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Assessment of Micronucleation and Abnormal Nucleation in the Peripheral Erythrocytes of the Fish *Mystus gulio* of Hooghly River Downstream as per Seasonal Variation

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Abstract: The aim of the study was to identify the frequencies of micro nucleations (MN) and nuclear abnormalities (NAs) in the peripheral erythrocytes of fish (*Mystus gulio* Ham. – Buch.) inhabiting downstream at three locations of river Hooghly, West Bengal, India. The study area mainly consists of three sampling sites, viz., Budge Budge (Bg), Batanagar (Bt) and Birlapur (Br), which were selected. The present study is a first-time endeavour to know the environmental status with particular reference to water pollution through genetic biomonitoring in the Hooghly River of a middle stretch from Batanagar to Birlapur near Diamond-Harbour coastal zone in the inhabiting fish (*M. julio*). The genotoxic effect, especially induction of MN and NAs in the peripheral erythrocytes, was done on the studied fish. In the case of MN frequencies (%), the values for sites Bt1 & Bt2, Bg1 & Bg2 and Br1 & Br2 were observed 1.92±0.10, 1.40±0.24 and 2.00±0.13, respectively were increased during pre-monsoon season compared to post-monsoon season (1.83 ±0.15, 1.36 ±0.04 and 1.91 ±0.12, respectively). The frequencies (%) of NA, such as lobed nuclei (LN), blebbed nuclei (BLN), notch nuclei (NN), bi-nuclei (BN), dumble-shaped nuclei (DSN), retracted nuclei (RN), nuclear caryolysis (NC), and fragmented nuclei (FN) values, were also observed in the fishes of three study sites. In the case of NA frequencies (%), the values for sites Bt1&Bt2, Bg1&Bg2 and Br1&Br2 were observed higher for BLN (1.54±0.09, 1.14±0.07 and 1.77±0.10, respectively), BN (1.09±0.04, 0.85±0.21 and 1.32±0.06, respectively), NN (1.02±0.09, 0.61±0.16 and 1.18±0.06, respectively), LN (1.86±0.08, 1.22±0.07 and 2.12±0.11, respectively), DSN (2.22±0.22, 1.69±0.08 and 2.56±0.11, respectively), RN (2.33±0.15, 1.82±0.13 and 2.73±0.05, respectively), FN (2.20±0.10, 1.72±0.08 and 2.56±0.13, respectively) and NC (3.01±0.06, 2.72±0.11 and 3.32±0.08, respectively) during pre-monsoon season when compared to post-monsoon season (BLN = 1.50 ±0.07, 1.06 ±0.03 and 1.73 ±0.07; BN = 0.83 ±0.11, 0.62 ±0.11 and 1.18 ±0.06; NN = 0.77 ±0.15, 0.43 ±0.11 and 1.05 ±0.03; LN = 1.42 ±0.06, 1.14 ±0.06 and 2.06 ±0.08; DSN = 1.76 ±0.06, 1.34 ±0.05 and 2.36±0.08; RN = 1.68 ±0.08, 1.30 ±0.06 and 2.31 ±0.08; FN = 1.59 ±0.08, 1.18 ±0.04 and 2.46±0.11 and NC = 2.58 ±0.53, 2.37 ±0.43 and 3.18 ±0.09, respectively). This study provides an important impact of mutagenic risk on the fish specimen, which may vanish due to the long-term effect of genotoxins or a combination of other pollutants. This specimen may show an alarming impact of water pollution, and this study is suggested for future study with other fish species to know the risk of vulnerability of mutation.

Introduction

The concentration of various metallic elements like Cu, Zn, Fe, Pb, Cd, Cr and Ni were evaluated by various researchers in surface water as well as in the sediment of the river Ganges at upstream sites followed by river Hooghly at downstream sites (Mitra, 1998; Gupta et al., 2009; Mitra et al., 2012; Goswami and Sharda, 2014; Sarkar et al., 2017; Mauryaa et al., 2019; Mandal, 2020; Mondal et al., 2021; Roy et al., 2022; Mondal et al., 2022; Mandal and Chatterjee, 2022; 2023). Heavy metal

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contamination in the water of the river Hooghly within the stretch of West Bengal has been reviewed by Paul (2017), while Bonnail et al. (2019) examined heavy metals containing sediments in the different depths of the river.

