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Genetic Ecotoxicology of Sarotherodon melanotheron from some Sénégal Hydrosystems Related to Level of Contamination by some Micropollutants

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Abstract

On the purpose to study the genetic ecotoxicology of Sarotherodon melanotheron, the degree of contamination due to two micropollutants (Polychlorinated biphenyls (PCBs) and Polybrominateddiphenyl ethers (PBDE)) on seven sites (Foundiougne, Kaolack, Missirah, Koular, HannBay and Niayes (1 and 2) was evaluated. Seven systems (ADH, AAT, IDHP, MDH, PGM, GPI and EST) were analyzed through the technical of enzymatic electrophoresis. The specimens and sediments used for the proportioning pollutants were sampled into 2009.The analysis of indicator PCBs was carried out by gaseous chromatography coupled to a detector with capture of electrons (GC-DCE).

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The analysis of the PBDE, was done by gaseous chromatography coupled to mass spectrometry high resolution (GC-MSHR). Highest concentrations of pollutants PCBs (19 ng/g) and PBDE (5 ng/g) were observed in Hann Bay. The analysis of allelic variability revealed an adaptive polymorphism at locus EST-14 and PGM at S. m. heudelotii; PGM at S. m. paludinosus. The presence of allele EST-14*110 in Hann Bay and weakest frequencies particularly of allele PGM*105 in populations of disturbed zones suggest her implication in the response to the undergone environmental stress. A negative correlation was observed between locus EST-14 and PBDE. The fast alleles from this locus (EST-14*110) could be selected against in populations living in contaminated environment.

Keywords: Genetic ecotoxicology; Sarotherodon melanotheron; Polychlorinated biphenyls (PCBs); Polybrominateddiphenyl ethers (PBDE); adaptatif polymorphism.

1. Introduction

The genetic ecotoxicology, also designed under the terms of " eco-genotoxicology" [1]or of toxicology of the evolution [2], is of a growing interest. The objective of genetic ecotoxicology is to seek changes induced by pollution on the genetic inheritance of the natural populations. The genetic modifications measured on populations subjected to a toxic stress can result in the deterioration of the genetic diversity and in changes of allelic and genotypic frequencies. Any modification of the genetic patrimony of a population can lead to a strong erosion of its evolutionary potential and as consequence, a reduction of its fitness particularly in an unstable, environment. This could lead in long term to the disappearance of this population. The preservation of the genetic diversity and structure of natural populations is thus of a major interest for the long-term maintenance of the ecosystems health and constitutes today a major worldwide preoccupation (Convention of Rio of 1993).

In order to quantify the genetic diversity of populations subjected to stress, species having a broad range of tolerance to their environment disturbances prove to be interesting for studies. Hence, an endemic euryhaline species of West Africa [3], Sarotherodon melanotheron that occurs in many aquatic ecosystems of West Africa was targeted for the present investigation. The weak dispersion of its larvae due to an original mode of reproduction (mouthbrooder) makes it possible to guarantee the link between the biological response observed locally and the environmental parameters. In Côte d'Ivoire, this species subject to intensive artisanal fishing in the lagoon systems [4].In the Ayamé Lake, it represents 50 % of the commercial captures[5].With a proportion of 29%, the species Sarotherodon melanotheron constitutes the main part of the captures of commercial fishing in this lake[6].

Several studies describing the variability and the genetic structure of Sarotherodon melanotheron were carried out [7, 8, 9, 10, 11, 12]. However, if many studies in ecotoxicology genetics were made [13, 14, 15, 16], less were interested in the coastal ecosystems and even less with the endemic species such as Sarotherodon melanotheron. It appears thus necessary to highlight the modifications of the genetic material of individuals subjected to pollution in the current context of global climatic changes.

The main goal of this work is to provide genetic and environmental data on the effects of pollution on the genes of S. melanotheron.

