trace element and genetic analyses on the same individuals could provide new insights into the introgression processes at work in wild populations sotcked with hatchery fish. Furthermore, it could be helpful for studying colonization or recolonisation processes in depopulated rivers.

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Chapitre VII. Recolonisation naturelle par le saumon Atlantique

L'objectif de ce chapitre est d'identifier l'origine de saumons recolonisant la Seine.

Historiquement, le fleuve Seine présentait une abondante population de saumons constituée de gros poissons de plusieurs hivers de mer. Conséquence de multiples facteurs, la régression progressive de cette espèce depuis le XVIIIe siècle a conduit à la disparition du saumon de la Seine. Depuis les années 1990, la qualité de l'eau de la Seine s'est fortement améliorée, notamment grâce aux efforts de traitement des effluents. Parallèlement, on a pu constater la recolonisation de nombreuses espèces de poissons migrateurs, dont le saumon Atlantique. Depuis le début des années 2000 des adultes de saumons sont capturés par pêche à la ligne et lors des programmes d'inventaires des espèces de poissons et de contrôle de la qualité de l'eau. En 2008, 162 poissons ont été observés par vidéocomptage dans la passe à poisson du barrage de Poses, située en amont de Rouen.

Une première analyse des caractéristiques biométriques, démographiques et génétiques d'un échantillon de sept saumons adultes capturés dans la Seine a été réalisée. L'origine de ces poissons a été déterminée par analyse génétique en les assignant à une collection d'échantillons de référence issus de 34 populations françaises ainsi que de stocks du Royaume-Uni et de Scandinavie.

Les saumons adultes analysés mesuraient entre 56 et 97 cm pour un poids compris entre 1,3 et 7 kg. Quatre étaient des castillons, deux étaient des petits saumons de printemps et le dernier était un grand saumon de printemps. Les analyses génétiques montrent que ces saumons semblent avoir des origines diverses. En effet, ils sont assignés soit aux stocks de rivières proches, de Basse-Normandie, soit à des stocks de bassins plus éloignés comme l'Allier ou des stocks étrangers.

Ce travail suggère qu'une amélioration de la qualité de l'eau et de l'habitat pourrait permettre de rendre une rivière précédemment dépeuplée, en partie à cause de la pollution, attractive pour les saumons des cours d'eau proches et distants. Ceci illustre le fait que le repeuplement n'est pas une méthode systématique de gestion et qu'une restauration de l'habitat peut-être à privilégier et peut permettre une recolonisation naturelle et peut être davantage pérenne.

Natural recolonization of the Seine River by Atlantic salmon (*Salmo salar*) of multiple origins

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Abstract

The restoration of previously extinct salmon populations is usually achieved with stocking programmes, but natural recolonization can also occur through the straying of individuals from nearby populations. Here we investigated the origin of Atlantic salmon (*Salmo salar*) that recently recolonized the Seine River (France). The degradation of this river had led to the extinction of the population, but since the 1990s, the water quality has greatly improved. Although no stocking was performed, 162 individual salmon were recently observed by video-counting. Seven fish were sampled for morphological and genetic analyses. These individuals were genotyped at 17 microsatellites markers and their probable source populations were identified using baseline samples from regional and distant populations. Four of the sampled individuals were grilse and three were multi-sea-winter fish. Genetic analyses revealed that the fish partly originated from a nearby stock but also from distant populations, suggesting long-distance straying. This natural recolonization of a large river by strayers from several origins is discussed in terms of population sustainability and management.

Key words: Seine River, recolonization, *Salmo salar*, genetic assignment, straying

Introduction

Many Atlantic salmon (*Salmo salar*) populations are critically endangered, especially in large rivers that are generally more impacted by human activities. In cases of extinct populations, recolonization can occur through the straying of individuals from nearby populations and (or) by the controversial use of stocking with hatchery reared fishes (Myers *et al.* 2004). The results of stocking programmes are variable and have been amply documented (for a review see Fraser 2008) while cases of natural recolonization by Atlantic Salmon are rare and have been only described in small or medium river systems (Vasemagi *et al.* 2001; Grandjean *et al.* 2009). In brown trout (*Salmo trutta)* there are some examples of recolonization of large drainages by anadromous sea trout, for instance the Rhine River (Schreiber and Diefenbach, 2005). However, there is apparently no case of natural recolonization by Atlantic salmon of a large river system where the population was previously extinct.

The Seine is a major French river with a basin area of 78.910 km^2. A large Atlantic salmon population, including a large component of multi-sea-winter fish, dwelt in this river until the beginning of the 19th century (Lavollée 1902). This population progressively collapsed until the end of the 19th century due to obstruction to migration, canalization, and chronic degradation of water quality by industrial and domestic pollution (Lavollée 1902; Belliard et al. 2009). The only recorded stocking operations were performed during the 19th century and remained unsuccessful (Lavollée 1902; A. Richard, Délégation Interrégionale Nord-Ouest, ONEMA, 188 rue carlet, 27310 Bourg-Achard, France, unpublished data). The water quality clearly improved since the beginning of the 1990s, allowing the return of nine migratory species to the lower Seine (Belliard et al. 2009). One hundred and sixty-two adult Atlantic salmon were counted in 2008 in the video trap of the Poses Dam (A. Richard, unpublished data), located 160 km from the sea (Fig. 1*a*). Seven of them were adipose fin clipped (a common practice on stocked fishes), suggesting a low proportion of hatchery-reared fishes. Some adult salmon were also caught by angling or during scientific monitoring in lower parts of the river but also near Paris (and even in Paris; see Table 1).

The aim of the present study was to identify the probable source populations of the salmon recolonizing the Seine River using genetic assignment methods. We genotyped seven individuals caught in the Seine at 17 microsatellite markers and inferred their origin using six baseline groups: the five major French genetic units (Fig. 1*b*) and a group of foreign samples. Finally, we discuss the management options that may allow the restoration of a self-sustaining salmon population in the Seine River.

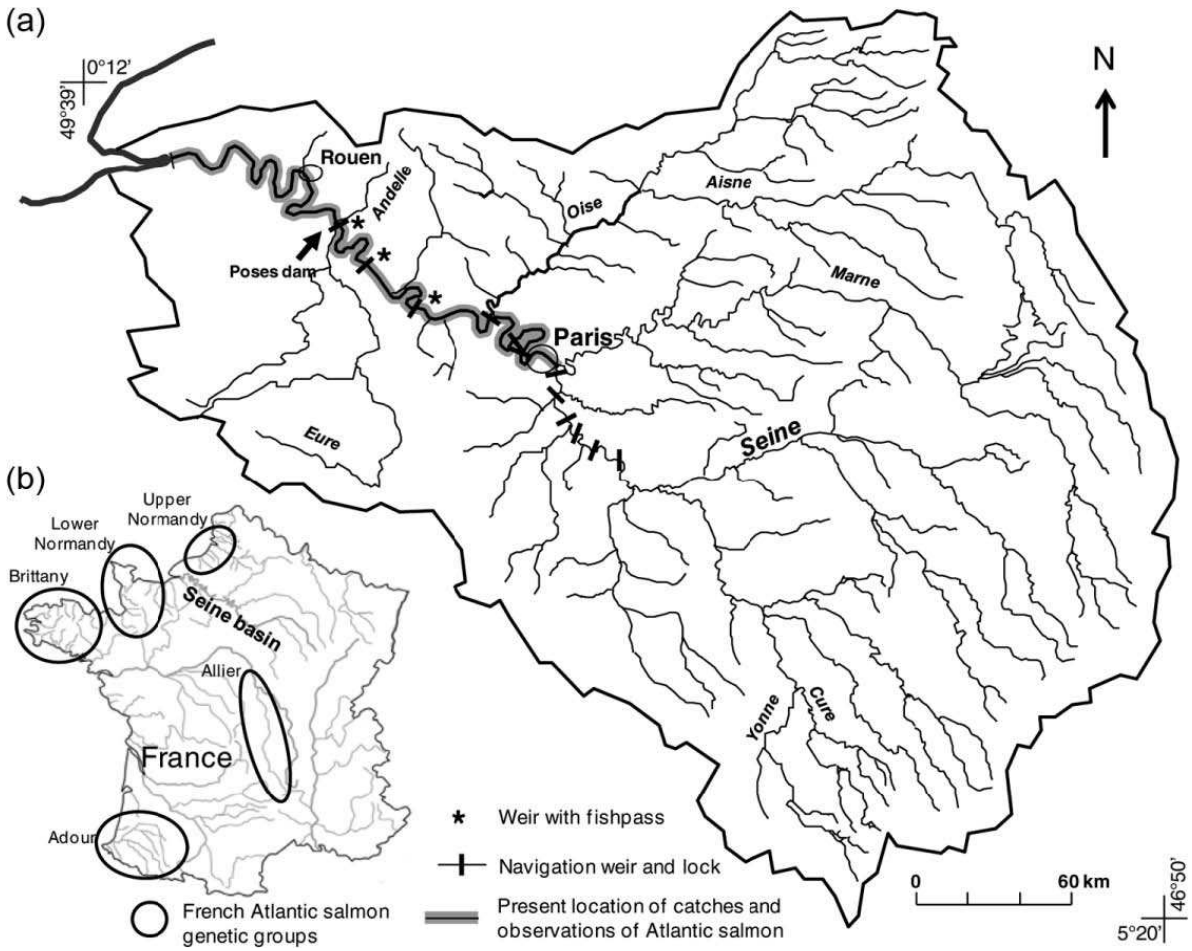

Figure 1. (a) Map of the Seine drainage with locations of the observed and sampled salmon (*Salmo salar*) and (b) location of the Seine basin in France, as well as the five French baseline groups used for the genetic assignment tests.

Material and methods

Sampling

Seven adult salmon caught by angling (salmon fishing is officially prohibited) or net fishing from 2001 to 2008 were sampled (Table 1). Total length and weight were measured and some scales were collected for age estimation and genetic analyses.

