



# Effect of polychlorinated biphenyls on osmoregulatory response and apoptosis in GIFT tilapia, *Oreochromis niloticus*

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**ABSTRACT.** In the present study, GIFT tilapia *Oreochromis niloticus* were exposed to polychlorinated biphenyls (PCBs) for 7, 14, and 21 days. Over the duration of the exposure, genotoxicity and the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) and Ca<sup>+</sup>/Mg<sup>+</sup>-ATPase (CMA) were measured in the gill, kidney, and intestine, to evaluate changes in osmoregulatory response in *O. niloticus*. Our results showed significant decreases in organic NKA (except in gill tissues after 0.5 mg/L PCB-exposure) and CMA activity. The results of the genotoxicity assay showed significant increases in *atp1a1a*, *nkcc2* (only in gill tissue), and *fxyd7* (except after

21 days of 5 mg/L PCB exposure). We found significant increases in caspase proteins in the liver in the 5-mg/L PCB exposure group, and the transcripts showed dose-dependent increases between treatment groups over the exposure duration. This study presents evidence that chronic exposure to PCB could result in organic osmoregulatory response and hepatic apoptosis in GIFT tilapia.

**Key words:** Apoptosis; *Oreochromis niloticus*; Osmoregulatory responses; Polychlorinated biphenyls

## INTRODUCTION

Our previous study indicated that hepatic antioxidant enzymatic activity significantly increased under methomyl exposure in *Oreochromis niloticus*, which is an ideal subject for toxicity experiments (Meng et al., 2014). The tropical GIFT tilapia (*O. niloticus*) is suitable for culture in warm waters, and is very sensitive to aquatic environmental factors (Gabriel et al., 2015). Fish genotoxicity, a more sensitive pollution indicator (Chakravarthy et al., 2014), might be a useful biomarker in ecotoxicological studies. The concentration of polychlorinated biphenyls (PCBs) in the aquatic environment ranges from 1.7 to 29.8 ng/L in the Yangtze River, China (Ge et al., 2014). PCBs function as oxidative stress inducers to produce excess reactive oxygen species, which was shown to result in hepatotoxicity in the Chinese rare minnow (*Gobiocypris rarus*) (Wu et al., 2014). Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) and Ca<sup>+</sup>/Mg<sup>+</sup>-ATPase (CMA) activity are the key endpoints in osmoregulatory pathway, while hepatic caspases are vital in apoptosis pathway. When fish exposed to toxicant, we hypothesized that osmoregulatory response and hepatic apoptosis would be impaired as measured by organic NKA and CMA activity and hepatic caspases, respectively, and that the related transcripts would be enhanced following PCB exposure. The main purpose of the present study was to investigate osmoregulatory responses (organic NKA and CMA activity), apoptosis (hepatic caspases) and transcriptional genotoxicity of freshwater *O. niloticus* in response to 0.5- and 5-mg/L PCB exposure.

Gill, kidney, and intestine were the main target organs associated with osmoregulatory response in *O. niloticus* exposed to heavy metals, which suggests that NKA and CMA activity can be used as sensitive biomarkers in metal contaminated waters (Atli and Canli, 2011). For example, NKA activity in the kidney was significantly increased in tilapia under cadmium exposure (Garcia-Santos et al., 2015), and it could therefore be used as a sensitive biomarker to assess the health status of tilapia populations (Wu et al., 2015). Tilapia gill NKA and Ca<sup>2+</sup>-ATPase could act as sensitive biomarkers in metal-contaminated waters (Eroglu and Canli, 2013). Caspases are a family of protease enzymes playing essential roles in programmed cell death and inflammation. Hepatic caspase 3, 8, and 9 activity in tilapia (*O. niloticus*) has been shown to increase in both perfluorooctane sulfonate and perfluorooctanoic acid exposed groups (Liu et al., 2007). Apoptosis has been found in *Oreochromis mossambicus* gill mitochondrion-rich cells in the form of osmoregulatory response under salt water stress (Inokuchi and Kaneko, 2012).

