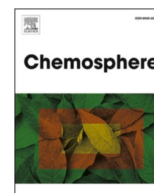
Chemosphere 351 (2024) 141236

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



Environmental bisphenol A disrupts methylation of steroidogenic genes in the ovary of Paradise threadfin *Polynemus paradiseus* via abnormal DNA methylation: Implications for human exposure and health risk assessment

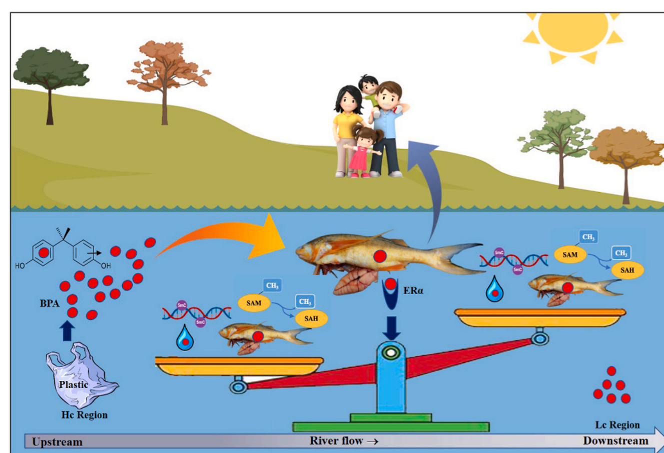
Sourav Kundu, Archisman Ray, Subhadeep Das Gupta, Ayan Biswas, Shreya Roy, Nitish Kumar Tiwari, V Santhana Kumar, Basanta Kumar Das *

ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata, 700 120, West Bengal, India

HIGHLIGHTS

- Compared to Lc region of Ganga, Hc region water and fish samples have more BPA.
- DNA methylation and *FSHR*, *20β-HSD*, and *cyp19a1* methylation density upregulated.
- Concentration of SAM and SAH was also in harmony with DNA methylation.
- BPA increased Hc-region DNMT1 and DNMT3A expression compared to Lc region.
- Human exposure to BPA from consuming Paradise threadfin was assessed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Giulia GUERRIERO

Keywords:
Endocrine-disrupting chemical
DNA methylation
Aromatase
Paradise threadfin
Human health risk

ABSTRACT

Bisphenol A, endocrine-disrupting chemicals (EDCs) impacting disease development via epigenetic modifications, is crucial in transcriptional regulation. However, ecotoxicology's limited exploration of epigenetics prompted our study's objective: examining the extended exposure of riverine Bisphenol A (BPA), a potent EDC, on DNA methylation during female paradise threadfin (*Polynemus paradiseus*) reproductive maturation. Assessing BPA contamination in riverine water, we collected fish samples from two locations with distinct contamination levels. In the highly contaminated region (Hc), we observed elevated DNA methylation in aromatase (7.5-fold), 20β-HSD (3-fold), and FSHR (2-fold) genes. Hormone receptor investigation highlighted an escalating connection between transcriptional hyper-methylation and contamination levels. Additionally, our study revealed a positive correlation between oocyte growth and global DNA methylation, suggesting BPA's potential to modify DNA methylation in female paradise threadfins. This effect likely occurs through changes in hormone receptor expression, persisting throughout oocyte maturation. Notably, our research, the first of its kind in estuarine

* Corresponding author.
E-mail address: basantakumard@gmail.com (B.K. Das).

https://doi.org/10.1016/j.chemosphere.2024.141236
Received 17 October 2023; Received in revised form 10 January 2024; Accepted 15 January 2024
Available online 17 January 2024
0045-6535/© 2024 Elsevier Ltd. All rights reserved.

areas, confirmed BPA contamination in paradise threadfins, raising concerns about potential health risks for humans.

1. Introduction

The concept of epigenetics pertains to mechanisms that induce heritable modifications in gene activity while leaving the fundamental DNA sequence unaltered. Epigenetic landmarks such as DNA methylation, histone modifications, and non-coding RNA activity exert their influence on gene expression primarily by inducing changes in chromatin structure at a localized level (Jablonka and Lamb, 2002). Epigenetic markers, unlike DNA, can be directly affected by the environment and have thus been identified as key mediators of phenotypic responses to environmental cues. Photoperiod, toxicity exposure, and nutrition have all been linked to phenotypic alterations and changes in DNA methylation in mammals (Weaver et al., 2004; Dolinoy et al., 2006; Azzi et al., 2014). Like mammals, fish DNA methylation patterns reveal a similar level of environmental sensitivity (Wang et al., 2009; Strömqvist et al., 2010; Campos et al., 2013; Artemov et al., 2017). It has been established that many epigenetic alterations caused by the environment are temporary but some of them may last throughout an organism's existence (Weaver et al., 2004; Dolinoy et al., 2006; Heijmans et al., 2008). Given that the majority of aquaculture operations are conducted in open or natural habitats that are susceptible to environmental fluctuations, it is imperative to examine the impact of epigenetics. Recent studies conducted on several aquaculture species, such as mussels, oysters, and salmonids, have yielded initial evidence indicating a connection between epigenetic pathways and economically valuable traits (Le Luyer et al., 2017; Fallet et al., 2020; Gawra et al., 2023; Dang et al., 2023; Wang et al., 2023). There exists a correlation between alterations in DNA methylation and changes in life-history features in salmonids, encompassing early male maturation, smoltification, anadromous migration, and growth capacity (Morán and Pérez-Figueroa, 2011; Morán et al., 2013; Baerwald et al., 2016; Burgerhout et al., 2017). According to a recent assessment by Wang and Bhandari (2020), medaka genome methylation reprogramming is alike in mice and humans, suggesting that medaka could be a good biological model for epigenetic studies. Bisphenol A (BPA) is an extensively used chemical which is harmful to aquatic life and European CLP regulation assigned BPA as a class 1B reproductive toxicant (Sogorb et al., 2019). BPA has been proven to cause several epigenetic changes in both animal and human cells. BPA possesses the capacity to influence both the methylation patterns of numerous genes that encode proteins associated with reproductive physiology, as well as the genes accountable for DNA methylation, particularly within the cyprinid group. BPA has been shown to affect reproductive processes via estrogenic mechanisms in breeding zebrafish, and environmentally relevant BPA doses are linked to modified transcription of critical enzymes engrossed in the maintenance of DNA methylation (Laing et al., 2016). The exposure to BPA resulted in a widespread alteration of DNA methylation in the gonads of zebrafish, as demonstrated by Liu et al., in 2016. The research conducted by Liu et al. (2014) demonstrated that exposure to BPA in the *Gobiocypris rarus* can lead to reproductive toxicity and alterations in global DNA methylation. Additionally, Zhang et al. (2018) found that BPA exposure in these minnows might cause irregular DNA methylation patterns in genes related to ovarian steroidogenesis, testicular DNA-hypermethylation (Yuan et al., 2019), epigenetic modulation via DNA and histone methylation leading to impaired ovary development (Liu et al., 2020) and by inducing oxidative stress, BPA altered DNA/histone methylation leading to reduced fertilization capacity and reproductive impairment (Zhu et al., 2021).

The species, *Polynemus paradiseus* (Linnaeus, 1758), being an amphidromous species, assumes a significant position within the intricate network of trophic interactions. The species is widely distributed in the eastern Indian Ocean and the western Pacific region and serves as a lucrative single-species fishery in the Hooghly-Matlah estuarine system. *Polynemus paradiseus*, locally known as '*Topshey*' holds a significant economic value for indigenous fish-eating populations residing within its geographical distribution. The species holds an important commercial significance due to its palatability, and nutritional composition of its flesh. The Ganga River after taking its name Hooghly passes through several cities of West Bengal before draining into the Bay of Bengal. The city sewage which includes a wide array of contaminants has been disposed into the main river channel for decades. Therefore, the examination of the species like *Polynemus paradiseus* can provide valuable insights into various aspects of contaminants persisting into the environment. The accumulation of plasticizers in biological organisms can give rise to prolonged health complications, perturb endocrine systems, and undermine the resilience of *Polynemus* populations. This prompted us to assess the presence of BPA in *Polynemus* specimens inhabiting water bodies affected with BPA.

The objective was to assess the potential impact of BPA on the alteration of DNA methylation patterns observed during premature gametogenesis, both in the Lc and Hc regions including global DNA methylation. The methylation status of genes implicated in early gonadal development and oocyte maturation was investigated using bisulfite-treated methyl-specific and semi-quantitative PCR analysis. We, in the present study also detected the methylation level of the gene encoding the follicle-stimulating hormone receptor (*FSHR*), a pituitary hormone that is well known for its involvement in fish oocyte growth and differentiation; the expression of 20β-hydroxy steroid dehydrogenase (*20β-HSD*), the enzyme accountable for the transformation of 17α-hydroxy progesterone into 17α, 20β-dihydroxy-4-pregnen-3-one, commonly referred to as DHP or maturation-inducing hormone (MIH), exhibits high efficacy in stimulating the completion of oocyte maturation in various fish species. Additionally, the expression of gonadal aromatase (*cyp19a1a*), a widely recognized physiological regulator of ovarian differentiation in fish, is also influenced. Our findings represent the initial scientific report on the health risks associated with ambient BPA for consumers of this species.

2. Materials and methods

2.1. Animal and water sampling

Wild, mature female threadfins (*P. paradiseus*) were collected (n > 50 samples per month) during commercial fish catch (Mean body wt: 35 g; total mean length: 155 mm) from two different sites. As both the site of collection points lie within the Diamond Harbour block of the district South 24 Parganas, the site locations were divided into two major locations designated as the "low contaminated" region or "Lc" region (22°11′33.72″N, 88° 11′22.2″E), and the second one (~20 km upstream) as "high contaminated" region or "Hc" region (22°16′3.91″N, 88° 5′0.61″E) in the present study. For each site, 12 individuals were used for each experiment. The sampling was done from the main channel of the estuary during early morning fishing hours during the commercial fish catch for human consumption from the period of March 2021 to May 2021. The fish sampling was performed on monthly basis and samples were collected in an ice-cold medium and RNAlater for further laboratory analysis. The ovaries of *P. paradiseus* are clearly distinguished into five phases of maturation (Refer to Supplementary Fig. S1) as per the methods elucidated by Kagwade (1970) in the species *Polynemus heptadactylus* (family: Polynemidae) and macroscopic identification of maturity stages for the Mediterranean fishery resources (Ungaro, 2008). Among the five phases, stage III (known as the Mature stage) was characterized by the increased size of the ovary, orangish-yellow in

colour, covering about 2/3 of the body cavity in length, and was chosen for the present study. Ova is transparent or translucent with one-to-many oil globules. Subsequently, along with fish sampling, water sampling was also performed from the two locations. River water samples were collected in triplicate particularly in autoclaved glass bottles to avoid the leaching effect and stored at 4 °C until further use. The fishes were dissected out individually and all tissue samples were kept in -80 °C freezer for future analysis.

