

Lake Megali Prespa basin trout biodiversity
Genetic structure of *Salmo peristericus*
sampling of 2005 and 2007

-
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1. Introduction

The Lake Prespa trout, mainly living in its tributaries, is considered as an endemic species, *Salmo peristericus*, not included in the *Salmo trutta* complex. This is a first hypothesis to test using molecular markers.

This species is living in four main rivers flowing into the lake, three in Macedoine (Goluma, Kranska and Brajcinska) and one in Greece (Agios-Germanos).

The conservation of this species needs biological knowledge that population genetics can partly provide. By analysing the nuclear DNA of these trout, it is possible to know if the different tributaries of the lake host similar populations or not. If a differentiation is observed among rivers, a separate management, river by river, can be necessary.

Another important information is the relative level of polymorphism which is dependant to the efficient size of the population and the existence of an eventual recent bottleneck. This parameter is considered as an image of the sensibility of the population to anthropic modification: with a lower polymorphism, a population is thought to resist less to ecological changes.

2. Sampling

In order to answer to these questions, a large sample of 533 trouts have been constituted during the 2006 and 2007 summers. It is composed of 2 individuals from the lake, 59 from Leva Reka River (Goluma basin), 103 from Kranska River, 250 from Brajcinska basin and 119 from Agios Germanos basin.

Each river have been sampled in several sites and at several dates. The table 1 gives this information in details.

All these fishes were anaesthetized, photographed and a small piece of fin conserved in 95° alcohol.

3. Molecular methods

The DNA was extracted from tissue samples (fin clips) using the Chelex method. DNA was then involved in amplifications (PCR) on 6 microsatellite markers called **Sfo1**, **SsoSL-311**, **Omm1105**, **Oneu9**, **Ssa197** and **Mst85**. The amplified molecules where then migrated in a denaturing poly-acrylamide gels in order to separate alleles of different size. The gels are then scanned in a FMBIO II scanner. The estimation of allele sizes is helped with an appropriate image analyser programme. A genotypes matrix is constructed, basis of all statistical next analyses.

4. Statistical methods

In a first step, the genotypes matrix is analysed using GENETIX software. The following basic analyses are done:

- a general picture of the trout diversity is obtained through multidimensional analyses (here Factorial Correspondences Analyses: FCA). This method is used to confirm the belonging of all samples to *Salmo peristericus*, then to look for intraspecific genetic differentiation among rivers.

- an estimation of genetic polymorphism (mainly H parameters) indicate the level of diversity linked to the population size and the eventual recent bottlenecks
- an estimation of the inter samples or inter rivers differentiations (Fst) and of intra-river panmixia (Fis)

In a second step, assignment tests are realized in order to understand the Fst estimation. This method, using here PARTITION ML programme, subdivide the whole sample into 3 or 4 subgroups with the best genetic equilibrium in term of panmixia and markers linkage in each part. Moreover, the two *S. peristericus* caught in the lake can be assigned to a river.