Genetic analyses

Genomic DNA was extracted from scale individual samples by heating in a 150 ml solution [proteinase K, TE (TrisEDTA) buffer and 5% Chelex] at 55 °C 2 hours and then at 100 °C for 10 min. We used an economic method called “M13 method” for the fluorescent labelling of PCR fragments. DNA was amplified using 17 microsatellites (BHMS176; BHMS179A;

BHMS184B; BHMS429; SSA85; SSA65; SSOSL85; SSA9; BHMS235; BHMS217; BHMS111; SSA197; SSA171; BHMS377; SSSP2216; SSA224; BHMS365; for more details on these markers, see Nikolic et al. 2009). PCR was carried out in a 10 ml reaction volume containing 1.5 mM MgCl2, 200 mM dNTPs, 0.1 mM forward primer, 0.15 mM reverse primer, 0.15 mM M13-Fluo, 25–50 ng DNA and 0.5 U Taq DNA polymerase. The amplification conditions were as follows: an initial denaturation for 5 min at 94 °C, then 42–45 cycles for 30 s at 94 °C, 30 s at annealing temperature, 30 s at 72 °C and a final synthesis for 30 min at 72° C.

Data analyses

Genetic assignment of the seven Seine samples was performed using two Bayesian methods implemented in the software GENECLASS 2.0 (Piry *et al.* 2004) and STRUCTURE (Pritchard *et al.* 2000). For both analyses, we considered as baselines one group of foreign fishes from several Scottish, Danish, and Norwegian rivers (29 individuals) and the five major French genetic units (Fig. 1*b*): Upper Normandy, Lower Normandy, Brittany, Allier River, and Adour drainage (34 fish per unit). These five French groups were previously identified based on the analysis of 975 adult individuals originating from 34 French rivers (C. Perrier, J.-L. Baglinière, R. Guyomard, and G. Evanno, unpublished data). GENECLASS was used by assigning individuals to each of the six baseline groups using the Baudouin and Lebrun approach. STRUCTURE was run six times (mean values are given), with a burn-in period of 50 000 steps followed by 500 000 Markov chain Monte Carlo (MCMC) replicates, assuming six populations (k = 6), and an admixture model (*i.e.*, allowing the genetic composition of individuals to be a mixture from the six different source populations).

Results

The adult salmon ranged in total length from 560 to 970 mm and weighed between 1300 and 7000 g (Table 1). Three fish were one-year-olds in freshwater and three were two-year-olds. Four salmon were grilse, two were two-seawinters, and the last one was three-sea-winters. Genetic assignment showed that these fish had different origins (Table 1). Three grilse were assigned with GENECLASS software to Lower Normandy stock with 100% scores, and one multi-sea-winter fish was assigned to the Allier population with a score of 100%. Two multi-sea-winter individuals were assigned to the foreign group with scores of 99% and 91%, whereas the last grilse could not be unambiguously assigned to any stock. Assignment with

STRUCTURE software gave similar results except that the last grilse was assigned to the foreign group with a score of 90%.

Discussion

Our results suggest a high diversity in the origin of the fish recolonizing the Seine River. As no stocking operation has occurred in this river since the end of the 19th century (Lavollée 1902), the salmon recolonization can only be explained by natural straying. The sample size is small but sufficient to reveal that source populations are not only in nearby rivers (Lower Normandy region), but also in distant rivers (Allier River) or foreign stocks. The results were not congruent between the two assignment methods for one individual, meaning that this fish probably originated from an unsampled foreign population. Clearly, more samples from the Seine River and from foreign stocks would be needed to make a thorough analysis of the source populations and their relative contributions to this recolonization event. In particular, individuals assigned to the foreign stocks might also come from foreign hatcheries, sea farms, and (or) a foreign river where they had been stocked. Indeed, straying rates of hatchery-reared fishes are higher than those of wild individuals, and stocking is a widespread practice in the British Isles. In addition, one sampled individual and seven of the 162 fishes observed by video-counting were adipose fin clipped, suggesting that stocking in other rivers may have also played an indirect role in the recolonization of the Seine River. Stocking operations also occur in several French rivers, but only salmon of national origin are used (Vauclin 2005).

The fact that many individuals were observed by videocounting in 2008 and that some fish were assigned to the Lower Normandy stock may also reveal the presence of a small local population actually in establishment in a tributary localized in the lower basin. Indeed, as salmon learn the location of their home river when moving to sea, most of the individuals born in the Seine River will come back to their natal river to spawn (Jonsson et al. 1990).

Table 1. Biological characteristics and results from genetic assignment for seven Atlantic salmon (*Salmo salar*) sampled in the Seine River.

Catch			Fish characteristics					Genetic assignment					
Date	Localization	Method	Sex	Total length (mm)	Weight (g)	Freshwater age	Sea age	Upper Normandy	Lower Normandy	Brittany	Allier R.	Adour	Foreign origin
25/08/2001	Duclair	Angling	f	560	1300	2	1+	0 ; 1	0 ; 0	6 ; 1	18 ; 7	27 ; 1	49 ; 90
04/10/2004	Duclair	Angling	-	580	1450	1	1+	0 ; 0	100 ; 72	0 ; 7	0 ; 16	0 ; 1	0 ; 4
29/08/2007	Hynville	Angling	-	630	2100	1	1+	0 ; 0	100 ; 92	0 ; 0	0 ; 0	0 ; 7	0 ; 1
03/08/2007	Hynville	Angling	-	610	2000	1	1+	0 ; 1	100 ; 99	0 ; 0	0 ; 0	0 ; 0	0 ; 0
26/05/2008	Guerne Dam	Net fishing	-	753	3840	2	2	0 ; 0	0 ; 0	0 ; 0	100 ; 100	0 ; 0	0 ; 0
20/07/2008	Bougival (Paris)	Angling	-	800	4200	-	2	0 ; 1	0 ; 2	1 ; 2	0 ; 2	0 ; 1	99 ; 91
03/10/2008	Suresnes (Paris)	Angling	m*	970	7000	2	3	9 ; 3	0 ; 1	0 ; 2	0 ; 1	0 ; 1	91 ; 92

Note: Genetic assignment tests were computed with the GENECLASS (left value) and STRUCTURE (right value) software. These results are expressed either in relative assignment scores to each baselinegroup (GENECLASS) or as the fraction of the genome inherited from each baseline group (STRUCTURE).

**Adipose fin clipped.*

The multiple origins that we observed could be a key factor for successful natural recolonization as they should result in higher genetic diversity within the settling population compared with a single-origin population. This higher genetic diversity should buffer the impact of losses of genetic variability linked to the low number of migrants during the early phases of recolonization. Alternatively, the fact that nearby Normandy populations could provide a large proportion of the founders may also facilitate the recolonization due to a better adaptation of the settlers to local environ-mental conditions (Dionne et al. 2008). This high overall evolutionary potential, in turn, would increase the probability of the new population reaching a self-sustainable stage.

The recolonization of the Atlantic salmon could be related to a general improvement of water quality in the estuary and lower parts of the river. More specifically, improving water quality resulted in recovering a good dissolved oxygen concentration (Belliard et al. 2009) and low ammonium concentrations. During the 19th century, known spawning grounds were restricted to some tributaries of the estuarine part of the Seine River and of the upper basin (Lavollée 1902). Currently, no spawning site was detected in the Seine drainage, but it seems that given the numerous obstacles to fish migration, only the tributaries located downstream could be used as spawning areas (e.g., the Andelle River).

Finally, fishpass management and water and habitat quality improvement seem the priorities to address to facilitate the sustainable natural recolonization of the Seine River by Atlantic salmon. More generally, this case study suggests that stocking may be superfluous for restoring previously extinct salmon populations in large river systems.

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Chapitre VIII. Discussion générale

L'objectif de cette thèse était d'approfondir les connaissances de la structure génétique des populations de saumon Atlantique en France, au travers de trois questionnements :

1- Quel est le niveau de structuration génétique des populations inféodées aux rivières françaises et comment varie la diversité au sein de ces populations ?

2- Comment a évolué cette structure génétique au cours des dernières décennies ?

3- Quel sont les influences de certains facteurs environnementaux (distance géographique entre les rivières et longueur des rivièeres) et de certaines activités humaines (repeuplement et amélioration de l'habitat) sur cette structuration et son évolution temporelle ?

Les résultats obtenus dans le cadre de ce travail répondent à ces questionnements en montrant :

- ✓ Une distribution spatiale de la diversité génétique des populations de saumons à l'échelle du territoire français permettant d'identifier cinq principaux groupes génétiques ;
- ✓ L'influence sur cette structure de deux facteurs environnementaux prépondérant à savoir la distance côtière entre les estuaires et de la longueur des rivières ;
- ✓ L'existence d'une population originale inféodée à la rivière Allier, attestant d'une adaptation du saumon à des conditions locales ;
- ✓ L'influence des repeuplements sur la structure génétique des populations. Cet impact apparaît d'autant plus fort qu'il a pu être démontré à l'aide de plusieurs approches : comparaisons d'échantillons récents et anciens, utilisation d'outil de modélisation, et couplage d'analyses microchimiques et génétiques ;
- ✓ L'origine de poissons recolonisant spontanément la Seine dont la qualité s'est récemment largement amélioré.

Distribution spatiale de la diversité génétique

Déterminer la structure génétique entre les populations ou groupes de populations est un enjeu majeur en génétique des populations (Waples & Gaggiotti 2006). Notamment, la détermination de ces patrons de différenciation génétique est de plus en plus utilisée à des fins de conservation des espèces menacées (Palsboll *et al.* 2007) (Schwartz *et al.* 2007).

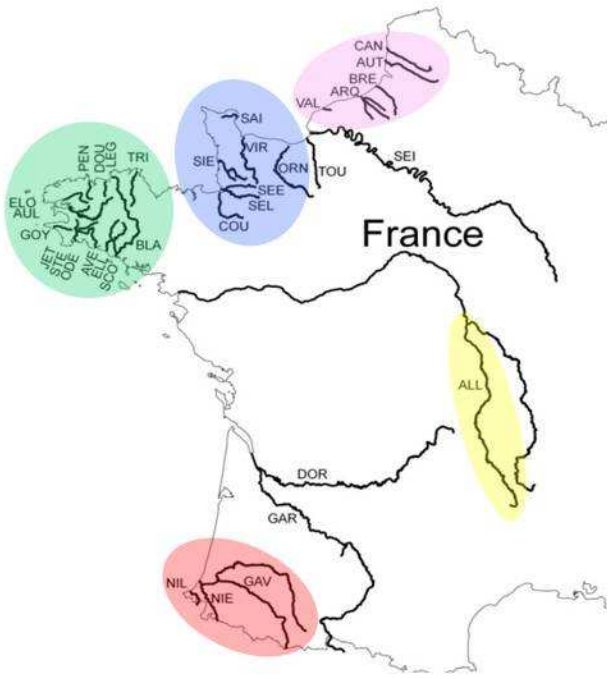

Figure 18 : Principaux groupes génétiques parmi les populations françaises de saumon Atlantique.