Figure 1. Satellite image of study area (source: Google Earth)

Metal pollution can cause genotoxicity, particularly the induction of micro nucleation (MN) and nuclear abnormalities (NAs) in numerous fish species (Al-Sabti and Metcalfe, 1995; Talapatra and Banerjee, 2007; Elgendy et al., 2017; Talapatra et al., 2014; Mandal, 2020; Mondal et al., 2021; Flores-Galván et al., 2020; Chakraborty et al., 2023). Fish variety *Liza parsia* lives in the Sundarbans' coastal regions, and this was found to have abnormal nucleation in their peripheral erythrocytes (Mondal et al., 2021). It was observed by them that MN and NAs such as lobed nuclei (LN), blebbed nuclei (BLN), notch nuclei (NN), bi-nuclei (BN), dumble-shaped nuclei (DSN), vacuolated nuclei (VN), retracted nuclei (RN), nuclear caryolysis (NC), and fragmented nuclei (FN) in fish inhabited in the Hatania-Doania river connected with river Hooghly of Sundarbans coastal zone during monsoon period. Their findings indicated that the frequency was much greater in the downstream location in comparison to the upstream location. Various researchers found that metal bioaccumulation in fish organs causes genotoxic consequences such as DNA damage, MN, Nas, etc. (Omar et al., 2012; Nagpure et al., 2015; 2016; Igbo et al., 2018). According to Hussain et al. (2018), fish genotoxicity is a genotoxic indicator, and blood is a useful biomarker for assessing the risk of water pollution and genotoxin.

Limited research has been conducted on fish genotoxicity in order to assess the genetic pollution caused by water contamination in the Ganges River (Nagpure et al., 2015; 2016; Mandal, 2020; Mondal et al., 2021). However, there is a dearth of information on the health state of the fish species in the downstream area of the Hooghly River, particularly in relation to the research of its genotoxic effects.

The study aimed to determine the occurrence rates of micronucleations and nuclear abnormalities in the peripheral erythrocytes of fish (*Mystus gulio* Ham. – Buch.) residing in the downstream regions of the Hooghly River at three specific places.

Materials and Methods

The study area mainly three sampling sites such as Batanagar as Bt (Latitude = 22º30′N and Longitude = 88º12′E), Budge Budge as Bg (Latitude = 22º28′N and Longitude = 88º08′E) and Birlapur as Br (Latitude = 22º26′N and Longitude = 88º08′E) were selected. Figure 1 shows the study regions' satellite image.

The chosen fish species, usually called Gulse tangra (scientific name: Mystus gulio) (Hamilton – Buchanan, 1822), is a catfish belonging to the family Bagridae of order Siluriformes. The species is commonly referred to as Gangetic Mystus and is found in India, Bangladesh, Pakistan, Nepal, Sri Lanka, Thailand, and Myanmar. The animal in question is a fish that exhibits euryomnivorous and predatory feeding behavior, meaning it has a diverse diet (Begum et al., 2008). It is much sought after because of its affordability and its significant nutritional content.

Each study site was divided into two sites. The Bt site was divided into Bt1 and 2, the Bg site was divided into Bg1 and 2, and the Br site was divided into Br1 and 2, respectively. In each study site, the 5 fish samples were collected as having just died during the pre-monsoon and post-monsoon seasons of the year 2020.

Blood was collected from the hearts of dead fish specimens using an insulin syringe. Genotoxicity was evaluated in a total of 10 fish samples. After blood collection, two slides were prepared for each fish to

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create smears. The slides were dried at room temperature and stored in a slide box for MN and NA test.

The genotoxicity screening, particularly MN and NAs screening, was executed according to the methods of Palhares and Grisola (2002) and Talapatra and Banerjee (2007) with some adjustments during pre- and post-monsoon seasons. The frequencies of micronuclei (MN) and nuclear anomalies (NAs) in the peripheral erythrocytes were thoroughly evaluated by using the methodology introduced by Fenech (1993).

The smeared slides were dried for 24 hours, stained with a 5% Giemsa solution, air-dried, and finally ready for long-term usage. Ten minutes in 100% methanol was used for fixation. Every specimen was analyzed using a bright field microscope (magnification: 1000X) with oil immersion. A total of 1000 erythrocytes per slide were examined and scored. The presence of micronuclei (MN) was determined by applying the criteria employed by Fenech et al. (2003). Additional nuclear abnormalities (NA) were individually documented based on the criteria established by Da Silva Souza and Fontanetti (2006) as well as Mondal et al. (2021). The identified nuclear abnormalities included a variety of aberrations such as lobed nuclei (LN), micronuclei (MN) found either independently or attached to the main nucleus, blabbed nuclei (BLN), notched nuclei (NN), nuclear fragmentation (NF), bi-nucleated erythrocytes (BN), vacuolated nuclei (VN), nuclear cariolysis (NC), Dumble shaped nuclei (DSN), and Retracted nuclei (RN).

