Genetic Adaptability of *Sarotherodon Melanotheron* to Polycyclic Aromatic Hydrocarbons (PAHs) in Some Senegal Hydrosystems

Ahou Nicole Yoboue
N'guessan Gustave Aliko
Université Jean Lorougnon Guédé,
UFR Environnement, Daloa, Côte d'Ivoire
Bi Boh Nestor Gore
Université Jean Lorougnon Guédé,
UFR Agroforesterie, Daloa, Côte d'Ivoire
Félix Koffi Konan
Université Jean Lorougnon Guédé,
UFR Environnement, Daloa, Côte d'Ivoire
Abouo Béatrice Adepo-Gourene
Université Nangui Abrogoua ex Abobo-Adjamé,
Laboratoire d'Ecologie et de Biologie Aquatique

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Abstract

The objective of this study is to evaluate the level of Polycyclic Aromatic Hydrocarbons (PAH) contamination at the Sine-Saloum (Foundiougne, Kaolack and Missirah), Hann Bay and Niayes (1 and 2) sites in Sénégal and genetic ecotoxicology of *Sarotherodon melanotheron* specimens from these sites.The genes of the specimens were studied by the enzymatic electrophoresis technique. Seven enzymatic systems (ADH, AAT, IDHP, MDH, PGM, GPI and EST) were analyzed. The analysis of the PAHs was carried out by gas chromatography coupled to mass spectrometry (GC-MS). Populations of *Sarotherodon melanotheron* and sediments used for PAH measurements were sampled in 2009. Chemical characterization of the sampling sites revealed a high concentration of PAHs at Foundiougne and Hann Bay. The high pollution of environment is characterized by PAH napht (Foundiougne (14 378 ng/g) ; Hann Bay (5856 ng/g). The analysis of allelic variability showed the existence of an adaptive polymorphism at the PGM locus in *S. melanotheron*. The particularly low frequencies of the PGM * 105 allele in populations of disturbed environment (Foundiougne, Kaolack, Hann Bay, Niayes 1 and Niayes 2) suggest its involvement in the response to environmental stress. A negative correlation was observed between the PGM, IDHP and MDH-1 locus and PAH. The presence of PAHs in the environmentcauses to a decrease in the frequency of the PGM * 105 alleleat*S. melanotheron*specimens.

Keywords: Hydrocarbures Aromatiques Polycycliques, genetic ecotoxicology, *Sarotherodon melanotheron*, adaptative polymorphisme, Sénégal.

Introduction

Estuarine and coastal ecosystems that are both spatially limited and densely populated are largely degraded by anthropogenic actions (Halpern *et al*., 2008, Levêque, 1994). In Senegal, some hydrosystems such as the Sine-Saloum Delta, Hann Bay and the Niayes do not escape this threat. Indeed, the Sine-Saloum Delta is weakened by strong anthropic pressure on resources but also by adverse weather conditions (Sarr, 2005). The mangrove of this delta undergoes several types of threats, some of which are of anthropic origin and can be summed up by an abusive and anarchic exploitation of this important element of the ecosystem (Diouf, 1996). At Hann Bay, fishing is one of the most important economic activities. This bay is the first industrial area of Senegal containing 70% of the industrial units of the peninsula of Cap Vert. Industry is the primary source of water pollution in this bay (MEPN, 2005). The Niayes ecosystem, because of these natural features, represents a favorable area for horticultural exploitation (Cissé 2000, N'diaye 2000 and Fall &Gueye, 2005). About 80% of Senegalese horticultural production is concentrated in the Niayes, which supply the population of Dakar with fresh produce (Diatta, 2008).

All these abusive exploitations are direct causes of the decline in productivity and the diversity of these ecosystems.

The genetic diversity that constitutes the raw material of evolution (Hedrick, 2001, Frankham, 2005) is involved in the ability of populations to adapt to various disturbances (Frankham & Kingslover, 2004). Genetic modifications measured in populations subject to toxic stress may result in changes in allele and genotype frequencies.

To quantify the genetic diversity of populations subjected to stress, species with a wide range of tolerance to environmental disturbances are interesting. A West African euryhaline endemic species (Faunce, 2000), *Sarotherodon melanotheron*, found in most of West Africa's aquatic ecosystems (lacustrian, estuarine, marine and hypersaline environments) was targeted. The small dispersion of its larvae due to an original mode of reproduction (oral incubation) makes it possible to guarantee the link between the biological responses observed locally and the environmental parameters.

