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Parental benzo[*a*]pyrene exposure and timing of reproduction determine the magnitude and persistence of transgenerational toxicity in marine medaka (*Oryzias melastigma*)

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ABSTRACT

Benzo[*a*]pyrene (BaP), a high-molecular-weight polycyclic aromatic hydrocarbon, is a persistent contaminant with well-documented developmental and endocrine-disrupting effects in aquatic organisms. This study examined how reproductive timing after exposure cessation influences transgenerational toxicity in marine medaka (*Oryzias melastigma*). Fish were exposed to environmentally relevant BaP concentrations (1, 4, and 8 μg/L) for 120 days, and F1 offspring were obtained from parents spawning 1, 30, and 60 days post-exposure. Offspring from 1-day post-exposure spawns showed up to 40 % reduced hatching, elevated mortality, malformations, and shorter body length, accompanied by downregulation of antioxidant (*sod, cat, gpx*) and steroidogenic (*cyp17a, 17βhsd, cyp11b*) genes and elevated thyroglobulin (*tg*) and vitellogenin (*vtg1, vtg2*). Partial recovery occurred in oxidative and apoptotic pathways at 30 days, while endocrine and growth disruptions persisted. By 60 days, most parameters normalized except thyroid- and growth-axis markers. Hormonal assays revealed increased adrenocorticotropic hormone, cortisol, elevated thyroxine, and suppressed growth hormone, indicating slow endocrine and growth recovery.

1. Introduction

Benzo[*a*]pyrene (BaP) is a model high-molecular-weight polycyclic aromatic hydrocarbon (PAH) formed during incomplete combustion and widely detected in air, sediments, and water worldwide (Bukowska et al., 2022). It is well-documented for its carcinogenic, mutagenic, and developmental toxic properties (Mo et al., 2025). Recognized as a potent endocrine-disrupting chemical (EDC), BaP induces a broad spectrum of toxic effects across multiple species and life stages, extending to transgenerational and multigenerational impacts in aquatic organisms (Chen et al., 2025; Corrales et al., 2014a, 2014b; Knecht et al., 2017; Mo et al., 2023, 2020; Pandelides et al., 2023; Rurale et al., 2022; Seemann et al., 2017; Sun et al., 2020; Wan et al., 2023, 2022; Yin et al., 2020; Zeb et al., 2025), leading to osteotoxicity, neurotoxicity, behavioral alterations, epigenetic regulation, among other adverse outcomes in unexposed offspring and indicating the transgenerational transmission of toxic effects.

Recent multigenerational and transgenerational studies on fish have highlighted the persistent consequences of parental BaP exposure on offspring. In Japanese medaka (*Oryzias latipes*), BaP exposure has been shown to induce multigenerational reproductive impairments, including alterations in reproductive development, endocrine function, and gene expression in offspring (Chen et al., 2025). Pandelides et al. (2023) reported that BaP exposure in zebrafish (*Danio rerio*) led to developmental and behavioral deficits in both F1 and F2 generations. Similarly, Wan et al. (2022) demonstrated that parental BaP exposure in Japanese medaka caused neurotoxicity and altered DNA methylation in unexposed F1 larvae. In marine medaka (*Oryzias melastigma*), life-cycle BaP exposure caused sex-specific endocrine disruption that persisted into the F2 generation, with signs of recovery only observed by the F3 generation (Zeb et al., 2025). Together, these studies underscore the transgenerational persistence of BaP's adverse effects.

Despite the extensive evidence demonstrating transgenerational impacts of BaP exposure, most existing studies have focused on collecting F1 eggs immediately after the end of parental exposure and then monitoring the subsequent offspring. However, the conventional

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experimental approach in these studies overlooks an important biological dimension, the timing of reproduction following exposure. In natural or managed populations, spawning may occur either directly after a contaminant event or after a period of recovery, during which depuration and biotransformation processes may reduce internal contaminant burdens. Toxicokinetic and depuration studies have shown that fish can eliminate PAHs from tissues once returned to clean water, with elimination rates (half-lives) differing among compounds and tissues (Möller et al., 2014; Wang et al., 2017; Zeb et al., 2024a). Such differences are likely to affect the level of maternal transfer of BaP and its metabolites to the developing eggs. Although previous parental exposure studies have provided important insights into the mechanisms of BaP-induced transgenerational toxicity, the impact of the interval between exposure cessation and reproduction on the inheritance of these effects remains unclear. To address this gap, the present study aimed to examine how reproductive timing after parental BaP exposure influences the transmission of toxicity to offspring.

