

Effects of Exposure to Diazepam on the Survival, Development, Behavior, and Transcriptomic Profiles in the Offspring of Japanese Medaka

Shuning QIU

Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University : Master' s Program

Xuchun QIU

School of the Environment and Safety Engineering, Jiangsu University

Sijing WANG

School of the Environment and Safety Engineering, Jiangsu University

Kun CHEN

School of the Environment and Safety Engineering, Jiangsu University

他

<https://doi.org/10.5109/7407632>

出版情報 : 九州大学大学院農学研究院紀要. 71 (1), pp.11-18, 2026-02-27. Faculty of Agriculture, Kyushu University

バージョン :

権利関係 :



Effects of Exposure to Diazepam on the Survival, Development, Behavior, and Transcriptomic Profiles in the Offspring of Japanese Medaka

Shuning QIU¹, Xuchun QIU^{2,3,*}, Sijing WANG², Kun CHEN², He GAO^{4,*}, Yuki TAKAI⁵, Yohei SHIMASAKI⁵, and Yuji OSHIMA⁵

Laboratory of Marine Environmental Science, Division of Animal & Marine Biosciences,
Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan

(Received October 24, 2025 and accepted November 5, 2025)

Diazepam (DZP), an emerging medicinal pollutant routinely identified in aquatic ecosystems, has been shown to accumulate significantly in the gonads of fish species. Nevertheless, the detrimental impacts stemming from parental DZP exposure on fish offspring remain insufficiently characterized. This study examined the effect of 4-week DZP exposure (0.8 µg/L) in F0 generation Japanese medaka on the survival capacity, ontogenetic development, behavioral responses, and transcriptional regulation of their offspring (F1 generation). Following over two weeks of exposure, F0 individuals exhibited a marked decline in daily egg production. Parental exposure can transmit DZP to offspring of Japanese medaka and significantly reduce the survival and hatching rates of F1 individuals. In the light/dark transition test, F1 larvae derived from DZP-exposed parents demonstrated lower average swimming velocity. Furthermore, transcriptome analysis identified 341 differentially expressed genes (DEGs, 261 up-regulated, and 80 down-regulated) in F1 larvae of DZP-exposed parents. Those DEGs are enriched within three critical KEGG pathways: Antigen processing and presentation, Endoplasmic reticulum protein processing, and Estrogen signaling pathway. Considering that residual DZP levels detected in aquatic ecosystems may exceed the experimental threshold (0.8 µg/L), the impacts of parental pharmaceutical exposure on fish offspring should be of greater concern.

Key words: Diazepam; Parental exposure; Offspring; Transcriptomic profiles; Japanese medaka

INTRODUCTION

Benzodiazepines (BZDs) are psychotropic agents that amplify inhibitory neurotransmitter gamma-aminobutyric acid (GABA) neurotransmission, resulting in sedative, hypnotic, anxiolytic, anticonvulsant, and muscle-relaxant properties (Fogliano et al., 2022). Due to their long-term and widespread use, BZDs have become significant environmental contaminants in aquatic ecosystems (Wilkinson et al., 2022). For instance, residual diazepam (DZP) has been identified across different water environments, such as wastewater treatment plant (WWTP) influent and effluent (Kosjek et al., 2012), along with riverine environments (Wilkinson et al., 2022), with concentrations spanning from ng/L to µg/L.

River water samples have shown maximum DZP concentrations of up to 0.85 µg/L (Wilkinson et al., 2022).

In the context of environmentally relevant concentration exposure conditions, BZDs exert a substantial influence on the growth and developmental processes, behavioral responses (Richendrfer et al., 2012), and gene expression (Oggier et al., 2010) in fish during their critical early life phases. For instance, exposure to delorazepam (at 5 µg/L for 4 days) significantly reduced the *Xenopus laevis* embryonic heart rate and locomotor performance (Fogliano et al., 2022). Regarding DZP, zebrafish larvae aged 7 days post-fertilization (dpf) exposed to 2-hour periods exhibited substantial alterations in their avoidance behavior and tactile activity (Richendrfer et al., 2012). Consequently, the adverse impacts of DZP contamination on aquatic organisms and their corresponding ecological hazards warrant considerable environmental attention.

Exposure to BZDs may impair reproductive capacity in aquatic organisms (Fogliano et al., 2024; Wang et al., 2023; Xuchun et al., 2025), thereby causing potential risks to their population dynamics and aquatic ecosystems. For example, exposure to oxazepam (0.8 µg/L for 2 months) significantly reduced the number of eggs of wandering snails (*Radix balthica*) (Lebreton et al., 2021). Exposure to delorazepam (1 µg/L for 48 h) reduced the fertilizing ability of sea urchin (*Paracentrotus lividus*) gametes and interfered with embryo development (Fogliano et al., 2024). Exposure to DZP (8 µg/L for 21 days) could impair oocyte maturation in zebrafish (Zhang et al., 2024b). Given widespread BZDs contamination, it is essential to investigate how

¹ Master's Program, Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China

² School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China

³ Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Suzhou University of Science and Technology, Suzhou 215009, China

⁴ Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013, China

⁵ Laboratory of Marine Environmental Science, Division of Animal and Marine Biosciences, Department of Bioresource Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan

* Corresponding Author: xuchunqiu@ujs.edu.cn (Xuchun QIU); gaoh@ujs.edu.cn (He GAO)

parental exposure to these contaminants influences the offspring of aquatic species. Nevertheless, research examining the impacts and mechanistic pathways of DZP exposure in parental fish on their offspring remains limited.