Results

The present observations recommend an alarming risk of genotoxicity in the peripheral erythrocytes of the fish, *M. gulio*, through the induction of MN and NA such as BLN, BN, NN, LN, DSN, VN, RN, NC, and FN.

The percentages of MN and various NA frequencies were recorded in fish species of three study sites (Table 1). In the case of MN frequencies (%), the values for sites Bt1&Bt2, Bg1&Bg2 and Br1&Br2 were observed at 1.92±0.10, 1.40±0.24 and 2.00±0.13, respectively, during pre-monsoon season.

The percentages of frequencies of NA such as BLN, BN, NN, LN, DSN, RN, FN and NC values were also observed in the fishes of three study sites (Table 1). In case of NA frequencies (%), the values for sites Bt1 & Bt2, Bg1 & Bg2 and Br1 & Br2 were observed for BLN(1.54±0.09, 1.14±0.07 and 1.77±0.10, respectively), BN (1.09±0.04, 0.85±0.21 and 1.32±0.06, respectively), NN (1.02±0.09, 0.61±0.16 and 1.18±0.06, respectively), LN (1.86±0.08, 1.22±0.07 and 2.12±0.11, respectively), DSN (2.22±0.22, 1.69±0.08 and 2.56±0.11, respectively), RN (2.33±0.15, 1.82±0.13 and 2.73±0.05, respectively), FN (2.20±0.10, 1.72±0.08 and 2.56±0.13, respectively) and NC (3.01±0.06, 2.72±0.11 and 3.32±0.08, respectively), during pre-monsoon season.

The frequencies (%) of MN and different NA were observed in fish species of three study sites (Table 2). In the case of MN frequencies (%), the values for sites Bt1&Bt2, Bg1&Bg2 and Br1&Br2 were observed at 1.83 ±0.15, 1.36 ±0.04 and 1.91 ±0.12, respectively, during post-monsoon season.

The frequencies (%) of NA, such as BLN, BN, NN, LN, DSN, RN, FN and NC values, were also observed in the fishes of three study sites (Table 2). In case of NA frequencies (%), the valuesfor sites Bt1&Bt2, Bg1&Bg2 and Br1 & Br2 were observed for BLN (1.50 ±0.07, 1.06 ±0.03 and 1.73 ±0.07, respectively), BN (0.83 ±0.11, 0.62 ±0.11 and 1.18 ±0.06, respectively), NN (0.77 ±0.15, 0.43 ±0.11 and 1.05 ±0.03, respectively), LN (1.42 ±0.06, 1.14 ±0.06 and 2.06 ±0.08, respectively), DSN (1.76 ±0.06, 1.34 ±0.05 and 2.36±0.08, respectively), RN (1.68 ±0.08, 1.30 ±0.06 and 2.31 ±0.08, respectively), FN (1.59

Table 1. Frequencies (%) of MN and NA in the peripheral erythrocytes of fish *Mystus gulio* inhabiting three sites of river Hooghly during pre-monsoon season.

Sites	MN	BLN	BN	NN	LN	DSN	RN	FN	NC
Bt1 & Bt2	1.92 ± 0.10	1.54 ± 0.09	1.09 ± 0.04	1.02 ± 0.09	1.86 ± 0.08	2.22 ± 0.22	2.33 ± 0.15	2.20 ± 0.10	3.01 ± 0.06
Bg1&Bg2	1.40 ± 0.24	1.14 ± 0.07	0.85 ± 0.21	0.61 ± 0.16	1.22 ± 0.07	1.69 ± 0.08	1.82 ± 0.13	1.72 ± 0.08	2.72 ± 0.11
Br1&Br2	2.00 ± 0.13	1.77 ± 0.10	1.32 ± 0.06	1.18 ± 0.06	2.12 ± 0.11	2.56 ± 0.11	2.73 ± 0.05	2.56 ± 0.13	3.32 ± 0.08

