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Research Paper

# Tetrabromobisphenol A induced reproductive endocrine-disrupting effects in mussel *Mytilus galloprovincialis*

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ABSTRACT

Tetrabromobisphenol A (TBBPA) pollution in marine environmental media poses great risks to marine organisms due to its potential endocrine-disrupting effects. However, limited attention of TBBPA's endocrine-disrupting effects has been paid on marine invertebrates. In this work, the reproductive endocrine-disrupting effects of TBBPA were evaluated by observing the gametes development, quantifying the gender-specific gene expression, and determining vertebrate sex hormones in mussels *Mytilus galloprovincialis* treated with TBBPA for 30 days. Additionally, transcriptomic profiling and enzymes activities were conducted to investigate the potential mechanisms of reproductive endocrine-disrupting effects. We found that promotion of gametogenesis and alterations of vertebrate sex hormones occurred in TBBPA-treated mussels of both sexes. Meanwhile, estrogen sulfotransferase (SULT1E1) and steroid sulfatase (STS) were up-regulated at transcript level as a result of TBBPA treatments, suggesting that TBBPA disrupted the steroidogenesis in mussels through promoting steroids sulfonation and hydrolysis of sulfate steroids. The induction of SULTs for TBBPA biotransformation might be responsible for the dysregulation of steroidogenesis and steroids metabolism. Overall, these findings provide a new insight into assessing impact of TBBPA as well as TBBPA biomonitoring in marine environment.

## 1. Introduction

With polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) banned as listed under Annex A of the Stockholm Convention on persistent organic pollutants, tetrabromobisphenol A (TBBPA) has become one of the brominated flame retardants (BFRs) with the largest market demand. TBBPA, its byproducts and derivatives could be introduced to the environment through production, use, and disposal (Liu et al., 2016b). Estuaries and coastal marine waters are typical areas of persistent organic pollutants (POPs) owing to anthropogenic activity from a burgeoning population in the coastal zone. TBBPA has been detected in aquatic organisms from coastal zone worldwide (Kotthoff et al., 2017; Liu et al., 2016a; Morris et al., 2004). With the increasing frequency and concentrations of TBBPA detected in marine environment, the environmental risk of TBBPA on marine animals is of great concern.

Due to molecular similarity to thyroid hormones (THs), TBBPA has been frequently reported to disrupt development (Zhang et al., 2014) and dysregulate lipid metabolism (Grasselli et al., 2014) by simulating THs. TBBPA also exhibited reproductive toxicity, indicated by decrease of sex hormone and abnormal gene expression of androgen receptor in the testes of *Rana nigromaculata* (Zhang et al., 2018) as well as increase of gonadotropin transcript levels in Atlantic cod *Gadus morhua* L. (von Krogh et al., 2019). In rodents, available evidence suggested that TBBPA might increase the levels of circulating estrogens by binding to estrogen sulfotransferases (SULT1E1) and competitive inhibition of estrogen conjugation, which was regarded as a plausible molecular initiating event of reproductive toxicity (Lai et al., 2015; Wikoff et al., 2016).

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So far, studies on endocrine-disrupting effects of TBBPA primarily focused on vertebrates. Limited attention has been paid on invertebrate such as marine bivalves which have been frequently used as marine environmental indicators. Clarifying the molecular basis of the action of endocrine disruptors on invertebrates is highly essential to elucidate the ecological effects of exposure to endocrine disruptors starting at the bottom of the food chain (Lguchi et al., 2006). It is therefore urgent to evaluate whether TBBPA could lead to endocrine-disrupting effects in invertebrates like in vertebrates. A previous work in our laboratory showed that TBBPA induced differential expression profiles of reproduction and development related proteins, including vitelline envelop receptor for lysin, zonadhesin, and meiosis-specific unclear structural protein, suggesting the underlying reproductive endocrine disruption of TBBPA on mussels Mytilus galloprovincialis (Ji et al., 2014). However, analyses on other reproductive endpoints were absent because the aim of our previous work was to investigate the global effects of TBBPA.

In order to unravel the potential reproductive endocrine-disrupting effects and its mechanism, in this work, we focused on a serial of endpoints associated with reproduction in marine mussel *M. galloprovincialis* treated with TBBPA for 30 d. Gametogenesis in mussels was evaluated by histological observation on gonads and analyzing the expression of gender-specific genes. Transcriptomic analysis on mussel digestive gland was performed to select responsive genes associated with reproductive endocrine. Vertebrate sex hormones and enzyme activities were also measured as supplementary to explore the potential mechanism of endocrine-disrupting effects by TBBPA. The investigation on the influences of TBBPA on mussel reproduction enables to better understand whether and how TBBPA exerted the potential reproductive endocrine-disrupting effects in marine invertebrates, which will provide new insights into the ecological risk evaluation of TBBPA and other emerging endocrine disruptors in marine environment.

## 2. Methods and materials

### 2.1. Animals culture and TBBPA exposure

Tetrabromobisphenol A (TBBPA, CAS # 79-94-7, purity > 98%) and its isotope-labeled internal standard, $^{13}C_{12}$-TBBPA (50 μg/mL in methanol, purity > 98%), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Cambridge Isotope Laboratories, Inc. (Andover, U.S.A.), respectively. TBBPA was dissolved in dimethyl sulfoxide (DMSO) as a stock solution. The final concentrations of DMSO for all treatments were consistently less than 0.01%. All solvents were of HPLC grade and other chemicals used were of analytical grade.

Mature mussels *M. galloprovincialis* (shell length: 5.67 ± 0.32 cm in length) were purchased from a culturing farm (Rizhao, China). After being transported to laboratory, the mussels were acclimatized in fresh seawater for at least 7 days. Meanwhile, monitoring of gonadal development was performed on mussels until the gametes were on the point of proliferation. Mussels were randomly divided into 6 groups, including DMSO control group and 5 TBBPA-treated groups (0.6, 3, 15, 75, and 375 μg/L TBBPA). The lowest concentration (0.6 μg/L) of TBBPA was designed for marine environmental relevance (Jiang et al., 2018). There were two replicate tanks for each treatment and each tank contained 20 individuals. Considering no significant influence of DMSO on mussels, we did not set seawater control group according to our previous study (Ji et al., 2013). During acclimation and exposure periods, the mussels were fed *Chlorella vulgais* and 100% of the test solution was renewed daily. After exposure for 30 days, mussels were averagely collected from each replicate tank. For each individual, the mantle tissue was used for sex identification and evaluation of gametogenesis stage. Histological observation and vertebrate sex hormones determination were conducted on mussel gonads. The digestive gland was used for transcriptomic analysis and enzyme activity measurement. All samples except histological tissue were snap-frozen in liquid nitrogen and stored at − 80 °C.

### 2.2. Quantification of TBBPA in whole soft tissue of mussel

TBBPA accumulation in whole soft tissues of mussel ($n$ = 10) was quantified by HPLC-ESI-MS/MS (Quattro Premier XE, Waters Corp., U.S.A.) with electrospray ionization (ESI) in negative ion mode. The detailed protocols for extraction, clean up, analysis, and quality assurance and quality control (QA/QC) are provided in the Supporting Information (SI). The limit of detections (LODs) and the limit of quantifications (LOQs) were 1 μg/kg and 2 μg/kg for TBBPA, respectively.

### 2.3. Sex identification

A female specific egg protein, vitelline envelop receptor for lysin (VERL), and a male specific sperm protein, vitelline coat lysin (VCL) were used for sex identification in mussels (Anantharaman and Craft, 2012; Hines et al., 2007). In details, total RNA was extracted from mussel mantle tissue, followed by cDNA synthesis. The reverse transcription quantitative PCR (RT-qPCR) was applied to evaluate the expressions of *VERL* and *VCL* transcripts. Samples with positive ΔCt (*VERL-VCL*) values were from male mussels, while those with negative values were from female mussel samples. Specific primer sequences used for the RT-qPCR are listed in Table S1. Only individuals with defined sex were used for subsequent analyses.

### 2.4. Evaluation of gametogenesis stages

The gonads (approximately 1 $cm^2$) were fixed in Bouin's fixative solution for 24 h and stored in 70% ethanol. After dehydrated in graded ethanol and paraffin-embedded, histological Section (5 μm) were stained with hematoxylin-eosin (H&E) and examined under a light microscope (Olympus BX61, Tokyo, Japan) at × 200 magnification (zoom on the camera was × 2.5).