Japanese medaka (*Oryzias latipes*) is a widely utilized model species in chemical risk assessment studies due to its small size, rapid growth, continuous daily egg production, short diapause cycle, and well-documented genetic information (Matsui, 2017). Our previous studies proved that exposure to DZP (0.8 and 8 µg/L for 28 days) induced gonadal accumulation of DZP in Japanese medaka (Qiu et al., 2023). Based on this, we hypothesized that parental DZP exposure negatively impacts Japanese medaka offspring. To validate this, mature Japanese medaka (F0) were exposed to DZP (0.8 µg/L) over four weeks and allowed to mate naturally. During the exposure period, we collected fish embryos and examined variations in their survival capacity, ontogenetic development, behavioral responses, and transcriptional regulation.

MATERIALS AND METHODS

Test chemicals

Diazepam (CAS 439–14–5; purity: ≥ 99.8%) was purchased from the Laiyao Biotechnology Co., Ltd. (Beijing, China). Methanol and other reagents (NaCl, KCl, CaCl₂•2H₂O, MgSO₄•7H₂O, NaHCO₃, and Methylene Blue) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Test fish

Sexually mature Japanese medaka (*Oryzias latipes*) aged 6 months used in this study were obtained from the Fish Experimental Platform of Jiangsu University (Zhenjiang, Jiangsu, China). The experimental organisms were reared in 16-L cylindrical glass tanks containing 12 L of culture water (dechlorinated and carbon-filtered tap water; pH: 7.2–7.6; temperature: 26 ± 1°C) under a 14-h light/10-h dark photoperiod. The fish received freshly hatched *Artemia* nauplii (< 24 h post-hatching) in two daily feedings at a total rate of 2% body weight per day. All animal experiments were performed in accordance with the associated relevant guidelines and regulations approved by the Animal Care and Use Committee of Jiangsu University.

Exposure of the F0 generation

DZP stock solution (1 mg/ml in methanol) was subjected to stepwise dilution, yielding a target concentration of 0.8 µg/L in the culture water. In the control groups, culture water containing methanol (0.008%) was used as the test solution. The concentration used in this study was based on the monitoring studies that detected the maximum DZP concentration (0.85 µg/L) in river water (Wilkinson et al., 2022). A total of 72 healthy adult Japanese medaka (6 months old, half males and half females) were used for the parental DZP exposure test. For either treatment group (i.e., control or DZP

exposure), 18 females and 18 males were housed in 20-liter glass tanks filled with 15 liters of prepared test solution. The exposure was conducted for four weeks, with systematic replacement of the test solution at 48-h intervals.

Over the four-week experimental duration, embryos in each experimental tank were collected and counted daily (8:00–8:30 AM), except on the days for experimental tank cleaning (i.e., days 6, 12, 18, and 24). In addition, water samples (10 ml) were collected to confirm the realistic DZP concentration in the exposure system following the method described by Zhang et al. (2024b). For embryos collected during the first three weeks, the outer chorionic layer was meticulously dissected away, followed by triple-rinse purification with culture water. The specimens were stored at –80 °C until used for determining the DZP content, following the method described by Qiu et al. (2023).

Maintenance and observation of the F1 generation

The embryos collected during the fourth week were cultured in a DZP-free embryo culture medium (Koenig et al., 2020) until 18 dpf. Within each experimental group, a total of 90 embryos were systematically distributed across triplicate culture dishes (30 embryos/plate; n = 3 replicates) for subsequent physiological assay. All culture dishes were incubated at 28 °C with a 14:10 light-dark cycle, accompanied by daily renewal of embryo culture medium. The mortality, deformity, and hatching time were confirmed and recorded daily, and the dead individuals were removed upon notice. At 6 dpf, 10 randomly sampled embryos from each culture dish were used to assess heart rate using an Olympus SZX16 stereomicroscope (Tokyo, Japan). Furthermore, the unused F1 embryos were maintained under identical culture conditions and subsequently designated for behavioral assay and transcriptomic profiling.

Behavioral responses assay

At 16 dpf, 24 F1 larvae were randomly sampled from each experimental group and subsequently underwent behavioral assessment using the light-dark transition test, following the method described by Qiu et al. (2024a). Briefly, F1 larvae were carefully transferred into individual wells of 24-well plates, with 800 µL embryo culture medium prefilled in each well. The movement trajectories of larvae under light-dark transitions (5 min per phase: dark-light-dark) were quantitatively assessed using a DanioVision System (Noldus, Wageningen, Netherlands). Subsequent data processing and behavioral parameter extraction were performed with EthoVision XT software (version 11.5, Noldus).

Transcriptomic Profiling in F1 larvae

At 18 dpf, 20 F1 larvae were harvested and pooled to constitute a single biological sample for transcriptomic profiling, with 3 replicates generated per experimental group. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) and subsequently purified through phase separation. Following established proto-

cols from the Beijing Genome Institute (BGI), high-quality cDNA libraries were constructed and subjected to sequencing on the BGISEQ-500 platform. Gene expression quantification was performed using RSEM (v1.2.12), followed by statistical identification of differentially expressed genes (DEGs) through DESeq2 (v1.4.5) with selection criteria (Q -value ≤ 0.05 ; $|\log$ fold change ≥ 1.5). We subsequently performed functional enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) platform. Furthermore, protein-protein interaction (PPI) networks of DEGs identified through KEGG pathway enrichment analysis were constructed using the STRING database and visualized with Cytoscape software. The key driver genes (hub genes) were identified using the plugin CytoHubba.

Statistical analysis

The statistical framework employed generalized linear models (GLM) to investigate associations between critical developmental parameters (survival rate, hatchability success, and heart rate) and behavioral responses in Japanese medaka. These quantitative evaluations were executed using the SPSS 16.0 software (SPSS Inc., Chicago, IL).

RESULTS

The realistic DZP concentrations in exposure systems were detected in the range of 0.72 to 0.93 $\mu\text{g/L}$. All

F0 Japanese medaka survived the entire exposure period without observable death. The body lengths (mean $\pm$ SE) of F0 females and males were measured as 2.13 ± 0.03 cm and 2.24 ± 0.03 cm, respectively. Their corresponding body weights (mean $\pm$ SE) averaged 0.17 ± 0.01 g and 0.20 ± 0.01 g.

