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Effect Of The Antropic Pressure On The Genetics Diversity And Structure Of *Sarotherodon Melanotheron Heudelotii* And *Sarotherodon Melanotheron Paludinosus*

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Abstract :

In order to know effect of anthropic pressure on genetic diversity of nine populations of *Sarotherodon melanotheron heudelotii* and *S. m. paludinosus*), some study was undertook in six polluted sites (Foundiougne, Kaolack, Guiers Lake, Bay Hann, Niayes 1 and Niayes 2) and three healthy sites (Missirah, Saint Louis and Koular) in 2009. The diversity of samples colleted were analyzed with seven enzymatic markers (ADH, AAT, IDHP, MDH, PGM, GPI and EST).

The result of analysis indicated a similar genetic diversity between the two subspecies, *S. m. heudelotii* (A= 2,19 ; H_E= 0,29 and $P_{(95\%)}$= 84,28) and *S. m. Paludinosus* (A= 2,15 ; H_E = 0,32 and $P_{(95\%)}$= 85).

Concerning the sampled sites, genetic diversity doesn't vary between population of polluted and healthy sites. The majority of the analyzed populations showed a deviation from Hardy-Weinberg equilibrium in the direction of a deficit of heterozygosis.

Also the analysis of population showed a deviation with Hardy-Weinberg equilibrium model in the direction of a deficit heterozygosis ($F_{IS} > 0$; $P < 0.05$). The essential of this deficiency was explained by locus EST-14.

Except Missirah and Saint-Louis, a genetic structure was revealed between population of Koular and populations of disturbed sites ($0.024 < F_{ST} < 0{,}168$; $P < 0.05$). Locus PGM contributes to this differentiation observed between Koular and these populations. This locus would undergo a selective pressure in contaminated site.

Keywords: *Sarotherodon melanotheron*, polluted site, healthy site, genetic diversity, genetic structure

Introduction

Estuarine and coastal ecosystems are well known to constitute favorable environments colonized by numerous aquatic species during one or several phases of their biological cycle (reproduction, development, growth, grown-up life…). These environments, by various functions (nursery, shelter or food supply) ensure the proliferation and maintaining of aquatic species or biodiversity (Costanza *et al*., 1997). However, many estuarine and coastal ecosystems, nowadays, undergo overexploitation and chronic chemical multi-contamination due to intense human activity. Anthropogenic pressures contribute to the impoverishment of biological diversity and genetic variability (Vituosek *et al*., 1997). Indeed, the increase in exploitation resulted in the degradation of ecosystems and the decline of biomass of target species that are often overexploited (Allison *et al.*, 2009, Lam *et al.*, 2012, Durand *et al.* 2013). Anthropogenic stressors, stemming from industrial, agricultural and urban waste are discharged in these ecosystems where they strongly affect, according to their nature, duration and importance, biological characteristics of aquatic species. So, the maintenance of healthy and productive costal and estuaries ecosystems is a critical issue and fishes are good indicators to estimate the system

health affected by anthropogenic pressure. Many investigations reported biological responses of fishes to aquatic contaminants (Mehdaoui *et al.*, 2000, Dailianis *et al.*, 2003, Marchands *et al*., 2003, Choi *et al*., 2009). Certain of them, used allozymes markers, to evaluate the impact of chemical stress on the genetics of fish populations settled in heavily contaminated rivers (Foré *et al.*, 1995a, 1995b, Gillespie & Guttman, 1999). Present study deals with genetic response of *Sarotherodon melanotheron*, endemic fish species of West Africa, population to contaminants in rivers of Senegal using allozymes markers. This species is the subject of intensive artisanal fishing in lagoon systems (Legendre & Ecoutin, 1989). In the Ayamé Lake, it represents 50 % of the commercial captures (Gourène *et al*., 1999).

In a specific way, it is question of comparing genetic diversity between populations of weakly contaminated sites and those of polluted sites and of describing the genetic structure between populations of apparently healthy environment and those of polluted sites.

2. Materials and methods

2.1. Choice and characteristics of sites

The experiment was carried out in 2009 on six West African hydrosystems : Saloum estuary, Gambie and Sénégal rivers, Guiers Lake, Hann Bay and the Niayes (Fig 1). The sites were selected according to their degree of pollution (table 1).