This study uses marine medaka, a small estuarine fish commonly applied in ecotoxicology and known for its easy culture conditions, fast life cycle, and high sensitivity to pollutants, making it suitable for transgenerational work (Chen et al., 2019). The fish were exposed to BaP and then allowed to spawn at different post-exposure intervals (1 day, 30 days, and 60 days). This experimental design differentiated the immediate offspring effects from those arising after parental depuration. The primary aims were to (i) quantify transgenerational effects of parental BaP exposure on F1 offspring when eggs were collected at various recovery times, (ii) determine which biological systems (developmental, oxidative stress, apoptosis, endocrine/growth) are most affected and which recover fastest, and (iii) identify endpoints that remain altered despite parental depuration.

2. Materials and methods

2.1. Chemicals

Benzo[*a*]pyrene (BaP, CAS No. 50-32-8) and ethyl 3-aminobenzoate methane sulfonate (MS-222) were obtained from Sigma-Aldrich (Thermo Scientific, USA). A stock solution of BaP was prepared by dissolving the compound in dimethyl sulfoxide (DMSO; Sangon Biotech, Shanghai, China). All solvents and reagents used throughout the study were of analytical grade, with a minimum purity of 98 %, ensuring reliability and consistency in experimental procedures.

2.2. Chemical analysis in water, ovaries and embryos

BaP concentrations in the exposure tanks were regularly monitored throughout the parental exposure period, following the established analytical procedures. The concentration ranges and methods are described in detail in the Supplementary information (Table S1). During the subsequent depuration phase, water samples were again analyzed for BaP, but no detectable levels were found; therefore, these data are not included. Ovarian samples were collected at 1, 30, and 60 days after exposure cessation and pooled from each group ($n = 15$/replicate), and F1 embryos were pooled at the same time points from each group ($n = 50$/replicate) or as required by the analytical protocol. Both ovarian and embryonic BaP detections were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Meimian Industry, China) specifically developed for BaP detection. Further methodological details and the corresponding BaP detection data for parental ovaries, F1 embryos are presented in the Supplementary information (Fig. S1).

2.3. Fish husbandry and parental exposure

The parental exposure experiment was conducted using both male and female marine medaka, maintained under stable laboratory conditions at 26 °C, salinity 28 ± 2 ‰, and a controlled photoperiod of 14 hour light and 10 hour dark. Fish were provided brine shrimp *Artemia nauplii* twice per day throughout the study. The exposure phase lasted for 120 days, during which groups of fish were subjected to BaP at nominal concentrations of 1, 4, and 8 μg/L, alongside a solvent control (DMSO ≤0.01 % v/v) and a blank control (without DMSO), which showed no statistically significant differences from the solvent control; therefore, only the solvent control was included in the analyses. The selected concentration range reflects environmentally realistic levels of BaP contamination reported from marine and estuarine environments (He et al., 2020; Leng et al., 2021; Lin et al., 2023; Meng et al., 2019; Yu et al., 2021). Each concentration treatment was maintained in three independent replicate tanks to ensure reproducibility. Tank management, water renewal, and feeding schedules followed standardized procedures established in our prior research (Yin et al., 2020; Zeb et al., 2025). All procedures involving fish were approved by the Xiamen University Institutional Committee for the Care and Use of Laboratory Animals (XMULAC20190066).

2.4. Parental depuration and eggs collection (time-resolved design)

At the end of the exposure period, both male and female adults from each treatment were moved to clean, BaP-free seawater (salinity 28 ± 2 ‰). Eggs (F1 embryos) were collected from these exposed parents at three intervals: spawn at 1, 30, and 60 days after exposure cessation. The spawning arrangement consisted of 50 fish per replicate (male-to-female ratio 1:2), and the same group of breeders was used for all three collection time points. For each time point, three replicates were obtained; each replicate consisted of eggs pooled and distributed equally.

2.5. F1 rearing and developmental assays

Fertilized F1 embryos were thoroughly rinsed three times with water to eliminate any residual medium or debris, and only healthy, fertilized embryos were selected to ensure developmental uniformity among samples. For each parental exposure group and corresponding spawning time point, eggs were randomly allocated into three replicate sets of 300 embryos each and incubated in glass plates containing clean artificial seawater. The incubation water was renewed daily for 21 consecutive days, during which no BaP or DMSO was introduced. Embryos were examined and photographed daily using a Leica DMi1 inverted microscope (Leica, Germany) equipped with a Leica DMC4500 digital camera, and any embryos showing signs of mortality (whitening) were promptly removed. Hatching activity was monitored from the onset of hatching until completion, and the hatching rate (%) was expressed as (number of hatched larvae / total number of embryos) × 100. The embryos that did not hatch by 21 days post-fertilization (dpf) were categorized as failed hatchings. For morphometric evaluation, 5 newly hatched larvae were randomly chosen from each replicate group. Prior to measurements, the larvae were anesthetized in 0.02 % MS-222 and positioned within 3 % methylcellulose to maintain a stable orientation during imaging. Body length was measured using Mshot image analysis software calibrated in millimeters. Morphological deformities were examined microscopically and classified following the criteria established in previous studies (Li et al., 2020; Zeb et al., 2024b). The larval mortality rate at 21 dpf was determined as (number of dead larvae / total number of hatched larvae) × 100, calculated from the beginning of larval emergence.