Two NKA α1 isoforms (α-1a and α-1b) are mainly expressed in the gill, and α-1a (NKA α1 polypeptide 1a, *atp1a1a*) is mainly expressed in freshwater tilapia (Baysoy et al., 2013). Gene transcriptional levels of ion pumps (such as NKA α3-subunit) have been found to show

significant increments in tilapia exposed to various stressors (Seale et al., 2014; Moorman et al., 2015). The key transporter associated with the NaCl transport process is thought to be Na<sup>+</sup>/K<sup>+</sup>/2Cl-cotransporter 1 (NKCC2, *nkcc2*) (Hwang and Lee, 2007). By interacting with NKA, the FXYP domain-containing ion transport regulator (*fxyp*) has been shown to be involved in teleost osmoregulation (Hu et al., 2014). FXYP7 (*fxyp7*) is mainly expressed in the brain, and a recent study found that FXYP7 could decrease K<sup>+</sup> affinity, thus, it contributes to enzyme stimulation at elevated extracellular K<sup>+</sup> concentrations (Li et al., 2013). Currently, the understanding of the function of FXYP7 in tilapia is limited.

## MATERIAL AND METHODS

### Experimental design

The parents for producing GIFT tilapia offspring (*O. niloticus*) were obtained from the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences, Yixing, where fertilized eggs were maintained. The one-month old *O. niloticus* juveniles that were used in the experiment were acclimatized to the aquarium facility with dechlorinated tap water at 28° ± 1°C, with 14 h:10 h light/dark cycle. The experimental fish were offered feed once a day (feed were purchased from Ningbo Tech-Bank Co. Ltd, Yuyao city, China). Fish (73.65 ± 3.24 g) were selected randomly for the exposure experiments. Throughout the experimental period, water samples were taken before and after each water change, the water conditions were as follows: pH, 7.0 ± 0.5; dissolved oxygen (tested by YSI 556MPS), 7.25 ± 0.11 mg/L; total phosphate, 2.05 ± 0.11 mg/L; total nitrogen and ammonia nitrogen (measured using Nessler's reagent spectrophotometry), 0.54 ± 0.12 and 0.46 ± 0.05 mg/L, respectively; total water hardness (ICP-OES, Optima 7000), 199.1 ± 10.5 mg/L CaCO<sub>3</sub>. The Animal Care and Use Committee of the Nanjing Agricultural University approved the experimental protocol, which was performed in accordance with the Guidelines for the Experimental Animals of the Ministry of Science and Technology (2006, Beijing, China).

The GIFT tilapia (N = 180) were divided into nine groups (N = 20 per aquarium, 1500 L, 1.5 m length x 1.0 m width x 1.0 m height). Each group of fish was exposed to 0, 0.5, or 5 mg/L PCB, respectively, in triplicate. PCBs were purchased from Sigma-Aldrich (containing PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180 at 10 mg/L per congener). The exposure period followed that used by Eroglu and Canli (2013), and the concentrations were based on previous studies (Wu et al., 2014; Zhao et al., 2015). Fish were exposed to test solutions for 21 days and all exposure solutions were replenished every 48 h with fresh exposure solutions at the same concentration during the exposure experiment. The control group was also kept for 21 days with fresh water changes but without the addition of PCBs every 48 h. There were no statistically significant differences in fish body weight or length among the different exposure groups. During the experiment, no fish mortality was observed.

### Fish sampling

All fish samples of the exposure and control groups were performed once a week. Samples for both gene expression (N = 3, gill, kidney, intestine, and liver) and biochemical analysis (N = 3, gill, kidney, intestine, and liver) were collected per replicate. The samples for the gene expression study were homogenized using Trizol reagent (Invitrogen), frozen

in liquid nitrogen, and immediately stored at  $-80^{\circ}\text{C}$ . Tissues for biochemical analysis were homogenized in ice-cold buffer containing 20 mM Tris-HCl, 0.25 M sucrose, and 1 mM EDTA (pH 7.7) with a ratio of 1/10 at 8273 g for 2-3 min. Homogenates were centrifuged at 13,000 g ( $4^{\circ}\text{C}$ ) for 20 min. The supernatants were collected for determination of total protein levels and ATPase activity.

### **Determination of osmoregulatory response**

The supernatant above was analyzed in triplicate for the NKA and CMA activity assays, using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Tests were quantified spectrophotometrically with a PowerWave XS2 (BioTek Instruments Inc., Winooski, VT, USA).