Dans le cadre de ce travail, il a été identifié cinq principaux groupes de populations génétiquement et géographiquement distincts actuellement en France: Haute-Normandie, Basse-Normandie, Bretagne, Allier, et Adour (Chapitres I et II) (Fig. 18). Les populations inféodées à la Garonne et à la Dordogne possèdent des caractéristiques intermédiaires aux groupes Allier et Adour et ne semblent pas pouvoir être identifiées comme un groupe à part entière (rappelons que ces rivière font l'objet de programme de restauration et donc de repeuplement). Par ailleurs, au sein de chacun de ces groupes, le niveau de différentiation est faible. Ces résultats sont en accord avec ceux de la littérature (Dionne *et al.* 2008; Tonteri *et al.* 2009). En effet, il est généralement observé un niveau élevé de différenciation intergroupes indiquant des flux de gènes limités alors que la structure génétique au sein des groupes est plus faible, indiquant des flux relativement importants entre des populations proches et localisées dans des zones géographiques aux caractéristiques proches. Ceci est particulièrement remarquable pour les populations de Bretagne qui sont réparties sur plus de 400 km de côtes et sont peu différenciées.

Néanmoins, de récentes études ont montré l'existence de différenciations génétiques significative entre différentes populations ou sous-populations d'une même grande rivière (Primmer *et al.* 2006; Vaha *et al.* 2007; Dillane *et al.* 2008; Dionne *et al.* 2009). Cette constatation a également été faite au sein du système l'Adour dans le Sud-ouest de la France (Le Gentil *et al.*, article en préparation, résumé présenté en annexe). Une étude similaire

visant à déterminer la distribution spatiale de la diversité génétique au sein de la rivière Allier est en cours (Le Cam *et al., données non publiées,*UMR Ecologie et santé des Ecosystèmes).

Influence de la distance côtière et de la longueur des rivières sur la différenciation des populations

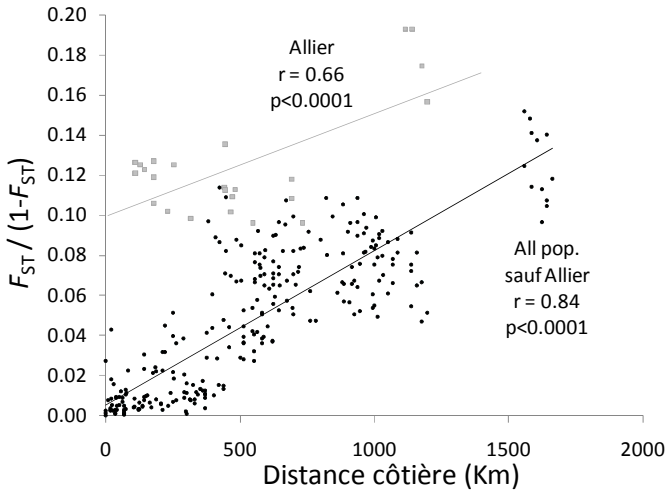

Figure 19 : Isolement par la distance entre les populations françaises de saumon Atlantique. Isolement par la distance plus important de l'Allier.

La distance côtière entre les populations ainsi que la longueur des rivières sont deux facteurs environnementaux influençant la différentiation des populations étudiées (Chapitre I) (Fig. 19).

Le premier facteur évoqué est un paramètre expliquant généralement bien les patrons de différentiation chez le saumon Atlantique (Verspoor *et al.* 2005; Dionne *et al.* 2008; Grandjean *et al.* 2009; Tonteri *et al.* 2009). De plus, très peu de poissons sont assignés à des groupes autres que celui correspondant au site dans lequel ils ont été capturés. Ces résultats suggèrent un homing important et un faible impact des repeuplements sur les rivières incluses dans l'analyse.

La longueur de la rivière est le second paramètre expliquant de façon significative la distance génétique entre les populations uniquement lorsque l'Allier était intégré dans l'analyse. La longueur de la rivière a été suggéré dans d'autres études comme un facteur influençant la diversité génétique à l'intérieur des rivières, avec notamment un gradient aval-amont d'appauvrissement de la diversité (Primmer *et al.* 2006; Vaha *et al.* 2007). En accord avec ces études, les analyses présentées montrent que la population de l'Allier, colonisant uniquement des sites de fraie très éloignés de l'estuaire de la rivière, possède une diversité génétique relativement faible. Cependant, l'absence du saumon sur la partie aval de l'Allier et sur la partie basse de la Loire, ne permet aucune comparaison de la diversité génétique sur un gradient aval-amont.

D'autres analyses réalisées dans le cadre de ce travail mais non présentées dans ce manuscrit suggèrent une influence possible de deux autres paramètres sur la différenciation

entre les populations : la nature du substratum géologique et la présence de larges zones côtières dépourvue de rivières favorables à la migration. Le substratum pourrait avoir une importance dans la reconnaissance de la rivière natale (Stabell 1984) et donc favoriser une différentiation locale. La présence de zone côtière sans cours d'eau accessible au saumon habitat favorable pourrait expliquer une importante différenciation entre les groupes de populations. En effet, la dispersion des saumons s'effectue principalement dans une zone restreinte de part et d'autre de leur rivière d'origine (environ dans les 60 premiers kilomètres (Jonsson *et al.* 2003)). Ainsi, la présence de zones côtières larges de plus de 60 km sans rivière accessible a pu engendrer à terme des différenciations importantes entre des populations localisées de part et d'autre de ces zones.

En perspectives, une analyse plus précise de l'influence de ces trois paramètres longueur de la rivière, nature du substratum géologique, et présence de larges zones côtières dépourvue de rivières favorables, pourrait permettre d'étudier plus finement la différence génétique entre des groupes contigus au niveau spatial. Ainsi, ces paramètres pourraient être à l'origine des taux de différenciation importants entre les populations de Bretagne et de Basse-Normandie et des taux très faibles au sein du groupe breton malgré les distances géographiques importantes entre les populations situées de part et d'autre de cette région.

Adaptation locale de la population de l'Allier

De nombreux travaux suggèrent des adaptations locales chez le saumon Atlantique (Taylor 1991; Garcia de Leaniz *et al.* 2007), exprimées notamment par des différences de tailles et d'âges des poissons adultes qui pourraient à leur tour être le reflet des difficultés de migration et d'accès aux sites de frais (Schaffer & Elson 1975) (Power 1981; Dionne *et al.* 2008). Cette difficulté de migration et d'accès aux sites de reproduction est généralement une fonction combinant longueur de la rivière, débit, pente et présence d'obstacles naturels ou d'origine anthropique, etc…

Les frayères à saumon de la rivière Allier sont aujourd'hui localisées sur une aire restreinte de la partie haute de la rivière située à plus de 700km de l'estuaire. Ainsi, les zones de reproduction du saumon sur l'Allier sont, comparativement plus difficiles à atteindre que celles situées sur les autres rivières françaises (de l'estuaire jusqu'à 150km en amont). De plus, elles restent actuellement les seules zones véritablement accessibles (présence de quelques sites colonisés sur la Gartempe, affluent de la Vienne) pour la reproduction du saumon sur le bassin Loire-Allier. Du point de vue phénotypique, les saumons capturés sur

cette rivière sont des individus de grande taille séjournant 2 à 3 ans en mer (Les individus de trois de mer constituaient la composante dominante des remontées de saumons adultes dans le passé) et remontant tôt en rivière (en hiver et jusqu'à quatorze mois avant leur reproduction dans la partie basse de la Loire). Il est particulièrement important de noter que les castillons sont presque totalement absents de cette rivière. En revanche, sur les autres rivières françaises, les poissons sont essentiellement des poissons séjournant un an en mer et remontant l'année où ils vont se reproduire (printemps et été). Signalons cependant que la rivière Adour possède également une composante d'adultes de deux et trois de mer mais dans des proportions beaucoup plus faibles que celles de l'Allier, et comprend un nombre important de castillons (Baglinière & Porchere, 1994). Par ailleurs, si cette composante de poissons de plusieurs hivers de mer peut être également élevée sur les cours d'eau du Nord de l'Europe (Anonyme, 2008) et au Canada (Baglinière, 1983), la période de remontée des adultes n'est jamais aussi précoce que sur l'Allier et il existe généralement une grande quantitée de castillons.

La population de l'Allier apparaît génétiquement très différenciée des autres populations françaises et bien plus que si le niveau de différentiation était prédit par la relation d'isolement par la distance (Chapitre I) (Fig. 17). Pour exemple, Le Fst entre le Blavet (Bretagne Sud) et l'Allier est de 0.11 alors que celui entre le Blavet et le Trieux (Bretagne Nord) est égal à 0.01 alors que la distance géographique entre l'Allier et le Blavet est quatre fois inférieure à celle entre le Blavet et le Trieux. Enfin, aucun individu immigrant issu d'une autre population n'a été identifié dans l'Allier, pas plus qu'un hybride entre un individu immigrant et un poisson de l'Allier. Ces résultats suggèrent une adaptation phénotypique de la population de saumon de l'Allier aux caractéristiques physiques de la rivière et à la difficulté de migration sur les zones de frayère. Ils suggèrent également des flux réduits d'individus et de gènes entre l'Allier et les autres populations accentuant l'originalité génétique de sa population de saumon.

L'Allier représente un site particulièrement intéressant pour étudier plus précisément les phénomènes d'adaptation locale. Il serait notamment possible de mesurer l'héritabilité des traits morphologiques des individus, tels que la taille, via des croisements in situ et des analyses de parenté, ainsi qu'en recherchant des QTL (Reid *et al.* 2005; Houston *et al.* 2009; Baranski *et al.* 2010). Par ailleurs, les caractéristiques exceptionnelles de cette population notamment en termes de résistance aux nombreux stress rencontrés durant la migration laissent supposer une forte pression de sélection chez les individus, notamment pour les gènes du Complexe Majeur d'Histocompatibilité (Dionne *et al.* 2007; Kekalainen *et al.* 2009). La

diversité génétique de cette population au CMH est actuellement étudiée dans l'Unité (UMR ESE, Rennes) et devrait permettre d'établir une première estimation du potentiel adaptatif de la population relativement aux autres populations françaises. De plus, l'analyse à 20 marqueurs microsatellite et à un gène du CMH d'une série chronologique d'échantillons commençant en 1965 devrait également permettre de déterminer les effets sur la population des changements récents apparus dans l'environnement de cette population.