Sample	Lab. n°	Field n°	Basin	River	Date of capture	N
1	T09112-T09150	A225-A263	Agios-Germanos	Agios-left	17/08/2005	39
2	T09151-T09160	A264-A273	Agios-Germanos	Agios-Right 6	19/08/2005	10
3	T10006-T10015	A291-A300	Brajcinska	main river 4 upstream	19/08/2006	10
4	T10016-T10025	A301-A310	Brajcinska	Rzanska 5	19/08/2006	10
5	T10026-T10035	A311-A320	Brajcinska	Drmisar 9	19/08/2006	10
6	T10096-T10098	A364-A366	Brajcinska	Baltanska 11	12/08/2006	3
7	T10036-T10045	A321-A330	Kranska	main 2 middle	21/08/2006	10
8	T10046-T10055	A331-A340	Kranska	main 3 downstream	21/08/2006	10
9	T10056-T10065	A341-A350	Kranska	main 4 downstream	21/08/2006	10
10	T10066-T10076	A274-A284	Goluma	Leva Reka 2 downstream	21/08/2006	11
11	T10077-T10085	A285-A353	Goluma	Leva Reka 3 middle	21/08/2006	9
12	T10086-T10095	A354-A363	Goluma	Leva Reka 4 upstream	21/08/2006	10
13	T10523-T10524	Presp1 & 2	lake	Megali-Prespa Lake	30/10/2006	2
14	T10199-T10223	A367-A391	Brajcinska	main river 4 upstream	23/07/2007	25
15	T10224-T10274	A392-A442	Brajcinska	Rzanska 5	25/07/2007	51
16	T10275-T10311	A524-A560	Brajcinska	Kriva Kobila 12	27/07/2007	37
17	T10312-T10342	A561-A591	Brajcinska	main river 8 middle	27/07/2007	31
18	T10343-T10371	A592-A620	Brajcinska	Baltanska 11	30/07/2007	29
19	T10372-T10399	A621-A648	Brajcinska	Drmisar 9	30/07/2007	28
20	T10400-T10415	A649-A664	Brajcinska	Drmisar/Marusica 17	31/07/2007	16
21	T10416-T10431	A443-A458	Kranska	main 2 middle	25/07/2007	16
22	T10432-T10452	A459-A479	Kranska	main 3 downstream	25/07/2007	21
23	T10453-T10472	A480-A494	Kranska	main 4 downstream	25/07/2007	15
24	T10453-T10472	A665-A669	Kranska	main 5 upstream	25/07/2007	5
25	T10473-T10488	A670-A685	Kranska	Srbino 6 [upstream/middle]	25/07/2007	16
26	T11931-T11940	A514-A523	Goluma	Leva Reka 2 downstream	26/07/2007	10
27	T11927-T11930	A510-A513	Goluma	Leva Reka 3 middle	26/07/2007	4
28	T11912-T11926	A495-A509	Goluma	Leva Reka 5 upstream	26/07/2007	15
29	T11890-T11911	734-851	Agios-Germanos	Agios-Right 3 downstream	08/08/2007	22
30	T11862-T11889	706-733	Agios-Germanos	Agios-Right 5 upstream R	03/08/2007	28
31	T11842-T11861	686-705	Agios-Germanos	Agios-Right 6 upstream L	03/08/2007	20

Table 1: Details of the 2005 then 2007 samplings all around the lake.

5. Results

5.1. Specific determination

This first part of results aims at the specific recognition of the samples coming from the Megali Prespa Lake basin. The sampling given in table 1 is opposed to local and European trout lineages in order to avoid any perturbation of the analyses due to misidentification of introduced foreign lineages.

