Dispersal capacities of anadromous Allis shad population inferred from a coupled genetic and otolith approach

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Abstract

Understanding dispersal capacities for migratory species is crucial for their management. By coupling otolith microchemistry and microsatellite genetic analyses, we provided information on snapshot and long-term dispersal capacity of Allis shad, an anadromous clupeid in decline throughout its distribution range. The allocation of natal origin was obtained from water chemistry, signatures in otoliths of juveniles and spawners within a Bayesian model. The majority of adults were assigned to a source river with high degrees of confidence, only 4% were undetermined. Otolith natal origins were used to define a population baseline by grouping individuals from the same natal river and not from the same sampling location as usually done. While *Alosa alosa* exhibited a high level of natal site fidelity, this species showed weak genetic structure which supported the evidence of a significant flow of strayers between river basins in vicinity or at longer distances. However, long distance straying was probable but not frequent. In a context of global change, straying would be a key mechanism to drive dispersal and allow resilience of Allis shad populations.

Keywords: Natal origins, Straying pattern, Allis shad, Otolith chemistry, Microsatellites.

Introduction

Understanding population structure and connectivity for migratory species across broad geographic areas (e.g., species' distribution) is of major interest for their management and conservation. Anadromous fish are defined by McDowall (2008) as fish which spend most of their life at sea and migrate to freshwater to reproduce. Philopatry to natal sites and straying behavior, defined as adult migration to non-natal rivers, are two fundamental life-history traits of most anadromous fishes that jointly convey adaptive advantages to their persistence. Selection in spatially heterogeneous environment leads to local adaptation which can be enhanced by natal site fidelity hence providing fitness benefits (Hendry et al. 2004). On the other hand, straying allows for colonization of new habitats, the maintenance of genetic diversity, and can mitigate spatial and temporal variation in habitat quality (Keefer and Caudill 2014). Regardless whether fish are straying by habitat choice or by mischance of returning home through inadequate imprinting, accumulating evidence suggests that the degree to which straying is undertaken probably varies within and among populations of many fish species (McDowall 2001). Thus, understanding this straying behavioral diversity at the individual scale may be necessary to ensure anadromous fishes resilience in the face of anthropogenic threats and large scale climatic and environmental changes (Hamann and Kennedy 2012).

The combination of otolith microchemistry and genetic markers has shown promise in identifying patterns of connectivity among populations at different scales (Bradbury et al. 2008; Selkoe et al. 2008; Barnett-Johnson et al. 2010; Hughes et al. 2014). Genetic studies provide insight on long-term connectivity patterns on evolutionary time scales whereas chemical tracers can reveal dispersal patterns within the time scale of an individual lifetime (Feyrer et al. 2007). However, magnitudes of dispersal or straying rate present particular challenges to genetic investigation of connectivity for anadromous species (Selkoe et al. 2008;

Humston et al. 2010). The use of otolith based techniques and genetic markers on the same individuals may provide valuable information about the dispersal pattern of a given fish during its lifetime, but also about longer term gene flow between populations (Fromentin et al. 2009).

Otoliths are paired aragonitic structures in the inner ear of bony fish that grow by continuous accretion of metabolically inert material (Campana 1999). Otolith chemical composition reflects, to some degree, the chemistry of ambient waters at the time of material deposition (Bath et al. 2000; Walther and Thorrold 2006). Otoliths from fish hatched in chemically distinct water masses will record unique signatures, reflecting their habitats. The use of trace element ratios recorded in otoliths has provided separation of anadromous fish according to their natal river (Barnett-Johnson et al. 2008; Walther et al. 2008). Strontium and Barium have been amongst the most powerful elements because their concentrations in otoliths are largely influenced by their respective metal-to-Ca ratios in the ambient water (Bath et al. 2000; Dorval et al. 2007; Elsdon et al. 2008; Martin et al. 2013b). In addition, natural Sr isotopic signatures have proved to be useful as fish markers, particularly for species that inhabit freshwater during their juvenile stages (Ingram and Weber 1999; Kennedy et al. 2000; Martin et al. 2013a). Otolith ^{87}Sr:^{86}Sr ratios directly reflect dissolved ambient ratios (Blum et al. 2000), and have been found to remain stable over the years, making them "ideal" spatial markers for characterizing natal sources (Kennedy et al. 2000). Fine-scale geographic discrimination of rivers could be achieved by the combined use of elemental and Sr isotope ratios.

Microsatellites are neutral genetic markers not affected by selective pressures. They consist of short tandem repeat sequences of nucleotides (2-5 base pairs repeated units) (O'Connell and Wright 1997) that undergo fast mutation rates and thus lead to high levels of polymorphism. They are among the most commonly used markers to test the level of among-

population connectivity (Bentzen et al. 1996; Selkoe and Toonen 2006; Bradbury et al. 2014) and have been useful for fine-scale population discrimination in anadromous fishes (Banks et al. 2000; Spidle et al. 2003). However, the success of genetics-based approaches to quantify natal homing is dependent on the levels of interbreeding among populations since even very low exchange rates can prevent populations' differentiation (Collins et al. 2013).

In the present study, we integrated multiple approaches (i.e. otolith microchemistry, and genetics) to assess the dispersal capacity of Allis shad (*Alosa alosa*), an anadromous clupeid in decline throughout its distribution range (Baglinière et al. 2003). This species has a pelagic marine existence but upon maturation (from 4 to 6 years) migrates to spawn in the higher middle watercourse of rivers. Eggs hatch at spawning site and seaward emigration takes place in the summer and fall of their first year of life (Lochet et al. 2009). Allis shad was distributed historically in latitude from the North of Morocco to Norway (Baglinière et al. 2003). Since the middle of the 20th century, the impact of human activities such as overfishing, damming of rivers and pollution, has led to a drastic restriction of its distribution area (De Groot 2002; Limburg and Waldman 2009). Climate change is also expected to shift the species range towards higher latitudes and southern populations are expected to be particularly affected by global warming (Lassalle et al. 2008a). The species was classified as 'vulnerable' and has been included in Appendix III of the Bern Convention as well as in Annexes II and V of the European Community Habitats Directive. Currently, the most successful breeding populations, excluding relict or residual ones, are distributed across the north-east Atlantic coastline in a few rivers in western France and Portugal (Baglinière et al. 2003).

Very little is known about Allis shad population structure in Europe. Divergence in life history characteristics among populations was observed with southern populations exhibiting larger length at maturity compared to basins further north (Lassalle et al. 2008b).

Studies reporting discrimination of natal origins of European Allis shad populations using otolith microchemistry are scarce and were restricted to the Gironde population, where Tomas et al. (2005) discriminated fish from Garonne and Dordogne Rivers. Genetic studies generally indicated low levels of genetic structure among populations but some authors (Alexandrino et al. 2006; Jolly et al. 2012) described a slight divergence between French and Portuguese populations and a process of isolation-by-distance over 2,000-4,000 km, suggesting that gene flow primarily takes place between neighbouring populations. Although there is a pressing need to understand the migratory dynamics of Allis shad throughout its range for management and conservation purposes, all previous studies have been restricted to a limited geographic area or have compared only a handful of populations.

The main goal of the present study was to couple otolith chemistry with genetic analysis on the same individuals to study population connectivity and dispersal of *A.alosa* over ecological and evolutionary time scales. This integrative approach was used to identify the Allis shad's natal rivers and assess the level of gene flow between populations, and thus determine the temporal and spatial scale over which straying occurs.