The final assay concentrations to measure tissue NKA and CMA activities (gill, kidney, and intestine) were 40 mM Tris-HCl, 120 mM NaCl, 20 mM KCl, 3 mM  $\text{MgCl}_2$ , 7.7 pH, and 1 mM ouabain. In addition, an incubation medium (pH 7.7) containing 40 mM Tris-HCl, 4 mM  $\text{MgCl}_2$ , 1 mM  $\text{CaCl}_2$ , and 1 mM EGTA was used for measuring the CMA activity. For measuring ATPase activity, 50  $\mu\text{L}$  enzyme suspension ( $\sim 100$   $\mu\text{g}$  protein) was added to 850  $\mu\text{L}$  incubation medium and was preincubated for 5 min at  $37^{\circ}\text{C}$ . The reaction was started by the addition of 100  $\mu\text{L}$   $\text{Na}_2\text{ATP}$  (3 mM) and 30 min incubation. The reaction was stopped by adding 500  $\mu\text{L}$  of ice-cold distilled water. Appropriate blanks were included with each assay to correct for non-enzymatic hydrolysis of ATP.  $\text{KH}_2\text{PO}_4$  (25-250  $\mu\text{M}$ ) was used as Pi standard and the spectrophotometric analysis was carried out at 390 nm using a Cecil 5000 series spectrometer. Specific NKA activity was calculated from the inorganic phosphate liberated from ATP using the differences between the presence (CMA activity) and absence (Total-ATPase activity) of the ouabain. CMA activity was measured as the absorbance difference between the presence and absence of  $\text{CaCl}_2$ . All assays were carried out in triplicate and ATPase activities were calculated as  $\mu\text{mol Pi/mg prot/h}$ . Total protein levels were determined according to Lowry et al. (1951) and bovine serum albumin was used as standard.

### **Determination of caspases**

Hepatic caspase 3, 8, and 9 activity was measured by using caspase 3, 8, and 9 Activity Assay Kits (Nanjing Kaiji Biotechnology, China), respectively, following the method of Genestier et al. (1998). Briefly, liver samples were homogenized in lysate buffer and the homogenate were centrifuged at 15,000 g for 20 min at  $4^{\circ}\text{C}$ . Then, the supernatants were collected and caspase 3, 8, and 9 activity were measured by using substrate peptides Ac-DEVD-pNA.

### **RNA extraction, reverse transcription (RT), and qRT polymerase chain reaction (PCR)**

Total RNAs were extracted from the gill, kidney, intestine, and liver of control and PCB exposed GIFT tilapia juveniles, using Trizol reagent and were subsequently treated with RNase-free DNase I (Fermentas, Canada). To investigate genomic DNA contamination and to verify the total RNA quality, the total RNA was loaded on a 1% agarose gel stained with EtBr (Sigma-Aldrich). We then investigated the 18/28S ribosomal RNA integrity using the spectrophotometric method (NanoDrop 1000, Thermo Scientific, USA). After determining RNA quality, cDNAs were synthesized from 3  $\mu\text{g}$  DNase I-treated total RNA, using the

M-MLV First Strand cDNA Kit (Invitrogen) with oligo (dT)<sub>12-18</sub> primers in a 20- $\mu$ L final volume, according to the manufacturer instructions. The cDNAs were used for cloning genes and carrying out gene expression analysis after normalization.

The qRT-PCR was performed using the CFX96 Real-Time PCR System (Bio-Rad, USA) with SYBR (TaKaRa, Japan). After normalization of each cDNA sample, the qRT-PCRs were carried out using 1X SYBR Premix Ex Taq<sup>TM</sup>, 0.4  $\mu$ M each primer, and 2.5  $\mu$ L RT reaction solution in a final volume of 25  $\mu$ L in triplicate. The reaction was initially denatured at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. A melt curve analysis was performed at the end of each PCR thermal profile to assess the amplification specificity.

*$\beta$ -actin* was the most stable reference gene under PCB exposure in our study, based on the selection method described by Zheng et al. (2014). The qRT-PCR primers for  *$\beta$ -actin*, *atp1a1a*, *nkcc2*, *fxyd7*, *caspase 3*, *caspase 8*, and *caspase 9* are shown in [Table S1](#). Caspase genes were identified in the liver, whereas *atp1a1a*, *nkcc2*, and *fxyd7* were observed in the gill, kidney, and intestine. Changes in expression levels of these genes after PCB exposure were calculated using the  $2^{-\Delta\Delta Ct}$  method with the formula,  $F = 2^{-\Delta\Delta Ct}$ ,  $\Delta\Delta Ct = (C_{t, \text{target gene}} - C_{t, \beta\text{-actin}})_{\text{PCBs}} - (C_{t, \text{target gene}} - C_{t, \beta\text{-actin}})_{\text{control}}$  (Livak and Schmittgen, 2001).