Effets des repeuplements sur la structure génétique des populations

Utilisation d'échantillons contemporains

Les « repeuplements » sont des pratiques courantes utilisées dans le but de soutenir la production des populations naturelles ou de restaurer une population éteinte. Cependant, ces pratiques sont à même de modifier les caractéristiques génétiques des populations. Ces effets des repeuplements sur la structure génétique des populations peuvent être mesurés en comparant la structure d'échantillons contemporains de populations repeuplées, de populations voisines de celles-ci et celles des souches (stocks) utilisées pour effectuer ces repeuplements. Les travaux réalisés en utilisant cette approche mettent bien en évidence les effets non négligeables des déversements de poissons d'origine bretonne dans les populations normande de la baie du Mont-Saint-Michel. Les taux d'introgression sont variables, s'échelonnant depuis un taux faible sur le stock de la rivière Sée jusqu'à un taux très élevé d'introgression celui de la rivière Couesnon. Ces observations restent relativement courantes (Hansen 2002; Campos *et al.* 2008; Finnegan & Stevens 2008; Sonstebo *et al.* 2008; Hansen *et al.* 2009). De plus, ces taux d'introgression semblent proportionnels à la quantité de poissons déversés sur les quatre rivières considérées, ce qui suggère fortement que les repeuplements effectués de 1989 à 2003 constituent la cause de l'introgression observée. Concernant les autres populations françaises, il n'est pas noté de distribution génétique particulière pouvant suggérer des effets de repeuplements récents utilisant des poissons natifs, ou encore des effets de repeuplements non natifs plus anciens.

Comparaison d'échantillons anciens et récents

D'une façon générale, les analyses comparant des échantillons anciens et récents montrent une réduction de la différenciation entre les populations entre les deux périodes échantillonnées (Fig. 20). Cette réduction est due à un brassage génétique plus important lié aux opérations de repeuplement qui utilisent des souches non natives c'est-à-dire provenant

d'un groupe génétique auquel n'appartient pas la population repeuplée. Cette tendance à l'homogénéisation entre populations a déjà été observée dans le cas de systèmes soumis à des repeuplements (Ayllon *et al.* 2006; Marie *et al.* 2010).

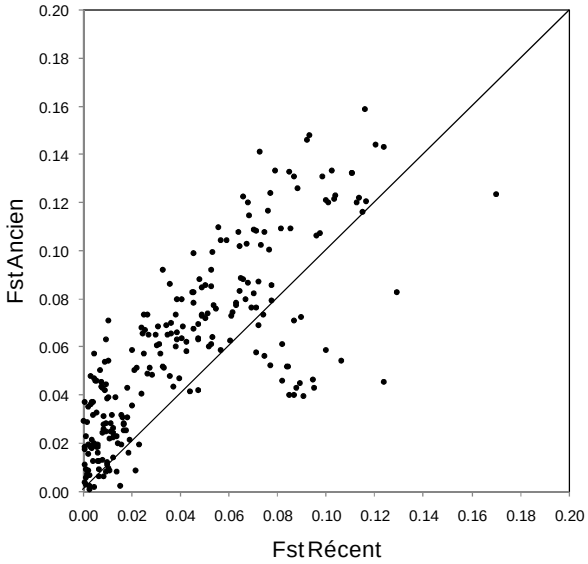

Figure 20 : Réduction de la différenciation génétique entre les populations de saumon.

Au niveau de la baie du Mont-Saint-Michel, alors que les échantillons contemporains étaient très introgressés, l'analyse d'échantillons antérieurs aux opérations de repeuplements montre de faibles taux d'introgression. Ceci confirme que l'introgression observée dans les échantillons récents est une conséquence des opérations de repeuplement. Ces résultats sont en accord avec les études comparant des échantillons de populations de saumon et de truite collectés avant et après des activités de repeuplements (Hansen 2002; Finnegan & Stevens 2008; Hansen *et al.* 2009).

A l'inverse, cette comparaison entre échantillons anciens et récents semble indiquer une dilution d'effets de repeuplements anciens, notamment sur la Bresle (Haute-Normandie) et en Bretagne. En effet, l'échantillon ancien de la Bresle est assez fortement introgressé, ce qui suggèrent une introgression ancienne de la population de la Bresle, conséquence des repeuplements effectués dés les années 1960 avec des souches principalement écossaises. A l'inverse, l'échantillon récent de la Bresle est très faiblement introgressée, ce qui semblerait indiquer une dilution de l'introgression. Cette dilution pourrait provenir du faible succès des opérations de repeuplements et/ou de l'immigration d'individus natifs depuis des rivières voisines non repeuplées.

Concernant la Bretagne, la diminution de la différenciation entre les populations ainsi qu'un isolement par la distance plus significatif au sein des échantillons contemporains pourrait indiquer une dilution d'effets de repeuplements anciens encore détectable dans les vieux échantillons mais non présente dans les récents. Il est également possible que cette structure génétique actuelle ait été influencée par de la dispersion des nombreux poissons natifs déversés dans l'Aulne et L'Elorn depuis les années 90.

Afin d'évaluer plus précisément l'impact des repeuplements anciens, il serait utile d'analyser davantage d'échantillons issus de populations potentiellement utilisées comme

source. En effet, outre l'Ecosse, il est fait mention de nombreuses origines de poissons provenant d'Irlande, de Norvège, de Suède, de Finlande et du Canada (Québec). En parallèle, des analyses de l'ADN mitochondrial devraient permettre de donner davantage de précision sur l'impact de ces repeuplements anciens (Tessier *et al.* 1995; Campos *et al.* 2008). En effet, la dilution de l'introgression pourrait aussi provenir en partie de l'utilisation des marqueurs microsatellites très polymorphes.

Utilisation de simulations

Les analyses d'échantillons anciens et récents des populations de la baie du Mont-Saint-Michel ont montré des impacts variables des repeuplements. La prise en compte de données démographiques et migratoires des populations repeuplées (abondance du stock, taux de survie en mer et de retour à la rivière natale), et relatives aux repeuplements (quantité et origine des individus déversés) a rendu possible au travers de simulations d'évaluer l'impact des opérations de repeuplement sur les populations naturelles. En accord avec la littérature, les résultats obtenus suggèrent :

1- Une fitness faible des individus repeuplés (Fig. 21) (Hansen 2002; Finnengan & Stevens 2008; Sonstebo *et al.* 2008), environ 20 fois inférieure ;

2- Des taux de dispersion relativement importants des poissons sauvages et déversés (Jonsson *et al.* 2003; Pedersen *et al.* 2007) ;

3- Une introgression plus forte dans la Sienne et la Sée que dans la Sélune et le Couesnon, pour une même intensité de repeuplement. Ceci pourrait être lié à des différences de qualité dans l'environnement où sont déversés les juvéniles. Cependant, ces différences de taux d'introgression peuvent être également fonction des fluctuations de taille de population.

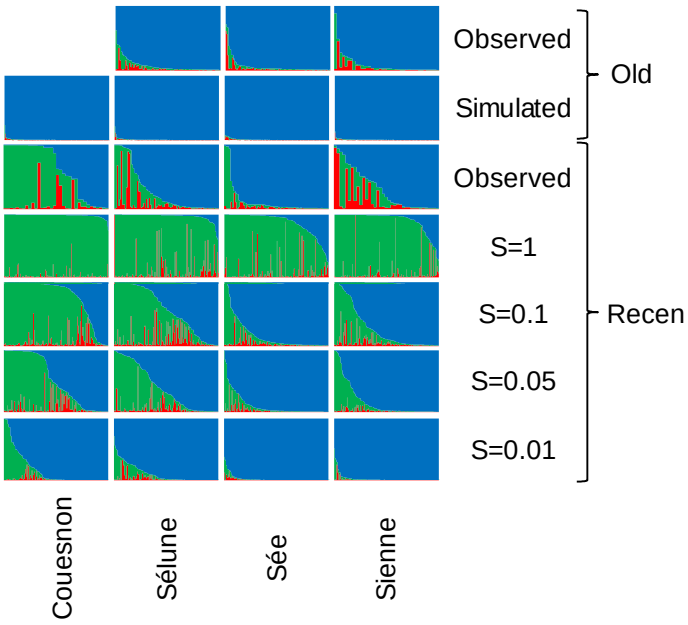

Figure 21 : Taux d'introgression (admixture) observés et simulés pour les quatre populations de la baie du Mont-Saint-Michel. Les simulations sont présentées pour quatre taux de survie différents et des taux de dispersion de 15%.

Afin d'approfondir l'étude des effets des repeuplements en baie du Mont-Saint-Michel, nous avons pour projet d'étudier comment se répartit l'introgression au sein de chacun des quatre bassins versants concernés. En effet, il possible qu'il existe une structuration spatiale de l'introgression, pouvant être due au différentes pressions de repeuplements selon la localité ou aux différences d'habitat (Finnengan & Stevens 2008) ou bien encore dans les comportements des individus (Hansen & Mensberg 2009). Il sera également intéressant de comparer les patrons d'introgression sur les échantillons d'adultes et de juvéniles afin de mettre en évidence d'éventuelles différences entre les survies des sauvages, repeuplés, et hybrides (Blanchet *et al.* 2008; Ford *et al.* 2008). Par ailleurs, nous projetons d'étudier l'évolution de l'introgression au sein de la population de la Sélune de 1987 à 2007, sur pas de temps annuel. Ce travail pourrait être complété d'une étude basée sur des simulations sur les bases de celle présentée dans cette thèse.

Utilisation d'analyses microchimiques des otolithes

Les analyses génétiques sont très utiles pour déterminer l'origine des individus et mesurer l'impact des repeuplements. Cependant, dans un système repeuplé depuis plusieurs années, un poisson possédant des caractéristiques génétiques proches voir identiques à celles de la souche utilisée pour les repeuplements, peut soit être issu du repeuplement actuel mais aussi de la reproduction locale de poissons précédemment repeuplés. Seule une méthode de marquage des individus, couplée à la génétique, pouvait permettre de différencier l'origine de ces individus.