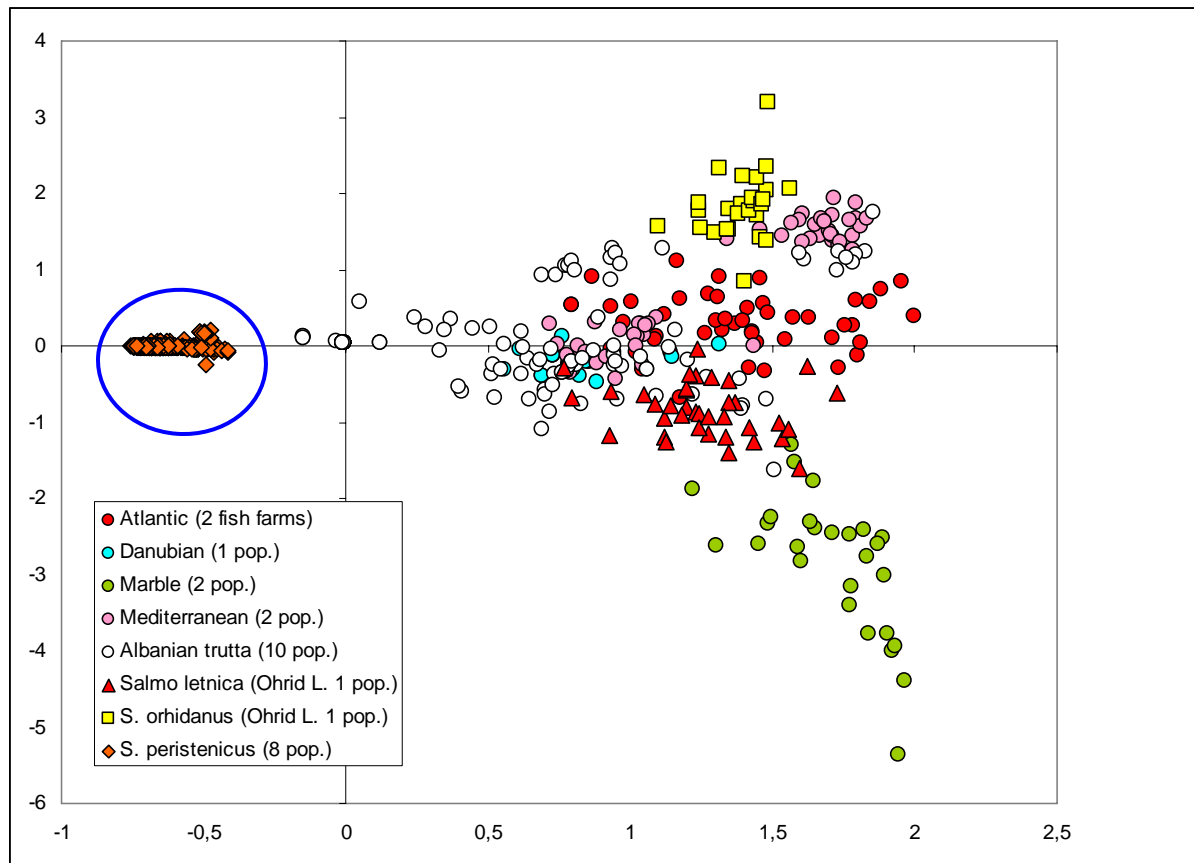


Figure 1: In this multi-lineages global analysis (Factorial Correspondences Analyse = FCA), the whole 875 Prespa Lake basin individuals are included in the independent ellipse in the middle left of the diagram. It is totally composed of *Salmo peristericus*.

5.2. Description of the inter-rivers differentiation

The figure 2 clearly indicates that the sampling is partly heterogeneous. The envelopes describing each river diversity, while partly overlapping, are separated, mainly for the samples of the rivers Kranska and Leva Reka... Brajcinska and Agios Germanos Rivers are mostly similar.

The two individuals captured in the lake seems to resemble to the Kranska type.

Multidimensional analyses are only indicative or qualitative methods. Statistical tests are necessary to demonstrate a genetic differentiation between samples.

5.3 Tests of differentiation

In a first step, F_{st} were calculated between rivers. For this, all the individuals of each rivers were grouped. This is necessary to include samples which are too small (see Table 1).

The F_{st} tests estimates if the genetic differentiation between samples is significant.

In the tables bellow, each sample correspond to a river, adding the captures of 2005 (for Agios Germanos only), 2006 and 2007.

The significance is estimated after 5000 permutations. The number of these permuted matrices which F_{st} is bellow the natural value determine the significance. If this number is equal or superior to 95%, that means that the natural value of F_{st} cannot be explained by