Materials and methods

Fish and water sampling

Mature Allis shad (n = 425) were collected from upstream spawning areas to tidal freshwater portions of 15 rivers along the Atlantic coast, from northern France to Portugal, between April and June 2009 to 2013 (supplementary Figs. S1, S2, Table S1). Spawners were sampled either by sport fishing, by commercial fishermen using a trammel net, or found dead after spawning during summer. Muscle tissues were removed and stored in 95% ethanol. The lengths of shad were measured to the nearest millimeter. Fifty decaying fish carcasses, sampled after spawning, were not available for length and weight measurements. All fish were frozen upon collection.

Juvenile shad (n = 26) were sampled in four French rivers (Blavet, Dordogne, Loire and Vilaine) between June and October 2013 (supplementary Table S1). Juveniles from the Minho River in Portugal (n = 20) were sampled between 2009 and 2012 from September to January. The Blavet, Dordogne, Loire, Vilaine and Minho Rivers have been sampled for both adults and juveniles. Fish were collected using bongo nets and seines in upper-estuarine regions of each river prior to their downstream migration to the sea.

Spatial variability in water chemistry was investigated across 17 major spawning rivers from throughout the native range of Allis shad. These rivers corresponded to the rivers where fish were sampled. The Charente, Oloron and Nive rivers were sampled despite no fish collection. At each river, water samples were collected, once a month, from late May to September 2013, close to well-known spawning area of Allis shad. At each location, 100 mL of river water was collected for Sr:Ca, Ba:Ca and ^{87}Sr:^{86}Sr analyses. Water samples were passed through 0.45 µm Nalgene polytetrafluoroethylene filters (http://www.vgdusa.com/Nalgene-Syringe-Filters-PTFE-25mm.htm) with syringes into acid-washed low-density polyethylene (LDPE) bottles and acidified (2%) using concentrated, ultrapure HNO_3 (JT Baker, Ultrex II; www.jtbaker.nl). Samples were kept on ice in the field and refrigerated upon return to the laboratory.

Microsatellite DNA genotyping

Genetic analyses were performed on all collected specimens except for Portuguese rivers where no fish tissue was available. Genomic DNA was extracted from ethanol-preserved muscle tissue using a modified Chelex protocol from Estoup et al. (1996). Genotyping was performed using 13 microsatellite markers: Alo1, Alo6, Alo7, Alo9, Alo15, Alo16, Alo17, Alo26, Alo29, Alo32, Alo33, Alo43 and Alo45 (Rougemont et al. 2014) specifically developed for *Alosa alosa* (and its closely related species *A. fallax*) by following the protocol recommended by the authors. We found no evidence of null alleles and scoring

errors due to large allele dropout or stutter peaks by using the software Micro-Checker (Van Oosterhout et al. 2004).

Otolith preparation and analysis

Frozen fish were thawed and dissected to remove pairs of sagittal otoliths. Otoliths were rinsed in distilled water, air-dried, and one sagitta per fish was embedded in epoxy resin (Araldite 2020, Escil) with the primordial surface downwards. Resin blocks were ground with ultrapure water and sandpaper (1200 – 4000 grit) until the primordium was reached. Finally, otoliths were rinsed with ultrapure water, and then air-dried before being stored in individually labeled plastic vials.

A C-shaped ablation trajectory centered on the primordium (40 µm away from the primordium, ablation width 60 µm; Fig. 1) was applied on otoliths of all individuals collected (adult and juvenile Allis shad), which avoided the maternal influence on the chemical signature (Kalish 1990; Rieman et al. 1994). We assume that the first feeding mark was always before 40 µm from the primordium for each sample of Allis shad (Lochet et al. 2008). Semi coronas of 60µm thick (difference between the inner 40 µm and outer radius 100 µm) were constructed by fast rotation of an 8 µm spot (using 8 concentric semicircles). A UV high-repetition-rate femtosecond laser ablation (fs-LA) system (Nexeya SA, Canejan, France) was employed (Pulse duration: 360fs; wavelength: 257 nm).

Otoliths were analyzed for Sr:Ca and Ba:Ca ratios on a High Resolution (Thermo Scientific, USA) inductively coupled plasma quadrupole mass spectrometer (HR-ICP-MS). A He gas stream carried ablated material to the HR-ICP-MS (carrier gas flow rate 0.68 $L \cdot min^{-1}$). Elemental ratios were quantified by monitoring ^{43}Ca, ^{86}Sr and ^{138}Ba. Calcium was used as an internal standard to improve the reliability of the concentration measurement (Campana 1999). Elements were standardized to calcium based on the stoichiometry of calcium carbonate (389 000 µg $Ca \cdot g^{-1}$ otolith) (Brown and Severin 2009): Sr:Ca ($mg \cdot g^{-1}$), Ba:Ca

202 ($\mu g \bullet g^{-1}$). Quantification of trace elements in otoliths was achieved by external calibration
203 using both carbonate pellets (Barats et al. 2007) and 3 NIST glass standards (610, 612, 616) to
204 ensure the best accuracy. An otolith Certified Reference Material (NIES 22, National Institute
205 for Environmental Studies) was also pelletized and used in the quality control of the analysis
206 of selected trace elements in the fish otolith. The limits of detection ($\mu g \bullet g^{-1}$ in otoliths)
207 achieved in this study were as follows: ^{86}Sr, 0.05 and ^{138}Ba, 0.01. They were based on a 3σ
208 criterion, where σ is the standard deviation of the mean blank count for each isotope. All the
209 elemental concentrations in the otolith were above the detection limits.

210 After elemental analysis, the same otoliths were used for ^{87}Sr:^{86}Sr ratio and the laser
211 traced out a semi corona of 60µm thick opposed to the one ablated for elemental ratio
212 measurements (Fig. 1). We used a Nu-Plasma multicollector ICP-MS (Nu Instruments, UK)
213 coupled to the same fs-LA system described above. The otoliths were analysed for ^{87}Sr:^{86}Sr,
214 following the method used by Martin et al. (2013a). The accuracy of this approach was
215 checked through the analyses of NIES 22 obtained from otoliths of a marine fish (*Lutjanus*
216 *sebae*). We analyzed NIES 22 pellets (n = 36) with the same ablation strategy applied to
217 otoliths and we obtained a mean value of 0.70927 ±0.00011 2 SD, for ^{87}Sr:^{86}Sr. The ^{87}Sr:^{86}Sr
218 ratios in pellets fell within the expected range for nearly constant modern sea water (0.70917)
219 (Allègre et al. 2010). Finally, the ^{87}Sr:^{86}Sr ratio of NIES 22 pellets was used as an in-house
220 marine carbonate standard and was measured every 6 samples as an external check of
221 reproducibility.

222 **Water sample preparation and analysis**

223 Water samples were analyzed using solution-based ultra-sensitive inductively coupled
224 plasma mass spectrometer (ICP-MS, Bruker Aurora Elite; www.bruker.com) to measure Ca,
225 Sr and Ba concentrations following protocol described by Martin et al. (2013a). The general
226 performance of the procedure was checked every 10 samples using the certified reference

freshwater SLRS-5 (NRCC; www.nrc-cnrc.gc.ca). External precision (% relative standard deviation, R.S.D.) for the laboratory standard (n = 10) were 3% for Ca and Ba, 4% for Sr.

Sr isotope analysis was performed using the Nu-Plasma MC-ICP-MS, and following protocol described by Martin et al. (2013a). Accuracy and precision were monitored with a Standard Reference Material (SRM 987; www.nist.gov/srm). The mean±s.d. value of ^{87}Sr:^{86}Sr in SRM 987 (n = 54) run throughout the analyses was 0.71034±0.00003, which compares favourably with the accepted value of 0.71034 ± 0.00026.