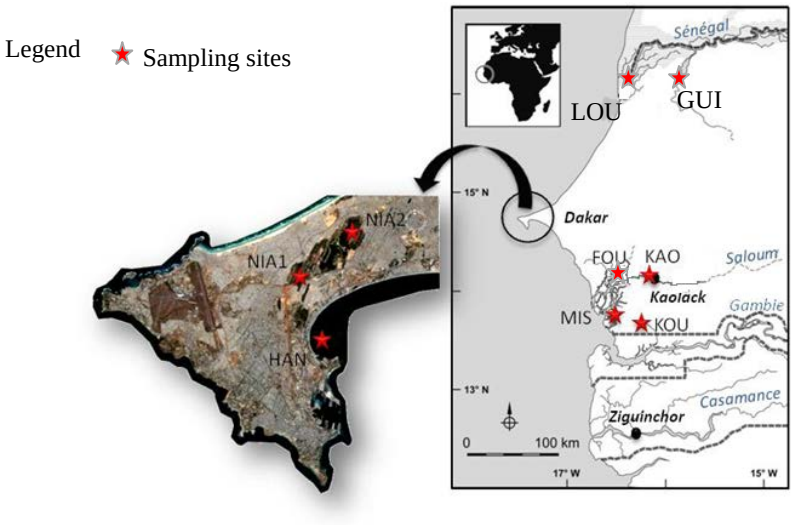

Fig 1: Map of sampling locations for *Sarotherodon melanotheron heudelotii and S. m. Paludinosus* in Sénégal hydrosystems.

Table 1: Characteristics of the sampled sites

Selected sites	Hydrosystems	Salinity (g/l)	Types of anthropic pressures
Saint Louis	Fleuve Sénégal		Strong pressure of fishing
Guiers Lake	Lac de Guiers	0,2	Polluted by the industrial activities, agricultural and the watery plants
Niayes 1	Niayes	58,6	Polluted
Niayes 2	Niayes	2,7	Polluted
Hann Bay	Baie de Hann	34,7	Polluted by the urban and industrial activities
Foundiougne	Siné-Saloum	51,4	Polluted
Missirah	Siné-Saloum	39,3	Not polluted
Kaolack	Siné-Saloum	102	Polluted by the urban effluents
Koular	Gambie	34,1	Not polluted

2.2. Field sampling

A total 221 specimens of the subspecies, *S. m. heudelotii* (173) and *S. m. paludinosus* (48) (Table 2), was fished using purse seine by experimental fishing. These specimens were captured in 2009. Nine populations of *Sarotherodon melanotheron* were collected. The sampled specimens were preserved in refrigerators and conveyed to the laboratory.

Table 2.Origin of *Sarotherodon melanotheron* populations

Subspecies	Hydrosystems	Country	Sites	Abbreviation	No. of individuals
S. m. heudelotii	Siné-Saloum Estuary	Sénégal	Foundiougne	FOU	30
S. m. heudelotii	Siné-Saloum Estuary	Sénégal	Kaolack	KAO	29
S. m. heudelotii	Siné-Saloum Estuary	Sénégal	Missirah	MIS	29
S. m. heudelotii	Guiers Lake	Sénégal	Guiers	GUI	16
S. m. heudelotii	Sénégal river	Sénégal	Saint Louis	LOU	14
S. m. heudelotii	Hann bay	Sénégal	Hann	HAN	27
S. m. heudelotii	Gambie estuary	Gambie	Koular	KOU	28
S. m. paludinosus	Niayes	Sénégal	Niayes 1	NIA 1	18
S. m. paludinosus	Niayes	Sénégal	Niayes 2	NIA 2	30

2.3. Electrophoresis

The liver from each specimen was preserved at -80◦C until processed. Homogenates for electrophoresis were obtained with fractions of liver crushed in distilled water. Electrophoresis was performed on gels composed of 12% hydrolysed starch. Extract from liver was screened with seven enzymatic systems (ADH, AAT, IDHP, MDH, PGM, GPI and EST) based on technical of enzymatic electrophoresis described by Pasteur *et al*. (1988).

Genetic nomenclature of Shaklee *et al.* (1990) was used. Alleles were designated by their mobility relative to the most common allele, which was designated 100 for each locus.