## Statistical analysis

All the experimental data are reported as means  $\pm$  SD. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) prior to analysis. The data were analyzed with one-way analysis of variance (ANOVA) followed by the least significant difference test (Ahmad et al., 2006) using SPSS Statistics 18.0 (SPSS Inc., Chicago, IL, USA), with  $P < 0.05$  indicating a significant difference. Data that did not conform to the assumptions of normality and homoscedasticity were transformed (log 10) and then analyzed as described above.

## RESULTS

### NKA and CMA activity

The tissue NKA and CMA activities (gill, kidney, and intestine) are shown in Table 1. Gill NKA activity in the 5 mg/L PCB exposure group were significantly decreased (after 7, 14, and 21 days) compared to both the control and 0.5 mg/L PCB exposure groups. Compared to the control group, both kidney and intestine NKA activity showed a significant decline both in the 0.5 and 5 mg/L PCB exposure groups at all three sample times. The kidney and intestinal NKA activity in the 5-mg/L PCB exposure group were also significantly lower than in the 0.5-mg/L PCB exposure group (Table 1).

The same patterns as found for NKA were found for kidney and intestinal CMA activity, which were significantly reduced in both the 0.5- and 5-mg/L PCB exposure groups in a dose-dependent manner. Compared to the control group, the gill CMA activity was significantly repressed in all PCB exposure groups, whereas no significant differences were found between the two PCB exposed groups.

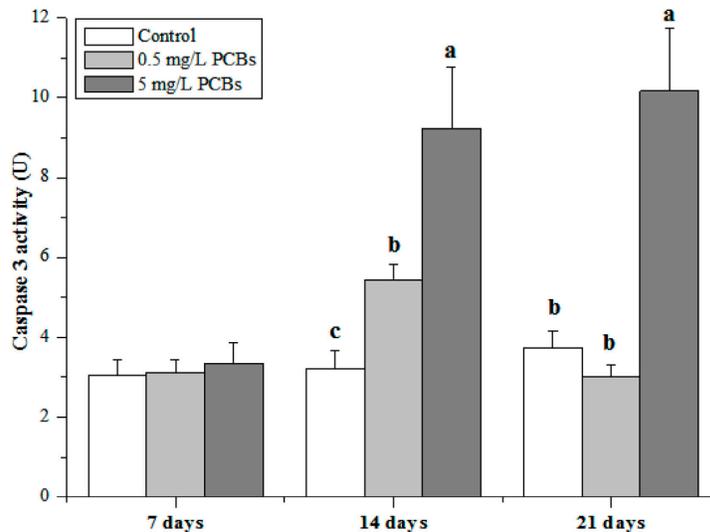
**Table 1.** Tissue NKA and CMA activity of *Oreochromis niloticus* under PCB exposure.

