Bull. Environ. Contam. Toxicol. (2003) 70:1071–1082

DOI: 10.1007/s00128-003-0092-3

Bulletin of Environmental Contamination and Toxicology

Nuclear Anomalies and Blood Protein Variations in Fish of the Hooghly-Matlah River System, India, as an Indicator of Genotoxicity in Water

P. Mallick, A. R. Khuda-Bukhsh

Department of Zoology, University of Kalyani, Kalyani 741 235, West Bengal, India

Received: 6 May 2002/Accepted: 10 March 2003

Fish can be useful models for analyzing the genotoxic potential of the aquatic environment as they are constantly in direct contact with water. Attempts have been made since the 1980s to assess genotoxic activity by examining the incidence of micronuclei (MN) in peripheral blood cells of fish (Hooftman and de Raat 1982; Manna et al. 1985). Subsequently this rapid-screening method became useful to test genotoxicity in various fishes as an indicator of possible pollution (Hose et al. 1987; Metcalfe 1988; Carassco et al. 1990; de Flora et al.1993; Al-Sabti 1994; Al-Sabti and Metcalfe, 1995; Minissi et al. 1996; Poongothai et al. 1996). The other protocols commonly used to assess genotoxicity in mammalian studies, such as chromosome aberrations and sister-chromatid exchanges (SCE), are not suitable for use in fish species due to their large number of small chromosomes and low mitotic index, particularly in brackish water fish (Khuda-Bukhsh and Chakrabarti 1999; Chakrabarti and Khuda-Bukhsh 2000). Therefore, in order to strengthen the basis for ascertaining genotoxic stress to which the fish may be exposed in their natural habitat, due to various pollutants/toxicants, it was felt necessary to assess if some other parameters of study like nuclear shape anomaly, nucleo-cytoplasmic ratio, and gel electrophoretic profiles of blood plasma and hemoglobin could be of any additional help. Thus, in the present study, apart from recording incidence of micronucleated erythrocytes and erythrocytes with abnormal nucleus in natural populations of seven brackish water species of fishes collected from mainly three strategic localities, gel electrophoretic profiles of two blood proteins, namely, plasma protein and hemoglobin (Hb) have also been critically analyzed for their possible correlation with the degree of aquatic pollution, if any.

Two of the three main collection spots, Canning and Kakdwip, are part of the Sunderban, a large mangrove ecosystem in the north-east coast of India. Haldia is located a little upstream of Kakdwip alongside the Hooghly river. The Hooghly river at Kakdwip generally carries industrial effluents and sewage discharges from the industrial belt of Calcutta, and Howrah districts in West Bengal. A lot of industrial effluents are discharged in the Hooghly river at Haldia from the growing petrochemical and fertilizer industries. Canning is situated on the Matlah river in the Sunderbans, about 35 km south-east of Calcutta. Apart from factory

Correspondence to: A. R. Khuda Bukhsh

discharges of a few tanneries and some amount of sewage discharge from a part of Calcutta, the Matlah river does not carry a heavy load of other pollutants as the area is devoid of any industry. Therefore, though information on the exact quantity of different toxicants existing in these three zones is not recorded, the river Matlah at Canning has been reported to carry relatively few toxic substances as compared to Kakdwip and Haldia in the Hooghly river (Ghosh 1990; Jhingran 1990; Guhathakurta and Kaviraj 2000), and thus Canning can be considered to be relatively clean among the three sites under study.

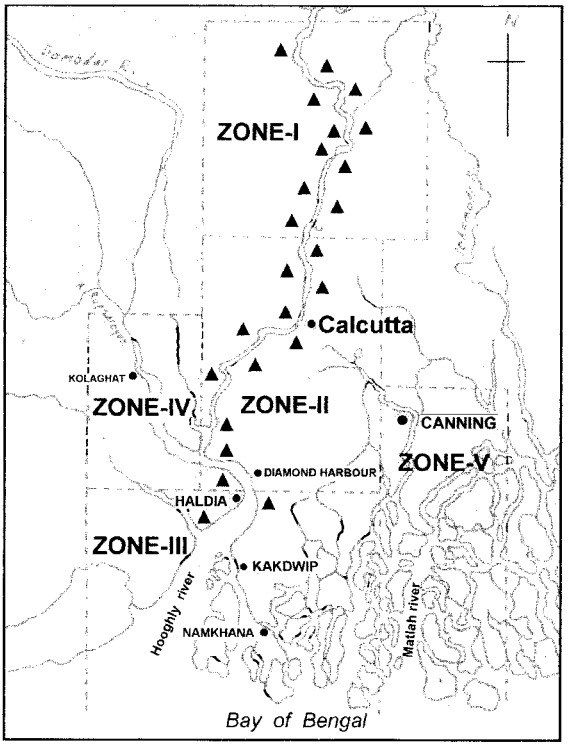


Figure 1. Showing study area.
▲ = indicates industrial area.