Data analysis

Water chemistry analyses

Measurements of elemental and Sr isotopic ratios in water were statistically analyzed using non-parametric multivariate tests as data were not normally distributed. Water chemistry differences among sites were tested using the non-parametric Mann–Whitney *U*-Wilcoxon tests with a Bonferroni adjustment. Geographic differences in multivariate signatures among locations were visualized using canonical discriminant analysis. Canonical variate coefficients provide a useful way to measure the relative importance of each variable to the observed separation among rivers (Walther et al. 2008). Finally, a quadratic discriminant function analysis (QDFA) was employed to discriminate among the different rivers. A QDFA was used because this procedure does not assume homogeneity of covariance matrices and tolerates modest deviations from normality (McGarigal et al. 2000). The discriminant function analysis used a jackknife cross-validation procedure to determine classification accuracy. Data analyses were performed using R software (R. Development Core Team 2013).

Bayesian hierarchical mixture model: model specification

Flash ablation is a laser strategy that allows ablating semi-coronas and sends the corresponding otolith powdered material at once to the ICP-MS (for elemental ratio

252 measurements) or the multicollector ICP-MS (for Sr isotope ratio measurements). Since we
253 ablated the same period of life on the otolith for both semi-coronas, we used Sr:Ca, Ba:Ca and
254 ^{87}Sr:^{86}Sr as a three dimensional value system to characterize the natal origin chemical
255 signature of a fish.

256 In the following section, braces were used to denote vectors while brackets were used
257 to denote matrices. An approach similar to Pflugeisen and Calder (2013) was used to infer
258 shad natal rivers based on their otolith microchemistry and habitat information. This approach
259 was based on a Bayesian clustering method using otolith elemental compositions and Sr
260 isotopic ratios as fish descriptors and similar measurements made on possible natal rivers as
261 habitat information.

262 Each adult shad *a*, was characterised by a river of capture $C_{ad}(a)$, its otolith elemental
263 composition $\{E_{ad}(a)\}$, and a Sr isotope ratio $IR_{ad}(a)$. The objective was to infer adult natal
264 rivers $N_{ad}(a)$ based on $\{E_{ad}(a)\}$ and $IR_{ad}(a)$. Similarly, a juvenile *j* was characterised by its
265 otolith elemental composition $\{E_{ju}(j)\}$, a Sr isotope ratio $IR_{ju}(j)$ and a natal river $N_{ju}(j)$ (which
266 was known contrary to adult fish). Each river *r*, was also characterised by its elemental
267 signature $\{E_{ri}(r)\}$ and Sr isotope ratio $IR_{ri}(r)$.

268 We assumed that $E_{ad}(a)$ and $IR_{ad}(a)$ given the fish was born in river *r* followed
269 respectively a multinormal (*MN*) and a normal (*N*) distribution:

$$(\{E_{ad}(a)\}|N_{ad}(a) = r) \sim MN(\{\alpha\} \cdot \{E_{ri}(r)\} + \{\beta\}, [\Sigma])$$

$$(IR_{ad}(a)|N_{ad}(a) = r) \sim N(IR_{ri}(r), \sigma_i)$$

270 with $\{\alpha\}$ and $\{\beta\}$ the partition coefficients of element assimilation (Farrell and Campana
271 1996; Bath et al. 2000). Uninformative priors were used for $[\Sigma]$ and σ_i (*i* for isotope):

$$\sigma_i \sim InvGamma(0.01,0.01)$$

$$[\Sigma] \sim InvWishart([I_n], n)$$

272 with $[I_n]$ the identity matrix and *n* the number of element considered in the study. Vague

uniform priors were used for {α} and {β}, respectively between [0, 2] and between [-3, 3]. No partition coefficients were required for isotopic ratio since otolith isotopic ratio is equal to the water ratio (Blum et al. 2000).

For juveniles, we assumed that:

$$\{E_{ju}\}(j) \sim MN\left(\{\alpha\} \cdot \{E_{ri}\}\left(N_{ju}(j)\right) + \{\beta\}, [\Sigma]\right)$$

$$IR_{ju}(j) \sim N\left(IR_{ri}\left(N_{ju}(j)\right), \sigma_i\right)$$

The natal origin of an adult caught in river $C_{ad}(r)$ was given by:

$$N_{ad}(a) \sim Categorical\left(\{\theta_{C_{ad}(a)}\}\right)$$

$\{\theta_r\}$ was a vector that contains the a priori probabilities $\{\theta_r(1), \ldots, \theta_r(n_r)\}$ that an adult caught in river *r* was born in each of the n_r rivers.

Similarly to Pflugeisen and Calder (2013), an uninformative prior was used for $\{\theta_r\}$:

$$\{\theta_r\} \sim Dirichlet(\{\gamma_k\})$$

with $\gamma_1 = \ldots = \gamma_{n_r} = \frac{1}{n_r}$

Model fitting and convergence diagnostics

Three independent parallel chains were run for 20 000 iterations after a burn-in period of 10 000 iterations. The model was implemented using the software JAGS (Plummer 2003) and was run using runjags (Denwood 2013), a package that facilitated the communication between JAGS and R statistical software. Convergence diagnostics were made out using the coda library (Plummer et al. 2006). Gelman and Rubin (1992) tests and visual inspection of a posteriori distribution were carried out. In addition, visual inspections of rivers reallocation were carried out to check consistency between the three chains. *{E_{ri}(r)}*, *{E_{ju}(j)}* and *{E_{ad}(a)}* were previously scaled and centered for each element. Centering covariates is useful in Bayesian inference to decrease the correlation between regression parameters. Scaling was required to have a common scale of variations among elements. $\{\alpha\}$, $\{\beta\}$, $[\Sigma]$, σ_i and $N_{ad}(a)$

were monitored. $\{\alpha\}$, $\{\beta\}$ provided information on the partition function. $[\Sigma]$, σ_i provided information on the relative influence of each element and Sr isotopes. Finally, $N_{ad}(a)$ corresponded to the adult shad reallocations.

Genetic data analysis

We first tested deviation from Hardy-Weinberg Equilibrium (HWE) and genotypic disequilibrium between pairs of loci for each population using Genepop (Rousset 2008). Basic indices of population genetic diversity, namely observed heterozygosity and unbiased expected heterozygosity (Ho and Hnb) were computed using Genetix (Belkhir et al. 2004). Allelic richness (Ar) averaged over loci was computed using Fstat (Goudet 2001) and this software was also used to compute population level genetic differentiation (F_{ST}) estimated by θ (Weir and Cockerham 1984). Significance was assessed using 1000 permutations in Fstat. We used Bonferonni correction to adjust significance level for multiple tests (Rice 1989). As F_{ST} estimation is dependent on within and between population allele frequency (Meirmans and Hedrick 2011), we also computed D_{ST} (Jost 2008) using SMOGD (Crawford 2010) and 999 bootstrap replicates. D_{ST} is a standardized measure of population differentiation, not dependent on allele frequencies within and among populations. Population genetic structure was further investigated using the DAPC approach (Jombart et al. 2010) using the Adegenet package (Jombart 2008) implemented in R software. We used the Bayesian method implemented in GeneClass (Piry et al. 2004) to assign individuals to their potential river of origin. In a first approach, the population baseline was defined based on the river the fish were sampled in. The likelihood that sampled fish came from one of the river was tested with the Paetkau et al. (2004) algorithm using 100 000 simulated individuals. We finally quantified the relationship between genetic differentiation (F_{ST}) and geographic distance by testing for a signal of isolation by distance (IBD) using a simple Mantel test in R with the package Vegan

(Oksanen et al. 2009). Population genetic distance was transformed following Rousset (1997) formulae (F_{ST} / (1-F_{ST})).