Day	Treatment (mg/L)	Gill activity (U/g prot)		Kidney activity (U/g prot)		Intestine activity (U/g prot)	
		NKA	CMA	NKA	CMA	NKA	CMA
7	Control	25.66 ± 6.32 <sup>a</sup>	7.95 ± 1.24 <sup>a</sup>	26.25 ± 4.92 <sup>a</sup>	5.12 ± 1.10 <sup>a</sup>	20.14 ± 3.22 <sup>a</sup>	6.62 ± 1.10 <sup>a</sup>
	0.5	24.14 ± 3.26 <sup>a</sup>	5.24 ± 1.02 <sup>b↓</sup>	22.65 ± 2.32 <sup>b↓</sup>	4.26 ± 0.45 <sup>b↓</sup>	18.62 ± 2.13 <sup>b↓</sup>	4.12 ± 0.87 <sup>b↓</sup>
	5	19.23 ± 3.49 <sup>b↓</sup>	4.67 ± 1.19 <sup>b↓</sup>	18.15 ± 3.09 <sup>c↓</sup>	3.01 ± 0.86 <sup>c↓</sup>	17.52 ± 1.94 <sup>c↓</sup>	3.12 ± 0.67 <sup>c↓</sup>
14	Control	25.45 ± 4.25 <sup>a</sup>	7.02 ± 1.47 <sup>a</sup>	24.18 ± 3.91 <sup>a</sup>	6.24 ± 0.58 <sup>a</sup>	20.47 ± 3.64 <sup>a</sup>	6.49 ± 0.96 <sup>a</sup>
	0.5	27.44 ± 3.64 <sup>a</sup>	5.16 ± 0.95 <sup>b↓</sup>	21.43 ± 2.52 <sup>b↓</sup>	4.15 ± 0.46 <sup>b↓</sup>	18.23 ± 0.96 <sup>b↓</sup>	5.03 ± 0.94 <sup>b↓</sup>
	5	18.79 ± 5.41 <sup>b↓</sup>	4.47 ± 0.89 <sup>b↓</sup>	17.69 ± 1.98 <sup>c↓</sup>	3.25 ± 0.51 <sup>c↓</sup>	16.47 ± 0.64 <sup>c↓</sup>	3.96 ± 1.00 <sup>c↓</sup>
21	Control	25.88 ± 3.21 <sup>a</sup>	7.61 ± 2.31 <sup>a</sup>	26.96 ± 2.43 <sup>a</sup>	6.24 ± 1.51 <sup>a</sup>	20.68 ± 2.65 <sup>a</sup>	6.75 ± 2.11 <sup>a</sup>
	0.5	26.93 ± 3.01 <sup>a</sup>	4.95 ± 0.92 <sup>b↓</sup>	23.01 ± 1.86 <sup>b↓</sup>	4.50 ± 0.61 <sup>b↓</sup>	17.29 ± 1.06 <sup>b↓</sup>	5.14 ± 0.64 <sup>b↓</sup>
	5	20.38 ± 3.66 <sup>b↓</sup>	5.48 ± 0.96 <sup>b↓</sup>	19.64 ± 0.98 <sup>c↓</sup>	3.20 ± 0.31 <sup>c↓</sup>	15.78 ± 1.04 <sup>c↓</sup>	3.58 ± 0.51 <sup>c↓</sup>

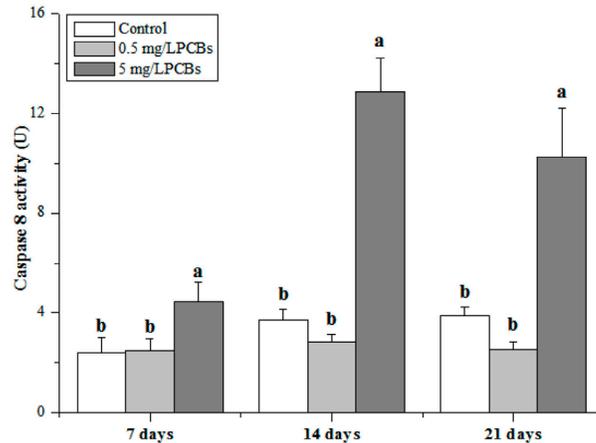
↑: up-regulation, ↓: down-regulation. Different lower-case letters indicate statistically significant differences ( $P < 0.05$ ).

## Caspase activity

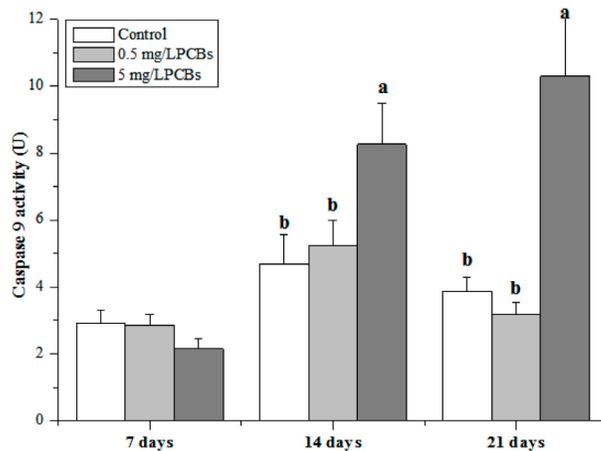
The activity of hepatic caspase 3 is shown in Figure 1. Caspase 3 activity was significantly increased compared to the control group in both the 0.5- (increased by 169% relative to the control group) and 5-mg/L (increased by 288%) PCB exposed groups at 14 days. In contrast, after 21 days, a significant increase was found only in the 5-mg/L PCB exposed group (which increased by 273%). In caspase 8, only the 5-mg/L PCB exposure groups showed a significant increase at all time points (at 7, 14, and 21 days the increase was 188, 346, and 264%, respectively) (Figure 2). Likewise, caspase 9 activity showed a significant increase in the 5-mg/L PCB exposure group, but only at 14 and 21 days (increased by 176 and 266%, respectively; Figure 3).