MATERIALS AND METHODS

Three sites (Fig. 1), Canning, Kakdwip and Haldia, of the Hooghly-Matlah estuary (between 21°32'-22°40'N and 88°85'-89°00'E) were selected strategically for the reasons stated above, and also for the easy accessibility and availability of sample collection and processing facilities at these three places.

Varying number of specimens of seven species, namely, *Lates calcarifer* (Family Centropomidae), *Liza parsia, Liza tade, Mugil cephalus, Rhinomugil corsula* (Family Mugilidae), *Terapon jarbua* (Family Teraponidae), and *Scatophagus argus* (Family Scatophagidae) collected from each of the three zones served as the materials for the present study. The sample size varied within the range of n=10 to 20, depending on the availability of a particular species of fish at a particular location. *Mugil cephalus* and *Scatophagus argus* could only be procured from Canning and Kakdwip.

Fish specimens were directly caught from the river. Blood samples (2-3 ml) were

collected from live specimens of different species by puncturing their caudal peduncle using 3.8% trisodium citrate as an anticoagulant (Jamieson and Turner 1978). 1-2 drops of blood were smeared on clean grease-free slides. Semidried slides were dipped in 90% methyl alcohol, air dried, and stained in May-Grunwald-Giemsa stain as per the routine procedure (Manna et al. 1985).

Generally, 3000 cells (600-1000 cells per slide) were scanned for each individual at random. The frequency of erythrocytes with micronuclei (MN) was scored for each individual of each species from a particular zone. Other than micronuclei the frequencies of erythrocytes with abnormal shaped nuclei (AN) viz. blebbed, lobed, notched, vacuolated or conical (Carrasco et al. 1990) were also scored. The radii of 25 normal cells per individual were measured with the help of an oculometer and the volumetric ratios between cytoplasm and nucleus (Cyt/Nu ratio) were calculated.

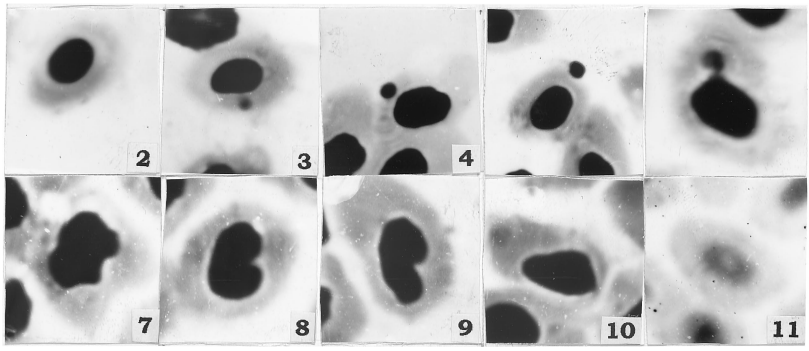
The mean data and the standard error were calculated for each sex/zone/species. Students' t-test was done to determine whether the differences in parameters i) between sexes of a particular species in a particular zone, ii) between same sex of a particular species of any two different zones and iii) between cumulative frequencies of both sexes of a particular species of any two different zones were significant or not.

Blood samples taken out from living individuals were centrifuged at 10000g for 30 min at 0°C and the supernatant (plasma protein) was electrophoresed following the method of Laemmli (1970) using Tris-Glycine buffer and 7.5% gel. The precipitations were washed thrice with 2.5% NaCl solution and hemolysed with cold distilled water. Then they were again centrifuged at 10000g for 30 min at 0°C. After centrifugation the supernatant (hemoglobin) was taken and subjected to electrophoresis.

The relative mobility (Rm), molecular weight (Mw) and nature and intensity of staining of different bands were scored for each sample as per routine procedure. For statistical analysis of band characteristics, Chi-Square test was performed.