Its proportion in estuarine and lagoon catch varies from 59% to 90% (Watanabe &Saito, 1998).In the current context of global climate change, we can question the medium-datedand long term of the populations of this species living in these anthropised sites. It is therefore necessary to highlight changes in the genetic material of individuals of *S. melanotheron* living in these environments subjected to pollution by polycyclic aromatic hydrocarbons (PAHs).

The general objective of this study is to provide genetic and environmental data that can be used to report the effects of pollution on *S. melanotheron* species. Specifically, it is: characterize the environment by the determination of Polycyclic Aromatic Hydrocarbons (PAH);

- describe genetic polymorphism to understand genetic strategies developed by specimens in response to environmental pressures;
- to highlight the effects of PAH on genes.

Materials and methods

Choice and characteristics of sites

Choiceof sites

The selected sites belong to some hydrosystems Ouest-africains. In 2009, samplings were done within the framework of the project ECLIPSES (Effets of change CLimatic and Pollution with the Sénégal in a fish Estuarian, tilapia, *Sarotherodon melanotheron*). Six sites located on the rivers of Sénégal and one in the Gambia river were selected on the basis of their level of contamination.

Characteristics of sites

Seven sites retained on the hydrosystems of Sénégal and the Gambia river are Kaolack (Saloum), Missirah and Foundiougne (estuary of Saloum), Niayes 1, Niayes 2, Hann Bay and Koular (Gambia river) (Fig.1). The characteristics of these sites are mentioned in table 1.

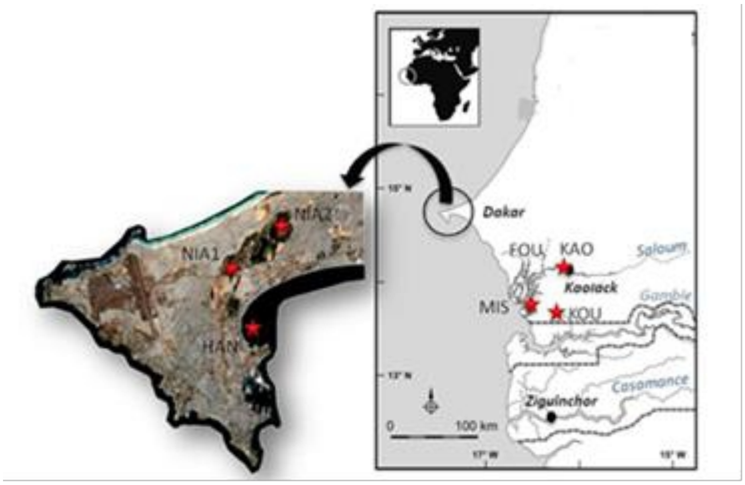

Fig 1: Map of sampling locations for *Sarotherodon melanotheron heudelotii*(BHAN = Hann Bay; KAO = Kaolack; KOU = Koular ; FOU = Foundiougne ; MIS : Missirah) and *S. m. Paludinosus* (NIA1 = Niayes 1 ; NIA2 = Niayes 2 (IRD du Sénégal) in Sénégal hydrosystems.

Table 1: Characteristics of the sampled sites

Selected sites	Hydrosystems	Salinity (g/l)	Types of anthropic pressures
Niayes 1	Niayes	58,6	Polluted
Niayes 2	Niayes	2,7	Polluted
HannBay	HannBay	34,7	Polluted by the urban and industrial activities
Foundiougne	Siné-Saloum	51,4	Polluted
Missirah	Siné-Saloum	39,3	Notpolluted
Kaolack	Siné-Saloum	102	Polluted by the urban effluents
Koular	Gambie	34,1	Notpolluted

Materials

Field sampling

A total 191 specimens of the subspecies, *S. m. heudelotii* (143) and *S. m. paludinosus* (48)(Table 2) was sampled. These specimens were captured in 2009. The sampled specimens were preserved in refrigerators and conveyed to the laboratory. In addition to these specimens, the sediments were also sampled on all sites.

Table 2.Origin of *Sarotherodon melanotheron* populations and numbers specimens sampled by site

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	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

Methods

Sampling of specimens and Sediments

Specimens of *S. m heudelotii* were fished using purse seine by experimental fishing in the basins of Saloum, Gambia and HannBay. The samples of *S. m. paludinosus*were also captured using purse seine by experimental fishing in Niayes of Senegal. Concerning the sediments, they were sampled on all seven sites.