Determination of gametogenesis stage in mussel gonads ($n$ = 6) was based on five-stage scale according to previous descriptions with some modifications (Seed, 1969; Smolarz et al., 2018): I-beginning of gametogenesis, II-developing stage, III-mature stage, IV-spawning stage, V-resting stage. The detailed description on five stages of mussel gonads and their representative photomicrographs (Fig. S1) are available in the **SI**. The following formula was used to calculate the gonadal index (GI) that represented the maturity of gametes:

$$GI = [n1 * 1 + n2 * 2 + n3 * 3 + n4 * 4 + n5 * 5]/N$$

"ni" represents the number of individuals in the i stage of gonadal development, "N" represents the total number of individuals.

The gamete development in mussels ($n$ = 6) was also evaluated by determining the relative expression levels of *VERL* and *VCL* transcripts which are proportional to the numbers of ova and sperm and are indicative of the stage of gametogenesis (Anantharaman and Craft, 2012). The relative expression levels were expressed as the values of ΔCt [*VERL* - (*β-actin*)] in females and ΔCt [*VCL* - (*β-actin*)] in males. Accordingly, the ΔCt values of *VERL* and *VCL* presented U-shaped curves during the whole gametogenesis in females and males with bottoms at mature stage.

### 2.5. Quantification of vertebrate sex hormones in gonads

After sex identification analysis, gonads from male and female individuals ($n$ = 6) were classified into separate bags and kept at − 80 °C. Concentrations of progesterone (P4), androst-4-ene-3–17-dione (AE), testosterone (T), estrone (E1), 17β-estradiol (E2) and estriol (E3) were determined using HPLC-ESI-MS/MS. The details are available in the SI.

### 2.6. Transcriptomic analysis

Total RNA was extracted from digestive gland samples ($n$ = 9) of

*M. galloprovincialis* using the TRIzol reagent. A total of 33 sequencing libraries (11 groups each containing 3 replicates, each replicate including 3 samples) were constructed using NEB Next® UltraTM RNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following manufacturer's instructions. Transcriptomic profiling was not performed on female samples in 0.6 μg/L TBBPA-treated group due to lack of plenty of samples. DESeq2 provided statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulted *P* values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with adjusted *P* value $< 0.05$ and $|\log_2$ (fold change)$| > 1$ were assigned as differentially expressed genes (DEGs). Validation of transcriptomic result was performed by DEGs quantification using RT-qPCR with *β-actin* as an endogenous reference gene.

Gene function was annotated based on the following databases: Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (A manually annotated and reviewed protein sequence database), KO (KEGG Ortholog database) and GO (Gene Ontology). In addition, enrichment analyses were implemented by the clusterProfiler R package (Version 2), in which gene length bias was corrected. GO terms and KEGG pathways with adjusted *P* values less than 0.05 together with at least 2 genes included were considered significantly enriched by DEGs.

### 2.7. Measurement of estrogen sulfotransferase and steroid sulfatase activities in mussel digestive gland tissues

Digestive gland samples ($n = 6$) were used for testing the activities of SULT1E1 and steroid sulfatase (STS). The activities of SULT1E1 and STS were measured following the manufacturer's protocols of commercial ELISA kits (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). The tissue was homogenized in PBS (0.01 M $Na_2HPO_4$-$KH_2PO_4$, pH 7.2) with w/v ratio of 1:4. The supernatant was collected for subsequent ELISA analysis. Specific antibodies were designed for SULT1E1 and STS, and only enzymes with active binding sites for antibodies could be detected. Both SULT1E1 and STS activities were expressed as U/g tissue (wet weight). The detailed description of SULT1E1 and STS measurements is available in SI.

### 2.8. Statistical analysis

The statistical differences in vertebrate sex hormones and enzyme activities between control and exposure groups were analyzed by two-way analysis of variance (ANOVA) with TukeyHSD test (for data that exhibited a normal distribution) or Kruskal-Wallis two-way ANOVA (for data that did not follow a normal distribution or failed the equal variance test). One-way ANOVA with TukeyHSD test (for data that exhibited a normal distribution) or Kruskal-Wallis one-way ANOVA (for data that did not follow a normal distribution or failed the equal variance test) was performed on TBBPA accumulation and gender-specific genes expression. Fisher's exact test was used to analyze the development stage distribution among treatments. For all of the indices studied, we had at least three values from each group. A *P* value less than 0.05 indicated a statistically significant difference at a confidence level of 95%.

## 3. Results

### 3.1. TBBPA accumulation in whole soft tissue of mussels

After TBBPA exposure for 30 days, significant ($P < 0.05$) TBBPA accumulation in whole soft tissue was observed in all TBBPA-treated groups compared to control group (Fig. 1). Ranging from 4.52 to 11,176.81 ng/g (dry weight, dw), TBBPA accumulation presented an exponential increase with increasing TBBPA concentration from 0.6 to 375 μg/L. It is noteworthy that as low as environmentally relevant concentration (0.6 μg/L) of TBBPA exposure even induced significant ($P < 0.05$) TBBPA accumulation in mussels, suggesting that *M. galloprovincialis* is a reliable bioindicator to TBBPA pollution.

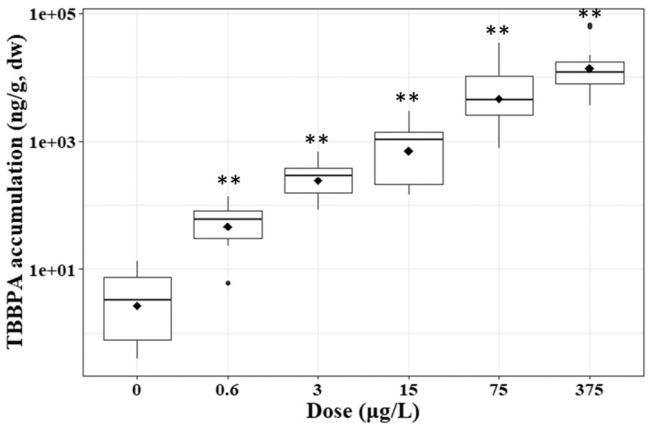


**Fig. 1.** TBBPA accumulation in whole soft tissues of mussels ($n = 10$) treated with TBBPA for 30 days. Data are presented as box plots indicating the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles; filled circles indicate outliers; solid lines and points inside the box indicate median and mean, respectively; ** indicates significant differences ($P < 0.01$) between control group and TBBPA-treated groups.

### 3.2. Influence of TBBPA on gamete development (gametogenesis)

The development stages of mussel gametes were determined based on histological observation and expression of gender-specific genes. Fig. S1 exhibits the typical graphics of five reproductive stages of mussel gonads, including beginning of gametogenesis, developing, ripe, spawning, and resting stages. Mussels during acclimation were at the onset of gametogenesis. After TBBPA exposure for 30 days, the development of gonads from TBBPA-treated groups preceded those from control group. In control group, no mussel developed to mature or spawning stages, while a large proportion of individuals in TBBPA-treated groups were under mature, spawning or resting stages. Especially, high concentration (375 μg/L) of TBBPA significantly ($P < 0.05$) promoted the development of gametes in both sexes (Fig. 2A and B). GI curves of both sexes showed a gross increase trend with increasing TBBPA dose (Fig. 2C).

The relative expression of *VERL* in females and *VCL* in males reflected the development stage of gametes. U-shaped curves exhibiting relative expression profiles of *VERL* and *VCL* occurred in females and males with TBBPA exposure dose, respectively. The lower ΔCt values of *VERL* and *VCL* in 3 and 15 μg/L TBBPA-treated groups meant that ripe gametes were dominant in these two groups, whereas the higher ΔCt values in control group and high concentrations (75 and 375 μg/L) of TBBPA-treated groups meant that gametes were at stages prior to or after ripe stage. The expression of gender-specific genes was basically consistent with the stages of gametogenesis based on histological observations.