RESULTS AND DISCUSSION

Normal erythrocytes (Fig. 2) contained mainly elliptical nuclei. The small non-refractile circular or ovoid particles lying in the cytoplasm and resembling a nucleus with respect to staining properties were considered as micronuclei (Figs. 3-5). The size of the micronuclei varied to some extent (between $^1/_5$th and $^1/_{25}$th that of nuclear size) but the number was always one, contrary to the one to several range reported by various authors (Manna et al. 1985; Rahman and Khuda-Bukhsh 1992). The position of the micronuclei in the cytoplasm also varied, some located very near to the nucleus or some located very far even at the periphery of the cell.



Figures 2-11. Photomicrographs of erythrocytes with normal nucleus (Fig.2), with micronuclei (Figs.3-5) and with anomalous nuclei (Figs.6-11): blebbed (Fig.6), lobed (Fig.7), notched (Figs.8 & 9), conical (Fig.10) and vacuolated (Fig.11).

Some of the nuclei clearly deviated from their normal shape and were either blebbed (Fig. 6), lobed (Fig. 7), notched (Figs. 8-9), vacuolated (Fig. 10) or conical (Fig. 11) or some with bizarre shape. All abnormalities of nuclei were scored.

In general, the percentages of the MN were found to be relatively high in all the species found at Haldia followed by those at Kakdwip and the lowest in the Canning populations (Fig. 12). In most of the cases these differences were highly significant ($p<0.001$) except for Canning and Kakdwip populations of *M. cephalus*. Both sexes showed more or less similar incidences of MN, for which the combined data of both males and females were taken together for analysis.

An analysis revealed that the occurrence of AN was in increasing order from Canning population to Kakdwip population and then to Haldia population and the differences were quite significant in most of the cases (Fig. 13). However, *S. argus* showed some opposite trend as observed for other species. There was a general tendency of occurrence of "lobed" and "notched" types in higher frequencies in all the species.

An increasing trend in the ratio of Cyt/Nu was found from Canning to Kakdwip and then to Haldia and also the differences between populations were quite significant in most of the cases (Fig. 14).

Micronuclei are formed by condensation of chromosomal fragments or by whole chromosomes that are not included in the main nuclei following anaphase (Heddle 1973; Schmid 1975). The mutagenicity of chemicals can be determined by MN studies along with chromosome aberration studies (Kar and Das 1987). In fact, MN assays provides an indirect measurement of the induction of structural

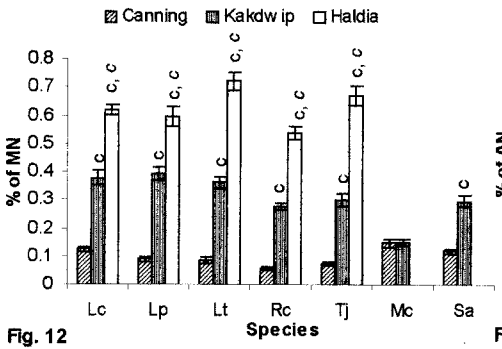


Fig. 12

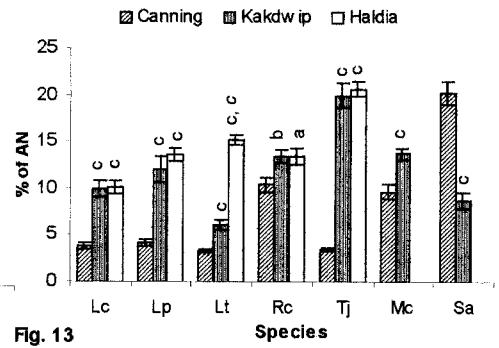


Fig. 13

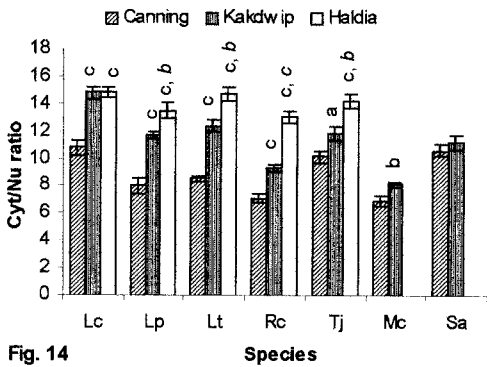


Fig. 14

Figures 12-14. Comparative data of % of MN (Fig.14), % of AN (Fig.15) and Cyt/Nu ratio (Fig.16) in seven species of fish collected from different sites. (significance levels of t-tests between Canning and Kakdwip and between Canning and Haldia denoted on the top of the bars, and between Kakdwip and Haldia by italics on the top of Haldia bar; a=p<0.05, b=p<0.01, c=p<0.001).