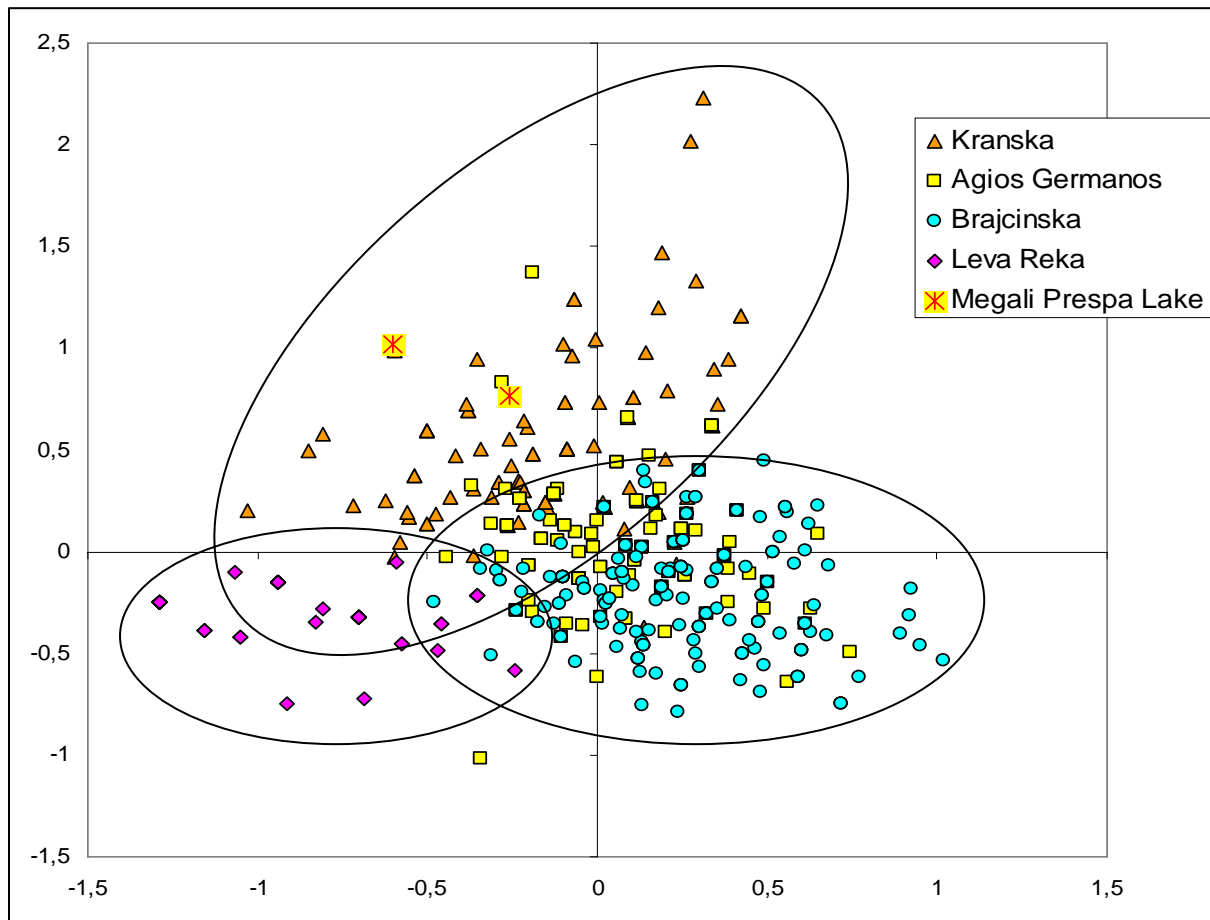


Figure 2 : The FCA presented here concerns *S. peristericus* alone. A clear but weak differentiation is detected between most basin samples. The genetic diversity is however partially described (other axis not represented here) and a complete description should be given using other methods.

chance. When the percentage of F_{st} calculated on permuted matrices bellow the true F_{st} is over 95%, the true value is significant. Here, with 100%, $p < 1/5000$.

In a second step, more detailed calculations are given in annexes 5.3.1 to 5.3.4.

FST	BR	KR	LR
AG	0.06768	0.06768	0.39984
BR		0.15655	0.40888
KR			0.41332

%val <	BR	KR	LR
AG	100.00	100.00	100.00
BR		100.00	100.00
KR			100.00

Table 2: The upper triangular matrix gives the estimations of F_{st} by pair of rivers. The lower one gives the percentage of F_{st} of randomized matrices below the true value. For all tests, the true value is never reach in permuted matrices (AG=Agios Germanos; BR=Brajcinska; KR=Kranska; LR=Leva Reka, Goluma basin)..

5.4. Assignation

The whole sample is tentatively divided into 3 (Table 3) then 4 (see Annex 5.4) subgroups, each partition being at a best panmictic and linkage equilibriums.