2.2. Chemicals and reagents

Cytosine, 5-MeC, s-adenosyl methionine (SAM), s-adenosyl homocysteine (SAH), dimethyl sulfoxide (DMSO), N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA), N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), chlorotrimethylsilane (TMCS), HPLC-grade methanol, acetonitrile, α-phosphoric acid (85% purity) and triethylamine were procured from Sigma-Aldrich (St. Louis, MO). Chemicals and reagents used for this study are mentioned above or else of analytical grade and obtained from reputed chemical companies.

2.3. BPA extraction from water and fish tissue

One of the most widely used techniques for extracting endocrine-disrupting substances (EDCs) from water samples before chemical analysis is solid-phase extraction (SPE). We, in our study, followed the method of Kim et al. (2014) with few modifications. Briefly, on a vacuum manifold, Fisher C18 cartridges were put in position (SPE 24-port, Fisher Scientific, Pittsburgh, PA). In order to remove any remaining bonding agents, the cartridges were treated with 40 mL of deionized water and 25 mL of methanol, which were both pulled through the cartridges at a low vacuum. Then, 1-L river water sample from each of the two regions was separately loaded onto the appropriate cartridges, and it was pumped through them while being slightly vacuumed (mean flow rate: 60 mL/min). The target compounds were transferred from the aqueous samples onto the SPE cartridges during the sample loading stage. Following loading, the cartridges were rinsed with 20 mL of deionized water, and any surplus water was then removed by vacuum drying the cartridges for 5 min. The adsorbed analytes were then extracted from the cartridges in 10 mL vials using 5 mL methanol (flow rate- 5 mL/ min). The samples were derivatized by methanol eluent from SPE using a rotary vacuum evaporator (Equitron Medica Private Limited, Mumbai, India) to remove the polar functional groups in BPA. Either BSTFA with 1 percent TMCS or MSTFA with 1 percent TMCS was used to derivatize the dry residues. Each reaction vial for either agent received 100 μL of the derivatization reagent. After that, the vials were sealed and heated to 65 °C for 25 min. Test samples (100 g/ L) were derivatized in triplicate at reaction durations of 10, 20, 25, 30, 35, and 40 min. No underivatized compounds were discovered in the findings for reaction times of 25 min or longer, hence this reaction time (25 min) was kept constant throughout the investigation.

Fish gonad aliquots that were defrosted were weighed, and a glass dounce homogenizer was used to homogenise them. Samples were homogenized before being put into a 15 mL BPA-free falcon centrifuge tube and extracted with 8 mL of acetonitrile using a vortex for 2 min and an ultrasonicator for 10 min. The samples were vibromixed repeatedly for 1 min, centrifuged for 10 min at RT at 2640 g, and then extracted again with 2 mL of acetonitrile. The mixed supernatants were then applied to the SPE cartridge or kept at room temperature until further usage after being evaporated using a vacuum evaporator and collected using 2 mL of acetonitrile. (Modified from Cerkvenik-Flajs, V., & Šturm, S., 2021).

The efficiency of extracting BPA from samples obtained from both the Lc and Hc areas was examined at concentrations of 12.5, 50, 100, and 200 ng/ mL. The average recovery rates from the samples were 76.88%, 87.32%, 96.73%, and 98.47% for the Hc region, and 75.43%, 86.01%, 95.87%, and 98.23% for the Lc region, respectively. The findings suggest that the extraction efficiency of BPA from both locations is comparable, and no significant variations in extraction efficiencies were identified.

2.4. Detection of BPA using Sandwich-ELISA

In this study, we have used a sandwich-ELISA kit (Cat. No. MBS2602664) procured from MyBiosource Inc. (San Diego, CA). The method for ELISA has been described in detail in Kundu et al. (2024). To state briefly, the kit contents were kept in RT for 30 min prior to the experiment. All the reagents along with standards were reconstituted prior to the experiment. For making the standard curve, 1.0 mL of standard diluent was added to the lyophilized standard vial and was allowed to sit for 30 min after proper mixing. After the standard has completely dissolved, the concentrations used to achieve the standard curve are as follows: 200, 100, 50, 25, 12.5, 6.25, 3.12 ng/ mL. Hundred (100) μL of standards, as well as samples, were loaded to assigned ab-coated wells including blank. The plates (wells) were covered with the adhesive sealer provided with the kit and incubated at 37 °C for 90 min. After incubation, the wells were washed twice with the appropriate wash buffer and 100 μL of biotinylated Ab was added to each well. The wells were sealed again and incubated at 37 °C for 60 min. The wells were washed thrice after incubation and 100 μL of enzyme conjugate was added to each well. The wells were again incubated for 30 min at 37 °C after sealing. After the incubation period was over, wells were washed five times and 100 μL of the prepared color reagent was added to individual wells (in dark) including the blank well, sealed, and incubated at 37 °C for 30 min. After the incubation, 100 μL of the color reagent C was added to individual wells, mixed, and OD was read at 450 nm using EPOCH 2 microplate spectrophotometer (Agilent, Santa Clara, CA).

2.5. Isolation of RNA and preparation of cDNA

Total RNA was isolated from isolated female gonad tissues. Tissues were minced and homogenized using a 2 mL glass dounce homogenizer in 1 mL of TRI® Reagent (Sigma-Aldrich, St. Louis, MO) and extracted by using the standard chloroform-phenol-isopropanol method (Chomczynski and Sacchi, 1987). The total RNA was further cleaned using a DNAseI digestion and the RNeasy® Kit (Qiagen, Valencia, CA). Following the manufacturer's instructions, OligotexTM mRNA purification system (Qiagen, Valencia, CA) was used to separate poly (A) + RNA from total RNA. After isopropanol precipitation, gonad samples

Table 1
List of primers used in BS-MSP PCR for *cyp19a1a, FSHR, 20β-HSD* and in sq-RT-PCR for *DNMT1, and DNMT3A* transcript.

Name of the gene	Forward Primer	Reverse Primer	Amplicon Size (bp)
Methylation Specific Primers			
cyp19a1a	GGATCAATGGTGAGGAAACT	TCAAGACTGAACTGATTGGG	982
FSHR	GAGCTCTCCGTCTATACTCT	ACTTTTTGCCAGAGGTTGTA	974
20β-HSD	CAAAAGACGCACGTTATTCA	GCTTGTGTATGTTGTGTAGC	519
Regular Oligonucleotide Primers			
DNMT1	TTACTTTGGGCAAGAGGAGA	TCACACTCATCCACTAGGAA	685
DNMT3A	CTATTTTTCGCCAACAACCA	AAGAACACAGGAAGGTTGGA	976
GAPDH	TGGTATTAACGGATTCGGTC	TCCCAGAATTCCTTTCATGG	808

were also treated with 4 M lithium chloride for the elimination of glycogen from the pellets. RNA integrity was assessed by electrophoresis on a 1.0 percent agarose gel in TAE buffer (40 nM Tris, 40 nM EDTA), visualized using a transilluminator; and RNA purity was calculated by the A260/A280 ratio using an EPOCH2 reader (Agilent, Santa Clara, CA). In accordance with the manufacturer's instructions, 5 μg of RNA was treated with 2 U of DNAase I and incubated at 37 °C for 30 min for enzyme activation, and then at 65 °C for 10 min to inactivate it. Using a standard protocol (Pal et al., 2018), RNA was finally used for reverse transcription with oligo (DT)18 primers using a commercially available reverse transcriptase first-strand cDNA synthesis kit (iScript Reverse Transcription Supermix, Cat. No. 1708841, BioRad). The consequential cDNA (2 μL) served as the prototype for consequent PCR.

2.6. Bisulfite conversion-specific methylation-specific PCR (BS-MSP) analysis of cyp19a1a, 20β-HSD, FSHR, and RT-PCR analysis of DNMT1, DNMT3A

To conduct BS-MSP, bisulfite-specific primers for cyp19a1a, 20β-HSD, and FSHR were designed using Methprimer software (https://www.urogene.org/methprimer/) for the detection of % DNA methylation density (Table 1). Regular oligonucleotide primers for DNMT1, and DNMT3A were designed (Table 1) using Primer3web software, ver. 4.1.0 (https://primer3.ut.ee/). The selection of the optimum annealing temperature, ranging from 53 to 57 °C, was based on the ovary-specific cDNA sequences of *Oncorhynchus mykiss*, *Carassius auratus*, and *Danio rerio*, which are members of the Actinopterygii family. These sequences were obtained from the GenBank database at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and presented in Table 4 (S2). Following the utilization of conventional nucleotide BLAST (https://www.blast.ncbi.nlm.nih.gov/) to assess all the primer sets that were accessible, a single pair of primers that exhibited the most optimal alignment was chosen for further gene expression studies. The gene that was utilized for housekeeping purposes in this study was GAPDH. PCR was conducted in a 50 μL volume by combining 2.5 U of Taq DNA polymerase (FastStart™ Taq DNA Polymerase, Roche) with a master mix consisting of 20 μM of each deoxyNTP, 20 pmol of each primer, and 20 μM of each reaction buffer (composed of 50 mM KCl, 10 mM Tris-HCl [pH 8.3], and 0.1 percent Triton X-100). Before the amplification cycles, an initial denaturing step was conducted at 94 °C for 5 min (except for the last cycle, which lasted 10 min). A total of 38 amplification cycles were performed in this study using a T100 thermal cycler (BioRad, Cat no. 186–1096). Each cycle consisted of a denaturation phase at 94 °C for 30 s (except for the first cycle, which lasted 5 min), followed by annealing of primers at a predefined temperature of 55 °C for 30 s, and finally an extension step at 72 °C for 30 s. The expression study employed reverse transcription-polymerase chain reaction (RT-PCR) products that were generated using specific primers. The PCR bands have been quantified using ImageJ software (https://imagej.net/ij/index.html).