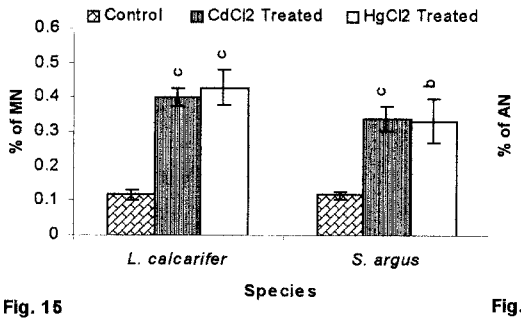


Fig. 15

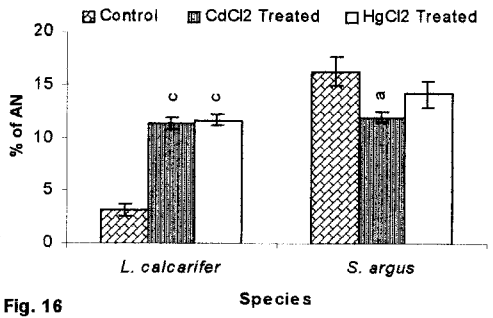


Fig. 16

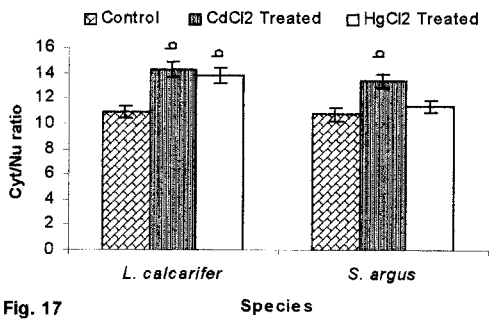


Fig. 17

Figures 15-17. Comparative data of % of MN (Fig.15), % of AN (Fig.16) and Cyt/Nu ratio (Fig.17) in *Lates calcarifer* and *Scatophagus argus* treated with $CdCl_2$ and $HgCl_2$ and their distilled water controls. (significance levels of t-tests between control and treated series denoted on the top of the respective bars; a=p<0.05, b=p<0.01, c=p<0.001).

Table 1. Shows total no. of SDS-PAGE plasma protein and hemoglobin band profiles in different species collected from different zones under study.