2. Materials and methods

2.1. Study sites

The selected sites belong to some west-africainshydrosystems. Six sites were located on the river Sénégal (Kaolack, Missirah and Foundiougne(estuary of Saloum), Niayes1, Niayes 2, Hann Bay) and one (Koular) on river Gambia (Figure 1). They were selected on the basis of their level of contamination. The characteristics of these sites are mentioned in table 1

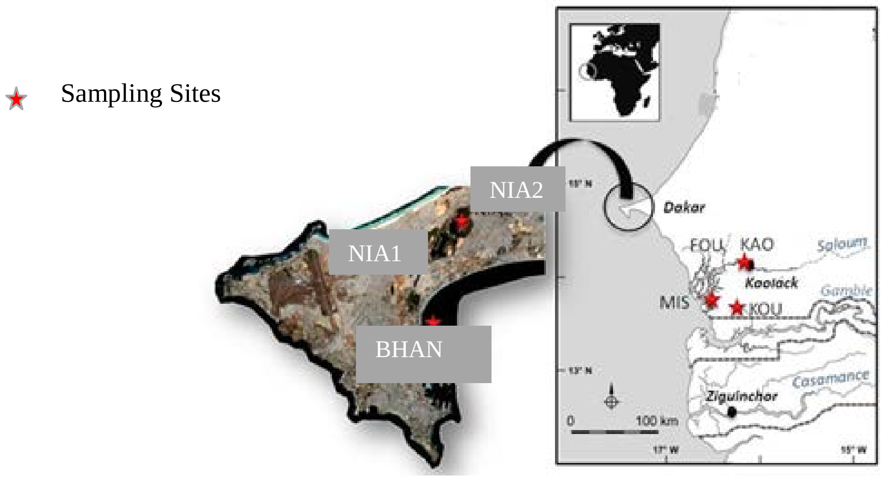


Figure 1: Map of sampling locations for Sarotherodon melanotheron heudelotii(BHAN = Hann Bay; KAO = Kaolack, KOU = Koular; FOU = Foundiougne; MIS: Missirah) and S. m. Paludinosus(NIA1 = Niayes1 ; NIA2 = Niayes 2 (IRD du Sénégal) in Sénégalhydrosystems.

2.2. Sampling in situ

A total 191 specimens of subspecies of S. melanotheron, S. m. heudelotii (143) and S. m. paludinosus (48)(Table 2) were collected. 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.

2.3. Fish and sediments sampling

Specimens of S. m.heudelotii and S. m. paludinosus were captured using purse seine through experimental fishing. Concerning the sediments, they were sampled on all seven sites.

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
Hann Bay	Hann Bay	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

Table 2.Origin of *Sarotherodon melanotheron* populations and number of 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

2.4. Chemical analysis

A total of nine pollutants were proportioned in sampled sediments. These proportioned pollutants are Polybrominateddiphenyl ethers (PBDE) and polychlorinated biphenyls or PCBs. The nine pollutants are distributed like this: two congener of PBDE (47 and 99) and seven composed of types PCBs (59+28, 52, 101, 118, 153, 138 and 180).

For the determination and the quantification of the pollutants, proportioning was made by gaseous chromatography coupled with the mass spectrometry. The analysis of the indicator PCBs was carried out by gaseous chromatography coupled to a detector with capture of electrons (GC-DCE). The analysis of the PBDE, was done by gaseous chromatography coupled to mass spectrometry high resolution (GC-MSHR).

2.5. Electrophoresis

The liver from each specimen was preserved at -80◦C until the process. 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 [17].

Genetic nomenclature was used[18]. Alleles were designated by their mobility relative to the most common allele, which were designated 100 for each locus.

2.6. Statistical analysis

2.6.1. 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.

2.6.2. Genetic data

The analysis and interpretation of 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[19].

3. Results

3.1. Description of the enzymatic polymorphism of Sarotherodon melanotheron

Genetic diversity, of 143 specimens of Sarotherodon melanotheron heudelotii and 48 individuals of paludinosus, was analyzed for seven enzymatic systems: AAT, ADH, IDHP, MDH, PGM, PGI and EST. The analyses was related to 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 of them are polymorphic with the level 95%. Alleles identified on these locus, their relative mobilities and their frequencies are presented in table 3. There were two alleles on the polymorphic locus ADH-2, AAT-2, IDHP, MDH-1, MDH-2, PGI-2, PGM and four on locus EST-1, EST-3 and EST-14.