Le couplage d'analyses génétiques et microchimiques d'otolithes est une approche originale qui a permis de déterminer l'origine des poissons : i) sauvages aux caractéristiques génétiques locales, ii) sauvages aux caractéristiques de repeuplement, iii) sauvages aux caractéristiques hybrides, et iiii) de repeuplement (Fig. 22). Par ailleurs, le travail réalisé montre que la microchimie permet de différencier les populations très proches de la baie du Mont-Saint-Michel (réassignation de près de 90% des individus à leur rivière natale) alors que l'utilisation d'outils génétique ne le permet pas. Cependant, l'analyse des otolithes est invasive et nécessite de tuer les individus collectés. De plus, le coût des analyses microchimiques est élevé, en particulier les analyses effectuées par ablation laser et non par dissolution des otolithes.

Pour poursuivre ce travail, il devient nécessaire de pouvoir disposer d'un plus grand nombre d'échantillons de saumon adultes provenant des différentes rivières de la baie. Ce

plus grand nombre d'échantillons devrait notamment permettre d'analyser les pourcentages relatifs de survie en mer des quatres types de poissons évoqués plus haut. Il serait également pertinent de statuer sur la stabilité temporelle des signatures microchimiques des stocks étudiés (Campana *et al.* 2000; Schaffler & Winkelman 2008).

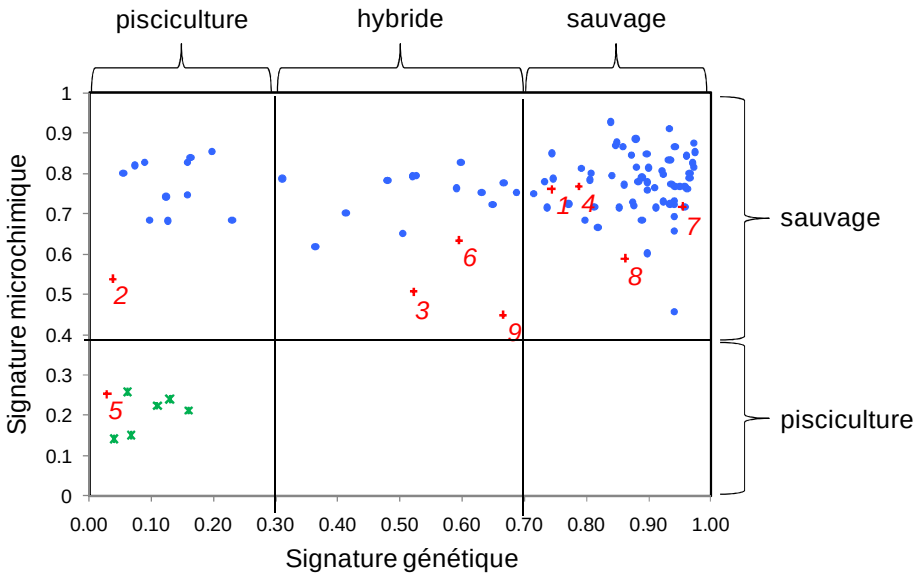

Figure 22 : Utilisation couplée des analyses génétiques et microchimiques pour déterminer l'origine géographique et le pédigrée des individus.

Recolonisation naturelle par le saumon

La restauration de populations éteintes est souvent envisagée *via* le repeuplement avec des individus provenant de populations encore viables. Cependant, les phénomènes de recolonisation naturelle existent. Même s'ils n'ont été décrits chez le saumon Atlantique que sur des rivières relativement petites (Vasemagi *et al.* 2001; Grandjean *et al.* 2009), il existe des exemples de recolonisation naturelle de fleuves chez la truite commune (*Salmo trutta*) (Schreiber and Diefenbach, 2005).

De fait, l'observation de la recolonisation naturelle d'un grand fleuve comme la Seine reste un fait marquant et intéressant d'autant que la population de saumon est éteinte de puis plus de 80 ans voir plus et que le fleuve a connu dans la décennie 1970 une dégradation très marquée de la qualité du milieu (réduction extrêmement forte de la diversité pisciaire). Les quelques saumons analysés semblent provenir à la fois de populations proches, de Normandie, et de populations plus distantes, comme l'Allier et d'autres populations européennes non déterminées avec précision (référence à des souches étrangères). De façon générale, ces phénomènes de recolonisation naturelle sont intéressants du point de vue de la gestion car mettent en avant le fait que le repeuplement ne constitue pas la seule option possible pour

restaurer une population. Il est même raisonnable de penser que des individus sauvages issus du milieu naturel auront davantage de chance de mettre en place une nouvelle population pérenne que des individus d'élevage. L'installation de cette nouvelle population pourrait bénéficier d'un avantage lié à la diversité génétique observée au travers de l'analyse de ces premiers échantillons, mais également lié au caractère sauvage des individus (non sujets aux effets négatifs inhérents à l'élevage en pisciculture et au déversement). Cependant les conditions d'une recolonisation durable passe par l'amélioration de la libre circulation (connectivité) des poissons sur le bassin permettant l'accès à certaines zones de fraye de qualité suffisante (Fraser *et al.* 2007; Kiffney *et al.* 2009). Ces zones existent déjà sur certains affluents de la partie aval de la Seine (Andelle) où des densités relativement importantes de juvéniles ont pu être estimées.

Cette étude a été réalisée sur un nombre limité d'individus et doit être étoffée par un échantillonnage plus important. Analyser davantage d'individus pourraient donner des informations plus solides quand aux origines des individus et aux traits de vie potentiellement particulier qu'ils pourraient posséder et par exemple expliquer leur comportement de dispersion. Afin d'obtenir davantage de précision dans l'origine des individus, il serait possible d'ajouter des échantillons de l'ensemble de l'Europe et en utilisant des analyses d'ADN mitochondrial. Des analyses de juvéniles pourraient également fournir des informations sur le succès reproducteur des individus aux origines diverses recolonisant la Seine. Ces succès reproducteur pourraient être reliés à des traits de vie ou des origines génétiques particulières. La microchimie pourrait permettre de différencier les migrants de leur descendant dans la nouvelle population, dont les caractéristiques génétiques pourraient être identiques.

Conclusion

Au plan scientifique, cette étude est la première permettant d'avoir une vision globale des caractéristiques et de la structure génétique des populations de saumon en France. Par ailleurs, elle apporte d'importants éléments de compréhension de la structure génétique des populations de saumon Atlantique en identifiant cinq grands groupes et en faisant ressortir l'originalité génétique de la population de la rivière Allier. Cette originalité est vraisemblablement la conséquence d'une adaptation de la population à des conditions locales particulières. De fait, la population de l'Allier constitue une opportunité unique d'étudier ces phénomènes d'adaptation locale étant donné les caractéristiques morphologiques et génétiques des poissons de cette population implantée sur un des derniers grands fleuves encore colonisé par le saumon. Ce travail met également en lumière l'impact des repeuplements sur la structure génétique des populations en montrant que ce type d'opération conduit à une plus faible différenciation des groupes identifiés. La Baie du Mont-Saint-Michel a constitué un lieu d'étude privilégié des effets des repeuplements sur les populations de saumons. En effet, cette baie comprend quatre rivières proches, appartenant au même groupe génétique et ayant fait l'objet de repeuplements d'intensités variables avec des individus non-natifs. Les résultats ont parfaitement mis en évidence le niveau d'introgression plus ou moins important des populations naturelles des quatre cours d'eau et la dispersion des individus repeuplés dans les rivières voisines.

Au plan appliqué, les résultats de ce travail ont d'importantes implications pour la gestion des populations de saumon Atlantique. En premier lieu, cette étude montre une fois de plus l'intérêt du suivi régulier des populations de saumon mais également du recueil de chroniques de données sur le long terme avec stockages d'échantillons de tissus tels que des écailles ou des fragments de nageoires qui peuvent être utilisés dans de nombreuses études à posteriori. Une meilleure conservation de ces populations passe par une gestion concertée à l'échelle de chacun des grands ensembles génétiques identifiés (ce qui est actuellement réalisé pour la plupart de ces ensembles, e.g. Allier et Adour). Cette structure suggère en effet une connectivité importante des populations au sein de ces grands groupes. Ainsi, une mesure de gestion appliquée à une population peut avoir des effets sur les populations voisines. Les repeuplements sont notamment à utiliser avec précaution étant donné leurs effets sur les populations ciblées mais également voisines. De plus, étant donné les possibles adaptations locales des populations, il est important d'éviter les pratiques de gestion introduisant des individus non-natifs, en préférant les repeuplements avec des poissons natifs issu du même groupe génétique ou de la même rivière, et en minimisant les processus de sélection en

pisciculture. Néanmoins, en dépit des impacts forts des repeuplements, leur efficacité demeure très faible. Enfin, les possibilités de recolonisation naturelle peuvent être importantes dans certains cas et permettre la restauration d'une population si celle de la qualité et de la connectivité des habitats est préalablement effectuée.

Travaux annexes

Un marqueur microsatellite pour distinguer *Salmo salar, Salmo trutta,* et leurs hybrides

Le but de ce travail était de discriminer saumon atlantique, truite fario, et leurs hybrides, à l'aide d'un marqueur microsatellite.

Le saumon Atlantique et la truite fario coexistent fréquemment dans les mêmes habitats. Les juvéniles des deux espèces peuvent parfois être confondus. Des phénomènes d'hybridation sont connus et les hybrides sont difficiles à identifier et peuvent présenter des phénotypes de type saumon ou truite (Verspoor 1988b; Hurrell & Price 1991; Beall *et al.* 1997). Ainsi différents marqueurs ont été développé pour discriminer ces espèces et leurs hybrides (Guyomard 1978; Vuorinen & Piironen 1984; Pendas et al. 1995; Elo et al. 1997; Susnik et al. 1997; Lee et al. 1998; Perez et al. 1999).

Dans cette étude nous présentons un marqueur microsatellite, SsAD486 (King *et al.* 2001), ayant deux allèles espèces spécifiques permettant l'identification des saumon Atlantiques, truites fario, et leurs hybrides, en Europe. Comme ce marqueur est polymorphique chez le saumon en Amérique du Nord, il peut également être utilisé pour différencier les saumons de l'Atlantique Ouest et Est.