**Figure 1.** Caspase 3 activity of *Oreochromis niloticus* under PCB exposure. Different lower-case letters indicate statistically significant differences ( $P < 0.05$ ).



**Figure 2.** Caspase 8 activity of *Oreochromis niloticus* under PCB exposure. Different lower-case letters indicate statistically significant differences ( $P < 0.05$ ).



**Figure 3.** Caspase 9 activity of *Oreochromis niloticus* under PCB exposure. Different lower-case letters indicate statistically significant differences ( $P < 0.05$ ).

## Genotoxicology

The gene expression profiles of the osmoregulatory response-signaling pathway (*atp1a1a*, *nkcc2*, and *fxyd7*) are shown in Table 2. The *atp1a1a* transcripts of the three tissues showed significant increases in a dose-dependent manner at all sample time points. The *fxyd7* transcripts revealed significant dose-dependent increases after 7 and 14 days' PCB exposure. In contrast, after 21 days of exposure, a significant increase was observed in the 0.5-mg/L PCB exposure group, whereas a decrease was found in the 5-mg/L treatment group. Gill *nkcc2* transcripts demonstrated dose-dependent increases in both the 0.5 and 5 mg/L PCB exposure groups at all sample times, whereas no differences were found in kidney or intestine *nkcc2* transcripts.

**Table 2.** Transcriptional osmoregulatory response-related gene expression profiles in different *Oreochromis niloticus* tissues under PCB exposure.

Day	Treatment (mg/L)	<i>atp1a1a</i>			<i>nkcc2</i>			<i>fyd7</i>		
		Gill	Kidney	Intestine	Gill	Kidney	Intestine	Gill	Kidney	Intestine
7	Control	1.05 ± 0.14 <sup>c</sup>	1.07 ± 0.10 <sup>c</sup>	1.09 ± 0.08 <sup>c</sup>	1.14 ± 0.12 <sup>c</sup>	1.12 ± 0.13	1.02 ± 0.06	1.16 ± 0.10 <sup>c</sup>	1.07 ± 0.13 <sup>c</sup>	1.06 ± 0.14 <sup>c</sup>
	0.5	1.65 ± 0.09 <sup>b†</sup>	1.42 ± 0.07 <sup>b†</sup>	1.87 ± 0.05 <sup>b†</sup>	2.46 ± 0.19 <sup>b†</sup>	1.21 ± 0.14	1.40 ± 0.18	2.15 ± 0.25 <sup>b†</sup>	3.08 ± 0.17 <sup>b†</sup>	1.88 ± 0.11 <sup>b†</sup>
	5	3.78 ± 0.16 <sup>a†</sup>	4.67 ± 0.13 <sup>a†</sup>	3.57 ± 0.19 <sup>a†</sup>	3.49 ± 0.11 <sup>a†</sup>	1.40 ± 0.10	1.37 ± 0.12	5.18 ± 0.16 <sup>a†</sup>	6.49 ± 0.15 <sup>a†</sup>	4.09 ± 0.25 <sup>a†</sup>
14	Control	1.04 ± 0.21 <sup>c</sup>	1.06 ± 0.07 <sup>c</sup>	1.08 ± 0.09 <sup>c</sup>	1.09 ± 0.13 <sup>c</sup>	1.10 ± 0.24	1.11 ± 0.14	1.14 ± 0.11 <sup>c</sup>	1.04 ± 0.08 <sup>c</sup>	1.05 ± 0.12 <sup>c</sup>
	0.5	2.25 ± 0.17 <sup>b†</sup>	3.46 ± 0.19 <sup>b†</sup>	2.96 ± 0.22 <sup>b†</sup>	4.68 ± 0.65 <sup>b†</sup>	1.24 ± 0.08	1.47 ± 0.40	4.27 ± 0.36 <sup>b†</sup>	2.49 ± 0.16 <sup>b†</sup>	3.45 ± 0.17 <sup>b†</sup>
	5	4.19 ± 0.11 <sup>a†</sup>	6.82 ± 0.15 <sup>a†</sup>	9.56 ± 0.30 <sup>a†</sup>	8.65 ± 0.11 <sup>a†</sup>	1.64 ± 0.10	1.33 ± 0.60	6.94 ± 0.10 <sup>a†</sup>	6.78 ± 0.22 <sup>a†</sup>	7.95 ± 0.44 <sup>a†</sup>
21	Control	1.10 ± 0.09 <sup>c</sup>	1.08 ± 0.12 <sup>c</sup>	1.16 ± 0.14 <sup>c</sup>	1.12 ± 0.07 <sup>c</sup>	1.05 ± 0.16	1.04 ± 0.17	1.13 ± 0.20 <sup>b</sup>	1.06 ± 0.10 <sup>b</sup>	1.09 ± 0.16 <sup>b</sup>
	0.5	5.21 ± 0.36 <sup>a†</sup>	6.21 ± 0.14 <sup>b†</sup>	2.08 ± 0.11 <sup>b†</sup>	2.69 ± 0.16 <sup>a†</sup>	1.25 ± 0.15	1.20 ± 0.16	4.36 ± 0.14 <sup>a†</sup>	2.64 ± 0.10 <sup>a†</sup>	3.26 ± 0.18 <sup>a†</sup>
	5	8.65 ± 0.17 <sup>a†</sup>	9.54 ± 0.55 <sup>a†</sup>	6.01 ± 0.16 <sup>a†</sup>	5.22 ± 0.30 <sup>a†</sup>	1.10 ± 0.16	1.30 ± 0.34	0.36 ± 0.16 <sup>↓</sup>	0.18 ± 0.07 <sup>↓</sup>	0.31 ± 0.05 <sup>↓</sup>