### 3.3. Vertebrate sex hormones in gonad tissues of mussels

Fig. 3 shows the responses of six vertebrate sex hormones, including AE, E1, E2, E3, T and P4, in mussel gonads. A two-way ANOVA was conducted that examined the effect of gender and TBBPA dose on levels of vertebrate sex hormones (Table S2). Significant interaction was only observed in the effects of gender and TBBPA dose on AE levels ($P < 0.01$). TBBPA induced statistically significant alterations of E3 ($P < 0.05$), E1 ($P < 0.01$), and AE ($P < 0.01$), while there were no

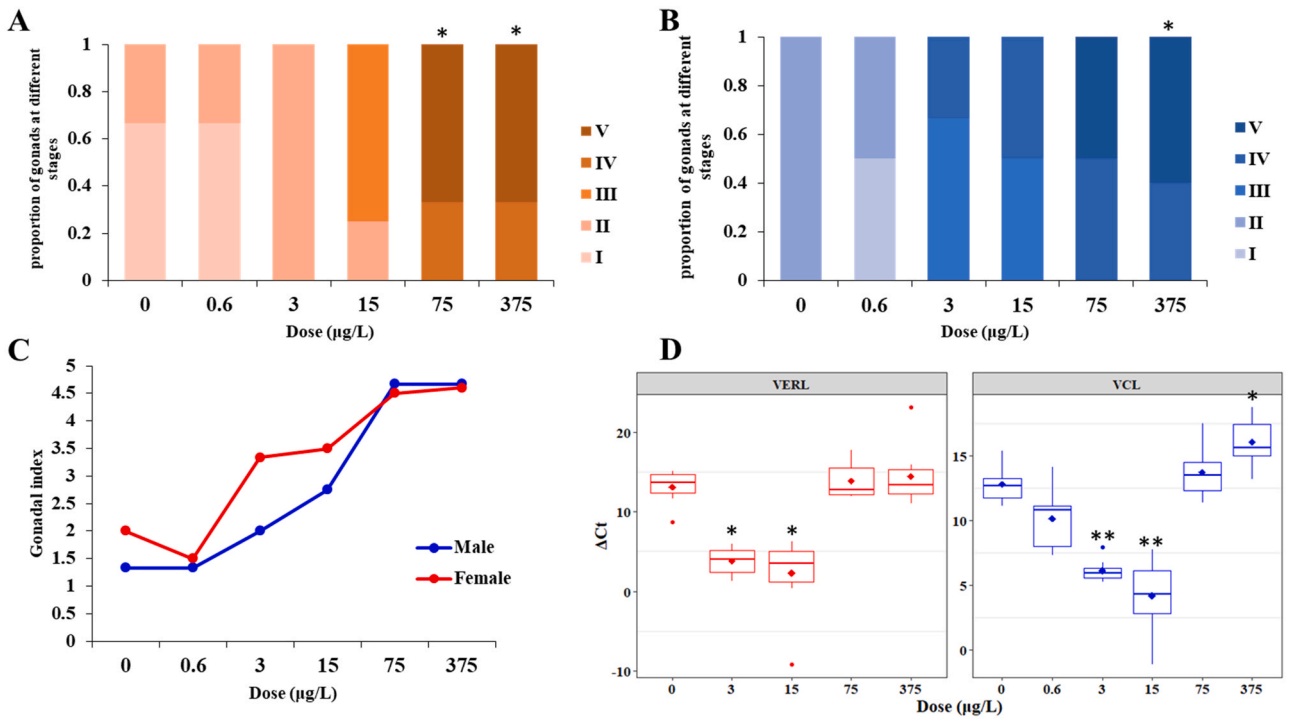


**Fig. 2.** Status of gametogenesis in mussel gonads ($n = 6$). Proportion of gonads at different gametogenesis stages (I-beginning of gametogenesis, II-developing stage, III-mature stage, IV-spawning stage, V-resting stage) in females(A) and males (B); data are presented as stacking histogram; * indicates significant differences $P < 0.05$ between control group and TBBPA-treated groups by Fisher's exact test. (C) Gonadal indices (GIs) of mussel gonads; red and blue indicate female and male, respectively. (D) Relative expression of *VERL* in females and *VCL* in males indicated by ΔCt [*VERL* or *VCL*-(*β-actin*)]; data are presented as box plots indicating the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles; filled circles indicate outliers; solid lines and points inside the box indicate median and mean, respectively; red and blue indicate female and male, respectively; * and ** indicate significant differences $P < 0.05$ and $P < 0.01$ between control group and TBBPA-treated groups.

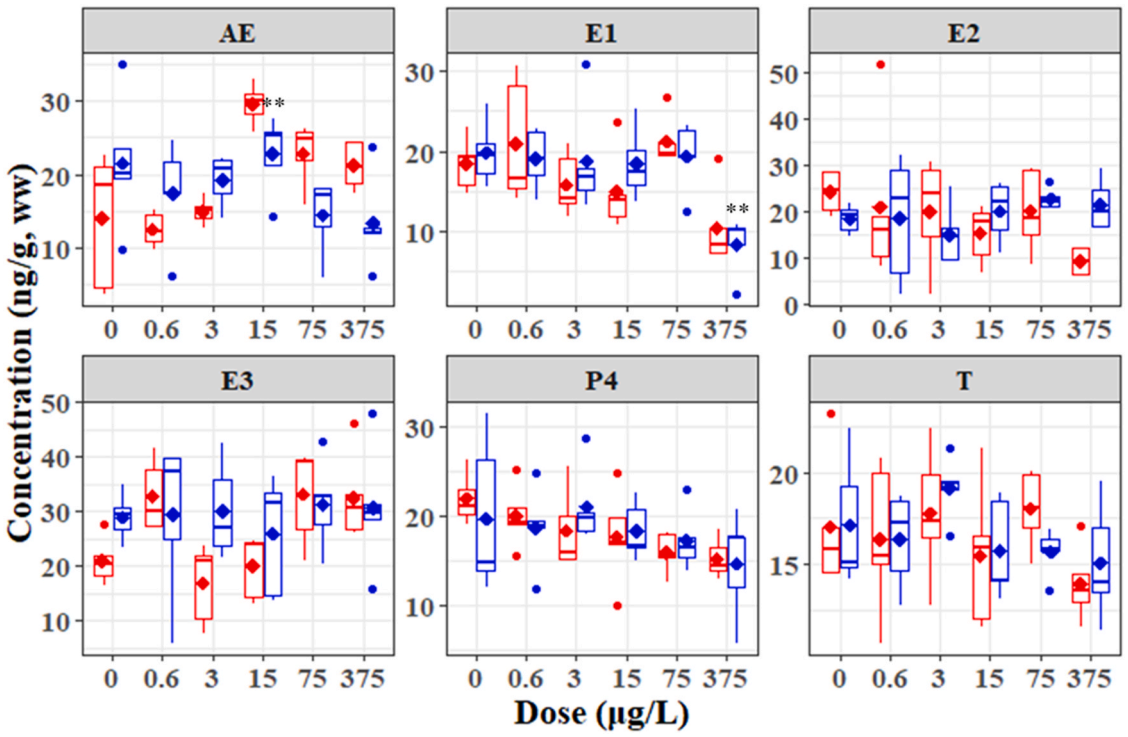


**Fig. 3.** The concentrations of vertebrate sex hormones in mussel gonads ($n = 6$). Abbreviation: AE, androst-4-ene-3–17-dione; E1, estrone; E2, 17β-estradiol; E3, estriol; P4, progesterone; T, testosterone. Data are presented as box plots indicating the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles; filled circles indicate outliers; solid lines and points inside the box indicate median and mean, respectively; red and blue indicate female and male, respectively; * and ** indicate significant differences $P < 0.05$ and $P < 0.01$ between control group and TBBPA-treated groups.

significant differences in all six vertebrate sex hormones between females and males. In details, AE presented inverted U-shaped curves in response to TBBPA treatments, and the highest AE was found in 15 μg/L TBBPA-treated group. The average level of AE was significantly higher ($P < 0.01$) in 15 μg/L TBBPA-treated females than those in control females. E3 presented multiphase alterations in female mussels with increasing TBBPA dose, while no marked changes occurred in male mussels. E1 showed polynomial changes in mussels, obviously marked with a sudden decrease in the highest concentration of TBBPA-treated group. The average E1 level in 375 μg/L TBBPA-treated males was significantly lower ($P < 0.01$) than those in control males. With the increase of TBBPA exposure concentration, a monotonic decrease of P4 was identified in mussels of both sexes. A sharp decrease of E2 was only found in female mussels treated with 375 μg/L TBBPA, whereas male mussels showed no significant alteration in E2 contents. T presented an inverted U-shaped curve in male mussels with top at 3 μg/L of TBBPA, and decreased in the highest concentration (375 μg/L) of TBBPA-treated mussels of both sexes.