Reproductive capacity of the F0 generation

The weekly egg production (over a 6-day counting period) by Japanese medaka during the DZP exposure experiment is shown in Fig. 1. Egg production declined significantly in the Japanese medaka exposed to DZP during the third and fourth weeks of the experiment. Furthermore, DZP accumulation was detected in embryos following parental DZP exposure, with concentrations ranging from 2.53 ± 0.29 $\mu\text{g/kg}$ to 3.98 ± 0.19 $\mu\text{g/L}$.

Survivorship and Ontogenetic Development of the F1 generation

Parental DZP exposure exerted detrimental effects on F1 offspring survivorship, as evidenced by both the pronounced elevation in mortality rates (Fig. 2A) and concurrent decline in hatching success (Fig. 2B). At 6 dpf, heart rates of F1 embryos in the control and DZP-exposed groups were recorded at 113.5 ± 1.33 beats/min and 109.9 ± 2.41 beats/min, respectively, with no significant difference (Fig. 2C).

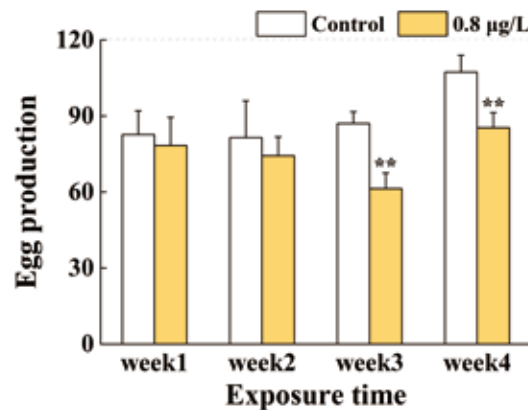


Fig. 1. Egg production of F0 generation Japanese medaka (*Oryzias latipes*) exposed to diazepam at 0 (control) and 0.8 $\mu\text{g/L}$. Data are shown as mean $\pm$ SD ($n = 6$), with asterisks denoting statistical significance (** $p < 0.01$).

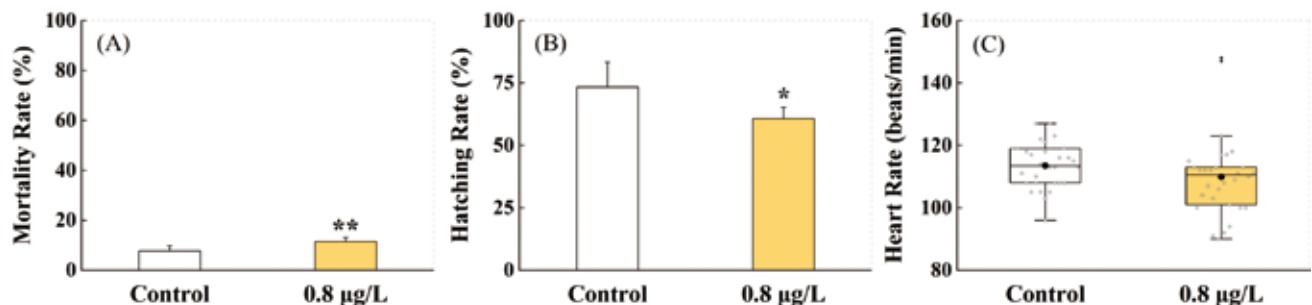


Fig. 2. Response in physiological parameters in F1 generation of DZP-exposed F0 Japanese medaka (*Oryzias latipes*). (A) The mortality rate at 16 dpf; (B) The hatching rate at 16 dpf; (C) The heart rate at 6 dpf. Each dish was regarded as a replicate (30 embryos, $n = 3$); Data are shown as mean $\pm$ SD, with asterisks denoting statistical significance (* $p < 0.05$; ** $p < 0.01$).

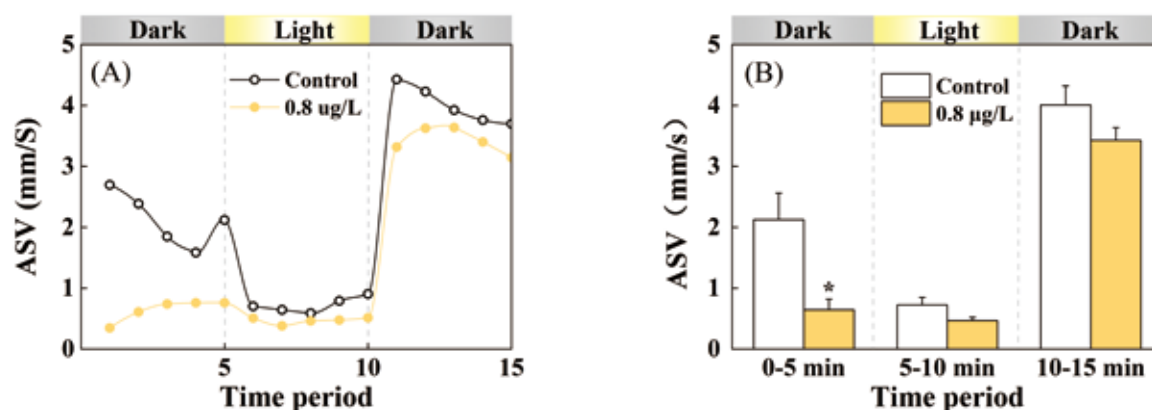


Fig. 3. The average swimming velocity (ASV) of F1 larvae derived from control and DZP-exposed F0 Japanese medaka (*Oryzias latipes*). **(A)** Line chart describing the ASV of F1 larvae in each group during 1 minute. **(B)** Bar chart with ASV data during 5 minutes. Expressed as mean $\pm$ SD. The dark and light periods are represented by the gray and yellow bars at the top of the graph; Data are shown as mean $\pm$ SD ($n = 6$), with asterisks denoting statistical significance ($*p < 0.05$).