2.6. Real-time quantitative PCR

From each treatment group at 21 dpf, 10 F1 larvae were pooled to form 1 biological replicate ($n = 3$ replicates / treatment). The samples were rapidly flash-frozen in liquid nitrogen and subsequently stored at −80 °C to preserve RNA integrity for downstream molecular analyses. Total RNA was extracted using TRIzol reagent (Invitrogen, USA), and RNA concentration and purity were evaluated with a NanoDrop 2000

spectrophotometer (Thermo Fisher Scientific, USA). RNA integrity was confirmed through agarose gel electrophoresis. Complementary DNA (cDNA) synthesis was carried out using the PrimeScript RT-PCR Kit (TaKaRa, China) according to the manufacturer's protocol. Gene expression profiling targeted multiple functional categories, including detoxification [superoxide dismutase 1 (*sod1*), superoxide dismutase 2 (*sod2*), catalase (*cat*), glutathione peroxidase (*gpx*)], apoptosis [tumor protein p53 (*p53*), Fas cell surface death receptor (*fas/tnfrsf6*), caspase 9 (*casp9*), caspase 3 (*casp3*)], steroidogenesis and endocrine regulation [cytochrome P450 17A1 (*cyp17a*), 17β-hydroxysteroid dehydrogenase (*17βhsd*), cytochrome P450 11β-hydroxylase (*cyp11b*), cytochrome P450 aromatase (*cyp19a*), vitellogenin 1 (*vtg1*), vitellogenin 2 (*vtg2*)], and growth and thyroid hormone pathways [insulin-like growth factor 1 (*igf1*), insulin receptor substrate (*irs*), insulin-like growth factor binding protein 3 (*igfbp3*), thyroglobulin (*tg*)]. Primer sequences were designed using NCBI Primer-BLAST (Table S2). All primers exhibited amplification efficiencies between 90 % and 110 % (Table S3). Real-time quantitative PCR (qPCR) was performed using the FastStart SYBR Green PCR Master Mix (Roche, USA) on an ABI 7500 real-time PCR system. The thermocycling protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 amplification cycles at 95 °C for 30 s and 60 °C for 1 min. Each treatment group included three biological replicates ($n = 3$), with each sample analyzed in triplicate to account for technical variation. Gene expression was normalized to *18 s* rRNA as the internal control, and relative transcript levels were calculated using the formula ($2^{-\Delta\Delta Ct}$) following Livak and Schmittgen (2001).

2.7. Hormonal assays

For each treatment group, 50 larvae were pooled to form one biological replicate ($n = 3$ replicates per treatment). Larvae were homogenized in 1× phosphate-buffered saline (PBS) using an electric homogenizer (Tiangen Biotech, China), and the resulting supernatants were collected after centrifugation. Total protein content in the supernatants was determined using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA). The protein concentrations of the neat homogenates were within the range of 1.5 to 3.0 μg/μL. For the hormone assays, all samples were diluted to a uniform final protein concentration of 1.0 μg/μL using 1× PBS. Hormone levels were then quantified using commercial ELISA kits (CUSABIO Biotech, China) for adrenocorticotropic hormone (ACTH; Cat No. CSB-E15926Fh), cortisol (Cat No. CSB-E08487f), growth hormone (GH; Cat No. CSB-E12121Fh), and thyroxine (T4; Cat No. CSB-E08489f), following the manufacturers' protocols. Optical densities were measured at 450 nm with a Tecan Infinite F200 Pro Microplate Reader (Tecan, Switzerland), and hormone concentrations were calculated from standard curves. The detection limits for ACTH, cortisol, GH, and T4 were 75 pg/mL, 2.3 pg/mL, 312.5 pg/mL, and 20 ng/mL, respectively. Final hormone concentrations are expressed as pg/mg of total protein.

2.8. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 7.0 (GraphPad Software, USA). Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. For comparisons involving multiple groups, two-way analysis of variance (ANOVA) was performed, followed by Dunnett's multiple-comparison tests. Statistical significance was defined as $p < 0.05$ and $p < 0.01$.