2.7. Global DNA methylation in gonad

2.7.1. Genomic DNA isolation from fish gonad

Genomic DNA from fish gonads was isolated using the protocol from Kumar et al. (2007) with slight modification. A glass Dounce homogenizer was used to homogenise 20 mg of minced fish ovarian tissue, which was then suspended in 500 μL of lysis buffer (20 mM Tris-Cl, 5 mM EDTA, 400 mM NaCl, 1% (w/v) SDS, and 400 g/ml Proteinase K). The mixture was then kept overnight at 55 °C in a shaking incubator with an oscillation of 200 rpm. The dissolved liquid was combined with an equal volume of Phenol, Chloroform, and Isoamyl Alcohol (25:24:1), which was then centrifuged at 14000 rpm for 5 min. An equivalent volume of pre-chilled isopropanol was carefully added to the upper aqueous layer in a fresh microcentrifuge, which was then centrifuged once more at 8000 rpm for 15 min. The remaining particle inside the tube was cleaned with 70% ethanol after the isopropanol layer was removed. The extracted DNA pellet was resuspended in nuclease-free water and kept at -80 °C for later usage after being air-dried in a laminar hood. Bisulfite conversion of the samples (250 ng) was executed with the EZ DNA Methylation-Lightning Kit (Zymo Research).

2.7.2. Global DNA methylation quantification

To quantify global methylation levels, the Imprint ® methylated DNA quantification ELISA kit (Cat. No. MDQ1, Sigma-Aldrich, St. Louis, MO) was employed according to the manufacturer's instructions. The amount of 5-methylcytosine (5-mC) was assessed using this kit. In summary, the DNA samples were diluted in a DNA binding solution, and afterwards, 30 μL of the diluted sample was introduced into the sample wells, with the DNA binding solution serving as a blank. The wells were securely closed and subjected to incubation at a temperature of 37 °C for a duration of 60 min. Following the incubation period, a volume of 150 μL of block solution was introduced into each well, and subsequently incubated once more at a temperature of 37 °C for 30 min. Subsequently, the samples were discarded and subjected to three washes using 150 μL of the designated working wash buffer. In the process of methylation DNA capture, the capture antibody (Ab) was diluted in a working wash buffer at a ratio of 1:1000. Subsequently, 50 μL of the diluted capture antibody was introduced into each well. The wells were securely sealed and placed in an incubator at room temperature for a duration of 60 min. Following the incubation period, the capture antibody was removed and the wells were subsequently washed four times with 150 μL of working wash solution. A volume of 50 μL of diluted detection antibody was introduced into the wells and subjected to an incubation period of 30 min at room temperature. The wells underwent a washing process consisting of five repetitions, each involving the addition of 150 μL of working wash buffer, subsequent to the removal of the detection antibody. A volume of 100 μL of developing solution was introduced into the wells and subjected to incubation at room temperature in the absence of light. During this temporal interval, a chromatic hue of blue manifested within the respective receptacles. In order to halt the reaction, 50 μL of stop solution was introduced into each well, resulting in a color change to yellow. The measurement of absorbance was conducted at a wavelength of 450 nm using the EPOCH2 microplate reader. The relative global DNA methylation was then calculated using the following equation:

$$\frac{(A450\ av\ sample - A450\ av\ blank)}{(A450\ av\ methylated\ control\ DNA - A450\ av\ blank)} x100$$

2.8. Detection of s-adenosyl methionine (SAM) and s-adenosyl homocysteine (SAH) by Sandwich-ELISA

To determine the index of methylation, SAM and SAH levels in fish gonadal tissue have been analyzed using a SAM and SAH combo sandwich-ELISA kit (Cat. No. MBS169604) procured from MyBiosource Inc. (San Diego, CA). Approximately 100 mg of fish ovarian tissue was homogenized in 500 μL ice-cold PBS and centrifuged at 10000×*g* for 15 min at RT. The supernatant was used for ELISA analysis. 50 μL of the sample, as well as standards, were added to the respective conjugate-coated plate and incubated inside an orbital shaker for 10 min. 50 μL of anti-SAM or anti-SAH Ab was added to each well and incubated using an orbital shaker for 60 min at RT. The wells were washed thrice with wash buffer solution, 100 μL of diluted secondary Ab HRP conjugate was added and incubated like the previous step. Wells were washed thrice with wash buffer and 100 μL of substrate solution was added immediately. The incubation period for this step varied from 2 to 30 min depending on the standardized protocol at RT. The reaction was stopped using a stop solution and the absorbance was read at 450 nm wavelength.

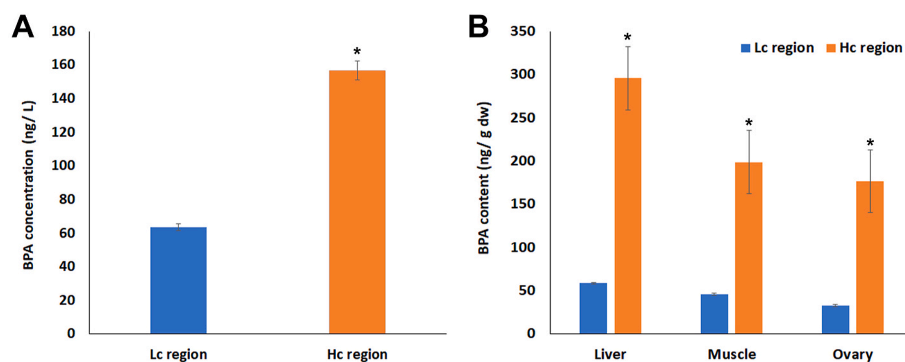


Fig. 1. BPA concentration was measured using ELISA from river water obtained from Lc and Hc region (A). BPA was extracted from water samples using solid-phase extraction. BPA content was measured from the liver, muscle and gonad samples from the fish collected from Lc and Hc region (B). Data is presented as mean ± SEM, n = 12 fish/group. *p < 0.05 vs. Lc region.

2.9. Exposure to BPA and risk assessment method

The estimation of central tendency exposures can be conducted by utilizing the mean values of the concentration of bisphenol A (BPA) in fish. Alternatively, a more cautious estimation of the mean concentration can be obtained by employing the 95% upper confidence limit of the mean concentration, as recommended by the U.S. Environmental Protection Agency (USEPA). Hence, the utilization of the mean and 95th percentile was employed to signify moderate and high levels of exposure in the assessment of exposure.

2.9.1. Estimated daily intake

The calculation of the Estimated Daily Intake (EDI) of individual BPA was based on the muscle concentration of paradise threadfins, as well as the recommended fish intake for adults (100–200 g of muscle/week) and children (25–40 g of muscle/week) as advised by the National Institute of Nutrition, ICMR (NII, 2011). The body weights used for the calculation were 60 kg for adult men, 55 kg for adult women, and 25.1 kg for children aged between 7 and 9 years (Manual, A, 2011). The EDI was calculated using the following equation (Jin et al., 2022):

$$EDI = \frac{C_m \times CR}{BW} \quad \text{[Eq. 1]}$$

Where EDI is the estimated daily intake (ng/kg-bw/day); *Cm* is the concentrations of BPA in fish muscle tissue (ng/g); CR is the consumption rates (g/day); BW is the individual's body weight (kg).

2.9.2. Target hazard quotient

The Target Hazard Quotient (THQ) is evaluated by using the potential health risks of BPA. The value is obtained by dividing the median exposure to BPA in ng/kg/day by the corresponding Reference Dose (RfD) to calculate the hazard quotient for BPA. If THQ< 1, there is limited or no clear evidence of significant health risks to the exposed population. However, if THQ> 1, it indicates the potential existence of negative health consequences. The THQ was calculated by the following equation (Jin et al., 2022):

$$THQ = \frac{EDI}{RfD} \quad \text{[Eq. 2]}$$

where 'EDI' is the estimated daily intake obtained from Eq. (1); RfD is the reference doses of BPA for non-carcinogenic effect (i.e., 0.2 ng/kg-bw/day).

2.10. Statistical analysis

The Shapiro-Wilk normality test was performed using PAST software, ver. 4.03, to assess the normal distribution and correctness of the data. The homogeneity of the data was confirmed using Levene's Test at a significance level of $p < 0.05$. Primer of Biostatistics 7.0 (McGraw-Hill, New York, NY) was used to carry out the statistical analysis. To compare the Hc and Lc groups, a one-way analysis of variance (ANOVA) was employed, followed by the Bonferroni correction. When $p < 0.05$, differences were deemed significant. The values are shown as the mean ± SEM (n = 12).

3. Results

3.1. Concentration of BPA in river water and fish tissues

We employed the sandwich-ELISA technique to determine the concentration of BPA in water samples collected from the river Ganga. The results indicated a significantly greater level of BPA concentration (p <

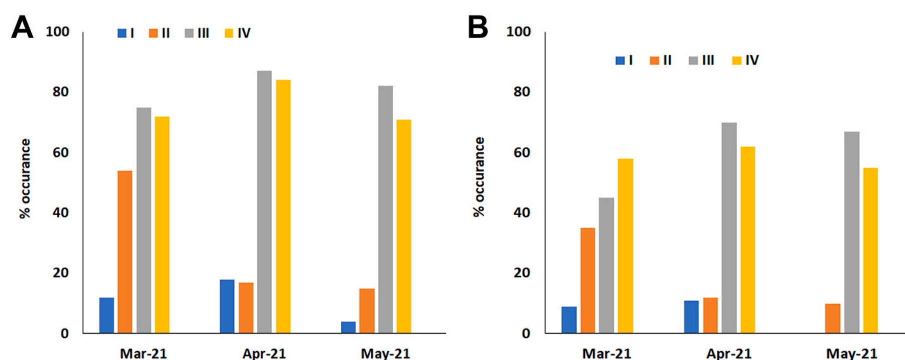


Fig. 2. Month-wise percentage occurrence of maturity stages of female Paradise threadfin found at Lc region (A) and Hc region (B). The samples were collected daily during the study period as per availability and the status of maturity stages was examined. Stage I: Immature, Stage II: Maturing, Stage III: Mature, Stage IV: Ripe.