2.3. Statistical analysis

The resulting data matrix of alphabetically designated allele phenotypes was analysed using GENETIX 4.3 software (Belkhir *et al.* 2004). Estimated genetic diversity parameters were observed: number of alleles per locus (*A*), proportion of polymorphic loci ($P_{(95\%)}$), observed (H_0) and expected heterozygosity (H_E). Wright's parameter (F_{IS}, F_{ST}) values for polymorphic loci were determined to evaluate intrapopulation differentiation (F_{IS}) and differentiation between populations (F_{ST}) (Wright, 1978). Significance of values of F_{IS} and F_{ST} was tested under the null

hypothesis by using the procedure of permutations (1000) (Weir & Cockerham, 1984). A locus was considered polymorphic if the frequency of the common allele is not greater than 0.95.

3. RESULTS

3.1. Intra-population diversity

3.1.1. Genetic Variability

In a general, the average values of diversity obtained are very high and relatively not very variable from one subspecies to another. They are of 2.19 (A), 0.29 (H_E) = 0.13 (H_O) and 84.28 ($P_{(95\%)}$) at *Sarotherodon melanotheron heudelotii* and 2.15 (A), 0.32 (H_E) , 0.12 (H_O) and 85 ($P_{(95\%)}$) for *Sarotherodon melanotheron paludinosus* (Table 3).

The number of allele (A) within populations of *S. m. heudelotii* is included between 1.9 (population of the Lake Guiers) and 2.4 (population of bay of Hann). The highest value of heterozygoties waited (H_E) and observed (H_O) are respectively 0.36 (Koular) and 0.18 (Louis Saint). But the lowest of the two parameters were recorded with the population from Lake Guiers (H_E = 0.20 and H_O = 0.09). The rate of polymorphism ($P_{(95\%)}$) was included between 60 % (Lake Guiers) and 100 % (Kaolack).

About population of *S. m. paludinosus*, the highest values of diversity was recorded in Niayes 1 (A = 2.2 ; H_E = 0.36 ; H_O = 0.14 and $P_{(95\%)}$ = 90) and the lowest value in Niayes 2 (A = 2.1 ; H_E = 0.27 ; H_O = 0.09 and $P_{(95\%)}$ = 80).

The genetic diversity doesn't vary between populations of polluted sites and health.

Table 3 Genetic diversity of populations of *S. melanotheron*

	Populations	*A*	H_E	H_O	$P_{(95\%)}$
	Guiers Lake	1.9	0.20	0.09	60
	Saint Louis	2.1	0.28	0.18	80
	Foundiougne	2.2	0.28	0.13	90
S. m. heudelotii	Kaolack	2.3	0.30	0.17	100
	Koular	2.2	0.36	0.14	90
	Missirah	2.2	0.33	0.11	90
	Hann Bay	2.4	0.30	0.12	80
Average values		**2.19**	**0.29**	**0.13**	**84.28**
S. m. paludinosus	Niayes 1	2.2	0.36	0.14	90
	Niayes 2	2.1	0.27	0.09	80
Valeurs moyennes		**2.15**	**0.32**	**0.12**	**85**

3.1.2. Fixation index (F_{IS})

The positive values of F_{IS} multilocus (0.409 to 0.694) revealed a significant deficiency in heterozygotes ($P < 0.001$) within all the populations analyzed with the two subspecies (Table 4). Also, the positive value F_{IS} of monolocus (0.345 to 0.760) and a value recorded with locus EST-14 showed a significant deficiency in heterozygotes within all the populations some with the two subspecies.

With *S. m. heudelotii the value* of F_{IS} obtained for locus ADH-2 are positive, high (0.663 to 0.931) and significantly different to zero ($P < 0.001$) at the specimens coming from the estuary from Saloum (Foundiougne, Kaolack and Missirah) and from Gambia (Koular). The locus of *S. m. paludinosus* revealed also a significant deficiency in heterozygotes ($F_{IS} = 0.868$; $P < 0.001$) in the population of Niayes 1. For locus IDHP, the positive, very high values ($F_{IS} = 0.792$ to 1.000) and significantly different from zero ($P < 0.001$) obtained at the populations of *S. m. heudelotii* (Koalack, Missirah and bay of Hann) and *S. m. paludinosus* (Niayes 1 and Niayes 2). Within the population of Kaolack, non significant excess in heterozygotes is observed with locus AAT-2 and EST-1 for values of F_{IS} negative.

The analysis of F_{IS} of the specimens reveals an imbalance of Hardy Weinberg in the direction of a deficiency within all the populations. Locus EST-14 contributes to this deficit.