Ce marqueur trouve une utilité dans toutes études portant sur des populations de truite ou de saumon pour lesquelles l'hybridation est possible, ou pour des échantillonnages de juvéniles qui sont alors plus difficile à discriminer. Ce marqueur permet ainsi d'exclure de l'analyse les hybrides ou poissons d'une espèce non désirée qui peuvent générer des erreurs d'analyse et d'interprétation. Ce marqueur peut aussi se révéler fort utile pour l'étude des processus d'hybridation.

A species-specific microsatellite marker to discriminate European Atlantic salmon, brown trout, and their hybrids

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Abstract

Atlantic salmon and brown trout frequently co-occur in the same habitats and juveniles of both species are difficult to discriminate. Hybridization between the two taxa has also been widely documented especially in endangered populations hence the need for species-specific molecular markers. Here we show that the microsatellite marker SsAD486 has two species-specific alleles allowing the identification of Atlantic salmon, brown trout, and their hybrids throughout the European range of these species. Since this marker is polymorphic in Atlantic salmon populations from Western Atlantic, it could also help discriminating between North American and European salmon.

Key-words: hybridization; microsatellite marker; *Salmo salar*; *Salmo trutta*

Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) often coexist in the same habitats and are difficult to discriminate at the juvenile stage. These species also frequently hybridize in the wild and hybrids present a salmon-like or a trout-like phenotype (Verspoor 1988b; Hurrell & Price 1991; Beall *et al.* 1997). This difficulty to recognize hybrids may lead to a biased sampling of *S. salar* or *S. trutta* juveniles since hybrids can represent up to 10% of salmonid juveniles in some rivers (Castillo *et al.* 2010). Such sampling errors may bias inferences of population genetic diversity, which are increasingly used in the management of endangered populations. Hybridization seems to occur more frequently in small populations (Deleaniz & Verspoor 1989) meaning that hybridization rates may help to identify vulnerable populations. Genetic introgression between the two species has been particularly documented in endangered *Salmo salar* and *S. trutta* populations subject to supplementation programs using domestic fish (Castillo *et al.* 2008). In this context, genetic methods of species and hybrid identification were developed using enzyme loci (Guyomard 1978; Vuorinen & Piironen 1984), RAPD markers (Elo *et al.* 1997), restriction analysis and histone-3 coding genes (Perez *et al.* 1999), a rDNA locus (Pendas et al. 1995), and transferrine gene (Lee et al. 1998). Castillo et al. (2008) also used the microsatellite marker BFRO 002 (Susnik et al. 1997) that is polymorphic in brown trout and monomorphic in Atlantic salmon (Ayllon et al. 2006; Castillo et al. 2008). However, no microsatellite marker with two species-specific alleles has been described so far to quickly identify hybrids and both parental species. In this study we show that the microsatellite marker SsAD486 developed by King et al. (2005) can be used for that purpose since European populations of Atlantic salmon and brown trout are fixed for two distinct alleles at this locus. Furthermore, this locus could be extremely useful for the identification of *S. salar* continental origin since it is polymorphic in North-American Atlantic salmon (King et al. 2005; Palstra et al. 2007).

We genotyped at the SsAD486 locus 630 S. salar and 90 S. trutta adults from 45 and 25 rivers respectively (see Supplementary material for localization of sampling sites). As controls, we genotyped at both the SsAD486 and the diagnostic 5srDNA markers (Pendas et al. 1995) six Atlantic salmon adults, six brown trout adults and 11 hybrid embryos resulting from artificial crosses between two S. salar females and two S. trutta males. Genomic DNA was extracted from fin tissues and scales by heating samples in a solution of proteinase K, TE buffer (TrisEDTA) and Chelex 5% at 55° C overnight and then at 105°C for 15 min. PCR analyses and alleles scoring were conducted using two different genotyping procedures. First, we used the economic “M13 method” according to a protocol described in Perrier et al. (2010). The second procedure (called “kit” in table 1) based on standard fluorescent primer labeling is presented in Grandjean et al. (2009).

Table S1: Sample size and coordinates (river mouth) for each of the 59 rivers sampled.

Country	*River*	*Atlantic Salmon*	*Brown Trout*	*N*	*W*
Canada	Bonaventure	2		48°02	65°28
	Cascapedia	2		48°09	65°55
	Jupiter	3		49°28	63°36
	Moisie	2		50°11	66°04
	Adour	32	4	43°31	1°31
	Aulne	9	1	48°24	4°21
	Authie	3		50°22	1°34
	Aven	9	1	47°48	3°44
	Blavet		1	47°42	3°22
	Boutonne		4	45°57	1°04
	Bresle	33	3	50°03	1°22
	Canche	2		50°32	1°36
	Couesnon	28		48°37	1°30
	Dordogne	12		45°34	1°06
	Douron	6		48°40	3°38
	Drennec		4	48°36	4°35
	Ellé	6		47°46	3°32
	Elorn	19		48°17	4°15
	Garonne	20		45°34	1°06
	Gouet		1	48°31	2°43
	Goyen	27		48°00	4°32
	Jet	17		47°52	4°06
	Leguer	15		48°43	3°33
	Loire	51	13	47°16	2°10
	Nivelle	13		43°23	1°40
	Odet	14		47°52	4°06
	Orne	68	4	49°17	0°14
	Vilaine		1	47°30	2°28
	Penzé	22		48°40	3°56
	Rhone		6	43°20	4°50
	Scorff	22	5	47°42	3°22
	Sée	23		48°39	1°29
	Seine	5		49°26	0°07
	Sélune	50	5	48°39	1°29
	Steir	12		47°52	4°06
	Touques	11	11	49°21	0°04
	Trieux	16	1	48°49	3°04
	Valmont	2		49°45	0°22
	Vire	1	1	49°21	1°07
Ireland	Moy	4		54°12	9°08
Norway	Eira	1		62°41	8°07
	Visa	4		62°43	7°55
Poland	Slupia		9	54°35	16°51
Spain	Cares	1		43°23	4°30
Sweden	Själsö		1	57°44	18°24
	Ire		1	57°50	18°35
	Gatarve		2	57°36	18°45
	Svajde		1	57°36	18°21
	Gothem		1	57°36	18°45
	Kopparsvik		2	57°38	18°16
United Kingdom	Dee	1		53°19	3°12
	Frome	6	6	50°41	1°56
	Grimersta	1		58°10	5°44
	Spey	3		57°39	3°38
	Tay	1		56°27	2°47
	Tweed	6		55°45	1°59
Total Brown	25		90		
Total Atlantic	45	630			
Total	59	720			

Table 1: Genotyping procedure, geographic origin and genotypes of the samples analyzed at the SsAD486 locus.

Species	Method	Country	Number of	Sample Size	Genotypes
Atlantic Salmon	KIT	Canada, QC	4	9	158-158; 170-174; 170-186; 170-190; 174-174;
		Europe	19	223	170-170
	M13	Europe	31	398	193-193
	Total		45	630	
Brown Trout	KIT	Europe	21	58	158-158
	M13	Europe	8	32	181-181
	Total		25	90	
Total			59	720	

The SsAD486 locus was monomorphic in all European Atlantic salmon samples (Table 1). King et al. (2005) and Grandjean et al. (2009) also found only one allele in respectively 32 individuals from a Scottish population and 365 individuals from ten French populations. We found five alleles in the nine Canadian samples (Table 1). Correspondingly, King et al. (2005) and Palstra et al. (2007) found respectively seven alleles in 48 individuals from one US population and 13 alleles in 1346 individuals from Newfoundland and Labrador regions. Interestingly, we also found one single allele in all brown trout samples, which was 12 pb shorter than the *S. salar* allele. Results of both genotyping procedures were similar except allele size that was logically longer with the M13 method. Artificial hybrids were all heterozygotes and parallel analyses made with both the SsAD486 and 5srDNA loci produced similar results (Table 2).

Table 2: Genotypes of Atlantic salmon, brown trout, and hybrid samples at the SsAD486 and 5srDNA loci.

Species	Sample Size	Genotypes	
		SsAD486	5srDNA
Atlantic	6	193-193	275-275
Brown	6	181-181	297-297
Hybrids	11	181-193	275-297

Homozygote individuals at the 170 bp allele of the SsAD486 locus can be accurately identified as Atlantic salmon. The continental origin of such individuals could be further determined using the SS1 microsatellite and / or the ND1/16sRNA region of the mitochondrial DNA (Gilbey et al. 2005). Given the extremely low proportion of 158/158 and 158/170 genotypes in North American Atlantic salmon (<0.0004, F. Palstra pers. com., data set from Palstra et al. 2007), individuals sampled in Europe with these genotypes can be identified with high confidence as brown trout and hybrids respectively. All other genotypes indicate an Atlantic salmon from North-America. To conclude, the SsaD486 locus represents an extremely valuable tool for Atlantic salmon and brown trout conservation genetic studies

since it can be used to identify both species and their hybrids in Europe, but also *S. salar* continental origin.

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Structure génétique des populations de l'Adour

Le but de ce travail était de déterminer la structure génétique existante au sein des populations de la région de l'Adour.

De récentes études ont montré l'existence de différenciation génétique significative entre différentes populations ou sous-populations d'une même grande rivière (Primmer *et al.* 2006; Vaha *et al.* 2007; Dillane *et al.* 2008; Dionne *et al.* 2009). Le bassin de l'Adour présente la particularité d'être relativement large et d'avoir des affluents de tailles importantes et rejoignant le cours principal très en aval. La forme de ce bassin et la localisation des frayères pourraient entrainer une importante structure génétique entre les populations de l'Adour. Les études menées par Perrier *et al.* suggèrent une différenciation génétique significative entre le Gave d'Oloron, la Nive, et un fleuve côtier très proche, la Nivelle. De plus, l'Adour fait l'objet de repeuplements avec des poissons natifs et aucun suivi de l'impact génétique n'est encore réalisé.

Les buts de cette étude étaient de définir la structure génétique des différentes (sous-) populations du bassin de l'Adour et déterminer l'impact des repeuplements effectués sur cette structure génétique. 924 juvéniles ont été échantillonnés sur 41 sites et génotypés à 12 microsatellites.

Les analyses de variance moléculaire, arbres de distances génétiques, et l'analyse bayesienne de clustering des individus ont révélé l'existence d'au moins 10 groupes génétiques. Trois principaux groupes correspondent aux rivières Nivelle, Nive, et Adour. Au sein de chacun de ces trois groupes, au moins trois sous-groupes ont pu être identifiés. Les tests de Mantel ont révélé un isolement par la distance significatif. Une analyse d'autocorrelogramme a suggéré une différenciation significative des individus à partir de 30km.