Chemical analysis

A total of 23Polycyclic Aromatic Hydrocarbons(benzofluoranthene; Pyrene; Fluoranthene; Phenanthrene; Benzo (a) Pyrene; Anthracene; Triphenylene chrysene; Benzo (a) anthracene, Benzo (e) pyrene; Dibenzo (a) anthracene; Di (a) Chrysene; Perylene; Indeno(1,2,3-cd) Pyrene; benzopyrene; Benzonaphtothiophene; Dibenzothiophene; Methylnaphthalene, Methylphenanthene, Fluorene, Acenaphthene, Acenaphthylene, Naphtha)were proportioned in sampled sediments. This chemical analysis was made with an aim of characterizing the pollution of the sampled sites. For the determination and the quantification of the pollutants, proportioning was made by gaseous chromatography coupled with the mass spectrometry (GC-MS).

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 withseven 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 were designated 100 for each locus.

Statistical analysis

Chemical data

The concentrations of pollutants obtained on the sites were treated using the software Statistica 7.1. To see the adaptive character of the locus, the correlation between pollutants and locus were calculated.

Genetic data

The analysis and the interpretation of the data made it possible to identify, for each locus enzymatic, the genotypes and the alleles present in the samples and to establish their frequency. These frequencies were established using the software of GENETIX 4.3 (Belkhir *et al.*, 2004). These identified frequencies make it possible to see the adaptive character of the locus according to the characteristics of each locality.

Results and Discussion

Results

Description of the enzymatic polymorphism of *Sarotherodon melanotheron*

Genetic diversity, of 143 specimens of *Sarotherodon melanotheron heudelotii* and 48 individuals of *Sarotherodon melanotheron paludinosus*, was analyzed for seven enzymatic systems: AAT, ADH, IDHP, MDH, PGM, PGI and EST. The analyses were carried out o only for the 10 locus which appeared polymorphic (AAT-2, ADH-2, IDHP, MDH-1, MDH-2, PGM, PGI-2, EST-1, EST-3 and EST-14). All are polymorphic with the level 95%. The various alleles identified on the level of these loci, their relative mobilities and their frequencies are presented in table 3. Two alleles were observed for locus ADH-2, AAT-2, IDHP, MDH-1, MDH-2, PGI-2, PGM and four alleles for locus EST-1, EST-3 and EST-14.

Within the populations of *S. m. heudelotii* all the 10 locus appeared polymorphic at the specimens of Koalack (Saloum). At the other populations of Saloum, alleles PGM*100 is fixed at Foundiougne and Hann Bay and PGI-2*80 is also fixed at Missirah. .Allele IDHP*105 was not expressed at the specimens of Koular. EST-1*110, EST-3*110 and EST-14*110 are alleles specific to the specimens of Bay of Hann (Table 3).

At *S. m. paludinosus* only locus PGM is monomorphe. Within this subspecies, alleles PGM*105, EST-1*110, EST-3*110 and EST-14*110 were not observed. At the specimens of Niayes 2, allele ADH-2*80 was not expressed.

The frequency of allele PGM*105 decreased considerably when one passes from a no polluted site (0.12 to 0.30) to a polluted site (0 to 0.05).

Table 3 : Allelic frequencies of the two subspecies of *S. melanotheron* sampled; FOU = Foundiougne ; KAO = Kaolack ; MIS = Missirah ; KOU = Koular ; HAN = Baie de Hann ; NIA1 = Niayes 1 ; NIA2 = Niayes 2.

	S. m.	*heudelotii*				*S. m. paludinosus*	
		Saloum		Gambie	Hann Bay	Niayes	
Populations	FOU	KAO	MIS	KOU	HAN	NIA1	NIA2
N Locus	30	29	29	28	27	18	30