MN = Micronucleus; BLN = Blebbed nuclei; BN = Binuclei; NN = Notch nuclei; NA = Nuclear abnormalities; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei; FN = Fragmented nuclei and NC = Nuclear cariolysis

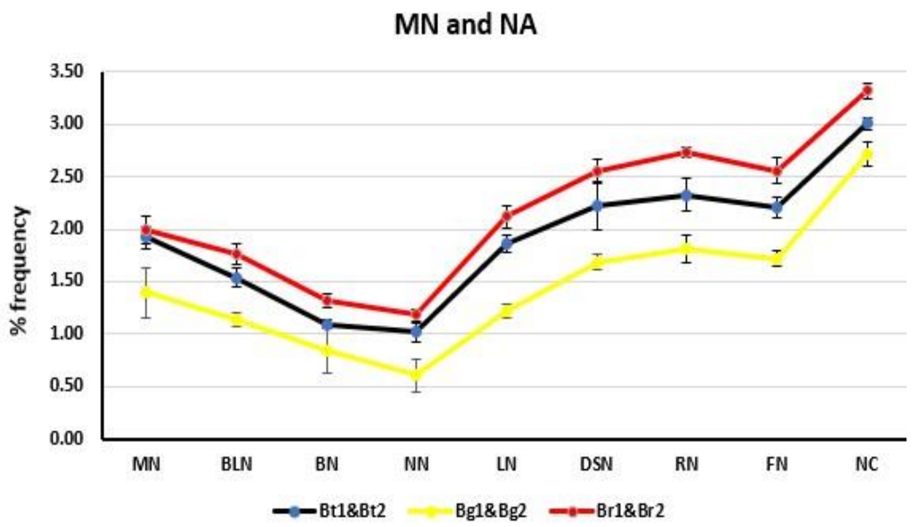

Figure 2. Frequencies (%) of MN and NA in the peripheral erythrocytes of fish *Mystus gulio* inhabiting in three sites of Hooghly River during the summer season (MN = Micronucleus; BLN = Blebbed nuclei; BN = Binuclei; NN = Notch nuclei; NA = Nuclear abnormalities; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei; FN = Fragmented nuclei and NC = Nuclear cariolysis).

±0.08, 1.18 ±0.04 and 2.46±0.11, respectively) and NC (2.58 ±0.53, 2.37 ±0.43 and 3.18 ±0.09, respectively) during post-monsoon season.

Table 2. Percentage frequencies of MN and NA in the peripheral erythrocytes of fish *Mystus gulio* inhabited three sites of Hooghly River during post-monsoon season

Sites	MN	BLN	BN	NN	LN	DSN	RN	FN	NC
Bt1 & Bt2	1.83 ± 0.15	1.50 ± 0.07	0.83 ± 0.11	0.77 ± 0.15	1.42 ± 0.06	1.76 ± 0.06	1.68 ± 0.08	1.59 ± 0.08	2.58 ± 0.53
Bg1 & Bg2	1.36 ± 0.04	1.06 ± 0.03	0.62 ± 0.11	0.43 ± 0.11	1.14 ± 0.06	1.34 ± 0.05	1.30 ± 0.06	1.18 ± 0.04	2.37 ± 0.43
Br1 & Br2	1.91 ± 0.12	1.73 ± 0.07	1.18 ± 0.06	1.05 ± 0.03	2.06 ± 0.08	2.36 ± 0.08	2.31 ± 0.08	2.46 ± 0.11	3.18 ± 0.09
MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blabbed nuclei; BN = Binuclei; NN = Notch nuclei; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei; FN = Fragmented nuclei and NC = Nuclear cariolysis									

