Genetic diversity of brown trout introduced in Japan

Report **JPNTROUT2 (step 2)**
October 2017

Mamashi (up) and Azusa (down) brown trout © K. Hasegawa

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1. Introduction

In Japan, the first introduction of brown trout is probably due to its erroneous presence among eggs of rainbow trout or brook trout from USA before 1900 (1877 according to Maruyama et al. 1987; 1892 according to Elliot 1989). The population of Lake Chuzenji in Nikko city, Tochigi prefecture (about 100km north of Tokyo) should be one of the oldest brown trout population in Japan (there is no official record, but it was maybe introduced around 1900's), and other brown trout in Japan are thought to be introduced via Lake Chuzenji.

Other introductions have been recorded later (see report JPNTROUT1).

The present step 2 report aims at analyzing and interpreting Japanese trout samples genotypes by adding four new samples (see Table 1).

In the step 1 report (JPNTROUT1), the first results can be summarized into five items:

i) Japanese introduced brown trout are not homogeneous, corresponding to several distinct introductions of different origins.

ii) The genetic geographic structure opposes Azusa trout to the remaining populations.

iii) While Azusa basin has been stocked by only one homogeneous strain, the differentiation of Kane, Odori and Lake Chuzenji trout populations can be due to different strains introduced or to long time of isolation after a common introduction.

iv) The Chuzenji hatchery releases are not found in any sampled population. The genetic diversity is very low. The brown trout in the scientific fish hatchery has been bred for more than 30 years without input of any fish from other populations. These fish has not been used for stocking, not even into the lake.

v) Several populations have high genetic diversity (Mamachi, Kane and Odori streams, Lake Chuzenji) while Azusa River numerous samples miss genetic diversity.

2. Sampling

The sampling was done by the Japanese part of the consortium. The characteristics of all analyzed trouts are given Table 1. Their geographic location is represented in Figure 1. Each river was given a map number between 1 and 19. Samples 16 to 19 are the new ones justifying the second step of the survey.

The new samples arrived in Montpellier the 21 June 2017. Genotyping were done the 30 August 2017.

Map	Station	N	date	Medium	Basin	ISEM N° of station	Report
1	Mamachi stream	25	03/09/2012	River	Ishikari (W)	F514	JPNTROUT1+JPNTROUT2
2	Monbetsu stream	25	03/09/2012	River	Ishikari (W)	F515	JPNTROUT1+JPNTROUT2
3	Jigoku (Lake Chuzenji)	22	05/01/2017	Lake	Tone (E)	F528	JPNTROUT1+JPNTROUT2
4	Chuzenji hatchery	25	02/12/2016	Hatchery	Tone (E)	F516	JPNTROUT1+JPNTROUT2
5	Kane stream	25	11&12/2016	River	Fuji (E)	F517	JPNTROUT1+JPNTROUT2
6	Odori stream	24	17/11/2016	River	Jinzu (W)	F518	JPNTROUT1+JPNTROUT2
7	Azusa (Kamikouchi) without name 1	24	26/06/2013	River	Shinano (W)	F519	JPNTROUT1+JPNTROUT2
8	Azusa (Kamikouchi) without name 2	18	27/06/2013	River	Shinano (W)	F520	JPNTROUT1+JPNTROUT2
9	Azusa (Kamikouchi) without name 3	19	25/09/2013	River	Shinano (W)	F521	JPNTROUT1+JPNTROUT2
10	Azusa (Kamikouchi) Zenrokusawa stream	1	06/11/2013	River	Shinano (W)	F522	JPNTROUT1+JPNTROUT2
11	Azusa (Kamikouchi) Shimizusawa stream	3	06/11/2013	River	Shinano (W)	F523	JPNTROUT1+JPNTROUT2
12	Azusa (Matsumoto city)	7	01/05/2008	River	Shinano (W)	F524	JPNTROUT1+JPNTROUT2
13	Azusa (Matsumoto city) YOY	13	5to11/2008	River	Shinano (W)	F525	JPNTROUT1+JPNTROUT2
14	Azusa (Matsumoto) Toyoshina	9	20/11/2016	River	Shinano (W)	F526	JPNTROUT1+JPNTROUT2
15	Azusa (Matsumoto) Shimauchi	21	25/11/2016	River	Shinano (W)	F527	JPNTROUT1+JPNTROUT2
16	Hekirichi stream	25	2013	River	Hekirichi (E)	F551	JPNTROUT2
17	Shiriuchi stream	25	2013	River	Shiriuchi (E)	F552	JPNTROUT2
18	Shizunai stream	25	2013	River	Shizunai (E)	F553	JPNTROUT2
19	Torizaki stream	25	2013	River	Torizaki (E)	F554	JPNTROUT2
20	Lees Athas hatchery	20	11/02/2014	Hatchery	French Dept. 64	L443	PA2
21	Babeau hatchery (Cauterets 2014 strain)	28	16/12/2014	Hatchery	French Dept. 34	L556	MAE
22	Isère hatchery	30	2008	Hatchery	French Dept. 38	L266	GSALM2