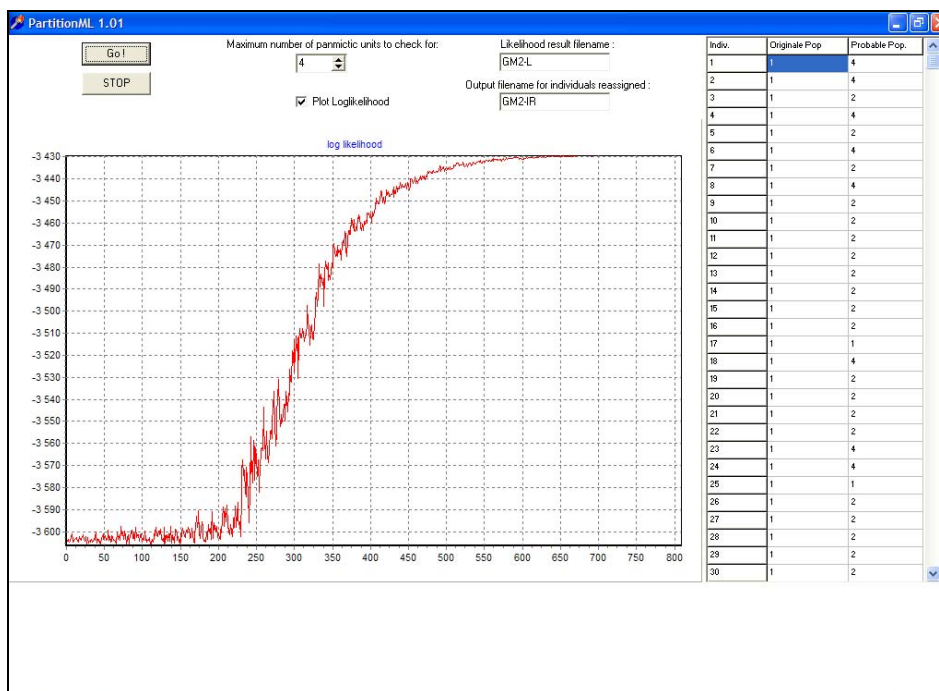


Figure 3: This is a picture of the successive attempts of partition of the whole sample into subgroups (here 4). At each tentative, the likelihood of the partition is calculated (in terms of genetic equilibriums) and plotted in the diagram. The learning algorithm permits to increase the likelihood until an asymptote is reached.

	N	part. 1	part. 2	part. 3
Agios Germanos	119	52	64	3
	%	43,7	53,8	2,5
Brajcinska	250	24	221	5
	%	9,6	88,4	2,0
Kranska	103	91	7	5
	%	88,3	6,8	4,9
Leva Reka	59	0	1	58
	%	0,0	1,7	98,3
Prespa Lake	2	2	0	0
	%	100,0	0,0	0,0

Table 3: When dividing the whole sample into 3 subgroups, the assignment method reach a comprehensive structure showing clear link between rivers and partitions.

Partition into 3, 4 and 5 subgroups were tested. The partition in 4 is significantly better (see Annex 5.4). However, the partition into 3 subgroups clearly assign Brajcinska River in the partition 2, Kranska River in the partition 1 and Leva Reka river (Goluma basin) in the partition 3. That means that these rivers are populated by differentiated trout.

Agios Germanis is not attributed to a unique partition (nor when 4 partitions are demanded, see Annex 5.4) but more or less equally distributed in the partitions 1 and 2 (Kranska and Brajcinska types). This is in perfect agreement with the FCA topology (Figure 2). This can be caused by a secondary foundation of the population of this river, founders coming from already differentiated neighbouring rivers.

Finally, the two lake specimens are assigned into the Kranska type (also present in Agios Germanos).

5.5. Panmixia

Panmixia is an equilibrium where each individual have the same probability to cross with any other individual. Panmixia disequilibria are generally observed when migrations occur or when a given sample is composed of several differentiated subgroups.

Here all the tests are not significant (see Annex 5.5)

5.6. Genetic diversity

Genetic diversity, well estimated through heterozygosity, is an important parameter for conservation. It is thought to represent the population capacity to overcome an ecologic change. This value is dependant of the population size, but also to its history and mainly past bottlenecks.

Here, a global calculation allow inter rivers comparison. Kranska River appears as the most diversified ($H_{nb}=0.39$), followed by Agios Germanos and Brajcinska (0.32 to 0.34) and finally, Leva Reka River (Goluma basin) population appears as largely less polymorphic (0.20).

A more detailed calculation, sample by sample, is given in Annex 5.6.

	Hexp.	H n.b.	Hobs.	P(0.95)	P(0.99)	Nbre Moyen d'allèles/locus
AG	0.3362	0.3377	0.3222	0.6667	0.6667	2.6667
Ecart-type :	0.2670	0.2681	0.2513			
BR	0.3236	0.3242	0.2310	0.6667	0.8333	3.1667
Ecart-type :	0.2451	0.2456	0.1793			
KR	0.3902	0.3918	0.3186	0.8333	0.8333	3.5000
Ecart-type :	0.2449	0.2459	0.1991			
LR	0.2006	0.2031	0.1874	0.5000	0.8333	2.6667
Ecart-type :	0.2103	0.2128	0.1950			

Table 4 : Several classical parameters describing the populations genetic polymorphism. For all parameters, Kranska is the most polymorphic and Leva Reka the less.

6. Interpretation

The populations inhabiting the tributaries of Lake Prespa, at least at the sampled station, are all representatives of the taxon *Salmo peristericus*, genetically distinct from the more common *Salmo trutta* lineage (Figure 1).

But this species, endemic of this watershed flowing to Lake Megali Prespa, living in a very limited area, is not homogeneous. Three genetic types have been evidenced: the Brajciska, Kranska and Goluma types. Agios Germanos River population is intermediate between Kranska and Brajciska types, but more investigations are necessary to know if this is a distinct type or if this river has been founded by both origin founders (mtDNA sequences could be useful if polymorphic).

Two specimens have been samples in the lake, they belong to the Kranska type.