ADH-2							
80	0.42	0.47	0.41	0.52	----	0.14	----
100	0.58	0.53	0.59	0.48	1	0.86	1
AAT-2							
80	0.87	0.91	0.91	0.84	0.88	0.64	0.75
100	0.13	0.09	0.09	0.16	0.12	0.36	0.25
IDHP							
100	0.90	0.86	0.85	1	0.81	0.61	0.70
105	0.10	0.14	0.15	----	0.19	0.39	0.30
MDH-1							
100	0.83	0.66	0.71	0.86	0.87	0.25	0.47
105	0.17	0.34	0.29	0.14	0.13	0.75	0.53
MDH-2							
100	0.87	0.93	0.79	0.88	0.78	0.78	0.93
105	0.13	0.07	0.21	0.12	0.22	0.22	0.07
PGI-2							
80	0.82	0.93	1	0.66	0.82	0.77	0.91
100	0.18	0.07	----	0.34	0.18	0.23	0.09
PGM							
100	1	0.95	0.88	0.70	1	1	1
105	----	0.05	0.12	0.30	----	----	----
EST-1							
90	0.17	0.15	0.22	0.28	0.13	0.14	0.06
100	0.69	0.70	0.62	0.55	0.63	0.72	0.79
105	0.14	0.15	0.16	0.17	0.18	0.14	0.15
110	----	----	----	----	0.06	----	----
EST-3							
90	0.05	0.09	0.14	0.09	0.04	0.19	0.14
100	0.74	0.72	0.68	0.71	0.61	0.67	0.66
105	0.21	0.19	0.18	0.20	0.28	0.14	0.20
110	----	----	----	----	0.07	----	----
EST-14							
90	0.09	0.18	0.14	0.21	0.11	0.14	0.06
100	0.86	0.76	0.76	0.72	0.67	0.72	0.79
105	0.05	0.06	0.10	0.07	0.13	0.14	0.15
110	----	----	----	----	0.09	----	----

Polycyclic Aromatic Hydrocarbon (PAH) Content

The concentrations of Polycyclic Aromatic Hydrocarbure (PAH) proportioned in the sediments are presentedin Table 4.

The highest overall PAH values were obtained at Foundioungne and Hann Bay. The high pollution of these environments is characterized in large part by the PAH napht group (Foundiougne (14378 ng/g), Hann Bay (5856 ng/g), among these PAHs napth, fluoranthene (Foundiougne (2132 ng/g), Hann Bay (643 ng/g) and Benzo fluoranthene (Foundiougne (2055 ng/g), Hann Bay (946 ng/g) have the highest concentrations.The contents obtained in the other five sites are relatively weak. However, the lowest levels were recorded in Koular and Missirah.

Table 4 : Concentrations of PAHs on the sampling sites.

	MISS	KOU	KAO	NIA 1	NIA 2	BHAN	FOU
Napht (ng /g)	nd	0,3	nd	nd	1,0	2,4	4,4
Acenaphthylene (ng /g)	nd	nd	nd	nd	0,2	2,9	27
Acenaphtene (ng /g)	nd	nd	0,4	nd	0,2	159	40
Fluorene (ng /g)	0,1	0,003	0,6	0,03	0,6	70	46
Phe (ng /g)	0,4	0,001	5,4	1,1	3,0	293	823
An (ng /g)	0,2	0,05	0,6	0,2	0,5	59	260
Fluo (ng /g)	1,2	0,2	8,2	2,9	3,8	643	2132
Pyr(ng /g)	1,1	0,3	6,9	2,7	3,5	509	1830
BaA(ng /g)	0,6	0,1	3,7	2,0	1,5	491	1213
triph+chrys(ng /g)	1,1	0,1	6,7	2,4	2,7	530	1171
BNT (ng /g)	0,1	< 0,1	1,3	0,4	0,4	109	249
BbF + BkF + BjF(ng /g)	1,5	0,1	8,5	3,7	2,8	946	2055
BeP(ng /g)	0,7	< 0,1	3,8	1,7	1,2	380	853
BaP(ng /g)	2,4	0,8	4,8	2,3	5,7	522	1157
Per (ng /g)	1,1	2,0	2,0	1,0	1,5	171	377
IP (ng /g)	0,9	< 0,1	4,0	1,4	1,4	500	1018
DaA+DaC(ng /g)	0,1	< 0,1	0,9	0,3	0,2	99	199
BP (ng /g)	0,8	0,04	4,4	1,5	1,5	372	929
SOMME HAP - Napht(ng /g)	**12**	**4**	**62**	**23**	**31**	**5856**	**14378**
2MN (ng /g)	nd	nd	0,4	nd	0,3	11	2
1MN (ng /g)	0,2	nd	0,3	nd	0,4	21	4
SOMMEMnaphts(ng /g)	**0,2**	**nd**	**0,7**	**nd**	**0,7**	**32**	**6**