Table 1: *Characteristics of the Japanese samples considered. In column 1, new samples are in red and French hatcheries references in black. In column "Basin", E = flowing to Pacific Ocean; W = flowing to Sea of Japan.*

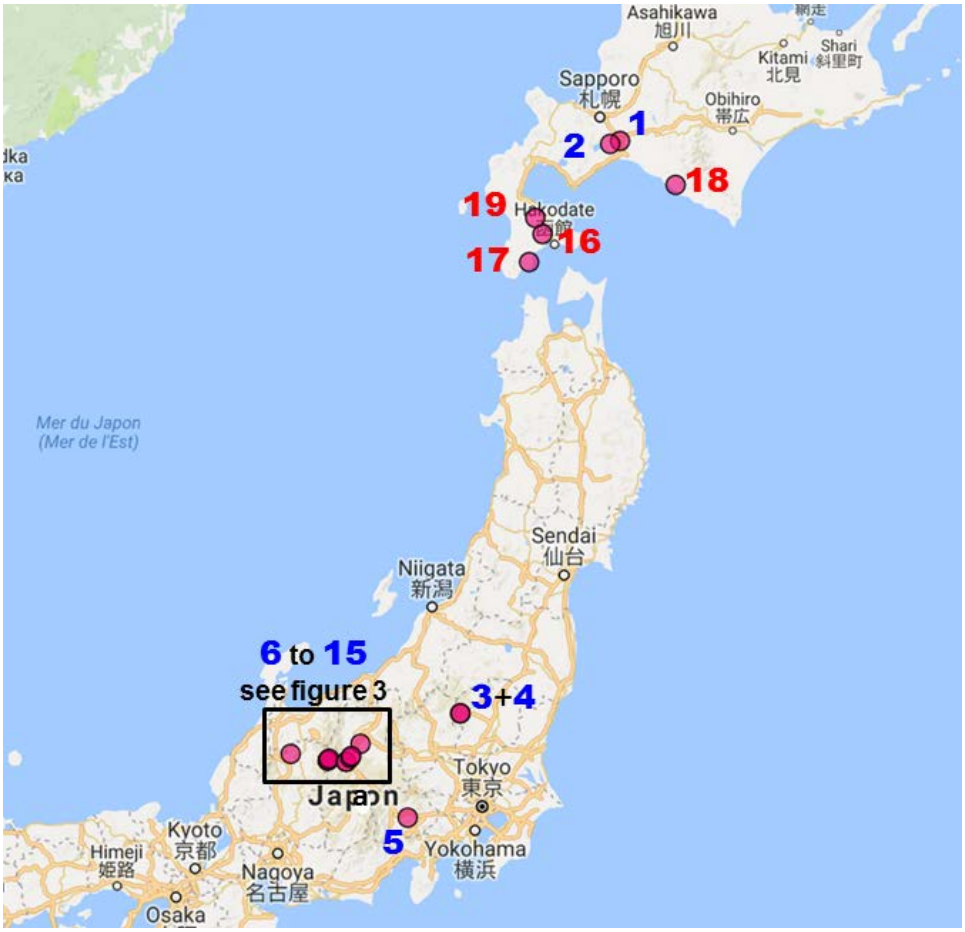

Figure 1: *Geographic position of the 19 Japanese samples analyzed. Numbers in red: the new samples.*