Combining population genetic and otolith natal origins

Otolith fingerprints were used to define a new population baseline by grouping individuals from the same natal river (and not from the same sampling location, as above). Measures of intra and inter-population differentiation (Ar, Ho, Hnb, pairwise F_{ST}) were computed as above for these reconstructed populations. Differences in allelic richness and observed heterozygosity were tested using Wilcoxon paired signed-rank test (using values per locus) in R software. We then used this new baseline to assign all fish for which genetic data was available to potential groups using Geneclass (Piry et al. 2004). These results were then compared to those obtained previously.

Results

Spatial differences in water signatures

Mean water Sr:Ca, Ba:Ca and $^{87}Sr:^{86}Sr$ ratios were significantly different among sites (Bonferroni adjustment, $P < 0.05$) (Fig. 2). Water $^{87}Sr:^{86}Sr$ ratios separated three groups of rivers: Portuguese rivers (Lima, Mondego and Minho rivers) with elevated $^{87}Sr:^{86}Sr$ ratios (range between 0.72261±1.03E-04 and 0.71571±1.81E-04), Normandy and Brittany's rivers (Vire, Blavet, Scorff, Aulne, Loire and Vilaine rivers) with lower ratios (range between 0.71160±1.00E-04, 0.71429±1.01E-04), and southern French rivers from Charente River to Nivelle River (range between 0.70823±5.70E-05 and 0.71037±1.22E-04). Ba:Ca and Sr:Ca ratios separated most of the rivers within the three groups with the exception of the Oloron Saison and Nive Rivers which had overlapping elemental and Sr isotopic ratios.

Canonical Discriminant Analysis (CDA) showed strong geographical separation of water samples based on the geochemical signatures in rivers (Fig. 3). The 95% confidence

ellipse around the mean value for each river suggested that the water chemistry did not change significantly over the course of the 2013 sampling season (from late May to September 2013). The first two canonical variates explained more than 80% of the variation within the data (Wilks' lambda = 0.419, $P < 0.01$). Rivers were generally separated along the first canonical variate, with the exception of the Saison, the Nive, the Oloron Rivers, and also, the Vire and the Vilaine Rivers. Canonical structure coefficients indicated the relative importance of the three variables to the separation in geochemical signatures amongst the rivers (Table 1). The first canonical variate was primarily driven by Sr isotopes ratios. Sr:Ca ratios also contributed to separation among rivers on the first canonical variate. Loadings on the second canonical variate were dominated by variation in Sr:Ca ratios with smaller contributions from ^{87}Sr:^{86}Sr ratios. Finally Ba:Ca ratios appeared to contribute moderately to signature separation along the first and second variates. Owing to differences in Sr:Ca, Ba :Ca and ^{87}Sr:^{86}Sr ratios, the cross-validation classification accuracies of rivers based on their geochemical signatures ranged from 80% to 100% (not presented). Among the seventeen rivers, misclassifications occurred in three neighboring rivers (the Oloron, the Saison and the Nive Rivers) and also between the Vilaine and the Vire Rivers. Those results likely arose because of similarities in chemical signatures between rivers as described in the Figure 2.

Determination of adult natal streams

For all samples tested, apparent convergence under the Gelman-Rubin diagnostic was achieved. The Bayesian hierarchical mixture model was used to analyze otolith microchemistry signatures and provided reliable estimates of the proportion of individuals that displayed natal site fidelity or reproduced outside of their watershed of origin, i.e., migrants (supplementary Table S2). Posterior conditional assignment probabilities (i.e. the probability of assignment to each natal river) were higher than 0.80 for 85% of fish, indicating that the majority of adults were assigned to a source river with high degrees of confidence. In

366 the present results, while most individuals seemed to return to their natal watersheds, their
367 fidelity to the natal river within the watershed of origin appeared less precise. The majority of
368 spawners captured in the Scorff River and the Garonne River originated from adjacent
369 spawning tributaries (i.e., the Blavet River and the Dordogne River respectively). Seventeen
370 adults were classified as "undetermined" in supplementary Table S2 indicating that those
371 individuals represent heterogeneous signatures that did not match those from the water and
372 juvenile reference data set. Some individuals captured in the Vilaine River (n = 3), the Loire
373 River (n = 3), the Garonne River (n = 14), the Saison River (n = 3), the Lima River (n = 2)
374 and the Mondego River (n = 11) strayed into non-natal rivers to spawn but originated from
375 neighbouring watersheds (distance between natal and spawning rivers were < 300 km) (Table
376 2). Some nonresident spawning adults captured in the Vire River (n = 34), the Adour
377 watershed (n = 2), the Minho River (n = 1) and the Mondego River (n = 4) travelled long
378 (300-700 km) and ultra-long distances (> 700 km) between natal and spawning river. The
379 Vire River, the Garonne River and the Saison River displayed immigrants originating from
380 the South (Table 2). The Vilaine River, the Loire River, the Adour watershed and all
381 Portuguese Rivers displayed immigrants originating from the North.

382 **Genetic analysis**

383 A total of 287 individuals were genotyped at 13 loci (supplementary Table S3).
384 Significant deviations from HW equilibrium (heterozygote deficiency) were found in the
385 Garonne population both in 2012 and 2013 (supplementary Table S3). Further investigation
386 showed no significant linkage disequilibrium in these populations. Allelic richness ranged
387 from 3.96 to 5.56 and averaged Hnb and Ho values were 0.61 and 0.60 respectively. Global
388 F_{ST} was 0.026 (95%Ci 0.019-0.033, P = 0.0001). 10 out of the 45 pairwise F_{ST} comparison
389 were non-significant with values ranging from -0.0004 to 0.0745 (supplementary Table S4).
390 The Nivelle showed the highest genetic differentiation with other rivers. Genetic

differentiation was similar using D_{ST} (0.032) with pairwise values ranging from -0.0132 to 0.745. The DAPC analysis indicated the existence of 5 genetically distinct groups based on the Bayesian Information Criterion. However, these groups were widely admixed (results not shown). Further investigations showed a significant pattern of isolation by distance ($r = 0.6359$, $P = 0.001$). Geneclass analysis assigned 81 out of 287 individuals at a threshold greater than 90% (Table 3). Individual assignment to their river of sampling was low and also supported a weak population structure and thus high connectivity. In five cases (Aulne, Scorff, Nivelle, Blavet and Vire), the majority of individuals were assigned to their respective sampling river, whereas for the remaining rivers, only few individuals were assigned to their river of sampling.

Coupling otolith microchemistry and genetic markers

The new baseline as defined by otolith natal origins was composed of eight groups representing the major French drainages. These new groups displayed similar level of genetic diversity (Ar, Ho, Hnb) when compared to populations defined according to their sampling site (supplementary Table S5). Levels of F_{IS} were also reduced (supplementary Table S5). Level of genetic differentiation was also similar (Table 4). When using the new baseline 17 other individuals were assigned to a potential group at a 90% probability (Table 5). According to the new baseline, a greater number of individuals were miss-assigned to other rivers than their sampling river except for the Aulne, Nivelle and Vire Rivers where high rate of self-assignment were already observed using the genetic baseline.

Discussion

Variations in water and otolith chemistry

Our analyses of water chemistry of Allis shad natal rivers revealed significant spatial variation from northern France to Portugal. This observed variation among rivers likely arose

from contrasting bedrock geology among respective drainage basins (www.brgm.fr). However, similarities in water chemical signatures were observed in the case of three neighboring rivers from southern France (the Oloron, the Saison and the Nive rivers) and two northern French Rivers (the Vilaine and the Vire rivers), making the discrimination of these rivers more difficult.