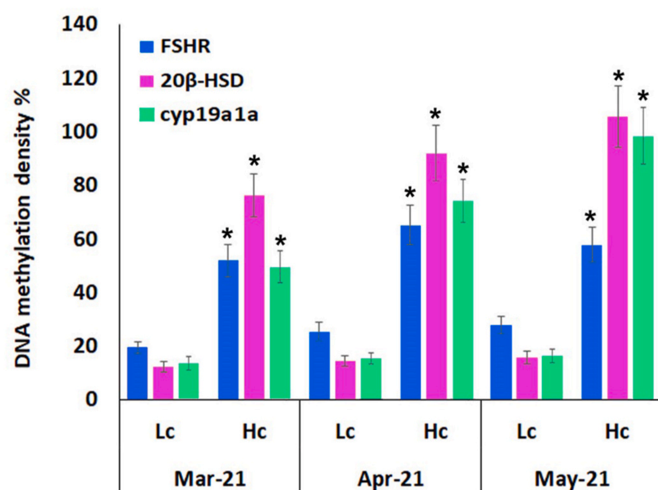


Fig. 3. Changes in the DNA methylation density %age levels of *FSHR, 20β-HSD* and *cyp19a1a* genes in gonads of female Paradise threadfins from Lc (Low contaminated region) and Hc (High contaminated region) during March to May 2021. Data is presented as mean ± SEM, n = 12 fish/group. *p < 0.05 vs. Lc region.

0.05) in the Hc region (156.8 ng/L) compared to the Lc zone (63.5 ng/L) (Fig. 1A). Likewise, a notable elevation in the quantity of BPA was seen in fish tissues. The content of BPA was determined in fish liver and muscle tissues. In the liver, BPA concentration was measured to be 58.6 ng/g-dw in samples obtained from the Lc region, whereas samples collected from the Hc region exhibited a BPA concentration of 295.63 ng/g-dw. The muscle samples yielded a value of 45.96 ng/g-dw when using the Lc area sample. Conversely, a notable rise in the BPA value (198.47 ng/g-dw) was seen in the samples acquired from the Hc region (Fig. 1B). The analysis of gonad samples revealed that the BPA concentration acquired from the Lc area sample was 32.75 ng/g-dw. Notably, a substantial rise in BPA content (176.59 ng/g-dw) was observed in the samples collected from the Hc region.

3.2. Month-wise percentage occurrence of maturity stages of female threadfin

The maximum frequent incidence of various development stages of female threadfin was observed throughout the study period spanning from March to May 2021. The obtained data indicated that there was a moderate level of availability of stage I (immature) and stage II (maturing) in the Lc region during the study period. The maximum prevalence of stage III (mature) and stage IV (ripe) was observed over the period from March to May 2021, whereas no instances of stage V (spent) were identified during this timeframe. The samples collected from the Hc region exhibited a comparable pattern of availability across different development stages, albeit with a lower percentage occurrence compared to the Lc region (Fig. 2).

3.3. DNA methylation status of genes involved in gonadal maturation

In this study, we employed reverse transcription polymerase chain reaction (RT-PCR) to investigate the expression of *cyp19a1a* and *FSHR* in mature gonadal tissue. To accomplish this, we utilized a primer set that specifically detects the methylation level in the exonic sections of the *FSHR* and *20β-HSD* genes, as well as the promoter area of the *cyp19a1a* gene. The findings of the study indicate that the methylation level of the exonic regions of the *FSHR* and *20β-HSD* genes exhibited a statistically significant increase in fish samples obtained from the Hc region compared to those from the Lc region during the months of March, April, and May 2021. Specifically, the methylation level in the Hc region was 2.65-fold and 6.18-fold higher for the *FSHR* and *20β-HSD* genes, respectively, in March 2021. Similarly, in April 2021, the methylation level was 2.56-fold and 6.37-fold higher for the *FSHR* and *20β-HSD* genes, respectively. In May 2021, the methylation level was 2.08-fold and 6.62-fold higher for the FSHR and *20β-HSD* genes, respectively (Fig. 3). It is noteworthy that the methylation level at the promoter region of the *cyp19a1a* gene exhibited a comparable pattern to that of *FSHR* and *20β-HSD*. Moreover, it was observed to be significantly elevated in the Hc region compared to the Lc region during the months of March 2021 (3.61-fold), April 2021 (4.82-fold), and May 2021 (5.97-fold) (Fig. 3). Furthermore, a significant association was seen between the methylation status of the DNA sequences of the *FSHR* and *20β-HSD* genes and the concentration of BPA in the gonads of fish collected from the Hc region. This finding provides evidence supporting the hypothesis that BPA contamination has an impact on the DNA methylation levels of

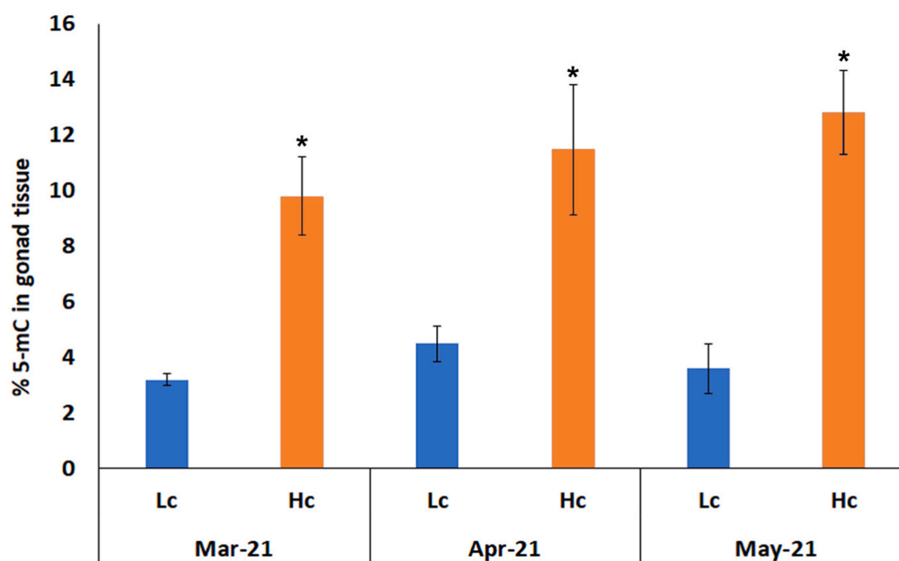


Fig. 4. Global DNA hypermethylation in mature female gonads from Lc and Hc region in the months of March, April, and May 2021. gDNA was isolated from the female gonads of the mature threadfins and used to measure the methylation. Data is presented as mean ± SEM, n = 12 fish/group. *p < 0.05 vs. Lc region.

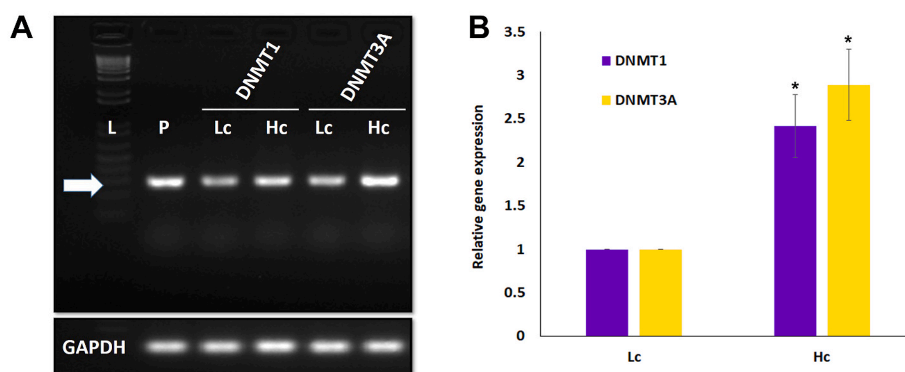


Fig. 5. RT-PCR gene expression analysis of DNMT1 and DNMT3A gene in mature female gonads from Lc and Hc region (A). GAPDH was used as a loading control. (B) The bar diagram represents fold change. L: DNA Ladder, P: +ve control, Lc: Low contaminated region, Hc: High contaminated region. Values are presented as mean ± SEM, n = 12 fish/group. *$p < 0.05$ vs. Lc region.

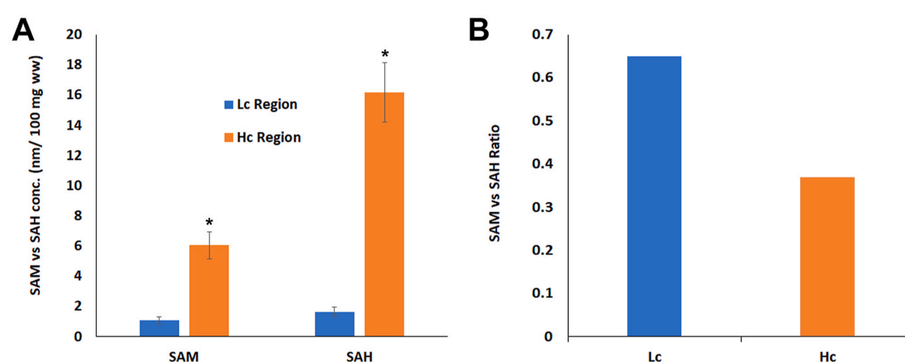


Fig. 6. SAM and SAH concentration in mature female gonads from Lc and Hc region (A). SAM and SAH ratio were calculated from the values obtained using the data from the experiment (B). Values are presented as mean ± SEM, n = 12 fish/group. *$p < 0.05$ vs. Lc region.

both the *FSHR* and *20β-HSD* genes.

3.4. Global DNA methylation status

To evaluate the extent of global hypermethylation in fish ovarian tissue caused by environmental BPA, the quantification of 5-methylcytosine (5-mC) was conducted on the DNA isolated from fish ovary. Based on the data depicted in Fig. 4, it is evident that there was a significant increase in methylation levels observed in the samples obtained from the Hc region when compared to the samples obtained from the Lc region. The statistics exhibited a 3.06-fold surge in values in the month of March 2021, succeeded by a 2.54-fold increase in April 2021. The most substantial increase was recorded in the month of May 2021, exhibiting a 3.55-fold surge in values. The observations were made based on the samples harvested from the Hc region, as opposed to the samples acquired from the Lc zone (Fig. 4).