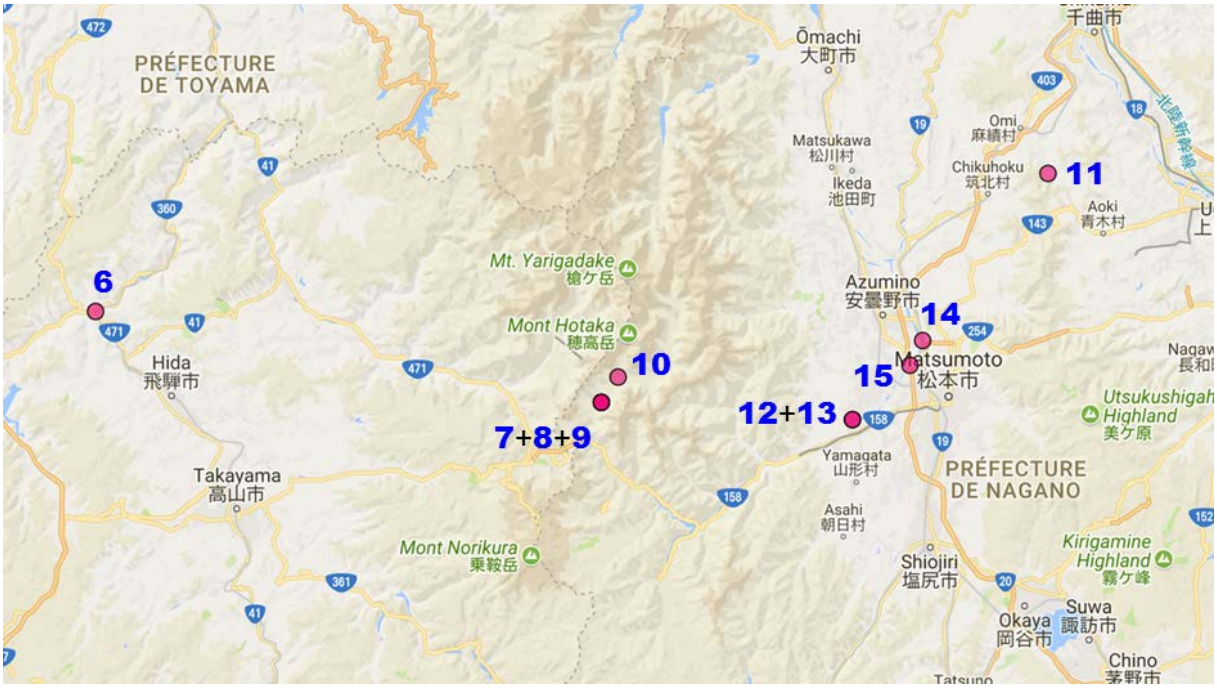

Figure 2: *Precisions on the positions of samples 6 to 15.*

3. Methods

The detailed methodology can be found in the report JPNTROUT1. Here, only the main features are reminded.

A set of 12 nuclear microsatellite loci were genotyped (*Mst543, MST85, Omm1105, Omy21Dias, Oneμ9, Sfo1, Ssa197, SsoSL311, SsoSL438, SsoSL417, Str591* and *StrBS131*) in order to constitute the data matrix.

A Factorial Correspondence Analysis allowed positioning each trout in a hyperspace according to its 24 alleles. Clusters (clouds) detected on the diagram correspond to homogeneous genetic lineages, independently of their geographic origin.

Assignment tests, using the Bayesian STRUCTURE 2.1 program, subdivided the whole sample into K = 2 to 15 subgroups characterized by the best genetic equilibrium in terms of panmixia and lower linkage. The estimation of the best K value (number of biological subgroups in the entire sample) was approached using the "Delta K method" of Evanno et al. (2005)

Population parameters were also calculated. Panmixia (all members of a sample are crossing at random) was estimated through Fis parameter. Genetic diversity was also tested through several methods. Differentiation between samples was estimated using the Fst parameter.