Nos analyses ne suggèrent pas d'effet significatif des repeuplements effectués sur l'Adour sur la structure génétique des populations de la Nive et de la Nivelle. Cependant, la relativement faible structure au sein de la rivière Adour pourrait suggérer une diminution de la structure génétique due aux déversements.

Ces résultats ont d'importantes implications pour la gestion des populations de la région Adour, en montrant l'existence d'une structure génétique à fine échelle géographique et en suggérant des effets homogénéisant des repeuplements. Nous avons pour perspective d'étudier plus en détail les effets des repeuplements *via* l'analyse comparée d'échantillons anciens et récents.

Fine scale population structure of Atlantic salmon *Salmo salar* in Adour Catchment, south-western France.

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In preparation

Extended abstract

Analyses of genetic structure within and among populations are crucial for the development of management plans. Atlantic salmon populations are generally genetically differentiated even at small spatial scales. Recent studies showed evidences of complex within rivers genetic structure (Primmer *et al.* 2006; Vaha *et al.* 2007; Dillane *et al.* 2008; Dionne *et al.* 2009). Adour spawning grounds were distributed in the mainstream and in several big tributaries, from the estuary to 200km from the sea. The geographic structure of this river and the locations of spawning grounds could have led to significant differentiation within this catchment. Studies of French populations (Perrier et al., *In prep.*) suggested significant differentiation within the Adour region, among Adour, Nive, and Nivelle rivers. In addition, stocking was performed in Adour River since 1984 with native individuals.

Also, the aims of this study were to investigate the genetic structure within the Adour catchment and the potential impact of native stocking. 924 juvenile salmon from 41 locations from Adour (Fig. 1, table 1) were genotyped at 12 microsatellite loci.

According to multidimensional scaling (Fig. 2), analyses of molecular variance (Table 2), and individual Bayesian clustering analysis (Fig. 3), we almost found 10 main genetic groups. The genetic structure was hierarchical and the three main groups corresponded to Nive, Nivelle, and Gaves. Thereafter, these groups could be divided in several other groups, suggesting very fine scale genetic structure. The Mantel test revealed significant isolation by distance ($r = 0.53$, $p < 0.01$). Allelic richness trend to decrease with geographic distance to the sea. According to autocorrelogramm analysis, genetic differences were significant from 30km. Our analyses did not suggest significant impact of stocking in Adour River on the genetic structure of Nive and Nivelle rivers. However, stocking may have lead to a decrease of fine scale genetic structure within Adour River. Indeed, we expected a clearer genetic structure among Adour river locations given their geographic distribution and given the high structure found within Nive and Nivelle. These results overall suggest the existence of a fine scale genetic structure within the Adour catchment but a decrease of differentiation due to stocking. We plan to evaluate more finely stocking impact on Adour catchment genetic structure by analyzing historical samples.

Key-words: Genetic diversity, microsatellite loci, *Salmo salar*, stocking

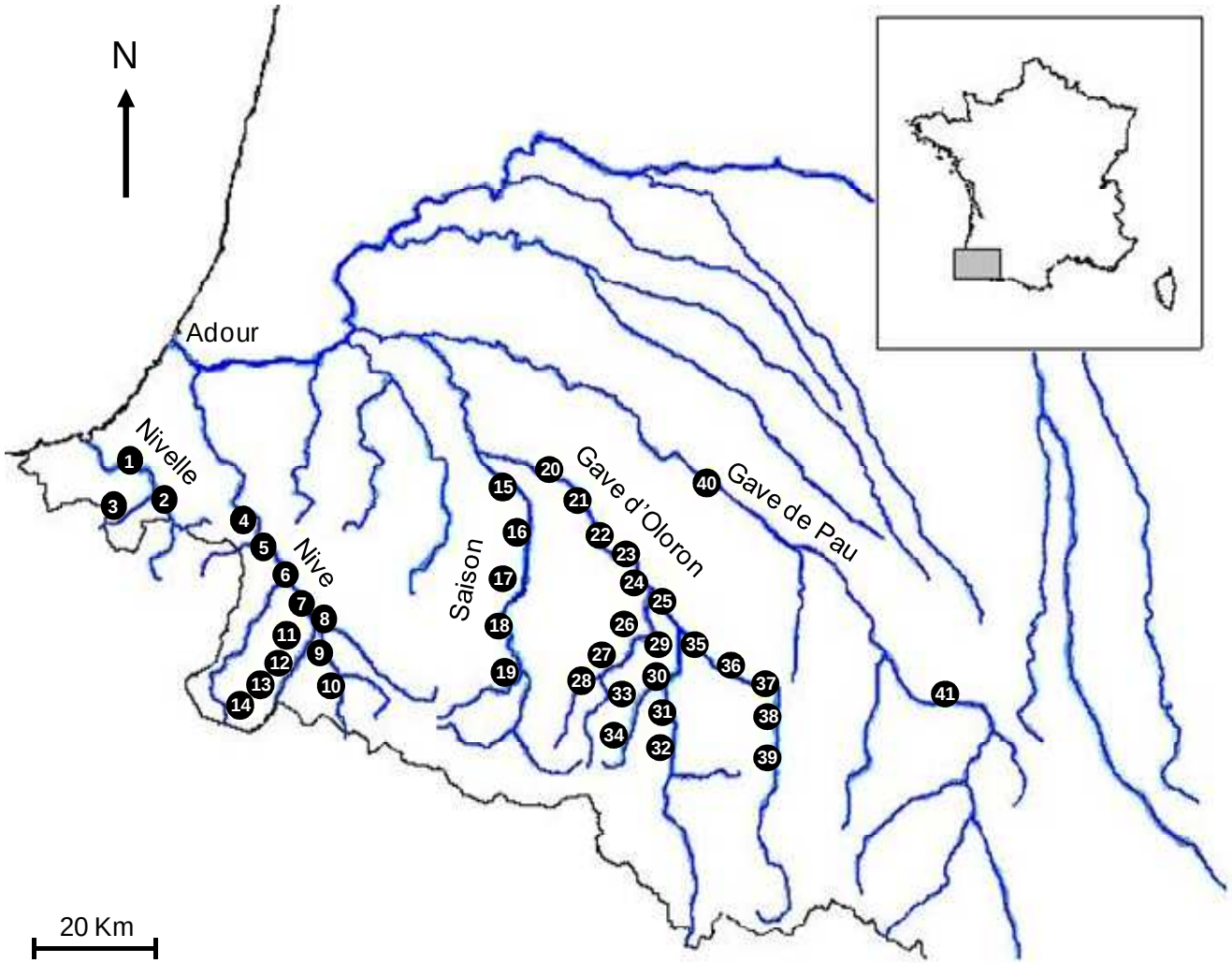

Figure1: Map of the locations of the study populations (see also Table 1).

Table 1: Sampling and genetic diversity data for the 41 location studied. N is the number of individuals, AR is allelic richness (based on 4 individuals), H_e is the unbiased expected heterozygosity, AN is the number of alleles found.

N°	Location	Geographic group	Coordinates	N	Distance from river mouth	He	AR	AN
1	Nivelle		43°20’52’’N / 01°33’06’’W	30	16.52	0.83	5.1	12
2	Nivelle	Nivelle	43°18’33’’N / 01°31’49’’W	45	22.11	0.83	5.1	15
3	Lurgorieta		43°18’28’’N / 01°33’58’’W	19	22.41	0.79	4.6	9.5
4	Nive		43°24’28’’N / 01°30’77’’W	10	52.31	0.80	4.7	7.8
5	Nive		43°20’44’’N / 01°26’53’’W	7	59.1	0.75	4.3	5.6
6	Nive		43°19’29’’N / 01°25’31’’W	8	61.6	0.79	4.6	6.4
7	Laurhibar		43°17’03’’N / 01°24’31’’W	6	64.65	0.81	4.8	6
8	Béhérobie		43°16’92’’N / 01°24’32’’W	50	64.8	0.80	4.7	12
9	Béhérobie	Nive	43°15’40’’N / 01°24’31’’W	15	67.18	0.77	4.4	7.9
10	Béhérobie		43°13’39’’N / 01°21’89’’W	45	70.06	0.80	4.7	11
11	Arnéguy		43°12’90’’N / 01°25’18’’W	46	64.6	0.80	4.7	12
12	Arnéguy		43°16’08’’N / 01°24’88’’W	47	65.71	0.78	4.5	11
13	Arnéguy		43°12’89’’N / 01°26’74’’W	44	70.08	0.79	4.6	12
14	Arnéguy		43°10’92’’N / 01°28’10’’W	37	72.79	0.75	4.3	8.9
15	Saison		43°31’27’’N / 00°87’19’’W	8	87.61	0.84	5.2	7.7
16	Saison		43°24’97’’N / 00°89’02’’W	19	96.46	0.84	5.1	10
17	Saison		43°19’51’’N / 00°91’33’’W	23	104.53	0.84	5.2	12
18	Saison		43°16’25’’N / 00°89’59’’W	51	109.2	0.83	5	13
19	Saison		43°11’92’’N / 00°86’68’’W	24	116.02	0.82	5	11
20	Gave d’Oloron		43°30’66’’N / 00°75’00’’W	4	95.31	0.79	4.7	4.7
21	Gave d’Oloron		43°27’00’’N / 00°71’53’’W	5	101.49	0.84	5.1	5.8
22	Gave d’Oloron		43°26’85’’N / 00°68’41’’W	12	105.64	0.82	4.9	8.2
23	Gave d’Oloron		43°24’52’’N / 00°67’84’’W	23	108.8	0.83	5.1	12
24	Gave d’Oloron		43°23’44’’N / 00°66’14’’W	21	110.78	0.81	4.9	11
25	Gave d’Oloron		43°23’11’’N / 00°63’70’’W	47	114.5	0.82	5	14
26	Vert	Gaves	43°19’51’’N / 00°65’68’’W	14	116.19	0.79	4.6	7.7
27	Vert		43°16’31’’N / 00°68’15’’W	15	122.93	0.82	4.8	8.3
28	Vert		43°13’83’’N / 00°69’27’’W	13	126.51	0.81	4.8	8.4
29	Gave d’Aspe		43°17’69’’N / 00°60’30’’W	5	143.8	0.84	4.9	5.6
30	Gave d’Aspe		43°14’99’’N / 00°58’98’’W	20	124.24	0.82	4.9	10
31	Gave d’Aspe		43°10’25’’N / 00°61’74’’W	14	131.82	0.78	4.3	7
32	Gave d’Aspe		43°03’88’’N / 00°60’42’’W	8	141.21	0.82	4.8	6.7
33	Lourdios		43°10’74’’N / 00°63’84’’W	16	132.94	0.83	5	9
34	Lourdios		43°07’69’’N / 00°66’98’W	14	134.37	0.87	5.5	11
35	Gave d’Ossau		43°11’98’’N / 00°47’76’W	20	138.15	0.83	5.2	11
36	Gave d’Ossau		43°10’57’’N / 00°42’20’W	36	145.8	0.81	4.7	10
37	Gave d’Ossau		43°15’23’’N / 00°56’03’W	23	125.83	0.83	4.9	10
38	Gave d’Ossau		43°12’66’’N / 00°51’12’W	20	134.59	0.81	4.9	10
39	Gave d’Ossau		43°08’60’’N / 00°42’10’W	11	149.16	0.79	4.7	7.7
40	Gave de Pau		43°30’46’’N / 00°43’64’W	4	117.9	0.81	4.7	4.7
41	Gave de Pau		43°09’60’’N / 00°17’10’W	45	157.01	0.80	4.7	11