↑: up-regulation, ↓: down-regulation. Different lower-case letters indicate statistically significant differences (P < 0.05).

The hepatic gene expression profiles of the apoptosis signaling pathway (*caspase 3*, 8, and 9) are shown in Table 3. Hepatic *caspase 3*, 8, and 9 transcripts were all significantly higher in the 5-mg/L PCB exposure group than in the 0.5-mg/L PCB exposure groups at all time points, and both the 0.5- and 5-mg/L PCB exposure groups were significantly higher than the control group, which suggest dose-dependent increments between treatment groups.

**Table 3.** Hepatic apoptosis-related gene expression profiles in *Oreochromis niloticus* liver under PCB exposure.

Day	Treatment (mg/L)	<i>caspase 3</i>	<i>caspase 8</i>	<i>caspase 9</i>
7	Control	1.06 ± 0.08 <sup>e</sup>	1.12 ± 0.09 <sup>e</sup>	1.10 ± 0.17 <sup>c</sup>
	0.5	2.16 ± 0.14 <sup>b†</sup>	3.11 ± 0.17 <sup>b†</sup>	2.67 ± 0.16 <sup>b†</sup>
	5	6.26 ± 0.18 <sup>a†</sup>	5.32 ± 0.24 <sup>a†</sup>	7.79 ± 0.57 <sup>a†</sup>
14	Control	1.02 ± 0.07 <sup>e</sup>	1.09 ± 0.07 <sup>e</sup>	1.14 ± 0.10 <sup>c</sup>
	0.5	3.49 ± 0.19 <sup>b†</sup>	2.66 ± 0.10 <sup>b†</sup>	4.78 ± 0.24 <sup>b†</sup>
	5	6.81 ± 0.44 <sup>a†</sup>	5.19 ± 0.46 <sup>a†</sup>	6.66 ± 0.77 <sup>a†</sup>
21	Control	1.16 ± 0.11 <sup>e</sup>	1.06 ± 0.10 <sup>e</sup>	1.11 ± 0.11 <sup>c</sup>
	0.5	3.67 ± 0.65 <sup>b†</sup>	3.51 ± 0.29 <sup>b†</sup>	3.09 ± 0.48 <sup>b†</sup>
	5	7.31 ± 0.44 <sup>a†</sup>	6.00 ± 0.80 <sup>a†</sup>	5.34 ± 0.30 <sup>a†</sup>

†: up-regulation, ‡: down-regulation. Different lower-case letters indicate statistically significant differences ( $P < 0.05$ ).