3. Results and discussion

3.1. Developmental impacts on the F1 generation depend on parental spawning time after exposure cessation

Parental BaP exposure significantly affected F1 hatching, survival, growth, and morphological development in a dose-dependent manner, and these effects were strongly influenced by the timing of spawning relative to exposure. The offspring from parents that spawned 1 day after exposure cessation exhibited significantly lower hatching rates in 4 and 8 μg/L groups compared to the controls ($p < 0.05$ and $p < 0.01$, respectively). In offspring from parents that spawned 30 days after exposure cessation, the hatching rates for these higher-dose groups remained depressed ($p < 0.05$). By contrast, the offspring from parents that spawned 60 days after exposure cessation showed hatching rates almost similar to the controls across most groups, indicating substantial recovery of developmental potential. Consistent with these findings, Corrales et al. (2014b) reported decreased hatching success in zebrafish embryos from parents exposed to BaP at 2.3 and 20 μg/g diet, attributing this effect to compromised embryonic development, while Sun et al. (2020) observed significant hatching delays and increased embryo mortality in marine medaka at parental BaP exposures of 20 and 200 μg/L. Larval survival followed a similar pattern, with elevated mortality in offspring from parents that spawned 1 day after exposure cessation in the 4 and 8 μg/L groups ($p < 0.05$ and $p < 0.01$, respectively), which remained high in the high-dose groups of offspring spawned 30 days after exposure cessation ($p < 0.05$). By 60 days after exposure cessation, survival largely normalized, except in the 8 μg/L group where mortality remained elevated ($p < 0.05$). A comparable trend was observed for larval growth, indicating that the developmental impairment extended beyond early survival. Larval body length was significantly reduced in offspring from parents that spawned 1 day after exposure cessation ($p < 0.05$ and $p < 0.01$, respectively, in the high-dose groups), partially recovered in the 30-day spawn offspring ($p < 0.05$), and was fully restored in the 60-day spawn offspring (Fig. 1A, B and C).

Morphological malformations, including pericardial and yolk-sac edema, craniofacial deformities, eye anomalies such as microphthalmia, abnormal pigmentation (hyperpigmentation), and spinal curvature, were most pronounced in offspring from parents that spawned 1 day after exposure cessation ($p < 0.05$ and $p < 0.01$, respectively, in the high-dose groups). The incidence of these malformations decreased progressively in offspring from parents that spawned 30 days after exposure cessation ($p < 0.05$) and was further reduced in offspring from parents that spawned 60 days after exposure cessation ($p < 0.05$) (Figs. 1, and S2). Comparable studies have demonstrated pronounced developmental defects in F1 offspring following parental BaP exposure, although these findings were based on eggs collected immediately after the exposure period (Corrales et al., 2014b; Mo et al., 2020). In the present study, malformations were not completely eliminated, indicating that some developmental effects persisted. Together, these results highlight the importance of parental spawning timing in modulating transgenerational developmental toxicity. The observed recovery in offspring phenotypes across the depuration timeline aligns with the measured decrease in BaP residues in maternal ovaries and F1 eggs (Fig. S1), providing a mechanistic explanation for the reduction in toxicity through maternal depuration. Detailed hatching success, larval mortality, malformation rates, and body length are summarized in Table S4.

3.2. Mechanistic basis of BaP-induced transgenerational molecular perturbations

Parental BaP exposure elicited distinct molecular responses in F1 offspring, with recovery patterns varying among detoxification, apoptotic, and endocrine/growth signaling pathways. In offspring from parents that spawned 1 day after exposure cessation, antioxidant genes *sod1* and *sod2* were significantly downregulated in the higher-dose groups ($p < 0.05$), along with suppression of *cat* and *gpx* ($p < 0.05$), indicating compromised oxidative defense. This oxidative imbalance aligns with previous findings that BaP induces reactive oxygen species and oxidative stress in fish (Sun et al., 2020; Zeb et al., 2024b; Zhao et al., 2013). In offspring from parents that spawned 30 days after