All the 10 locus appeared polymorphic with S. m. heudelotiispecimens from Koalack (Saloum). In the other populations from Saloum, alleles PGM*100 and PGI-2*80 are fixed respectively at Foundiougne and Missirah. Allele IDHP*105 was not expressed on specimens from Koular. Alleles EST-1*110, EST-3*110 and EST-14*110 appeared specific to the specimens from Hann Bay (Table 4).

Within S. m. paludinosus populations onlylocus PGM were monomorphe. Alleles PGM*105, EST-1*110, EST-3*110 and EST-14*110 were not observed in this subspecies. Similarly, allele ADH-2*80 was not expressed in specimens from Niayes 2. The frequency of allele PGM*105 decreases considerably when pollution level increases.

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	30	29	29	28	27	18	30
Locus							
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	----	----

3.2. Sediment contents in inorganic pollutant compounds

The concentrations in organic compounds (PCBs and PBDE) proportioned in the sediments are presented both in the table 4 and in figure 2.

According to ecotoxicological evaluation criteria's suggested by the convention of Oslo-Paris(OSPAR), the normal average concentrations in polychlorinated biphenyls (PCBs) lie between 1 and 10 ng/g PS. The average highest concentrations were observed in sediments from HannBay (19 ng/g) and the weakest at Koular (0.2 ng/g). Most of the congener contents are lower than those of the witness in all the sites except for PCB-153 (6 ng/g), PCB-138 (5 ng/g) and PCB-180 (4 ng/g) whose average concentration are very high at HannBay. In addition, PCB-180 presents an average concentration higher than the normal at Missirah, Kaolack and Niayes 2. At Saloum, all the seven PCBs congeners analyzed were observed at the site of Foundiougne, six were detected in the sediments of the two other sites (Missirah and Kaolack). AtNiayes, two congeners (PCB-52, PCB-180) were detected. In Koular, in the estuary of Gambia, only PCB-52 was detected.

The highest concentration of PBDE was obtained at HannBay (0,5ng/g) and the weakest at Missirah(0.06 ng/g) (Table4). The highest concentrations of the two congeners Polybrominateddiphenyl ethers(BDE 47: 0.5 ng/g and PBDE-99: 0.1 ng/g)) were registered at HannBay. At Saloum, the two congener of PBDE were detected at Kaolack with high concentrations. However, only one congener was revealed respectively at Foundiougne (PBDE-47) and Missirah (PBDE-99). PBDE-47 and PBDE-99 were revealed in the sediments of Niayes 2 with concentrations higher than the normal. No congener, of Polybrominateddiphenyl, was observed in sediments of Koular and Niayes 1.

Overall, the chemical characterization of the seven sites shows that the Hann Bay seems to be the most polluted. Indeed, the analysis of the types of pollutants reveals that the highest contents of PCBs and PBDE were obtained in the sediments of this bay. On the other hand the analysis of the concentrations shows that the site of Koular is not polluted.

3.3. Correlation between analyzed pollutants and locus

The analysis shows that there is significant correlations between some pollutants and locus (Table 5). The significant values of correlations are mentioned in black. Negative significant correlations were observed between the PCBs and locus AAT-2, MDH-1 and PGI-2; and also between the PBDE and the EST-14.

Correlations were significant and positive between PCBs and ADH-2; PDBE and locus AAT-2, IDHP and MDH-1.

Table 4: Concentrations of PBC and PBDE in the sediments : ns = not specified.