It is likely that the otolith signatures measured in the present study had enough stability through time to enable the correct inference from 2013 signatures of the natal origin of fish hatched five or six years before (2007-2008). While previous works have shown that otolith ^{87}Sr:^{86}Sr ratios remained very stable from year to year as reported for American shad (Walther and Thorrold 2009), interannual stability was not well established in the case of trace elements. Both intra-and interannual variability in otolith elemental fingerprints may potentially confound spatial variation (Gillanders 2002). Thus, pooling juvenile otoliths and/or water samples collected from several years was recommended to account for the range of element values likely to be found in the adult cohorts (Walther and Thorrold 2009). Owing to a lack of water chemistry data and no juveniles' collection over the past few years in each study river, we did not know if ambient concentrations of Sr and Ba have fluctuated over years. However, comparison between recent water chemistry (data presented in this paper) and previous sampling years in 2001 (Tomas et al. 2005), in 2010 to 2013 in the Garonne River, the Dordogne River (F. Daverat, unpublished data) and tributaries of the Adour watershed (Martin et al. 2013a) indicated minor variation in Sr and Ba concentrations. This variability was neglectable compared to strong spatial variation in water chemistry among rivers. Moreover, significant interannual variability in otolith element signature was less frequently reported in freshwater species than estuarine or marine species (Hamer et al. 2003; Rooker et al. 2003; Patterson et al. 2004; Bergenius et al. 2005; Patterson et al. 2005; Elsdon and Gillanders 2006; Martin et al. 2013a). It appeared that strontium isotopes in otoliths

mainly drove the allocation of natal origins of Allis shad spawners. To illustrate its importance we re-ran the Bayesian hierarchical mixture model without including $^{87}Sr:^{86}Sr$ ratios. We found that posterior conditional assignment probabilities (i.e. the probability of assignment to each natal river) dropped from 0.80 for 85% of fish to 0.6 for 90% of fish. It indicated that the majority of adults were assigned to a natal river with low degrees of confidence when Sr isotopes ratios were excluded from the model. First, the $^{87}Sr:^{86}Sr$ ratio varies among rivers according to underlying geology (Kennedy et al. 1997; Ingram and Weber 1999; Hobbs et al. 2005) and is temporally stable (Kennedy et al. 2000; Walther and Thorrold 2009; Walther and Limburg 2012). Second, Sr isotopes are not trophically fractionated and the $^{87}Sr:^{86}Sr$ ratios in otoliths closely match that of stream water (Kennedy et al. 2000; Martin et al. 2013a). These properties highlighted the utility of Sr isotope ratios to distinguish natal origins of Allis shad at a local level. Further, given its strong spatial variability, Sr:Ca was also a key ratio for discriminating among natal rivers. Otolith Sr:Ca reflect ambient water composition (Bath et al. 2000), particularly in freshwater systems (Wells et al. 2003). Sr provided another temporally stable signature (Martin et al. 2013a) as its concentration in water is primarily controlled by the weathering of different lithologies and related soils (Rondeau et al. 2005). Therefore, we assumed that the assignation of natal origin using the water and otolith signatures cannot be impaired by minor temporal variability of chemical signatures.

The value of a Bayesian framework

Bayesian tools were especially useful to determine geographical origins of individuals as they can incorporate several sources of uncertainty in a single analysis (Pella and Masuda 2001; Munch and Clarke 2008; Smith and Campana 2010; Pflugeisen and Calder 2013). In our study, we used water composition and otolith juvenile composition as additional sources of information to build the Bayesian hierarchical mixture model. Based on the linear

relationship between water and otolith signatures, the model allowed us to predict otolith chemistry for rivers where juveniles were not available (Pflugeisen and Calder 2013). Our ability to classify natal origins of Allis shad (85% overall accuracy) compared reasonably well with other studies on freshwater natal habitats of anadromous species (Thorrold et al. 1998; Milton and Chenery 2001; Tomas et al. 2005). A key assumption for inferring natal origins by the use of classification-based methods is that the signatures of all potential spawning rivers are known (i.e., exhaustive sampling) (Neubauer et al. 2013). Although we did not sample all potential natal rivers across the distribution range of Allis shad, our sampling was representative of the main spawning sites and likely exhaustive. Unsampled rivers were limited and also exhibited low natural reproductive success for Allis shad (Baglinière et al. 2003). Thus, we did not use the Bayesian models described by Neubauer et al. (2013) which inferred dispersal from incomplete geochemical baselines, taking into account potential un-sampled sources. Our modelling approach assumed that most potential natal rivers have been characterized and that the assignment of individuals to a finite set of sources was non erroneous. Of the 410 individuals analyzed, 96% were assigned to rivers with high probability and only 4% were undetermined indicating that those individuals represented heterogeneous signatures not well established in the database. Those adults of unknown origin were probably from rivers outside the database. Thus, immigration of individuals from those rivers was likely low. Nevertheless, the unidentified origins of those individuals remain to be determined by expanding this work in geographical coverage. Some secondary rivers could deserve to be included in the signature references set to strengthen the power of origin detection such as the Vienne, the Creuse and the Mayenne rivers in the Loire River Basin, the Trieux and the Elorn River in Britany, and in a southerly direction the Sèvre Niortaise River.

Patterns of straying and philopatry

Our genetic and otolith-based analysis suggested different patterns of philopatry and straying behavior between natal and spawning rivers for Allis shad. We found a significant proportion of individuals hatched and grown in a different watershed than the one they were collected from. Even if, the true probability of straying can only be estimated based on the population sizes in each location (Munch and Clarke 2008), that are not available for all the rivers we sampled, our study provided a valuable qualitative view on the dispersal and homing patterns of Allis shad. While *A.alosa* exhibited a high fidelity to the natal site on ecological timescales, as inferred from otolith microchemistry, this species on the other hand showed modest genetic differentiation between collection sites, and followed an isolation by distance pattern, which imply moderate but nevertheless sufficient straying to uniformize the Allis shad population genetic structure. Extensive gene flow between populations of Allis shad at this spatial scale were already suggested in French rivers using allozymes markers (Alexandrino et al. 2006) and mtDNA (Faria et al. 2012). Supporting this high rate of gene flow, genetic assignment success to natal sources remained relatively modest, even when using the otolith natal origin to define new baseline genetic groups. To date, the extent of intraspecific population structure is still unclear over the entire geographic range of Allis shad (Jolly et al. 2012).

In contrast, genetic analyses for *A. sapidissima* (Waters et al. 2000; Hasselman et al. 2013) and geochemical signatures from otolith (Walther et al. 2008) established a higher degree of homing behavior which induced significant but subtle population structuring. Conventional tagging (Melvin et al. 1986; Hendricks et al. 2002) also revealed that more than 90% of American shad spawners return to natal rivers. In our study, some rivers displayed more than 10% of spawners; up to 23% of spawners originated from other watersheds. Such a proportion of immigrants from neighbouring sites may explain the weaker genetic differences between discrete spawning populations in *Alosa alosa* (Wright 1950) as compared to *Alosa*

514 *sapidissima*. In comparison, anadromous salmonids tend to show a greater proportion of
515 homing behavior than *Alosa alosa* (Jonsson and Jonsson 2011).