### 3.4. Transcriptomics profiles

A total of 2530 DEGs were identified in TBBPA-treated mussels (Table S3). Fig. S3 shows the numbers of DEGs in each TBBPA-treated group from both sexes. In general, there were more DEGs identified in

**Table 1**
The relative expression of key genes in mussel digestive glands from TBBPA-treated groups.

| Sex/TBBPA doses (μg/L) / Gene Description | Male | | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.6 | 3 | 15 | 75 | 375 | 3 | 15 | 75 | 375 |
| **Steroid hormone biosynthesis** | | | | | | | | | |
| Carbohydrate sulfotransferase 11-like isoform X2 | -1.96 | -1.19 | -2.80 | -1.93 | -0.95 | 5.97 | 7.50* | 3.14 | 4.96 |
| Cytosolic sulfotransferase 1 | 0.19 | 1.33 | -0.29 | 2.87 | 5.32* | 1.43 | -0.32 | 2.39 | 4.73* |
| Sulfotransferase family cytosolic 1B member 1-like | -0.61 | -2.06 | -0.90 | -1.57 | -2.31* | -0.40 | 0.75 | -0.31 | -0.56 |
| Sulfotransferase family cytosolic 1B member 1-like isoform X2 | -0.65 | -2.43 | -1.28 | -1.24 | -2.03 | -0.64 | 0.45 | -0.61 | -0.99 |
| Estrogen sulfotransferase-like | -2.14 | -1.51 | -1.16 | 0.42 | 1.55 | 0.44 | 0.93 | 1.35 | 3.52* |
| Sulfotransferase 1C2-like | 0.02 | -0.01 | 0.93 | 2.53* | 4.61* | 0.60 | 1.63 | 3.82* | 5.42* |
| Sulfotransferase family cytosolic 1B member 1-like | 0.29 | 0.25 | 1.31 | 4.16* | 6.51* | 1.70 | 2.80* | 5.35* | 7.68* |
| Arylsulfatase I | 0.34 | -0.22 | 0.28 | 1.64 | 2.78* | 0.13 | -0.08 | 1.55 | 3.35* |
| Steryl-sulfatase | 3.04 | 0.83 | NA | 2.46 | 8.11* | 1.69 | 1.65 | 4.09 | 7.47* |
| Steryl-sulfatase | 1.09 | 0.23 | 0.89 | 4.04* | 5.72* | 1.42 | 0.89 | 3.11 | 6.19* |
| Arylsulfatase D | 4.62 | 4.00 | 3.84 | 7.20* | 8.88* | 0.57 | -0.20 | 1.92 | 5.24* |
| **Biotransformation and transport** | | | | | | | | | |
| Cytochrome P450 2H1 | 0.25 | 1.05 | 1.40 | 3.31* | 4.30* | 0.52 | 1.26 | 3.34* | 4.37* |
| Cytochrome P450 2D15 | 0.44 | 0.97 | 1.42 | 3.49* | 4.59* | 0.57 | 1.21 | 3.51* | 4.42* |
| Cytochrome P450 2E1 | 0.44 | 1.54 | 1.88 | 3.71* | 4.87* | 0.85 | 1.51 | 3.65* | 4.71* |
| Cytochrome P450 2H2 | 0.59 | 0.55 | 0.45 | 3.54* | 5.77* | 0.91 | 0.66 | 2.55 | 5.91* |
| Cytochrome P450 2U1 | 0.20 | -0.01 | 0.60 | 3.29* | 5.38* | 0.66 | 0.58 | 2.39 | 5.61* |
| Cytochrome P450 2J1 | 0.52 | 1.46 | 1.61 | 6.26* | 8.74* | 0.11 | 1.76 | 4.16 | 8.52* |
| Cytochrome P450 2C7 | 0.44 | 0.78 | 0.27 | 1.72 | 1.90 | 0.36 | 0.32 | 0.67 | 1.83* |
| Cytochrome P450 2G1 | 0.50 | 0.79 | 0.42 | 1.93 | 2.26* | 0.81 | 0.67 | 1.13 | 2.19* |
| Cytochrome P450 2U1 | 0.40 | 0.74 | 0.37 | 1.85 | 2.26* | 0.66 | 0.43 | 1.28 | 1.99* |
| Omega class glutathione-s-transferase | -0.31 | -0.52 | -0.26 | 0.74 | 1.47 | 0.21 | -0.10 | 0.79 | 2.12* |
| Glutathione S-transferase theta-1 | -0.74 | -0.77 | -0.88 | 1.92 | 3.14* | -0.53 | 0.42 | 1.86 | 4.47* |
| P-glycoprotein | -0.60 | 0.13 | 0.38 | 1.35 | 2.67* | 0.90 | 0.69 | 1.86 | 3.16 |
| P-glycoprotein | -0.50 | 0.55 | 0.66 | 1.67 | 2.84* | -0.51 | -0.29 | 1.10 | 2.44 |
| P-glycoprotein | -0.62 | 0.13 | 0.18 | 1.77 | 2.86* | 0.96 | 0.22 | 1.18 | 3.21 |
| P-glycoprotein | -0.07 | 0.48 | 0.26 | 1.42 | 2.42* | 0.95 | -0.16 | 0.95 | 2.70* |
| ATP-binding cassette transporter sub-family A | 0.68 | 0.37 | 0.08 | 0.79 | 1.79* | -0.24 | 0.15 | 0.76 | 2.05 |
| **Non-genomic pathway** | | | | | | | | | |
| Caveolin-1 | 1.09 | -1.07 | -0.90 | -1.64 | -5.40* | 1.69 | 0.79 | NA | 0.69 |
| Caveolin-1 | -0.47 | -1.74 | -1.78 | -5.98 | 2.16 | 0.71 | -0.95 | 1.10 | -3.54 |
| Breast carcinoma amplified sequence 2 | -0.45 | -1.80 | -2.51 | -9.25* | -0.52 | 1.25 | 0.79 | -0.37 | 1.78 |
| **PPAR signaling pathway and lipid metabolism** | | | | | | | | | |
| Peroxisomal acyl-coenzyme A oxidase 1 | 0.57 | -0.30 | 0.56 | 2.48 | 3.62* | 1.14 | 1.23 | 1.91 | 4.39* |
| Peroxisomal acyl-coenzyme A oxidase 1 | 0.92 | -0.19 | 1.22 | 2.71* | 4.16* | 1.69 | 1.90 | 2.12 | 4.90* |
| Polyubiquitin (Fragment) | 6.15 | 5.78 | 4.56 | 6.31 | 6.83* | -1.77 | -0.12 | -0.40 | -0.65 |
| Palmitoyl-CoA oxidase | 0.57 | -0.30 | 0.56 | 2.48 | 3.62* | 1.14 | 1.23 | 1.91 | 4.39* |
| Cytochrome P450 2B11-like isoform X2 | 0.20 | -0.01 | 0.60 | 3.29* | 5.38* | 0.66 | 0.58 | 2.39 | 5.61* |
| **Lysosome** | | | | | | | | | |
| Polyketide synthase 1 | -3.05 | -2.35 | 0.33 | -7.12* | -7.02* | -23.27 | -5.83 | -3.69 | -5.27 |
| Glycosyl hydrolase family 2, TIM barrel domain protein | -3.12 | -1.68 | -0.57 | -6.18* | -7.99* | -0.90 | -0.39 | -6.57 | -6.77 |
| Dipeptidyl peptidase 1-like isoform X1 | -2.06* | -2.80* | -1.12 | -2.22 | -3.12* | -1.91 | -0.17 | -2.25 | -2.40 |
| Dipeptidyl peptidase 1 | -2.19* | -3.04* | -1.00 | -2.18 | -3.55* | -1.92 | 0.04 | -2.18 | -2.19 |
| Lysosomal protective protein-like isoform X2 | -1.94 | -2.65 | -1.23 | -2.96* | -3.19* | -1.91 | -0.04 | -2.35 | -1.54 |
| Alpha-N-acetylgalactosaminidase-like | -0.83 | -1.56 | -0.51 | -1.27 | -2.41* | -1.36 | 0.17 | -1.78 | -1.98 |
| Alpha-N-acetylgalactosaminidase-like | -1.00 | -1.57 | -0.28 | -1.26 | -2.33* | -1.44 | -0.20 | -1.47 | -1.69 |
| Cathepsin L1-like | -1.32 | -1.96 | -1.25 | -1.80 | -4.03* | 0.81 | 1.67 | 0.73 | 0.57 |
| Cathepsin-like protein-3, partial | -1.74 | -1.79 | -0.74 | -1.97 | -2.37* | -1.22 | 0.02 | -1.31 | -1.32 |
| Cathepsin L1 | -1.83* | -2.75* | -1.29 | -2.77* | -3.48* | -1.02 | 0.71 | -1.28 | -1.34 |
| Dipeptidyl peptidase 1-like isoform X1 | -3.63 | -3.42 | -1.64 | -3.03 | -3.47* | -1.76 | 0.36 | -2.86* | -0.91 |
| Cathepsin-like protein-3, partial | -1.80 | -1.70 | -0.60 | -2.05 | -2.68* | -1.32 | -0.22 | -1.71 | -1.70 |
| Cathepsin L1-like isoform X1 | -2.27* | -3.28* | -1.34 | -3.61* | -3.31* | -1.69 | 0.46 | -2.17 | -1.94 |
| Digestive cysteine proteinase 2 | -2.02 | -3.22* | -0.79 | -2.44 | -3.73* | -2.59* | 0.55 | -2.50* | -2.12 |
| Cathepsin C | -1.95 | -2.61* | -0.42 | -1.88 | -3.32* | -1.55 | -0.07 | -2.20 | -2.63 |