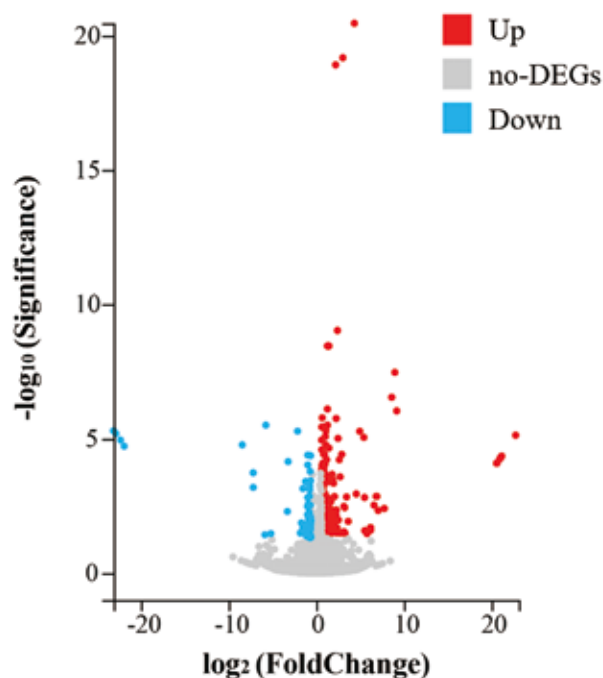


Fig. 4. Scatterplot of the differentially expressed genes (DEGs) in F1 larvae parentally exposed to diazepam.

Behavioral responses during the light/dark transition test of F1 larvae

Light–dark transition–induced behavioral responses in F1 larvae (16 dpf) are depicted in Fig. 3. Relative to the control, parental exposure to DZP reduced the response of F1 larvae to the light-switching stimulus (Fig. 3A). Notably, larvae of DZP-exposed parents exhibited a marked reduction in average swimming velocity (ASV) compared with control groups (Fig. 3B).

Transcriptional responses in F1 larvae of DZP-exposed parents

Relative to control groups, transcriptomic profiling identified 341 DEGs in F1 larvae of DZP-exposed parents, of which a total of 261 genes were up-regulated in expression, and 80 genes were down-regulated (Fig. 4). The top five enriched KEGG pathways of DEGs are

shown in Fig. 5. Most of the DEGs were significantly enriched in pathways of Antigen Processing and Presentation, Leukocyte transendothelial migration, and the Estrogen Signaling Pathway (Fig. 5). In addition, significant enrichment in the Protein Processing in the Endoplasmic Reticulum was also identified.

Analysis of the PPI network derived from DEGs identified 6 key driver genes in response to parental DZP exposure (Fig. 6). Among them, 5 DEGs (i.e., *hspa1s*, *hspa4*, *hspa5*, *hsppb1*, and *cd74*; all up-regulated) were identified as key driver genes within the molecule networks governing Antigen Processing and Presentation, Protein Processing in the Endoplasmic Reticulum, and Estrogen Signaling Pathway. In addition, *gart* (down-regulated) was identified as the key driver gene within the molecular networks governing metabolism pathways.