516 Allis shad site fidelity was not spatially precise, as adults returned to spawn within the
517 river basins of origin rather within the river of origin in the Garonne-Dordogne Basin and
518 Blavet-Scorff Basin. This suggests again, that the imprinting and homing behavior was not as
519 strong in *Alosa alosa* as in other species of anadromous fish such as sockeye salmon (Stewart
520 et al. 2003; Quinn et al. 2006; Quinn et al. 2012). Nevertheless, several authors showed that
521 straying is not spatially random and fish are more likely to stray in adjacent watersheds (with
522 similar environmental settings) than to enter more distant river drainages (Candy and
523 Beacham 2000; Correa and Gross 2008; Hamann and Kennedy 2012).

524 While within our sample, straying occurred more frequently between neighbouring
525 river basins, the lack of genetic structure but significant pattern of isolation by distance along
526 the whole Atlantic coast (at the exception of Nivelle river) also supported the evidence of a
527 significant flow of strayers between river basins in vicinity or at longer distances (two
528 individuals captured in the Adour watershed seemed to originate from the Aulne River in
529 Brittany located 600 km northward). However, if long distance straying is probable it seems
530 not frequent, as we found that five individuals moved from France (natal area) to Portugal
531 (spawning destination), and three individuals from Blavet toward the Vire River. The low
532 probability of long distance straying between Portugal and northern populations was
533 supported by the significant genetic differentiation of Portuguese and French populations
534 (Alexandrino et al. 2006) and the lack of observations of abnormally large bodied shad
535 spawners in the northern part of our study. As a matter of fact, Portuguese shad spawners
536 compared to other populations, achieve a larger size at reproduction, which was attributed to
537 the location of their marine nursery in an upwelling zone (Lassalle et al. 2008b). Furthermore,
538 the IBD patterns suggest that long distance dispersal is limited and is probably stronger

between adjacent rivers. However, even a small number of migrants per generation may be enough to homogenize allele frequencies between populations (Allendorf 1983) and the impact of these few migrating individuals should not be neglected.

As the Vilaine and the Vire water signatures were very close to each other, the assignation of the Vire spawners as Vilaine River origin (n = 31) can be doubtful. Hence, results were interpreted with caution concerning the reallocation of individuals sampled in the Vire River which originated from the Vilaine River. The confusion between the Vilaine and Vire otolith chemical signatures did not allow drawing definite conclusions on the intensity of flow of Allis shad straying from southern Brittany out to the Manche area, even though few spawners originating from Blavet were found in the Vire River.

Whether straying occurs in the early stage of sea nursery phase or when the growth is completed at sea remains unclear. Allis shad were found to inhabit shallow waters (< 150 m) and aggregate at low-salinity areas of coastal regions (river mouths of most important watersheds) (Taverny and Elie 2001). A later study, Trancart et al. (2014), demonstrated that Allis shad populations remained in the same geographic area throughout the year, but the study lacked to be extended to the southern part of the Atlantic coastal area below the Gironde estuary mouth. Lassalle et al. (2008b) also concluded that the marine growing areas are probably located near the natal river. Straying would then occur between the coastal nursery grounds corresponding to different river basins origins. The genetic differences observed between the French and Portuguese Allis shad populations may be due to the lower ability of shad to cross over large coastal distances and deep sea bathymetric barriers such as Capbreton canyon (Bay of Biscay, SW France) (Carlsson et al. 2011). *Salmo salar* disperse as a spawner from a single marine nursery located offshore and in consequence, strayers may originate from rivers far away (Perrier et al. 2011). It seems that a precise homing behavior especially in salmonids that undertake long distance migrations, made the successful evolution of

anadromy possible, by maximizing returns of individuals back to suitable habitats where they were spawned (McDowall 2001). As marine growing areas of Allis shad seem to be located near their natal rivers, the ability to home could be an evolutionary feature of clupeid biology that is not as critical as compared to salmonid species. Moreover, straying may be more frequent for Allis shad compared to other anadromous fish through inadequate juvenile imprinting due to the shorter freshwater growing period (only two months). Twaite shad also has a short freshwater growing period but genetic studies provided strong evidences for a greater segregation of populations compared to Allis shad (Jolly et al. 2012). The marine distribution of *Alosa fallax* is more coastal (water depths < 50 m) than for *Alosa alosa* (Taverny and Elie 2001) and the species is clumped in aggregations around the major catchments for reproduction, which could be consistent with a higher fidelity to spawning grounds and/or limited migration during the adult phase.

In a context of global change, straying would be a key mechanism to drive dispersal and allow resilience of Allis shad populations. Such behavioral diversity may be critical to long-term persistence of the species by linking local populations that independently would be vulnerable to extinction or by refunding those that do go extinct (Schlosser and Angermeier 1995; Rieman and Dunham 2000; Rieman and Allendorf 2001). The recovery of a depleted population relies on the size and proximity of neighboring populations, as well as on the proportion of immigrants that contribute to the depleted population (Hamann and Kennedy 2012; Keefer and Caudill 2014). Our result suggested that a river may act as a source of spawners when the other rivers had experienced a lower recruitment success. The disproportionate contribution of the Dordogne River and the Blavet River to the adult population entering both river basins may be due to fish in these habitats having more successful recruitment to the adult population. Similarly, Minho River provided a source of migrants into neighbouring rivers (i.e., the Lima and Mondego Rivers). Whether the provision

589 of spawners may be sufficient to restore a population remains doubtful when habitats may not
590 be suitable for juveniles. The Minho River is the only Portuguese River which has free access
591 to suitable spawning grounds for Allis shad since the first downstream barrier is located far
592 from river mouth (Mota and Antunes 2012). In the Lima and Mondego Rivers, dams blocked
593 access to historical spawning reaches, causing severe fish population declines. In the specific
594 case of Dordogne-Garonne river basin, within our entire sample, no spawner with a Garonne
595 otolith signature was identified for two consecutive years (2012 and 2013) suggesting either
596 that the survival of juveniles or the spawning activity in the Garonne river five years earlier
597 might have been critical. Despite the immigration of Dordogne and other river spawners into
598 the Garonne River, no sign of population recovery could be observed. A significant F_{IS} value
599 was observed for the Garonne population in both 2012 and 2013, suggesting a Wahlund
600 effect. This effect suggests that the depleted populations may not be sufficiently supported by
601 straying itself. The depletion of Allis shad populations along the Atlantic coast may not allow
602 the recovery of specific stocks if an Allee effect is confirmed for this species (Rougier et al.
603 2012).

604 Lassalle et al. (2009), showed that under global warming the environment would be
605 more favorable for shad towards the north of its distribution area. Our results did not conclude
606 on the predominance of a northward dispersal of Allis shad with more strayers going
607 northward. However, the recent increase of spawners abundance in the Vire River at the
608 northern part of our sampling, may be the combined result of a larger abundance of strayers
609 and a more successful recruitment. Thus the analysis of straying data together with data on the
610 relative abundance of river populations would bring insight on a northward predominance of
611 dispersal. This suggests a possibility for the stock to regain its historical distribution area by
612 recolonizing rivers of the North of Europe, such as the Seine or the Rhine, providing that their
613 longitudinal connectivity and spawning habitats are accessible, suitable and/or restored.

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Table captions

Table 1. Canonical coefficients for the first two canonical variates (CV) performed on elemental and Sr isotopic ratios in water samples.

Table 2. Distance travelled by Allis shad strayers between natal and spawning river measured by a straight-line between the two river mouths. Distance classes were: short (20-100 km), Middle (100-300 km), Long (300-700 km) and Ultra long (> 700 km).

Table 3: Number of individuals from each river assigned to a sampling river. All individuals (n = 287) were used in the baseline and in assignment tests. Only individuals with P > 90% were reported here.

Table 4: Pairwise F_{ST} values between Allis shad populations (populations defined by otolith natal origin).