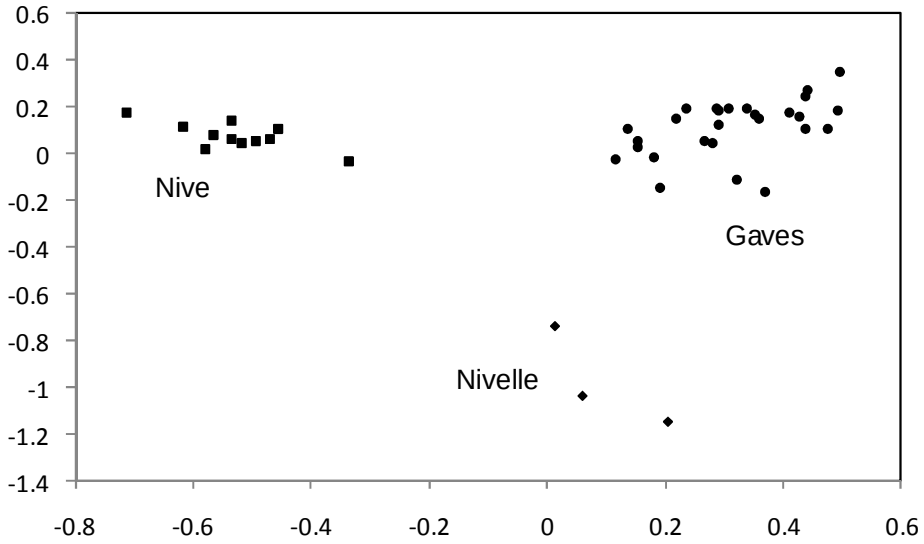

Figure 2: Multidimensional scaling analysis.

Table 2: Results of AMOVA considering as groups: Nivelle, Nive, and Gaves.

	% of variation explained
Among groups	2.99
Among populations within groups	2.36
Within populations	94.65

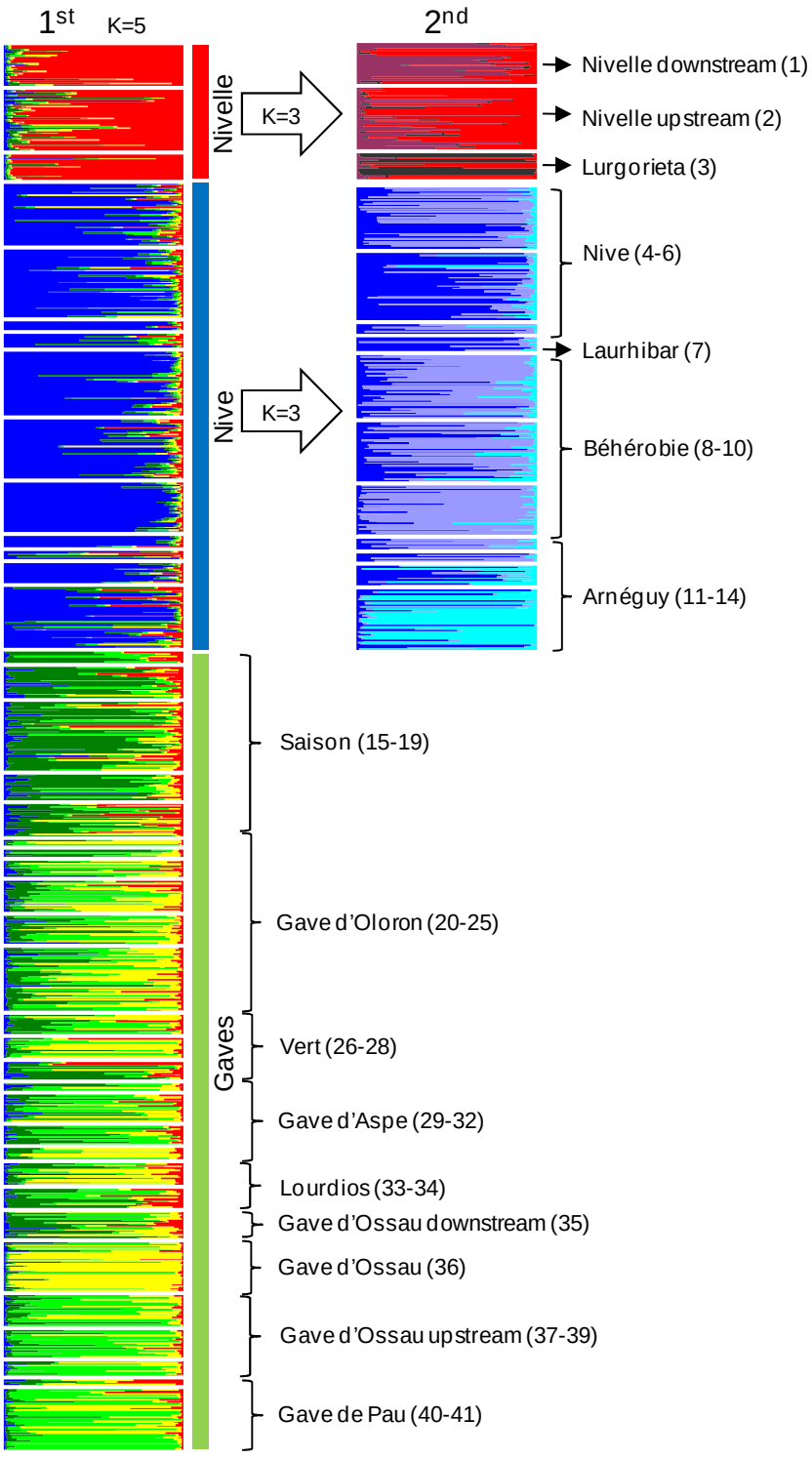

Figure 3: Bayesian individual clustering results with STRUCTURE for two rounds of individual clustering analysis. Colored bars represent proportions of membership of each individual to each cluster represented by different colors.

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Structure génétique des populations de saumon Atlantique en France.

Cette thèse étudie la distribution spatiale de la diversité génétique des populations françaises de saumon Atlantique, s'intéressant notamment à l'effet de facteurs environnementaux et des repeuplements. 1739 individus échantillonnés dans 34 rivières françaises et provenant de cohortes anciennes (à partir 1965) et récentes ont été génotypés à 17 marqueurs microsatellites. Les résultats montrent l'existence de cinq groupes de populations génétiquement et géographiquement distincts. La distance côtière entre les populations ainsi que la longueur des rivières expliquent largement la structure observée. La grande taille des poissons adultes de l'Allier, ainsi que la différenciation importante de cette population inféodée à des sites de frais très éloignés de la mer suggèrent une adaptation locale à la difficulté de montaison. La comparaison des échantillons anciens et récents montre des taux d'introgression variables par les souches utilisées pour les repeuplements. Dans certaines rivières dépeuplées et non sujettes au repeuplement, nous avons observé des recolonisations spontanées par des poissons provenant de stocks voisins ou distants. Afin de quantifier l'impact des repeuplements dans les populations de la baie du Mont-Saint-Michel, nous avons développé des simulations individus centrées dont les résultats suggèrent une faible survie des poissons déversés. Enfin, nous avons également réalisé des analyses microchimiques sur les otolithes d'individus issus de populations repeuplées. Le couplage des analyses microchimiques et génétiques a permis de déterminer si les poissons ayant des caractéristiques génétiques de pisciculture provenaient de repeuplement ou de reproduction *in natura* de poissons précédemment déversés.

Genetic structure in Atlantic salmon populations from France.

This thesis investigates the genetic structure among Atlantic salmon populations from France. We more specifically studied the influence of environmental factors and stocking on the spatial distribution of genetic diversity. We genotyped at 17 microsatellite markers 1739 individuals from 34 French rivers, from old (1965-1987) and recent (1998-2006) cohorts. Analyses of recent samples classed individuals into five genetically and geographically distinct groups. Distance among estuaries and river length were strong predictors of population structure. Moreover, the positive trend between body size and river length and the higher differentiation of the population having farthest spawning grounds off the river mouth suggest local adaptation to upstream migration difficulty. Comparison of recent and old samples showed a general reduction of differentiation among populations and some high introgression rates most probably resulting from stocking. In some depopulated rivers were no stocking was performed we observed natural recolonization by fish from neighbouring and distant stocks. To quantify the impact of stocking on some populations for which it was precisely documented, we developed an approach using temporally explicit simulations. This study suggests low fitness of stocking fish. In parallel to genetic analyses, we carried on microchemistry analyses of otolith from some fish from stocked populations. Coupling genetic and microchemistry analyses on the same individuals allowed identifying river-born fish with hatchery pedigrees, discriminating them from hatchery-born fish with same genetic characteristics.

Mots clefs, indexation Rameaux : empoissonnement, animaux--adaptation, simulations, microchimie, variabilité génétique, génétique moléculaire

Mots clefs, indexation libre : adaptation locale, introgression, marqueurs microsatellites, microchimie, otolithe, repeuplements, saumon Atlantique, simulations, structure génétique.

Discipline : Physiologie, Biologie des Organismes, Populations, Interactions

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