3.5. Gene expression of DNA methyl transferases

In order to elucidate the fundamental molecular mechanism responsible for the worldwide hypermethylation induced by BPA in relation to both maintenance and de novo methylation processes, we conducted measurements of the mRNA expression levels of DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3A (DNMT3A) in both groups. The outcome is illustrated in Fig. 5. It has been observed that the expression of DNMT1 in the samples collected from the Hc region was 2.4 times higher compared to the Lc region. It is worth noting that the expression of DNMT3A, which is responsible for de novo methylation, exhibited a statistically significant increase ($p < 0.05$) in the samples collected from the Hc region compared to the Lc region (Fig. 5 A & B).

3.6. s-adenosyl methionine (SAM) and s-adenosyl homocysteine (SAH) concentration

The threadfins that experienced higher exposure to ambient BPA exhibited a substantial rise in the levels of SAM and SAH, as well as a decrease in the SAM/SAH ratio in the gonad samples from the Hc region, in comparison to the Lc region ($p < 0.05$) (Fig. 6). The amount of SAM was observed to have grown by around 5.6% in the Lc region, whereas the contents of SAH exhibited a 9.8% increase. In Fig. 6A, it can be observed that there was a reduction in the SAM/SAH ratio. This reduction was found to be statistically significant, as depicted in Fig. 6B. The monthly content of SAM and SAH in the gonad exhibited a comparable pattern. The concentration of SAM content exhibited an increase from 1.2% in March 2021 to 2.3% in April 2021 and further rose to 3.47% in May 2021. In a like manner, the content of SAH was observed to be 3.96%, 7.22%, and 16.49% for the months of March, April, and May 2021, respectively (data not presented).

3.7. Health risk assessment: estimated daily intake, target hazard quotient

BPA has been the subject of numerous studies and health risk assessments due to concerns about its potential effects on human health. We in the present study, calculated the EDI and THQ for paradise threadfins collected from Lc and Hc region.

3.7.1. Estimated daily intake

The daily intake of BPA was estimated and compared with the recommended daily intake (RDI) and tolerable daily intake (TDI) levels,

Table 2
Estimated daily intake (EDI) and target hazard quotient (THQ) of Bisphenol A (BPA) estimated for adult men, adult women and children.

Sampling Point	Estimated Daily Intake of BPA (EDI) (ng/kg body weight/day) [a]						Target hazard quotient (THQ)					
	Adult Men		Adult Women		Children (7–9 yrs)		Adult Men		Adult Women		Children (7–9 yrs)	
	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile
Lc	22.84	1.434	24.92	1.564	10.92	0.685	114.2	7.17	124.6	7.82	54.6	3.425
Hc	94.35	1.396	102.93	1.523	45.11	0.667	471.75	6.98	514.65	7.615	225.55	3.335

[a] Recommended by European Food Safety Authority (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids et al., 2023).

which are considered good monitoring parameters for human exposure to BPA. The manual of "Dietary Guidelines for Indians" published by the National Institute of Nutrition, Indian Council of Medical Research (2011) suggested consuming fish frequently with a weekly recommendation of a minimum of 100–200 g/week which renders a value of 28.57 g/day fish muscle intake. We calculated the EDI value based on this and according to Table 2, the EDI of *P. paradiseus* muscles in the current study is found to be the lowest (22.84 ng/kg-bw/day in men, 24.92 ng/kg-bw/day in women and 10.92 ng/kg-bw/day in children) at Lc region and highest (94.35 ng/kg-bw/day in men, 102.93 ng/kg-bw/day in women and 45.11 ng/kg-bw/day in children) at Hc region.

3.7.2. Target hazard quotient (THQ)

The THQ is a quantitative metric employed to evaluate the potential health hazards linked to the exposure of BPA. According to the European Food Safety Authority (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids et al., 2023), the Reference Dose (RfD) or Acceptable Daily Intake (ADI) for BPA is established as 0.2 ng/kg-bw/day, representing the presently deemed permissible level of BPA exposure. Table 2 showed that the highest THQ was found in adult women (514.6) at the Hc region followed by adult men (471.7) and the least (225.5) was found among the children of 7–9 years of age. In the Lc region, the Total Hazard Quotient (THQ) values observed for adult men, women, and children were 114.2, 124.6, and 54.6 respectively.

4. Discussion

The current investigation demonstrated the concentration of environmental BPA in the water sample obtained from a comparatively less polluted stretch of the river. The highest concentration of BPA was noticed in the Hc region and the value was 156.8 ng/L. Previous research by Chakraborty et al. (2021) also reported similar results in the lower stretch, 7 out of the 13 sites surpassed the threshold of 1 μg/L. Mukhopadhyay and Chakraborty (2021) also observed that the concentration of bisphenol A (BPA) in the lower segment of the Ganga River varied between 43 and 8800 μg/L. The BPA concentration in the lower stretch of the present study corroborates with the previously reported studies. This suggests the increasing usage of plastics and waste disposal in the river which needs proper management measures. The elevated levels of BPA detected upstream of Diamond Harbour can be ascribed to the absence of effective waste management methods in the surrounding areas, the prevalence of foam and fragments resulting from the excessive use of disposable packaging materials, and the fragmentation of big plastic trash including plastic debris from fishing activities (damaged nets, strings, lines, etc.). The relatively lower amounts of BPA found downstream of DH may be attributed to the lack of significant industrial effluents or household trash, which have minimal impact on pollution levels. The BPA accumulation in fish tissues also showed a similar pattern. The levels of BPA in the liver, muscle, and gonads of paradise threadfin were considerably higher in the Hc region in comparison to the Lc region, as illustrated in Fig. 1B. This is mainly due to the bioavailability of BPA to fish from the existing water bodies. Earlier studies also showed that the accumulation of BPA in fish tissues depends upon the exposure concentration and a strong positive correlation between the tissue concentration and BPA concentration in the water was also noticed (Kundu et al., 2024).

The accumulation pattern of BPA in fish was noticed as Liver > Muscle > Gonads, which is comparable to the earlier studies on various brackish water fishes collected from the North-East Atlantic Ocean viz., *Dicentrarchus labrax, Scomber colias, Platycephalus indicus* (Barboza et al., 2020). The results were also comparable to the BPA accumulation in tissue of Hilsa in the river Ganga (Kundu et al., 2024). Liver is the main organ for BPA detoxification and digestion in which BPA gets metabolized into enzyme conjugates. This might be the reason for higher accumulation in the liver and low accumulation in muscle. Similarly, numerous studies noticed a higher accumulation of BPA in liver when compared to the fish muscle. A study from the Pearl River system was collected 10 different fish species and found high accumulation in the liver when compared to muscle. In our study, BPA accumulation in gonads ranged between 32.75 and 176.59 ng/g-dw of gonad. The high accumulation in gonads is mainly due to their high affinity towards estrogen and androgen receptors of the gonads (Faheem and Bhandari, 2021). The accumulation of BPA in fish muscle ranged from 45.96 to 198.47 ng/g dw which is similar to the findings showed by us in *Tenualosa ilisha* (19.8–198.34 ng/g-dw) where the higher values were obtained in the samples from the estuarine zone (Kundu et al., 2024).

The present study also observed disparities in the proportions of individuals in the developing and mature stages between the two regions, it can be deduced that the spawning activity is more pronounced in the less contaminated zone compared to the highly contaminated region. The high prevalence of mature fish in the Lc zone suggests that juvenile migration to less contaminated water may be driven by a desire for enhanced food opportunities, enabling them to reach adult size and reproductive readiness. Exposure of BPA at high concentrations in the female brown trout delayed the ovulation time (Faheem and Bhandari, 2021).

A plethora of epigenetic alterations have been observed in both animal and human subjects subsequent to their exposure to BPA. Specifically, BPA has the potential to directly impact the genes responsible for regulating DNA methylation, as well as induce changes in the methylation patterns of other genes that encode proteins important in reproductive physiology. The findings of our study revealed variations in the methylation levels of the exonic regions of *FSHR* and *20β-HSD*. Notably, a statistically significant increase in methylation was observed in threadfins obtained from the Hc region in comparison to the Lc region throughout March–May 2021. The increase in methylation might be due to gonadal hypomethylation or hypermethylation caused by environmental BPA exposure. The study conducted by Pierron et al. (2014) observed notable variations in the methylation level at exon 1 of the *FSHR* gene in female silver eels (*Anguilla anguilla*) across various sample groups originating from places with varying pollution levels. However, no significant changes were observed in the value of *11β-HSD*. Consistent with previous research, our study revealed an increase in methylation levels within the exonic regions of both *FSHR* and *20β-HSD* across different months and from places with different BPA levels. The enzyme *20β-HSD* is responsible for the synthesis of 17α, 20β-dihydroxy-4-pregnen-3-one (DHP), which is the maturation-inducing steroid necessary for the restart of prophase-I arrested oocytes in teleost fishes. There is a

lack of published evidence on the methylation status and expression patterns of the β-HSD genes in species other than humans, mice, and zebrafish.

In this study, we have demonstrated, for the first time, that there is an upregulation of methylation in the exonic region of *20β-HSD* in wild-caught threadfins from the Hc region across different months. This finding suggests that there is a strong influence of environmental BPA on methylation of steroidogenic genes. The study conducted by Zhang et al. (2018) showed that female rare minnows, *Gobiocypris rarus*, exhibited DNA hypermethylation of *hsd11b2* and DNA hypomethylation of *hsd3β* as a result of *in vitro* exposure to BPA. Additionally, it was observed that the methylation level at the promoter region of the *cyp19a1a* gene exhibited a statistically significant increase in the Hc area in comparison to the Lc region within the period of March to May 2021. Hence, the data we have obtained aligns with previous findings in female *Anguilla anguilla*, which demonstrated a notable increase in the promoter region of the *aro* gene in samples collected from the contaminated site compared to those from the clean site (Pierron et al., 2014). Exposure to BPA has been demonstrated to induce changes in global DNA methylation and significantly affect the methylation patterns of the *cyp19a1a* gene in the ovaries of adult (Liu et al., 2014) and juvenile (Zhu et al., 2021) rare minnows (*Gobiocypris rarus*), even at lower doses (Zhang et al., 2014). Additionally, BPA exposure has been found to upregulate E2 levels and impact the gonads of male zebrafish (Zhao et al., 2017). It is noteworthy that the *cyp17a1* and *cyp11a1* enzymes, which play a crucial role in the production of steroids in fish, have been observed to undergo changes following exposure to BPA. Till date, no research has demonstrated the role of environmental BPA on DNA methylation of the steroidogenic genes crucial for reproduction.