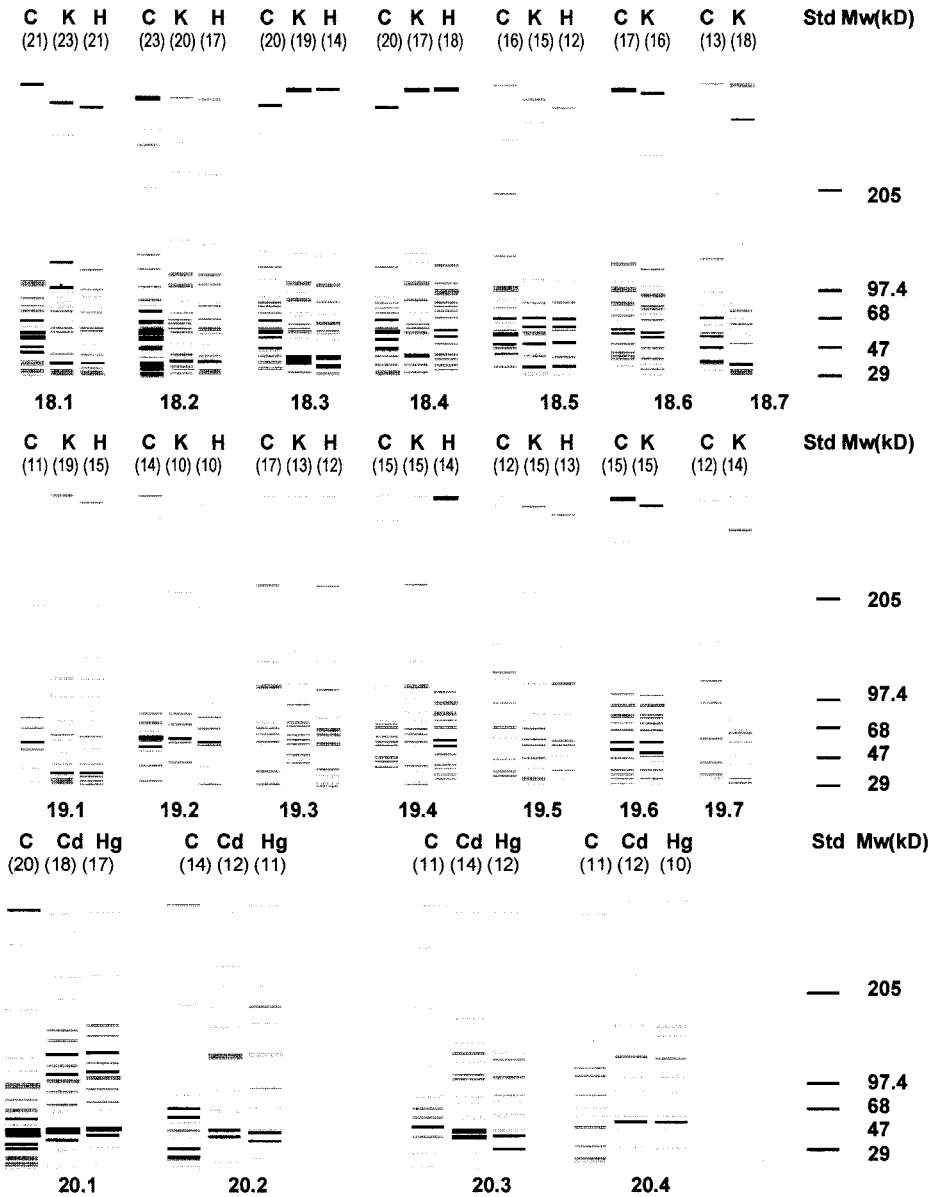
Species	Plasma protein						Hemoglobin					
	C		K		H		C		K		H	
	M	F	M	F	M	F	M	F	M	F	M	F
Lates calcarifer	21	20	23	19	21	19	11	10	19	18	15	14
Liza parsia	23	22	20	18	17	16	14	15	10	11	10	10
Liza tade	20	19	19	19	14	16	17	16	13	14	12	14
Rhinomugil corsula	20	20	17	17	18	17	15	14	15	13	14	13
Terapon jarbua	16	17	15	15	12	12	12	12	15	14	13	12
Mugil cephalus	17	18	16	14	-	-	15	15	15	14	-	-
Scatophagus argus	13	14	18	15	-	-	12	11	14	15	-	-

C=Canning, K=Kakdwip, H=Haldia.
M=Male, F=Female.

(acclimated for 15 days in laboratory condition in pollution-free salt water) to be close to the "standard" band features. This became necessary because none of these zones was absolutely pollution-free, though Canning was only relatively clean. This analysis clearly showed statistically significant differences between individuals inhabiting different zones in terms of specific bands being compared for their presence or absence and also for their differences with regard to their specific band features.

The hemoglobin (Hb) profiles and their characteristics have been provided in Table 1 and Fig. 19. Though the band profiles were fairly similar between the zones, some palpable differences in band characteristics could be observed in some cases, e.g. in *L. calcarifer* between Canning and Kakdwip, between Canning and Haldia and between Kakdwip and Haldia. Similar findings of either some bands in excess or missing were also noted for *L. tade, L. parsia* and *T. jarbua.* Detailed band characteristics of the Hb profile in *L. calcarifer* collected from different sites have been provided in Table 2 for convenience of direct comparison.

In our laboratory experiment, qualitative changes of band profiles of plasma protein and Hb were also noted in both *L. calcarifer* and *S. argus* as a result of individual treatments of $HgCl_2$ and $CdCl_2$ (Mallick and Khuda-Bukhsh, unpublished) (Fig. 20) against Dw-treated controls. This would tend to support that the exposure of fish to pollutants in natural habitat could cause damaging effects on blood in fish almost similar in nature to that observed in our lab experiments (Hooftman and deRaat,1982, Metcalfe, 1988).



Figures 18-19: Diagram of gel electropherograms of plasma protein (Fig. 18) of *L. calcarifer* (Fig. 18.1), *L. parsia* ((Fig. 18.2), *L. tade* (Fig. 18.3), *R.. corsula* (Fig. 18.4), *T. jarbua* (Fig. 18.5), *M. cephalus* (Fig. 18.6) and *S. argus* (Fig. 18.7) collected from Canning (C), Kakdwip (K) and Haldia (H), and their hemoglobin (Fig. 19), respectively (Fig 19.1-19.7). The total number of bands are given in parentheses.