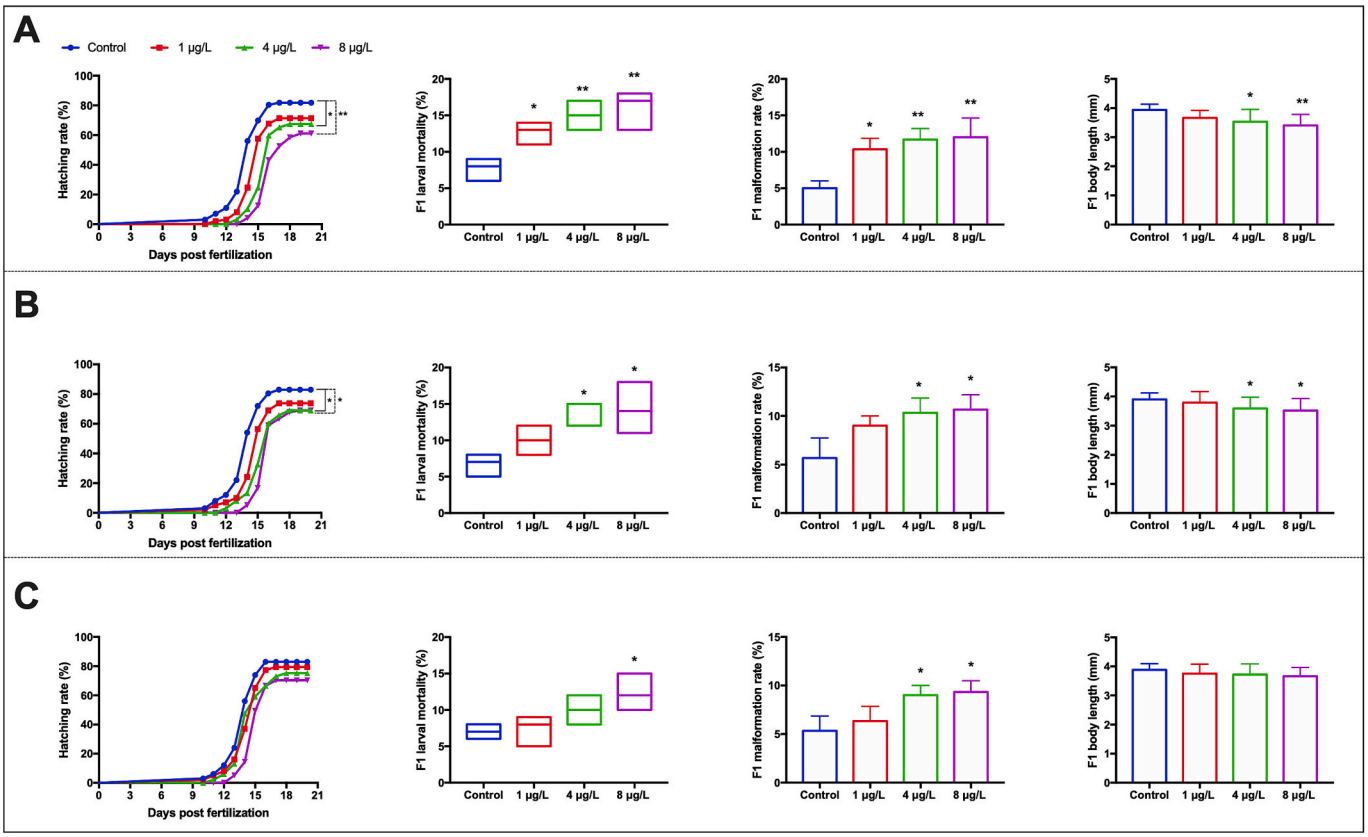

Fig. 1. Hatching rate, larval mortality, malformation incidence, and body length in F1 offspring derived from BaP-exposed parents. A, B, and C correspond to offspring produced by parents that spawned 1, 30, and 60 days after termination of BaP exposure, respectively. Parental exposure concentrations were 1, 4, and 8 μg/L BaP. Data are presented as mean ± SD, and asterisks denote statistically significant differences relative to the control group (*$p < 0.05$, **$p < 0.01$).

exposure cessation, *sod1* expression returned to control levels, whereas *sod2* and *gpx* remained suppressed in the higher-dose groups ($p < 0.05$). By 60 days after exposure cessation, all detoxification-related transcripts had normalized, suggesting that redox homeostasis recovers relatively quickly compared with other molecular systems. The apoptotic pathways showed slower recovery. Pro-apoptotic genes *p53*, *fas* (*tnfrsf6*), *casp9*, and *casp3* were upregulated ($p < 0.05$) in offspring from parents that spawned 1 day after exposure cessation, reflecting activation of programmed cell death in the higher-dose groups. Most apoptotic transcripts returned to baseline in the offspring from parents that spawned 60 days after exposure cessation, however *casp9* and *casp3* elevations persisted at the highest exposure ($p < 0.05$), indicating prolonged cellular stress (Fig. 2A, B and C). These apoptotic responses are in line with zebrafish findings where oxidative stress was shown to trigger programmed cell death during embryonic development (Aparna and Patri, 2021; Wan et al., 2022).