4. Results

4.1. Multidimensional pictures

This method, the Factorial Correspondent Analysis or FCA, places each trout in a diagram according to all its genetic components. Because based on the Khi-2 distances, it favors the exceptions in respect to the Gaussian distribution of the variables.

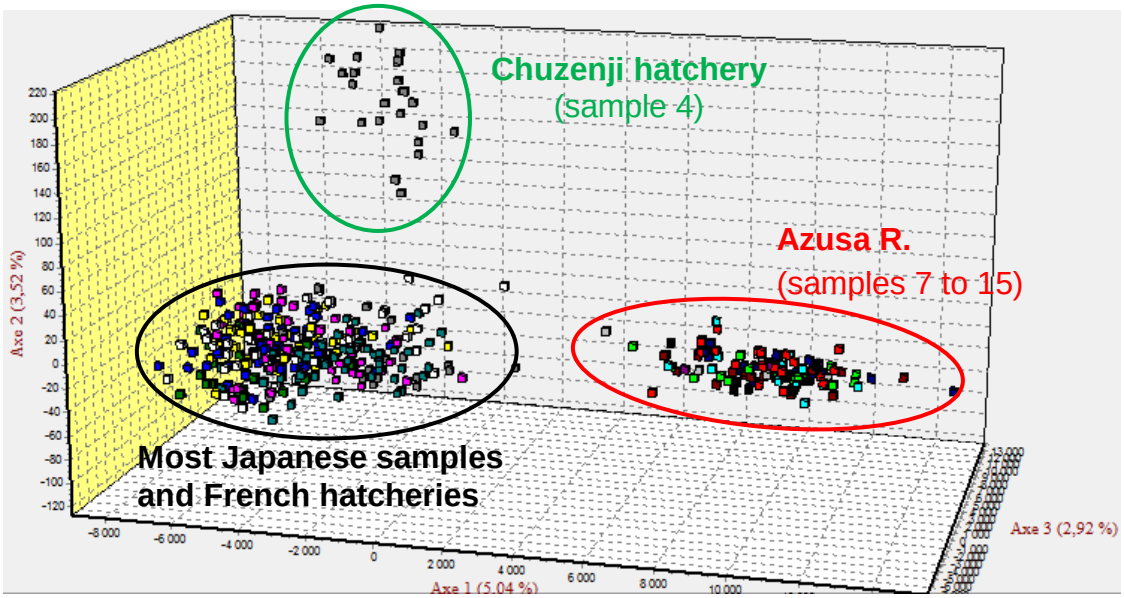

Figure 3: *This diagram represents all the analyzed individuals clearly forming three clouds (or clusters).*

In Figure 3, most samples are clustered in the black ellipse, in a way very similar to the Figure 5 of the report JPNTROUT1. At this scale, there is a similitude between the French hatcheries and the dominant lineage in Japan.

The second step consists in removing the Chuzenji hatchery (mostly experimental) and the Azusa very homogeneous samples, in order to have a better resolution of the black ellipse.