Figure 20: Plasma protein patterns of *L. calcarifer* (Fig. 20.1) and *S. argus* (Fig. 20.2) in distilled water, $CdCl_2$ and $HgCl_2$ treated series and their hemoglobin patterns (Fig. 20.3-20.4) , respectively. The total number of bands are given in parentheses.

Table 2. A comparative account of gel-electrophoretic band profiles of plasma protein and hemoglobin in male (M) and female (F) *Lates calcarifer* collected from 3 different zones on the basis of molecular weight classes.

Class Intervals	Plasma Protein						Hemoglobin					
	Canning		Kakdwip		Haldia		Canning		Kakdwip		Haldia	
	M	F	M	F	M	F	M	F	M	F	M	F
21-25												
26-30	1	1	1	2	1	2	1		2	2	1	1
31-35	3	2	4	2	4	3	1	2	3	4	2	3
36-40	2	2	1	1	1	1	1	2	2	1	2	1
41-45	1	2	1	1	1	1				1		1
46-50	1	1	1	1	1	1	1		1		1	
51-55	1	1	1	2	1	1	1	1	1	1		
56-60	1		1		1	1			1	1	1	1
61-65			1	1	1	1						
66-70	1	1	1	1	1	1	1	1	1	1	1	1
71-75	1		1	1	1	1			1	1	1	
76-80	1	2	1	1	1		1	1				1
81-85	1	1	1	1	2	1						1
86-90	1		2			1			1	1	1	
91-95		1	1	1	1		1					
96-100				1	1	1		1				
101-105	1		1						1	1	1	
106-110		1										1
111-115				1								
116-120			1		1	1			1	1	1	
121-125	1	1										
126-130												
131-135												
136-140	1		1	1	1	1						
141-145		1								1	1	1
146-150									1			
.												
.												
.												
191-195	1											1
196-200		1					1	1			1	
201-205									1	1		
206-210												
.												
.												
.												
261-265			1									
266-270							1					
271-275	1	1							1			
276-280												
281-285												
286-290												
291-295					1	1						
296-300			1	1								
301-305												
306-310	1	1					1	1			1	1
311-315									1	1		

Table 3. Frequency distribution of bands arbitrarily categorized in terms of band width and staining intensities for plasma protein and hemoglobin of *Lates calcarifer* in natural populations (C=Canning;K=Kakdwip;H=Haldia) and in laboratory experimental series (Dw=Distilled water treated; $CdCl_2$=Cadmium chloride treated; $HgCl_2$ =Mercuric chloride treated). Significance (Sig) tested by Chi-square analysis.

	B+	B++	B+++	M+	M++	M+++	T+	T++	T+++	L+	L++	L+++	Sig
Plasma protein													
Dw		1				1	7	7	1	2	1		
C		1				2	5	7	4	2			0.20
K							2	7	2	9	3		-
H							5	7	1	4	3	1	0.50
$CdCl_2$			1	1	1		4	5	3	3			0.50
$HgCl_2$				1	1	1	4	5	3	2			0.50
Hemoglobin													
Dw							5	2	1	2	1		
C							4	3	1	3			0.70
K					2		4	5	1	6	1		0.20
H				1			5	5	1	2	1		0.95
$CdCl_2$				1		2	6	3		2			0.70
$HgCl_2$				2			5	1	1		2	1	0.30

B=Broad, M=Medium, T=Thin, L=Linear. One '+' indicates one unit of staining intensity.

Estuaries throughout the world have high population densities with the resulting impacts of pollution in its various forms (Spellerberg 1991). The Sunderban estuaries are no exception to this and carry a huge amount of metal load. Riverine drainage is the main source of metal contamination in these coastal areas (Mitra 1998). The Hooghly river is the main contributor of metallic pollutants in Sunderban apart from the other pollutants joining as run-off from the adjacent cities of Calcutta, Howrah and the developing industrial town Haldia (Guhathakurta and Kaviraj 2000). Further, considerable amounts of various metals, like Cd, Zn, Pb, Fe, Hg etc., also enter into these areas from sea (Bay of Bengal) through tidal waters (Chattopadhyay and Saha 1982; Jhingran 1990). Therefore, the metal pollutants downstream of Hooghly river are quite remarkable. On the other hand, Canning is situated about 20 Km away from the coast and instead of receiving direct fluxes from the Hooghly estuary receives tidal waters from the Matlah estuary. Thus metal load is less at Canning except for Fe (Guhathakurta and Kaviraj 2000).