Kranska River is the most polymorphic population (Hnb=0.39), and that of the Goluma basin the less (Hnb=0.2). This order is not directly parallel with the basin size order, showing that the Leva Reka population should be the most endangered local population. Moreover, these values can be considered as low. In French Pyrenees for instance, Hnb is generally between 0.6 and 0.8.

For management purpose, no inter-basin translocation should be done in order to maintain this structure.

Montpellier, May 20th, 2008

ANNEXES

Annex 5.3.1 - Between Agios-Germanos samples:

FST	02	28	29	30
01	0.18092	0.04655	0.06384	0.12130
02		0.24540	0.10446	0.08284
28			0.06140	0.08561
29				0.05927
% val <				
01	100.00	99.38	100.00	100.00
02		100.00	99.78	97.04
28			99.78	99.76
29				99.54

Annex 5.3.2 - Between Brajcinska samples:

FST	04	05	06	14	15	16	17	18	19	20
03	0.33601	0.28114	0.45876	0.03650	0.29102	0.11292	0.07241	0.64831	0.24368	0.55547
04		0.39936	0.47488	0.37726	0.03845	0.31832	0.19047	0.63358	0.42164	0.59285
05			0.15302	0.13450	0.31466	0.09328	0.08362	0.34083	-0.00394	0.12538
06				0.36467	0.33571	0.33770	0.21660	0.00359	0.20373	0.14094
14					0.33497	0.04139	0.06032	0.51650	0.09421	0.37666
15						0.29932	0.18870	0.43973	0.34729	0.43450
16							0.02621	0.47995	0.07628	0.33026
17								0.38040	0.09376	0.26807
18									0.34620	0.28772
19										0.20484
% val <										
03	99.84	99.82	98.12	89.64	100.00	99.84	99.24	100.00	100.00	99.98
04		99.66	98.24	100.00	95.64	100.00	99.98	100.00	100.00	99.94
05			94.66	99.94	100.00	99.02	99.28	99.98	54.30	99.38
06				99.10	99.70	99.32	99.28	58.92	97.76	91.82
14					100.00	99.18	99.86	100.00	100.00	100.00
15						100.00	100.00	100.00	100.00	100.00
16							97.46	100.00	99.90	100.00
17								100.00	99.98	100.00
18									100.00	100.00
19										100.00

Annex 5.3.3. Between Kranska samples:

FST	08	09	21	22	23	24	25
07	0.01555	0.06963	-0.00930	0.00804	0.05474	0.16581	0.13590
08		0.05221	0.05065	-0.00779	0.00846	0.31312	0.18040
09			0.07021	0.03411	-0.01483	0.30803	0.20022
21				0.04063	0.05942	0.14978	0.17157
22					0.02624	0.26898	0.14280
23						0.26554	0.19873
23B							0.39145
% val <							
07	74.00	95.88	37.44	71.98	96.74	98.86	99.72
08		90.72	95.98	45.36	70.44	99.08	99.98
09			98.04	91.06	33.20	98.96	99.98
21				97.68	98.86	98.92	99.98
22					91.58	99.82	100.00
23						99.64	100.00
24							99.62

Annex 5.3.4. Between Leva Reka samples:

FST	11	12	25	26	27
10	-0.01150	0.01000	0.00811	-0.05359	-0.01360
11		0.12442	-0.00501	-0.03876	-0.02180
12			0.11321	-0.01747	0.07340
25				-0.06892	-0.01185
26					-0.03887
	% val <				
10	50.62	70.10	70.54	27.54	40.62
11		95.52	55.82	38.54	29.04
12			92.40	51.30	92.70
25				7.64	42.96
26					21.40

Annexes 5.3.1 to 5.3.4: In the different tables, the upper triangular matrix is the Fst estimation, and the lower one the significativity estimation (significant when the permutation value is over 95). The calculations compare elementary samples.

- the significant Fst are **in yellow**
- the samples of 2006 are **in grey**
- the underlined samples are bigger than 15
- the very small samples (5 and less) are **in blue**

Most of the comparisons are significant, showing a high isolation even inside a river, and/or a high genetic change from a year to the other.

We however noticed that:

- no change from 2006 to 2007 in the main Brajcinska main river (3=14), the Baltanska River (6=18) and the Drmisar River (5-19)
- no change between the main Baltanska River sample of 2006 and the Marusica, Dsmisar tributary (6=20) but this tributary is different from the Drmisar River in the same year (19≠20)
- in Leva Reka River, only 11≠12 i.e. in 2006 between middle and upstream. No difference in 2007.