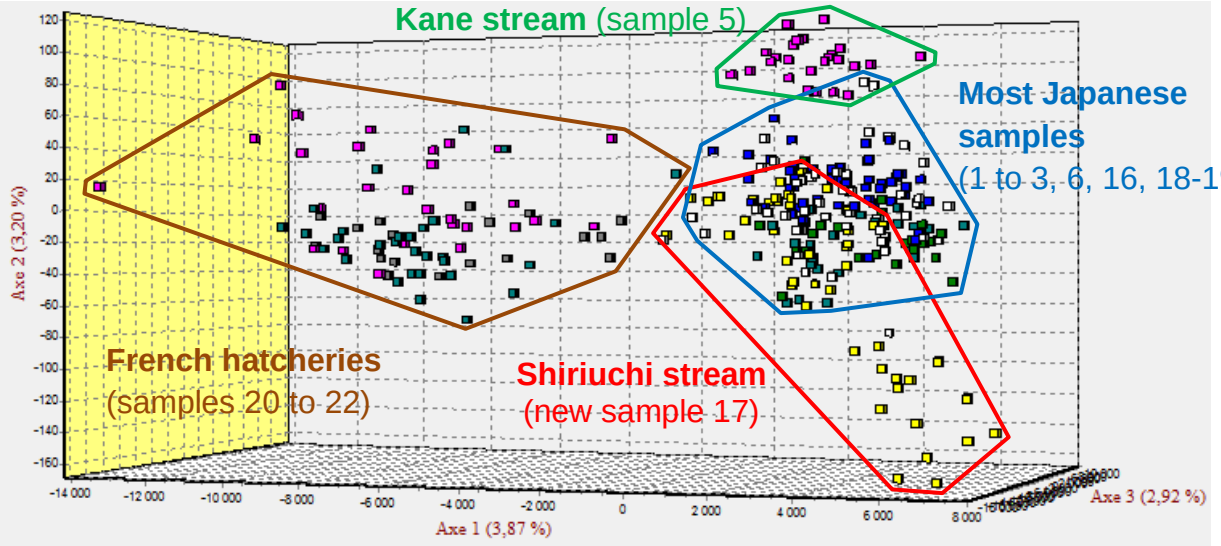

Figure 4: *Inside the black ellipse of the Figure 3, French hatcheries are clearly separated; the remaining rivers samples constitute a dominant cluster (blue polygon) and two partly differentiated populations: those of Kane stream and Shiriuchi new sample.*

4.2. Assignment structure

Assignment tests are powerful methods able find coherent clusters of individuals independently to their origin. The previous report highlighted the clear distinction between Japanese trout and French hatchery ones, plus the distinction between the Azusa trouts, the Chuzenji hatchery strain and the remaining sampling all around the country.

Here, several river samples have been added in addition to all the samples analyzed in JPNTROUT1.

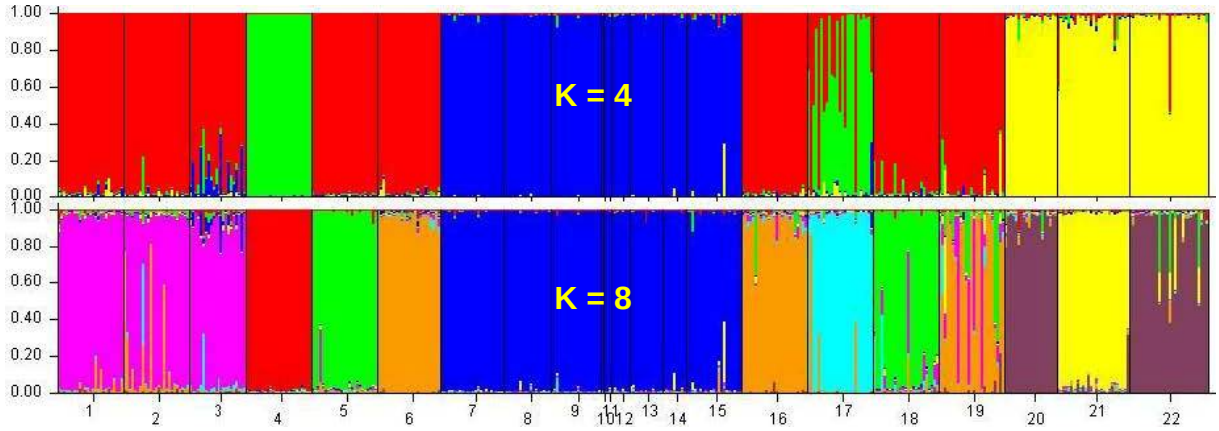

Figure 5: *STRUCTURE output for K=4 and K=8 in order to compare with the results of the report JPNTROUT1.*

Figure 5 gives two histograms produced by the assignment software STRUCTURE for K=4 and K=8 in order to compare with the results obtained for the report JPNTROUT1. In fact, the Evanno method designated K=2, then 5, then 7, then 9, then 11, in this order, as the best partitions. Samples 16 to 19 are new and according to K=4, Shiriuchi stream seems close to the Chuzenji experimental hatchery (but that link disappears for K=8). The remaining samples gather into 1, 2, 3 / 5, 18 / 6, 16, 19 / 17, but this is only slightly comparable to the JPNTROUT1 analysis indicating that these sub-groupings describe very weak structures and should be taken with care.