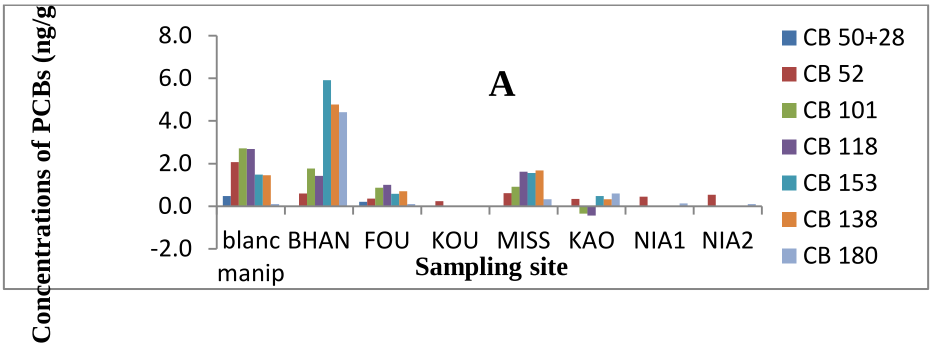
	Blanc manip	BHAN *1.1057 g*	FOU *2.3754 g*	KOU *2.2106 g*	MISS *1.1118 g*	KAO *1.1447 g*	NIA1 *1.1794 g*	NIA2 *1.0165 g*
	ng	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
PCBs 50+28	0.5	Ns	0.2	ns	ns	Ns	ns	ns
PCBs 52	2.1	0.6	0.4	0.2	0.6	0.3	0.4	0.5
PCBs 101	2.7	1.8	0.9	ns	0.9	-0.3	ns	ns
CB 118	2.7	1.4	1.0	ns	1.6	-0.4	ns	ns
PCBs 153	1.5	6	0.6	ns	1.6	0.5	ns	ns
PCBs 138	1.5	5	0.7	ns	1.7	0.3	ns	ns
PCBs 180	0.1	4	0.1	ns	0.3	0.6	0.1	0.1
∑ TOT (ng/g)	11	19	4	0.2	7	1	0.6	0.6
PBDE 47	0.01	0.4	0.1	ns	ns	0.05	ns	0.1
PBDE 99	0.02	0.1	ns	ns	0.06	0.04	ns	0.08
∑ TOT (ng/g)	0.03	0.5	0.1	ns	0.06	0.09	ns	0.18

4. Discussions

The results obtained following the analysis of pollutants (PCBs and PBDE) in the different sites showed a significant pollution level particularly at Hann Bay. The strong concentration of PCBs and PBDE in the sediments of this bay would be due to the industrial pollution. Indeed, HannBay is the main industrial park of Senegal. It contains 70% of the production facilities of CapVertisland. PCBs are produced by industries for their great chemical and thermal stability[20]. Concerning PBDE, they are abundantly used in industry, in particular for their fireproof properties [21].

The 2.2' 4.4' tetrabrominated diphenyl ethers (PBDE-47) and the 2.2',4.4'.5-penta brominated diphenyl ethers (PBDE-99) commonly called penta-mix are abundant in this bay. These results corroborate those of [22]. According to these authors, the principal congeners in the sediments are PBDE-209 followed by the PBDE-47 and PBDE-99. The weak contents of pollutants at Koular (Gambia river) shows that this site is indeed a site of reference, free from pollution. The Gambia river would be then not very disturbed contrarly to Missirah of Saloum that seems a threatened hydrosystem. At Niayesthe pollutants showed inconsiderable values. So the level of pollution reported by previous studies would be due to other pollutants.

The analysis of allelic diversity on each locus enzymatic revealed a private allele (allele 110) in each esterase analyzed on specimens of S. m. heudelotii captured at HannBay. In addition, the study of the correlations showed that locus EST-14 is negatively correlated with the PBDE. It supposes that this locus would probably intervene in the phenomena of maintenance and survival of this fish in the stressed environments. The esterases are implied in the catabolism of certain organic molecules [15]. The amplification of a gene esterase would be responsible for resistance to insecticides in the mosquitoand for the tolerance to cadmium and copper in the natural population of Drosophila, Drosophila melanogaster [23].