Endocrine and growth-related genes were particularly sensitive and exhibited the slowest recovery in this time-resolved transgenerational study. Steroidogenic pathways play a central role in regulating key physiological processes in vertebrates, including embryonic development, sex differentiation, metabolism, and reproductive function, and their activity is controlled by specific transcription factors and biosynthetic enzymes (Tokarz et al., 2015). In particular, genes such as *cyp17a* and *cyp11b* are critical for the production of sex steroids and corticosteroids: *cyp17a* mediates both 17α-hydroxylase and 17,20-lyase activities required for androgen and estradiol synthesis, while *cyp11b* catalyzes 11β-hydroxylation, a crucial step in the formation of corticosteroids and 11-ketotestosterone (Lv et al., 2020; Payne and Hales, 2004; Zhou et al., 2007). In the present study, expression of *cyp17a*, *17βhsd*, and *cyp11b* was reduced in offspring from parents that spawned 1 day after exposure cessation across all dose groups ($p < 0.05$). These reductions persisted in offspring from parents that spawned after 30 and 60 days, primarily in the higher-dose groups ($p < 0.05$), with the exception of *cyp11b*, which exhibited recovery over time. *Cyp19a* (aromatase) is essential for sex differentiation and reproductive function, and yolk protein genes *vtg1* and *vtg2* serve as reliable biomarkers for estrogenic chemicals (Cao et al., 2019b, Cao et al., 2019a; Kazeto et al., 2004). In this study, *cyp19a* and *vtg1* and *vtg2* were upregulated in higher-dose offspring from parents that spawned 1 day and 30 days after exposure cessation ($p < 0.05$), suggesting estrogenic shifts in steroid signaling. In the offspring from parents that spawned 60 days after exposure cessation, only *vtg2* expression level remained elevated in the higher-dose groups ($p < 0.05$), while the others returned to nonsignificant levels (Fig. 2A, B and C). Transgenerational modulation of steroidogenic genes has been observed in other studies, reflecting persistent endocrine-related alterations across generations (Chen et al., 2025). However, contrasting patterns have also been documented, such as reduced *cyp19a* and *vtg1* expression in offspring, indicative of anti-estrogenic effects (Sun et al., 2020). Such inconsistencies among studies may result from differences in exposure regimes and BaP concentrations, as varying exposure contexts can shift BaP's action between estrogenic and anti-estrogenic modes (Booc et al., 2014; Patel et al., 2006).

Furthermore, in this study, *igf1*, *irs*, and *igfbp3* were suppressed in parallel with reduced larval body length in higher-dose offspring from parents that spawned 1 day and 30 days after exposure cessation ($p < 0.05$). While *igf1* expression returned to control levels in the offspring from parents that spawned 60 days after exposure cessation, *irs* and *igfbp3* showed only partial recovery (Fig. 2A, B and C). Previous studies have shown that *igf1* regulates somatic growth in fish (Moriyama et al., 2000), and suppression of *igf1* and its binding proteins can inhibit larval growth (Dang et al., 2018). Additionally, *tg* transcripts were elevated

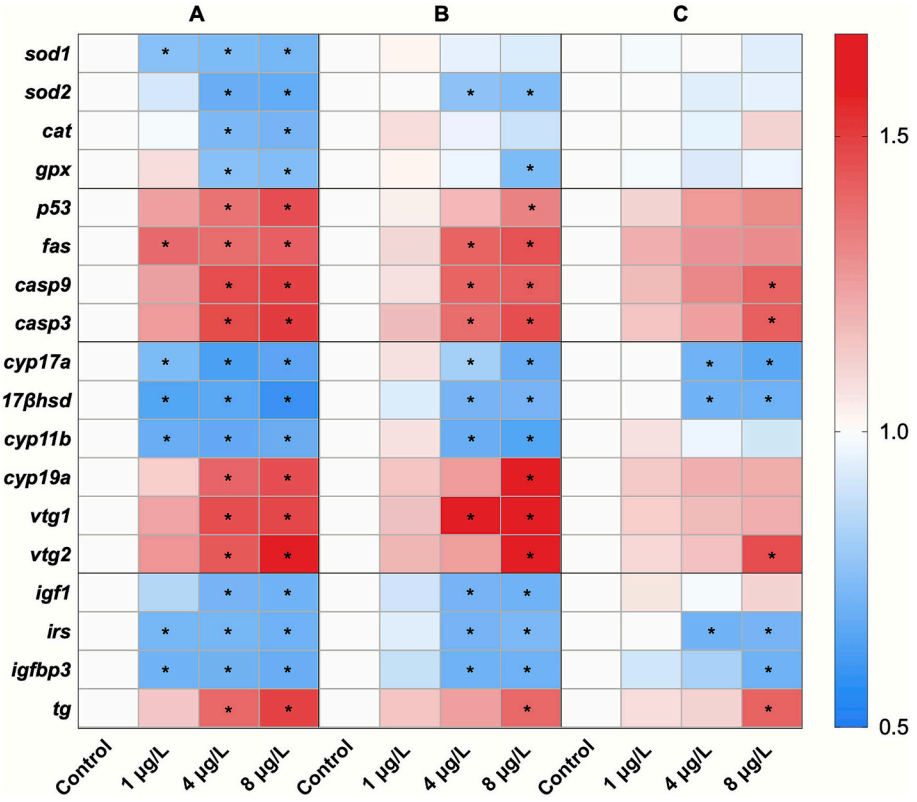

Fig. 2. Gene expression alterations in F1 offspring derived from BaP-exposed parents. A, B, and C correspond to offspring produced by parents that spawned 1, 30, and 60 days after termination of BaP exposure, respectively. Parental exposure concentrations were 1, 4, and 8 μg/L BaP. Data are presented as mean ± SD, and asterisks denote statistically significant differences relative to the control group (*$p < 0.05$).