Given the well-documented status of BPA as a strong endocrine disruptor, extensive utilization of this compound has been undertaken to ascertain its impact on worldwide DNA methylation. Alterations in DNA methylation levels have been documented in various species belonging to the teleost group, including zebrafish, Bluegill, False kelpfish, and Nile tilapia, as well as the aquatic invertebrate *Daphnia magna*, in response to exposure to industrial solid waste or endocrine-disrupting chemicals (EDCs) (Shugart, 1990; Vandegehuchte et al., 2010; Wang et al., 2009; Flohr et al., 2012; Qiao et al., 2012). The findings of our study for the first time indicate a statistically significant increase ($p < 0.05$) in global DNA methylation levels caused by environmental BPA in ovarian samples obtained from the Hc region, as compared to the Lc region. The percentage of methylation exhibited a 3.06-fold increase in March 2021, a 2.54-fold increase in April 2021, and a 3.55-fold increase in May 2021. All reported studies were based on the *in vitro* effect of BPA. For e.g., there was a significant rise in the global DNA methylation level in the ovarian tissue of adult uncommon minnows following prolonged exposure to BPA (Liu et al., 2014). In a study conducted by Fan et al. (2020), low dose of BPA resulted in an increase in the overall level of 5-methylcytosine in grass carp ovarian cells. Liu et al. (2016) showed that BPA has a species-specific impact on the overall levels of DNA methylation, i.e. higher doses or prolonged periods of BPA exposure led to a reduction in overall DNA methylation levels in the reproductive organs of zebrafish, namely the ovary, and testis. Hence, akin to numerous other species, the outcomes of our study offer fresh data indicating that environmental BPA exerts a substantial influence on the overall levels of DNA methylation in fully matured female threadfin gonads. Moreover, variations in the impacts of BPA were noted based on the particular time intervals selected for sampling throughout the annual cycle.

The role of DNA methyl transferase enzymes (DNMTs) in facilitating the crosstalk between DNA methylation and histone modifications has been extensively documented. A comprehensive investigation utilizing the zebrafish genome was conducted, which unveiled a total of eight DNMT genes (Goll and Halpern, 2011). Among these genes, DNMT1 and DNMT3 were found to play crucial roles in the processes of DNA maintenance and de novo methylation, respectively. There was a strong association observed between the mRNA expression of DNMT1 or DNMT3 family members and alterations in the overall methylation status in both human and zebrafish specimens. The process of generating new DNA methylation patterns and maintaining existing DNA methylation patterns is believed to be facilitated by the DNMT1 and DNMT3 families, respectively. Furthermore, the translation capacity of the DNMT1 and DNMT3 family may be influenced by exogenous chemicals (Bönsch et al., 2006; Xiang et al., 2008; Vinken et al., 2010; Vivekanandan et al., 2010; Qiao et al., 2012). Based on the results of our research, it was observed that the exposure to environmental BPA resulted in a notable increase in the expression of DNMT isoforms responsible for the preservation of methylation, specifically DNMT1, as well as the initiation of new methylation, DNMT3A, in the mature female threadfin gonads obtained from the Hc region. This effect was not observed in the sample obtained from the Lc region. The study conducted by Fan et al. (2020) demonstrated that a lower dosage of BPA exposure resulted in a direct increase in DNMT transcripts in the ovarian cells of grass carp. Conversely, Liang et al. (2016) found that a higher dosage of BPA led to a decrease in the expression of the DNMT1 enzyme, accompanied by a significant reduction in overall DNA methylation levels, in both male and female gonads of the breeding zebrafish, *Danio rerio*. In comparison to threadfins, which are amphidromous and marine inhabitants, hence experiencing elevated levels of BPA in their natural habitat, minnows and Zebrafishes are freshwater species. These freshwater species have been found to have a notable impact on abnormal DNA methylation patterns following exposure to BPA *in vitro* but no research to date reported the role of environmental BPA on any fresh or marine water fish species *in vivo*.

The methylation index, commonly referred to as the S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratio and indicative of the cellular methylation capacity (Cuyàs et al., 2018), was shown to be reduced in our study of threadfin ovarian samples. This decrease suggests a fall in transmethylation processes. Additionally, an examination was conducted on the levels of SAM and SAH, which are critical intermediates in the process of methylation, specifically in the Hc and Lc regions. The results revealed a substantial rise in the levels of both intermediates in the Hc region when compared to the Lc region. The levels of SAM and SAH exhibited a consistent upward trajectory throughout the sampling period spanning from March to May 2021. The data unequivocally demonstrates the ongoing conversion of SAM to SAH, indicating the consistent progression of DNA methylation at the Hc region during the whole sampling period.

With the exception of the findings reported by Fan et al. (2020), which demonstrated a decrease in SAM and SAH levels in grass carp ovarian cells following exposure to BPA, there is currently a lack of specific information regarding the detection of SAM and SAH levels in the mature fish ovaries. In summary, our research elucidated the potential mechanism by which environmental BPA may promote global DNA methylation in threadfins captured from a location naturally contaminated with BPA. One potential constraint of the current study is the lack of data from additional river segments where the presence of the fish species in question has been documented, apart from the experimental location specifically examined in this study. Ongoing research is being conducted to investigate the involvement of additional enzymes from the DNMT family in the process of DNA methylation, as well as the state of histone changes associated with this mechanism.

The study has identified that the river Ganga contains heightened concentrations of ambient BPA, which have been observed to elicit alterations in DNA methylation patterns in the course of gonadal development. The current observation is additionally supported by changes in the SAM ratio to SAH. Elevated amounts of environmental BPA buildup were also detected in the muscle, liver, and gonads. The present work provides a cautionary signal of the environmentally relevant hazardous concentration of BPA on fish reproduction, while also revealing fundamental insights into the reproductive toxicity of BPA. These findings possess potential significance in informing the formulation of

Table 3
A comparison of EDI and THQ obtained from consuming fish across the world.

Country	Studied Waterbody	Fish/Species	Conc. of BPA (ng/g) in Muscle	EDI			THQ			References
				Adult Men	Adult Women	Children	Adult Men	Adult Women	Children	
Iran	Persian Gulf	*Epinephelus coioides*	0.02–28.29	8.24E-01 μg/kg-bw/day		1.83 μg/kg-bw/day	1.65E-02		3.66E-02	Akhbarizadeh et al. (2021)
		Platycephalus indicus	BDL-12.48	4.61E-01 μg/kg-bw/day		1.02 μg/kg-bw/day	9.22E-03		2.05E-02	
Portugal	North-East Atlantic Ocean	*Dicentrarchus labrax*	9.1 ± 21.4	5.966 μg/kg-bw/day		5.917 μg/kg-bw/day	1.492		1.479	Barboza et al. (2020)
		Trachurus trachurus	1.4 ± 8.7							
		Scomber colias	1.0 ± 3.9							
China	Huangpu River	*Ctenopharyngodon idellus*	2	1.09 ng/kg-bw/day		1.09 ng/kg-bw/day				Jin et al. (2022)
		Parabramis pekinensis	2	BDL-1.09 ng/kg-bw/day		BDL-1.36 ng/kg-bw/day				
		Aristichthys nobilis	1.66	BDL-1.09 ng/kg-bw/day		BDL -0.81 ng/kg-bw/day				
		Carassius auratus	2.33	BDL-1.63 ng/kg-bw/day		BDL-1.63 ng/kg-bw/day				
		Pelteobagrus fulvidraco	3	BDL-1.63 ng/kg-bw/day		BDL -1.9 ng/kg-bw/day				
		Monopterus albus	2.66	BDL-0.81 ng/kg-bw/day		BDL -1.09 ng/kg-bw/day				
		Siniperca chuatsi	2	BDL-1.36 ng/kg-bw/day		BDL -1.36 ng/kg-bw/day				
Iran	Sea Water	*Pampus Argenteus*	2.99							Soltani Nejad et al. (2023)
		Thunnus tonggol	11.55							
China	Taiwanese major river	*Tilapia zilli*	0.52 (ww)				1.17	1.07		Lee et al. (2015)
		Chanos chanos	1.23 (ww)							
		Psenopsis anomala	1.39 (ww)				3.58	3.35		
		Trichiurus lepturus	0.9 (ww)							
		Wild freshwater fishes	1.62 (ww)				0.76	0.73		
Iran	Persian Gulf	*Penaeus semisulcatus*	ND-7.17 (dw)	0.0011 μg/kg-day		0.0025 μg/kg-day	2.21E−05		4.90E−05	Akhbarizadeh et al. (2020)
		Portunus armatus	4.99–6.67 (dw)	0.0013 μg/kg-day		0.0029 μg/kg-day	2.61E−05		5.80E−05	
		Liza klunzinger	5.11–8.94 (dw)	0.0018 μg/kg-day		0.0040 μg/kg-day	3.56E−05		7.91E−05	
		Platycephalus indicus	ND-18.29 (dw)	0.0022 μg/kg-day		0.0050 μg/kg-day	4.47E−05		9.93E−05	
		Epinephelus coioides	7.33–21.45 (dw)	0.0029 μg/kg-day		0.0065 μg/kg-day	5.83E−05		1.29E−04	
China	Northern rivers of Taiwan	Fish	1.5 ± 2.2 (dw)	5.62×10^{-7} ng/kg bw/day	5.28×10^{-7} ng/kg bw/day	1.13×10^{-6} ng/kg bw/day	6.74×10^{-6} ng/kg bw/day	5.81×10^{-6} ng/kg bw/day		Lu et al. (2021)
China	Pearl river Delta	Freshwater fish	0.5–2 ng/g (ww)	1.1×10^{2} ng/kg bw/day			<1			Wei et al. (2011)
		Marine water fish	ND-1.1	2.2×10^{2} ng/kg bw/day						
India	Ganga	*Tenualosa ilisha*	Male: 36.71–200.18 (dw) Female: 40.71–196.45 (dw) Juvenile: 1.95–19.19 (dw)	13.357–64.91 ng/kg bw/day	14.571–70.811 ng/kg bw/day	31.93–155.164 ng/kg bw/day	66.785–324.55	30.68–354.05	159.65–775.82	Kundu et al. (2024)
India	Ganga	*Polynemus paradiseus*	47.97–198.15 (dw)	58.59 ng/kg-bw/day	63.92 ng/kg-bw/day	28.01 ng/kg-bw/day	292.97	319.62	140.07	Present study

ND- Not detected, BDL- Below detection level.

conservation strategies aimed at the preservation of paradise threadfins.