> *Remark: there is a possibility to found a better partition among what has been called the "dominant group". New methods said "hierarchical assignment" can be tested.*
>
> *This time-consuming tentative is an obligation for the next step: publishing the results in an international journal.*
>
> *There are two results to reconsider: in Figure 3, French hatcheries are clustered with the dominant Japanese brown trout lineage and in Figure 5, for K=4, Shiriuchi stream is close to the Chuzenji experimental hatchery. These similarities must be explained, especially since they are not confirmed in the next step (respectively Figures 4 and 5 with K=8).*

Only the partition K=4 is detailed in terms of percentages in the Table 2. It describes the main structure of the sampling analyzed.

Map	Station	N	dominant group	Shuzenji hatchery	Azusa basin	Atlantic French domestic
1	Mamachi stream	25	96	1	1	2
2	Monbetsu stream	25	97	1	1	1
3	Jizoku (Lake Chuzenji)	22	86	4	9	1
4	Chuzenji hatchery	25	0	99	0	0
5	Kane stream	25	98	1	0	1
6	Odori stream	24	97	1	1	1
7	Azusa (Kamikouchi) without name 1	24	0	1	99	0
8	Azusa (Kamikouchi) without name 2	18	0	0	99	0
9	Azusa (Kamikouchi) without name 3	19	0	1	99	0
10	Azusa (Kamikouchi) Zenrokusawa stream	1	0	0	99	0
11	Azusa (Kamikouchi) Shimizusawa stream	3	0	1	99	0
12	Azusa (Matsumoto city)	7	0	1	99	0
13	Azusa (Matsumoto city) YOY	13	0	1	99	0
14	Azusa (Matsumoto) Toyoshina	9	0	0	99	1
15	Azusa (Matsumoto) Shimauchi	21	1	1	97	2
16	Hekirichi stream	25	98	1	1	1
17	Shiriuchi stream	25	27	69	2	2
18	Shizunai stream	25	95	4	1	1
19	Torizaki stream	25	94	2	1	3
20	Lees Athas hatchery	20	1	1	1	97
21	Babeau hatchery (Cauterets 2014 strain)	28	3	1	1	95
22	Isère hatchery	30	2	1	0	97

Table 2: *Percentage of each lineage detected by assignment in each sample for K=4 (the colors of the headings correspond to that of the Figure 5, upper histogram). Orange cells show dominant lineage of each sample. A frequency of 0.05 and below is considered as not significant.*

4.3. Population parameters

It is considered that more a population is polymorphic, more it can overcome future modifications (anthropization, global warming…). The best descriptor of genetic diversity is the Hnb parameter (non-biased heterozygosity, because pondered according to the sample size): see Table 3.

Map	Station	N	Hnb	Ho	A	Fis	signif.
1	Mamachi stream	25	0.6607	0.6563	5.5833	0.00696	ns
2	Monbetsu stream	25	0.5889	0.5817	4.7500	0.01261	ns
3	Jizoku (Lake Chuzenji)	22	0.7086	0.6854	6.3333	0.03340	ns
4	Chuzenji hatchery	25	0.3905	0.4267	2.2500	-0.09480	ns
5	Kane stream	25	0.6541	0.6800	4.0000	-0.04037	ns
6	Odori stream	24	0.6127	0.5859	4.6667	0.04464	ns
7	Azusa (Kamikouchi) without name 1	24	0.4779	0.4618	3.7500	0.03441	ns
8	Azusa (Kamikouchi) without name 2	18	0.4643	0.4679	3.8333	-0.00792	ns
9	Azusa (Kamikouchi) without name 3	19	0.4354	0.4474	3.0833	-0.02828	(*)
10	Azusa (Kamikouchi) Zenrokusawa stream	1	0.7273	0.7273	1.7273	0.00000	***
11	Azusa (Kamikouchi) Shimizusawa stream	3	0.5833	0.5278	2.7500	0.11628	ns
12	Azusa (Matsumoto city)	7	0.4644	0.4802	2.8333	-0.03596	ns
13	Azusa (Matsumoto city) YOY	13	0.4951	0.5385	3.7500	-0.09150	ns
14	Azusa (Matsumoto) Toyoshina	9	0.4670	0.4213	3.5000	0.10297	ns
15	Azusa (Matsumoto) Shimauchi	21	0.4509	0.3974	4.5000	0.12127	*
16	Hekirichi stream	25	0.6837	0.7129	6.1667	-0.04368	(*)
17	Shiriuchi stream	25	0.6349	0.5667	4.0000	0.10946	*
18	Shizunai stream	25	0.5967	0.6133	4.9167	-0.02853	(*)
19	Torizaki stream	25	0.6828	0.6567	6.3333	0.03902	ns
20	Lees Athas hatchery	20	0.6355	0.5500	4.9167	0.13755	ns
21	Babeau hatchery (Cauterets 2014 strain)	28	0.7765	0.7629	8.0833	0.01782	ns
22	Isère hatchery	30	0.6705	0.6694	6.0833	0.00164	ns