## DISCUSSION

The present study focused on investigating the organic osmoregulatory response and hepatic apoptosis in tilapia during PCB exposure. Our results showed that NKA and CMA activity (in the gill, kidney, and intestine) were significantly reduced, with the exception of the 0.5-mg/L PCB exposure group in gill tissue. These results agree with results observed in tilapia following Cu-exposure (Wu et al., 2015) as well as following Zn, Cd, and combined Zn-Cd exposure (Eroglu and Canli, 2013). Lerner et al. (2007) reported that juvenile Atlantic salmon (*Salmo salar*) exposed to PCB-1 or PCB-10 during smolting exhibited a dose-dependent reduction in preference for seawater. The current study implies that dose-dependent changes also in organic NKA activity (as for the related genes, except for kidney and intestinal *nkcc2* transcripts), CMA activity, and hepatic caspase transcripts. We speculate that NKA/CMA activity and hepatic caspase transcripts could act as sensitive biomarkers for different concentrations of PCB exposure in tilapia. Further studies are needed to explore the actual mechanism of how PCBs work in different pathways (based on different responses at various exposure time points).

When the fish gill energy faces acute transfer, phosphocreatine acts as an energy source to meet the osmoregulatory demand through the activity of NKA activity. In the present study, *atp1a1a* (NKA), *nkcc2* (NKA transporter) and *fxyd7* (NKA transport regulator) were detected in three osmoregulatory response-related tissues. When faced with a hyperosmotic challenge, the transcripts of organic osmoregulatory response-related genes (gill *nkcc2*, *atp1a1a*, and *fxyd7*) were found to be induced. This is consistent with the results found in a previous study in which tilapia were transferred from freshwater to seawater (Moorman et al., 2015). Kidney and intestinal *nkcc2* transcripts were unchanged in the present study. The cause for this may be that either *nkcc2* transcripts were generally lower in the middle segments of the intestine, or are generally more highly expressed in seawater-acclimated fish, compared with freshwater-acclimated fish (Seale et al., 2014). Our results suggested that PCB exposure not only accelerates the acute transfer of ions in the gill, but also ion uptake/excretion in osmoregulatory response-related organs. Hu et al. (2014) reported that gill and kidney *fxyd11/12* transcripts were negatively correlated with NKA activity in spotted scat

(*Scatophagus argus*), whereas intestinal *fxyd11/12* correlated positively with NKA activity. In the present study, *fxyd7* transcripts were significantly up-regulated, suggesting that *fxyd7* is involved in the osmoregulatory response in tilapia following PCB exposure (Hu et al., 2014).

Free ceramide production, resulting from osmoregulatory response, serves as an important stress-signaling molecule in apoptosis (Mizushima et al., 1996). In caged *O. niloticus*, Franco et al. (2010) found caspase 3 activation in polluted sites of industrial cities in southern Brazil. Kumar et al. (2009) demonstrated that up-regulation of serum caspase 3 of *O. mossambicus* might contribute to the decreased phagocytic activity upon exposure to hyperosmotic solutions. It has also been reported that prolonged apoptosis occurred in *O. mossambicus* exposed to elevated salinity *in vitro* (Kammerer and Kültz, 2009), which suggests that caspase proteins are usually induced when fish are faced with long-term exposure to osmoregulatory stress. Yadetie et al. (2014) demonstrated that genes associated with apoptosis pathways were significantly enriched in juvenile Atlantic cod (*Gadus morhua*) exposed to PCB153 (at 0.5, 2, and 8 mg/kg body weight) for two weeks. The current study revealed a significant activation of caspase 3 (except for at 7 days), 8, and 9 (except for at 7 days) in the 5-mg/L PCB exposure groups, and that hepatic caspase (3, 8, and 9) transcripts were also significantly enhanced. This suggests that hepatic apoptosis was impaired in GIFT tilapia under PCB exposure.

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## Supplementary material

**Table S1.** Tilapia primers used for qRT-PCR in the study of *Oreochromis niloticus*.