*indicates significant difference ($P < 0.05$) between TBBPA-treated groups and control group; Red- and blue-coded table cells indicate up- and down-regulation, respectively, and the colour depth was positive with the expression levels of gene.

male mussels than in female mussels from each TBBPA-treated group. Especially, in the highest concentration (375 μg/L) of TBBPA-treated group, the number of DEGs in males was twice over those in females. It was noteworthy that there were many DEGs commonly altered between sexes and among TBBPA-treated groups. Tables S4 and S5 summarized the enriched GO terms and KEGG pathways with key DGEs listed in Table 1. Concerning KEGG pathways, significant (corrected $P < 0.05$) enriched pathways were only found in 75 and 375 μg/L TBBPA-treated groups. In 75 μg/L TBBPA-treated group, up-regulated genes enriched in steroid hormone biosynthesis were only identified in male mussels, while down-regulated genes were enriched in lysosome in both female and male mussels. In 375 μg/L TBBPA-treated group, enrichment of up-regulated steroid hormone biosynthesis, biosynthesis of unsaturated fatty acids, and arachidonic acid metabolism were found in both female and male mussels (Fig. 4), indicated by up-regulation of *cytochrome P450*s (*CYPs*) and *steroid sulfatase* (*STS*). In contrast, down-regulated genes enriched in antigen processing and presentation, apoptosis, and lysosome were only found in male mussels. Concerning GO terms, TBBPA-treated groups were significantly enriched in molecular functions, such as sulfuric ester hydrolase activity, iron ion binding and heme binding. Significant enrichment of sulfuric ester hydrolase activity was attributed to alterations of *STS* and *arylsulfatase B-like*, which were commonly altered in both female and male mussels. The sequencing results of two typical genes, *STS* and *SULT1B1*, were validated by qRT-PCR with Pearson correlation coefficient of 0.814 and *P* value < 0.01 (Fig. S2).

### 3.5. Activities of SULT1E1 and STS in mussel digestive gland

Statistically significant differences in average SULT1E1 activities were found in TBBPA-treated groups for both sexes ($P < 0.01$) and TBBPA treatments ($P < 0.01$), though the interaction between these terms was not significant (Table S6). In details, the activities of SULT1E1 in male samples were significantly higher than those in female samples ($P < 0.01$). SULT1E1 activities in both female and male mussels decreased with increasing TBBPA exposure dose, and significant alteration of SULT1E1 activity was only found in females from 375 μg/L TBBPA-treated group (Fig. 5). Concerning STS, there was a statistically significant difference in average STS activities among TBBPA-treated groups ($P < 0.05$). Though no significant difference existed in average STS activities between males and females, the interaction between sex and TBBPA dose was significant ($P < 0.05$). In details, the STS activities in male mussels decreased with increasing TBBPA dose, and significant difference of STS ($P < 0.05$) was found in the high concentration (375 μg/L) of TBBPA-treated group. In contrast, no significant alterations of STS activities occurred in female mussel groups.

## 4. Discussion

TBBPA has been detected in multiple environmental media including wild marine bivalves (Liu et al., 2016a). It has been well documented that TBBPA could induce reproductive endocrine-disrupting effects in vertebrates. However, it is still unknown whether TBBPA could cause reproductive endocrine-disrupting effects in invertebrates like bivalves. In this work, we found that TBBPA exposure for 30 days induced acceleration of gametogenesis and alterations of vertebrate sex hormones in mussels of both sexes. Transcriptomic analysis together with bioinformatic analysis revealed that steroid hormone biosynthesis was significantly up-regulated in TBBPA-treated mussels of both sexes, supported by the up-regulation of *SULT*, *STS* and *CYP2*. To the best of our knowledge, this is the first report on whether and how TBBPA induces reproductive endocrine-disrupting effects in bivalves.

Bivalves are abundant in marine ecosystems and perform important ecological functions through the movement of nutrient and energy (Vaughn and Hoellein, 2018). Considering its ecological importance and ease of pollutants accumulation, bivalves are frequently used for marine environmental biomonitoring (Fernandez-Tajes et al., 2011). So far, most vertebrate sex steroids have been identified in invertebrate (Janer and Porte, 2007). It also has been documented that vertebrate sex steroids are involved in gonadal and gamete development in bivalves (Croll and Wang, 2007; Gauthier-Clerc et al., 2006). Furthermore, a recent study by Blalock et al. (2018) identified nearly total genes of steroidogenesis in marine mussel *Mytilus edulis* and found that steroidogenesis pathway and nongenomic estrogen signaling pathway were likely related to the mechanisms of action for endocrine disruptors. All these findings indicate that mussels are reliable bio-indicators for endocrine disruptors in marine environment. In addition, we found that mussel *M. galloprovincialis* was a sensitive bioindicator to TBBPA pollution due to its ease of TBBPA bioaccumulation.

TBBPA exposure markedly promoted the gametogenesis in both male

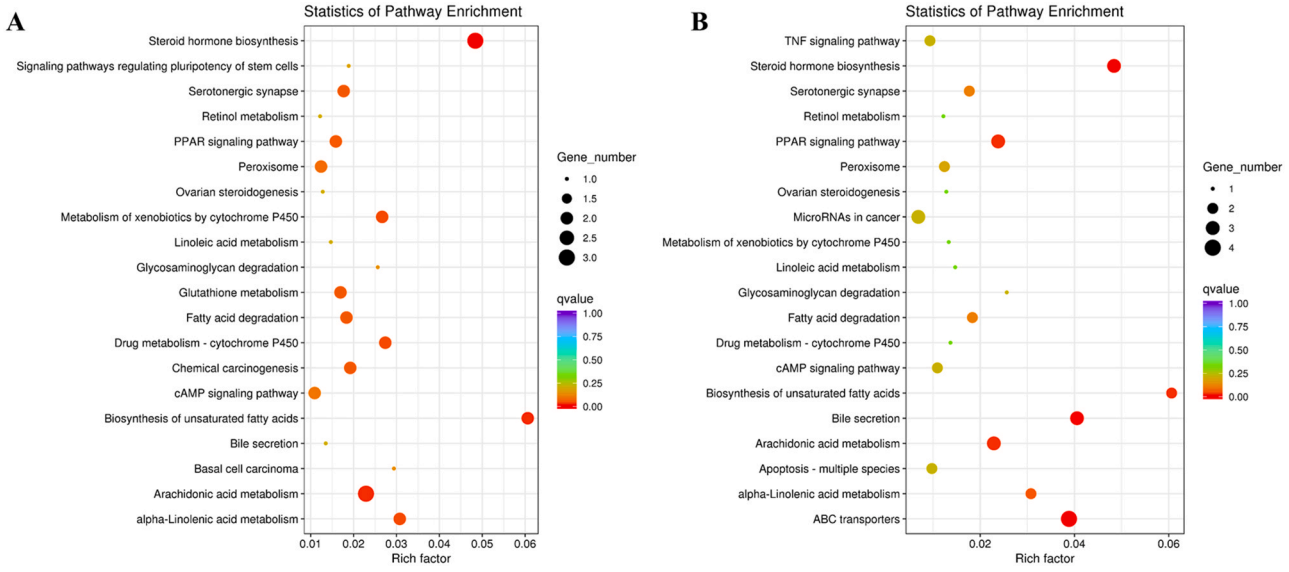


**Fig. 4.** Scatter plot of enriched KEGG pathways in the highest concentration (375 μg/L) of TBBPA-treated group. The enriched KEGG pathways in female groups (A) and male groups (B); rich factor is the ratio of the differentially expressed gene number to the total gene number in a certain pathway; *Q*-value is corrected *P*-value ranging from 0 ~ 1; the color and size of the dots represent the range of the *Q*-value and the number of DEGs mapped to the indicated pathways, respectively. Top 20 enriched pathways are shown in the figure.