Table 3: *The population parameters describe the samples genetic diversity (green columns titles) and their panmictic equilibrium (in pink). Orange cells designate the highly polymorphic estimations, the yellow one the moderate polymorphic ones. Grey cells highlight the very low polymorphism.*

*Panmixia is globally respected. * and *** are levels of departure-from-zero significance. Ns = not significant = respects the panmixia. Very small letters indicate the too small samples.*

Map	Station	N	1	2	3	4	5	6	7	8	9	12	13	14	15	16	17	18	19	20	21	22
1	Mamachi stream	25	0	0.09989	0.08475	0.32144	0.17348	0.14896	0.23127	0.23112	0.22696	0.19660	0.20226	0.21311	0.24995	0.10197	0.15192	0.17817	0.09936	0.12509	0.10898	0.10968
2	Monbetsu stream	25		0	0.12814	0.37267	0.17068	0.13700	0.25314	0.25943	0.26549	0.25029	0.25713	0.24595	0.26831	0.07992	0.16468	0.16031	0.09353	0.15357	0.14421	0.13684
3	Jizoku (Lake Chuzenji)	22			0	0.29428	0.16783	0.15614	0.20421	0.21095	0.21061	0.16755	0.18141	0.19703	0.22121	0.11410	0.12909	0.15644	0.12403	0.14075	0.09069	0.13807
4	Chuzenji hatchery	25				0	0.34014	0.37959	0.43465	0.46381	0.46499	0.45462	0.45178	0.47464	0.46457	0.35641	0.35301	0.37956	0.33395	0.38772	0.30898	0.36011
5	Kane stream	25					0	0.20000	0.30922	0.31008	0.32645	0.29565	0.29689	0.29993	0.31712	0.15485	0.21486	0.17786	0.13764	0.20588	0.12902	0.17672
6	Odori stream	24						0	0.26328	0.26083	0.27815	0.25765	0.27080	0.24547	0.27636	0.07865	0.17099	0.21095	0.08547	0.16102	0.15027	0.15650
7	Azusa (Kamikouchi) without name 1	24							0	0.00367	0.01197	0.08564	0.06618	0.03696	0.04895	0.24289	0.28847	0.29043	0.25832	0.23583	0.21507	0.23140
8	Azusa (Kamikouchi) without name 2	18								0	0.02578	0.10393	0.07546	0.03001	0.04677	0.23694	0.29461	0.30366	0.25664	0.24203	0.21719	0.22449
9	Azusa (Kamikouchi) without name 3	19									0	0.09417	0.06089	0.06753	0.07960	0.25331	0.29098	0.31722	0.25846	0.23664	0.22052	0.23106
12	Azusa (Matsumoto city)	7										0	-0.00031	0.03006	0.03191	0.22935	0.25907	0.30062	0.25184	0.19824	0.17049	0.20257
13	Azusa (Matsumoto city) YOY	13											0	0.02904	0.04058	0.23683	0.28176	0.31130	0.25406	0.19691	0.17681	0.19928
14	Azusa (Matsumoto) Toyoshina	9												0	-0.00154	0.22084	0.29638	0.30696	0.24316	0.21634	0.19448	0.20432
15	Azusa (Matsumoto) Shimauchi	21													0	0.24684	0.30729	0.32453	0.27419	0.23997	0.21907	0.22908
16	Hekirichi stream	25														0	0.12651	0.14859	0.05370	0.12859	0.11566	0.10995
17	Shiriuchi stream	25															0	0.20149	0.14093	0.18504	0.13711	0.18416
18	Shizunai stream	25																0	0.15564	0.18772	0.17532	0.18008
19	Torizaki stream	25																	0	0.12726	0.11076	0.10513
20	Lees Athas hatchery	20																		0	0.10422	0.03347
21	Babeau hatchery (Cauterets 2014 strain)	28																			0	0.08941
22	Isère hatchery	30																				0