Similar elevated frequencies of fish micronuclei and anomalous nuclei have earlier been suggested to be potent parameters to denote toxicity of the

chromosomal aberrations (Mavournin et al. 1990). While some of the various nuclear abnormalities may actually represent natural senility of the blood cells, some of them possibly represented environmental "insults". Nuclear anomalies presumably led fish to eliminate these defective cells in a survival bid, because the damaged cells could further increase the genotoxicity in unaffected cells.

The results of the present study show that the environmental stress in various fish inhabiting these habitats is also different. The occurrence of MN and AN in different fishes occurring in these zones would lead to such a conclusion. The seven species of fish collected from three strategic zones differed quite significantly from their counterparts in almost all the blood parameters examined. A definite trend could be substantiated with respect to the occurrence of nuclear abnormalities. Fishes collected from Canning in the Matlah river generally showed the least occurrence of MN and AN as compared to their counterparts collected from Kakdwip and Haldia in the Hooghly river, with the exception of *S. argus* which showed more AN in the Matlah population than in the Kakdwip population. Interestingly enough, in laboratory experiments when some toxic metallic compounds like $HgCl_2$ and $CdCl_2$, two known constituents of pollution in the Hooghly river estuaries (Jhingran 1990), were separately injected in low doses (0.007% $HgCl_2$ and 0.005% $CdCl_2$) into *L. calcarifer* and *S. argus*, two of the seven species under investigation, qualitatively similar nuclear changes were recorded (Mallick and Khuda-Bukhsh –unpublished data) in the toxic chemical treated fish at an appreciably enhanced rate than in their double distilled water (Dw) treated controls (Figs.15-17). If the occurrence of MN and AN was critically analyzed and compared, it would be revealed that along with the increase of MN, there was a decrease in the frequency of AN. One hypothesis to explain this differential response with respect to occurrence of MN and AN in these species could be that most of the anomalous nuclei might actually be precursors of MN and were more readily extruded as MN in course of combating the toxicity.

Some salient gel band characteristics of plasma protein in both males and females of different natural populations of the seven species of fishes have been summarized in Table 1 and Fig. 18. In most cases, there were differences in band number between populations and in general, the Canning populations showed a greater number of bands, particularly visible in *L. parsia*. As a representative case, the detailed band characteristics of plasma protein in *Lates calcarifer* collected from the different sites have been presented in Table 2. In some cases, however, there were some differences in the molecular weight and staining properties of individual bands although the different populations did not apparently differ much in their band numbers (Table 1, Fig. 18). The band number being same, there could still be significant differences in the band width and staining intensities apart from their difference in molecular weights. Therefore, to substantiate whether the differences in gel band characteristics of plasma protein and Hb exhibited by individuals inhabiting different zones, their band characteristics such as band width and staining intensities were compared (Table 3) by taking into consideration the patterns exhibited by Dw-treated controls

environment where the fish inhabited (Hose et al. 1987; Carrasco et al. 1990; Al-Sabti and Metcalfe 1995; Minissi et al. 1996). From the genotoxic standpoint, the occurrence of elevated frequencies of nuclear anomalies in natural populations of Kakdwip and Haldia in the Hooghly river would possibly imply that the increased level might actually be due to the presence of greater toxicity in their immediate environment. Further, the differences in the other endpoints like plasma protein and hemoglobin profiles would also lead one to suggest that the expression of different protein profiles in these populations could be due to origin of some new species of protein, which might include some "stress proteins". Alternatively, these altered proteins in blood plasma or hemoglobin could reflect subtle eco-physiological adaptations in response to various degrees of aquatic pollution prevailing locally in the different zones. Thus, the two-step study involving both cytogenetical and biochemical endpoints in different natural populations of fishes can be useful in approximating and formulating bio-monitoring strategies with regard to aquatic pollution in any given area.

Acknowledgments. Grateful acknowledgements are made to the Department of Environment and Forests, Government of India, New Delhi, for funding the project.