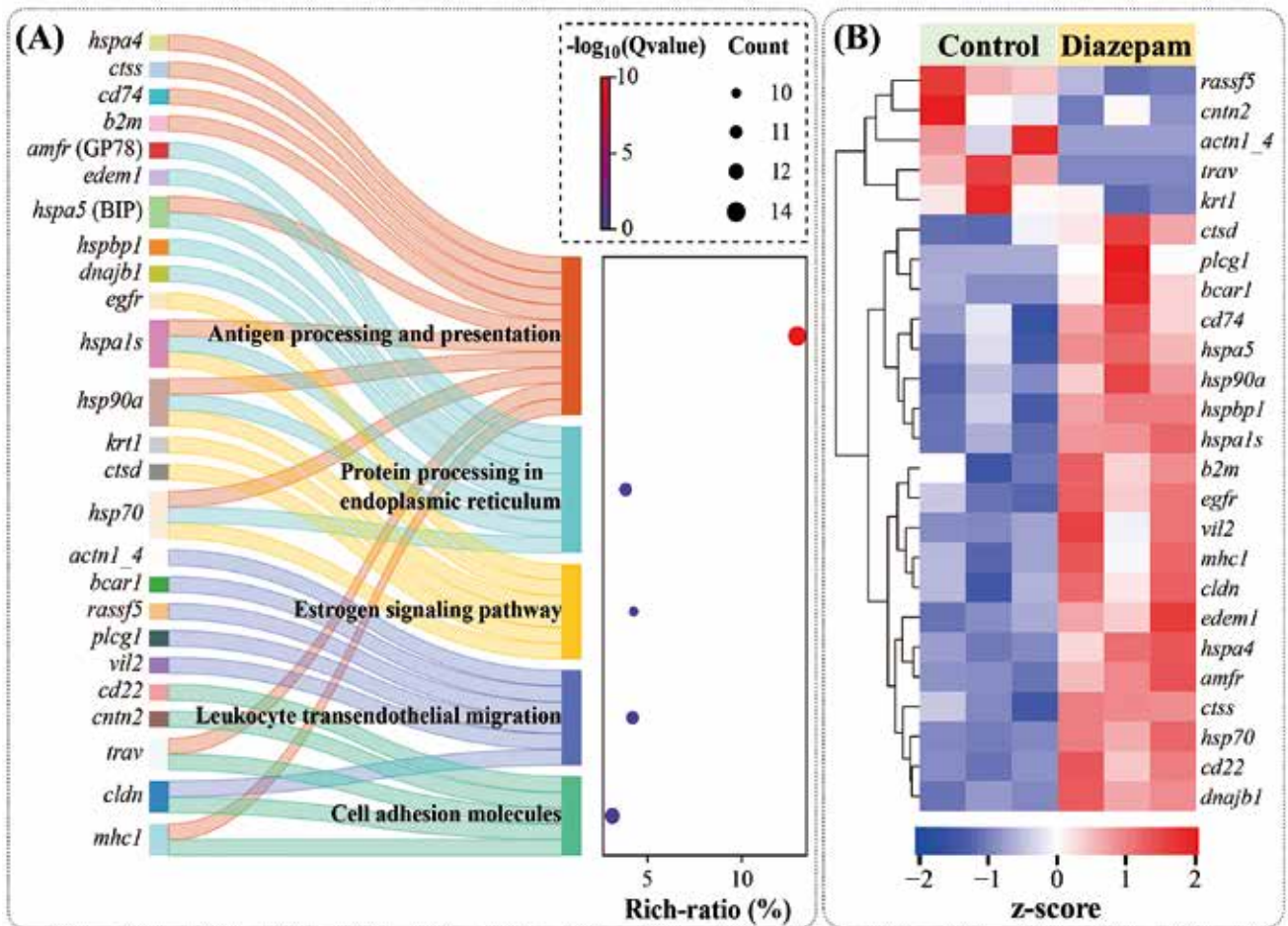


Fig. 5. (A) Sankey-bubble diagram depicting associations between DEGs and the top five significantly enriched KEGG pathways identified through comparative transcriptomic analysis of F1 larvae derived from control and DZP-exposed F0 Japanese medaka (*Oryzias latipes*). Connecting flows represent genes with significant co-enrichment across multiple pathways. (B) Heatmap showing the expression profiles of DEGs associated with the top 10 KEGG-enriched pathways in F1 larvae derived from control and DZP-exposed F0 medaka.

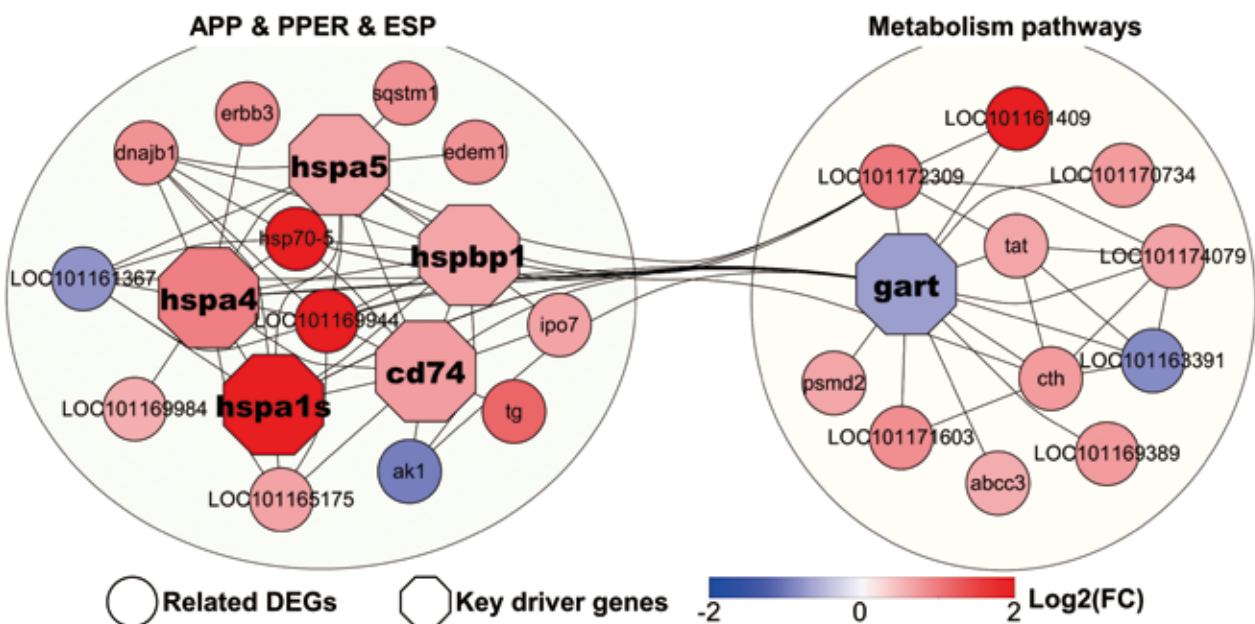


Fig. 6. Protein-protein interaction (PPI) network showing the key driver genes and related differentially expressed genes (DEGs) identified in F1 larvae of DZP-exposed F0 Japanese medaka (*Oryzias latipes*). APP: Antigen Processing and Presentation; PPER: Presentation and Protein Processing in the Endoplasmic Reticulum; ESP: Estrogen Signaling Pathway.

DISCUSSION

The results revealed that parental exposure to DZP disrupted survival, developmental processes, behavioral responses, and gene expression profiles in Japanese medaka offspring, thereby corroborating our initial hypothesis. DZP has been evidenced to impinge on the reproductive capability of aquatic species. For example, exposure to DZP (8 µg/L for 4 weeks; 12 µg/L for 3 weeks) significantly disrupted the courtship-associated behavioral traits in fish, suggesting a reduction in reproductive attempts (Chen et al., 2021b; Wang et al., 2024). Furthermore, exposure to DZP could dysregulate the pathways related to oocyte maturation in female zebrafish, which may provide evidence for its impact on reproductive capacity at the molecular level (Zhang et al., 2024b).