Our results which are depicted in Table 2 reveal that the consumption of fish from the Lc region leads to an increase in the Estimated Daily Intake (EDI) exposure ranging from 10.92 to 24.92 (ng/kg bw/day). Conversely, consuming fish from the Hc region is associated with a higher EDI exposure, ranging from 45.11 to 102.93 (ng/kg bw/day). It's noteworthy that adult women exhibit a higher EDI from fish consumption compared to adult men and children, consistent with previous research findings (Barboza et al., 2020). However, it's essential to note that the level of risk associated with consuming paradise threadfin or other fish species can vary, as different fish may have different efficiencies in accumulating chemicals, either reducing or increasing the overall risk associated with fish consumption (Wang et al., 2020).

It is the first study to report THQ values using the European Food Safety Authority (EFSA) guideline 2023 and it is very obvious that all our values are >1. In the previous studies using wild-caught fish, the THQ values were shown to be < 1 (Akhbarizadeh et al., 2020, 2021; Lu et al., 2021) as the researchers have used a t-TDI for BPA of 4 μg/kg bw/day issued by EFSA Panel on Food Contact Materials, Enzymes and Processing Aids et al. (2023). When experts at the EFSA found several data gaps and uncertainties, they re-evaluated TDI in 2023 based on a 2-year research by the US National Toxicology Program and recommended the new TDI value as 0.2 ng/kg bw/day which is 20,000 times lower than the 2015 value (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids et al., 2023). A comparison table of EDI and THQ on human beings by consuming different types of fish around the world has been incorporated in Table 3. It is pretty clear from our study that wild-caught fishes are more exposed to BPA present in their environment than preserved seafood where BPA exposure is insignificant in terms of THQ values which were <1 (Lin et al., 2022).

5. Conclusion

In the gonads of adult female paradise threadfin fish captured from their natural habitat, this study examined gene transcription changes related to DNA methylation and demethylation and the effects of environmental BPA pollution. Overall, the nomenclature Hc and Lc were chosen based on the comparative examination of environmental BPA concentrations in water samples from the Hc and Lc locations from March to May 2021. Significantly, female paradise threadfin liver, muscle, and gonad tissues from Hc region had higher environmental BPA levels. Furthermore, environmental BPA affected the distribution of fish at different maturity stages in the Hc region. Due to DNA hypermethylation, upregulation of FSHR, 20β-HSD, and cyp19a1a genes was seen in methylated areas. Monthly analysis showed a statistically significant increase in global DNA methylation in the Hc region compared to the Lc region. SAM, SAH, and the SAM: SAH ratio matched global DNA methylation data. DNMT1 and DNMT3A gene expression in the Hc region may be upregulated by environmental BPA. Through hormone receptor transcriptional changes, environmental BPA can affect DNA methylation patterns in female paradise threadfins throughout oocyte development. The outcomes of this study showed that environmental BPA harms edible fish models, arguing for its substitution in consumer goods to minimize environmental BPA levels. The results showed that global DNA methylation, nucleic acid derivative indices, and EDC detection are accurate ecotoxicological markers.

Funding

The mentioned research work is funded by the National Mission for Clean Ganga, Ministry of Jal Shakti, Government of India.

CRediT authorship contribution statement

Sourav Kundu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Archisman Ray:** Data curation, Investigation, Methodology, Writing – review & editing. **Subhadeep Das Gupta:** Data curation, Formal analysis, Visualization. **Ayan Biswas:** Formal analysis, Software. **Shreya Roy:** Methodology, Software, Validation, Writing – review & editing. **Nitish Kumar Tiwari:** Data curation, Formal analysis, Software. **V Santhana Kumar:** Investigation, Methodology, Validation, Writing – review & editing. **Basanta Kumar Das:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgment

The National Mission for Clean Ganga (NMCG), Ministry of Jal Shakti, Government of India, is gratefully acknowledged by the authors for providing the necessary funding to carry out the study. The authors are appreciative of the local Ganga River fisherman, particularly Mr. Buddhadev Naskar, for his kind assistance with sampling during the research time. The authors express their deep sense of gratitude towards the esteemed reviewers for their immense evaluation and diligent help in enhancing the manuscript. The authors are thankful to Dr. Sagarika Das for her immense help during the revision of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141236.

References

Akhbarizadeh, R., Moore, F., Monteiro, C., Fernandes, J.O., Cunha, S.C., 2020. Occurrence, trophic transfer, and health risk assessment of bisphenol analogues in seafood from the Persian Gulf. Mar. Pollut. Bull. 154, 111036.

Akhbarizadeh, R., Russo, G., Rossi, S., Golianova, K., Moore, F., Guida, M., et al., 2021. Emerging endocrine disruptors in two edible fish from the Persian Gulf: occurrence, congener profile, and human health risk assessment. Mar. Pollut. Bull. 166, 112241.

Artemov, A.V., Mugue, N.S., Rastorguev, S.M., Zhenilo, S., Mazur, A.M., Tsygankova, S. V., et al., 2017. Genome-wide DNA methylation profiling reveals epigenetic adaptation of stickleback to marine and freshwater conditions. Mol. Biol. Evol. 34 (9), 2203–2213.

Azzi, A., Dallmann, R., Casserly, A., Rehrauer, H., Patrignani, A., Maier, B., Kramer, A., Brown, S.A., 2014. Circadian behavior is light-reprogrammed by plastic DNA methylation. Nat. Neurosci. 17 (3), 377–382.

Baerwald, M.R., Meek, M.H., Stephens, M.R., Nagarajan, R.P., Goodbla, A.M., Tomalty, K.M., et al., 2016. Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. Mol. Ecol. 25 (8), 1785–1800.

Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O., Guilhermino, L., 2020. Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. J. Hazard Mater. 393, 122419.

Bönsch, D., Lenz, B., Fiszer, R., Frieling, H., Kornhuber, J., Bleich, S., 2006. Lowered DNA methyltransferase (DNMT-3b) mRNA expression is associated with genomic DNA hypermethylation in patients with chronic alcoholism. J. Neural. Transm. 113 (9), 1299–1304.

Burgerhout, E., Mommens, M., Johnsen, H., Aunsmo, A., Santi, N., Andersen, Ø., 2017. Genetic background and embryonic temperature affect DNA methylation and expression of myogenin and muscle development in Atlantic salmon (Salmo salar). PLoS One 12 (6), e0179918.

Campos, C., Valente, L., Conceição, L., Engrola, S., Fernandes, J., 2013. Temperature affects methylation of the myogenin putative promoter, its expression and muscle cellularity in Senegalese sole larvae. Epigenetics 8 (4), 389–397.

Cerkvenik-Flajs, V., Šturm, S., 2021. A rapid analysis of Bisphenol A using MISPE coupled with HPLC-FLD in tissues of food-producing animals. MethodsX 8, 101351.

Chakraborty, P., Shappell, N.W., Mukhopadhyay, M., Onanong, S., Rex, K.R., Snow, D., 2021. Surveillance of plasticizers, bisphenol A, steroids and caffeine in surface water of River Ganga and Sundarban wetland along the Bay of Bengal: occurrence, sources, estrogenicity screening and ecotoxicological risk assessment. Water Res. 190, 116668.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162 (1), 156–159.

Cuyàs, E., Fernández-Arroyo, S., Verdura, S., García, R.Á.F., Stursa, J., Werner, L., et al., 2018. Metformin regulates global DNA methylation via mitochondrial one-carbon metabolism. Oncogene 37 (7), 963–970.

Dang, X., Lim, Y.K., Li, Y., Roberts, S.B., Li, L., Thiyagarajan, V., 2023. Epigenetic-associated phenotypic plasticity of the ocean acidification-acclimated edible oyster in the mariculture environment. Mol. Ecol. 32 (2), 412–427.

Dolinoy, D.C., Weidman, J.R., Waterland, R.A., Jirtle, R.L., 2006. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ. Health Perspect. 114 (4), 567–572.

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Lambré, C., Barat Baviera, J.M., Bolognesi, C., Chesson, A., Cocconcelli, P.S., Van Loveren, H., 2023. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 21 (4), e06857.

Faheem, M., Bhandari, R.K., 2021. Detrimental effects of bisphenol compounds on physiology and reproduction in fish: a literature review. Environ. Toxicol. Pharmacol. 81, 103497.

Fallet, M., Luquet, E., David, P., Cosseau, C., 2020. Epigenetic inheritance and intergenerational effects in mollusks. Gene 729, 144166.

Fan, X., Hou, T., Jia, J., Tang, K., Wei, X., Wang, Z., 2020. Discrepant dose responses of bisphenol A on oxidative stress and DNA methylation in grass carp ovary cells. Chemosphere 248, 126110.

Flohr, L., Fuzinatto, C.F., Melegari, S.P., Matias, W.G., 2012. Effects of exposure to soluble fraction of industrial solid waste on lipid peroxidation and DNA methylation in erythrocytes of Oreochromis niloticus, as assessed by quantification of MDA and m5dC rates. Ecotoxicol. Environ. Saf. 76, 63–70.

Gawra, J., Valdivieso, A., Roux, F., Laporte, M., de Lorgeril, J., Gueguen, Y., Vidal-Dupiol, J., 2023. Epigenetic variations are more substantial than genetic variations in rapid adaptation of oyster to Pacific oyster mortality syndrome. Sci. Adv. 9 (36), eadh8990.

Goll, M.G., Halpern, M.E., 2011. DNA methylation in zebrafish. Progress in Molecular Biology and Translational Science 101, 193–218.

Heijmans, B.T., Tobi, E.W., Stein, A.D., Putter, H., Blauw, G.J., Susser, E.S., et al., 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc. Natl. Acad. Sci. USA 105 (44), 17046–17049.