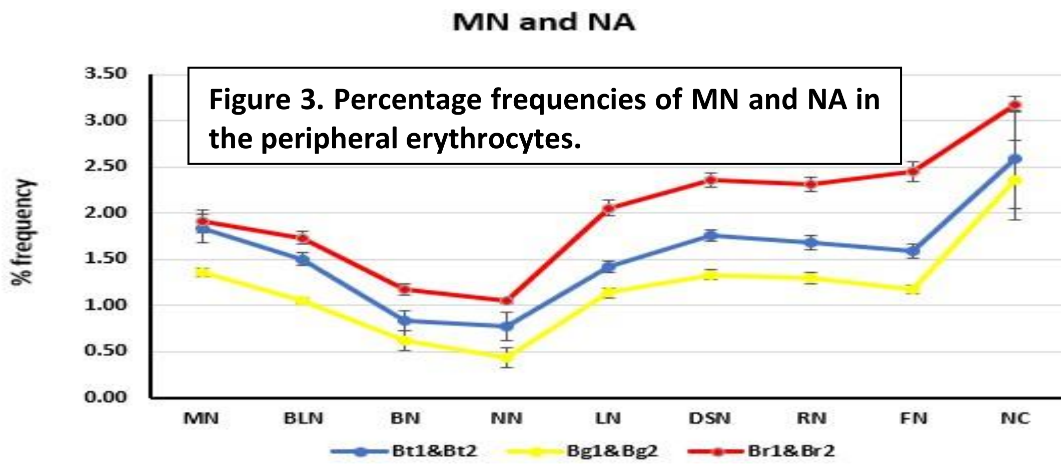

Figure 3. Percentage frequencies of MN and NA in the peripheral erythrocytes.

Figure 4. Percentage frequencies of MN and NA in the peripheral erythrocytes of fish *Mystus gulio* inhabited in three sites of Hooghly River during winter season (MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blebbed nuclei; BN = Binuclei; NN = Notch nuclei; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei; FN = Fragmented nuclei and NC = Nuclear cariolysis).

Discussion

The increased frequencies of MN and NAs were reported to cause the reduction of 96% of the population of fish species (*Labeo rohita*) of the river Chenab in Pakistan (Hussain et al., 2018). This study indicated single and double micro nucleation as well as NA in the peripheral erythrocytes of fish, *Labeo rohita*. Interestingly, the fish specimen from this polluted site (experimental) of the river significantly ($P<0.05$) induced mean frequencies (%) of single micronucleus (50.00 ± 6.30), double micronucleus (14.40 ± 2.56) and different NA as 150.00 ± 2.92 compared to non-polluted site 04.20 ± 0.13, 0.60 ± 0.40, 40.40 ± 1.21, respectively, which is comparatively lower values compared to this study. In agreement with earlier studies, similar observations were found for MN and NAs induction in other fish species inhabiting river Hooghly (Mandal, 2020; Mondal et al., 2021).

It was observed in this current study that MN and all parameters for NA were induced in the erythrocytes of fish species. However, these values were relatively lower compared to other earlier cyto-genotoxicity studies (Omar et al., 2012; Nagpure et al., 2016; Bhattacharya et al., 2016). To date, the mechanism of a few NAs is unclear (Braham et al., 2017). However, this study may be a concerning warning of genotoxic danger in the fish specimens, and this model might potentially induce mutagenic effects in individual metals or combinations with other metals or genotoxins. Interestingly, in a recent *in vivo* study, Abdullah et al. (2021) emphasized that lead, chromium and cadmium-induced genotoxicity in the fish (*Wallago attu*) and Mandal and Chatterjee (2023) observed Pb accumulation in the muscles of the fish (*Mystus gullio*) inhabiting Hooghly river. Hence, the Pb may pose genotoxicity in the present study.

This is a first-time genetic biomonitoring to determine the genotoxic risk with this test specimen. However, more investigation is necessary in this context with other inhabiting fish species to understand the genotoxic effect on edible food like fish. Generally, individual metals or combinations of metals or chemicals may alter the nuclear shape as genotoxic stress in fish (Al-Sabti and Metcalfe, 1995; Talapatra and Banerjee, 2007; Talapatra et al., 2014; Elgendy et al., 2017; Mandal, 2020; Flores-Galván et al., 2020). Interestingly, the induction of MN and NAs was found to have an increasing trend in pre-monsoon season compared to post-monsoon season, which is supported by Inayat et al. (2023), where the elevated levels of heavy metals were obtained in the summer season due to increased temperature of river Jhelum in Punjab, Pakistan.

Conclusion

The present study is a first-time endeavour to know the genotoxic risk, especially the induction MN and NAs in the peripheral erythrocytes of fish specimen (M. gulio) inhabiting river Hooghly in the middle stretch from Batanagar to Birlapur near Diamond-Harbour coastal zone.

Moreover, there is an alarming genotoxic effect, especially the induction of MN and NAs in the peripheral erythrocytes of studied fish. This study provides an important impact on the possibility of mutagenic risk on the fish specimen, which may vanish due to the long-term effect of metal pollution or a combination of other pollutants. This study is suggested for future study with other fish species to know the risk of vulnerability of mutation.

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Conflict of interest

The author declares that no conflict of interest is associated with this work's publication.

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