Annex 5.4:

	N	part. 1	part. 2	part. 3	part. 4
Agios Germanos	119	6	68	3	42
	%	5,04	57,14	2,52	35,29
Brajcinska	250	73	163	5	9
	%	29,20	65,20	2,00	3,60
Kranska	103	1	16	6	80
	%	0,97	15,53	5,83	77,67
Leva Reka	59	1	1	57	0
	%	1,69	1,69	96,61	0,00
Prespa Lake	2	0	0		2
	%	0,00	0,00	0,00	100,00

Annex 5.4: When PartitionML subdivide the whole sample into 4 parts, the significance of each sub-group is less clear than the partition into 3 (see table 3)

Annex 5.5.

Two methods of panmixia checking can be employed. The **bootstrap method** change the loci used in the calculation by random sorting. A given locus can be used two time or not used among the 5000 attempts. According to the table above, all samples are in equilibrium.

The second method (next page) is the permutations: only one sample is in disequilibrium, which can be omitted: there is a general panmixia in Lake Prespa tributaries.

6 Loci
5000 Bootstraps
Fis values after changing the loci involved.

Calculations applied to the whole data (6 loci) :

Populations	Real value	(CI 95%)	Significance
<u>01</u>	-0.07890	(-0.22462 - 0.03579)	ns
<u>02</u>	0.21053	(-0.19118 - 0.49533)	ns
<u>03</u>	0.05263	(-0.28940 - 0.30149)	ns
<u>04</u>	0.02703	(-0.22727 - 0.24211)	ns
<u>05</u>	-0.23270	(-0.50000 - -0.04598)	ns
<u>06</u>	-0.20000	(-1.00000 - 0.00000)	ns
<u>07</u>	-0.19084	(-0.45833 - 0.00277)	ns
<u>08</u>	0.08163	(-0.40462 - 0.37600)	ns
<u>09</u>	-0.10695	(-0.51007 - 0.07455)	ns
<u>10</u>	-0.02564	(-0.28114 - 0.11504)	ns
<u>11</u>	0.24269	(-0.21212 - 0.43385)	ns
<u>12</u>	0.37209	(-0.12500 - 0.66667)	ns
<u>13</u>	-0.40000	(-1.00000 - -0.40000)	ns
<u>14</u>	0.04000	(-0.15845 - 0.20906)	ns
<u>15</u>	0.06916	(-0.04232 - 0.16530)	ns
<u>16</u>	0.10922	(-0.07376 - 0.26602)	ns
<u>17</u>	0.04901	(-0.13427 - 0.18726)	ns
<u>18</u>	-0.20172	(-0.51515 - 0.10909)	ns
<u>19</u>	0.06766	(-0.11724 - 0.23368)	ns
<u>20</u>	-0.09756	(-0.25000 - -0.02740)	ns
<u>21</u>	-0.09957	(-0.30312 - 0.03615)	ns
<u>22</u>	0.02498	(-0.18837 - 0.20089)	ns
<u>23</u>	0.15934	(-0.04830 - 0.30047)	ns
<u>24</u>	-0.03967	(-0.23529 - 0.08451)	ns
<u>25</u>	-0.18563	(-0.57500 - 0.20000)	ns
<u>26</u>	0.40000	(-0.50000 - 0.66667)	ns
<u>27</u>	-0.25561	(-0.51174 - -0.01818)	ns
<u>28</u>	0.08795	(-0.12752 - 0.25431)	ns
<u>29</u>	-0.07427	(-0.22129 - 0.03165)	ns
<u>30</u>	-0.08126	(-0.30122 - 0.08439)	ns

6 Loci

1000 & 5000 permutations

Fis values after permutations of alleles inside each population

Calculations applied to the whole data (6 loci) :