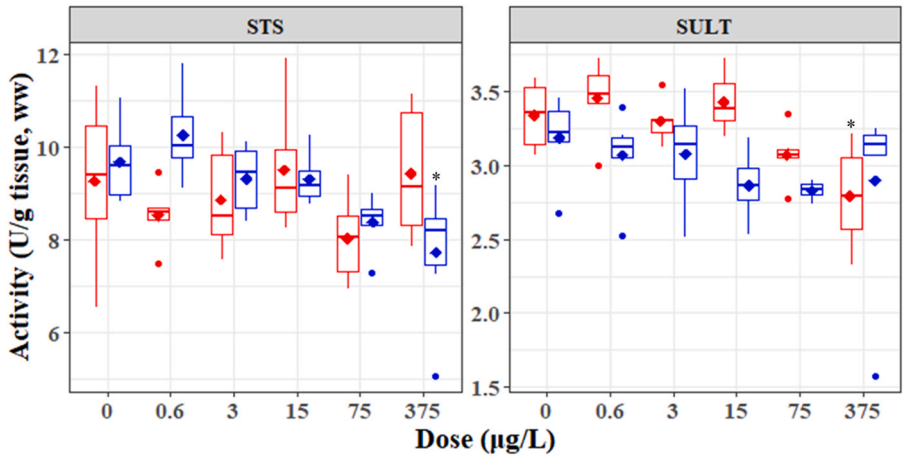


**Fig. 5.** Activities of estrogen sulfotransferases (SULT) and steroid sulfatase (STS) in mussel digestive glands ($n = 6$). Data are presented as box plots indicating the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles; filled circles indicate outliers; solid lines and points inside the box indicate median and mean, respectively; red and blue indicate female and male, respectively; * indicate significant differences $P < 0.05$ between control group and TBBPA-treated groups.

and female mussels supported by histological observation and gender-specific gene (*VERL* and *VCL*) expression. As the transcripts of *VERL* and *VCL* were uniquely expressed in female and male mussels, respectively, they were reliable biomarkers to indicate the events of reproduction in mussels (Anantharaman and Craft, 2012). Meanwhile, the decreased steroids in TBBPA-treated mussels were closely associated with the post-spawning stage of gonad. Yan et al. (2011) found that E2 and T decreased markedly in razor clam Sinonovacula constricta after spawning. Rapid decrease in E2 in females and T in males also occurred in Zhikong scallop Chlamys farreri after spawning (Liu et al., 2014). The correlation between vertebrate sex hormones and gametogenesis stages in clam and scallop was consistent with our finding. The acceleration of gametogenesis together with the changes of vertebrate sex hormones induced by TBBPA confirmed the reproductive endocrine-disrupting effects of TBBPA on mussels.

In general, modes of action of reproductive endocrine disruptors in vertebrates consist of simulating the action of the naturally produced hormones, inhibiting the action of natural hormones, changing the function and synthesis of hormone receptors, or altering the synthesis, transport, metabolism, and elimination of hormones (Reinen and Vermeulen, 2015). In this work, we found that steroid hormone biosynthesis (ko00140) was the target of TBBPA, supported by significant alterations of *STS*, *SULT*, and *CYP2*. As it is known, the sulfonation reaction by SULT1E1 and the hydrolysis of steroid sulfates by STS are responsible for modulating the biological activity of steroid hormones (Reed et al., 2005). Hence, steroidogenesis disruption by regulating steroid sulfonation and hydrolysis of the steroid sulfates might be the mode of action for TBBPA-induced reproductive endocrine-disrupting effects in mussels. A recent study on genetic responses in *M. edulis* exposed to 17β-ethinylestradiol and 4-nonlyphenol also found that steroidogenesis was affected based on the induction of steroidogenic acute regulatory protein (*StAR*) and adrenodoxin reductase (*Adx*) transcripts (Blalock et al., 2018), which presented a different mechanism of steroidogenesis dysregulation from those in TBBPA-treated mussels.

Steroidogenesis disruption in TBBPA-treated mussels might be attributed to TBBPA biotransformation. The metabolic process of xenobiotics (e.g. environmental toxicants) and unnecessary endobiotics (e.g. hormones) is basically comprised of three essential steps: phase I by enzymatic transformation, phase II by enzymatic conjugation, and phase III by transport (Xenotiotic metabolism). The up-regulation of genes of phase I enzyme CYP2, phase II enzymes GSTs and SULTs, and phase III enzyme P-glycoprotein suggested the induction of TBBPA biotransformation, which also tended to cause the adverse effects on sex hormones biosynthesis or metabolism since they shared similar metabolic pathways. Taking SULTs as an example, it has been reported that sulfonation by SULTs was one of the means for TBBPA detoxification in rats (Hakk et al., 2000; Knudsen et al., 2014), humans (Ho et al., 2017) and zebrafish (Kacew and Hayes, 2020). The superfamily of cytosolic *SULT* comprises 13 *SULT* genes and spans four families according to their substrates (Rizner, 2016). SULT1 consists of three phenol SULT subfamilies (SULT1A, SULT1B, and SULT1C) and an estrogen SULT (SULT1E), while SULT2s catalyze sulfonation of the hydroxyl groups of steroids (SULT2A1 and SULT2A2) (Lindsay et al., 2008). In this work, the up-regulation of *SULT1B* and *SULT1C* probably resulted from TBBPA's phenolic rings. However, there is often overlapping substrate selectivity among the SULTs. The common up-regulation of four *SULT1* isoforms was also observed in zebrafish liver cell lines treated with TBBPA (Yang and Chan, 2015). This means that if the activity of one form of SULT is altered (either inhibited or enhanced) by the presence of a xenobiotic, the sulfonation of endogenous and xenobiotic substrates for other isoforms may be affected (James and Ambadapadi, 2013). This could explain why *SULT1E1* was also activated in TBBPA-treated mussels. That is to say, the induction of *SULTs* for TBBPA biotransformation was responsible for the regulation of steroidogenesis and steroids metabolism (Monostory and Dvorak, 2011).

A recent study on crystallographic analysis has confirmed that TBBPA could bind to the steroid-metabolizing enzyme SULT1E1 (Gosavi et al., 2013). By competing for binding to the steroid-metabolizing enzyme estrogen SULT, TBBPA therefore acts as an inhibitor of SULT1E1 activity (Borghoff et al., 2016). Sulfonation of E2 by SULT1E1 is an important pathway for E2 inactivation, and inhibition of SULT1E1 may lead to an increase bioavailability of E2, which was regarded as a plausible molecular initiating event of reproductive toxicity caused by TBBPA (Lai et al., 2015; Wikoff et al., 2016). Therefore, the decrease in SULT1E1 activity suggested that the binding sites of SULT1E1 for estrogen were reduced because of TBBPA competing for binding. Similarly, the inhibition of SULT activity by TBBPA was also observed in scallop even at the low concentration (0.2 mg/L) (Hu et al., 2015). However, SULT1E1 activities in TBBPA-treated mussels were negatively related to its gene expression. The lack of correlation between SULT1E1 transcript levels and its activities implied that post-translational modifications might play vital roles in regulation of proteins (Rizner, 2016).

Apart from sulfonation reaction by SULT that was frequently discussed, the hydrolysis of the sulfate steroids into active steroids by STS also plays essential roles in modulating the biological activity of steroid hormones (Reed et al., 2005). The responses of STS to TBBPA were reported for the first time. The inhibition of STS activities occurred only in male individuals, suggesting the gender-specific effects of TBBPA on STS. The gender-specific transcriptional regulation of STS was also found in rodents with unknown mechanism (Garbacz et al., 2017). In

oyster *Crassostrea virginica*, Janer et al. (2005) found that STS might interfere with in vitro determination of sulfotransferase activity. The mechanism of STS response to TBBPA and its interaction with SULT deserve to be further investigated.

Non-genomic estrogen signaling pathway was regarded as another strategy in bivalves in response to endocrine disruptors (Blalock et al., 2018). Differing from genomic action, nongenomic pathway is activated through vertebrate sex hormones binding to receptors on plasma membrane or in cytosol. The downstream responses always rely on G-protein linked to MAPK/ERK or PI3-kinase pathways and occur with short lag time (Losel and Wehling, 2003). In this work, activation of genes involved in non-genomic pathways, such as GTPase related proteins and caveolin-1, was absent in TBBPA-treated mussels. Especially, caveolin-1 has been documented as a key hub gene within estrogen receptor signal transduction network and the most highly differentially expressed biomarkers in mussels treated with E2 (Blalock et al., 2018). Differing from the up-regulation of caveolin-1 in mussels treated with E2, caveolin-1-like was down-regulated in male mussels treated with the highest concentration (375 μg/L) of TBBPA. Taken together, non-genomic pathway seemed not to be the main target of TBBPA in mussels.