and remained high in high-dose progeny at all the three time points ($p < 0.05$), indicating persistent thyroid-axis disruption, whereas lower-dose groups returned to baseline at later intervals. Given thyroglobulin's role in thyroid hormone synthesis and growth regulation (Deal and Volkoff, 2020; Zhang et al., 2018), these alterations further implicate endocrine dysregulation in the observed developmental impairments. Overall, these findings demonstrate a hierarchical recovery pattern in F1 offspring from BaP-exposed parents. While antioxidant defenses recover first and apoptotic responses resolve more slowly, the most persistent perturbations in endocrine and growth pathways pose the highest risk for long-term adverse outcomes.

3.3. Hormonal outcomes and implications for transgenerational endocrine disruption

Parental BaP exposure produced marked hormonal alterations in F1 offspring, linking endocrine disruption to observed developmental outcomes. At 21 dpf, the offspring from parents that spawned 1 day after exposure cessation exhibited elevated ACTH and cortisol in all dose groups ($p < 0.05$), indicating activation of the hypothalamic-pituitary-interrenal (HPI) axis in response to parental chemical stress. ACTH and cortisol gradually declined in the offspring from parents that spawned 30 days after exposure cessation, with levels in the high-dose group remaining significantly elevated ($p < 0.05$), and normalized in the 60-day spawn cohort (Fig. 3A, B and C). This recovery paralleled the normalization of detoxification and apoptotic markers observed in the corresponding offspring cohorts. In teleost fish, cortisol is synthesized in the interrenal cells of the head kidney and mediates the physiological stress response, with production regulated by pituitary ACTH and hypothalamic corticotropin-releasing hormone (Hontela, 2005; Waring et al., 1992). The results indicate that parental BaP exposure activates the HPI axis in F1, with stress markers showing a tendency to return toward control levels after a 60-day depuration interval.

GH plays an important role beyond direct growth promotion, including stimulating insulin-like growth factor 1 production, which is essential for normal development (Björnsson et al., 2018). In this study, GH was significantly suppressed in the higher-dose offspring from parents that spawned 1 day after exposure cessation ($p < 0.05$) and partially recovered in the 30-day spawn cohort, with full recovery observed only in the 60-day spawn group. These patterns align with the partial restoration of growth-axis gene expression (*igf1, irs, igfbp3*) and correspond to reduced larval body length, indicating that parental BaP exposure impairs growth regulation in F1. Alongside GH, thyroid-axis disruption was evident, with T4 levels showing persistent elevation (Fig. 3A, B and C). Environmental pollutants are known to disturb the hypothalamic-pituitary-thyroid (HPT) axis, leading to abnormal thyroid hormone levels and developmental consequences (Chan and Chan, 2012; Deal and Volkoff, 2020; Kim and Ji, 2019). In many teleost species, early embryos depend on thyroid hormones already present in the egg, and these maternally sourced hormones influence key developmental processes before the onset of endogenous thyroid activity (Campinho et al., 2014; Da Silva Rodrigues et al., 2025; Power et al., 2001; Vergauwen et al., 2018; Wei et al., 2018). In the present study, T4 was elevated in offspring from the 1-day and 30-day spawn groups ($p < 0.05$) and remained significantly higher in the high-dose 60-day spawn offspring ($p < 0.05$), consistent with sustained upregulation of *tg* transcripts. These findings demonstrate that T4 dysregulation underlies thyroid-axis perturbation, which in turn drives delayed growth and morphological anomalies such as hyperpigmentation as observed in zebrafish and Japanese flounder (*Paralichthys olivaceus*) (McMenamin et al., 2014; Walpita et al., 2009; Yoo et al., 2000). The elevated cortisol level under chronic stress may further interact with T4, exacerbating pigmentation changes (Ruane et al., 2005; Vissio et al., 2021; Yamada et al., 2011). Collectively, these results demonstrate that persistent HPT axis