Table 4 : Continuation

	MISS	KOU	KAO	NIA 1	NIA 2	BHAN	FOU
2MA (ng /g)	0,2	0,04	0,5	0,1	0,3	10	71
3MP +2MP (ng /g)	0,5	nd	6,9	0,6	1,4	58	222
9MP+1MA+1MP (ng /g)	nd	nd	5,5	nd	0,4	37	177
SOMME Mphe(ng /g)	**0,7**	**0,04**	**12**	**0,7**	**1,8**	**95**	**399**
DBT (ng /g)	0,4	0,2	0,9	0,4	0,6	17	51

nd = not determinated ; BbF = Benzo(b)fluoranthene ; BkF = benzo(k)fluoranthene ;BjF = benzo(j)fluoranthenePyr = Pyrene ; Fluo = Fluoranthene ; Phe = Phénanthrene ; BaP = Benzo(a)Pyrene ; An = Anthracene ; Tryph-chrys = Triphénylene-chrysene ; BaA = Benzo(*a*)anthracene, BeP = Benzo(*e*)pyrene; DaA = Dibenzo(a)Anthracene; DaC = Di(a)Chrysene ; Per =Pérylene ; IP = Indeno (1,2,3-cd)Pyrene; BP = Benzopyrene; BNT = Benzonaphtothiophene; DBT = : Dibenzothiophene ; MN= Métylnaphtalene ; MP = Méthylphénanthene.

Correlation between analyzed pollutants and locus

The analysis of table 5shows that some of the significant correlations between the PAHs and the locus are negative . They are those given between the PAHs (napth, DBT) and the locus IDHP, MDH1-1 and PGM. The suggests that as PAH concentrations increase at the sites, the frequencies of the alleles of locus responsible for adaptation of specimens to PAHs are decreasing.

Table5: Correlation between analyzed pollutants and locus

	ADH-2	AAT-2	IDHP	MDH-1	MDH-2	PGI-2	PGM	EST-1	EST-3	EST-14
HAP - Napth	-0,03	-0,09	**-0,19**	**-0,19**	0,07	0,02	**-0,15**	0,08	0,07	0,09
DBT	-0,05	-0,08	**-0,19**	**-0,18**	0,06	0,05	**-0,15**	0,07	0,06	0,08

Discussion

The results obtained from the analysis of PAHs have shown that significant pollution has been identified and particularly affects Hann Bay and Foundiougne. This pollution is characterized by PAH napth (Hann Bay (5856 ng / g) and Foundiougne (14378 ng / g)).

This pollution would be due to anthropogenic activities carried out on these sites. Indeed, Hann bay is the first industrial zone of Senegal. Indeed, the Hann bay is the first industrial park of Senegal. It contains 70% of the production facilities of the island of Carpe Vert. The National Observation Networkstudies indicate that chronic contamination of the Gascogne gulf by PAHs is attributable to urban, port and industrial coastal activities.The mangrove of the Sine-Saloum delta is subject to several types of threats, some of which the anthropogenic origin can be summed up by the abusive and anarchic exploitation of this important element of the ecosystem (Diouf, 1996).In this delta of Sine-Saloum, fishing constitutes the second economic activity after agriculture (Kébé, 1994). This artisanal fishing activity practiced on this ecosystem with traditional gear is a means of pollution of the waters of this estuary.

The analysis of PAHs by compound reveals that high concentrations were recorded with compounds such as Fluoranthene, BaF + BbF + BjF, benzo (a) anthracene (BaA), pyrene, BaA, Triph + chrys, BaP and IP in Foundiougne. andFluoranthene, BaF + BbF + BjF and BaA at Hann Bay. The presence of these PAHs represents a danger to the biological and genetic diversity of these environments. Indeed, all these PAHs are part of the list of priority PAHs of the US-EPA (American Agency for Environmental Protection). According to EPA-TSCA (Environmental Protection Agency-Toxic Substances Control Act) and IARC (International Association for Research on Cancer), fluoranthene has a mutagenic effect for humans and a moderate toxicity. On the other hand, EPA-TSCA and IARC mention that BaF and BbF are mutagenic, carcinogenic with high toxicity. In addition, BaF, BbF and BjF, IP, BaA and BaP have been shown to be chemical carcinogenesis (NTP, 1999). Some studies have shown that mutagenicity and carcinogenicity of PAHs also affect marine life (Belkin *et al.*, 1994, Moore &Myers 1994, Myers *et al*., 1998).