Table 5: Number of individuals from each river assigned to the otolith baseline. Only known origin individuals inferred from otolith chemistry (n = 275) were used in the baseline. All individuals (n = 287) were used in assignment tests. Only individuals with P > 90% were reported here.

975 # Figure captions

976

977 Fig. 1. Picture of adult Allis shad otolith showing the two areas ablated by the laser prior to
978 ICP-MS (right semi corona) and MC-ICP-MS analysis (left semi corona). Semi coronas of 60
979 µm thick (difference between the inner 40 µm and outer radius 100µm) are centered on the
980 primordium and correspond to the juvenile freshwater period of growth only.

981

982 Fig. 2. Box plot of mean (a) Sr:Ca, (b) Ba:Ca, and (c) $^{87}Sr:^{86}Sr$ ratios of water samples from
983 the sampling locations. Abbreviations are used for three French rivers: Adour river (Adour
984 R.), Garonne river (Gar.), and Dordogne river (Dord.). Interquartile ranges (25th and 75th
985 percentile) are shown by extent of boxes, and horizontal lines represent medians (50th
986 percentile). Whiskers range from 10th to 90th percentiles, and values outside this range are
987 plotted with circles.

988

989 Fig. 3. Canonical discriminant plot of isotope ($^{87}Sr:^{86}Sr$) and elemental (Sr:Ca and Ba:Ca)
990 signatures from water samples collected from 17 rivers in 2013. Symbols represent water
991 samples, and ellipses are 95% confidence intervals around each group. Grid scale is given (d
992 = 1).

Ratios	CV1	CV2
$^{87}Sr:^{86}Sr$	-0.880	0.362
Sr:Ca	0.151	-0.518
Ba:Ca	0.034	-0.205

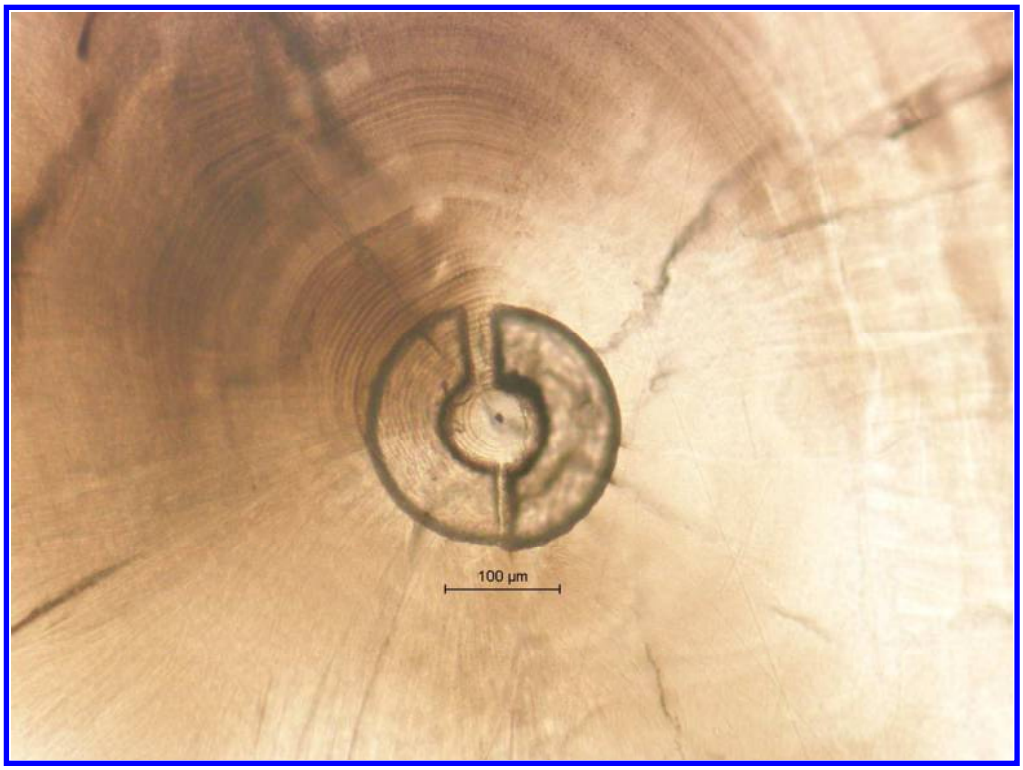
Collection site	Individuals displaying northward movements	Individuals displaying southward movements	Distance between natal and spawning river			
			Short	Middle	Long	Ultra Long
Vire (34)	34 (100%)				34	
Aulne (12)						
Scorff (10)						
Blavet (7)						
Vilaine (19)		3 (16%)	3			
Loire (28)		3 (11%)	3			
Dordogne (71)						
Garonne (64)	14 (22%)			14		
Adour R. (6)		1 (17%)			1	
Adour E. (31)		1 (3%)			1	
Saison (6)	3 (50%)		3			
Nivelle (16)						
Minho (87)		1 (1%)				1
Lima (4)		2 (50%)	2			
Mondego (15)		15 (100%)		11		4

	Reference populations based on geographic origins									
River	Adour2013	Aulne2013	Dordogne2013	Blavet2013	Loire2013	Nivelle2009	Scorff2013	Vire2013	Garonne2012	Garonne2013
Adour (46)	9 (20%)					1				
Aulne (14)		9 (64%)								
Dordogne (69)			1 (1.4%)							
Blavet (17)				8 (47%)						
Loire (24)					8 (33%)					
Nivelle 2009 (17)						16 (94%)				
Scorff (10)							6 (60%)			
Vire (29)								17 (58%)	1	
Garonne 2012 (25)									4 (16%)	
Garonne 2013 (36)										1 (3%)

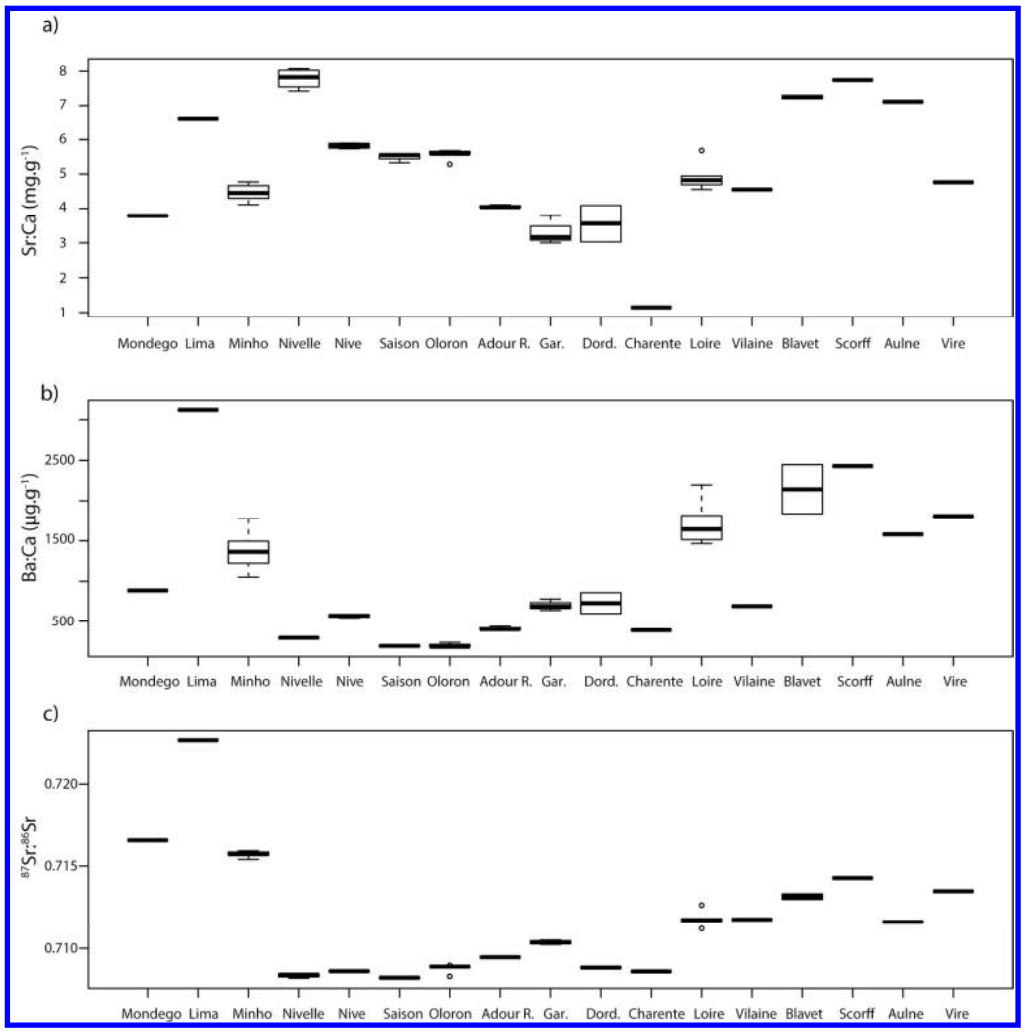
	Aulne	Blavet	Dordogne	Loire	Vilaine	Nivelle	Oloron
Adour	0.0354	0.0151	***0.0053***	***0.0131***	0.0305	0.036	***0.0037***
Aulne		***0.006***	0.0414	0.0415	0.0562	0.0447	0.035
Blavet			0.0278	0.0244	0.026	0.0409	0.0305
Dordogne				0.0109	0.027	0.0476	***0.017***
Loire					0.0372	0.0443	***0.0192***
Vilaine						0.0473	0.0588
Nivelle							0.0399