It was noteworthy that the promotion of gametogenesis, decrease in vertebrate sex hormones and enzymes activities, as well as the enrichment of many KEGG pathways were only identified in higher concentrations (75 and 375 μg/L) of TBBPA-treated groups. These TBBPA concentrations were far higher than those in waters from field environment such as the Chaohu Lake (4.87 μg/L) (Yang et al., 2012) and the Bohai Sea (0.336 μg/L) (Jiang et al., 2018). The environmental concentrations of TBBPA in field environment are not likely to induce such remarkable reproductive endocrine-disrupting effects like promotion of gametogenesis and alterations of vertebrate sex hormones in wild mussels. However, significant alterations of some DEGs such as *VERL*, *VCL* and other metabolic enzymes transcripts indeed occurred in mussels treated with environmentally relevant concentration (3 μg/L) of TBBPA. Due to its sensitiveness, the response at transcript level provided us with early warning signals of reproductive endocrine disruption. Hence, the potential ecological risk induced by chronic exposure to environmental TBBPA concentrations deserves to be paid more attention, since low concentration of TBBPA exposure covers the whole life stage of organisms and might induce more serious adverse effects.

## 5. Conclusions

In summary, the reproductive endocrine-disrupting effects and its potential mechanisms of TBBPA were reported in mussel *M. galloprovincialis* for the first time. We found that mussel *M. galloprovincialis* is a reliable bioindicator to TBBPA because of its ease of TBBPA bioaccumulation. TBBPA exposure for 30 days induced reproductive endocrine-disrupting effects in mussels of both sexes supported by the promotion of gametogenesis and alterations of vertebrate sex hormones. Influences on steroids sulfonation and hydrolysis of the sulfate steroids in mussels seemed to be the mode of action of TBBPA inducing reproductive endocrine-disrupting effects. All these responses might be the side effects of SULTs induction for TBBPA biotransformation. It is noteworthy that significant alterations of some DEGs such as *VERL*, *VCL* and other metabolic enzymes transcripts were even observed in mussels treated with environmentally relevant concentration (3 μg/L) of TBBPA, which reminds us that the potential ecological risk caused by chronic exposure to lower concentration of TBBPA deserves constitute concerns. Hence, it is necessary to determine the endpoints associated with reproduction in mussels collected from TBBPA pollution sites and evaluate the ecological risk of TBBPA in marine environment.

## Ethical statement

All procedures were strictly performed according to the Guidelines of the Chinese Council on Laboratory Animal Care (2001), which was approved by the Animal Research Ethics Board of Chinese Academy of Sciences.

## CRediT authorship contribution statement

**Shuang Wang:** Formal analysis; Writing - original draft. **Chenglong Ji:** Supervision, Writing - review & editing, Project administration. **Fei Li:** Writing - review & editing. **Junfei Zhan:** Formal analysis. **Tao Sun:** Formal analysis. **Tang Jianhui:** Writing - review & editing. **Huifeng Wu:** Supervision, Writing - review & editing, Project administration.

## 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126228.

## References

Anantharaman, S., Craft, J.A., 2012. Annual variation in the levels of transcripts of sex-specific genes in the mantle of the common mussel, *Mytilus edulis*. Plos One 7. https://doi.org/10.1371/journal.pone.0050861.

Blalock, B.J., Robinson, W.E., Loguinov, A., Vulpe, C.D., Krick, K.S., Poynton, H.C., 2018. Transcriptomic and network analyses reveal mechanistic-based biomarkers of endocrine disruption in the marine mussel, *Mytilus edulis*. Environ. Sci. Technol. 52, 9419–9430. https://doi.org/10.1021/acs.est.8b01604.

Borghoff, S.J., Wikoff, D., Harvey, S., Haws, L., 2016. Dose- and time-dependent changes in tissue levels of tetrabromobisphenol A (TBBPA) and its sulfate and glucuronide conjugates following repeated administration to female Wistar Han Rats. Toxicol. Rep. 3, 190–201. https://doi.org/10.1016/j.toxrep.2016.01.007.

Croll, R.P., Wang, C., 2007. Possible roles of sex steroids in the control of reproduction in bivalve molluscs. Aquaculture 272, 76–86. https://doi.org/10.1016/j.aquaculture.2007.06.031.

Fernandez-Tajes, J., Florez, F., Pereira, S., Rabade, T., Laffon, B., Mendez, J., 2011. Use of three bivalve species for biomonitoring a polluted estuarine environment. Environ. Monit. Assess. 177, 289–300. https://doi.org/10.1007/s10661-010-1634-x.

Garbacz, W.G., Jiang, M., Xie, W., 2017. Sex-dependent role of estrogen sulfotransferase and steroid sulfatase in metabolic homeostasis. Adv. Exp. Med. Biol. 1043, 455–469. https://doi.org/10.1007/978-3-319-70178-3_21.

Gauthier-Clerc, S., Pellerin, J., Amiard, J.C., 2006. Estradiol-17 beta and testosterone concentrations in male and female *Mya arenaria* (Mollusca bivalvia) during the reproductive cycle. Gen. Comp. Endocrinol. 145, 133–139. https://doi.org/10.1016/j.ygcen.2005.08.004.

Gosavi, R.A., Knudsen, G.A., Birnbaum, L.S., Pedersen, L.C., 2013. Mimicking of estradiol binding by flame retardants and their metabolites: a crystallographic analysis. Environ. Health Perspect. 121, 1194–1199. https://doi.org/10.1289/ehp.1306902.

Grasselli, E., Cortese, K., Fabbri, R., Smerilli, A., Vergani, L., Voci, A., Gallo, G., Canesi, L., 2014. Thyromimetic actions of tetrabromobisphenol A (TBBPA) in steatotic FaO rat hepatoma cells. Chemosphere 112, 511–518. https://doi.org/10.1016/j.chemosphere.2014.03.114.

Hakk, H., Larsen, G., Bergman, A., Orn, U., 2000. Metabolism, excretion and distribution of the flame retardant tetrabromobisphenol-A in conventional and bile-duct cannulated rats. Xenobiotica 30, 881–890. https://doi.org/10.1080/004982500433309.

Hines, A., Yeung, W.H., Craft, J., Brown, M., Kenned, J., Bignell, J., Stentiford, G.D., Viant, M.R., 2007. Comparison of histological, genetic, metabolomics, and lipid-based methods for sex determination in marine mussels. Anal. Biochem. 369, 175–186. https://doi.org/10.1016/j.ab.2007.06.008.

Ho, K.L., Yuen, K.K., Yau, M.S., Murphy, M.B., Wan, Y., Fong, B.M.W., Tam, S., Giesy, J. P., Leung, K.S.Y., Lam, M.H.W., 2017. Glucuronide and sulfate conjugates of tetrabromobisphenol A (TBBPA): chemical synthesis and correlation between their