Jablonka, E., Lamb, M.J., 2002. The changing concept of epigenetics. Ann. N. Y. Acad. Sci. 981 (1), 82–96.

Jin, Y., Yuan, T., Li, J., Shen, Z., Tian, Y., 2022. Occurrence, health risk assessment and water quality criteria derivation of six personal care products (PCPs) in Huangpu River, China. Environ. Monit. Assess. 194 (8), 577.

Kagwade, P.V., 1970. Maturation and spawning in Polynemus heptadactylus cuv. And val. Indian J. Fish. 17 (1&2), 76–89.

Kim, W.S., Do, A., Yeh, D., Cunningham, J., 2014. Extraction of bisphenol-A and 17β-estradiol from water samples via solid-phase extraction (SPE). Rev. Anal. Chem. 33 (1), 59–77.

Kumar, R., Singh, P.J., Nagpure, N.S., Kushwaha, B., Srivastava, S.K., Lakra, W.S., 2007. A Non-invasive Technique for Rapid Extraction of DNA from Fish Scales.

Kundu, S., Biswas, A., Ray, A., Roy, S., Gupta, S.D., Ramteke, M.H., et al., 2024. Bisphenol A contamination in Hilsa shad and assessment of potential health hazard: a pioneering investigation in the national river Ganga, India. J. Hazard Mater. 461, 132532.

Laing, L.V., Viana, J., Dempster, E.L., Trznadel, M., Trunkfield, L.A., Uren Webster, T.M., et al., 2016. Bisphenol A causes reproductive toxicity, decreases dnmt1 transcription, and reduces global DNA methylation in breeding zebrafish (Danio rerio). Epigenetics 11 (7), 526–538.

Le Luyer, J., Laporte, M., Beacham, T.D., Kaukinen, K.H., Withler, R.E., Leong, J.S., et al., 2017. Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. Proc. Natl. Acad. Sci. USA 114 (49), 12964–12969.

Lin, N., Ma, D., Liu, Z., Wang, X., Ma, L., 2022. Migration of bisphenol A and its related compounds in canned seafood and dietary exposure estimation. Food Quality and Safety 6, fyac006.

Lee, C.C., Jiang, L.Y., Kuo, Y.L., Chen, C.Y., Hsieh, C.Y., Hung, C.F., Tien, C.J., 2015. Characteristics of nonylphenol and bisphenol A accumulation by fish and implications for ecological and human health. Sci. Total Environ. 502, 417–425.

Liu, Y., Wang, L., Zhu, L., Ran, B., Wang, Z., 2020. Bisphenol A disturbs transcription of steroidogenic genes in ovary of rare minnow Gobiocypris rarus via the abnormal DNA and histone methylation. Chemosphere 240.

Liu, Y., Yuan, C., Chen, S., Zheng, Y., Zhang, Y., Gao, J., Wang, Z., 2014. Global and cyp19a1a gene specific DNA methylation in gonads of adult rare minnow Gobiocypris rarus under bisphenol A exposure. Aquat. Toxicol. 156, 10–16.

Liu, Y., Zhang, Y., Tao, S., Guan, Y., Zhang, T., Wang, Z., 2016. Global DNA methylation in gonads of adult zebrafish Danio rerio under bisphenol A exposure. Ecotoxicol. Environ. Saf. 130, 124–132.

Lu, I.C., Chao, H.R., Mansor, W.N.W., Peng, C.W., Hsu, Y.C., Yu, T.Y., et al., 2021. Levels of phthalates, bisphenol-A, nonylphenol, and microplastics in fish in the estuaries of northern Taiwan and the impact on human health. Toxics 9 (10), 246.

Manual, A., 2011. Dietary guidelines for Indians. Nat. Inst. Nutrition 2, 89–117.

Morán, P., Pérez-Figueroa, A., 2011. Methylation changes associated with early maturation stages in the Atlantic salmon. BMC Genet. 12 (1), 1–8.

Morán, P., Marco-Rius, F., Megías, M., Covelo-Soto, L., Pérez-Figueroa, A., 2013. Environmental induced methylation changes associated with seawater adaptation in brown trout. Aquaculture 392, 77–83.

Mukhopadhyay, M., Chakraborty, P., 2021. Plasticizers and bisphenol A: emerging organic pollutants along the lower stretch of River Ganga, north-east coast of the Bay of Bengal. Environ. Pollut. 276, 116697.

Pal, P., Moulik, S.R., Gupta, S., Guha, P., Majumder, S., Kundu, S., et al., 2018. Estrogen-regulated expression of P450arom genes in the brain and ovaries of adult female Indian climbing perch, Anabas testudineus. J. Exp. Zool. Part A: Ecological and Integrative Physiology 329 (1), 29–42.

Pierron, F., Bureau du Colombier, S., Moffett, A., Caron, A., Peluhet, L., Daffe, G., et al., 2014. Abnormal ovarian DNA methylation programming during gonad maturation in wild contaminated fish. Environ. Sci. Technol. 48 (19), 11688–11695.

Qiao, K., Liu, Y.Y., Li, Y.H., Wang, Y.Z., Ding, X.C., Li, W.H., Li, Y.S., 2012. Epigenetic influences of bisphenol A on male reproductive toxicity in zebrafish. Lab. Anim. Comp. Med. 32, 30–37.

Shugart, L.R., 1990. 5-Methyl deoxycytidine content of DNA from bluegill sunfish (Lepomis macrochirus) exposed to benzo [a] pyrene. Environ. Toxicol. Chem.: Int. J. 9 (2), 205–208.

Sogorb, M.A., Estévez, J., Vilanova, E., 2019. Case study: is bisphenol S safer than bisphenol A in thermal papers? Arch. Toxicol. 93, 1835–1852.

Soltani Nejad, Y., Tahmasebi Birgani, Y., Mohammadi, M.J., Dehghan, A., Babaei, A.A., 2023. Bisphenol A in fish, seawater, and fishermen's urine: measurement and health risk assessment in southern Iran. Int. J. Environ. Sci. Technol. 20 (4), 3891–3900.

Strömqvist, M., Tooke, N., Brunström, B., 2010. DNA methylation levels in the 5′ flanking region of the vitellogenin I gene in liver and brain of adult zebrafish (Danio rerio)—sex and tissue differences and effects of 17α-ethinylestradiol exposure. Aquat. Toxicol. 98 (3), 275–281.

Ungaro, N., 2008. Field manual on macroscopic identification of maturity stages for the Mediterranean fishery resources. GCP/RER/ITA/MSM-TD-21, 21. MedSudMed Technical Documents No, pp. 34–pp.

Vandegehuchte, M.B., Lemière, F., Vanhaecke, L., Berghe, W.V., Janssen, C.R., 2010. Direct and transgenerational impact on Daphnia magna of chemicals with a known effect on DNA methylation. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 151 (3), 278–285.

Vinken, M., Snykers, S., Fraczek, J., Decrock, E., Leybaert, L., Rogiers, V., Vanhaecke, T., 2010. DNA methyltransferase 3a expression decreases during apoptosis in primary cultures of hepatocytes. Toxicol. Vitro 24 (2), 445–451.

Vivekanandan, P., Daniel, H.D.J., Kannangai, R., Martinez-Murillo, F., Torbenson, M., 2010. Hepatitis B virus replication induces methylation of both host and viral DNA. J. Virol. 84 (9), 4321–4329.

Wang, J., Shan, Q., Liang, X., Guan, F., Zhang, Z., Huang, H., Fang, H., 2020. Levels and human health risk assessments of heavy metals in fish tissue obtained from the agricultural heritage rice-fish-farming system in China. J. Hazard Mater. 386, 121627.

Wang, X., Cong, R., Li, A., Wang, W., Zhang, G., Li, L., 2023. Experimental DNA demethylation reduces expression plasticity and thermal tolerance in pacific oysters. Mar. Biotechnol. 1–6.

Wang, X., Bhandari, R.K., 2020. DNA methylation reprogramming in medaka fish, a promising animal model for environmental epigenetics research. Environmental Epigenetics 6 (1), dvaa008.

Wang, Y., Wang, C., Zhang, J., Chen, Y., Zuo, Z., 2009. DNA hypomethylation induced by tributyltin, triphenyltin, and a mixture of these in Sebastiscus marmoratus liver. Aquat. Toxicol. 95 (2), 93–98.

Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7 (8), 847–854.

Wei, X., Huang, Y., Wong, M.H., Giesy, J.P., Wong, C.K., 2011. Assessment of risk to humans of bisphenol A in marine and freshwater fish from Pearl River Delta, China. Chemosphere 85 (1), 122–128.

Xiang, N., Zhao, R., Song, G., Zhong, W., 2008. Selenite reactivates silenced genes by modifying DNA methylation and histones in prostate cancer cells. Carcinogenesis 29 (11), 2175–2181.

Yuan, C., Wang, L., Zhu, L., Ran, B., Xue, X., Wang, Z., 2019. N-acetylcysteine alleviated bisphenol A-induced testicular DNA hypermethylation of rare minnow (Gobiocypris rarus) by increasing cysteine contents. Ecotoxicol. Environ. Saf. 173, 243–250.

Zhang, T., Guan, Y., Wang, S., Wang, L., Cheng, M., Yuan, C., et al., 2018. Bisphenol A induced abnormal DNA methylation of ovarian steroidogenic genes in rare minnow Gobiocypris rarus. Gen. Comp. Endocrinol. 269, 156–165.

Zhang, Y., Gao, J., Xu, P., Yuan, C., Qin, F., Liu, S., et al., 2014. Low-dose bisphenol A disrupts gonad development and steroidogenic genes expression in adult female rare minnow Gobiocypris rarus. Chemosphere 112, 435–442.

Zhao, F., Wei, P., Wang, J., Yu, M., Zhang, X., Tian, H., et al., 2017. Estrogenic effects associated with bisphenol a exposure in male zebrafish (Danio rerio) is associated with changes of endogenous 17β-estradiol and gene specific DNA methylation levels. Gen. Comp. Endocrinol. 252, 27–35.

Zhu, L., Liu, Y., Xue, X., Yuan, C., Wang, Z., 2021. BPA's transgenerational disturbance to transcription of ovarian steroidogenic genes in rare minnow Gobiocypris rarus via DNA and histone methylation. Sci. Total Environ. 762, 143055.