In bold-italic: non-significant pairwise F_{ST} value after Bonferroni correction.

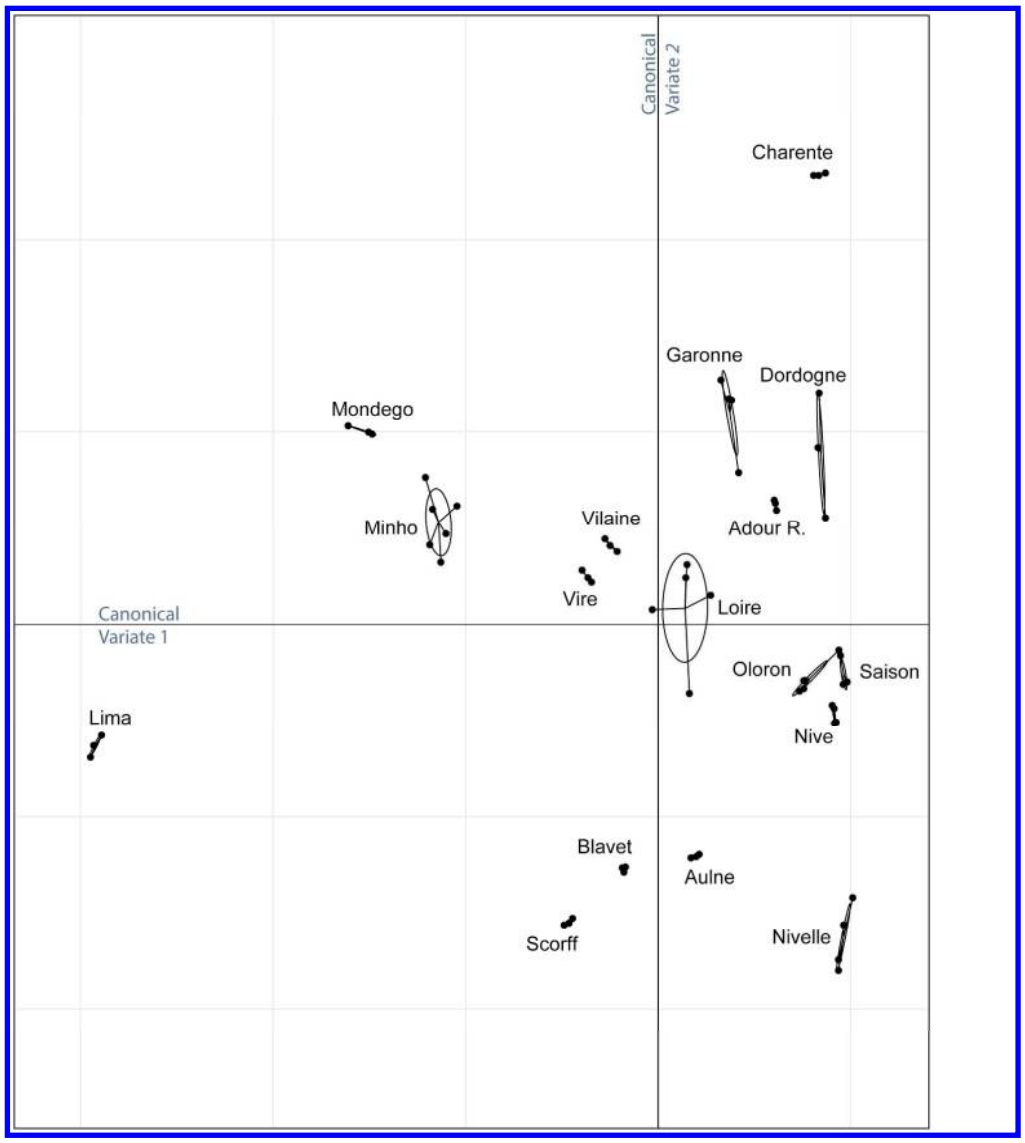
	Reference populations based on otolith natal origin							
River	Adour R.	Oloron	Aulne	Dordogne	Blavet	Loire	Nivelle	Vilaine
Adour (46)	6 (13%)	9 (20%)	1				1	
Aulne (14)		1	9 (64%)		1			
Dordogne (69)	1	4 (6%)		3 (4%)		2		1
Blavet (17)					7 (41%)			
Loire (24)						11 (46%)		
Nivelle 2009 (17)							16 (64%)	
Scorff (10)					2			1
Vire (29)								18 (62%)
Garonne 2012 (25)								
Garonne 2013 (36)		1		5 (14%)				



Picture of adult Allis shad otolith showing the two areas ablated by the laser prior to ICP-MS (right semi corona) and MC-ICP-MS analysis (left semi corona). Semi coronas of 60 µm thick (difference between the inner 40 µm and outer radius 100µm) are centered on the primordium and correspond to the juvenile freshwater period of growth only.
484x361mm (72 x 72 DPI)



Box plot of mean (a) Sr:Ca, (b) Ba:Ca, and (c) 87Sr:86Sr ratios of water samples from the sampling locations. Abbreviations are used for three French rivers: Adour river (Adour R.), Garonne river (Gar.), and Dordogne river (Dord.). Interquartile ranges (25th and 75th percentile) are shown by extent of boxes, and horizontal lines represent medians (50th percentile). Whiskers range from 10th to 90th percentiles, and values outside this range are plotted with circles.
192x194mm (300 x 300 DPI)



Canonical discriminant plot of isotope (87Sr:86Sr) and elemental (Sr:Ca and Ba:Ca) signatures from water samples collected from 17 rivers in 2013. Symbols represent water samples, and ellipses are 95% confidence intervals around each group. Grid scale is given (d = 1).
239x267mm (300 x 300 DPI)