In addition to reducing egg production of Japanese medaka, exposure to DZP also caused the accumulation of DZP in their F1 embryos. This may partly contribute to the significantly reduced survival rate and hatching rate of F1 embryos of DZP-exposed parents. Although waterborne exposure to BZDs has been demonstrated to interfere with embryonic development in aquatic species (Chen et al., 2021a; Fogliano et al., 2022), there has been limited research investigating the developmental consequences of in-ovo BZDs exposure on fish offspring. Our results also suggested that parental DZP exposure significantly reduced the swimming velocity of their offspring, which may further reduce the environmental adaptability of fish larvae. For example, diminished locomotor activity can lower predator encounters but frequently results in reduced feeding, growth, and reproductive opportunities (Brodin et al., 2014; Perreault et al., 2003). Given that Fish larvae and juveniles serve a critical role in aquatic ecosystems as both consumers and converters (Yen et al., 2022), the decline in their viability will not only reduce their populations but also disrupt the ecological balance of the aquatic environment.

Transcriptional analysis revealed that parental exposure of Japanese medaka to DZP could cause disturbances in the gene expression profile of their offspring. Functional enrichment analysis revealed that parental exposure to DZP could dysregulate the molecular networks related to the immune system, endoplasmic reticulum stress, and endocrine system, with *hspa1s*, *hspa4*, *hspa5*, *hspbp1*, and *cd74* as the key driver genes.

Antigen presentation helps the immune system recognize whether a cell is healthy or abnormal, which is an essential process in acquired immunity (Soler-Agosta et al., 2023). In teleosts, dysregulation of this pathway is associated with autoimmune response efficiency (Salvador-Mira et al., 2025). Moreover, most of the DEGs involved in the Antigen Processing and Presentation were up-regulated in the F1 larvae of parents exposed to DZP. Among them, the *cd74* gene encodes a molecule that interacts with class II major histocompatibility complex (MHC), serving as a critical molecular chaperone that facilitates antigen processing and presentation during immune responses (Qiu et al.,

2024b). Up-regulated expression of *cd74* could be observed in mammals under viral stress (Zhang et al., 2024a). On the other hand, 70-kDa heat shock proteins (Hsp70s, including *hspa1s*, *hspa4*, and *hspa5*) play critical roles as mediators of cellular protein folding and remodeling processes (Rosenzweig et al., 2019). In rainbow trout (*Oncorhynchus mykiss*) embryos, thermal adaptation-induced up-regulated expression of *hsp70a* may disrupt cardiac and pharyngeal arch structure development, thereby potentially contributing to malformations (Hosseinpour et al., 2024). Therefore, impaired antigen presentation in offspring following parental DZP exposure may compromise immune organ development, leading to persistent immune dysfunction.

The endoplasmic reticulum (ER) is a central organelle governing lipid biosynthesis and protein processing in eukaryotic cells (Molinari, 2024). Parental exposure of Japanese medaka to DZP dysregulated the protein processing in endoplasmic reticulum in their F1 larvae, which may induce ER stress. In mammals, the association between ER stress and neurodegenerative disorders has been well-established (Hasan et al., 2024). In zebrafish, ER stress has been observed to be associated with larval locomotor abnormalities (Zeng et al., 2016). Meanwhile, ER stress serves as a critical regulatory node in antigen presentation function (Salvador-Mira et al., 2025). In addition, up-regulated expression of HSPs (e.g., *hspa5*) represents a classic biomarker of ER stress (Fernández et al., 2024), and up-regulated expression of *hsp90* may exacerbate ER stress-associated apoptotic signaling (Raghunath et al., 2018). Collectively, our findings suggest that parental exposure to DZP may trigger ER stress in offspring, thereby contributing to neurodevelopmental disorders and resulting in disrupted behavioral responses.

Estrogen plays a critical role in regulating various physiological and pathological processes within the body (Khan et al., 2022). Disruption of its signaling by environmental pollutants can lead to developmental abnormalities and endocrine disruption in zebrafish offspring (Zhang et al., 2023). For *hsp90a*, it is instrumental in facilitating the maturation process of estrogen receptors, enabling its binding to estrogen and subsequent initiation of downstream transcriptional activation (Lee et al., 2012). HSP70 (including *hspa1s* and *hspa5*) mediates crosstalk between heat shock and estrogen signaling by stabilizing steroid receptor complexes, thereby enhancing germ cell stress tolerance and providing cytoprotective support during spermatogenesis and oocyte maturation (Silveira et al., 2023). Furthermore, the epidermal growth factor receptor (EGFR) serves as a pivotal regulator in developmental processes and tissue homeostasis maintenance, orchestrating cellular repair mechanisms and regenerative processes in organs (Uribe et al., 2021). Upregulation of *egfr* may accelerate the retraction of transzonal projections, leading to premature decoupling between germ cells and somatic cells, thereby compromising oocyte maturation and quality (Abbassi et al., 2021). Collectively, these findings demonstrate that parental DZP exposure may compromise

reproductive capacity by impairing oocyte maturation, ultimately leading to reduced fertility.

Dysregulation in the pathways of Leukocyte transendothelial migration and Cell adhesion molecules was also observed in F1 larvae of DZP-exposed parents. Leukocyte transendothelial migration is the critical process whereby leukocytes exit the bloodstream by traversing the endothelial cell layer to infiltrate surrounding tissues (Schwartz et al., 2021). Cell adhesion molecules constitute transmembrane glycoproteins that orchestrate biological interactions through facilitating cellular recognition (intercellular and cell-matrix associations), establishing adhesive connections, and initiating downstream signaling cascades (Harjunpää et al., 2019). Both are core mechanisms underlying immune response and inflammatory regulation. The dysregulation of either may lead to impaired immune response capacity during embryonic development, thereby affecting organogenesis. The *gart* encodes phosphoribosylglycinamide formyltransferase, a critical enzyme required for the de novo purine biosynthesis pathway (Ng et al., 2009). The down-regulation of *gart* compromises purine synthesis, impairing cell proliferation and survival in zebrafish embryos. These findings were similarly associated with reduced survival rates, hatching success, and locomotor activity in the F1 generation following DZP exposure.