Table 4: Values of F_{IS} for each population of two subspecies of *Sarotherodon melanotheron*; Significance of permutation test: $***P < 0.001, **0.01 \leq P < 0.05$.

	Po	multilocus	ADH-2	AAT-2	IDHP	MDH-1	MDH-2	PGI-2	PGM	EST-1	EST-3	EST-14
	GUI	0.564***				0.651	1.000		0.454	0,559**	0,502**	0.544*
	LOU	0.409***		0.303		0.187	0.649		0.772*	0.387*	0.081	0.699**
S. m.	FOU	0.567***	0.800***	0.151	0.272	0.767***	1.000***	0.892***		0.434**	0.250	0.455**
heudelotii	KAO	0.441***	0.663***	-0.077	1.000***	0.704***	0.477	1.000**	0.659	-0.118	0.252	0.345*
	MIS	0.662***	0.862***	0.359	0.873***	0.831***	0795***		0.843***	0.498***	0.359*	0.606**
	KOU	0.628***	0.931***	0.091		0.431	0.842***	0.923***	0.918***	0.509***	0.212	0.605***
	HAN	0.608***	0.329	0.521*	0.792***	0.521*	0.793***	0.614*		0.499***	0.536***	0.653***
S. m.	NIA1	0.628***	0.862***	0.655**	1.000***	0.575*	0.382	0.825**		0.336	0.574**	0.760***
paludinosus												
	NIA2	0.694***	0.329	0.600**	1.000***	0.740***	0.477	1.000**		0.323*	0.656***	0.682***

3.2. Genetic parameter of differentiation (F_{ST})

The values of F_{ST} multilocus (0.038 to 0.168) recorded between populations of *S. m. heudelotii* were low but significantly different from zero ($P < 0.05$) (Table 5).

Bay of Hann site is different from all populations except those of Louis-Saint and Guiers Lake site ($P > 0.05$). The number of migrants associated with this differentiation is very low (1.99 to 5.03). A genetic differentiation is also recorded between population of the estuary of Gambia (Koular) and all the other populations (F_{ST} = 0.065 with 0.168 ; $P < 0.05$) except for Missirah and Saint-Louis. Nevertheless, the F_{ST} calculated between the populations of Saloum (Koalack,

Foundiougne and Missirah) and between the populations of Louis-Saint and of the Guiers Lake highlight a genetic absence of differentiation between these samples ($P > 0.05$).

With these populations try to be precise speak about what population not differentiated numbers of very high migrants are associated. They are the Missirah-Foundiougne couples (*Nm*= 382.41), Saint Louis-Lake of Guiers (*Nm*= 8), Kaolack-Foundiougne (*Nm*= 8), Missirah-Kaolack (*Nm*= 8) and bay of Hann-Saint-Louis (*Nm*= 8).

Between the two populations of *Sarotherodon melanotheron paludinosus* no genetic difference is recorded ($P > 0.05$). A significant degree of connectivity is observed between these populations, Niayes 1-Nayes 2 (*Nm*= 32.64).

About monolocus, the locus PGM (Table 6), a strong genetic difference is noted between Koular ($0.161 < F_{ST} < 0.274$) and all the other populations except for that of Saint-Louis and Missirah.

The analysis, of the F_{ST} between populations of *S. m. heudelotii* does not reveal genetic differentiation intrabasins. To interbasin, a genetic differentiation is observed between the population of Koular and all the populations of disturbed site. The F_{ST} recorded between Koular and these populations show that locus PGM contributes to this differentiation.

Table 5 : Values of *the* F_{ST} and a number of migrants (Nm) exchanged by generation between pairs of populations of *Sarotherodon melanotheron*. The Values of F_{ST} are presented above the diagonal and those of *Nm* below. Significance of permutation test :: ***$P < 0.001$, **$0.001 \leq P < 0.01$,*$0.01 \leq P < 0.05$.