Of all these major compounds, fluoranthene is the pollutant obtained with the highest concentration. This strong presence of this compound is due to industrial activities. Indeed, fluoranthene is a product used for industrial purposes and is one of the main constituents of heavy cords made from coal (Bisson *et al*., 2005).

These substances in the majority in this bay and Foundiougne show that these sites are endangered.

The very low PAH content in Koular (Gambia River) and Missirah (Sine-Saloum) confirms that these sites are indeed reference sites, free of pollution. The Gambia River would then be undisturbed. At the level of the Niayes, NIA1 and NIA2 (polluted environment), Kaolack (Polluted by the urban effluents) the PAHs analyzed in our study are negligible. This shows that on these sites the PAHs dosed would not be responsible for the mentioned pollution.

The analysis of the allelic diversity at each enzymatic locus revealed a private allele (110 at each loci (EST-1, EST-3 and EST-110) analyzed in *S. m. heudelotii* from Hann Bay. This fact might suggest that this locus would probably intervene in the phenomena of maintenance and survival of this fish in stressed environments. Larno (2004) mentions that esterases are involved in the catabolism of some organic molecules. Amplification of an esterase gene is believed to be responsible for insecticide resistance in the mosquito (Mouche *et al*., 1986) and tolerance to cadmium and copper in the natural Drosophila population, Drosophila melanogaster (Maroni *et al*., 1987).

The frequency of the PGM * 105 allele is relatively high (0.12 to 0.30) in low-disturbance localities (Missirah and Koular), whereas it is practically nil in disturbed environments (Foundiougne, Kaolack, Baie of Hann, Niayes 1 and 2). In addition, a negative correlation is observed between the MDH-1, IDHP, PGM, and PAH. This shows that these loci are cons selected in environments polluted by PAHs. In addition, studies (Moraga *et al*., 2002, Patarnello *et al*., 1991, Nevo *et al*., 1981, Nevo & Lavie, 1989) in various environments show that in the presence of pollutants, the frequency of certain alleles PGM locus decreases. Our observations and arguments in the literature suggest that this allele would be contraindicated in disturbed environments. This argument has also been developed by Moraga *et al*. (2002). These authors suggest, moreover, that it can be used as a genetic marker of pollution. Contaminants appear to select individuals with resistant enzyme genotypes resulting in a decrease or gain in genetic variability in the population.

The polymorphisms observed at the EST-14 and PGM locus in *S. melanotheron* would then be adaptive. Thus, the impact of pollution would result in a change in the genotypic composition of the affected population. Indeed, the sensitivity of allozymes to environmental stresses would reflect the adaptive nature of individuals (Moraga *et al*., 2002). Anthropogenic factors (such as pollution) thus seem to favor the selection of individuals with a particular genetic makeup within fish populations through differential survival (Thorpe *et al*., 1981, smith *et al*., 1983; Nevo *et al*., 1984, Gillespie & Guttman, 1989 and Duan *et al*., 2001). In addition, according to Kopp *et al*. (1994), genotypes that survive exposure to pollution or salinity may be the most tolerant individuals to environmental stress. in addition, Chagnon & Guttman (1989) and Gillespie & Guttman (1989) mention that the degree of genetic variation maintained by a population may be evidence of its ability to survive in future environmental alterations by moderating or modulating the stressful effects of pollutants and providing the population with the necessary genetic plasticity.

Conclusion

This study allowed us to characterize sites of Sine saloum, Hann Bay and Niayes of Senegal and to understand the behavior of genes in *Sarotherodon melanotheron* against PAH. For site characterization, the highest levels of PAHs were found in Foundiougne sediments and Hann Bay. The low levels of PAHs in Koular (Gambia River) and Missirah confirm that these sites are indeed reference sites, free from PAH pollution.

With respect to enzymatic polymorphism, a particular polymorphism was revealed at the EST-14 and PGM locus in *S. m. heudelotii*, and PGM in *S. m. paludinosus*. The particularly low frequencies of the PGM * 105 allele in populations from disturbed areas (Foundiougne, Kaolack, Hann Bay, Niayes 1 and Niayes 2) show that this locus is involved in the response to stress. A negative correlation was observed between the PGM locus and PAHs. This shows that the decrease in the frequency of the PGM * 105 allele in disturbed environments is due to the presence of PAHs in these environment. The PGM * 105 allele is cons selected in contaminated environment.

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