AUTHOR CONTRIBUTIONS

S. QIU and S. WANG performed the exposure test, analyzed the data, and wrote the paper. X. QIU designed the study, wrote the paper, and provided facilities and resources. K. CHEN and H. GAO designed the study, wrote the paper, and provided facilities and resources. Y. TAKAI, Y. SHIMASAKI, and Y. OSHIMA designed the study, wrote the paper, and provided resources. All authors assisted in editing the manuscript and approved the final version.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 32071623) and JSPS KAKENHI (Grant No. JP24K20939).

REFERENCE

- Abbassi, L., El-Hayek, S., Carvalho, K.F., Wang, W., Yang, Q., Granados-Aparici, S., et al. 2021 Epidermal growth factor receptor signaling uncouples germ cells from the somatic follicular compartment at ovulation. *Nat. Commun.*, **12**: 1438
- Brodin, T., Piovano, S., Fick, J., Klaminder, J., Heynen, M., Jonsson, M. 2014 Ecological effects of pharmaceuticals in aquatic systems—impacts through behavioural alterations. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, **369**: 20130580
- Chen, C., Li, L., Li, M., Wu, M., Liang, W., Takai, Y., et al. 2021a Impacts of diazepam on the survival, development, and response to light stimulation in early-life stages of Zebrafish (*Danio rerio*). *J. Fac. Agr. Kyushu U.*, **66**: 205–210
- Chen, K., Wu, M., Chen, C., Xu, H., Wu, X., Qiu, X. 2021b Impacts of chronic exposure to sublethal diazepam on behavioral traits of female and male zebrafish (*Danio rerio*). *Ecotox. Environ. Safe.*, **208**: 111747
- Fernández, L., Kong, C.-S., Alkhoury, M., Tryfonos, M., Brighton, P.J., Rawlings, T.M., et al. 2024 The endoplasmic reticulum protein HSPA5/BiP is essential for decidual transformation of human endometrial stromal cells. *Sci. Rep.*, **14**: 25992
- Fogliano, C., Carotenuto, R., Cirino, P., Panzuto, R., Ciaravolo, M., Simoniello, P., et al. 2024 Benzodiazepine Interference with fertility and embryo development: A preliminary survey in the sea urchin *Paracentrotus lividus*. *Int. J. Mol. Sci.*, **25**: 1969
- Fogliano, C., Motta, C.M., Venditti, P., Fasciolo, G., Napolitano, G., Avallone, B., Carotenuto, R. 2022 Environmental concentrations of a delorazepam-based drug impact on embryonic development of non-target *Xenopus laevis*. *Aquat. Toxicol.*, **250**: 106244
- Harjunpää, H., Lloret Asens, M., Guenther, C., Fagerholm, S.C. 2019 Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment. *Front. Immunol.*, **10**: 1078
- Hasan, S.-A.-M., James, A.W., Fazili, F.M., Tarabishi, S., Sheikh, N.M., Shah, Z.A. 2024 Endoplasmic reticulum stress in neurodegenerative diseases. *J. Dement. Alzheimer's Dis.*, **1**: 87–97
- Hosseinpour, F., Vazirzadeh, A., Farhadi, A., Sajjadi, S.H. 2024 Acclimation to higher temperature and antioxidant supplemented diets improved rainbow trout (*Oncorhynchus mykiss*) resilience to heatwaves. *Sci. Rep.*, **14**: 11375
- Khan, M.Z.I., Uzair, M., Nazli, A., Chen, J.-Z. 2022 An overview on Estrogen receptors signaling and its ligands in breast cancer. *Eur. J. Med. Chem.*, **241**: 114658
- Koenig, J.A., Acon Chen, C., Shih, T.-M. 2020 Development of a larval zebrafish model for acute organophosphorus nerve agent and pesticide exposure and therapeutic evaluation. *Toxics*, **8**: 106
- Kosjek, T., Perko, S., Zupanc, M., Zanoški Hren, M., Landeka Dragičević, T., Žigon, D., et al. 2012 Environmental occurrence, fate and transformation of benzodiazepines in water treatment. *Water Res.*, **46**: 355–368
- Lebreton, M., Malgouyres, J.-M., Carayon, J.-L., Bonnafé, E., Gélet, F. 2021 Effects of the anxiolytic benzodiazepine oxazepam on freshwater gastropod reproduction: A prospective study. *Ecotoxicology*, **30**: 1880–1892
- Lee, H.-R., Kim, T.-H., Choi, K.-C. 2012 Functions and physiological roles of two types of estrogen receptors, ER α and ER β , identified by estrogen receptor knockout mouse. *Lab. Anim. Res.*, **28**: 71–76
- Matsui, H. 2017 The use of fish models to study human neurological disorders. *Neurosci. Res.*, **120**: 1–7
- Molinari, M. 2024 ER remodelling by ER-phagy in neurogenesis. *Nat. Cell Biol.*, **26**: 316–317
- Ng, A., Uribe, R.A., Yieh, L., Nuckels, R., Gross, J.M. 2009 Zebrafish mutations in *gart* and *paics* identify crucial roles for de novo purine synthesis in vertebrate pigmentation and ocular development. *Development*, **136**: 2601–2611
- Oggier, D.M., Weisbrod, C.J., Stoller, A.M., Zenker, A.K., Fent, K. 2010 Effects of diazepam on gene expression and link to physiological effects in different life stages in zebrafish *Danio rerio*. *Environ. Sci. Technol.*, **44**: 7685–7691
- Perreault, H.A.N., Semsar, K., Godwin, J. 2003 Fluoxetine treatment decreases territorial aggression in a coral reef fish. *Physiol. Behav.*, **79**: 719–724
- Qiu, X., Wang, S., Zhuo, M., Shi, Y., Chen, K. 2023 Diazepam at environmentally relevant concentrations disturbed social interactions and brain neurotransmitters in adult Japanese medaka (*Oryzias latipes*). *Aquat. Toxicol.*, **265**: 106773
- Qiu, X., Zhang, Y., Gao, J., Cui, Y., Dong, K., Chen, K., Shi, Y. 2024a Exposure to thimerosal induces behavioral abnormality in the early life stages of zebrafish via altering amino acid homeostasis. *J. Hazard. Mater.*, **478**: 135548
- Qiu, Z., Khalife, J., Ethiraj, P., Jaafar, C., Lin, A.-P., Holder, K.N., et al. 2024b IRF8-mutant B cell lymphoma evades immunity through a CD74-dependent deregulation of antigen processing and presentation in MHCII complexes. *Sci. Adv.*, **10**: eadk2091
- Raghunath, A., Panneerselvam, L., Sundarraj, K., Perumal, E. 2018 *Heat Shock Proteins and Stress*. Springer Cham, pp. 39–78
- Richendrfer, H., Pelkowski, S.D., Colwill, R.M., Creton, R. 2012 On