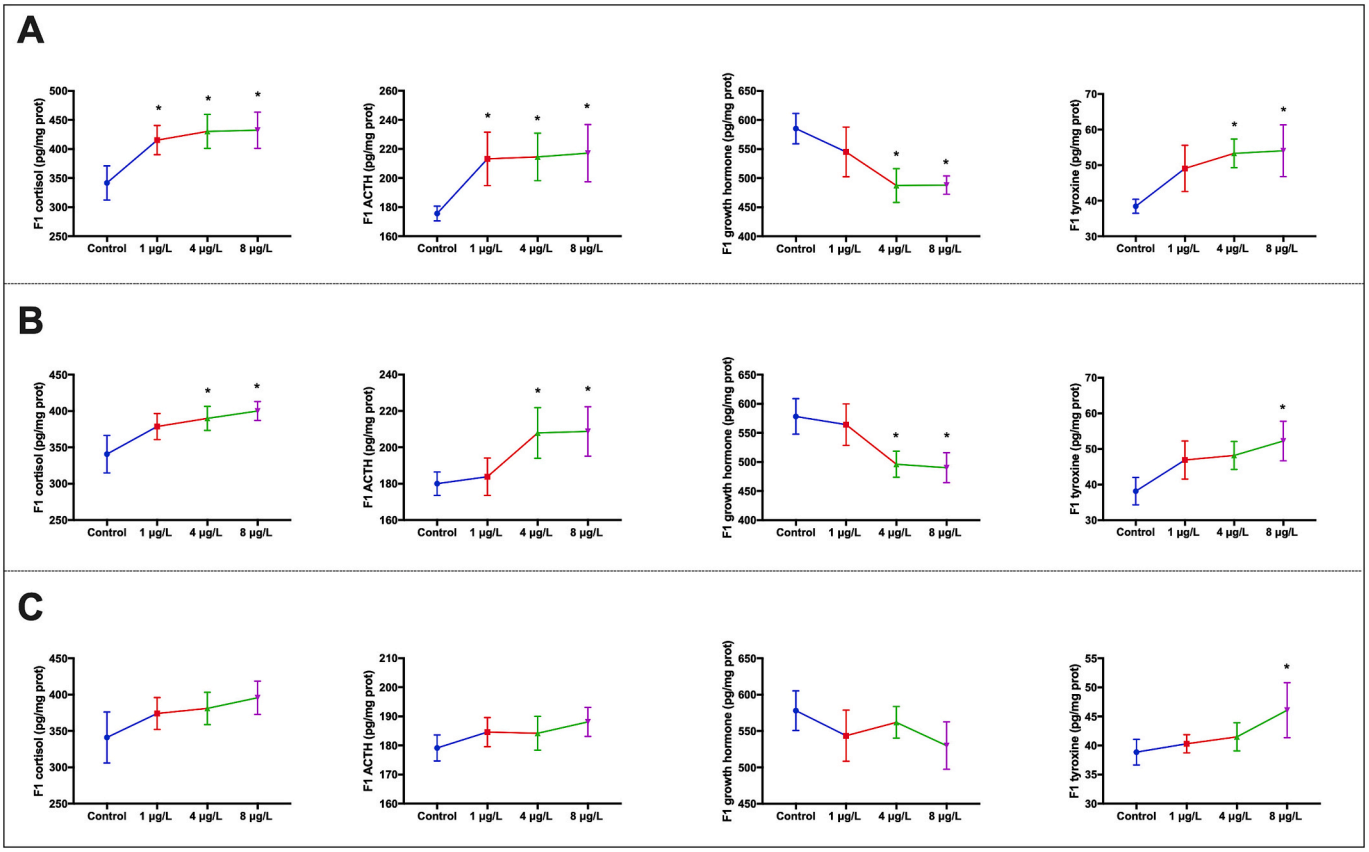

Fig. 3. Cortisol, ACTH (adrenocorticotropic hormone), GH (growth hormone), and T4 (thyroxine) levels in F1 offspring derived from BaP-exposed parents. A, B, and C correspond to offspring produced by parents that spawned 1, 30, and 60 days after termination of BaP exposure, respectively. Parental exposure concentrations were 1, 4, and 8 µg/L BaP. Data are presented as mean ± SD, and asterisks denote statistically significant differences relative to the control group ($^*p < 0.05$).

disruption parallels the observed molecular and developmental effects in F1 larvae.

4. Conclusion

Parental BaP exposure triggered a cascade of molecular disturbances in F1 offspring in this study, with endocrine function showing the slowest recovery in the time-resolved design. The timing of parental spawning after exposure appeared to influence the magnitude and persistence of these effects, with parental recovery time likely contributing to residual developmental changes in offspring from higher exposure groups. These findings provide insight into the potential transgenerational consequences of parental BaP exposure and highlight the importance of considering reproductive timing when assessing inheritance of chemical-induced effects.

CRediT authorship contribution statement

Rabia Zeb: Writing – review & editing, Writing – original draft, Software, Methodology, Data curation. **Xiaohan Yin:** Validation, Methodology. **Fangyi Chen:** Visualization, Validation. **Jun Bo:** Writing – review & editing, Validation, Funding acquisition. **Ke-Jian Wang:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2025.119211.

Data availability

Data will be made available on request.

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