Populations	GUI	LOU	FOU	KAO	KOU	MIS	HAN	NIA1	NIA2
GUI	----	-0.015	0.077**	0.079**	0.098**	0.052*	0.021	0.193***	0.114***
LOU	∞	----	0.051*	0.058**	0.038	0.032	-0.002	0.115**	0.055*

FOU	3.01	4.66	----	-0.003	0.024*	0.001	0.047**	0.129***	0.101***
KAO	2.92	4.04	∞	----	0.065*	0.014	0.075**	0.099***	0.080***
KOU	2.31	3.58	9.94	6.32	----	0.033	0.098***	0.168***	0.162***
MIS	4.58	7.43	382.41	∞	7.23	----	0.052**	0.096***	0.080***
HAN	11.68	∞	5.03	3.06	2.29	4.58	----	0.111***	0.059*
NIA1	1.05	1.92	1.69	2.29	1.29	2.36	1.99	----	0.008
NIA2	1.95	4.29	2.24	2.87	1.24	2.87	3.99	32.64	----

Table 6 : Values of the F_{ST} and a number of migrants (Nm) exchanged by generation between pairs of populations of *Sarotherodon melanotheron :* Significance of permutation test :: ***$P <$ 0.001, **$0.001 \leq P < 0.01$,*$0.01 \leq P < 0.05$.

Populations	GUI	LOU	FOU	KAO	KOU	MISS	HAN	NIA1	NIA2
GUI	----		0.141		0.161*c		0.130	0.093	0.141
LOU		----	0.220	0.047			0.204*	0.151	0.220
FOU			----	0.025	0.287**	0.094			
KAO				----	0.170**		0.021	0.005	0.025
KOU					----	0.065	0.274**	0.230**	0.287**
MIS						----	0.087	0.061	0.094
HAN							----	-0.047	-0.010
NIA1								----	0.032
NIA2									----

4. **Discussion**

Genetic variability is globally similar with the two subspecies, *Sarotherodon melanotheron heudelotii* (A= 2.18 ; H_E = 0.29 and $P_{(95\%)}$ = 84.28) and *Sarotherodon melanotheron paludinosus* (A= 2.15 ; H_E= 0.32 ; H_O= 0.12 and $P_{(95\%)}$= 85) in spite of the significant number of individuals analyzed at *S. m. heudelotti* (173) comparatively to those of *S. m. paludinosus* (48). This result suggests that the two subspecies are genetically very near. There existed a genetic affinity between them. A similar result was observed by Pouyaud (1994) with the both subspecies.

The lowest genetic variability of *S. m. heudelotii* was observed with the Guiers Lake. This value of diversity would be related to the construction of dam hydroelectric on this part of Sénégal river. Indeed, the start-up of the dams Diama (1985) and Manantali (1985) has deeply modified the hydrological functioning of the lake and it water quality, which involved, a strong development of aquatic vegetation in a area less deep (Arfi *et al.*, 2003). Nielsen *et al.* (1997) and Neraas & Spruell (2001) showed that the segmentation of the rivers by the dams isolates the populations from salmonidés, which reduced genic flows and can induce the demographic decline and the loss of genetic diversity of the populations.

However, on the level of the other populations of the two subspecies (*S. m. heudelotii* and *S. m. paludinosus*), genetic diversity doesn't vary between disturbed and health sites. A similar tendency was underlined by Larno *et al.* (2001) and Foré *et al.* (1995a), (1995b) respectively at *Leuciscus cephalus* and *Campostoma anomalum* coming from healthy and contaminated environment. At the populationnel level, at *S. melanotheron* this relative maintenance of diversity could be ascribable to the fact that, for some locus, one can consider an increase in the heterozygoty whereas the tendency would be reversed for other locus, this result in the stability of the heterozygoty multilocus. Moreover, the absence of difference in diversity genetic between populations of healthy environment as contaminated would be probably due to the weak biodisponibility of the contaminants and/or the complexity of the contaminant mixture. This report was also made by Klerks *et al.* (1997) with the populations of the fish *Gobionellus boneosoma* exposed and not exposed to HAPs contamination. According to these authors, the weak biodisponibility of the contaminants, the complexity of the contaminating mixture and the heterogeneity of distribution of HAPs which makes it possible the organizations to avoid pollution could justify the apparent absence of difference in genetic diversity.

The values of F_{IS} multilocus showed significant deviations from Hardy-Weinberg equilibrium. These F_{IS} estimates revealed the existence of a heterozygote deficiency within these populations. Analysis of the F_{IS} calculated by locus highlighted that this deficit was marked particularly more with locus EST-14. The contamination could thus exert a selective pressure on some loci but not on others, creating a certain imbalance in the allelic frequencies compared to the law of Hardy Weinberg.