- the edge: Pharmacological evidence for anxiety-related behavior in zebrafish larvae. *Behav. Brain Res.*, **228**: 99–106
- Rosenzweig, R., Nillegoda, N.B., Mayer, M.P., Bukau, B. 2019 The Hsp70 chaperone network. *Nat. Rev. Mol. Cell Biol.*, **20**: 665–680
- Salvador-Mira, M., Gimenez-Moya, P., Manso-Aznar, A., Sánchez-Córdoba, E., Sevilla-Diez, M.A., Chico, V., et al. 2025 Viral vaccines promote endoplasmic reticulum stress-induced unfolding protein response in teleost erythrocytes. *Eur. J. Cell Biol.*, **104**: 151490
- Schwartz, A.B., Campos, O.A., Criado-Hidalgo, E., Chien, S., del Álamo, J.C., Lasheras, J.C., Yeh, Y.-T. 2021 Elucidating the biomechanics of leukocyte transendothelial migration by quantitative imaging. *Front. Cell Dev. Biol.*, **9**: 635263
- Silveira, M.A.D., Khadangi, F., Mersaoui, S.Y., Naik, D., Masson, J.-Y., Bilodeau, S. 2023 HSP70 mediates a crosstalk between the estrogen and the heat shock response pathways. *J. Biol. Chem.*, **299**: 102872
- Soler-Agesta, R., Anel, A., Galluzzi, L. 2023 Mitochondrial control of antigen presentation in cancer cells. *Cancer Cell*, **41**: 1849–1851
- Uribe, M.L., Marrocco, I., Yarden, Y. 2021 EGFR in cancer: Signaling mechanisms, drugs, and acquired resistance, *Cancers*, p. 2748
- Wang, S., Dong, J., Chen, K., Shi, Y., Qiu, X. 2024 Effects of chronic diazepam exposure on the behaviors and oxidative stress homeostasis in the eyes and brains of female Japanese medaka. *Comp. Biochem. Phys. C*, **276**: 109812
- Wang, S., Zhuo, M., Chen, K., Shi, Y., Qiu, X., Takai, Y., et al. 2023 Bioconcentration and behavioral interference effect of diazepam on adult Japanese medaka (*Oryzias latipes*). *J. Fac. Agr. Kyushu U.*, **68**: 135–141
- Wilkinson, J.L., Boxall, A.B.A., Kolpin, D.W., Leung, K.M.Y., Lai, R.W.S., Galbán-Malagón, C., et al. 2022 Pharmaceutical pollution of the world's rivers. *Proc. Nat. Acad. Sci.*, **119**: e2113947119
- Xuchun, Q., Mengcheng, Z., Yanhong, S., Kun, C., Takai, Y., Shimasaki, Y., Oshima, Y. 2025 Impacts of diazepam on the locomotor activity and brain gene expression of female Japanese medaka (*Oryzias latipes*). *J. Fac. Agr. Kyushu U.*, **70**: 13–19
- Yen, K.-W., Pan, C.-I., Chen, C.-H., Lien, W.-H. 2022 Spatiotemporal characteristics of fish larvae and juveniles in the waters around Taiwan from 2007 to 2019. *Animals*, **12**: 1890
- Zeng, C.-W., Kamei, Y., Wang, C.-T., Tsai, H.-J. 2016 Subtypes of hypoxia-responsive cells differentiate into neurons in spinal cord of zebrafish embryos after hypoxic stress. *Biol. Cell*, **108**: 357–377
- Zhang, J.-G., Shi, W., Ma, D.-D., Lu, Z.-J., Li, S.-Y., Long, X.-B., Ying, G.-G. 2023 Chronic paternal/maternal exposure to environmental concentrations of imidacloprid and thiamethoxam causes intergenerational toxicity in zebrafish offspring. *Environ. Sci. Technol.*, **57**: 13384–13396
- Zhang, L., Woltering, I., Holzner, M., Brandhofer, M., Schaefer, C.-C., Bushati, G., et al. 2024a CD74 is a functional MIF receptor on activated CD4⁺ T cells. *Cell Mol. Life Sci.*, **81**: 296
- Zhang, Y., Shi, Y., Tang, J., Chen, K., Wu, M., Wu, X., Qiu, X. 2024b A transcriptomics-based analysis of mechanisms involved in the sex-dependent effects of diazepam on zebrafish. *Aquat. Toxicol.*, **275**: 107063