Population	Real Fis	% val. >	% val. <	
			1000 perm.	5000 perm.
01	-0.07890	85.4000	14.2000	14.9200
02	0.21053	4.0000	77.6000	77.8600
03	0.05263	25.4000	59.5000	57.9800
04	0.02703	24.8000	49.2000	46.5200
05	-0.23270	83.8000	1.7000	1.5400
06	-0.20000	18.0000	0.0000	0.0000
07	-0.19084	88.8000	6.3000	4.7400
08	0.08163	22.0000	66.9000	68.0200
09	-0.10695	71.0000	15.5000	14.4200
10	-0.02564	41.7000	32.5000	32.9000
11	0.24269	5.5000	88.7000	85.6000
12	0.37209	0.0000	84.8000	84.1200
13	-0.40000	58.1000	0.0000	0.0000
14	0.04000	30.1000	59.7000	62.7600
15	0.06916	10.1000	86.8000	86.4000
16	0.10922	9.5000	89.4000	89.6600
17	0.04901	21.6000	77.6000	76.6200
18	-0.20172	76.6000	6.9000	5.8400
19	0.06766	17.7000	68.0000	68.6200
20	-0.09756	34.5000	0.0000	0.0000
21	-0.09957	82.3000	15.7000	17.3200
22	0.02498	36.5000	53.8000	55.7200
23	0.15934	3.8000	96.0000	94.9000
24	-0.03967	50.6000	28.8000	27.5800
25	-0.18563	78.0000	19.3000	17.7400
26	0.40000	0.0000	57.1000	58.0200
27	-0.25561	87.9000	1.7000	1.1200
28	0.08795	16.1000	78.8000	76.9400
29	-0.07427	79.0000	14.5000	15.1600
30	-0.08126	77.1000	19.8000	19.6800

Annex 5.6. Genetic diversity

Lots	N	Basin	River	Hexp,	H n,b,	Hobs,	P(0,95)	P(0,99)	A
1	39	Agios-Germanos	Agios-left	0,31	0,31	0,34	0,67	0,67	2,00
2	10	Agios-Germanos	Agios-Right 6	0,20	0,21	0,17	0,67	0,67	1,67
29	22	Agios-Germanos	Agios-Right 3 downstream	0,32	0,33	0,30	0,67	0,67	2,17
30	28	Agios-Germanos	Agios-Right 5 upstream R	0,33	0,33	0,36	0,67	0,83	2,33
31	20	Agios-Germanos	Agios-Right 6 upstream L	0,31	0,32	0,35	0,67	0,67	1,83
3	10	Brajcinska	main river 4 upstream	0,28	0,30	0,28	0,67	0,67	2,00
4	10	Brajcinska	Rzanska 5	0,20	0,21	0,20	0,67	0,67	2,33
5	10	Brajcinska	Drmisar 9	0,21	0,22	0,27	0,67	0,67	1,67
14	25	Brajcinska	main river 4 upstream	0,27	0,27	0,26	0,67	0,67	2,00
15	51	Brajcinska	Rzanska 5	0,27	0,28	0,26	0,83	0,83	2,67
16	37	Brajcinska	Kriva Kobilica 12	0,26	0,26	0,23	0,67	0,67	2,00
17	31	Brajcinska	main river 8 middle	0,35	0,35	0,34	0,67	0,67	2,33
18	29	Brajcinska	Baltanska 11	0,09	0,10	0,11	0,17	0,33	1,33
19	28	Brajcinska	Drmisar 9	0,23	0,24	0,22	0,67	0,67	1,83
20	16	Brajcinska	Drmisar/Marusica 17	0,08	0,09	0,09	0,50	0,50	1,50
10	11	Goluma	Leva Reka 2 downstream	0,17	0,18	0,18	0,50	0,50	2,00
12	10	Goluma	Leva Reka 4 upstream	0,07	0,08	0,05	0,50	0,50	1,50
26	10	Goluma	Leva Reka 2 downstream	0,15	0,16	0,18	0,33	0,33	1,50
28	15	Goluma	Leva Reka 5 upstream	0,17	0,18	0,22	0,50	0,50	1,50
7	10	Kranska	main 2 middle	0,35	0,37	0,43	0,83	0,83	2,17
8	10	Kranska	main 3 downstream	0,34	0,36	0,33	0,67	0,67	2,33
9	10	Kranska	main 4 downstream	0,33	0,35	0,38	0,67	0,67	2,33
21	16	Kranska	main 2 middle	0,35	0,36	0,39	0,83	0,83	2,33
22	21	Kranska	main 3 downstream	0,32	0,33	0,32	0,67	0,67	2,50
23	15	Kranska	main 4 downstream	0,37	0,38	0,35	0,67	0,67	2,33
25	16	Kranska	Srbino 6 [upstream/middle]	0,31	0,32	0,34	0,83	0,83	1,83

Annex 5.6: This table gives the different classical parameters describing intra-samples diversity (no sample grouping).

The *H* parameters, mostly used in literature, are interpreted. Different colours indicate the levels of polymorphism. As in the global analysis (Table 4), there is a clear difference between rivers and an homogeneity between samples of the same basin.