This deficiency could be due to technical and/or biological factors. The presence of null alleles may cause an underestimation of the heterozygotes in a sample because heterozygous individuals in the sample may be incorrectly identified as homozygotes. However previous studies, carried out by Adépo-Gourène *et al.* (1993), Pouyaud (1994), Pouyaud *et al.* (1999) and Adépo-Gourène (2008) with the same species, revealed significant heterozygote deficiency. Thus, we could say that the biological causes may explain the recorded deficiency. The deficit observed with this species may be due to the mode of primarily endogamic reproduction which generated consanguineous siblings. Indeed the consanguineous individuals were more homozygotes on the unit of the locus than the non consanguineous individuals (Castric *et al.* 2002). This suggested that consanguinity contributed to explain the heterozygote deficiency observed in this study. In addition, the heterozygote deficiency observed for the octocoralliaire, *Pseudopterogorgia elisabethae* was explained by consanguinity following a local recruitment due to stray capacitances of the larvae (Guttierrez-Rodriguez & Lasker, 2004). The same assumptions were advanced to explain the heterozygote deficiency observed at the red coral with the allozymes (Abbiati *et al.*, 1993) and the microsatellites (Costantini *et al*, 2007). Moreover, an effect Wahlund (i.e. the presence of groups of individuals genetically differentiated within a sample) can also be one of the biological causes involving a heterozygote deficiency. It is very probable

that all of these factors (presence of null alleles, consanguinity and Wahlund effect) contributed to the deficiency observed (Costantini *et al.*, 2007) and in a variable way according to populations what would suggest that all the populations do not have the same functioning mode. This showed that the pairing of the gametes was not carried out a hazard within the populations.

For the analyzed populations, no significant difference intra-basin was observed between populations of *S. m. heudelotii* and also between populations of *S. m. paludinosus*. However study of Pouyaud (1994) revealed a significant genetic differentiation between populations of Saloum (Foundioungne-Kaolack ; Foundiougne-Missirah and Kaolack-Missirah) associated to a low number of migrants (Nm = 0.30 to 0.46). The report made in our study on the genetic structure of the populations of Saloum was due, mainly, to the important genetic flow (Nm = 382.41 to 8) recorded between these populations. Indeed, when the number of migrants exchanged by generation was raised, the genetic pools of the populations tended to be homogenized with the wire of time. According to Larno (2004), it is probable that high genetic flows was the explanation for the maintenance of a genetic diversity and ensured the genetic homogeneity between populations taken in the same river. Genetic flow can be regarded as a force which supports the evolution. The contribution of new genes in a population can limit the effects of consanguinity and contribute to the maintenance of genetic diversity essential with the conservation of the adaptive potential (Hubert-Vincent, 2007).

However, a significant genetic difference ($P < 0.05$) was observed between the relatively clean site, Koular of Gambia, and all the populations of disturbed sites (Lake Guiers, Hann Bay, Kaolack, Foundiougne, Niayes 1 and 2). This differentiation may be due to the characteristics of this site. Indeed, Koular is a site of reference of the estuary of Gambia. The F_{ST} monolocus showed that locus PGM was the main source of the differentiation observed between Koular and

the others populations coming from disturbed environment. Indeed, the significant F_{ST} (0,161 < F_{ST} < 0,287) recorded with locus PGM between Koular and these populations revealed a strong genetic differentiation. That reinforced our assumption of a selective pressure undergone by this locus in contaminated environment. The structuring of different basins observed, indicated the potential role that geographical isolation can play in speciation.

Conclusion

This study made it possible to apprehend at the genetic level the operation of nine populations of *Sarotherodon melanotheron* including 7 of the subspecies *S. m. heudelotii* and 2 of *S. m. paludinosus* in some hydrosystems of West Africa subjected to anthropic pressures.

The analysis of genetic diversity, revealed a similar genetic variability between the subspecies *S. m. heudelotii* and *S. m. paludinosus*. These two subspecies were genetically near. Genetic variability was identical in the populations from healthy and contaminated environments except for the Lake Guiers.

The majority of analyzed populations showed a deviation from the balance of Hardy-Weinberg in direction of a deficit in heterozygotes.

For the analysis of the genetic structuring, on the intra-bassin level, no genetic differentiation was observed between the three populations of Siné-Saloum.

Concerning the structuring inter-bassin, the site of Koular (a relatively healthy environmental of Gambia) differed from all the disturbed sites (Lake Guiers, Baie of Hann, Foundiugne, Kaolack, Niayes 1 and 2).

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