urinary levels and plasma TBBPA content in voluntary human donors. Environ. Int. 98, 46–53. https://doi.org/10.1016/j.envint.2016.09.018.
Hu, F., Pan, L., Xiu, M., Jin, Q., Wang, G., Wang, C., 2015. Bioaccumulation and detoxification responses in the scallop *Chlamys farreri* exposed to tetrabromobisphenol A (TBBPA). Environ. Toxicol. Pharmacol. 39, 997–1007. https://doi.org/10.1016/j.etap.2015.03.006.
James, M.O., Ambadapadi, S., 2013. Interactions of cytosolic sulfotransferases with xenobiotics. Drug Metab. Rev. 45, 401–414. https://doi.org/10.3109/03602532.2013.835613.
Janer, G., Porte, C., 2007. Sex steroids and potential mechanisms of non-genomic endocrine disruption in invertebrates. Ecotoxicology 16, 145–160. https://doi.org/10.1007/s10646-006-0110-4.
Janer, G., Mesia-Vela, S., Kauffman, F.C., Porte, C., 2005. Sulfatase activity in the oyster *Crassostrea virginica*: Its potential interference with sulfotransferase determination. Aquat. Toxicol. 74, 92–95. https://doi.org/10.1016/j.aquatox.2005.05.004.
Ji, C., Wu, H., Wei, L., Zhao, J., Yu, J., 2013. Proteomic and metabolomic analysis reveal gender-specific responses of mussel *Mytilus galloprovincialis* to 2,2 ',4,4 '-tetrabromodiphenyl ether (BDE 47). Aquat. Toxicol. 140, 449–457. https://doi.org/10.1016/j.aquatox.2013.07.009.
Ji, C., Wu, H., Wei, L., Zhao, J., 2014. iTRAQ-based quantitative proteomic analyses on the gender-specific responses in mussel *Mytilus galloprovincialis* to tetrabromobisphenol A. Aquat. Toxicol. 157, 30–40. https://doi.org/10.1016/j.aquatox.2014.09.008.
Jiang, T., Zhu, L., Han, C., Wang, C., Bu, Y., Chen, X., 2018. The study on accumalation of tetrabromobisphenol A in zooplankton of Bohai Sea and Yellow Sea (Abstract in Chinese). Period. Ocean Univ. China, Nat. Sci. Ed. 48, 51–58. https://doi.org/10.16441/j.cnki.hdxb.20170057.
Kacew, S., Hayes, A.W., 2020. Absence of neurotoxicity and lack of neurobehavioral consequences due to exposure to tetrabromobisphenol A (TBBPA) exposure in humans, animals and zebrafish. Arch. Toxicol. 94, 59–66. https://doi.org/10.1007/s00204-019-02627-y.
Knudsen, G.A., Sanders, J.M., Sadik, A.M., Birnbaum, L.S., 2014. Disposition and kinetics of tetrabromobisphenol A in female Wistar Han rats. Toxicol. Rep. 1, 214–223. https://doi.org/10.1016/j.toxrep.2014.03.005.
Kotthoff, M., Ruedel, H., Juerling, H., 2017. Detection of tetrabromobisphenol A and its mono- and dimethyl derivatives in fish, sediment and suspended particulate matter from European freshwaters and estuaries. Anal. Bioanal. Chem. 409, 3685–3694. https://doi.org/10.1007/s00216-017-0312-z.
von Krogh, K., Ropstad, E., Nourizadeh-Lillabadi, R., Haug, T.M., Weltzien, F.A., 2019. *In vitro* effects of bisphenol A and tetrabromobisphenol A on cell viability and reproduction-related gene expression in Pituitaries from sexually maturing Atlantic cod (*Gadus morhua* L.). Fishes 4, 48. https://doi.org/10.3390/fishes4030048.
Lai, D.Y., Kacew, S., Dekant, W., 2015. Tetrabromobisphenol A (TBBPA): Possible modes of action of toxicity and carcinogenicity in rodents. Food Chem. Toxicol. 80, 206–214. https://doi.org/10.1016/j.fct.2015.03.023.
Lguchi, T., Watanabe, H., Katsu, Y., 2006. Application of ecotoxicogenomics for studying endocrine disruption in vertebrates and invertebrates. Environ. Health Perspect. 114, 101–105. https://doi.org/10.1289/ehp.8061.
Lindsay, J., Wang, L.L., Li, Y., Zhou, S.F., 2008. Structure, function and polymorphism of human cytosolic sulfotransferases. Curr. Drug Metab. 9, 99–105. https://doi.org/10.2174/138920008783571819.
Liu, A., Qu, G., Yu, M., Liu, Y., Shi, J., Jiang, G., 2016a. Tetrabromobisphenol-A/S and nine novel analogs in biological samples from the Chinese Bohai Sea: implications for trophic transfer. Environ. Sci. Technol. 50, 4203–4211. https://doi.org/10.1021/acs.est.5b06378.
Liu, J., Zhang, Z., Zhang, L., Liu, X., Yang, D., Ma, X., 2014. Variations of estradiol-17 beta and testosterone levels correlated with gametogenesis in the gonad of Zhikong scallop (*Chlamys farreri*) during annual reproductive cycle. Can. J. Zool. 92, 195–204. https://doi.org/10.1139/cjz-2013-0202.
Liu, K., Li, J., Yan, S., Zhang, W., Li, Y., Han, D., 2016b. A review of status of tetrabromobisphenol A (TBBPA) in China. Chemosphere 148, 8–20. https://doi.org/10.1016/j.chemosphere.2016.01.023.
Losel, R., Wehling, M., 2003. Nongenomic actions of steroid hormones. Nat. Rev. Mol. Cell Biol. 4, 46–56. https://doi.org/10.1038/nrm1009.
Monostory, K., Dvorak, Z., 2011. Steroid regulation of drug-metabolizing cytochromes P450. Curr. Drug Metab. 12, 154–172. https://doi.org/10.2174/138920011795016854.
Morris, S., Allchin, C.R., Zegers, B.N., Haftka, J.J.H., Boon, J.P., Belpaire, C., Leonards, P. E.G., Van Leeuwen, S.P.J., De Boer, J., 2004. Distributon and fate of HBCD and TBBPA brominated flame retardants in North Sea estuaries and aquatic food webs. Environ. Sci. Technol. 38, 5497–5504. https://doi.org/10.1021/es049640i.
Reed, M.J., Purohit, A., Woo, L.W.L., Newman, S.P., Potter, B.V.L., 2005. Steroid sulfatase: molecular biology, regulation, and inhibition. Endocr. Rev. 26, 171–202. https://doi.org/10.1210/er.2004-0003.
Reinen, J., Vermeulen, N.P.E., 2015. Biotransformation of endocrine disrupting compounds by selected phase I and phase II enzymes - formation of estrogenic and chemically reactive metabolites by cytochromes P450 and sulfotransferases. Curr. Med. Chem. 22, 500–527. https://doi.org/10.2174/0929867321666140916123022.
Rizner, T.L., 2016. The important roles of steroid sulfatase and sulfotransferases in gynecological diseases. Front. Pharmacol. 7, 1–6. https://doi.org/10.3389/fphar.2016.00030.
Seed, R., 1969. The ecology of *Mytilus edulis* L. (Lamellibranchiata) on exposed rocky shores: I. Breeding and settlement. Oecologia 3, 277–316. https://doi.org/10.1007/bf00390380.
Smolarz, K., Zabrzanska, S., Konieczna, L., Hallmann, A., 2018. Changes in steroid profiles of the blue mussel *Mytilus trossulus* as a function of season, stage of gametogenesis, sex, tissue and mussel bed depth. Gen. Comp. Endocrinol. 259, 231–239. https://doi.org/10.1016/j.ygcen.2017.12.006.
Vaughn, C.C., Hoellein, T.J., 2018. Bivalve impacts in freshwater and marine ecosystems. Annu. Rev. Ecol. Syst. 49, 183–208. https://doi.org/10.1146/annurev-ecolsys-110617-062703.
Wikoff, D.S., Rager, J.E., Haws, L.C., Borghoff, S.J., 2016. A high dose mode of action for tetrabromobisphenol A-induced uterine adenocarcinomas in Wistar Han rats: a critical evaluation of key events in an adverse outcome pathway framework. Regul. Toxicol. Pharmacol. 77, 143–159. https://doi.org/10.1016/j.yrtph.2016.01.018.
Yan, H., Li, Q., Liu, W., Ke, Q., Yu, R., Kong, L., 2011. Seasonal changes of oestradiol-17β and testosterone concentrations in the gonad of the razor clam *Sinonovacula constricta* (Lamarck, 1818). J. Mollusca Stud. 77, 116–122. https://doi.org/10.1093/mollus/eyq045.
Yang, J., Chan, K.M., 2015. Evaluation of the toxic effects of brominated compounds (BDE-47, 99, 209, TBBPA) and bisphenol A (BPA) using a zebrafish liver cell line, ZFL. Aquat. Toxicol. 159, 138–147. https://doi.org/10.1016/j.aquatox.2014.12.011.
Yang, S., Wang, S., Wu, F., Yan, Z., Liu, H., 2012. Tetrabromobisphenol A: tissue distribution in fish, and seasonal variation in water and sediment of Lake Chaohu, China. Environ. Sci. Pollut. Res. 19, 4090–4096. https://doi.org/10.1007/s11356-012-1023-9.
Zhang, H., Liu, W., Chen, B., He, J., Chen, F., Shan, X., Du, Q., Li, N., Jia, X., Tang, J., 2018. Differences in reproductive toxicity of TBBPA and TCBPA exposure in male *Rana nigromaculata*. Environ. Pollut. 243, 394–403. https://doi.org/10.1016/j.envpol.2018.08.086.
Zhang, Y., Xu, W., Lou, Q., Li, Y., Zhao, Y., Wei, W., Qin, Z., Wang, H., Li, J., 2014. Tetrabromobisphenol A disrupts vertebrate development via thyroid hormone signaling pathway in a developmental stage-dependent manner. Environ. Sci. Technol. 48, 8227–8234. https://doi.org/10.1021/es502366g.