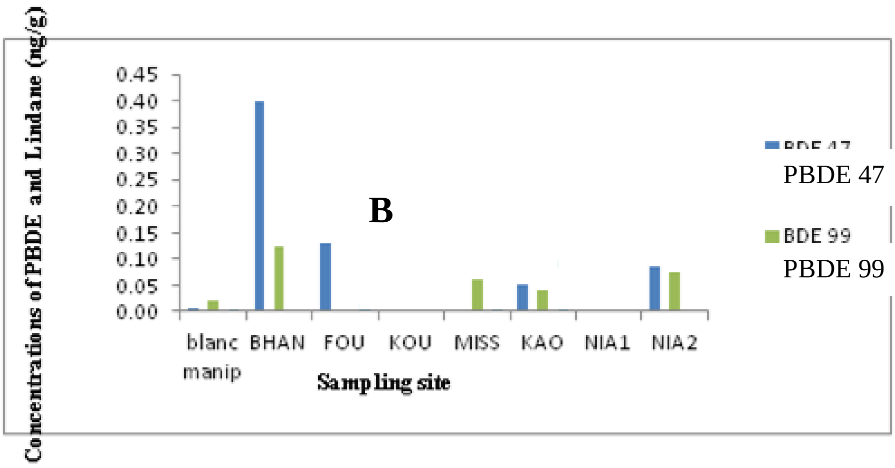


Figure 2: Concentration of the sediments in pollutants; A : Concentration in PCBs (ng/g), CB=PCBs; B : Concentration in PBDE (ng/g).

Table 5: Correlation between analyzed pollutants and locus.

	ADH-2	AAT-2	IDHP	MDH-1	MDH-2	PGI-2	PGM	EST-1	EST-3	EST-14
PCBs	**0.23**	**-0.16**	-0.03	**-0.20**	0.14	**-0.29**	-0.11	0.08	0.07	0.08
PDBE	0.08	**0.22**	**0.14**	**0.37**	-0.14	-0.01	-0.07	-0.08	-0.12	**-0.15**

The frequency of allele PGM*105, is relatively very high (0.12 to 0.30) in localities less disturbed (Missirah and Koular) whereas it appears practically absent in the disturbed environment (Foundiougne, Kaolack, HannBay, Niayes 1 and 2). Moreover, several studies[24, 25, 26, 27]in various environments show that in the presence of pollutants, the frequency of certain alleles of locus PGM decreases. These observations suppose that this allele would be selected against in the disturbed environment [24]. These authors suggest elsewhere, that this allele can be used as genetic marker of pollution.

The polymorphisms observed with locus EST-14 and PGM withS. m. heudelotii and PGM with S. m. paludinosus would be then adaptive. Thus, the impact of pollution would involve a modification of the genotypic composition of the exposed population. Indeed, the sensitivity of the allozymes to environmental stresses could reflect the adaptive nature of individuals [24]. The anthropogenic factors (such as pollution) thus seem to support the selection of individuals having a particular genetic composition within fish populations by the way of survival differential [28, 29, 30, 31, 32]. Moreover, the genotypes which survive an exposure to pollution or salinity can represent the most tolerant individuals to environmental stresses [33]. Moreover, the genetic degree of variation maintained by a population can be the proof of its capacity to survive during future environmental deteriorations.

5. Conclusion

This study enabled us to characterize sites of Sinésaloum, Hann Bay and Niayes from Sénégal and to understand the behavior of genes of Sarotherodon melanotheron under micro pollutants. The highest contents of PCBs and PBDE were obtained in the sediments of Hann Bay. These results show that pollution observed in this bay is due to the industrial wastes in PCBs and PBDE.

With regard to enzymatic polymorphism, a particular polymorphism was revealed with locus EST-14 and PGM with S. m. heudelotii and PGM with S. m. paludinosus. The presence of allele EST-14*110 in Hann Bay (highly polluted environment) and the negative correlation between this locus and PDBE reveal that this locus is implied in the response to the stresses. In addition, the particulary weak frequencies of allele PGM*105 in populations coming from disturbed zones show that they are also implied in the response to the stresses. Allele PGM*105 could be selected against in contaminated environments.

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