REFERENCES

Al-Sabti K (1994) Micronuclei induced by selenium, mercury, methyl mercury and their mixtures in binucleated blocked fish erythrocyte cells. Mutat Res 320:157-163

Al-Sabti K, Metcalfe CD (1995) Fish micronuclei for assessing genotoxicity in water. Mutat Res 343:121-135

Carrasco KR, Tilbury KL, Myers MS (1990) Assessment of the piscine micronucleus test as an *in situ* biological indicator of chemical contaminant effects. Canadian J Fish Aquat Sci 47:2123-2136

Chakrabarti J, Khuda-Bukhsh AR (2000) Chromosome banding studies in an Indian mullet: Evidence of structural rearrangements from NOR locations. Indian J Exp Biol 38:467-470

Chattopadhyay GN, Saha MS (1982) Distribution of diethylenetriamine pentaacetic acid extractable iron, manganese, copper and zinc in some brackish water mullet pond sediments of West Bengal, India. J Inland Fish Soc India 12:56-62

de Flora S, Vigano L, D'Agostini F, Camoirano A, Bagnasco M, Bennicelli C, Melodia F, Arillo A (1993) Multiple genotoxicity biomarkers in fish exposed *in situ* to polluted river water. Mutat Res 319:167-177

Ghosh A, Chakraborty PK, Naskar KR, Chattopadhyay GN, Nath D, Bhowmick ML (1990) Ecology and faunal association of intertidal mangrove habitats in the Hooghly-Matlah estuarine system. J Inland Fish Soc India 22:31-37

Guhathakurta H, Kaviraj A (2000) Heavy metal concentration in water, sediment, shrimp (*Paenaeus monodon*) and mullet (*Liza parsia*) in some brackish water ponds of Sunderban, India. Marine Pollution Bulletin 40:914-920

Heddle JA (1973) A rapid *in vivo* test for chromosomal damage. Mutat Res 18:187-190

Hooftman RN, deRaat WK (1982) Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminnow *Umbra pygmaea* by ethyl methane sulphonate. Mutat Res 104:147-152

Hose JE, Cross JN, Smith SG, Diehl D (1987) Elevated circulating erythrocyte micronuclei in fishes from contaminated sites of southern California. Mar Environ Res 22:167-176

Jamieson A, Turner RJ (1978) The extended series of Tf alleles in Atlantic cod *Gadus morhua* L. In: Jamieson, A., Turner, RJ (eds) Marine Organisms, Plenum Press, New York, p 699

Jhingran VG (1990) Aquatic pollution with special reference to Ganga river system. In: Manna GK, Jana, BB (eds) Impacts of Environment on Animals and Aquaculture, Publ. University of Kalyani, Kalyani, p 35.

Kar RN, Das RK (1987) Dominant lethality and spermatocytic chromosome aberrations in mice treated with chlordiazepoxide. Caryologia 40:267-273

Khuda-Bukhsh AR, Chakrabarti J (1999) Indication of sex chromosome pair bearing Ag-NORs in a brackish water fish, *Scatophagus argus* (Fam: Scatophagidae) showing male heterogamety. Indian J Exp Biol 37:793-797

Laemmli UK (1970) Cleavage and structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685

Manna GK, Banerjee G, Gupta S (1985) Micronucleus test in the peripheral erythrocytes of the exotic fish *Oreochromis mossambica*. Nucleus 28:176-179

Mavournin KH, Blakey DH, Cimino MC, Salamone MF, Heedle JA (1990) The *in vivo* micronucleus assay in mammalian bone marrow and periferal blood. A report of the US Environmental Protection Agency genetox program. Mutat Res 239:29-80

Metcalfe CD (1988) Induction of micronuclei and nuclear abnormalities in the erythrocytes on the midminnows (*Umbra limi*) and brown bullheads (*Ictalurus nebulosus*). Bull Environ Contam Toxicol 40:489-495

Minissi S, Ciccotti E, Rizzoni M (1996). Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, Pisces) from two natural environments: a bioassay for the *in situ* detection of mutagens in freshwater. Mutat Res 367:245-251

Mitra A (1998) Status of coastal pollution in West Bengal with special reference to heavy metals. J Indian Ocn Std 5:135-138

Poongothai K, Shayin S, Usharani MV (1996) Induction of micronuclei in fish by polluted water and heavy metals. Cytobios 86:17-22

Rahman A, Khuda-Bukhsh AR (1992) Genotoxic potential of some industrial effluents and chemical pollutants in fish by a rapid screening method. J Freshwater Biol 4:39-44

Schmid W (1975) The micronucleus test. Mutat Res 31:9-15

Spellerberg IF (1991) Monitoring Ecological Change. Cambridge University Press, Cambridge, UK.