Table 4: *Fst estimation between samples of 7 individuals at least (samples n°10 and 11 have been removed because too small). White cells designate significantly different compared samples (***). Yellow cells correspond to slightly different samples (** and *). Green cells are not-significant differentiation (soft green are not significant after Bonferroni correction only). Samples considered as identical (not significant differences) are observed mainly between Azusa River populations and between small Azusa samples (n°12=7 and n°14=9 and other ones.*

5. Interpretation and discussion

This is the second step of molecular analysis on brown trout populations stemming from ancient introductions in Japan. For this, four new samples from the south of Hokkaido Island have been added to the 15 former ones.

According to the first report JPNTROUT1:

i) Japanese introduced brown trout are not homogeneous, probably a consequence of several introductions.

ii) There is no geographic logic in the samples clustering, opposing Azusa trout to the remaining populations.

iii) The Chuzenji hatchery (sample 4) breeds a very distinct strain for scientific purposes for more than 30 years without input of any fish from other populations.

vi) Several populations have high genetic diversity (Mamachi, Kane and Odori streams, Lake Chuzenji) while Azusa River numerous samples miss polymorphism.

The analysis of the new four samples does not modify this general observation with one exception in the Shiriuchi stream (sample 17).

The scientific strain of the Chuzenji hatchery have probably be used in the Shiriuchi stream according to the assignment analysis given in Figure 5 for K=4. In this river, the dominant lineage and the Chuzenji strain are respectively estimated at about 30/70%.

The remaining 3 samples clearly belong to the dominant lineage introduced in Japan.

6.1. Genetic diversity

The four new samples are highly polymorphic, as was the Mamachi, Kane and Odori rivers and the Lake Chuzenji free population. This diversity is similar to that of the domestic French strains considered as highly polymorphic. This observation is opposed to the clearly too low polymorphism observed on the brown trout living in Azusa River.

The present report is called "step 2". A third step should need a complete description of European hatcheries in order to detect the origins of dominant and Azusa lineages established in Japan.

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7. References

Maruyama T., Fujii K., Kijima T., Maeda H.1987. Introduction of foreign fish species into Japan. Fisheries Agency of Japan. (in Japanese)

Elliott J.M. 1989. Wild brown trout *Salmo trutta*: an important national and international resource. Freshwater Biology 21:1-5.

Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Berrebi P., Cherbonnel C. 2009. Cartographie génétique des populations sauvages de truites françaises - Programme Genesalm - tome 1 - version du 15 décembre 2009. Rapport GSALM2. In: Université Montpellier 2, rapport de contrat du projet Genesalm, 22p.

Berrebi P., Schikorski D., Masseboeuf F. 2015. Composition génétique des truites de la Bidouze - Étude complémentaire au rapport Genetrutta de 2013 dans les Pyrénées-Atlantiques - Rapport PA2. In: Rapport d'analyse pour la FD64, Université de Montpellier, p. 9p.

Berrebi P. 2015 Structure génétique des truites naturelles et domestiques de l'Orb amont -marqueurs microsatellites- Rapport MAE1 (juin 2015). In: Rapport d'étape pour la thèse de Maëva Leitwein. Université de Montpellier. 9p.

Hasegawa K., Schikorski D., Berrebi P. 2017. Genetic diversity of brown trout introduced in Japan. Report JPNTROUT step1. April 2017. In: Université Montpellier 2, 17p.